TGA High Tunnel Cover Crop Study: Winter annual legumes and mixes in on-farm rotations

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Introduction/Background

High tunnels on farms provide a covered growing space with warmer day and night temperatures, as well as protection from wind and precipitation. In the harsh climate of the Upper Midwest, farmers can especially benefit from high tunnel season extension potential. Due to large initial costs for high tunnel construction, they are generally used for intensive high value crop production by small-acreage specialty crop farmers in order to maximize the use of the protected space and benefit from premium prices for off-season produce. Prolonged intensive cultivation may lead to depletion of soil nutrients and reduced yields, which farmers address by adding nutrient-dense inputs, including manure and compost. Over time, high levels of these inputs may lead to nutrient imbalances and excesses, and decrease plant and soil health. Legume cover crops can be an alternative source of soil fertility; replacing nitrogen fertilizers in high tunnel systems through symbiotic nitrogen fixation (SNF). Legumes are capable of SNF due to a unique relationship with rhizobia soil bacteria housed in nodules on legume roots. Overwintered legumes in high tunnels have been shown to contribute nitrogen (N) to summer cash crops and offer other soil health improvements like increased soil carbon and microbial activity without adding to nutrient imbalance (Perkus 2018; Rudisill et al. 2015). More studies on overwintered cover crops in tunnels are needed to better understand potential benefits, management challenges, and farmer perspectives on these techniques.

Understanding the seasonal change in growth, nodulation, and SNF of overwintered high tunnel legumes may help to predict fertility contributions. In the Upper Midwest, average outdoor winter temperatures typically remain below freezing, but in high tunnels overwintering plants are challenged by large diurnal temperature fluctuations. Maximum daily temperatures of 20°C (68°F) and minimums of -24°C (-11°F) observed during mid-winter have little-known effects on plant growth, and may reduce SNF, leading to lower N contributions to following cash crops. Study of common cover crop legumes in Upper Midwest high tunnels during the winter season could lead to more informed recommendations of suitable species for high tunnel production, as well as a greater understanding of SNF under tunnel conditions.

The aim of this study was to build and share knowledge about cover crop use, winter annual legumes as cover crops, and SNF in high tunnels. Our objectives were to quantify 1) legume cover crop productivity, 2) nodulation, and 3) SNF throughout the winter annual season in Upper Midwest high tunnels to better understand legume cover crop performance, potential fertility contributions, and best practices in high tunnel cover cropping for widespread use by growers.

Materials and Methods

Experimental design

This study was conducted in high tunnels at five sites in Minnesota and Wisconsin, which spanned three plant-hardiness zones, from 4a to 3a. The study was designed as a Mother and Baby trial, an approach developed for on-farm participatory research (Snapp, 1999). Two mother sites were replicated to allow for statistical analysis, including the North Central Research and Outreach Center in Grand Rapids, MN and the Horst-Rechlebacher farm in Osceola, WI. Three demonstration sites, Page and Flowers Farm, HAFA Farm, and Cala Farm, were un-replicated baby sites, each located on working farms (Table 1).

Table 1. High tunnel location, climate, regional and spatial metrics

High tunnel location	**Orientation**	**Replications**	**Plot size (m^2)**	**Plant Hardiness zone**	**Average frost-free days**	**Soil type**
Grand Rapids (GR)	N-S	3	5.9	3b	118	Shooker sandy loam/ Rosy sandy loam
Osceola (Horst)	N-S	4	15.1	4a	140	Hubbard sandy loam
Saint Paul (PF)	E-W	1	4.5	4b	160	Urban land-Waukegan complex
Rosemount (HAFA)	N-S	1	3.9	4b	144	Wadena loam
Turtle Lake (Cala)	N-S	1	7.4	4a	139	Crystal Lake silt loam

Site preparation

In late August and early September 2017 seed beds in the high tunnels were prepped for cover crop planting. Baseline soil samples were collected prior to planting, and aggregate samples sent to the soil testing lab at the University of Minnesota for analysis (results of baseline soil tests are in Table 2).

Table 2. Results of baseline soil tests for all sites, Fall 2017.

