Investigating the Effects of Grass-legume Winter Cover Crop Mixtures on Soil Nitrogen Supply in Rolling Agricultural Landscapes

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Project Information

Summary:

Sustainable cereal production systems rely on healthy soils and biologically based nitrogen. Previous research has shown that grass-legume cover crop mixtures can provide soil conservation benefits while also increasing the plant available nitrogen to a subsequent corn (*Zea mays* L.) crop. However, our understanding of the multifunctional benefits of cover crop mixtures is based largely on research conducted in uniform, plot-scale settings that are not representative of farmers' fields. Rolling-hill landscapes make up a large portion of farmland in the Southeastern United States and present a challenge for grain crop producers in the region because they possess areas of high soil fertility interspersed with regions of low fertility. We hypothesized that grass-legume cover crop mixtures would reduce this spatial variability. In particular, we hypothesized that a legume species would produce more biomass, add more nitrogen, and release nitrogen more quickly in lower-fertility sloping positions, whereas grass species would produce more biomass, scavenge nitrogen, and release it more slowly in higher-fertility summit and depressional positions. We tested these hypotheses using a field experiment at two on-farm locations and two research farm locations. We measured the biomass, species composition, nitrogen fixation, and nitrogen release of cereal rye (*Secale cereale* L.)/crimson clover (*Trifolium incarnatum*) mixtures as compared to monoculture cereal rye at different landscape positions (summit, backslope, and toeslope). In general, the toeslope position was the most productive in terms of cover crop biomass and nitrogen accumulation. The crimson clover biomass and fixed N increased with increasing slope and sand percentage. The rate of cover crop nitrogen release was similar among landscape positions and between the rye and

mixture cover crops. However, there tended to be more nitrogen released by the mixture than the rye monoculture on the backslope position. The results of this study were integrated into presentations, journal articles, and an extension publication to share with producers, agricultural professionals, students, and other researchers. We believe these findings will help farmers in the region align their use of specific cover crop species with portions of their farm or fields where those species will be most beneficial.

Project Objectives:

- Quantify the biomass production and species composition of cereal rye-crimson clover cover crop mixtures as compared to cereal rye monocultures and winter fallow at different landscape positions.
- Quantify the amount of nitrogen taken up from the soil nitrogen pool and produced through symbiotic nitrogen fixation by a cereal rye-crimson clover winter cover crop mixture at different landscape positions.
- Quantify the rates of decomposition of a cereal rye winter cover crop and a cereal rye-crimson clover winter cover crop mixture at different positions throughout the landscape under a corn cash crop.

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Research

Materials and methods:

Study Sites

Field trials for these experiments were conducted at two locations in Central Kentucky. Field sites were selected to represent hillslope settings found in row crop production areas in the Southeastern United States, and three landscape position treatments were identified at each field site based on slope and elevation (Table 1). One site was located at the University of Kentucky Spindletop Farm (ST; 38.123˚ N, -84.490˚ W), an experimental farm managed by the University of Kentucky College of Agriculture, Food, and Environment (Sites ST1 and ST2). The average annual rainfall at the ST location is 1240 mm, and the average temperature is 13.3 ℃ (1990 – 2020, http://weather.uky.edu). The second site was located in Hardin County, KY (HC; 37.602˚ N, -85.906˚ W) as a part of an on-farm collaboration with a local producer (Sites HC1 and HC2). The average annual rainfall at the HC location is 1321 mm, and the average temperature is 13.2 ℃ (2009 – 2020; http://weather.uky.edu).

Soils at the ST sites varied across landscape positions, but the arrangement of mapped soil series was consistent between years. At the summit positions, soils were classified as fine-silty, mixed, active mesic Typic Paleudalfs (Bluegrass Series). Soils at the backslope positions were classified as fine, mixed, active, mesic Mollic Hapludalfs (McAfee Series). The toeslope position soils were classified as fine-silty, mixed, active mesic Fluventic Hapludolls (Huntington Series). At the HC field sites, soils did not vary across landscape positions according to the soil map. The dominant soil at both HC sites was classified as fine silty, mixed, active, mesic Typic Paleudalfs (Crider Series; Soil Survey Staff, 2020). Soil depth varied across hillslopes, with toeslope positions having deeper soils and backslope soils noticeably shallower. Soil depth was determined via reported soil survey data in summit and toeslope (Soil Survey Staff, 2020), and hand probing in backslope positions. Selected soil fertility, physical components, and topographic attributes for each of the field sites are presented in Table 1.

Table 1: Soil and topographical characteristics for hillslope fields in 2019 and 2020

Site	Year	Landscape Position	Soil Characteristics[1]								Topographic Characteristics	
			Silt	Clay	Soil Depth	Soil pH	Organic Carbon	P	K	Inorganic N[2]	Slope	Elevation
			%	%	cm		%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	Deg.	masl
Spindletop	2019	Summit	72	17	100	5.9	2.01	370	146	9.2	2.03	905
Spindletop	2019	Backslope	54	26	35	6.6	2.40	472	239	7.5	5.18	899
Spindletop	2019	Toeslope	73	14	150	5.8	2.23	364	125	7.7	1.38	887
Spindletop	2020	Summit	64	17	90	6.6	3.27	395	352	9.2	1.48	903
Spindletop	2020	Backslope	64	17	40	6.7	3.16	366	284	16.5	5.14	891
Spindletop	2020	Toeslope	78	8	150	6.1	2.49	254	117	10.2	1.04	883
Hardin County	2019	Summit	68	16	100	6.2	1.09	38	284	4.2	2.68	668
Hardin County	2019	Backslope	64	16	30	6.3	1.11	28	261	3.9	5.13	661
Hardin County	2019	Toeslope	70	12	150	6.4	1.25	62	248	4.8	2.68	656

Hardin County	2020	Summit	68	20	100	6.1	1.17	12	91	7.0	1.94	674	
Hardin County	2020	Backslope	68	17	30	6.1	1.19	9	104	6.9	4.04	668	
Hardin County	2020	Toeslope	78	11	150	6.0	1.16	18	103	11.4	1.65	664	

1: Soil characteristics, unless noted, are from samples from 0 – 20 cm depth.
2: Soil inorganic N was measured to a 60 cm depth near the time of cover crop planting.

