ABSTRACT

Title of Thesis: Quantifying the impacts of climate-smart farming practices for improved management and long-term carbon storage

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Master of Science
2023

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Within agricultural production there is tension between feeding a rapidly growing population and conserving the finite resources at the foundation of our agroecosystems. Fortunately, in recent decades there has been a growing focus on farming practices that promote long-term soil health, land productivity, and resilience to climate change. The term ‘conservation agriculture’ encompasses practices that 1) promote minimum soil disturbance, 2) maintain permanent soil cover, and 3) diversify plant species. This research evaluated several conservation agriculture practices for their ability to deliver desired agroecosystem services across the Northeastern US. In the first study, a cover crop mixture field experiment was implemented in seven states to evaluate how climatic, edaphic, and management conditions affected the performance of cover crop bicultures that included species with varying functional traits. Seeding rate recommendations for mixtures are typically developed

at the regional level, thus cover crop performance is highly variable due to site-level conditions and competition among species. Our results indicated that expected spring growing degree days and baseline soil fertility (i.e., inorganic N) are the most significant variables to consider when designing site-specific cover crop mixtures. The second study assessed the effects of long-term management on soil organic carbon (SOC) dynamics in mid-Atlantic grain cropping systems. At the time of sampling, five unique systems (two conventional, three organic) had been continuously managed for 25 years, representing a range of tillage and fertility practices and rotational complexities. Results showed SOC loss in all systems over time regardless of management, likely because of high baseline SOC stocks from long-term perennial forage production prior to research plot establishment. However, cropping systems that best maintained SOC over time included management with minimal soil disturbance, frequent manure inputs, and/or greater rotational diversity through perennial cropping or cover cropping. Both studies increase our understanding of the ability of specific conservation practices to support agroecosystem biodiversity, long term soil health, and potential carbon sequestration.

QUANTIFYING THE IMPACTS OF CLIMATE-SMART FARMING PRACTICES FOR IMPROVED MANAGEMENT AND LONG-TERM CARBON STORAGE

by

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2023

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Dedication

To my parents, for their unwavering support of my passions and pursuits since the beginning.

Acknowledgements

Thank you to the talented, hardworking crew in both the Mirsky and Tully laboratories, for the knowledge and assistance they provided from start to finish of these projects. I especially appreciate the help of Resham Thapa, Gwen Bagley, Cara Peterson, Alison Schulenburg, Brian Moyer, Ana Breach, Sam Hasselhoff, and Sophie Smith.

Thank you to the NECCC Research Committee and the many research teams throughout the Northeast that made the NECCC region-wide mixture study a success.

Thank you to Kate White and Chris Rasmann of the BARC Farming Systems Project team for their resources and knowledge.

I also appreciate the dedicated work of Jen Showalter, George Meyers, and Stew ‘MixMaster’ McMaster of the BARC field research team.

Special thank you to my family and friends for their love and support during both the highs & lows of my graduate school journey. Especially Jack, Caroline, Nora, David, Jen, and Kelsey.

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Abstract

Cover crop mixtures that include functionally complementary species can increase resource use efficiency, total cover crop biomass, and agroecosystem benefits. However, current regional-based seeding rate recommendations may limit the extent of potential benefits due to influence of climatic, environmental, and management conditions. The development of site-specific seeding rates may be necessary to optimize cover crop mixture services and increase farmer adoption. The aim of this study was to understand how site conditions influence mixture performance across the northeastern US, with total biomass, species evenness (yield distribution of constituent species), and seed cost used as metrics of performance. A field experiment was implemented at seven research farms across the region, from Maryland to Maine. Monocultures and bicultures were established at various seeding rates: 0%, 25%, 50%, 100%, and 150% of the recommended rate of that species in monoculture. Winter cover crops were planted from three functionally diverse plant families: cereal rye (grass), hairy vetch (legume), and forage rape (brassica), which were selected for their differing functional traits and popularity among northeastern growers. Exploratory statistics showed that climate variables (spring growing degree days, hardiness zone) and soil conditions (soil nitrogen, pH, organic matter) were more influential on cover crop mixture performance than seeding rate proportions between the constituent species. Findings also indicated that growers have more flexibility in species selection and can use lower seeding rates in temperate than colder climates to produce high yielding, multi-functional mixtures. Cereal rye dominated at sites with colder winter seasons due to its winter hardiness compared to the other species. Evenness among mixtures that included hairy vetch was primarily influenced by baseline soil nitrogen levels, and slightly less influenced by spring growing degree days. As expected, forage rape biomass was highly dependent on climate

and performed poorly at colder sites; this study was helpful to show the geographical reach of this species as a winter cover crop. Our results suggest that anticipated growing degree days in the cover crop season and baseline soil fertility should be considered before selecting species and seeding rate proportions for multi-functional mixtures.

Introduction

Long-term tillage, continuous monocropping, and limited use of carbon-based amendments have left agricultural soils degraded. Lower performing soils make food production systems more vulnerable to extreme weather events caused by changing climates (Page et al., 2020). Agroecosystem resilience through improved soil health and biodiversity has been identified as a key solution to mitigate the detrimental effects of climate change (Basche et al., 2020). Small increases in agroecosystem biodiversity have been shown to trigger large increases in ecosystem services, such as building beneficial biological communities (i.e., mycorrhizal fungi), disrupting pest cycles, and improving soil structure (Blesh et al., 2019; Romdhane et al., 2019; Snapp et al., 2005). Planting winter cover crops into an existing crop rotation is one practical method to reintroduce diversity into an agroecosystem as it allows growers to utilize a window of time during the year that is typically not economically viable for crop production (Blesh et al., 2019). Cover crops have seen a steady increase in adoption across the US compared with other conservation strategies (USDA NASS, 2019), largely due to government-funded financial incentives aimed at promoting this practice (Basche et al., 2020; Sawadgo & Plastina, 2021). However, most cover crops are themselves planted in monoculture due to uncertainty among farmers about implementing multi-species cover crops (i.e., mixtures; USDA NASS, 2019). Studies have shown that cover crop mixtures that incorporate multiple functional traits provide more agroecosystem services due to better resource use efficiency among species (Blesh, 2018; Holmes et al., 2017; Wendling et al., 2017; Yu et al., 2015). Fine-tuning cover crop recommendations to farmers that optimize both multi-functional mixtures and low cost could encourage more widespread adoption.

Seed costs for cover crop mixtures depend on species number, plant functional type, and seeding rate of each component species in a mixture (CTIC et al., 2020). Quantifying how cover crop species compete with each other is key to managing for potential outcomes and ensuring high return on investment. Ecosystem services such as weed suppression, erosion control, and reduced nitrate leaching positively correlate with biomass production (Schipanski et al., 2014), and relatively even amounts of biomass from each species in a mix is essential for each to perform their affiliated services to a sufficient degree (Bybee-Finley et al., 2022; Finney & Kaye, 2017). A fall-sown mixture including a brassica species that grows deep taproots with a grass species that grows more fibrous roots allows both species to maximize their belowground biomass with minimal competition, due to complementary root architecture (Finney et al., 2016). Nitrogen accumulating grasses planted with nitrogen-fixing legumes can promote more efficient fixation, retention, and release of nitrogen (N) to subsequent crops compared to either species planted in monoculture (Hayden et al., 2014; Thapa et al., 2018). Selecting complementary species and appropriate seeding rate proportions is essential to deliver desired ecosystem services from multi-functional mixtures.

Farmers consult with county-level Soil Conservation District offices, state agricultural extension agents, or local seed dealers to obtain recommendations on single and multi-species cover crop selection and seeding rates (CTIC et al., 2020). However, current recommendations are made on a regional-level and typically do not consider site-specific climate and soil conditions. These non-optimized recommendations therefore may limit the extent of potential benefits due to the unequal performance of component species, i.e., asymmetric competition (Bybee-Finley et al., 2022; Murrell et al., 2017; Poffenbarger et al., 2015). Due to asymmetric competition, fast-growing grasses like cereal rye (*Secale cereale* L.) have been shown to produce more biomass than desired in a mixture compared to a monoculture; on the other hand, fall-growing brassicas such as forage rape (*Brassica napus* L.) have been shown to produce less biomass in a mixture compared to a monoculture (Murrell et al., 2017). Multiple studies have found that changing seeding rates of hairy vetch (*Vicia villosa* R.) and cereal rye in a mixture significantly influences stand density, species

composition, and total biomass (Poffenbarger et al., 2015; Thapa et al., 2018). Proper species selection and seeding rate proportions will vary based on climate, soil fertility, and field management. Cover crop seeding rate studies are important for understanding the interaction between site-specific variables and mixture composition.

The northeastern US encompasses agroecosystems with variable climatic, environmental, and management conditions, making it an ideal study region to help researchers and growers understand what site-specific variables are most influential on cover crop mixture success. To evaluate variability in mixture performance across the region, field experiments were conducted at seven sites distributed across the northeastern US. Study objectives included 1) assessing the relative importance of intrinsic (climatic, edaphic) site-specific variables compared to management variables (species selection, seeding rate) on mixture performance, and 2) determining which species and seeding rate combinations yielded the most successful mixtures across sites based on total biomass, species evenness, and seed cost in order to quantify productivity and multi-functionality. Quantifying how climate, soil, and management influence cover crop mixture performance will improve the ability of growers to link future management with desired outcomes, helping to reduce potential risks of adoption. The adoption of multi-functional cover crop mixtures can help to increase agroecosystem resilience across the US, an essential tool to help protect our food systems against the growing threat of a changing climate.

Methods

Participating sites

A field experiment was implemented in eight states throughout the northeastern US to assess how climate and soil influence cover crop mixture performance (Fig. 1.1). The participating research institutions, from south to north, included the USDA-ARS Beltsville Agricultural Research Center (BARC; Beltsville, MD), Pennsylvania State University (PSU; State College, PA), Cornell University (CU; Ithaca, NY), University of Massachusetts (UMass; Amherst, MA), University of New Hampshire (UNH; Durham, NH), University of Vermont

(UVM; Burlington, VT), and University of Maine (UME; Orono, ME). These sites collectively represent a range of weather conditions, soil types, and site management histories found across the northeastern US (Table 1.1). Average winter minimum temperatures (Dec-Feb) at these locations range from -12.2 °C to -2.6 ℃, and mean annual precipitation for the region ranges from 973 mm to 1215 mm. These variations in climate as well as soil and management conditions provide a broad range of soil × climate × management conditions that can impact cover crop mixture performance.

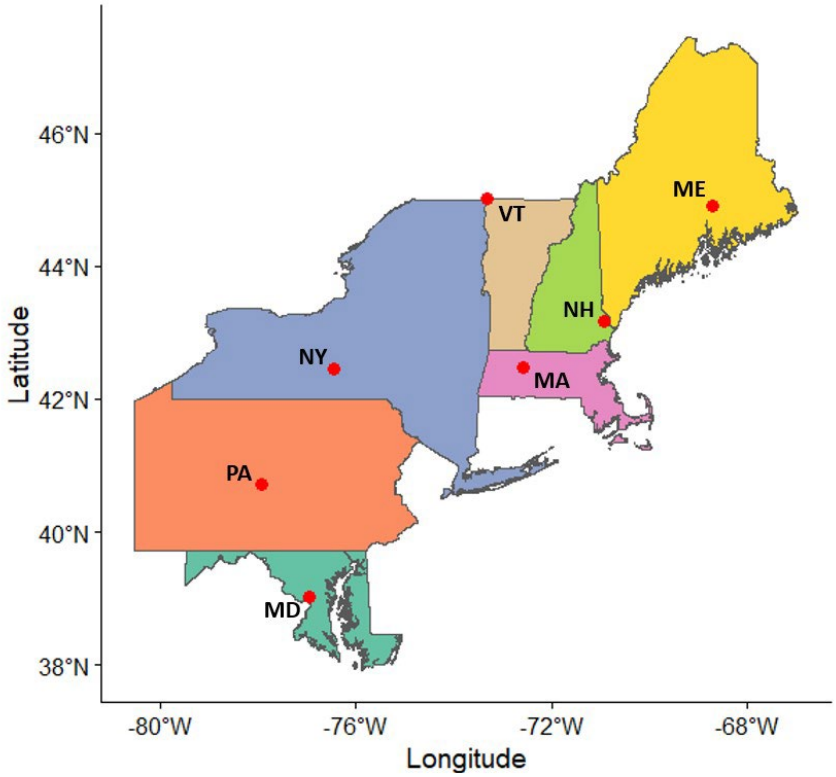

Fig. 1.1. Participating states across the northeastern US. Red dots represent location of field sites.

Table 1.1. Characteristics of the experimental sites across seven northeastern US states.

Site	USDA Beltsville Agricultural Research Center Beltsville, MD (MD)	Pennsylvania State Univ. Russell E. Larson Agricultural Research Center Pennsylvania Furnace, PA (PA)	Cornell Univ. Musgrave Research Farm Aurora, NY (NY)	Univ. of Massachusetts-Amherst South Deerfield, MA (MA)
Coor.	39.019, -76.944	40.719, -77.93	42.448, -76.459	42.475, -72.585
Soil series*	Codorus and Hatboro silt loams	Murrill channery silt loam	Niagara silt loam	Winooski silt loam
Clay content 0-30 cm (%)*	16	30	18	8
Typical crop rotation	No typical rotation	Corn / Soybean / Oats	No typical rotation	No typical rotation
Preceding crop (2020)	Cover crop trials (clover, pea, vetch, rye)	Corn silage	Wheat and barley trials	Corn / summer buckwheat
Avg min. temp. (℃)#	-2.6	-5.2	-8.2	-8.5
Avg max. temp. (℃)#	29.6	26.2	25.6	27.2
Avg annual precip. (mm)	1118	1055	973	1183
Planting date	9/25/2020	9/8/2020	9/7/2020	9/4/2020
Termination date	4/28/2021	5/11/2021	5/13/2021	5/19/2021

Table 1.1 Continued

Site	Univ. of New Hampshire Kingman Farm Madbury, NH (NH)	Univ. of Maine Orono, ME (ME)	Univ. of Vermont Borderview Research Farm Alburgh, VT (VT)
Coor.	43.171, -70.934	44.907, -68.69	45.011, -73.307
Soil series*	Hollis-Charlton fine sandy loams	Nicholville very fine sandy loam	Benson rocky silt loam
Clay content 0-30 cm (%)*	4.5	6	20
Typical crop rotation	Cucurbit / various cover crops	Corn silage / small grain + red clover	Corn silage / small grains / various cover crops
Preceding crop (2020)	Cucurbits / sudex	Fallow	Wheat
Avg min. temp. (℃)#	-8	-12.2	-10.1
Avg max. temp. (℃)#	25.8	24.8	26.5
Avg annual precip. (mm)	1215	1129	973
Planting date	9/9/2020	9/4/2020	8/30/2020
Termination date	5/19/2021	5/18/2021	5/4/2021

* Soil data collected from NRCS Web Soil Survey. Average clay content estimated for 0-30 cm.

Weather data collected from NOAA Northeast Regional Climate Center weather stations. Based on 1991-2020 monthly averages. Average minimum temperature taken from Dec-Feb; average maximum temperature taken from June-Aug

/ Indicates transition between crops for typical rotation

Experimental design

At each experimental site, a series of restricted response surfaces were laid out containing cover crop monocultures and bicultures (i.e., mixtures). The response surface method design (Inouye, 2001) allows researchers to examine the relationship between multiple input variables and one or more response variables. In this case, a response surface method was used to evaluate the effects of cover crop species selection, seeding rate, and site-specific conditions on total biomass and relative evenness among species in the mix. Only one replicate of each treatment was planted to maximize the number of mixture combinations to better evaluate the influence of competition within and among species in the non-linear regression models developed from this dataset (Inouye, 2001).

Common methods for studying seeding rates (i.e., replacement or additive approaches) obscure dynamics within and among species in a mixture because the seeding rates of the component species are not changed independently, but rather depend on the monoculture seeding rate used for each species (Inouye, 2001). Instead, a series of restricted response surfaces were used in this study, which enabled us to effectively investigate interactions in multi-species mixtures by varying seeding densities of the different species separately. Manipulating seeding densities independently in mixture studies is a helpful tool to distinguish between complementarity and competition in species dynamics (Bybee-Finley et al., 2022).

Winter cover crop varieties used in this study include ‘AU Merit’ hairy vetch, a legume, ‘Barsica rape’ forage rape, a brassica, and variety not specified (VNS) cereal rye, a grass. Cereal rye, the most common grass cover crop across the US (CTIC et al., 2020), is known for its high biomass production, prolific root system, and winter hardiness (Mirsky et al., 2017). Forage rape is known for its high fall biomass production and large taproots that mine residual nutrients and help alleviate soil compaction (Clark, 2008; Neely et al., 2015). Hairy vetch is popular due to its rapid spring growth and ability to biologically fix N in low fertility soils (Clark, 2008). For both monocultures and bicultures, five different seeding rates were used for each species at all sites, presented as a percent of the recommended rate for

each species when planted in monoculture (0%, 25%, 50%, 100%, or 150%). Seeding rates for each species in monoculture were provided and recommended by a regional seed dealer (King's Agriseeds Inc., Rocks, PA). Standard monoculture seeding rates used include: 89.67 kg ha^{-1}, 42.46 kg ha^{-1}, and 8.97 kg ha^{-1} for cereal rye, hairy vetch, and forage rape, respectively. A full factorial design was used for both monoculture (n = 12) and biculture (n = 48) treatments, with all possible species combinations and seeding rates. This approach allowed us to observe the impact of seeding density on total biomass and species evenness. Four no cover crop control plots were also included to measure weed pressure and composition at each site (total of 64 plots per site).

Field experiments were planted between late August and late September 2020, depending on field management and weather conditions (Table 1.1). Since forage rape requires a longer fall growing season for adequate biomass establishment to reduce the possibility of winter-kill (28 ℃) (Ackroyd et al., 2012), planting dates were chosen at northern sites to account for this. The selected forage rape variety ('Barsica rape') was bred to be more winter-hardy compared to other cultivars, but some degree of winter-kill was expected in the colder states participating in the study (e.g., New Hampshire, Maine, Vermont). Cover crops were planted using a cone seeder at approximately 13 mm depth and plot dimensions were roughly 0.5 m by 6.1 m, varying slightly across sites based on cone seeder dimensions. Specific site plot layouts varied based on field dimensions, and treatment placement was randomized throughout the field to account for spatial heterogeneity.