Site	**NO3 (mg kg^{-1}) (Soil nitrate)**	**% Organic Matter**	**Bray P (ppm)**	**NH4-K (ppm)**	**Soil water pH**
GR	11.8	3.0	120	311	6.7
Horst	178.7	4.1	85	344	6.9
Cala	21.3	2.6	80	160	6.6
HAFA	11.0	2.6	263	360	6.2
PF	43.1	8.9	105	207	7.0

Cover crop treatments

Mother high tunnels had four cover crop treatments:

- CC, a monoculture of crimson clover (*Trifolium incaratum*)
- RC, a monoculture of red clover (*Trifolium pratense*)
- VM, a mix of tillage radish, winter rye and hairy vetch (*Raphanus sativus, Secale cereale, Vicia villosa)*
- PM, a mix of winter rye and Austrian winter pea (*S. cereale, Pisum sativum*)

Baby high tunnels had three cover crop treatments: RC, VM, and PM.

Inoculation and seeding

Before planting, legume seeds were inoculated with a compatible rhizobia inoculant. See Table 3 for seeding rates, sources, and rhizobia inoculant species.

Table 3. Seed and rhizobia inoculant data

Seed variety	**Source**	**Seeding rate ($kg\ ha^{-1}$)**	**Rhizobia species in Inoculant**
Crimson clover	Johnny's Selected Seeds, LOT: 54643	28.0	*Rhizobium leguminosarum* biovar *trifolii*
Medium red clover	Johnny's Selected Seeds, LOT: 16A-32IL	13.5	pre-inoculated (*Rhizobium leguminosarum* biovar *trifolii)*
Austrian winter pea	Albert Lea, LOT: 2017	42.0	*Rhizobium leguminosarum* biovar *viceae*
Winter rye	Johnny's Selected Seeds, LOT: 57277	42.0	N/A
Winter max Mix (Radish, Vetch, Rye)	Albert Lea Seed, LOT: 2017-OCC3	84.1	*Rhizobium leguminosarum* biovar *viceae* (for vetch)

Seeds were broadcast and raked to ensure seed-to-soil contact and burial. Plots were watered as-needed until late October/early November. Beds were then covered in floating row-cover and tunnel sides and doors closed. Row cover and tunnel closure were efforts to prevent desiccation and frost damage to cover crops.

Sample Collection and Measurements

Legume roots, shoots, and nodules were sampled at four seasonal time-points to determine impact of winter high tunnel conditions on cover crop persistence and SNF indicators. Sampling times were early November 2017, January 2018, March 2018, and at cover crop termination in late April/early May 2018. Four legumes from each plot were sampled at each time-point. Living, green tissue of legume shoots was collected along with the roots and surrounding soil. Shoots were dried and weighed. Roots were washed and nodules were counted, removed, dried and weighed. Roots were also dried and weighed separately. Before drying, fresh nodules were observed for signs of active SNF, and were scored as "pink" (actively fixing N) or "not pink" (not actively fixing N).

Sampled legume biomass was used for SNF analysis. Adjacent winter rye biomass was also collected, dried and weighed at each sampling for use as reference plant material for SNF analysis. Dried shoot biomass of legumes and rye was ground and analyzed for N content and N isotope ratio. The difference in N isotope ratio between the non-N-fixing rye and the N-fixing legumes allowed us to calculate the percent of N the legumes derived from the atmosphere, or %Ndfa, a quantitative measurement of SNF.

In addition to collecting legume roots and shoots, measurements of stand biomass were collected in 0.10 m^2 quadrats at the final sampling in April/May 2018 prior to termination. Above-ground biomass of all plants within the quadrat was cut and separated by species. Biomass was dried and weighed to quantify relative cover crop species and weed abundance at termination.

Air temperature was collected every 30 minutes by four temperature sensors in each tunnel. Temperature data was used to calculate accumulated heat units in the form of Growing Degree Days (GDD), with 4°C used as a base temperature.

A note about the Cala Farm trial: Cover crops at Cala Farm were seeded in early November due to late fall use of the tunnel by the farmer. The seeds broadcast in this trial did not germinate until late winter/early spring, and cover crop mixes had very little biomass until late March/early April, so all cover crop measurements were collected at the final sampling time.