Study Design

Plots were arranged in a split-plot randomized complete block experimental design with three replicates at all field sites. At the ST locations, all replicates were located on one hillslope each year, with cover crop treatments randomly assigned within a landscape position-replicate combination (Figure 1). At the HC sites, each replicate was placed on a separate hillslope within the producer's field each year, and cover crop treatments were continuous strips across landscape positions (Figure 1). As such, at the ST sites, landscape position was treated as the main plot effect, and cover crop treatment was the subplot effect. At the HC sites, this was reversed, with cover crop treatment acting as the main plot effect, and landscape position as the subplot effect.

SARE report figure 1

Field Plot Management

Three cover crop treatments were established at the beginning of the experiment for all field sites, a cereal rye (*Secale cereale L.*) sole crop, a cereal rye-crimson clover (*Trifolium incarnatum*) mixture, and no cover. The cereal rye sole crop was drill seeded at a target seeding rate of 73 kg ha^{-1} at both locations. The mixture treatment was drill seeded at a target seeding rate of 45 kg ha^{-1} of cereal rye and 13 kg ha^{-1} of crimson clover at the ST locations, and 45 kg ha^{-1} of cereal rye and 22 kg ha^{-1} of crimson clover at the HC locations. The no cover treatments were not chemically controlled during the cover crop growth period. Consistent with recommended practices for the region, the cover crop was chemically terminated at least two weeks prior to optimal maize, and left on the soil surface (Quinn et al., 2021). At termination, the cereal rye was between the Feekes 7 and Feekes 8 growth stage (Knott, 2016), and the crimson clover had not yet begun to flower. Maize was no-till planted following cover crop termination on 76 cm rows at a target population of 78,000 plants ha^{-1}. Nitrogen fertilizer was managed as a split application, with 45 kg N ha^{-1} as 32% UAN fertilizer subsurface banded (5 cm below and 5 cm to the side of the seed) at planting, and 225 kg N ha^{-1} as 32% UAN fertilizer dribbled on the surface at the V5 growth stage.

Soil Sampling and Analysis

Soil samples were taken to a depth of 60 cm at the time of cover crop planting for soil inorganic N analysis. In the spring, prior to cash crop planting, soils were sampled to 20 cm to determine Mehlich 3-extractable nutrients, soil organic C and N, and soil texture. Samples were taken using a hand probe, separated at depths of 0-5, 5-15, 15-30, and 30-60 in the fall, and 0-10 cm and 10-20 cm in the spring. Each sample represent a composite of 9 subsamples taken randomly throughout the entire plot area. An 8 g dry equivalent subsample of fresh soil from the fall sampling was extracted using 40 ml of 1 M KCl and analyzed for total inorganic N using a colorimetric method (Crutchfield and Grove, 2011). The spring samples were subsampled, dried, and ground to 2 mm, then sent to the University of Kentucky Regulatory Services Soil Lab for Mehlich 3 extractable nutrients, texture, and C and N concentrations (Table 1). Soil texture analysis was done using the pipette method, and C and N were analyzed via dry combustion.

Cover Crop Sampling and Analysis

Cover crop samples were collected immediately prior to chemical termination. Either 2 or 4 subsamples were taken from each cover crop plot, processed individually, and then averaged to obtain a value for each plot. Cover crop samples were obtained by randomly placing a 0.25m^2 frame into the plot and removing all living biomass as close to the soil surface as possible. Fresh biomass samples were sorted into individual clover, rye, and weed groups, placed in the dryer at 65 ℃ for approximately one week, and then weighed to obtain a dry matter weight. In 2019, four subsamples were taken from each plot at the ST1 location. At the HC1, ST2, and HC2 locations, two subsamples were taken from each plot. Cover crop aboveground biomass was analyzed for C, N, and ^{15}N abundance in the University of Kentucky Stable Isotope Geochemistry Laboratory via dry combustion analysis interfaced with isotope ratio mass spectrometry. The amount of N derived from atmospheric fixation for the crimson clover was calculated using the natural abundance method described in Equation 1:

SARE report equation 1

where *Reference* ^{15}N is the ^{15}N value of the rye monoculture for a given landscape position and replicate, *Clover* ^{15}N is the ^{15}N value of the clover at a given landscape position and replicate, and β is a correction value for the fractionation of the N isotope that occurs between above and belowground biomass (Schipanski and Drinkwater, 2012). Here we use a β value equal to -1.55 based on the results from Blesh (2018).

Litterbag Decomposition

We used a litterbag method to measure cover crop decomposition during the maize growing season at the ST1 and ST2 sites. The litterbags measured 40 cm × 18 cm and were made using 1 mm nylon mesh. The amount of fresh plant material placed in each bag was constant across landscape positions in each year and was determined based on the average aboveground biomass across landscape positions, scaled to the area of one litterbag. In 2019, the rye sole crop litterbags contained 21 g dry weight equivalent (DWE) fresh rye material, and the rye-crimson clover mixture litterbags contained 17 g DWE fresh rye, and 2 g DWE fresh clover. In 2020, rye sole crop bags contained 24 g DWE fresh rye, and the mixture litterbags contained 22 g DWE fresh rye, and 2 g DWE fresh clover. Following litterbag preparation, the residue present at the site of litterbag placement was removed, and the litterbags were secured to the soil surface using landscape staples. Litterbags were installed prior to maize planting, approximately two weeks after chemical termination of the cover crop (Table 2). The litterbags were removed briefly during maize planting and pre-emergent herbicide application, after which they were installed in their final position in the middle of the center rows of the plot. Six litterbags were prepared per plot (n = 108). One litterbag per plot served as an indication of initial conditions of the residue and the residue was removed from the litterbag, placed in a paper bag, and placed directly into the drying oven at 65 ℃; the other five were removed sequentially throughout the maize growing season (Table 2). In 2020, two additional litterbags were added - one at the initial installation and one at the final removal date. These additional bags contained rye and clover amounts that were representative of landscape position average biomass levels, instead of field averages. Following removal from the field, the residue was dried at 65 ℃, weighed, ground, and analyzed for C and N via dry combustion. A 1 g subsample was ashed for 4 h at 450 ℃ to account for possible soil contamination of the cover crop residue, and dry weight and C and N content were adjusted to an ash free basis.