Data collection

Between late August and mid-October 2020, participants counted plant densities in each treatment plot after the first true leaves of the cover crop developed. A representative sampling area of 0.5 m^2 was selected in each plot to count the number of each individual species that emerged (Hume & Shirriff, 1995). Corners of the 0.5 m^2 sample area were flagged to ensure cover crop biomass samples in the spring were collected in the same area of the plot. The number of days between planting date and density counts varied by site (13-35 days).

Soil samples were collected from each field site at the time of density counts (late August and mid-October 2020). Sixteen cores (0-30 cm) were collected at random across the field experiment using a 1.9-cm diameter soil corer. Soil cores were homogenized by hand and air-dried at room temperature. Once dried, all soil samples were shipped to BARC for soil inorganic nitrogen (iN) analysis. Samples were ground to < 2 mm and analyzed by 2M potassium chloride extraction at the University of Maryland Agroecology Laboratory in a 1:5 soil to solution ratio. Extracts were frozen overnight and analyzed colorimetrically the next day after thawing on a LACHAT QuikChem (LACHAT Instruments, Loveland, CO, USA) using the salicylate-nitroprusside method for NH_4-N (detection limit 0.02 mg NH_4-N L-1; Hood-Nowotny et al., 2010) and the sulfanilamide method for NO_3-N (detection limit 0.025 mg NO_3-N L-1; Keeney & Nelson, 1983). Organic matter content was measured through loss on ignition; a 2-g subsample was oven-dried at 105°C for one hour and then weighed to determine moisture content, then placed in a 500°C oven for two hours and weighed again. Soil pH was measured by combining a 10-g subsample with 10-mL of ultrapure water to form a soil slurry, which was then left to sit for approximately one hour before a pH level was recorded (Mclean, 1982). Concentrations of other essential nutrients (i.e., P, K, Mg, Ca, Na, Fe) were estimated using a Mehlich-3 extraction (Mehlich, 1984); 20-mL of a Mehlich-3 solution was added to a 20-g subsample, shaken for 20-min and then the extract was filtered. Sample extracts were than analyzed using inductively coupled plasma mass spectrometry (Dairy One Co-Op Inc., Ithaca, NY).

Biomass samples were collected from each plot between late-April 2021 and mid-May 2021, before brassica seed maturation. Plants were clipped within 1 cm from the soil surface inside the pre-established 0.5 m^2 quadrats. Samples were separated by species, and placed in paper bags to be oven-dried. All weed species were separated from cover crop species but were not identified to the species-level. In the control plots, weed biomass was collected within representative sampling areas (0.5 m^2), identified to the species-level, and dried separately using the same methods as described above. All biomass was oven-dried (60 ℃) for at least one week before recording dry weights.

Spring growing degree days (GDD) were calculated from weather stations within 12 km of each field site. A base temperature of 4℃ was used since it is the minimum temperature required for growth of cereal rye, the most winter hardy cover crop species included in our study (Elhakeem et al., 2019; Stoskopf, 1985). Weather data were retrieved using CLIMOD2, an online weather resource provided by NOAA, Northeast Regional Climate Center at Cornell University (http://climod2.nrcc.cornell.edu/). Growing degree days were calculated for each day (Eq. 1.1) and the sum of these scores were recorded (1 Jan 2021 to biomass sampling date).

$$\frac{Daily\ min\ temperature + daily\ max\ temperature}{2} - Base\ temperature\ (4℃) \qquad \text{(Eq. 1.1)}$$

USDA hardiness zone was also recorded for each site location, a standard by which growers can determine what crops will grow best in their area based on the average annual minimum winter temperature, divided into 10℉ zones (Daly et al., 2012).

To determine the multifunctionality of each biculture at each site, total biomass and species evenness were quantified. Spring biomass of cover crops is a simple and effective measurement to estimate the magnitude of production and the delivery of several desired ecosystem services (weed suppression, erosion control, nitrate retention; Schipanski et al., 2014). A productive cover crop should yield roughly 4,500 kg ha^{-1} or more of total biomass at spring termination (Clark, 2008). The biomass (kg ha^{-1}) of each component species was summed to determine the total biomass of a biculture combination. Evenness among species indicates a relatively equal contribution of multiple desired services and is an important target when growing cover crop mixtures. The *vegan* package in R (Oksanen et al. 2019) was used to calculate species evenness based on biomass using Pielou's species evenness equation (Pielou, 1966). To obtain this value, Shannon's diversity index was first calculated (Eq. 1.2).

$$Shannon\ Weiner\ diversity\ index\ (H') = -\Sigma[(p_i) \times ln(p_i)] \qquad \text{(Eq. 1.2)}$$

Where p_i = proportion of biomass of the *i*-th species in a whole sample (biomass of species / total biomass of mixture). This value (H) is then divided by the natural log of the total number of species in the mixture (Eq. 1.3).

$$Pielou's\ species\ evenness\ (J) = \frac{H'}{ln(S)} \qquad \text{(Eq. 1.3)}$$

where S is equivalent to the observed number of species in the sample.

Pielou's measure of species evenness is set between 0 and 1, with 1 representing a community with equal components of each species. An evenness range of 0.6 - 1 has been linked to higher ecosystem productivity in forest and grassland ecosystems (Kirwan et al., 2007; Zhang et al., 2012). A value of 0.8 or higher has been shown in the literature to represent a community with relatively equal proportions of species (Tracy & Sanderson, 2004).

Statistical Analysis

To determine if individual species differed in biomass production across sites, the biomass of monoculture treatments was compared using three separate linear mixed effects (LME) models (one for each cover crop species), with site as the main effect and seeding rate as the random effect (*lme4* package for R; Bates et al. 2013). Prior to creating LME models, I calculated Spearman's rank correlation coefficients using the *cor.test()* command from the *stats* package (R Core Team, 2022) to determine the relationship between monoculture seeding rate and biomass at each site. I found no significant correlation between seeding rate and biomass for any species ($p > 0.05$), therefore, the various seeding rates were used as replicates. When needed, biomass data were square-root transformed before analysis based on the distribution of model residuals. Tukey post-hoc tests were used to examine significant differences among sites (*multcomp* package for R; Hothorn et al. 2008), determined at $P < 0.05$.

To quantify the primary associations between our target response variables (total biomass and evenness) and both observed and manipulated predictor variables, I used Classification and Regression Trees (CART; Breiman et al., 1984) in R using the *rpart*

package (Therneau & Atkinson, 2019). The CART analysis is widely used in the field of ecology as it helps to explain the variation of a response factor based on multiple predictors (numerical and/or categorical), yielding interpretable results from complex datasets (De'ath & Fabricius, 2000). The technique uses automatic stepwise regression to identify mutually exhaustive and exclusive subgroups of the dataset to create a decision tree that includes the most influential predictor variables (Machuca et al., 2017). Each tree node is split using the predictor variable that minimizes the "impurity" of the resulting nodes. Node impurity is a way to measure homogeneity, with a value of zero representing a completely homogeneous node (all observations in a node have the same response value), and node impurity increases as homogeneity decreases (De'ath & Fabricius, 2000). Primary splits in the tree are selected based on which predictor minimizes the sum of squares of the mean response value, that is, minimizes the amount of deviation from the mean. In addition, competitive variables are selected to show which factors could also split the node into two more homogenous subsets if the primary split variable was absent, although competitive variables have slightly higher impurity (Therneau et al., 2022). In other words, selected competitive variables explain some degree of variance within a node but are not as influential as the primary split variable. Therefore, the variable importance of a predictor in the final model is determined by both primary splits and competitive variables, and predictors can be considered competitive and highly influential to the dataset without being selected as a primary split (Therneau et al., 2022). This aspect of CART helps to prevent the masking of variables due to nonlinear correlation between predictors. Six regression trees were created, two for each cover crop combination (cereal rye and hairy vetch, cereal rye and rapeseed, and hairy vetch and rapeseed), considering both total biomass and species evenness as response variables. Predictor variables included in the CART models were climate variables (spring GDD, USDA hardiness zone), soil fertility variables (soil iN, organic matter content (OM), pH), and manipulated variables (seeding rates of component species) (Table 1.2).

Table 1.2. Site values for intrinsic predictor variables used in regression tree analysis

Site	MD	PA	NY	MA	NH	ME	VT
USDA hardiness zone	7A	6A	6A	5B	5B	5A	4B
Spring GDD+	767	757	470	739	647	488	467
Soil iN (kg ha^{-1})	18.94	91.58	47.44	80.43	36.85	149.73	170.94
pH	6.34	6.23	6.18	6.78	6.22	6.06	7.44
Organic matter (%)	2.14	2.67	2.22	2.63	4.89	3.98	4.59

+ GDD (growing degree days) collected from NOAA Northeast Regional Climate Center weather stations. Based on 1991-2020 monthly averages; base temperature of 4°C.

To train the model a leave-one-out cross-validation approach was used (Sammut & Webb, 2010); the model was run seven times, and each time a unique site subset was removed from the full dataset. The most parsimonious regression trees were chosen based on the complexity parameter, an advisory parameter for decision trees that weighs both the complexity of the tree and predictive power (Therneau et al., 2022). To capture the overall fit of each model, the root mean squared error (RMSE) and scatter index (SI) were reported. The calculated RMSE indicates the absolute fit of the model to the actual data. In other words, it helps to measure how close the observed data points are to the model's predicted values. Since the RMSE is reported in the same units as the response variable (i.e., total biomass or species evenness), SI was calculated to normalize the RMSE values across all regression trees. The scatter index is calculated by dividing the RMSE value of a model by the mean value of the response variable for the whole dataset and multiplying by 100. A lower SI value (%) indicates a better fit to the model. All statistical analyses were conducted in the R environment for Windows (v1.3.1073).

Determination of best performing mixtures

After quantifying how total biomass and species evenness of bicultures responded to changes in site conditions, the top performing bicultures at each site were identified using a qualitative approach, considering our three selected performance metrics of total biomass, species evenness and total seed cost. Top mixtures were identified to determine which two-species combinations performed best based on measured site variables, and to provide another comparison on how total biomass and species evenness at time of spring sampling differed across the study sites. Seed cost was calculated to consider the investment required for growers to plant a specific biculture combination. Although a mixture may produce high levels of biomass with evenly distributed species, a high seed cost will make adoption unrealistic for growers.

The first filter applied to each site was to determine high yielding bicultures relative to overall site biomass production. For each site, a subset containing bicultures within the top 50th percentile for total biomass was created to eliminate any treatments that produced below-median biomass over the growing season. Seed cost values for the cover crop bicultures were calculated based on 2020 seed costs provided by King's Agriseeds Inc. (Ronks, PA). Grain producers throughout the northeastern US spend similar amounts on their cover crop seed, with at least 75% of growers spending less than \$100 ha^{-1} on cover crop seed (CTIC et al., 2020). Therefore, each subset was further reduced by eliminating any mixtures that cost more than \$100 ha^{-1}. The remaining treatments in each site subset were ultimately sorted in descending order by Pielou's evenness index (Eq. 2, 3), as species evenness indicates the multifunctionality of the resulting cover crop mixture and the return on investment for growers. The five mixture treatments that were ranked at the top of this final subset were then noted as top performing species and seeding rate combinations for each site.

Results

Monoculture biomass across sites

Biomass accumulation of monocultures differed significantly by species ($p < 0.05$; Fig. 1.2), therefore each species was analyzed separately. Cereal rye biomass was more consistent across sites compared to the other two species, with average biomass ranging from 3,053 kg ha^{-1} (MD) to 6,210 kg ha^{-1} (PA) (Fig. 1.2-A; Table 1.3). Cereal rye monocultures produced the greatest biomass in PA and ME (4,902 kg ha^{-1}). High variability in biomass across sites was found for forage rape monocultures (Table 1.3), with average biomass by site ranging from 158 kg ha^{-1} (VT) to 5,699 kg ha^{-1} (PA). Pennsylvania, MA (5,416 kg ha^{-1}) and MD (4,389 kg ha^{-1}) produced relatively high forage rape biomass compared to the other sites (Fig. 1.2-B). In VT, the site with the most consecutive days with negative GDD (Fig. 1.3-G), spring forage rape biomass was nearly nil. Other northern sites (i.e., ME, NH, NY) also had low yields for rapeseed monocultures, and when averaged by site, produced less than 2,000 kg ha^{-1}. Across sites, high biomass variability was found for hairy vetch monocultures (Fig. 1.2C; Table 1.3); average biomass, by site, ranged from 380 kg ha^{-1} (VT) to 4,315 kg ha^{-1} (MD). Sites that produced relatively high biomass for hairy vetch monocultures included MD, NH (4,011 kg ha^{-1}) and MA (2,946 kg ha^{-1}). Other sites produced less than 2,250 kg ha^{-1}.

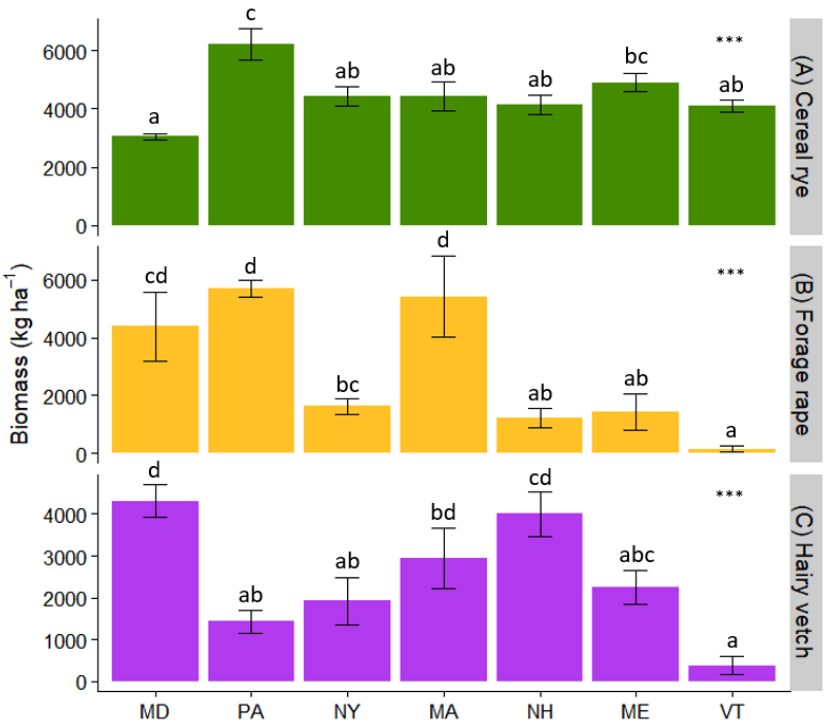

Fig. 1.2. Mean monoculture yields (kg ha-1) across study sites for each species: A) cereal rye, B) forage rape, and C) hairy vetch. Mean biomass was calculated for each species from the four seeding rate treatments (i.e., 0.25x, 0.50x, 1.0x, 1.5x the standard monoculture seeding rate). Error bars are the standard error of the mean (n = 4). Within a species, bars sharing the same letter are not significantly different at P < 0.05. Asterisks (***) indicate significance by site at P<0.001 in Tukey post hoc pairwise comparisons.

Table 1.3. ANOVA results from LME models testing the effects of site on monoculture biomass for each species, followed by Tukey's honestly significant difference (HSD) test results indicating statistical significance between sites. Significance was determined at $P < 0.05$.