Results and Discussion

Baby sites/Farm sites

Unreplicated baby site cover crop data are reported as observational data, averaged across cover crop species (see Table 5). Cover crops in these tunnels served important non-quantitative purposes as farmer-to-farmer learning and demonstration sites during on-farm workshops. For researchers, these farm sites were ideal for observing practical challenges of high tunnel cover cropping for working farms, such as issues with planting timing and termination equipment. Statistical differences reported are from the mother sites at Grand Rapids and Horst, with the exception of temperature measurements, which were replicated at all sites.

Seasonal temperature fluctuations and heat accumulation in high tunnels

- Temperature fluctuation in high tunnels was large, even during the shortest days of winter (Figure 1). During the month of December at Grand Rapids in Northern Minnesota, daily high temperatures were consistently 14°C greater than low temperatures. Overall, mean daily temperatures at Grand Rapids were lower than those at Horst.

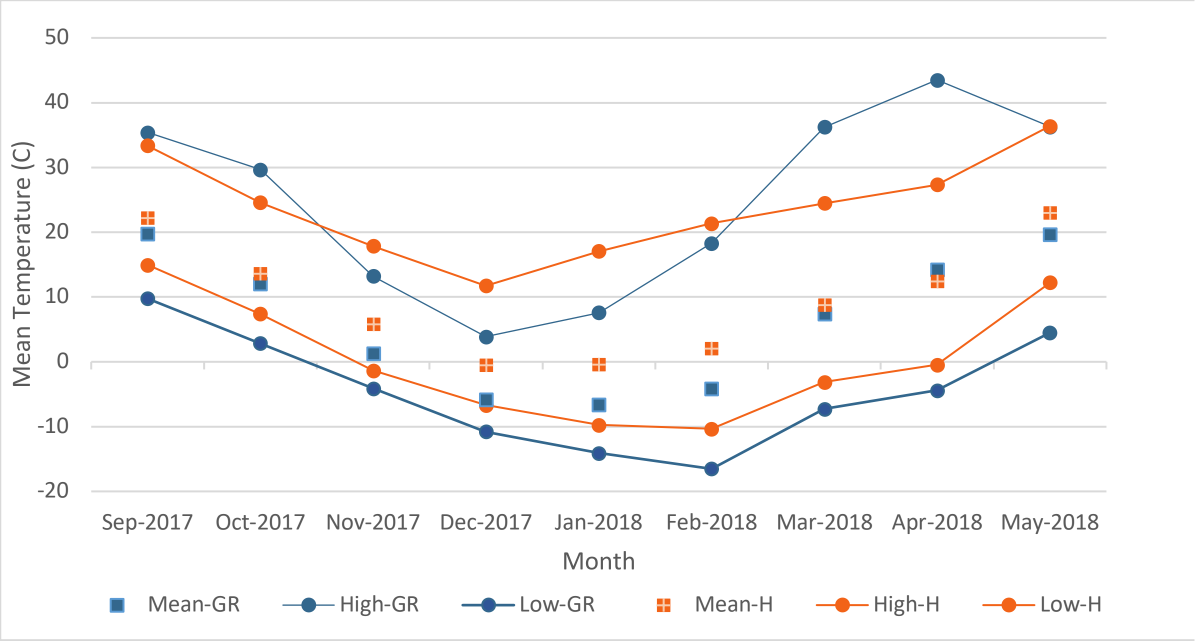

Figure 1. Average daily minimum, mean, and maximum temperatures (°C) at Grand Rapids (GR) and Horst (H) for each month.

- Growing Degree Days (GDD) accumulated most from late August to mid-October, and then again from late March to termination in May. See Table 4 for Cumulative GDD between each sampling time-point.

Table 4. Cumulative GDD for all sites during each period between samplings. For comparison across different sampling dates, estimates were standardized to match the earliest sampling date from each time-point. True cumulative GDD at each site at the time of termination are reported as Total GDD.