Table 2 – Removal dates and days after installation of litterbags in 2019 and 2020

Removal Date	Date		Days after installation		Growing Degree Days		Decomposition Days	
	2019	2020	2019	2020	2019	2020	2019	2020
0	April 29	April 16	0	0	0	0	0.0	0.0
1	May 13	April 27	14	11	253	120	3.0	1.5
2	May 29	May 11	30	25	625	293	7.5	2.9
3	June 30	June 15	62	60	1355	1015	16.1	8.0
4	July 30	July 16	92	91	2141	1759	23.5	18.2
5	September 6	August 29	130	132	3085	2770	29.9	25.6

Weather and Soil Microclimate Data

At the ST1 and ST2 sites, daily minimum and maximum air temperature and precipitation were measured at the Spindletop Farm station of the University of Kentucky Ag Weather Center, located within 1 km of both field sites. In both 2019 and 2020, temperature soil temperature sensors (HOBO TidbiT v2 Temperature Loggers; ONSET, MA USA) were installed after maize planting at 5 cm depth in the center rows of all plots in which litterbags were present, adjacent to the position of the litterbags. Sensors logged soil temperature at a 10-minute time interval throughout the maize growing season. Volumetric soil water content was measured at 10 cm depth increments down to 100 cm or bedrock, depending on the soil profile, using a Sentek Diviner 2000 Capacitance probe (Sentek Technologies, South Australia). In the data presented here, we focus on only the top 10 cm moisture measurements. Each measurement was taken as the average from three readings per sampling. Moisture measurements were calibrated for our site by estimating parameters for the non-linear equation relating volumetric moisture measurements taken at probe installation and scaled frequency measurements taken at the same time for each field site (see Paltineanu and Starr, 1997 for further discussion of calibration methods). Growing Degree Days (GDDs) were calculated from the mean daily air temperature data and accumulated over the period between initial litterbag installation and the final litterbag removal, using a base temperature of 0 ℃. A second set of GDDs was also calculated from the average daily soil temperature at each landscape position to capture the difference in thermal energy across the landscape (sGDD). Additionally, we calculated Decomposition Days (DCD) on a daily time-step following the methods described by Steiner et al. (1999) and Quemada (2004) to estimate the limiting factor to decomposition on each day during the growing season. Decomposition Days take both daily precipitation and temperature into account to calculate what fraction of decomposition occurs on a given day, relative to optimum conditions. Two coefficients are calculated, one for temperature and one for moisture. These daily environment coefficients are constrained between 0 and 1, with 0 indicating no decomposition occurring, and 1 indicating optimum decomposition conditions. The temperature factor was calculated as:

SARE report equation 2

where TC is the temperature coefficient, T_{mean} is the average daily air temperature, and $T_{Optimum}$ is the optimum air temperature for decom- position (defined as 32 ℃). The moisture coefficient (MC) was calculated based on rainfall; if on a given day total rainfall > 4 mm, MC was set equal to 1. If precipitation occurred on a given day, but was not > 4 mm, the MC was equal to the precipitation divided by 4. If no precipitation occurred, the MC was calculated as half the MC of the previous day. The DCD for a given day was then equal to the lower of the two values, indicating which was more limiting to decomposition. For both GDD and DCD values, the values of a given day represented an accumulation of value over the course of the season, i.e., the calculated value of a given day was added to the values of all prior days to arrive at a final value.

Statistical Analysis

Spatial data analysis and cover crop statistics

Digital elevation models were retrieved from the KentuckyFromAbove data repository (Kentucky Division of Geographic Information, 2017). Elevation data were collected using light detection and ranging (LiDAR) at a 1 m point spacing and presented as 1.5 m raster grid cells. ArcMap v 10.7.1 was used to calculate the mean elevation, slope, flow accumulation, profile curvature, and planar curvature, at the plot level for all site locations. Zonal statistics were then calculated for each plot from the raster datasets. Data for cover crop biomass, N uptake, and N derived from fixation were analyzed in R v 4.0.2 (R Core Team, 2020) using linear mixed models from the lme4 package v 1.1-23 (Bates et al., 2015). In the linear mixed models for the ST sites, landscape position, cover crop treatment, and the interaction between landscape position and cover crop treatment were considered fixed effects, replicate and the interaction between landscape position and replicate were considered random effects. In the linear mixed models for HC sites, landscape position, cover crop treatment, and the interaction between landscape position and cover crop treatment were considered fixed effects, replicate and the interaction between cover crop treatment and replicate were considered random effects. A Type III sums of squares ANOVA and a post-hoc Tukey Test were used to identify mean responses that were significantly different between treatments using the multcomp package in R (v. 1.1-13, Hothorn et al., 2008). Due to the heterogeneity in weather, soil, and the limited number of replications possible at each field site, significance was assigned at p-values < 0.10. Site effects and site by year interactions were significant. As such, we elected to analyze and present the treatment responses separately for all site years.

To identify significant relationships and trends in cover crop performance and landscape topographic variables, we conducted a principal component analysis using the prcomp function in the base R stats package (v 4.0.2; R Core Team, 2020). Data were pooled across site years and scaled using Z-scores prior to analysis. Biplots were constructed to visualize variable loading for the first two principle components using the factoextra package (Kassambara and Mundt, 2020). Following this analysis, individual regressions of the unscaled, pooled data were conducted to elucidate relationships between factors identified in the PCA to be major drivers of variation or to be highly correlated. In the PCA biplots, variables with acute angles between them were interpreted to be positively correlated, whereas variables arranged in obtuse angles were interpreted to be negatively correlated.