ANOVA

Cereal rye	*df*	Sum of Squares	Mean Square	*F*	*p-value*
Site	6	21841514	3640252	7.06	< 0.001

Tukey HSD

Site1	Site2	Mean Difference	Standard Error	*p-value*
PA	MD	3157	507.84	< 0.001
NY	MD	1374	507.84	0.097
MA	MD	1379	507.84	0.09
NH	MD	1104	507.84	0.31
ME	MD	1849	507.84	0.005
VT	MD	1049	507.84	0.37
NY	PA	-1783	507.84	0.008
MA	PA	-1778	507.84	0.008
NH	PA	-2053	507.84	< 0.001
ME	PA	-1308	507.84	0.13
VT	PA	-2109	507.84	< 0.001
MA	NY	5	507.84	1.00
NH	NY	-270	507.84	0.998
ME	NY	475	507.84	0.97
VT	NY	-326	507.84	0.995
NH	MA	-275	507.84	0.998
ME	MA	470	507.84	0.97
VT	MA	-330	507.84	0.995
ME	NH	745	507.84	0.76
VT	NH	-55	507.84	1.00
VT	ME	-800	507.84	0.70

Table 1.3. Continued

ANOVA					
Forage rape	*df*	Sum of Squares	Mean Square	*F*	*p-value*
Site	6	14123	2354	10.97	< 0.001

Tukey HSD				
Site1	Site2	Mean Difference	Standard Error	*p-value*
PA	MD	11.05	10.36	0.94
NY	MD	-24.53	10.36	0.21
MA	MD	7.13	10.36	1.00
NH	MD	-30.34	10.36	0.05
ME	MD	-32.35	10.36	0.03
VT	MD	-54.49	10.36	< 0.001
NY	PA	-35.58	10.36	0.01
MA	PA	-3.92	10.36	1.00
NH	PA	-41.38	10.36	0.0013
ME	PA	-43.39	10.36	< 0.001
VT	PA	-65.54	10.36	< 0.001
MA	NY	31.66	10.36	0.04
NH	NY	-5.81	10.36	1.00
ME	NY	-7.82	10.36	1.00
VT	NY	-29.96	10.36	0.06
NH	MA	-37.47	10.36	0.01
ME	MA	-39.48	10.36	0.003
VT	MA	-61.62	10.36	< 0.001
ME	NH	-2.01	10.36	1.00
VT	NH	-24.16	10.36	0.23
VT	ME	-22.15	10.36	0.33

Table 1.3. Continued

ANOVA					
Hairy vetch	*df*	Sum of Squares	Mean Square	*F*	*p-value*
Site	6	47267810	7877968	8.72	< 0.001

Tukey HSD				
Site1	Site2	Mean Difference	Standard Error	*p-value*
PA	MD	-2889	672	< 0.001
NY	MD	-2397	672	0.01
MA	MD	-1369	672	0.39
NH	MD	-304	672	1.00
ME	MD	-2066	672	0.03
VT	MD	-3935	672	< 0.001
NY	PA	491	672	1.00
MA	PA	1520	672	0.26
NH	PA	2585	672	0.002
ME	PA	823	672	0.88
VT	PA	-1046	672	0.71
MA	NY	1028	672	0.73
NH	NY	2094	672	0.03
ME	NY	332	672	1.00
VT	NY	-1538	672	0.25
NH	MA	1065	672	0.69
ME	MA	-697	672	0.95
VT	MA	-2566	672	0.002
ME	NH	-1762	672	0.12
VT	NH	-3631	672	< 0.001
VT	ME	-1870	672	0.08

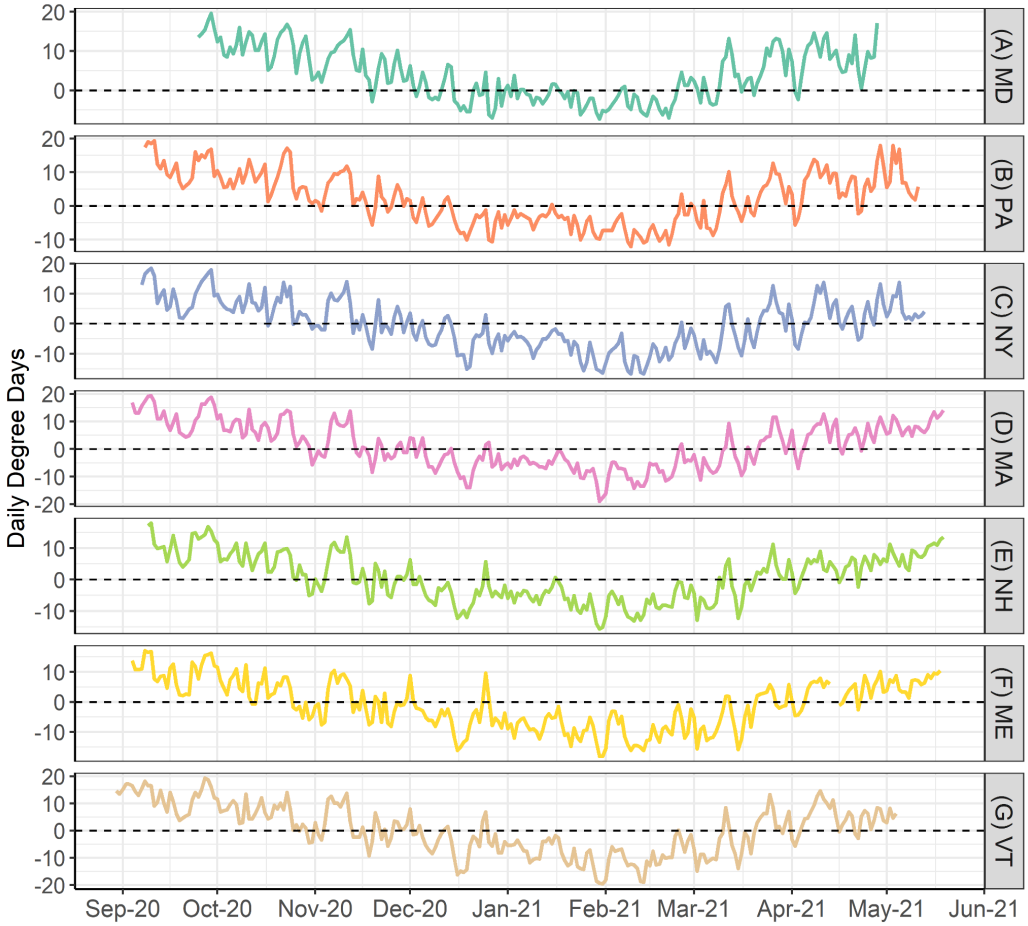

Fig. 1.3. Daily growing degree days (GDD) from fall planting to spring termination, using a base temperature of 4℃ for each site. The following weather stations were used: (A) MD - Beltsville, MD; (B) PA - State College, PA; NY - Ithaca, Cornell Univ; (D) MA - Amherst, MA; (E) NH - Durham 2N, NH; (F) ME - Old Town 2W, ME; (G) VT - Burlington International Airport, VT. Negative GDD are only included in the figure to serve as an indicator of winter severity, not included in the final spring GDD calculation.

Regression tree results

I) Cereal rye + hairy vetch

A) Total biomass

The average total biomass of cereal rye and hairy vetch mixtures across sites was 5,225 kg ha^{-1}, with values ranging from 1,270 kg ha^{-1} (MA) to 10,627 kg ha^{-1} (PA). The most parsimonious regression tree to explain variability in total biomass contained four primary splits using two weather variables (spring GDD, hardiness zone), one soil fertility variable (soil inorganic N), and one manipulated variable (cereal rye seeding rate) (Fig. 1.4-A, Table 1.3). Spring growing degree days (GDD) was shown to be the most influential factor across all sites (32% variable importance; Table 1.3). Sites with less than 479 spring GDD (NY, VT) yielded the least biomass and, on average, cereal rye made up 93.6% of all mixtures in this subset (Fig. 1.4-A). For sites with greater than 479 spring GDD, the next primary split was soil inorganic N (22% variable importance). Sites containing soil inorganic N levels less than 58.6 kg ha^{-1} (MD, NH) produced lower cereal rye + hairy vetch biomass compared to sites with higher soil inorganic N levels (Fig. 1.4-A). Three of five study sites contained soil inorganic N levels of 58.6 kg ha^{-1} or greater (PA, MA, ME). The next primary split for these relatively high fertility sites was cereal rye seeding rate (8% variable importance). Cereal rye + hairy vetch mixtures within each site that were seeded at less than 0.375 times the standard monoculture rate yielded less total biomass compared to mixtures with higher cereal rye seeding rates (Fig. 1.4-A). For cereal rye + hairy vetch mixtures that contained higher cereal rye seeding rates, the final primary split was based on hardiness zone (23% variable importance). Mixtures that were grown in a hardiness zone colder than 6A (MA, ME) yielded less biomass compared to warmer hardiness zones (PA; Fig. 1.4-A). Pennsylvania mixtures that were included in the right-most terminal node yielded greater biomass compared to all other sites and treatments ($p < 0.05$); however, the mixtures were largely dominated by cereal rye (Fig. 1.4-A).

B) Evenness

The average evenness of cereal rye + hairy vetch mixtures across sites was 0.51, with mixtures ranging from 0 (MA, VT) to 0.99997 (MD). The most parsimonious regression tree to explain variation in species evenness for cereal rye + hairy vetch mixtures contained only one split: soil inorganic N (33% variable importance; Fig. 1.4-B, Table 1.3). Sites that had baseline soil inorganic N levels of 86 kg ha^{-1} or greater resulted in extremely low evenness among cereal rye and hairy vetch (PA, ME, VT; mean Pielou's index = 0.21), and on average, cereal rye biomass was 19 times more than hairy vetch biomass (Figure 1.3-B). Sites with lower soil inorganic N levels had more equal representation among cereal rye and HV, with biomass of the two component species having an average ratio of 60:40, respectively (Fig. 1.4-B). Hardiness zone and organic matter content were included as competitive variables (both 22% variable importance; Table 1.3). It is important to note that this tree had a relatively high SI value (79.3%), indicating poor model fit to the dataset.

Although lower soil inorganic N levels resulted in less biomass compared to the highest yielding cereal rye + hairy vetch mixtures, species evenness was much higher in sites with lower soil inorganic N (Fig. 1.4-A, B).

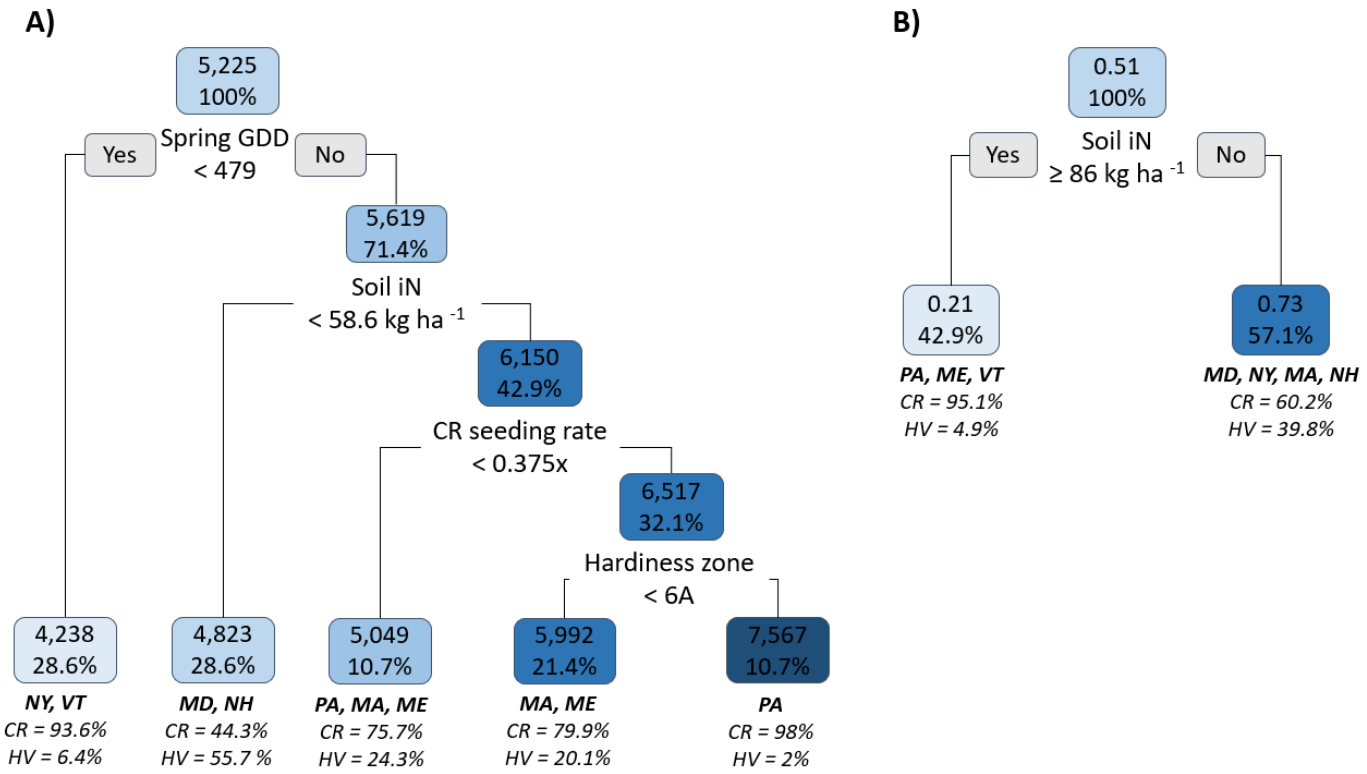

Fig. 1.4. Classification and regression tree (CART) results for cereal rye (CR) and hairy vetch (HV) mixtures for response variables A) total biomass (kg ha^{-1}) and B) species evenness (Pielou's evenness index). Top value in each box indicates the mean response value for that node, the percentage value in each box indicates the proportion of the complete dataset included in that node. Lines stemming from the left side of each primary split lead to mixtures that satisfy the split threshold (YES), and lines stemming from the right side of the primary split lead to mixtures that do not satisfy the split threshold (NO). States listed below each terminal node indicate what site is included in that subset. Average species proportions (%) for each subset are listed below each terminal node.

Table 1.4. Summary of classification and regression tree (CART) results for cereal rye (CR) and hairy vetch (HV) mixtures based on response variables total biomass and species evenness. cp = complexity parameter; RMSE = root mean squared error, SI = scatter index.

Mixture	*Response*	*cp*	*RMSE*	*SI*	*Predictor**	*Variable importance (%)*^
Cereal rye + Hairy vetch	Total biomass	0.032	1651.5	31.6%	Spring GDD	32
					Hardiness zone	23
					Soil iN	22
					pH	9
					CR seeding rate	8
					OM	7
	Species evenness	0.192	0.401	79.3%	Soil iN	33
					Hardiness zone	22
					OM	22
					pH	11
					Spring GDD	11

* Predictors are selected variables that were used as both primary splits and/or competitive variables in the final regression tree.

^ The model calculates variable importance to the final tree by considering the contribution of each selected predictor as both a primary split and/or competitive variable. The complete set of predictor variables considered in the regression analysis included climatic (spring growing degree days (GDD), hardiness zone), edaphic (soil inorganic nitrogen (iN), pH, organic matter (OM)), and seeding rate (CR and HV seeding rate).

II) Cereal rye + forage rape

A) Biomass

The average total biomass of cereal rye and forage rape mixtures across sites was 4,220 kg ha^{-1}, with mixtures ranging from 42 kg ha^{-1} (VT) to 9486 kg ha^{-1} (MA). The most parsimonious regression tree to explain variation in total biomass for cereal rye + forage rape mixtures contained two primary splits: hardiness zone and soil inorganic N (19% and 31% variable importance, respectively; Fig. 1.5-A, Table 1.4). Hardiness zone was the first tier primary split, with zones colder than 5A resulting in lower total biomass for cereal rye + forage rape mixtures (Fig. 1.5-A). The next primary split for sites within hardiness zones warmer than or equal to 5A was soil inorganic N. If soil inorganic N levels were less than 63.9 kg ha^{-1} (MD, NY, NH), cereal rye + forage rape mixtures yielded less biomass compared to

sites with higher soil inorganic N (Fig. 1.5-A). Competitive variables for this tree included pH and spring GDD (both 19% variable importance; Table 1.4).

B) Evenness

The average evenness of cereal rye + forage rape mixtures across sites was 0.41, with evenness ranging from 0 (MA, NH, ME, VT) to 0.999 (MD, PA). The most significant predictor to explain variability of species evenness within cereal rye + forage rape mixtures was hardiness zone (32% variable importance; Fig. 1.5-B, Table 1.4). Sites located within a hardiness zone of 6A or warmer (MD, PA, NY) yielded more even cereal rye + forage rape mixtures compared to colder locations (Fig. 1.5-B). Sites located in hardiness zones colder than 6A were further split by cereal rye seeding rate (MA, NH, ME, VT). If a mixture included cereal rye at a seeding rate less than 0.75 times its standard rate in monoculture, it generated a slightly higher species evenness score compared to mixtures with higher cereal rye seeding rates (Fig. 1.5-B). The average evenness scores for the two nodes on the left side of the tree are much lower than desired, and both subsets formed from the primary split of cereal rye seeding rate consist of rye-dominant mixtures. Competitive variables for this tree include OM and spring GDD (both 22% variable importance; Table 1.4).

Hardiness zone was the top tier primary split for both total biomass and species evenness of cereal rye + forage rape mixtures. Sites in zones warmer than or equal to 6A yielded mixtures with relatively high species evenness and high biomass (MD, PA, NY; Fig. 1.5-A, B).

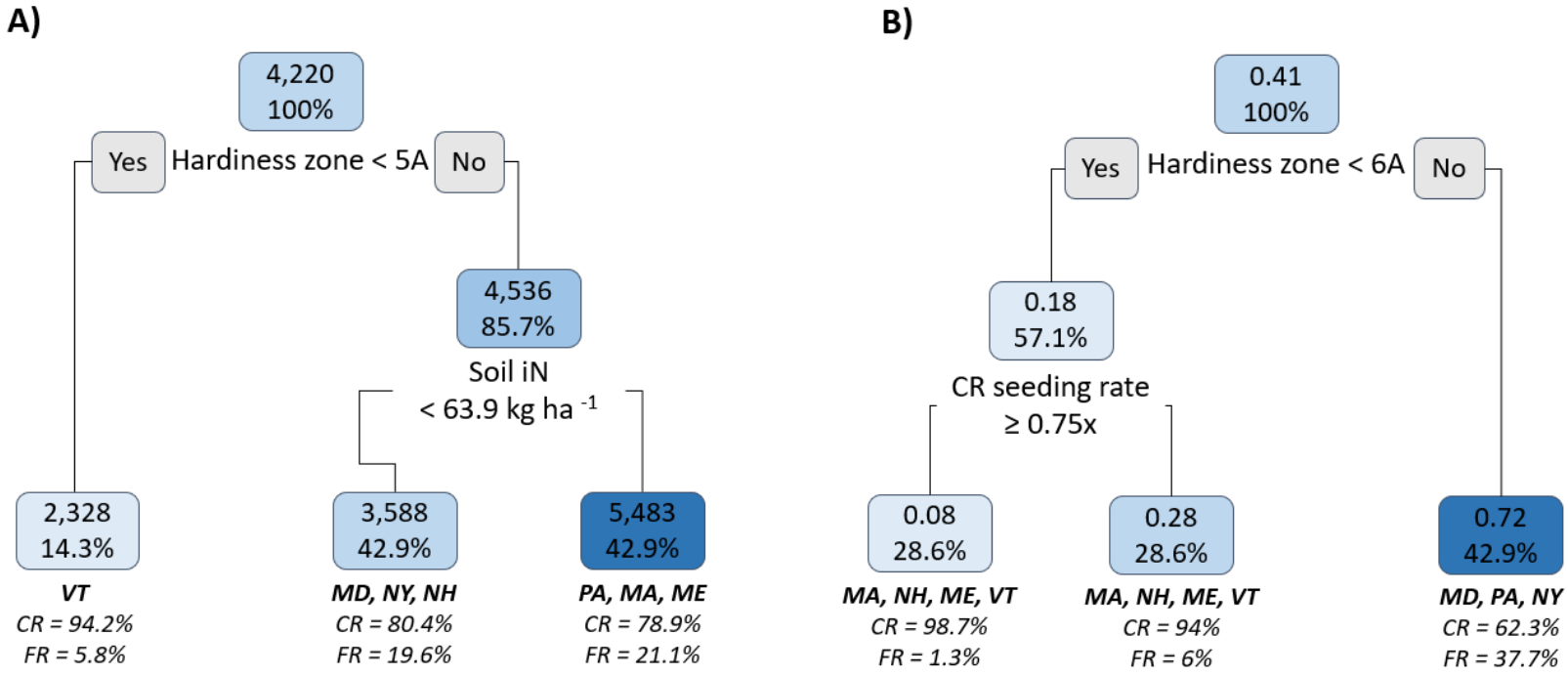

Fig. 1.5. Classification and regression tree (CART) results for cereal rye (CR) and forage rape (FR) mixtures for response variables A) total biomass (kg ha-1) and B) species evenness (Pielou's evenness index). Top value in each box indicates the mean response value for that node, the percentage value in each box indicates the proportion of the complete dataset included in that node. Lines stemming from the left side of each primary split lead to mixtures that satisfy the split threshold (YES), and lines stemming from the right side of the primary split lead to mixtures that do not satisfy the split threshold (NO). States listed below each terminal node indicate what site is included in that subset. Average species proportions (%) for each subset are listed below each terminal node.