Site	Page and Flowers	HAFA	Cala	Grand Rapids	Horst
Sampling time					
			------------------- Accumulated GDD -------------------		
1 (Sep1-Nov2)	892.1	994.9	ND[1]	937.3	982.5
2 (Nov2-Jan10)	34.2	223.6	39.0	55.4	167.4
3 (Jan11-Mar15)	68.4	337.7	72.5	179.2	177.8
4 (Mar16-Apr27)	304.9	549.8	404.9	609.2	352.8
Total GDD (Non-standardized)	1417.2	2153.9	720.0	2327.4	1830.0

[1] Not measured due to late cover crop seeding.

- Mean daily temperatures at the three baby sites were compared for November through April (Cala was not planted until November). HAFA had the highest temperatures overall, followed by Cala and then Page and Flowers (PF). In the other months, HAFA also had greater mean temperatures than PF (Figure 2).

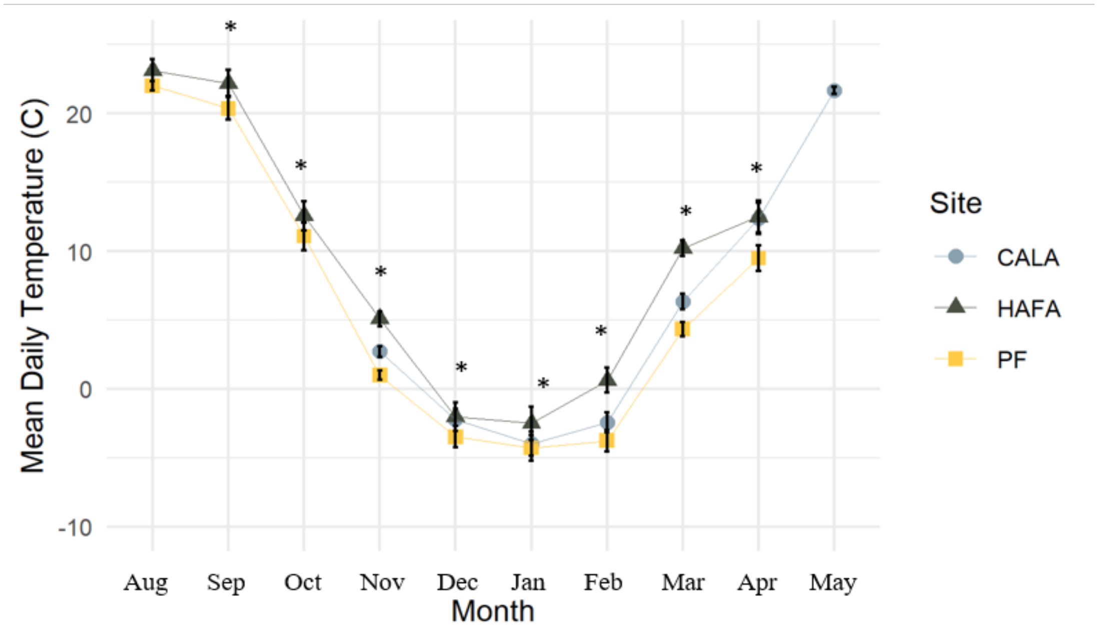

Figure 2. Mean daily temperatures at each month during the winter annual season for three baby sites. Stars indicate differences between sites within month (Tukey's HSD, $p<0.05$).

Seasonal changes in legume cover crop biomass and survival

- Overall, legume biomass was lower in March than in November, January, and May. Biomass followed a seasonal pattern that mirrored mean temperature changes in the tunnel. displaying a pronounced dip following the coldest period of the winter-annual season.
- Sites differed in legume biomass over time: at GR red clover and hairy vetch had more biomass in the fall, died back in the winter and showed spring re-growth, but Austrian winter pea and crimson clover died back heavily by the March sampling and did not recover biomass in the spring. No Austrian winter pea survived the winter in Grand Rapids. At Horst, likely due to warmer overall temperatures, crimson clover and winter pea had greater survival and individual spring biomass (Figure 3).
- Red clover and hairy vetch seem to be the best legume cover crops for over-wintering in high tunnels, with greater cold tolerance and more spring growth compared to crimson clover and winter pea.