Litterbag Statistics

The percentages of ash-free cover crop mass remaining (MR) and the N remaining (NR) of our measured field samples at a given litterbag removal date were calculated as:

SARE report equation 3

where X_{RD} is the mass of residue or the amount of N in the biomass at a given removal date, and X_{Int} is the mass of residue or the mass of residue N at Time 0. To calculate the percentage of N released, the percentage of N remaining was subtracted from 100. The percentage of mass remaining of the cover crop residue over time was modeled using a two- component asymptotic exponential decay model:

SARE report equation 4

where PMR is the predicted percentage of litter remaining at a given time point, k_m is the decomposition rate of the residue, t is the time since the litterbags were placed in the field and a_m is the (asymptotic) percentage of mass that is resistant to further decomposition. The model is therefore composed of two pools, a decomposable pool defined by (1- a_m), and a non-decomposable pool (a_m). Each year was analyzed separately. For both years of the study, the two-component asymptotic exponential decay model (Eq. 4) was fitted using three different predictor variables (i.e., timescales) to define when litter bags were removed: days after installation, GDDs, and DCDs. Model fitting was performed in R v. 4.0.2 (R Core Team, 2020), using nlraa v 0.73 and nlme v. 3.1–148 (Miguez, 2021; Pinheiro et al., 2021). The final nonlinear mixed model also included the fixed effects of cover crop treatment, landscape position treatment, the interaction between the two, and a random effect due to replicate. Including fixed and random effects in the nonlinear model structure allowed us to perform statistical testing of treatment effects and properly propagate uncertainty (Oddi et al., 2019). Model inference was based on the parameters of the non-linear model: the decomposition rate (k_m) and resistant fraction (a_m) were analyzed for significance using pairwise comparisons, with significant differences assigned when $\alpha < 0.10$. To analyze N release of the cover crop residue over time, we used an exponential rise to maximum model:

SARE report equation 5

where PNR represents the predicted percentage of N released at a given point in time, a_n is the maximum percentage of N released over the growing season, k_n is a release constant, and t is the time since the litterbags were initially placed in the field. A procedure of non-linear mixed model evaluation similar to the method described above was used to evaluate treatment effects on the rate of N release from the cover crops at different landscape positions.

Soil Microclimate Statistics

Measurements of soil temperature and volumetric moisture exhibited temporal autocorrelation, meaning that the value of a given day was highly dependent on the value of the previous day. To account for this autocorrelation when examining differences among landscape position and cover crop treatments on these microclimate variables, we used general additive models (GAMs), which have been shown to be a robust method of analyzing this type of soil moisture data (Basche et al., 2016b). All GAM analyses were performed in R v. 4.0.2 (R Core Team, 2020) using the mgcv v 1.8 (Wood, 2011) and the itsadug v 2.4 (van Rij et al., 2020) packages. In our models, the interaction between cover crop and landscape position was used to generate separate splines for each possible combination. An additional spline term was added to include the random effect of replicate in the model. Post-hoc comparisons of model predictions for each treatment were performed using a non-parametric Wald test, with an α level of 0.10. To account for different weather conditions during our two field seasons, we analyzed our microclimate data separately for each year. The soil moisture measurements at one replicate x landscape position combination (ST2 Summit, Block 1) had results outside the bounds of what could be expected for soils in this region. Point measurements indicated poor agreement with measured gravimetric water samples, and high variability compared to replicates 2 and 3. As such, this replicate x landscape position combination was not included in the final analysis of topsoil moisture presented here.

Research results and discussion:

Total cover crop biomass production and N uptake

SARE report figure 2

Cover crop biomass production varied in response to landscape position and site-year. The ST sites were more productive than the HC sites in both years of the study. Across landscape positions and cover crop treatments, Spindletop sites (ST1 and ST2) produced between 40 and 45% more overall biomass than the Hardin County sites (HC1 and HC2) (Figure 2A). The average biomass produced at the HC sites was 1.90 Mg ha^{-1} while the average biomass produced at the ST sites was 3.26 Mg ha^{-1}. The lowest biomass production occurred on the backslope position at HC2 (1.55 Mg ha^{-1}), and the highest biomass production occurred in the summit position at ST1 (3.54 Mg ha^{-1}). Cover crop N uptake mirrored trends in biomass production, with 53 – 54% greater N uptake by the cover crop in the ST sites than the HC sites. The average N uptake by the cover crop was 59.7 kg ha^{-1} at the ST sites, and 28.0 kg ha^{-1} at the HC sites.

The effect of landscape position varied by site-year and was more consistent at the Hardin County field locations than at the Spindletop field locations. When averaged across cover crop treatments, the toeslope produced the greatest amount of biomass in 3 out of 4 site-years. However, this effect was only significant the HC locations (p-values = 0.06 for HC1, and 0.03 for HC2), and only showed a numerical trend at the ST2 site (p-value = 0.89). In the HC sites, cover crop biomass production in the toeslope was significantly greater than in the summit and the backslope positions in both years (Figure 2A). Backslope and summit positions at the HC sites were not significantly different from each other. In ST1, the toeslope had significantly lower biomass than the backslope and summit positions, but there was no effect of landscape position in ST2 (Figure 2A). At the HC sites, the cover crops in the toeslope position assimilated significantly more N than cover crops in the backslope or summit positions in 2019, and significantly more than cover crops in the backslope position in 2020 (Figure 2B). At ST1, cover crops in the toeslope position took up significantly less N than those in the summit position.

Cover crop treatment was only significant in 1 of the 4 site years, at HC1. Averaged across landscape positions in this site-year, the mixture treatment produced 32% more biomass than the cereal rye monoculture treatment (p-value = 0.03). At all other experimental locations, cover crop treatment did not have a significant effect on biomass production. Additionally, there was not a significant interaction effect between landscape position and cover crop treatment in any site year. Nitrogen uptake was also not significantly impacted by cover crop treatment ($p > 0.10$ in all cases; Figure 2B).

Percentage clover and nitrogen fixation

SARE report figure 3

In addition to differences in the amount of biomass produced and the N recovered among landscape positions, the performance of the clover within the mixture varied considerably among landscape positions. In general, the backslope position had a greater percentage of clover within the cover crop biomass compared to the summit and toeslope positions at the ST sites, and the backslope and toeslope had a greater percentage of clover biomass than the summit at the HC sites. The lowest percentage of clover occurred at the toeslope position at the ST2 site, where the total biomass in the mixture treatment was 3% clover. The highest percentage of clover within the mixture was observed in HC1 at the backslope and toeslope positions, where clover accounted for 22.5-23.3 % of the total cover crop biomass (Figure 3B). At the ST sites, the backslope and summit positions were significantly different in 2019, but not in 2020. In both years, the percentage of clover on the backslope position at the ST sites was higher than the percentage present at the toeslope (Figure 3B).

The percentage of N derived from atmospheric fixation (NDFA) showed a different pattern than that of the proportion of clover and was more consistent between sites than the biomass production or proportion clover. Averaged across sites, NDFA was highest in the sloping areas, followed by the summit position, and then the toeslope (77%, 72%, 60%, respectively). At the HC sites, the percentage of NDFA at summit and backslope positions were similar in both years, and the toeslope position was lower (Figure 3A). This difference was significant in 2019, but not in 2020. At the ST sites, clover in the backslope position acquired significantly more N via fixation than the summit in 2019, but there were not significant results in 2020. However, a trend persisted across all years that backslope positions consistently had as high or higher percentages of NDFA than the other landscape positions.