Table 1.5. Summary of classification and regression tree (CART) results for cereal rye (CR) and forage rape (FR) mixtures based on response variables total biomass and species evenness. cp = complexity parameter; RMSE = root mean squared error; SI = scatter index.

Mixture	*Response*	*cp*	*RMSE*	*SI*	*Predictor**	*Variable importance (%)^*
Cereal rye + Forage rape	Total biomass	0.135	2159.4	51.2%	Soil iN	31
					Hardiness zone	19
					pH	19
					Spring GDD	19
					OM	12
	Species evenness	0.045	0.207	50%	Hardiness zone	32
					OM	22
					Spring GDD	22
					pH	11
					Soil iN	11
					CR seeding rate	3

* Predictors are selected variables that were used as both primary splits and/or competitive variables in the final regression tree.

^ The model calculates variable importance to the final tree by considering the contribution of each selected predictor as both a primary split and/or competitive variable. The complete set of predictor variables considered in the regression analysis included climatic (spring growing degree days (GDD), hardiness zone), edaphic (soil inorganic nitrogen (iN), pH, organic matter (OM)), and seeding rate (CR and FR seeding rate).

III) Hairy vetch + forage rape

A) Biomass

The average total biomass of hairy vetch + forage rape mixtures across sites was 3,209 kg ha^{-1}, with values ranging from 0 kg ha^{-1} (VT) to 7,647 kg ha^{-1} (PA). The most parsimonious regression tree to explain total biomass variability for hairy vetch + forage rape mixtures contained five primary splits using two weather variables (spring GDD, hardiness zone) and one manipulated variable (FR seeding rate; Fig. 1.6-A, Table 1.5). A combination of high spring GDD (≥ 568), relatively warm hardiness zone (≥ 6A), and forage rape seeded at less than 0.75 times the standard rate in monoculture (8.97 kg ha^{-1}) resulted in mixtures with the highest total biomass (mean biomass = 6,103 kg ha^{-1}; MD, PA). Sites with low spring GDD (< 568) had extremely low yields for hairy vetch + forage rape mixtures (VT, NY, ME),

with VT experiencing nearly complete winterkill for this species combination (mean biomass = 131 kg ha^{-1}; Fig. 1.6-A). The top tier primary split, spring GDD, explained the most variance in the dataset, followed by hardiness zone (29% and 20%, respectively; Table 1.5). Competitive variables for this tree included pH and soil inorganic N (both 19% variable importance; Table 1.5).

B) Evenness

The average evenness of hairy vetch + forage rape mixtures across sites was 0.49, with mixtures ranging from 0 (PA, NY, MA, ME, VT) to 0.999 (VT). The most parsimonious regression tree that captured variability in species evenness for hairy vetch + forage rape included three primary splits, using two soil variables (soil inorganic N, pH) and one weather variable (spring GDD; Fig. 1.6-B; Table 1.5). A combination of low soil inorganic N (< 86 kg ha^{-1}) and high spring GDD (≥ 693) resulted in hairy vetch + forage rape mixtures with the highest species evenness (mean Pielou's index = 0.80; MD, MA). On the other hand, a combination of relatively high soil inorganic N (≥ 86 kg ha^{-1}) and slightly more alkaline pH (≥ 6.15) resulted in mixtures with the lowest species evenness for this combination (mean Pielou's index = 0.15; PA, VT). Out of all predictor variables, pH and soil inorganic N explained nearly 50% of the variance (25% and 24%, respectively), and spring GDD explained 16% (Table 1.5). Hardiness zone and organic matter were competitive variables (both 17% variable importance). Overall, this regression tree had a poor model fit (SI = 124.4%; Table 1.5), likely due to insufficient growth of both component species at the colder study sites (NH, ME, VT).

Model fit for the hairy vetch + forage rape biomass regression tree was much better than model fit for species evenness (SI = 46.8% and 124.4%, respectively). Spring GDD was a common primary split used in both trees (Fig. 1.6-A, B; Table 1.5).

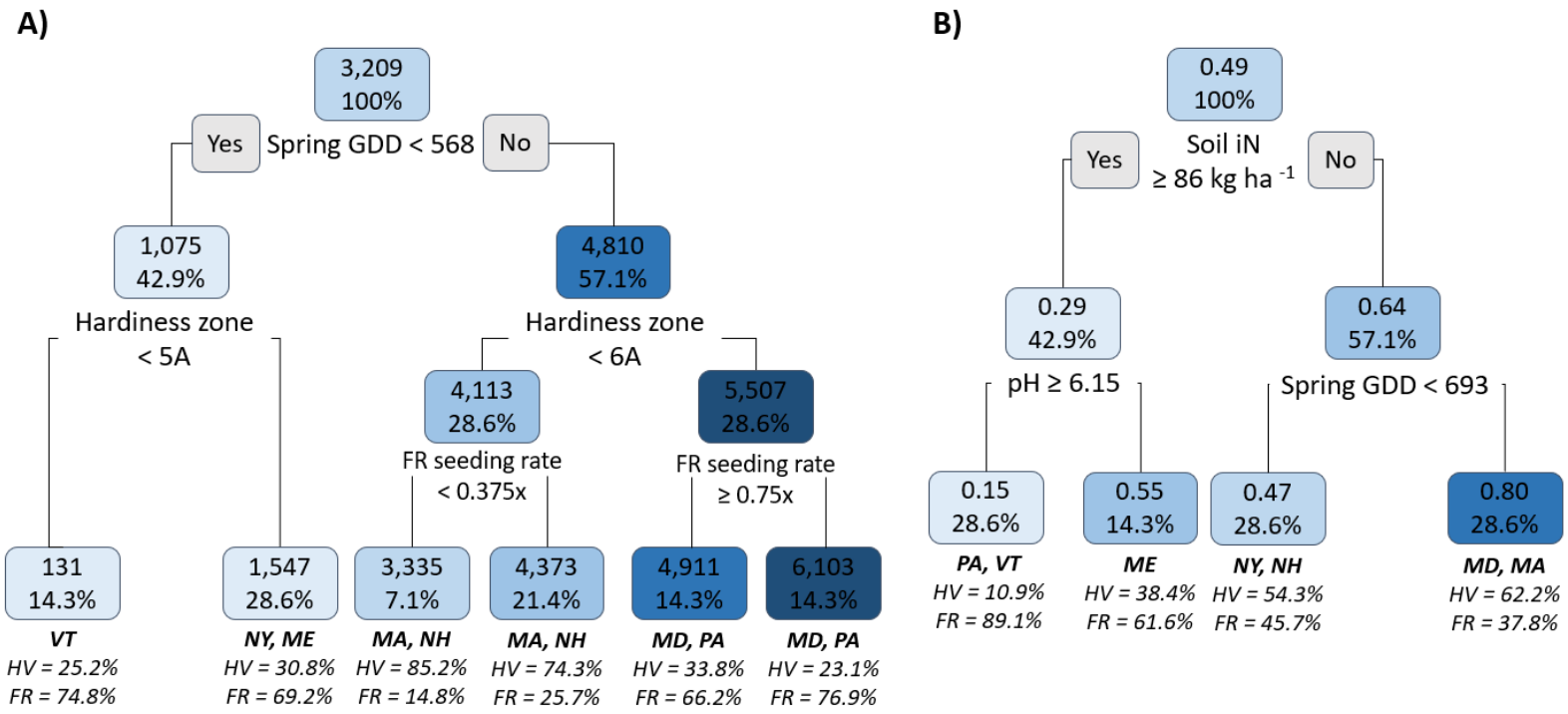

Fig. 1.6. Classification and regression tree (CART) results for hairy vetch (HV) and forage rape (FR) mixtures for response variables A) total biomass (kg ha-1) and B) species evenness (Pielou's evenness index). Top value in each box indicates the mean response value for that node, the percentage value in each box indicates the proportion of the complete dataset included in that node. Lines stemming from the left side of each primary split lead to mixtures that satisfy the split threshold (YES), and lines stemming from the right side of the primary split lead to mixtures that do not satisfy the split threshold (NO). States listed below each terminal node indicate what site is included in that subset. Average species proportions (%) for each subset are listed below each terminal node.

Table 1.6. Summary of classification and regression tree (CART) results for hairy vetch (HV) and forage rape (FR) mixtures based on response variables total biomass and species evenness. cp = complexity parameter; RMSE = root mean squared error; SI = scatter index.

Mixture	*Response*	*cp*	*RMSE*	*SI*	*Predictor**	*Variable importance (%)^*
Hairy vetch + Forage rape	Total biomass	0.01	1503.6	46.8%	Spring GDD	29
					Hardiness zone	20
					pH	19
					Soil iN	19
					OM	11
					FR seeding rate	1
	Species evenness	0.092	0.606	124.4%	pH	25
					Soil iN	24
					Hardiness zone	17
					OM	17
					Spring GDD	16

* Predictors are selected variables that were used as both primary splits and/or competitive variables in the final regression tree.

^ The model calculates variable importance to the final tree by considering the contribution of each selected predictor as both a primary split and/or competitive variable. The complete set of predictor variables considered in the regression analysis included climatic (spring growing degree days (GDD), hardiness zone), edaphic (soil inorganic nitrogen (iN), pH, organic matter (OM)), and seeding rate (HV and FR seeding rate).

Top performing mixtures at each site based on biomass, evenness, and cost.

Based on our selected primary metrics of performance, the qualitative ranking of treatments at each site indicated large variability in the total biomass (2,441 to 8,659 kg ha^{-1}) and species evenness (0.04 to 0.9998) of top-performing bicultures across the Northeast (Table 1.6). At sites with colder growing seasons (NY, ME, VT), all best-performing mixtures contained cereal rye, and most of the selected mixtures had lower evenness among species compared to the temperate sites because of cereal rye outcompeting the other component species. Mixtures with the highest degree of evenness (>0.8) at these colder sites did not qualify as top-performers due to poor biomass establishment. Sites with warmer spring growing seasons (MD, PA, MA, NH) produced several high yielding (>4500 kg ha^{-1}) and evenly distributed (>0.8) bicultures.

At the MD site, high-yielding, top-performing bicultures were composed of cereal rye + forage rape and hairy vetch + forage rape (Table 1.6). Interestingly, the mixture with the most biomass production out of these top performers was a hairy vetch + forage rape combination (7,044 kg ha^{-1}), with both species planted at their lowest rate, resulting in a low overall seed cost ($36.45 ha^{-1}; Table 1.6). The PA site had similar weather to the MD site (757 and 767 spring GDD, respectively), but soil inorganic N levels were higher (91.58 kg ha^{-1} in PA vs 18.94 kg ha^{-1} in MD). Based on the performance criteria, PA had high success with cereal rye + forage rape mixtures and, similar to MD, the lowest seeded mixture for this combination yielded the most biomass (8,659 kg ha^{-1} at $19.48 ha^{-1}; Table 1.6).

Both the MA and NH sites were located within the same hardiness zone, but a warmer spring growing season in MA likely led to the relatively higher biomass as compared to that in NH. Although they differed in overall mixture performance, both sites yielded bicultures with high biomass (> 4,500 kg ha^{-1}) and high evenness (>0.8) at low seed costs (Table 1.6). However, mixtures containing forage rape had greater evenness at MA compared to NH; for example, the cereal rye + forage rape mixture included as a top-performing mixture at the NH site only generated an evenness score of 0.04 (Table 1.6).

Table 1.7. Top five bicultures at each site that satisfied the biomass (2,500 kg ha-1) and seed cost ($100 ha-1) thresholds, sorted by decreasing evenness. CR = cereal rye, HV = hairy vetch, FR = forage rape. Seeding rates are expressed as a proportion of the standard monoculture rate for that species. Standard monoculture seeding rates used include CR = 89.67 kg ha^{-1}, HV = 42.46 kg ha^{-1}, FR = 8.97 kg ha^{-1}.

		Proportion of monoculture rate (%)					
Site	Mix	CR	HV	FR	Total Biomass (kg ha^{-1})	Pielou's Evenness	Cost ($ ha^{-1})
MD	CR+FR	0.5	--	1.5	4888	0.999	63.09
	HV+FR	--	0.25	0.25	7044	0.99	36.45
	HV+FR	--	0.5	1	4232	0.98	84.96
	CR+HV	0.5	0.25	--	4788	0.96	57.32

Table 1.7. Continued

	HV+FR	--	0.5	0.25	6046	0.95	66.86
PA	CR+FR	1	--	1	6922	0.99	77.93
	CR+FR	1	--	0.25	7210	0.97	59.83
	CR+FR	0.25	--	0.25	8659	0.96	19.48
	CR+FR	0.5	--	0.25	7623	0.93	32.93
	CR+FR	1	--	0.5	6266	0.90	65.87
NY	CR+FR	0.25	--	0.5	3672	0.94	25.52
	CR+HV	0.25	0.5	--	3897	0.72	74.28
	CR+FR	1	--	1	4576	0.63	77.93
	CR+FR	0.25	--	1.5	3901	0.58	49.64
	CR+FR	0.25	--	0.25	4178	0.57	19.48
MA	CR+HV	0.5	0.5	--	5574	0.94	87.73
	CR+HV	1	0.25	--	6020	0.92	84.22
	HV+FR	--	0.25	1.5	6110	0.90	66.61
	HV+FR	--	0.5	0.5	5974	0.87	72.90
	CR+FR	0.5	--	0.5	6128	0.84	38.97
NH	CR+HV	0.5	0.5	--	5157	0.9998	87.73
	CR+HV	1	0.25	--	5101	0.89	84.22
	CR+HV	0.25	0.5	--	5447	0.88	74.28
	HV+FR	--	0.5	1	4335	0.71	84.96
	CR+FR	1.5	--	0.25	4169	0.04	86.74
ME	CR+HV	0.25	0.5	--	4524	0.49	74.28
	CR+FR	0.5	--	0.25	5706	0.29	32.93

Table 1.7. Continued

	CR+HV	0.25	0.25	--	7818	0.27	43.87
	CR+FR	1	--	1	4476	0.27	77.93
	CR+FR	1	--	0.5	4922	0.25	65.87
VT	CR+FR	0.5	--	0.5	2858	0.71	38.97
	CR+FR	0.25	--	0.5	2441	0.55	25.52
	CR+FR	1	--	1	3007	0.42	77.93
	CR+FR	1	--	0.5	3979	0.25	65.87
	CR+HV	0.5	0.5	--	4266	0.23	87.73

Discussion

Biomass of monocultures of hairy vetch and forage rape varied considerably compared to that of cereal rye across the Northeast US

Cereal rye is the primary cover crop grown throughout the US because of its adaptability to both climate and management factors (Mirsky et al., 2017a; CTIC et al., 2020). Results from this study reflect findings from existing literature showing the reliability of cereal rye to provide high biomass throughout the winter season (Blesh et al., 2019; Clark, 2008). Despite differences in soil and weather conditions across the Northeast US, cereal rye monoculture biomass production was the most consistent compared to the other species across study sites.

The performance of forage rape monocultures is more dependent on weather, with higher levels of biomass in warmer than colder sites. For brassica cover crop species to overwinter, they require sufficient fall growing days to become established before the onset of freezing temperatures (< 28 ℃), therefore an earlier planting date is recommended for

brassicas than most grass or legume species (Ackroyd et al., 2012; Clark, 2008). However, planting too early can result in increased exposure of brassica plant tissue to freezing temperatures and higher rates of winterkill in colder climates (Ananga et al., 2012; Baraibar et al., 2020). Sites with higher relative fall GDD for this study accumulated between 1,223-1,363 fall GDD and, by increasing order, included PA, MA, MD, and VT. Vermont had unseasonably high cumulative fall GDD, and it is likely that forage rape fall biomass, which was not measured in this study, was higher at VT than at sites with similar winter conditions for this study (NY, ME). Throughout the winter months, resulting aboveground biomass established in the fall at VT was more susceptible to plant tissue damage, and a high accumulation of consecutive negative GDD likely led to increased winterkill and the lowest spring biomass recorded for forage rape across sites (Fig. 1.2-B).

The performance of hairy vetch monoculture was more influenced by baseline soil fertility than growing season weather conditions. These findings echo past studies that show hairy vetch growth is negatively correlated with soil inorganic N concentrations (Blesh, 2018; Clark, 2008; Plumhoff et al., 2022). The two sites with low soil inorganic N in this study (MD, NH) yielded the highest biomass for hairy vetch monocultures (Fig. 1.2-C; Table 1.2), suggesting that low baseline soil fertility favored vetch production and increased the amount of spring biomass compared to other sites. Overall, hairy vetch was the lowest yielding monoculture compared to the other two species (Fig. 1.2). This finding agrees with previous studies that found cover crop monocultures of grass and brassica species produced higher biomass compared to legume species in temperate climates and, as a result, were able to deliver desired ecosystem services, such as nitrate leaching reduction, weed suppression, and water conservation more effectively (Baraibar et al., 2018; Finney et al., 2016). Regardless of their relative performance, legumes are highly desired in mixtures because of their unique ability to fix atmospheric N and supply it to the subsequent cash crop.