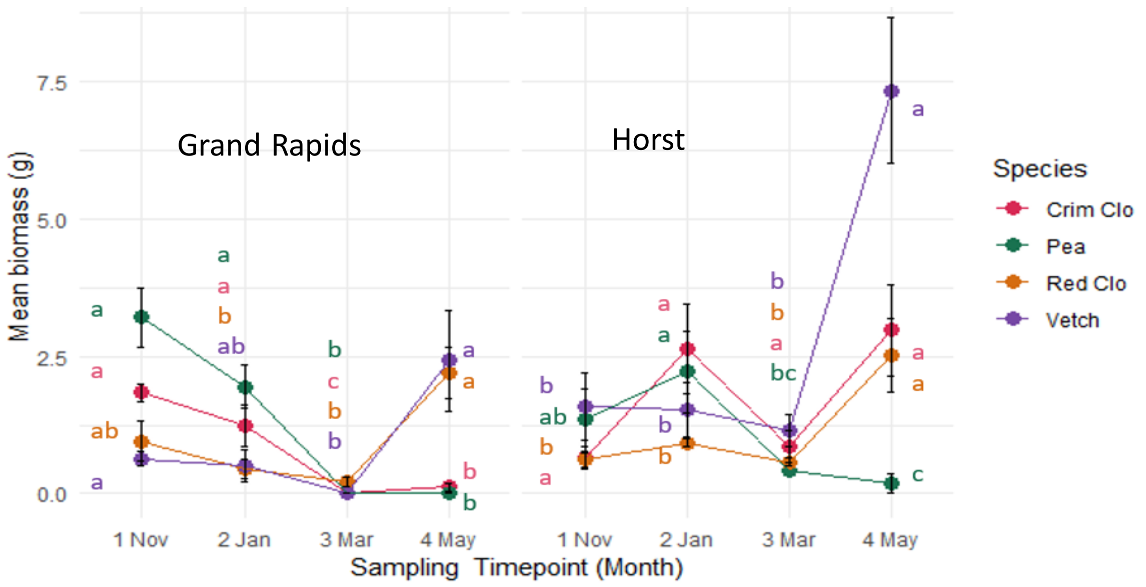

Figure 3. Aboveground legume biomass of the two mother sites at four sampling time-points. Error bars are 1 SE. Letters indicate significant differences within species and site, means were separated by Tukey's HSD, α = 0.05.

Spring biomass of cover crop stands

- The different cover crop treatments had equal amounts of spring biomass (kg ha^{-1}) at termination in May. Farmers could get similar total biomass from any of the mixes/monocultures tested in this study.
- Winter annual cover crops in high tunnels produced more spring biomass earlier than the same crops in the field. High tunnel cover crop biomass at Grand Rapids in mid-May was comparable to or greater than biomass measured in the field in early summer at the same site. We found over 2500 kg ha^{-1} of combined vetch and rye biomass in the VM mix and 123 kg ha^{-1} red clover in RC, while only 1950 kg ha^{-1} vetch/rye mix and 80 kg ha^{-1} red clover were measured under field conditions by Liebman et al. (2019). In high tunnels in warmer zones such as St. Paul and Rosemount, similar or greater biomass could be produced as early as April. Farmers growing cover crops in high tunnels get a "head start" on cover crop productivity in the spring.
- Using cover crop mixes may have reduced pea and vetch individual biomass and biomass in kg ha^{-1} through competition from other species, especially winter rye. Winter rye was the dominant species in PM and VM plots. Greater seeding rates of legumes in these mixtures may improve legume biomass and potential N contributions for growers.

- Cover crop biomass was equal or greater than weed biomass in all mixes and monocultures, suggesting cover crops suppressed weeds. Weed suppression is an important function of winter cover crops for farmers, especially in organic systems.
- Despite greater overall temperatures at HAFA, it did not have the highest final cover crop biomass and had no surviving Austrian winter pea (Figure 4.2). This could be due to large temperature fluctuation at HAFA; the day temperatures were very high, raising the mean daily temperatures, but night temps were similarly cold to the other farm sites.
- Late germination did not hinder spring cover crop growth. At Cala Farm, where cover crops were seeded in November and did not germinate until March, there was substantial biomass at termination. These results may indicate that an early spring seeding of cover crops in a high tunnel could be beneficial for farmers who have missed the timing for fall-planting.