Landscape controls on cover crop dynamics

SARE report figure 4

SARE report figure 5

To analyze the relationships among landscape variables, soil properties, and cover crop biomass and N accumulation, we used principal component analysis, a multivariate data analysis technique that allows for comparisons of factors across unitless, multidimensional data (Figure 4). Our results indicated several strong correlations between topographic and edaphic characteristics, and cover crop performance and dynamics. In analyses for both the rye sole crop and the mixture treatments, the variability in the first two principal components was explained largely by the same variables. In both analyses, PC1 was defined chiefly by variables related to soil fertility and cover crop performance, such as soil C percentage, soil P, rye N uptake, rye biomass production, and total biomass production. In contrast, the highest contributing variables to PC2 were related to landscape position and edaphic characteristics, such as slope, soil pH, and texture components, and in the mixture treatments, clover biomass and performance. In the rye monoculture, landscape position groups were primarily spread along PC2, and rye biomass was most correlated to inorganic N stocks, soil P, and soil C. In the mixture treatment, landscape positions were more closely clustered, with less spread along PC2. The rye grown in the mixture treatment showed similar relationships to the soil fertility traits that it was heavily correlated with in the monoculture. However, high correlations occurred between attributes of the clover within the mixture, such as clover biomass and proportion clover, and the topographic and edaphic characteristics such as slope and soil sand content. Further, variable loadings for the clover biomass were positioned perpendicular to the loadings for the rye biomass and associated soil fertility factors, indicating no significant relationship between clover and soil fertility factors, or rye performance.

Based on the PCA results, we plotted relationships between cereal rye and crimson clover biomass and key explanatory variables (Figure 5). The relationship between rye biomass and slope was insignificant, with similar biomass levels occurring at slopes ranging from 0 – 6 degrees. However, clover biomass was significantly correlated with slope ($p = 0.004$), showing an increase of 0.05 Mg ha^{-1}, or 19% of the average clover biomass observed in our study, with each 1 degree increase in slope. Similarly, as sand content increased, clover biomass increased significantly ($p < 0.001$). An increase in sand content of 10% led to an increase of 0.2 Mg ha^{-1} in total clover biomass. Rye biomass was not significantly impacted by soil sand content ($p = 0.446$). The total amount of rye biomass showed strong correlations with soil C % ($p < 0.001$) and total inorganic N at planting ($p = 0.006$). Crimson clover biomass was not related to either of these factors, showing numerically negative relationships that were not statistically significant.

Soil temperature and moisture response to landscape position and cover crop treatment

SARE report figure 6

SARE report figure 7

Overall, soil temperature at 5 cm depth followed the trends in air temperature in both 2019 and 2020 (Figure 6). Soil temperature ranged from 15.5 to 31.3 ℃ in 2019, and between 9.3 and 28.7 ℃ in 2020. The highest soil temperatures occurred at the beginning of August in 2019 (31.3 ℃) and in mid-July in 2020 (28.7 ℃). In both years, the highest temperature was observed at the backslope position. Landscape position, cover crop, and their interaction had a significant influence on soil temperature during both years of the study (Figure 6). The frequency of significant differences was greater in 2019 than in 2020, but the trends in temperature were consistent between years. Based the results of the GAM analysis, the backslope position was on average 0.5 ℃ warmer than the toeslope and the summit positions in both years. Across years, the backslope was warmer than the summit position 33% of the measurement period, and warmer than the toeslope position 29% of the measurement period. The cover crop influenced soil temperature at different time points at each landscape position. In both years however, the rye/crimson clover mixture was significantly warmer than the rye grown alone in the toeslope but cooler than the rye grown alone treatment in the summit at the beginning of the season. Similarly, in both years, the mixture treatment was periodically significantly cooler than the rye sole crop in all landscape positions at the in the middle and end of the season (Figure 6). Soil volumetric water content in the top 10 cm ranged from 10% to 23% in 2019 and from 11% to 21% in 2020. In both years of our study, the highest volumetric water content of the top ten centimeters of the soil profile occurred in the spring, with soil water content decreasing throughout the course of the season (Figure 7). Landscape position had a significant impact on topsoil volumetric water in the spring of 2019 (p- value < 0.001), and in 2020 (p-value < 0.001), however the effect was much more subdued in 2020 (Figure 7). The toeslope position was on average 12% wetter than the backslope and 8% wetter than the summit throughout the season in 2019. The toeslope was 4% wetter on average than the summit position in 2020. Cover crop treatment did not have a significant effect on topsoil moisture in either year, though landscape position effects were slightly different between the rye sole crop and mixture at different times throughout the season.

Initial litterbag properties

At the time of litterbag installation, the average C:N ratio of the cover crop residue in the 2019 litterbags for the rye sole crop treatments was 19.2, and for the mixture treatment was 17.2. The initial N content of the cover crop residue was 2.3% for the cereal rye sole crop treatment and 2.5% for the mixture treatment. In 2020, the average C:N ratio of the litterbag residue for the cereal rye sole crop treatment was 20.7, and for the mixture treatment was 20.1. The initial N content of the cover crop residue was 2.0% for the cereal rye sole crop treatment, and 2.1% for the mixture treatment in 2020.

Litterbag decomposition and nutrient release

SARE report figure 8

Table 3 - Decomposition rate (k) and asymptote (a) estimates and standard errors from non-linear models of cover crop residue decomposition in 2019 and 2020. No parameters were significantly different according to pairwise comparisons of model contrasts, except for the k_n parameter for nitrogen release in the backslope position in 2020, and the a_n parameter in 2019.