Consideration of soil fertility conditions outweighed weather conditions when targeting even mixtures containing hairy vetch

Both weather and soil fertility variables were primary splits for four out of the six regression trees for biomass and evenness, indicating that both factors are crucial when designing site-specific mixture recommendations. However, to achieve evenness within mixtures that include hairy vetch, soil fertility was more important to consider compared to weather or seeding rate. Sites with the highest baseline soil inorganic N concentrations (> 90 kg ha^{-1}) yielded cereal rye + hairy vetch mixtures with the lowest species evenness, showing a clear dominance of rye (Fig. 1.4-B; Table 1.2). The greater availability of soil N at these sites likely enhanced cereal rye productivity and N content, providing limited resources (e.g., water, light) for hairy vetch growth in the spring (Thapa et al., 2018a). Legumes like hairy vetch thrive under conditions of limited soil inorganic N because of their N-fixation abilities. At some of the sites with moderate baseline soil inorganic N levels (MD, MA, NH), both species complemented each other by optimizing atmospheric N fixation by hairy vetch and residual soil N retention by cereal rye, leading to overyielding when grown in a mixture compared to in a monoculture (Fig. 1.4-B; Table 1.2). Planting hairy vetch in combination with cereal rye has been shown to lead to higher rates of N fixation then when planted in monoculture, as cereal rye is able to stimulate the N-fixation capacity of legumes due to rapid uptake of residual soil N in the fall (Suter et al., 2015; Thapa et al., 2018b). Due to their higher seed cost relative to other cover crop families, planting legumes in a mixture instead of a monoculture can cut costs significantly, while still providing similar N fixation rates (Clark, 2008; Reiss & Drinkwater, 2020).

The evenness dynamics for hairy vetch + forage rape mixtures were not as easily explained, with the final evenness regression tree having low predictive power (SI = 124%; Table 1.5). This poor model performance is possibly a result of the low biomass production found for both species at colder study sites (NY, ME, VT; Fig. 1.2-B, C), as neither hairy vetch nor forage rape are as tolerant to freezing winter temperatures compared to cereal rye (Clark, 2008; Mirsky et al., 2017b). In addition, both species have different soil fertility

requirements: hairy vetch grows well in soils with low inorganic N stocks, while forage rape favors soils with higher inorganic N stocks (Baraibar et al., 2020; Clark, 2008). Both species require sufficient fall GDD to become established before winter temperatures set in, however forage rape is more susceptible to winterkill compared to hairy vetch (Clark, 2008; Duiker, 2014; Kucek et al., 2019). The cold-season cultivar of forage rape used in this study ('Barsica rape') is able to survive temperatures down to -12.2 ℃ (Clark, 2008). However, consecutive freezing degree days in NY, ME, and VT (Fig. 1.3-C, F, G) indicated more extreme winter conditions compared to other sites and led to higher rates of forage rape winterkill. Due to relatively high baseline soil inorganic N concentrations in the fall (Table 1.2), forage rape was able to establish more quickly compared to hairy vetch at the PA, VT, and ME sites. This resulted in rape-dominant hairy vetch + forage rape mixtures in the spring (Fig. 1.6-B), but extremely low total biomass for this species combination in ME and VT (Fig. 1.6-A) due to the increased rates of forage rape winterkill.

Sites with milder winter seasons have more flexibility in cover crop mixture composition

Regardless of seeding rate proportions, the total biomass of mixtures containing cereal rye was much higher compared to hairy vetch + forage rape mixtures at three sites with limited spring GDDs (NY, ME, VT). Like other cover crop studies, these results show the adaptability of cereal rye as a winter cover crop, as well as its tendency to dominate over less competitive species (Mirsky et al., 2017a; Reiss & Drinkwater, 2020). Evenness values for the top-performing mixtures at these colder sites were inconsistent and leaned towards dominance of CR, especially in ME and VT (Table 1.6). These two sites had the most negative GDD, indicating greater winter severity (Fig. 1.3-F, G). The relatively higher performance of cereal rye grown solely or in mixtures at these northern sites emphasizes the need for mixtures to include grass species in regions with more extreme winter conditions. Proper species selection ensures that growers are maximizing their return on investment when it comes to planting and managing cover crops. Extending the cover crop growing season by terminating later in the spring can encourage spring biomass production and

associated services of non-grass component species, especially legumes (Clark, 2008; Mirsky et al., 2017b). However, delaying cover crop termination can be challenging in northern climates due to potential yield reductions from a shorter cash crop season compared to more temperate locations (Mirsky et al., 2017b). Management accommodations for later cover crop termination include planting shorter season crop varieties, or planting green to delay cover crop termination until the day of cash crop planting (Antichi et al., 2022; Weyers et al., 2021).

No strong seeding rate trends were observed among regression trees, and the seeding rate contribution to model variance was minor compared to other predictor variables (0-8%; Tables 1.2-1.4). One common observation found in both the regression trees and top performing mixture selection was that total biomass did not consistently increase with seeding rate proportions of component species in the mixtures. This is likely a result of intraspecific competition, i.e., when an increase in seeding density inhibits plant growth due to crowding and resource limitation among the same species (Bybee-Finley et al., 2022; Wendling et al., 2017). Results from the final selection of top-performing mixtures show the capability of cover crop mixtures to be highly productive in temperate climates when seeded at low rates. Out of the top performing mixture treatments, the highest yielding mixture in both MD and PA was composed of the lowest seeding rate for the two component species (Table 1.6). This in turn resulted in significantly lower seed costs while simultaneously providing more ecosystem services than when planting either species in monoculture. Low seeding rates combined with high fall and spring GDD (Table 1.6; Table 1.2) allowed for rapid establishment and greater biomass production throughout the growing season, resulting in highly productive mixtures. Previous cover crop studies in the northeastern US have also shown that in milder climates there is greater flexibility with seeding rate to achieve sufficient biomass, but that biomass is more positively influenced by seeding rate in colder climates (Haramoto, 2019; Mirsky et al., 2017b; Ryan et al., 2017).

Warm spring growing seasons in MD and MA resulted in high yielding and even mixtures of all three species combinations (Table 1.6). Our results align with the findings by

Baraibar et al. (2020) suggesting that species selection for mixtures is also more flexible in temperate regions (Baraibar et al., 2020). Regardless of high GDD in PA, however, hairy vetch biomass was low compared to sites with similar GDD and baseline soil fertility. The regression analysis was not able to explain the poor performance of hairy vetch at this site based on included predictor variables, so it is likely that a factor not considered was inhibiting legume growth. Other possible management factors that could have affected hairy vetch biomass include germination rate or other soil nutrient concentrations. Relative to other species, hairy vetch has high P and K requirements, and legumes in general require sufficient levels of sulfur for chlorophyll production and root nodule development, among other functions (Clark, 2008; Weil & Brady, 2015). Predicting weather conditions for the cover crop growing season can be difficult, but selecting species based on previous performance in similar climates as well as predetermined soil fertility levels at a given site can reduce the associated risks when designing cover crop mixtures.

Conclusion

This region-wide study of cover crop mixture performance provides invaluable knowledge regarding what site variables are most influential on the performance of mixtures that include functionally diverse species. Our results showed that expected growing degree days and baseline soil fertility are primary factors to consider when selecting species and seeding rate combinations to produce multifunctional mixtures at low seed costs. When desiring mixtures that include legumes such as hairy vetch, initial soil inorganic N concentrations should be considered. As soil inorganic N levels increased, hairy vetch competitiveness and overall biomass decreased compared to cereal rye or forage rape. In colder climates, cereal rye was critical to producing high biomass cover crop mixtures due to its quick establishment and winter hardiness. Overall, sites with more temperate winter seasons showed greater flexibility in species selection and were able to produce multifunctional mixtures at lower seeding rates, cutting seed costs substantially. Understanding the link between climate, soil, and management is key to understanding the

outcomes of cover crop performance and how to adjust cover crop seeding rates to better achieve targeted objectives.

Chapter 2: Soil organic carbon dynamics influenced by organic input diversity and tillage intensity after 25 years in Mid-Atlantic US grain cropping systems

Abstract

There is a pressing need to identify management strategies that best maintain, or even increase, soil organic carbon (SOC) stocks in agroecosystems. Proper management of SOC can improve soil fertility and structure, making soils more resilient to extreme weather brought on by climate change as well as potentially increase C sequestration rates. In the Mid-Atlantic region, cultivation has caused greater SOC loss than in many other regions of the US due to high rainfall and mild temperatures. Therefore, evaluating soil and crop management strategies that can help farmers adapt to and mitigate climate change has been a topic of great interest among researchers and policy makers. In this study, I quantified SOC dynamics at the Farming Systems Project (FSP) in central Maryland, USA, after 25-years of management. The FSP includes five grain cropping systems that vary in: conventional vs. organic management, tillage, crop rotation complexity, cover crop use, and fertility inputs. Permanganate oxidizable carbon (POX-C) and soil aggregate distribution using the wet sieving method (both measures of carbon stabilization) were compared across systems. I compared SOC stocks in soils collected in 1996 and 2021, and results showed SOC loss in all systems regardless of management. At the 0-20 cm depth, a 6-yr organic rotation that included a perennial forage crop had minor SOC loss (-1.6 Mg ha^{-1} ± 7.7) compared to a conventional 3-yr chisel-till rotation (-11.4 Mg ha^{-1} ± 5.4). Greater frequency of poultry litter applications in a 3-yr organic system led to significantly higher labile C quantities at the 10-20 cm depth, with POX-C concentrations being 22-50% greater than in other cropping systems. Aggregate-associated C data suggest C storage mechanisms differ among cropping systems, with systems that received higher quality C inputs (i.e., poultry litter, legume cover crops) or reduced soil disturbance showing larger SOC quantities protected within micro- or macroaggregates. Overall, trends suggest that SOC maintenance was greatest in systems with reduced tillage, extended cropping system complexity (either through cover crops or

perennial crops), and poultry litter inputs. This research is unique due to its long study duration and diversity of treatments compared. Findings will help inform management strategies for growers to better enhance SOC stocks for agroecosystem health and long-term sustainability of Mid-Atlantic grain production systems.

Introduction

Globally, soils are estimated to hold more than three times as much carbon (C) as the atmosphere, yet anthropogenic disturbances, such as agriculture, have led to significant soil organic C (SOC) losses and an imbalance in the global C cycle (Sanderman et al., 2017). Soil organic carbon dynamics in agricultural soils are dependent on many variables, including fixed factors like inherent soil properties and climate, or factors affected by management like organic matter inputs, microbial community composition, and soil N availability (Devine et al., 2022; Lehmann et al., 2020; Sanderman et al., 2017). For example, cropland soils in the Mid-Atlantic US are more prone to SOC loss compared to other regions due to mild temperatures and high rainfall accumulation (Cavigelli et al., 2018). Conventional management practices that involve routine tillage, winter fallow, and minimal organic amendments not only lead to a loss of SOC, but also to related soil health problems such as accelerated erosion, increased compaction, and declines in soil fertility (Crews et al., 2018; Sanderman et al., 2017). As the world looks for solutions to mitigate the effects of climate change, there is growing interest in agricultural practices that increase stocks of SOC, since more C in the soil means less carbon dioxide (CO_2) in the atmosphere. The process of C sequestration involves transforming atmospheric CO_2 into a more stable solid or dissolved form, first through storage in plant matter via photosynthesis and then ultimately via storage in the soil profile (e.g., SOC; Lal et al., 2015). Encouraging management practices that increase C sequestration rates can simultaneously mitigate climate change, build and maintain SOC, thereby improving soil health to increase farmer return on investment (Devine et al., 2022; Mayer et al., 2018), and make agroecosystems more resilient to extreme weather events brought on by climate change (Page et al., 2020; Silici et al., 2011). Due to the complex and highly

dynamic nature of SOC and the impacts of fixed factors on these dynamics, the rate of SOC accrual is highly variable from site-to-site, and regional-level research is needed to identify effective management practices that build and maintain soil C under particular conditions.

Soil organic carbon consists of a complex heterogeneous mixture of substances at various stages of decomposition, ranging from readily degradable organic matter accessible to microbes (labile C) to organic matter bound to soil minerals or physically protected by aggregates (stable C) (Weil & Brady, 2017). Within managed cropping systems, the size of the SOC pool is driven by the balance between inputs (e.g., plant residues, root exudates, organic amendments) and outputs via mineralization of this organic material (Meersmans et al., 2012). Several management practices have been identified as feasible methods to maintain or enhance SOC stocks, such as no-till or reduced tillage, the use of cover crops or perennial forages, and application of organic amendments (Lehmann & Kleber, 2015; Lehmann et al., 2020: Jilling et al., 2020; Page et al., 2020; Rahmati et al., 2020; Tully & McAskill, 2020). However, the form and persistence of SOC in response to these practices can vary based on climatic and environmental conditions and management history (Rui et al., 2022). In a 29-year study on soil carbon dynamics in Mollisol soils of Wisconsin, Rui et al. (2022) observed that persistent SOC pools were only enhanced in well-managed perennial grasslands, and that no differences were found between a conventional continuous corn system and systems that included reduced tillage, diversified crop rotations, and manure additions. A global meta-analysis of pairwise comparison studies between monoculture corn rotations and extended crop rotations on SOC dynamics found that extended crop rotations were more likely to increase SOC in regions with intermediate mean annual temperatures (8-15 ℃) and precipitation (600-1000 mm) compared to regions with other climate types (Liu et al., 2022). Overall, their results showed that local climatic conditions and inherent soil properties outweighed the influence of management factors on SOC content (Liu et al., 2022), making SOC management highly site-specific. Accruing SOC also requires long-term, continuous implementation of C-building practices, and increases may not be evident within the first several years of adoption (Gubler et al., 2019; Weil & Brady, 2017). Evaluating the

long-term effects of management factors on SOC stocks is necessary to fully understand the impact of management on SOC dynamics.

Soil texture is an important factor modifying agroecosystem management effects on SOC and soil health. Soil mineralogy and texture determine the potential for organo-mineral complexation and soil aggregate formation, two drivers of SOC stability (Cotrufo et al., 2019; Six et al., 2000). The soil aggregate hierarchy defined in the conceptual model by Tisdall and Oades (1982) describes the varying ability of different aggregate size classes to protect SOC fractions from further decay. Organo-mineral complexes (i.e., microaggregates) form when small organic matter particles tightly bind to primary mineral particles, aided by flocculation (Weil & Brady, 2017). The stable structure of microaggregates can withstand mechanical and environmental stress, making them a persistent SOC pool (Totsche et al., 2017). In agricultural systems with minimal soil disturbance, microaggregates can be bound together further to form macroaggregates (Six et al., 2000; Tisdall & Oades, 1982). Organic material such as root exudates and microbial byproducts function as the biological binding agents in the formation of these macroaggregates (Six et al., 2000). In a four-year study examining the effects of organic amendments on aggregate-associated C and microbial biomass C, Zhang et al. (2014) found improved soil aggregate stability and SOC protection in continuous corn systems receiving chicken manure or corn residues compared to a system receiving no inputs of manure or residues, indicating an increase in biological binding agents like microorganism exudates that promote aggregation.

While the quantity of organic inputs affects SOC sequestration, the quality (i.e., C:N ratio, lignin content) of organic inputs also impacts biological cycling, soil aggregation, and ultimately SOC occlusion (Cotrufo et al., 2013). Organic amendments such as green manures from terminated cover crops and animal manures or compost have a higher C quality compared to plant residues left in the field after cash crop harvest. A high-quality organic input has a low C:N ratio and fast decomposition rate, allowing more of the residue-derived C to become stabilized into SOC due to enhanced microbial carbon-use efficiency (Thapa et al., 2022). Adding a continuous supply of diverse, high quality organic inputs

provides a wider array of substrates to feed a diverse microbial community, resulting in the increased protection of SOC compounds through mineral complexation or aggregation (Lehmann et al., 2020; Maillard et al., 2015). Increasing the fraction of time with living plants during a crop rotation, such as using cover crops or perennial crops, also increases the rate of belowground inputs via root biomass and rhizodeposition (Liang et al., 2022). Jackson et al. (2017) suggests that the proportion of belowground biomass contributing to soil organic matter formation in agricultural systems is about five times greater than aboveground biomass. Overall, the quantity and quality of C inputs, combined with climate and environmental conditions, determine the SOC dynamics in agroecosystems (Thapa et al., 2022).

Studies evaluating the effect of management on soil carbon are common; however, most tend to focus on only one or two management factors. The Farming Systems Project (FSP) is unique in that it has been continuously managed for 25 years and includes five diverse grain and forage cropping systems: two conventional and three organic. Management factors I was able to compare with this study system include reduced or no tillage, increased crop diversity, and addition of organic amendments. The main objective of this study was to evaluate long-term SOC dynamics by (1) comparing total SOC content in 1996 and 2021, and (2) assessing SOC quality by quantifying aggregate-associated C (i.e., physical protection of C within aggregates) and labile C in each cropping system in 2021. I hypothesize that (1) extended crop rotations that include perennial crops will encourage SOC stabilization due to enhanced soil aggregation and physical protection of SOC from reduced tillage and increased belowground C inputs, and (2) more frequent additions of manure will increase SOC stocks due to a higher quantity and quality of C inputs.

Methods

Study system

The Farming Systems Project (FSP) is a long-term cropping systems study that is part of the Lower Chesapeake Bay Long-Term Agroecological Research (LTAR) site at the Beltsville Agricultural Research Center in Beltsville, MD. Established in 1996, the FSP consists of five cropping systems representing a range of management practices that reflect grain cropping systems in the mid-Atlantic region. The 6.9 ha study site is located at the western edge of the Atlantic Coastal Plain. Soils include Christiana (fine, kaolinitic, mesic Typic Paleudults), Matapeake (fine-silty, mixed, semi-active, mesic Typic Hapludults), Keyport (fine, mixed, semi-active, mesic Aquic Hapludults), and Mattapex (fine-silty, mixed, active, mesic Aquic Hapludults) silt loams. The field was in conventional no-till corn production for three years before plot establishment in 1996, and prior to that was primarily under perennial forage management from 1972-1992. Annual precipitation, based on a 30-yr average, is 1063 mm (NOAA, 2021), and the mean annual minimum and maximum temperatures are 7.2 ℃ and 18.7 ℃, respectively (NOAA, 2021).