(See Figures 4.1 and 4.2 for spring biomass data)

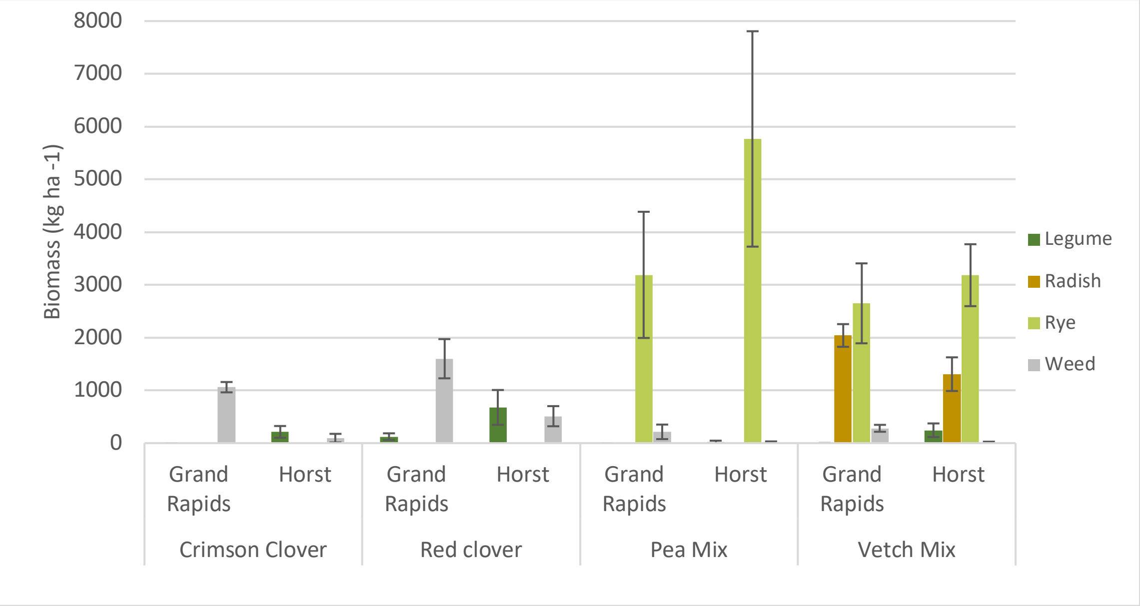

Figure 4.1 Biomass of cover crop species and weeds at the mother sites at the time of termination. Error bars are 1 SE of the mean.