Decomposition (% mass remaining in response to days after installation)							Nitrogen Release (% N released in response to days after installation)						
2019							2019						
Landscape Position	Cover Crop	k_m	k_m SE	a_m	a_m SE		Landscape Position	Cover Crop	k_n	k_n SE	a_n	a_n SE	
Summit	Rye	0.036	0.0047	0.253	0.052		Summit	Rye	0.032	0.006	0.809	0.0329	
	Mix	0.027	0.0068	0.368	0.036			Mix	0.046	0.007	0.711	0.0257	
Backslope	Rye	0.035	0.0067	0.364	0.039		Backslope	Rye	0.048	0.008	0.663^{+}	0.0271	
	Mix	0.034	0.0064	0.355	0.038			Mix	0.059	0.009	0.697^{+}	0.0229	
Toeslope	Rye	0.033	0.0060	0.318	0.038		Toeslope	Rye	0.048	0.007	0.798	0.0249	
	Mix	0.028	0.0052	0.306	0.046			Mix	0.045	0.007	0.795	0.0259	
2020							2020						

Landscape Position	Cover Crop	k_m	k_m SE	a_m	a_m SE		Landscape Position	Cover Crop	k_n	k_n SE	a_n	a_n SE	
Summit	Rye	0.014	0.0035	0.134	0.110		Summit	Rye	0.017	0.004	0.827	0.0987	
	Mix	0.022	0.0042	0.239	0.057			Mix	0.020	0.005	0.811	0.0774	
Backslope	Rye	0.022	0.0041	0.223	0.057		Backslope	Rye	0.025*	0.005	0.772	0.0568	
	Mix	0.015	0.0035	0.122	0.098			Mix	0.011*	0.004	1.061	0.2034	
Toeslope	Rye	0.018	0.0037	0.165	0.072		Toeslope	Rye	0.023	0.005	0.783	0.0640	
	Mix	0.013	0.0034	0.092	0.129			Mix	0.018	0.004	0.864	0.0924	

*: Cover crop treatments were significantly different in the backslope position for the k_n.
+: The backslope position a_n value was significantly less than the summit and toeslope position in 2019.

Averaged across landscape positions and cover crop treatments, the decomposable pool of residue lost mass more quickly in 2019 than in 2020. The decomposable pool decomposed at an average rate (k_m) of 3.2% per day in 2019, and 1.7% per day in 2020 (Table 3). In 2019, it took 45 days to lose 50% or more of the initial residue mass, whereas it took 56 days in 2020, though the date at which 50% was reached was similar, between June 10 and 12. The most rapid decomposition occurred between the installation and the first removal date, a period of 14 days during which 25% and 15% of the residue decomposed in 2019 and 2020, respectively (Figure 8). The percentage of residue resistant to further decomposition (i.e., the nondecomposable pool, model parameter a_m) averaged 32% of the original mass across all treatments in 2019, and 16% in 2020 (Table 3). The lower a_m parameter in 2020 relative to 2019 partially explains why the decomposition curve for 2020 appears steeper overall than what was observed in 2019, despite having a lower average k_m constant (Figure 8, Table 3). The average percentage cover crop residue remaining across treatments measured at the end of the growing season was 34% in 2019, and 25% in 2020, indicating that the model estimated asymptote was close to the final value in 2019, but not in 2020. Pairwise comparisons between the landscape position treatments did not indicate significant differences between any two landscape positions in the decomposition rate (k_m) or percentage of residue resistant to decomposition (a_m) in 2019 or 2020 (Table 3). Similarly, pairwise comparisons of the cover crop treatments at individual landscape positions did not indicate significant differences in either the km or am parameters. The average nitrogen release constants (k_n) were 4.6% per day in 2019 and 1.8% per day in 2020. The average model-predicted asymptotic N release (an) was lower in 2019 than in 2020 (74% and 85%, respectively; Table 3). Despite differences in a_n parameters, the measured percentage of residue N released was similar in 2019 and 2020, with 74% of biomass N released by the end of the growing season. in 2019, and 77% in 2020. Nitrogen release from cover crop residue was largely unaffected by cover crop treatment or landscape position in both the 2019 and 2020 growing seasons (Table 3, Figure 8). The rye sole crop released N more rapidly than the mixture treatment in 2020, but only in the backslope position (p-value = 0.02; Table 3). The actual magnitude of this effect was moderate (k_n = 1.1% and 2.5% for the mixture and rye sole crop, respectively), and balanced by a lower a_n value for the rye treatment than mixture treatment. This cover crop effect was not repeated at any other landscape position in either site year. In 2019, N release proceeded faster than mass loss. Averaged across landscape positions and cover crop treatments, 50% of the biomass N release occurred by the 25th day after installation, on May 24th, which was 20 days earlier than 50% of mass loss occurred. Similar to the decomposition model, the most rapid N release occurred at the beginning of the season; 35% of the N in the cover crop residue was released between installation and the first removal date 14 days later (Figure 8). The N release reached the model predicted asymptote (74%) by end of the season, 130 days after installation on September 6th, consistent with the pattern observed in decomposition which also reached the asymptote by the end of the final removal date. In 2020, N release from the litterbags was not more rapid than mass loss. Model predictions averaged across all treatments indicated that 50% of the biomass N was released by June 7th, 52 days after installation. The N release, like mass loss, did not reach the model-predicted asymptote in 2020 (Figure 8).

Table 4 - Predictions of N release and mass remaining at the final removal date (i.e., 130 or 132 days after installation for ST1 and ST2, respectively) for each landscape position based on the application of non-linear models to initial biomass measurements.

Year	Landscape Position	Cover Crop	N Release Estimate (kg ha^{-1})	SE	Mass Remaining Estimate (kg ha^{-1})	SE
2019	Summit	Mix	57.5	3.3	1365	120
2019	Summit	Rye	49.3	3.0	975	123
2019	Backslope*	Mix	49.4	4.4	1189	107
2019	Backslope*	Rye	42.4	2.2	1248	112
2019	Toeslope	Mix	42.0	2.4	764	81
2019	Toeslope	Rye	40.5	2.1	921	95
2020	Summit	Mix	41.1	2.5	801	110
2020	Summit	Rye	45.9	2.7	1207	216
2020	Backslope*	Mix	52.3	4.6	982	173
2020	Backslope*	Rye	39.0	2.0	828	121
2020	Toeslope	Mix	46.3	2.6	848	164
2020	Toeslope	Rye	49.2	2.8	800	127

* : Significant differences in N release estimates occurred between rye and mixture treatments at the backslope position (p-value = 0.02).