The study is a randomized complete block design with four replicates. There are five treatments: a 3-year conventional no-till corn-soybean-wheat/soybean rotation (NT); a 3-year conventional chisel-till corn-soybean-wheat/soybean rotation (CT); a 2-year organic corn-soybean rotation (Org2); a 3-year organic corn-soybean-wheat rotation (Org3); and a 6-year organic corn-soybean-wheat-perennial alfalfa rotation (Org6) (Table 2.1; Cavigelli et al., 2008). Each rotation phase is present every year. Detailed descriptions of management can be found in Cavigelli et al. (2008) and White et al. (2019). The NT and CT systems are conventionally managed using mineral fertilizers and herbicides for weed control. The organic systems rely on poultry litter and legume cover crops or forages for fertility and cultivation (rotary hoeing, between row cultivation) for weed control (Cavigelli et al., 2008). All systems utilize cover crops to varying intensities, with a cereal rye cover crop planted after corn in each system. A hairy vetch and cereal rye cover crop mixture is planted after soybean and wheat in Org2 and Org3, respectively.

Table 2.1. Summary of typical FSP cropping systems management (White et al., 2019).

	Cropping system				
Management practice	NT	CT	Org2	Org3	Org6
Crop rotation	C-r-S-W/S	C-r-S-W/S	C-r-S-v+r	C-r-S-W-v+r	C-r-S-W-A-A-A
Primary tillage	None	Chisel	Chisel	Chisel	Moldboard or Chisel
Weed control	Herbicides	Herbicides	Cultivation	Cultivation	Cultivation
Fertility	Mineral Fertilizer	Mineral Fertilizer	PL, Legumes	PL, Legumes	PL, Legumes

C = corn; S = soybean; W = wheat; W/S = wheat followed by double-cropped soybean; A = alfalfa; r = rye cover crop; v = hairy vetch cover crop; PL=poultry litter.

Soil sampling & processing

Soil samples were collected April 2021 from the corn phase of the rotations. Each system had completed their respective final rotation year in 2020, with Org6 being under no-till, perennial forage management for three years. At least four intact soil cores (0-50 cm depth) were collected per plot with a 5-cm diameter probe controlled using a tractor-mounted hydraulic soil sampling system. Cores were extracted in plastic sleeves and refrigerated overnight (4° ± 2℃) prior to further processing. Cores from each plot were subdivided into depth increments (0-5, 5-10, 10-20, 20-30, and 30-50 cm) and combined by depth. Fresh weights were recorded for each combined depth increment. The combined samples were refrigerated until further analysis. Soils were homogenized by sieving through a 6.35-mm screen and four 10-g subsamples from each depth increment were weighed and dried (60 ℃) for at least 5 days and then reweighed to determine gravimetric soil moisture. The remaining sieved soil was air-dried for at least 7 days and refrigerated until further analysis.

Bulk density was determined by dividing the soil dry weight by the soil core volume for each depth increment. The mass and volume of roots and rocks greater than 6.35-mm were subtracted from the fresh soil mass and volume, respectively, before calculating bulk density (Blake & Hartge, 1986). Since the process of collecting a hydraulic core may compact

soil layers, additional soil samples for bulk density analyses were collected from the 0-5 and 5-10 cm depth increments using the standard ring method (Arshad et al., 1997). Management effects on soil microbial activity and nutrient cycling are often confined to the surface layer (Epp Schmidt et al., 2022), therefore an accurate estimate of soil bulk density at these depths is necessary to properly estimate SOC dynamics. Within one week of soil core sampling, a 5-cm diameter metal ring was pushed into the soil vertically, and then was carefully excavated using a trowel and soil knife to collect bulk density measurements for the 0-5 cm depth. An additional ring was carefully placed in the same hole and used to excavate a sample from the 5-10 cm depth using the same steps as above. Four cores were collected per plot (two for each depth increment). The fresh weight of cores was recorded, and soils were placed in an oven (60 ℃) for at least 3 days to determine the dry weight. Bulk density of these surface soils (0-5 cm and 5-10 cm) was calculated by dividing the dry weight of soil by the volume of the sampling ring. Gravel and root content in these surface soils was < 5% based on the deep soil cores collected, so roots and rocks were not separated from the bulk soil for the bulk density ring method. This method was compared to the bulk density measurements from the hydraulic probe method and data from previous years to determine the best measure of soil bulk density for the surface depths.

Soil samples were collected to a depth of 50 cm in 1996, prior to the start of the study. Soil cores were separated into two depth increments: 0 cm-Ap (plow layer depth) and Ap-50 cm. The Ap depth ranged between 20-30 cm. Bulk density was not measured during sampling in 1996 but was measured during preliminary soil characterization in 1994. Therefore, I used these data as bulk density would not be expected to change significantly over this short period under consistent management (the entire site was managed as a no-till corn field from 1993-1995.

Soil analysis

I. Total organic carbon (TOC)

An ~1 g subsample from each plot and depth increment (n = 100) was ground to pass through a 0.25-mm sieve; rocks that did not pass through the sieve were removed, subtracted from the total aggregate mass, and archived. Subsamples were then oven-dried at 105°C or one hour and 0.25-g was prepared for elemental analysis. Total organic C for samples collected in 2021 was determined by dry-combustion with an elemental analyzer (LECO TruMac N, St. Joseph, MI). The 1996 archived soil was analyzed for TOC content in 2016 using the dry-combustion method with a similar elemental analyzer (LECO TruMac Series, St. Joseph, MI).

Prior to TOC analysis, soils were tested for carbonates by placing a few drops of 1 *M* HCl onto a ~2 g subsample of bulk soil and observing if bubbles formed on the soil surface (Nelson & Sommers, 1983). No carbonates were detected. The TOC and bulk density values were used to calculate bulk SOC stocks for each depth increment using Equation 2.1, and then stocks were summed to get the total 0-50 cm SOC stock for each plot.

$$Bulk\ SOC\ stock\ (Mg) = \%C * bulk\ density\ \left(\frac{g}{cm^3}\right) * depth\ increment\ (cm) \qquad \text{(Eq. 2.1)}$$

II. Permanganate oxidizable carbon

Permanganate oxidizable C (POX-C) was used to quantify labile C concentrations (Weil et al., 2003) for only the top three depth increments. This C fraction is composed of partially degraded particulate organic C, associated with smaller, heavier particulate fractions that are known to have longer soil residence times compared to less degraded fractions (Culman et al., 2012). Compared to TOC, POX-C is more sensitive to management changes (i.e., tillage, cover crop use, C inputs, land-use changes; Culman et al., 2012), therefore it can be a useful metric to compare management effects on SOC quality since biological

activity is greater in topsoil than lower depths (Biswas & Naher, 2019), likely making management effects most prevalent in these depth increments. Duplicate 2.5-g soil samples were placed in 50 mL centrifuge tubes and treated with a 0.02 M $KMnO_4$ solution for exactly 2 min at 120 oscillations per minute. Samples were then allowed to settle in the dark for 10 min at which time 0.5-mL of supernatant was removed and diluted to 50 mL with deionized water, and absorbance measured at 550 nm with a Pocket Colorimeter II, Wavelength Specific model (HACH Co., Singapore). Permanganate concentrations remaining following sample oxidation were determined by comparison with a standard curve prepared from diluted stock KMnO4 solution. The amount of C oxidized was determined assuming stoichiometric oxidation of 9000 mg C for each 1 mol of MnO_4 changing from Mn^{7+} to Mn^{2+}.

III. Aggregate size separation

A 50-g subsample from each depth increment in each plot was fractionated into four aggregate size classes using the slaking method developed by Six et al. (2000). Soils were wet-sieved through 2-mm, 0.25-mm, and 0.053-mm sieves to separate the bulk soil into four size classes: (1) large macroaggregates (>2 mm); (2) small macroaggregates (0.25 mm - 2 mm); (3) microaggregates (0.053 mm - 0.25 mm); (4) silt and clay (< 0.053 mm). Each sieve was placed inside a separate plastic wash bin, to catch the soil particles that fall through during the sieving process. First, the wash bin containing the 2-mm sieve was filled with ultrapure water to a depth of roughly 5 cm, so that the water reached about 2.5 cm above the sieve screen. The 50-g subsample was then placed on the 2-mm sieve and submerged in ultrapure water for 5 minutes to break apart any unstable soil aggregates. Soil was slaked at 25 repetitions for 2 min, allowing the water to pass through the sieve at each repetition. The portion of soil too large to pass through the 2-mm sieve was transferred into a pre-weighed tin and classified as large macroaggregates. Using the same slaking method, soil and water that passed through the 2-mm sieve into the wash bin was sequentially passed through a 0.25-mm sieve to separate small macroaggregates and a 0.053-mm sieve to separate microaggregates, with the smallest silt and clay particles passing through all sieve sizes. The

water and silt and clay fraction remaining at the end of slaking was transferred to a large aluminum tin. Once soil was settled, floating particulate organic matter was skimmed off the top of the large and small macroaggregate portions and was removed from further analyses. Once most water was evaporated from the tins, soils were oven-dried (60 ℃) for at least 4 days. Oven-dried fractions were weighed and stored until further analysis. As sand particles can range from 0.053 - 2-mm in size, their presence can falsely inflate aggregate size classes (Six et al., 2000). Therefore, a 2-g sub-sample from large macroaggregate, small macroaggregate, and microaggregate fractions in each plot was separated and set aside to determine sand content using the pipette method (de la Reguera & Tully, 2021). The mass of sand (g) within each aggregate sample was calculated by multiplying the sand proportion by the total aggregate mass (g) (Eq. 3). To determine the sand-free aggregate mass (g), the sand mass (g) was then subtracted from the total aggregate mass (g).

The remaining aggregate soil was then ground with mortar and pestle to pass through a 0.25-mm sieve to remove any small rocks or roots, which were weighed separately and subtracted from initial aggregate weight. Approximately 0.25 g of ground, oven-dried soil fraction was then prepared for TOC analysis using the dry combustion method (LECO TruMac N, St. Joseph, MI). If an aggregate size fraction represented < 2% of the total bulk soil sample, it was not analyzed for TOC, since this is a negligible amount of aggregate soil and there was not adequate sample for a reliable analysis. The percentage of each aggregate size class within a soil sample was determined by calculating the aggregate proportion (Eq. 2.2).

$$Aggregate\ proportion\ (\%) = \frac{sand\ free\ aggregate\ mass\ (g)}{\Sigma(sand\ free\ aggregate\ mass\ of\ all\ soil\ fractions)(g)} \quad \text{(Eq. 2.2)}$$

I then calculated the total aggregate C of a particular aggregate size class by multiplying the associated C concentration by aggregate mass and then dividing by a conversion factor of 1000 (Eq. 2.3).

$$Total\ aggregate\ C\ (g) = \frac{C\ concentration\ (\%) \times\ sand\ free\ aggregate\ mass\ (g)}{1000} \quad \text{(Eq. 2.3)}$$

IV. Comparing 1996 to 2021 soil organic carbon stocks

Changes in SOC stocks were evaluated by comparing the SOC stocks between 1996 and 2021 for each cropping system. Since soil samples in 1996 were collected at only two depth increments (0 cm-Ap, Ap-50 cm), finer depth increments were combined in the 2021 samples to make two relative depth increments (0-20 cm and 20-50 cm) for better comparison between the study years. The depth increments were separated at 20 cm as most plow depths in the 1996 soil samples were closer to 20 cm than 30 cm. All 1996 SOC data collected, regardless of Ap depth, were normalized to 0-20 cm and 20-50 cm depth increments. For example, if the Ap depth in 1996 was 25 cm, the SOC stock was normalized to 20 cm by applying a weight of 80% (i.e., 20/25 = 0.8). The same steps were followed to convert the 1996 Ap-50 cm SOC stock estimates to 20-50 cm stock estimates.

Historical C inputs

Farming Systems Project grain and forage crop yields, cover crop biomass, and poultry litter application rates and chemical analyses were used to estimate C inputs to soil in each sampled FSP plot (1996-2020). The amount of grain crop residue returned to soil each year was estimated using grain yield and harvest index calculations. Harvest index is the ratio of harvested biomass or grain to total aboveground biomass of the crop. Harvest index values used to calculate grain crop residue biomass were 0.5, 0.45, and 0.61 for corn, soybean, and wheat, respectively (Dai et al., 2016; Hay, 1995). All crop residues were returned to the soil except for conventional wheat straw, which was harvested. Cover crop biomass was sampled prior to termination, and samples were dried in an oven at 60˚C and dry weights converted to kg ha^{-1}. For 2015, only vetch cover crop biomass data were available, so rye cover crop biomass was estimated based on the ratio of vetch to rye biomass the following year (Kate White, personal communication). Forage biomass was measured from full plots during mechanical harvest by weighing a dump truck on a truck scale before and after the biomass from each plot was blown into it using a forage harvester. Plant C concentrations for most crops and cover crops were estimated based on values

provided by existing literature (Al Kaisi et al., 2005; Ma et al., 2018; Rannells & Wagger, 1996), and the following C concentrations were used: 45% for corn, 38.7% for soybean, 44.7% for wheat, oat, cereal rye, and red clover-orchardgrass hay, and 30.9% for hairy vetch. Forage alfalfa was analyzed for C concentration in several years over the life of the study, so these values were averaged to estimate alfalfa C concentration for the years when TOC was not quantified. Belowground root inputs for all crops were estimated using shoot:root ratios and root exudate estimates from Bolinder et al. (2007). Shoot:root ratios used ranged from 0.7 (red clover-orchardgrass hay mixture) to 4.7 (fertilized corn), (Bolinder et al., 2007). Root exudates were estimated by multiplying the plant root biomass by 0.65, which is a conservative estimate generated from previous C tracer studies (Bolinder et al., 2007; Kuzyakov & Schneckenberger, 2004). Applied poultry litter was only analyzed for C/N content from 2008 to 2011, however, total nitrogen (N) was analyzed each year. Therefore, C inputs from poultry litter were estimated using total N analyzed each year by taking the average C:N ratio from samples analyzed for both total C and N from 2008 to 2011.

Statistical analysis

Initially, I determined if there was an effect of soil depth on all soil analyses C concentrations (%) in 2021 bulk soils as SOC stocks were calculated from the bulk soil C concentrations. I used a linear mixed effects (LME) model (*lme4* package for R; Bates et al. 2013) with cropping system, depth, and their interaction as main effects and block as the random effect. As I observed significant differences in soil C concentration by depth (p<0.001), which might mask the effects of cropping system, I examined each soil depth increment separately in further analyses. Thus, I used five LME models (one for each depth) with cropping system as the main effect and block as the random effect to examine the effect of cropping system on both soil C concentration and SOC stocks. Tukey's honest significance post-hoc tests were used to examine significant differences among cropping systems when LME models were significant (*multcomp* package for R; Hothorn et al. 2008).

To determine the effect of cropping system management on SOC change overtime, I used a LME model with cropping system, depth, and their interaction as main effects and

block as the random effect. Significant differences were observed in SOC change by depth (p<0.05), therefore three separate LME models were used with system as the main effect and block as the random effect, to examine SOC change at 0-20 cm, 20-50 cm, and for the whole soil profile (0-50 cm). Due to large variance in SOC change across replicates for all cropping systems at the 0-20 cm, 20-50 cm, and 0-50 cm depths, one-sample t-tests were used to determine if SOC change in each system at each depth increment was significantly different than 0 (*stats* package; R Core Team, 2022).

An initial LME model for POX-C data was run with cropping system, depth, and their interaction as main effects and block as the random effect. I observed significant changes in POX-C concentrations by depth (p<0.001), therefore, soil depth increments were then analyzed separately. A separate LME model was used for each analyzed depth increment with cropping system as the main effect and block as the random effect. Tukey's honest significance post-hoc tests were used to examine significant differences in POX-C among cropping systems when LME models were significant.

To determine the aggregate distribution within each depth increment, I initially used five LME models (one for each depth increment) with cropping system, aggregate size class, and their interaction as main effects, and block as the random effect. The weight of aggregates varied significantly across aggregate size classes (p<0.001); therefore, aggregate size class proportions were compared across systems using four separate LMEs (one for each size class) to ensure that any cropping system effects were not masked by interaction effects. To measure the effect of cropping system management on aggregate-associated C, I initially evaluated aggregate C pools (g C; Eq. 3) of size classes using five LME models (one for each depth increment), with cropping system, aggregate size class, and their interaction as main effects and block as the random effect. The amount of C stored within aggregate size classes varied significantly within each cropping system (p<0.001), therefore aggregate-associated C was analyzed separately for each size class (four different LMEs) at each depth so that differences among systems would not be masked by any interaction effects. In addition, the amount of C stored among all aggregate size classes for a single system and

depth increment was summed and differences in the quantity of total aggregate-associated C were evaluated using five LME models (one for each depth increment), with cropping system as the main effect and block as the random effect. Tukey's honest significance post-hoc tests were used to examine significant differences across cropping systems for all aggregate analyses when LME models were significant.

Carbon input sources varied by cropping system, therefore total C inputs (Mg ha^{-1}) for each block in each system were summed and differences in the total quantity of C inputs among cropping systems was evaluated using a LME model, with cropping system as the main effect and block as the random effect. Tukey honest significance post-hoc tests were used to examine significant differences in total C inputs across cropping systems when LME models were significant.

I used Box-Cox (Box & Cox, 1964) or square root transformations before analysis when necessary to satisfy the assumptions of the statistical models. Significance for all tests was determined at $P < 0.05$ using Type II-Analyses of Variance (ANOVAs) for LME models without interaction effects, and Type III ANOVAs for LME models with interaction effects (*car* package; Fox & Weisberg, 2019). All statistical analyses were conducted in the R environment for Windows (v1.3.1073).

Results

Effect of cumulative carbon inputs on soil organic carbon stocks.