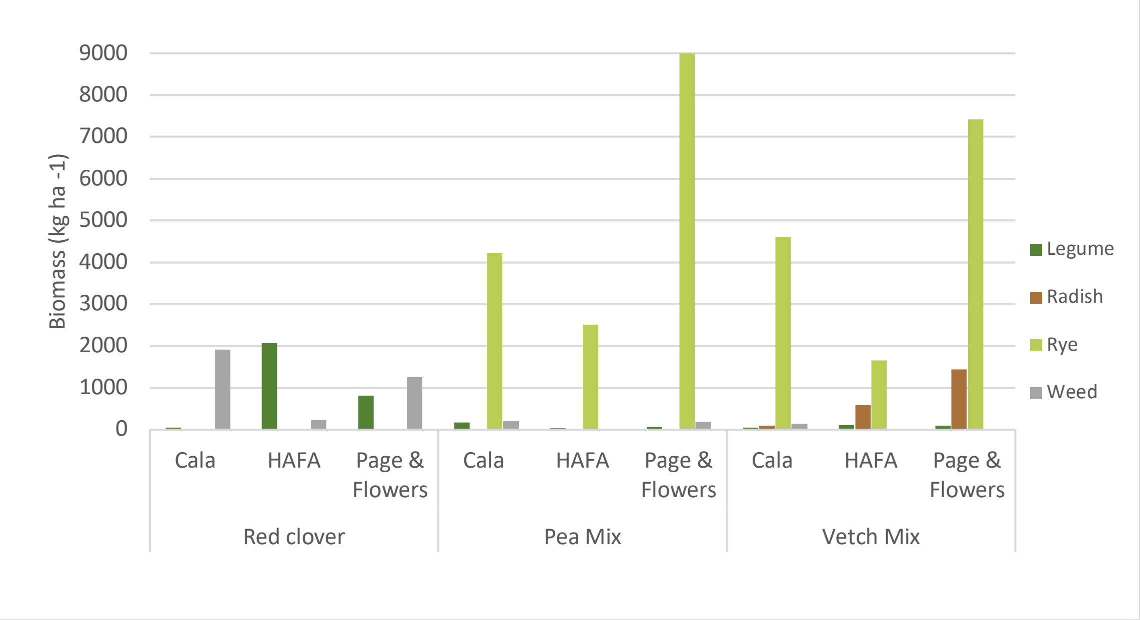

Figure 4.2. Biomass of cover crop species and weeds at the baby sites at the time of termination.

Seasonal changes in nodulation

- Nodule number on legume roots decreased from fall to winter and then recovered in spring. Red clover had the greatest nodule number of the legume species tested (an average of 165 nodules per plant, compared to less than 25 nodules per plant on average for vetch, pea and crimson clover).

- All species had greater nodule mass later in the season, at the March and May sampling times. Austrian winter pea had the greatest nodule mass (27 mg per plant on average) and crimson clover had the least (4.2 mg per plant on average).
- Pink nodules, indicating active SNF, occurred more frequently in the spring and fall than the winter, but were present at all sampling time-points at these sites.
- Seasonal fluctuation of nodulation and nodule activity indicated that legumes had lower N-fixing activity during the coldest period of the winter, but that they may still have been actively fixing N at low levels. This mid-winter legume activity may be due to the warmer day temperatures in high tunnels, and could potentially lead to greater N fertility for farmers in the spring.

Seasonal changes in symbiotic nitrogen fixation or %Nitrogen derived from the atmosphere

- Symbiotic nitrogen fixation, measured as %Ndfa, decreased over the winter season. At Grand Rapids, SNF was similar at November and January sampling, 56.9% and 65.8 %Ndfa, respectively. By May and termination, SNF had dropped to 27.9 %Ndfa. At Horst, SNF at November sampling was 33%, greater than in January and March (9%). By May, SNF was still lower than 15 %Ndfa, it had not risen back to fall levels. Horst had lower SNF values than GR overall.
- Greater levels of soil N at Horst (seen in baseline soil samples, Table 2) was likely the cause of lower %Ndfa overall at Horst compared to GR. Legumes will preferentially use available soil N rather than expend larger amounts of energy on SNF.
- Legumes were slow to recover SNF capacity following the winter, potentially due to very cold winter temperatures. Extreme temperature fluctuations in early spring in the high tunnels may have also reduced SNF. However, in late fall and early winter, warm day temperatures in the tunnel, plus less extreme temperature fluctuation possibly allowed for cover crops to continue growth and SNF past November and into the winter. This extension of legume activity could help to contribute more N to the system in the spring, even if SNF recovery in spring is slow.
- At the baby sites SNF (%Ndfa) was only calculated at termination (Table 5). SNF was lowest at Cala and greater at Page and Flowers (PF) and HAFA. High SNF values at PF

and HAFA were potentially due to warmer overall temperatures in these tunnels, as well as low levels of initial soil N that promoted SNF in the legumes.

Table 5. Mean values of response variables at each sampling time-point for Baby sites.

	Legume Biomass(g)			Nodule Number			Nodule mass(mg)			% Ndfa		
TP Site	PF	HAFA	Cala	PF	HAFA	Cala	PF	HAFA	Cala	PF	HAFA	Cala
1 (Nov)	2.4±0.7	14.5±5.6	ND	25.3 ± 0.5	133.9 ± 37.5	ND	5.3 ± 2.4	63.8 ± 38.3	ND	ND	ND	ND
2 (Jan)	2.2±1.4	8.0±2.4	ND	3.5 ± 0.9	18.8 ± 10.8	ND	0.38 ± 0.26	37.2 ± 0.6	ND	ND	ND	ND
3 (Mar)	1.3±0.3	2.0±1.7	ND	33.3 ± 21.2	253 ± 209.2	ND	18.7 ± 11.7	18.5 ± 10.6	ND	ND	ND	ND
4 (Apr/May)	2.3±0.8	9.1±6.3	2.5± 0.8	110.3 ± 90.1	399.3 ± 358.3	15.3± 8.4	12.6± 5.1	42.3 ± 28.8	2.3±1.5	64.5 ± 11.4	80.9 ± 14.6	8.8 ± 37.8

[1] Means are across species, within Site and Time-point, plus or minus 1 SE.