Landscape position-adjusted litterbags

In 2020, a secondary set of litterbags was added to evaluate the effect of initial residue mass on decomposition and N release using only an initial (Removal Date 0) and final removal date (Removal Date 5). For these bags, we used the average biomass of each landscape position rather than the average biomass of the overall site year to determine the mass of residue placed in each bag/ We did not observe a significant effect of landscape position (p-value = 0.86), cover crop treatment (p-value = 0.96), or the interaction of the land- scape position and cover crop treatments (p-value = 0.13) on the total amount of residue decomposed over the season. The average full season residue decomposition for the position averaged litterbags was similar to the site-averaged residue decomposition for 2020; 75% of the residue had decomposed between installation and the final removal date. Observing no effect of initial biomass on the results of the decomposition rate, we applied our non-linear models to the initial biomass and N content observed at each landscape position to estimate the mass of surface residue remaining and the amount of N released from the cover crop at the end of the maize growing season (Day after installation 130 and 132 for 2019 and 2020, respectively; Table 4). The total estimated amount of residue remaining ranged from 800 to 1365 kg ha^{-1} across sites and treatments, though no significant differences were detected between landscape position treatments (p-value = 0.12), cover crop treatment (p-value = 0.89), or the interaction effect between landscape position and cover crop (p-value = 0.93). Generally, there was more residue remaining in 2019 than 2020. Averaged across treatments, 1080 kg ha^{-1} of surface residue remained at the end of the maize growing season in 2019, and 905 kg ha^{-1} remained at the end of 2020. Overall, cover crop and landscape position effects did not show noticeable trends across years in regard to the amount of mass remaining. The average total amount of N released was more similar between years. In 2019, across landscape positions and cover crop treatments, 47 kg N ha^{-1} was released; 46 kg N ha^{-1} was released on average in 2020. The estimated total amount of N released from the residue varied numerically by landscape position and cover crop, though at the summit and toeslope position these differences were not significant. We observed that the mixture treatment, on average, exhibited a trend of releasing more overall N (48 kg ha^{-1} vs. 44 kg ha^{-1}, for the mix and the rye, respectively). This effect was strongest on the backslope position (51 kg ha^{-1} vs 41 kg ha^{-1}, for the mix and the rye, respectively), and examinations of the contrasts between the mixture and rye indicated a significant effect of cover crop at this position (p-value = 0.02). In contrast, at the toeslope position the rye and mixture treatments were similar; the rye treatment released 45 kg ha^{-1} N, and the mixture 44 kg ha^{-1} N (p-value = 0.67). The summit varied in its response to cover crop treatment between years but did not show a significant effect of cover crop overall (p-value = 0.62). The mixture released 14% more N than the rye sole crop in 2019 at the summit position (58 kg N ha^{-1} for the mix, 49 kg N ha^{-1} for the rye), but the cover crop treatments were more similar in 2020, with the rye numerically higher (41 kg N ha^{-1} vs 46 kg N ha^{-1} for the mix and rye, respectively).

Cover crop mixtures are a popular recommendation for producers attempting to capitalize on several cover crop benefits, such as reduced N leaching and N return to the subsequent crop. We found that cover crops produced similar levels of biomass whether grown in a mixture or monoculture. Landscape position had a significant effect on biomass production, with depression areas often producing more biomass than sloping and summit areas. We observed that the crimson clover within the mixture was significantly impacted by topography. The proportion clover was greatest in sloping areas, and the amount of NDFA was reduced in depression areas relative to slopes and summits. An analysis of the primary drivers of variations indicated that cereal rye was highly correlated with soil fertility factors such as soil C and inorganic N, while clover biomass was more related to soil texture and field slope. Our results indicate the heterogeneity in cover crop response to landscape factors and provide a baseline for improving cover crop management in areas of rolling hill topography. We also found that landscape position did significantly impact topsoil moisture and soil temperature, but that the results varied in intensity by weather year and hillslope orientation. Despite differences in microclimate factors, we did not observe significant differences among landscape positions in regard to the decomposition rate of cover crop residue. In addition, we did not observe an effect of cover crop species composition on decomposition rate. Our results suggest that in cover crop-maize sequences in the southeastern U.S., decomposition of surface residue in no-till fields is likely controlled by larger scale factors such as weather, tillage, and cover crop developmental stages and termination date, rather than topography within a field. When our non-linear models were applied to field data of measured residue amounts, we observed significantly more N release from mixture treatments compared to rye sole crop treatments on sloping positions. Producers should leverage the consistency in decomposition rates to focus on cover crop production in areas of need; increasing legumes in N poor areas to improve N release or increasing total biomass in weed prone areas to improve weed control, for instance. Future work focused on using a combination of targeted cover crop monocultures and mixtures across a field in response to soil and topographic characteristics variables could yield a method of improving environmental and economic sustainability, while still maximizing cover crop derived ecosystem benefits.

Participation Summary

1 Farmer participating in research

Educational & Outreach Activities

2 Curricula, factsheets or educational tools

2 Journal articles

1 Published press articles, newsletters

9 Webinars / talks / presentations

2 Workshop field days

1 Other educational activities: The results of this study were integrated into a guest lecture delivered to ~40 undergraduate students at the University of Kentucky.

PARTICIPATION SUMMARY:

50 Farmers

100 Ag professionals participated

Education/outreach description:

The knowledge gained from this study was integrated into a University of Kentucky extension publication about cover crop opportunities and challenges in Kentucky: http://www2.ca.uky.edu/agcomm/pubs/AGR/AGR240/AGR240.pdf. We are also working on a newsletter article for the University of Kentucky Corn and Soybean Newsletter about the effects of cover crops on nitrogen supply to corn in different landscape positions.

We published two peer-reviewed journal articles:

- Leuthold, S., D. Quinn, F. Miguez, O. Wendroth, M. Salmeron, and Poffenbarger, H. 2020. Topographic effects on soil microclimate and surface cover crop residue decomposition in rolling cropland. *Agriculture, Ecosystems, and Environment:* 107609.
- Leuthold, S., M. Salmeron, O. Wendroth, and Poffenbarger, H.J. 2021. Cover crops decrease maize yield variability in sloping landscapes through increased water during reproductive stages. *Field Crops Research 265*: 108111.

We presented the results of our experiments to a number of groups, including other researchers, the Kentucky Corn Board, Kentucky farmers at University of Kentucky Field Days, and members of the Tri-State Soil Health Initiative. Each of these events was a structured as a short talk, followed by a question and answer session, during which practitioners were able to ask questions and apply the research findings to their own experience and land.