Estimated cumulative C inputs from 1996-2020 differed across cropping systems ($p < 0.001$; Fig. 2.1-A; Table 2.2). Cumulative C inputs were greater (137 Mg ha^{-1}) and more diverse in Org6 than the other cropping systems. Grain crop residues (corn, soy, wheat) accounted for more than 85% of total C inputs in the conventionally managed systems, but only accounted for 61%, 55%, and 28% of total C inputs for Org2, Org3, and Org6, respectively. Manure applications, perennial crops and more diverse cover crops in the organic systems increased total cumulative C inputs compared to the conventionally managed systems by up to 26% (NT vs. Org6). Carbon inputs from three years of perennial alfalfa in Org6 contributed 58% of the total C inputs for this system, with a large portion of these inputs coming from below-ground root biomass and root exudates (73.1 Mg ha^{-1}).

Table 2.2. Type I ANOVA results from LME model testing the effect of cropping system on cumulative C inputs from 1996 to 2020, followed by Tukey's honestly significant difference (HSD) test results. Significance was determined at $P < 0.05$.

ANOVA	*df*	Sum of Squares	Mean Square	*F*	*p-value*
Systems	4	2369504130	592376033	15.63	< 0.001

Tukey HSD

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-6836	4354	0.52
Org2	CT	7946	4354	0.36
Org3	CT	9639	4354	0.17
Org6	CT	25559	4354	< 0.001
Org2	NT	14781	4354	0.01
Org3	NT	16475	4354	0.001
Org6	NT	32395	4354	< 0.001
Org3	Org2	1694	4354	1.00
Org6	Org2	17614	4354	< 0.001
Org6	Org3	15920	4354	0.002

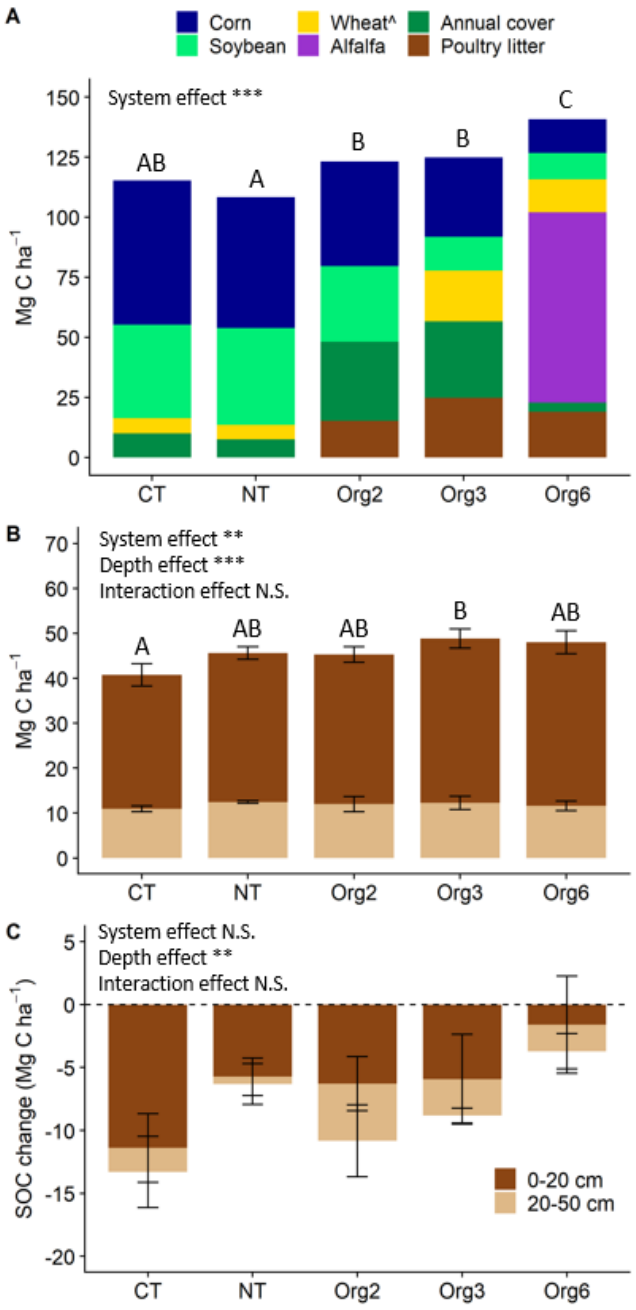

Fig. 2.1. A) Cumulative above- and below-ground carbon inputs estimated over 25 years. ^ On occasion, other small grain crops were substituted for wheat when conditions were not suitable for fall planting. B) Total soil organic carbon (SOC) stocks in 2021. SOC stocks are separated into topsoil (0-20 cm) and subsoil (20-50 cm) depth increments. C) SOC change after 25 years (1996-2021), separated by depth increment. 1996 SOC stocks were normalized for the 0-20 and 20-50 cm depth, regardless of depth to plow layer. 1996 SOC stocks estimated for 0-20, 20-50 cm depths; original calculation was for defining plow layer. CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, Org6 = 6-year organic. Error bars are the standard error of the mean (n = 4). Asterisks indicate significant effects at *P<0.05, **P<0.01, ***P<0.001 in Tukey post hoc

pairwise comparisons. Different letters indicate significant differences among cropping systems.

After 25 years of management, no significant interaction effects were found between cropping system and depth ($p > 0.05$; Fig. 2.1-B), therefore differences among systems for each depth increment were not evaluated. When SOC stocks over the whole 0-50 cm profile were compared, SOC stocks in Org3 were 18% greater than CT (48.8 Mg C ha^{-1} and 40.8 Mg C ha^{-1}, respectively; $p = 0.02$). The Org2 (45.3 Mg C ha^{-1}), NT (45.6 Mg C ha^{-1}), and Org6 (48.0 Mg C ha^{-1}) systems had intermediate SOC stocks ($p > 0.05$).

I further examined cropping system effects on SOC stocks at finer depth increments for the 2021 data (Table 2.2). No-till (11.49 Mg C ha^{-1}) and Org6 (11.10 Mg C ha^{-1}) had significantly greater SOC stocks compared to CT (8.64 Mg C ha^{-1}) and Org2 (8.81 Mg C ha^{-1}) at 0-5 cm ($p < 0.01$), while Org3 stocks were intermediate (9.66 Mg C ha^{-1}). No-till had similar SOC stocks to CT below 5 cm. The 6-year organic system had a significantly greater SOC stock compared to CT and NT at the 5-10 cm depth (22% more, on average; $p < 0.001$) while Org3 had significantly greater SOC stocks compared to both CT and NT at the 10-20 cm depth (28% more, on average; $p < 0.01$).

Table 2.3. Soil organic carbon (SOC) stocks for sampled depth increments across five diverse cropping systems in the Farming Systems Project after 25 years. Where CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, and Org6 = 6-year organic. Different letters in the same row indicate significant differences among cropping systems.

	SOC stocks (Mg C ha^{-1})				
Depth (cm)	CT	NT	Org2	Org3	Org6
0-5	8.64a	11.49c	8.81a	9.66ab	11.10bc
5-10	8.15a	8.31a	9.01ab	9.45ab	10.28b

Table 2.3. Continued.

10-20	13.04a	13.36a	15.50ab	17.45b	15.03ab
20-30	4.95	5.96	6.33	6.05	6.19
30-50	5.99	6.49	5.65	6.23	5.41

In the total sampled soil profile (0-50 cm), significant loss in SOC stocks was found over time in all systems except Org6 (Fig 2.1-C; Fig. 2.2); Org6 SOC change was not significantly different from 0 (t = -0.89; N.S.) . However, when comparing change across systems using LME models, no significant cropping system effect could be found at either 0-20 cm, 20-50 cm, or 0-50 cm depth (Fig. 2.1-C; p>0.05). Based on one sample t-test results, SOC change at the 0-20 cm depth for Org3 and Org6 were not significantly different from 0 (t = -1.66 and -0.41, respectively; N.S.). For the 20-50 cm depth, one sample t-test results showed that SOC change for Org2, CT, and NT was equivalent to 0 (t = -1.59, -0.68, and -0.35, respectively; N.S.).

Table 2.4. Type I ANOVA results from LME models testing the effect of cropping system on soil organic carbon (SOC) change over time for the following depth increments: 0-20 cm, 20-50 cm, 0-50 cm. Significance was determined at P < 0.05.

Depth	*df*	Sum of Squares	Mean Square	*F*	*p-value*
0-20 cm	4	194.08	48.52	2.10	0.14
20-50 cm	4	33.83	8.46	0.67	0.63
0-50 cm	4	225.78	56.44	1.26	0.34

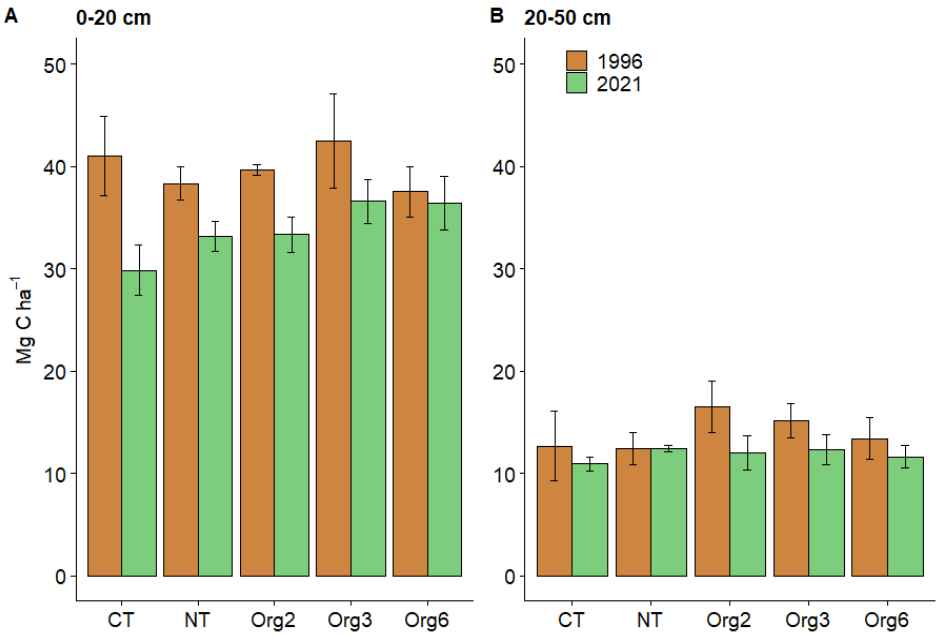

Fig. 2.2. Comparison of soil organic carbon stocks (Mg C ha^{-1}) in 1996 (brown bars) and 2021 (green bars) across systems in the Farming Systems Project at the A) 0-20 cm depth and B) 20-50 cm depth. 1996 SOC stocks were normalized for the 0-20 and 20-50 cm depth, regardless of depth to plow layer. CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, and Org6 = 6-year organic. Error bars are the standard error of the mean.

Table 2.5. One-sample t-test results comparing SOC change over time to 0 (no significant change) for each system and depth. System and depth increments with significant p-values indicate SOC change over time was not different than 0. Significance was determined at $P < 0.05$.

System	Depth (cm)	*t*	*df*	*p-value*
CT	0-50	-2.77	3	0.035
	0-20	-4.20	3	0.012
	20-50	-0.68	3	0.274
NT	0-50	-3.11	3	0.026
	0-20	-3.87	3	0.015
	20-50	-0.35	3	0.373
Org2	0-50	-2.43	3	0.047
	0-20	-2.92	3	0.031
	20-50	-1.59	3	0.105
Org3	0-50	-2.74	3	0.036
	0-20	-1.66	3	0.098
	20-50	-4.93	3	0.008
Org6	0-50	-0.89	3	0.219
	0-20	-0.41	3	0.353
	20-50	-1.51	3	0.114

Cropping system effect on soil organic carbon (SOC) and permanganate oxidizable carbon (POX-C) concentrations by depth

At 0-5 cm, NT (18.3 g C kg soil^{-1}) and Org6 (17.9 g C kg soil^{-1}) had the highest total SOC concentrations (Fig. 2.2-A; p<). Total SOC concentrations for NT and Org6 were significantly higher than in the CT and Org2 (12.7 g C kg soil^{-1} and 13.6 g C kg soil^{-1}), respectively, and Org3 had intermediate concentrations (15.3 g C kg soil^{-1}) ($p < 0.001$; Fig. 2.2-A). At the 0-5 cm depth, total SOC concentration was 36% greater in NT than CT ($p < 0.001$). Within organic systems, total SOC concentrations at the 0-5 cm depth were 27% greater in Org6 compared to Org2 ($p < 0.001$).

Total SOC concentrations at the 5-10 cm depth in CT (11.7 g C kg^{-1}) and NT (12.4 g C kg^{-1}) systems were significantly lower than those observed in Org3 (14.5 g C kg^{-1}) and Org6 (15.1 g C kg^{-1}) ($p < 0.05$, Fig. 2.2-B) but not Org2 ($p > 0.05$). Total SOC concentration in Org6 at the 5-10 cm depth was 25% higher than in CT, 20% higher than in NT, and 15% higher than in Org2 ($p < 0.02$). Compared to CT and NT, SOC concentration in Org3 was at least 16% higher at the 5-10 cm depth and 24% higher at the 10-20 cm depth ($p < 0.05$). At the 20-30 and 30-50 cm depths, total SOC concentrations were similar among all cropping systems and substantially lower than in other depths ($p > 0.05$, Fig. 2.2-D, E).

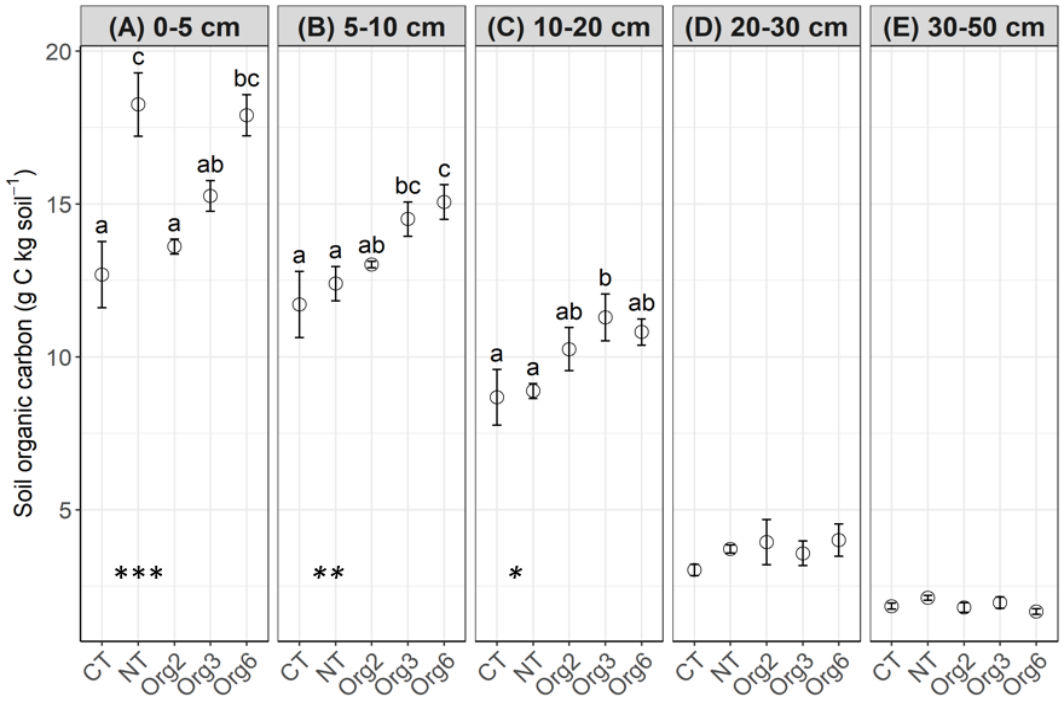

Fig. 2.3. Total soil organic carbon (SOC) concentrations (g C kg soil^{-1}) among cropping systems after 25 years at the Farming Systems Project (FSP) at five depth increments: (A) 0-5 cm, (B) 5-10 cm, (C) 10-20 cm, (D) 20-30 cm, and (E) 30-50 cm. Where CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, Org6 = 6-year organic. Different letters within each panel indicate significant differences among cropping systems for that depth. Error bars are the standard error of the mean. Asterisks (*) indicate significance by cropping system within a depth increment at *P<0.05, **P<0.01, ***P<0.001 in Tukey post hoc pairwise comparisons.

Table 2.6. Type I ANOVA results from LME models testing the effect of cropping system on C concentration for each of the five depth increments, followed by Tukey's honestly significant difference (HSD) test results. Significance was determined at $P < 0.05$.

Depth	*df*	Sum of Squares	Mean Square	*F*	*p-value*
0-5 cm	4	99409231	24852308	10.5	< 0.001
5-10 cm	4	31851275	7962819	8.89	0.001
10-20 cm	4	21547653	5386913	4.09	0.03
20-30 cm	4	2399678	599920	1.42	0.29
30-50 cm	4	448378	112094	1.66	0.22

Tukey HSD

0-5 cm

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	5557	1088	<0.001
Org2	CT	912	1088	0.92
Org3	CT	2566	1088	0.13
Org6	CT	5209	1088	<0.001
Org2	NT	-4645	1088	<0.001
Org3	NT	-2991	1088	0.05
Org6	NT	-348	1088	1.00
Org3	Org2	1654	1088	0.55
Org6	Org2	4297	1088	<0.001
Org6	Org3	2643	1088	0.11

5-10 cm

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	679.7	669	0.85
Org2	CT	1308.2	669	0.29
Org3	CT	2792.2	669	<0.001
Org6	CT	3347.2	669	<0.001
Org2	NT	628.5	669	0.88
Org3	NT	2112.5	669	0.01
Org6	NT	2667.5	669	<0.001
Org3	Org2	1484	669	0.17
Org6	Org2	2038.9	669	0.02
Org6	Org3	554.9	669	0.92

Table 2.6. Continued

10-20 cm				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	210	811	1.00
Org2	CT	1579	811	0.29
Org3	CT	2617	811	0.01
Org6	CT	2136	811	0.06
Org2	NT	1368	811	0.44
Org3	NT	2406	811	0.03
Org6	NT	1926	811	0.12
Org3	Org2	1038	811	0.70
Org6	Org2	557	811	0.96
Org6	Org3	-481	811	0.98

Significant differences in POX-C concentrations were found among cropping systems at all three depth increments measured ($p<0.05$; Fig. 2.3; Table 2.7). At the 0-5 cm depth, Org6 (548 mg C kg^{-1}), Org3 (491 mg C kg^{-1}), and NT (530 mg C kg^{-1}) had significantly higher POX-C compared to CT (360 mg C kg^{-1}) ($p<0.05$; Fig. 2.3-A). Concentrations in Org6 were 24% higher than in Org2 (430 mg C kg^{-1}) and 42% higher than in CT ($p<0.05$). At the 5-10 cm depth, POX-C concentrations in Org3 (482 mg C kg^{-1}) were more than 25% higher than the conventional CT (365 mg C kg^{-1}) and NT (374 mg C kg^{-1}) ($p<0.04$). At the 10-20 cm depth, POX-C concentrations were significantly greater in all organic systems compared to CT (220 mg C kg^{-1}; $p<0.03$), with values 29% greater in Org6 (295 mg C kg^{-1}), 30% greater in Org2 (298 mg C kg^{-1}), and 50% greater in Org3 (369 mg C kg^{-1}). The 3-year organic system had at least 24% higher POX-C concentration compared to the other organic systems at this depth ($p<0.04$).