Conclusions

- In terms of biomass, winter survival/persistence, and nodulation at replicated Mother sites, red clover and hairy vetch best over-wintered in our high tunnels, and are most likely to do well for Upper Midwest farmers hoping to add legume cover crops into their winter annual rotation.
- Monocultures of legumes and cover crop mixes produced similar biomass, and sites in different climatic zones had similar spring cover crop biomass as well. These are encouraging results for farmers in the Upper Midwest, they may see cover crop spring growth in high tunnels equal to that of growers in more southerly regions of the Midwest.
- Legumes were crowded out by strong competitors such as rye in species mixes, suggesting that higher legume seeding rates may be necessary for farmers interested in mixes for nitrogen provision.
- Cover crops suppressed weeds. Cover crop mixes that included rye suppressed more weeds than clover monocultures.
- Despite SNF reduction over the winter, results suggested that legumes fix nitrogen in high tunnels throughout the winter-annual season. Additionally, the active period of cover crop growth extended later into the fall and began earlier in the spring than for

plants in the field. However, temperature fluctuations in high tunnel environments appeared to delay recovery of SNF in the spring.

- Slow spring recovery of SNF suggests farmers should wait to terminate winter annual cover crops as long as their rotations will allow to increase legume N contributions.
- Legumes had low %Ndfa in several tunnels with high initial soil nitrate levels. This may be beneficial in the high tunnel context, where in some cases, inputs may create high residual soil N as well as greater levels of phosphorus and salinity. In these cases, legumes planted will not initiate high levels of SNF. The high tunnel soil, which needs no additional N, can still benefit from the organic matter, soil biological activity, and moisture retention provided by a legume cover crop.

Challenges in this study

- Farmer management practices may change or reduce the likelihood of legume SNF. For example: Low SNF at Cala may be partially attributed to late seeding and spring germination of plants at this site, but also to unexpectedly high soil N fertility. Baseline soil N was not excessive at Cala, but later in the season the farmer applied 54 kg/ha of N to the tunnel, which is higher than the recommended rate (Sustane Natural Fertilizer Inc. 2016), and this applied N likely reduced SNF.
- At all sites, there was high above-ground plant mortality for both crimson clover and Austrian winter pea, especially following the January sampling time-point, these species had lowest biomass in March. Having few surviving plants to sample may have affected our biomass and %Ndfa results for these species.

Further questions for future work

- More work on climate and temperature is needed. Why did colder sites have similar nodulation and spring biomass (kg ha^{-1}) to warmer sites in this study? Does soil temperature matter more than air temperature for growth and survival in these legumes? A replicated study of soil temperature in tunnels as well as air temperature could help explain the influence of soil temperatures in high tunnels on over-wintering cover crops. Comparing temperatures inside and outside the tunnels using the same measurement equipment could also help elucidate differences between the tunnel environment and the adjacent field environment and how these differences effect plants in tunnels during the winter annual season.

- Recent breeding has produced specific winter-hardy selections of some of these cover crop legume species. Would more cold-tolerant cultivars of these legumes have greater biomass and SNF in high tunnels?
- Soil N was tested at the beginning of this experiment, but not after cover crop termination. How much N is actually being released into the soil from these cover crops? How much N is plant-available at later times in the season when farmers need it to support their cash crops?
- Which of these cover crop monocultures or mixes would farmers use again, considering the tradeoffs of using the different crops? (i.e. the drawbacks of difficulty with terminating cover crops and sacrificing some season extension time to grow them vs. the gains of soil fertility and weed suppression?)

References

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