- Leuthold, S., Wendroth, O., Salmeron, M., Poffenbarger, H. (2020) Can cover crops reduce spatial variability in yield in areas of complex topography? 2020 ASA/CSSA/SSSA Annual Meeting. 8-12 November. Virtual Conference due to COVID-19.
- Poffenbarger, H.J. and S. Leuthold. (2020) Understanding and addressing spatial variability in corn yield. University of Kentucky Corn, Soybean, and Tobacco Field Day. 27 July, Princeton, KY.
- Leuthold, S., Poffenbarger, H., Salmeron, M., Wendroth, O., Haramoto, E. (2020) Do cover crops increase or decrease spatiotemporal variability in maize yield? Poster presentation at iCROPm 2020. 1 February. Montpellier, France. (H. Poffenbarger presenting author)
- Poffenbarger, H., Leuthold, S., Salmeron, M., Wendroth, O., and Haramoto, E. (2020) Corn, complex topography, and cover crops. Kentucky Corn CORE farmer program, 10 January, Versailles, KY.
- Leuthold, S., Wendroth, O., Salmeron, M., Haramoto, E., Poffenbarger, H. (2020) Corn, cover crops, and complex topography. Oral presentation presented at Southern ASA Regional Meeting. 2 February. Louisville, KY.
- Leuthold, S., Wendroth, O., Salmeron, M., Haramoto, E., Poffenbarger, H. (2019) Interactive effects of cover crops and nitrogen rate across complex landscape topography on maize yield stability., 10-13 November, Oral presentation at 2019 ASA/CSSA/SSSA Annual Meeting. San Antonio, TX.
- Poffenbarger, H., Leuthold, S., Salmeron, M., Wendroth, O., and Haramoto, E. Investigating nitrogen rates across areas of complex topography and the interaction with cover crops. Kentucky Corn Growers Annual Meeting, 5 November, Elizabethtown, KY.
- Leuthold, S., Wendroth, O., Salmeron, M., Haramoto, E., Poffenbarger, H. (2019) Cover crops response to landscape topography, and the effect on maize yield stability. 2019 ASA-CSSA-SSSA Annual Meeting, 10-13 November, San Antonio, TX.
- Leuthold, S., Wendroth, O., Salmeron, M., Haramoto, E., Poffenbarger, H. (2019) Cover crop growth varies by landscape position but does not affect spatial variability of maize yield. North Central Soil Fertility Conference, 5-6 November, Des Moines, IA.

- Leuthold, S., Poffenbarger, H.J. (2019) Quantifying decomposition rates and nitrogen fixation ability of leguminous cover crops in production fields characterized by complex topography. Southern Cover Crops Council Fall Meeting, July 16-17, Auburn, AL.
- Poffenbarger, H.J. (2019) University of Kentucky Corn, Soybean, and Tobacco Field Day. 23 July, Princeton, KY.

In addition, the results of this study were integrated into a guest lecture delivered to ~40 undergraduate students at the University of Kentucky.

- Poffenbarger, H. (2021) Understanding and managing soil variability in agroecosystems. Guest lecture for University of Kentucky ABT 201 course. 16 September, Lexington, KY.

Lastly, this project was featured in a University of Kentucky news article about farmer-researcher collaborations: http://news.ca.uky.edu/article/farmer-researcher-partnerships-are-backbone-agricultural-advance

Project Outcomes

2 Farmers reporting change in knowledge, attitudes, skills and/or awareness

3 Grants received that built upon this project

3 New working collaborations

Project outcomes:

The project we presented here has wide ranging implications for the use of sustainable agricultural practices in areas that have rolling hill topography, including large portions of the Central and Southeastern United States. Our findings that leguminous cover crops tend to compete more readily, fix more nitrogen, and subsequently release more nitrogen back to the system in areas of the landscape that have higher slopes is a useful thing to understand for farmers. Because the cost of legume seed can be much more expensive than that of grass, concentrating legumes where they can be most effective and produce the most benefit is a useful and important thing to highlight for farmers. In addition, some of the other work associated with this project has highlighted the benefits of cover cropping to yield and yield stability in areas of rolling hills, increasing the likelihood of adoption of these practices for farmers who may be averse because of the possibility of yield decreases.

Knowledge Gained:

I (S. Leuthold) learned several new skills throughout the course of these experiments. My data analysis skills improved; I learned a number of new statistical analysis tools, such as generalized additive models and principal component analysis. The coordination between collaborators and myself improved my communication skills and planning, and the opportunity to present research in both academic and applied settings gave me a chance to improve my public speaking and communication skills as well. In relation to my understanding of sustainable agriculture, the opportunity to work on this project was invaluable. I learned a great deal about the constraints on sustainable practice success, and how heterogeneous the response of certain practices can be across the landscape. I also was able to see first-hand how beneficial the implementation of practices such as cover cropping can be in certain situations. I think the biggest change in my case was an increased understanding of what it takes to make a farm run– working with Richard and watching the way he has to plan and coordinate across a large acreage made the research we do feel much more real, and much more exciting.

I (H. Poffenbarger) acquired a new understanding of soil constraints on sloping ground, including shallow depth, clayey textures, and vulnerability to hot and dry weather. My attitude about cover cropping has become more positive because I recognize the risk of soil erosion on sensitive sloping landscapes and the importance of soil protection now more than ever. I gained new knowledge about the suitability of certain cover crop species for certain soil types – specifically that crimson clover seems to perform well on sandy soils and sloping ground whereas cereal rye responds well to high soil organic matter and soil N availability. In talking with farmers about this project, I learned that some farmers prefer wheat over cereal rye as a cover crop and I am thinking of integrating wheat cover crops into my research program.

Recommendations:

Future studies that look at precision cover crop management would be beneficial to increasing our ability to recommend fiscally responsible practices to farmers. Ideally these studies could include a cost-benefit analysis, and recommendations for how management should be implemented when variable rate seeding different cover crop species across a given field. We also recommend developing crop models that capture landscape position effects on rye cover crops and mixture cover crops, so that a model can be used to evaluate cover crop growth and decomposition in a larger range of environments.

Information Products

- Cover Crop Benefits and Challenges in Kentucky (Bulletin)
- Farmer-Researcher Partnerships are Backbone of Agricultural Advances (Article/Newsletter/Blog)

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