Table 2.7. Type I ANOVA results from LME models testing the effect of cropping system on POX-C concentration for each of the three depth increments analyzed, followed by Tukey's honestly significant difference (HSD) test results. Significance was determined at $P < 0.05$.

Depth	*df*	Sum of Squares	Mean Square	*F*	*p-value*
0-5 cm	4	95160	23790	6.21	0.01
5-10 cm	4	37799	9450	3.48	0.04
10-20 cm	4	51556	12889	9.78	< 0.001

Tukey HSD

0-5 cm

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	169.68	43.75	<0.001
Org2	CT	70.39	43.75	0.49
Org3	CT	130.99	43.75	0.02
Org6	CT	188.33	43.75	<0.001
Org2	NT	-99.29	43.75	0.16
Org3	NT	-38.69	43.75	0.90
Org6	NT	18.65	43.75	0.99
Org3	Org2	60.6	43.75	0.64
Org6	Org2	117.94	43.75	0.05
Org6	Org3	57.34	43.75	0.68

5-10 cm

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	8.39	36.83	1.00
Org2	CT	66.66	36.83	0.37
Org3	CT	116.54	36.83	0.01
Org6	CT	73.65	36.83	0.27
Org2	NT	58.27	36.83	0.51
Org3	NT	108.15	36.83	0.03
Org6	NT	65.26	36.83	0.39
Org3	Org2	49.88	36.83	0.66
Org6	Org2	6.99	36.83	1.00
Org6	Org3	-42.89	36.83	0.77

Table 2.7. Continued

10-20 cm				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	29.84	25.68	0.49
Org2	CT	77.85	25.68	0.02
Org3	CT	149.64	25.68	< 0.001
Org6	CT	75.52	25.68	0.02
Org2	NT	48.02	25.68	0.25
Org3	NT	119.81	25.68	< 0.001
Org6	NT	45.68	25.68	0.25
Org3	Org2	71.79	25.68	0.03
Org6	Org2	-2.33	25.68	0.93
Org6	Org3	-74.12	25.68	0.02

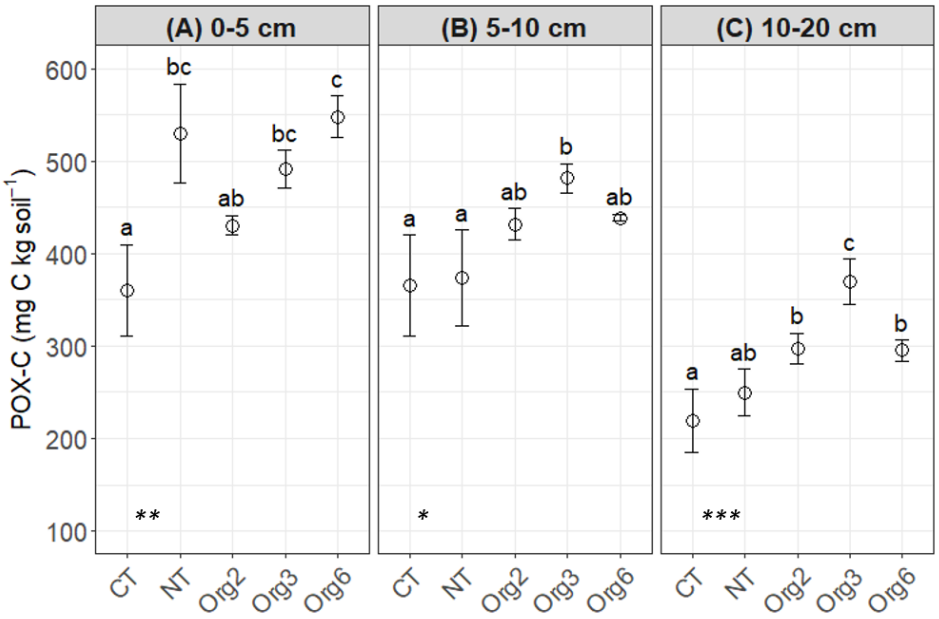

Fig. 2.4. Permanganate oxidizable carbon content (POX-C; mg C kg soil^{-1}) among cropping systems after 25 years at the Farming Systems Project (FSP) at the following depth increments: (A) 0-5 cm, (B) 5-10 cm, and (C) 10-20 cm depth. Where CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, Org6 = 6-year organic. Different letters within a panel indicate significant differences among cropping systems for that depth. Error bars are the standard error of the mean. Asterisks (*) indicate significance by cropping system at *P<0.05, **P<0.01, ***P<0.001 in Tukey post hoc pairwise comparisons.

Distribution of aggregate size classes and associated C among cropping systems

Significant differences in aggregate size class among cropping systems were observed at all depths except for 20-30 cm (p<0.001; Table 2.8; Fig. 2.4-A, B). At the 0-5 cm depth, NT and Org6 had larger proportions of small macroaggregates compared to Org2 (p<0.03), and the NT small macroaggregate proportion was also larger than Org3 (p<0.05; Figure 2.4-A). At the 5-10 cm depth, both NT and Org6 had larger proportions of small macroaggregates compared to Org2 (p<0.04; Figure 2.4-B). The Org2 system had the largest proportion of silt and clay sized particles compared to other cropping systems at 0-5 cm (p<0.03) and was only similar to Org3 at 5-10 cm (p<0.05). In the surface soil, Org3 had a larger proportion of microaggregates compared to Org2 (p<0.02). At the 10-20 cm depth, Org6 had a greater proportion of large macroaggregate fractions compared to either Org2 or Org3 (p<0.05). At the 30-50 cm depth, Org6 had a greater proportion of silt & clay fractions compared to either Org2 or NT (p<0.03). Most of the bulk soil among all cropping systems and depth increments was dominated by microaggregate and silt and clay size classes (Fig. 2.4), which represented 34.4 ±1.4% and 47.4 ±2.9% of the bulk soil, respectively.

Table 2.8. Type I ANOVA results from LME models testing the effect of cropping system on aggregate size class distribution for each aggregate size class within each of the five depth increments, followed by Tukey's honestly significant difference (HSD) test results. Significance was determined at $P < 0.05$.

Depth (cm)	Size class	*df*	Sum of Squares	Mean Square	*F*	*p-value*
0-5	LM	4	8.89	2.22	3.12	0.06
	sM	4	203.87	50.97	5.17	0.01
	m	4	207.20	51.80	4.05	0.01
	sc	4	443.19	110.80	4.38	0.02
5-10	LM	4	14.09	3.52	2.28	0.12
	sM	4	93.40	23.35	4.87	0.01
	m	4	171.95	42.99	2.40	0.11
	sc	4	448.36	112.09	4.71	0.02
10-20	LM	4	15.58	3.90	3.58	0.04
	sM	4	40.64	10.16	2.51	0.10
	m	4	12.82	3.21	0.09	0.99
	sc	4	81.42	20.36	0.46	0.77
20-30	LM	4	2.34	0.59	0.42	0.79
	sM	4	9.68	2.42	0.96	0.46
	m	4	220.44	55.11	2.20	0.13
	sc	4	290.07	72.52	2.08	0.15
30-50	LM	4	260.98	65.25	2.02	0.16
	sM	4	60.81	15.20	1.40	0.29
	m	4	110.43	27.61	2.10	0.14
	sc	4	340.06	85.01	5.28	0.01

Tukey HSD

0-5 cm - small macroaggregates

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	5.60	2.22	0.09
Org2	CT	-3.67	2.22	0.46
Org3	CT	-0.59	2.22	1.00
Org6	CT	3.13	2.22	0.62
Org2	NT	-9.27	2.22	< 0.001
Org3	NT	-6.18	2.22	0.04
Org6	NT	-2.46	2.22	0.80
Org3	Org2	3.09	2.22	0.63
Org6	Org2	6.81	2.22	0.02
Org6	Org3	3.72	2.22	0.45

Table 2.8. Continued

0-5 cm - microaggregates

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-4.77	2.53	0.33
Org2	CT	-6.37	2.53	0.09
Org3	CT	2.04	2.53	0.93
Org6	CT	-4.78	2.53	0.32
Org2	NT	-1.61	2.53	0.97
Org3	NT	6.81	2.53	0.06
Org6	NT	-0.02	2.53	1.00
Org3	Org2	8.41	2.53	0.01
Org6	Org2	1.59	2.53	0.97
Org6	Org3	-6.82	2.53	0.05

0-5 cm – silt & clay

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-1.96	3.56	0.98
Org2	CT	10.84	3.56	0.02
Org3	CT	-0.80	3.56	1.00
Org6	CT	-0.53	3.56	1.00
Org2	NT	12.80	3.56	0.003
Org3	NT	1.17	3.56	1.00
Org6	NT	1.43	3.56	0.99
Org3	Org2	-11.63	3.56	0.01
Org6	Org2	-11.37	3.56	0.01
Org6	Org3	0.27	3.56	1.00

5-10 cm – small macroaggregates

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	3.62	1.55	0.13
Org2	CT	-2.85	1.55	0.35
Org3	CT	-0.32	1.55	1.00
Org6	CT	1.74	1.55	0.79
Org2	NT	-6.46	1.55	< 0.001
Org3	NT	-3.94	1.55	0.08
Org6	NT	-1.88	1.55	0.74
Org3	Org2	2.53	1.55	0.48
Org6	Org2	4.59	1.55	0.03
Org6	Org3	2.06	1.55	0.67

Table 2.8. Continued

5-10 cm – silt & clay				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-2.69	3.45	0.94
Org2	CT	11.24	3.45	0.01
Org3	CT	3.79	3.45	0.81
Org6	CT	1.39	3.45	0.99
Org2	NT	13.93	3.45	< 0.001
Org3	NT	6.48	3.45	0.33
Org6	NT	4.08	3.45	0.76
Org3	Org2	-7.45	3.45	0.20
Org6	Org2	-9.85	3.45	0.04
Org6	Org3	-2.40	3.45	0.96
10-20 cm – large macroaggregates				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-0.0023	0.74	1.00
Org2	CT	-0.94	0.74	0.71
Org3	CT	-0.89	0.74	0.74
Org6	CT	1.50	0.74	0.25
Org2	NT	-0.94	0.74	0.71
Org3	NT	-0.89	0.74	0.75
Org6	NT	1.50	0.74	0.25
Org3	Org2	0.05	0.74	1.00
Org6	Org2	2.44	0.74	0.01
Org6	Org3	2.39	0.74	0.01
30-50 cm – silt & clay				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-3.67	2.84	0.70
Org2	CT	-7.27	2.84	0.08
Org3	CT	-0.57	2.84	1.00
Org6	CT	5.15	2.84	0.36
Org2	NT	-3.60	2.84	0.71
Org3	NT	3.10	2.84	0.81
Org6	NT	8.82	2.84	0.02
Org3	Org2	6.70	2.84	0.13
Org6	Org2	12.42	2.84	< 0.001
Org6	Org3	5.72	2.84	0.26

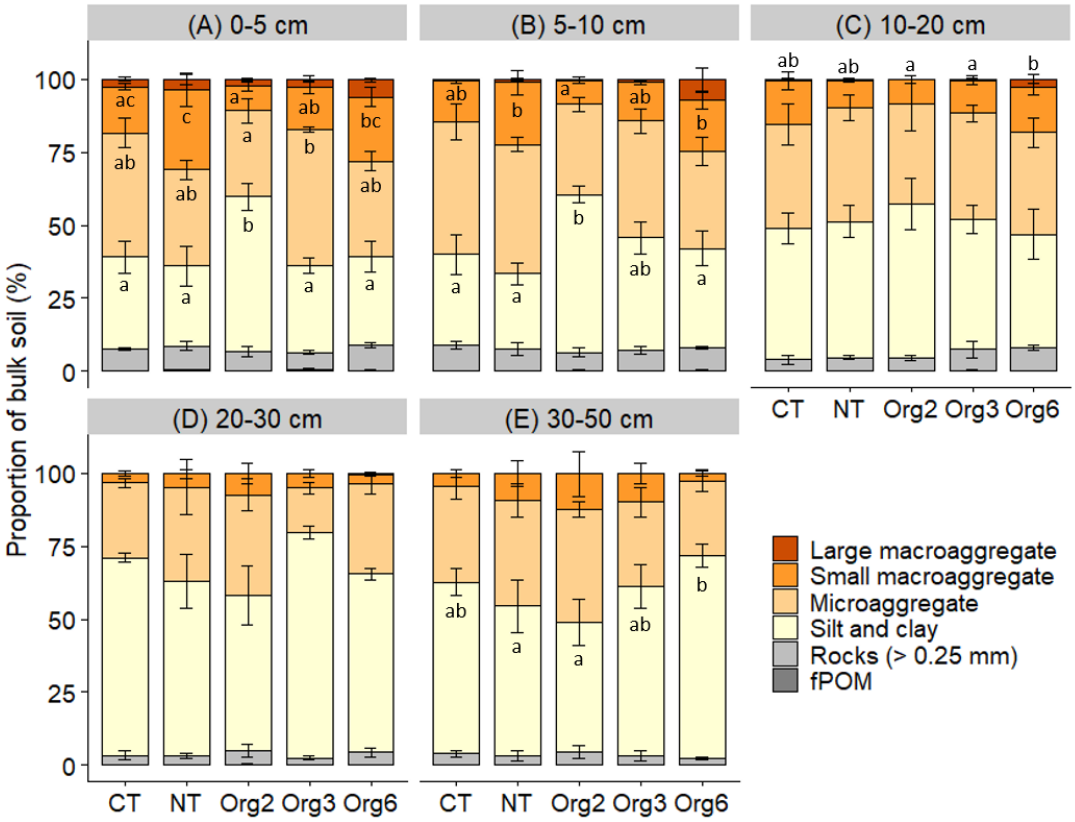

Fig. 2.5. Soil aggregate size distribution as a proportion of bulk soil (%) among cropping systems after 25 years at the Farming Systems Project (FSP) at the following depth increments: (A) 0-5 cm, (B) 5-10 cm, (C) 10-20 cm, (D) 20-30 cm, and (E) 30-50 cm depths. Where CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, and Org6 = 6-year organic. Bars of the same color with different letters within each panel indicate significant differences among cropping systems for the same aggregate size class. Error bars are the standard error of the mean.

Total aggregate-associated C was summed for each depth across all cropping systems (Table 2.3). Total aggregate-associated C varied significantly across cropping systems only at the 0-5 cm depth ($p < 0.05$), where NT (0.66 g C) had significantly higher total aggregate-associated C compared to CT (0.46 g C; $p<0.02$) and all three organic systems were not significantly different from CT and NT.

Table 2.9. Sum of aggregate-associated carbon (g C sand-free aggregate^{-1}) in each cropping system in the Farming Systems Project at five depth increments. Where CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, and Org6 = 6-year organic. Different letters in the same row indicate significant differences among cropping systems.

Depth (cm)	CT	NT	Org2	Org3	Org6
0-5	0.46_{a}	0.66_{b}	0.52_{ab}	0.60_{ab}	0.66_{ab}
5-10	0.41	0.45	0.47	0.58	0.54
10-20	0.37	0.35	0.42	0.47	0.38
20-30	0.09	0.18	0.20	0.18	0.20
30-50	0.09	0.12	0.13	0.12	0.11

The effect of cropping systems on the amount of C associated with each aggregate size class for each depth increment is shown in Fig. 2.5. Across all cropping systems and depth increments, large macroaggregates contributed the least to total aggregate-associated C due to being a small proportion of bulk soil. At the 0-5 cm depth, Org6 had greater average aggregate-associated C across aggregate size classes (0.39 g C) compared to CT and Org2 (0.32 g C, on average; $p<0.01$). A significant interaction effect of cropping systems and aggregate size classes on aggregate-associated C was also found at the 0-5 cm depth ($p<0.001$, Fig. 2.5-A). Small macroaggregates held significantly more C in NT and Org6 (0.23 g C and 0.20 g C, respectively) compared to the other systems (0.09 g C, on average; $p<0.05$). Microaggregates stored significantly more C in Org3 compared to CT and Org2 (55% and 68% more, respectively; $p<0.03$), while microaggregates in NT and Org6 had intermediate C values.

At the 5-10 cm depth, NT contained significantly more SOC in small macroaggregates (0.13 g C) compared to CT and Org2 (0.07 g C, on average; $p<0.001$), with intermediate values found in Org3 and Org6. In the silt and clay fraction, aggregate C was 72% higher in Org2 compared to NT (0.29 g C and 0.14 g C, respectively; $p < 0.01$), with all other systems containing intermediate C levels. Below 10 cm, there was no significant interaction effect between cropping systems and aggregate size classes ($p > 0.05$). However,

C stocks differed significantly among aggregate size classes ($p < 0.001$; Fig. 2.5-B, C, D, E), with the silt and clay fraction containing the highest quantities of aggregate C across all systems, followed by microaggregates. There was also a significant system effect at the 20-30 cm depth ($p < 0.05$; Fig. 2.5-D), with Org2 having a higher average aggregate-associated C across size classes (0.17 g C) compared to CT (0.12 g C).

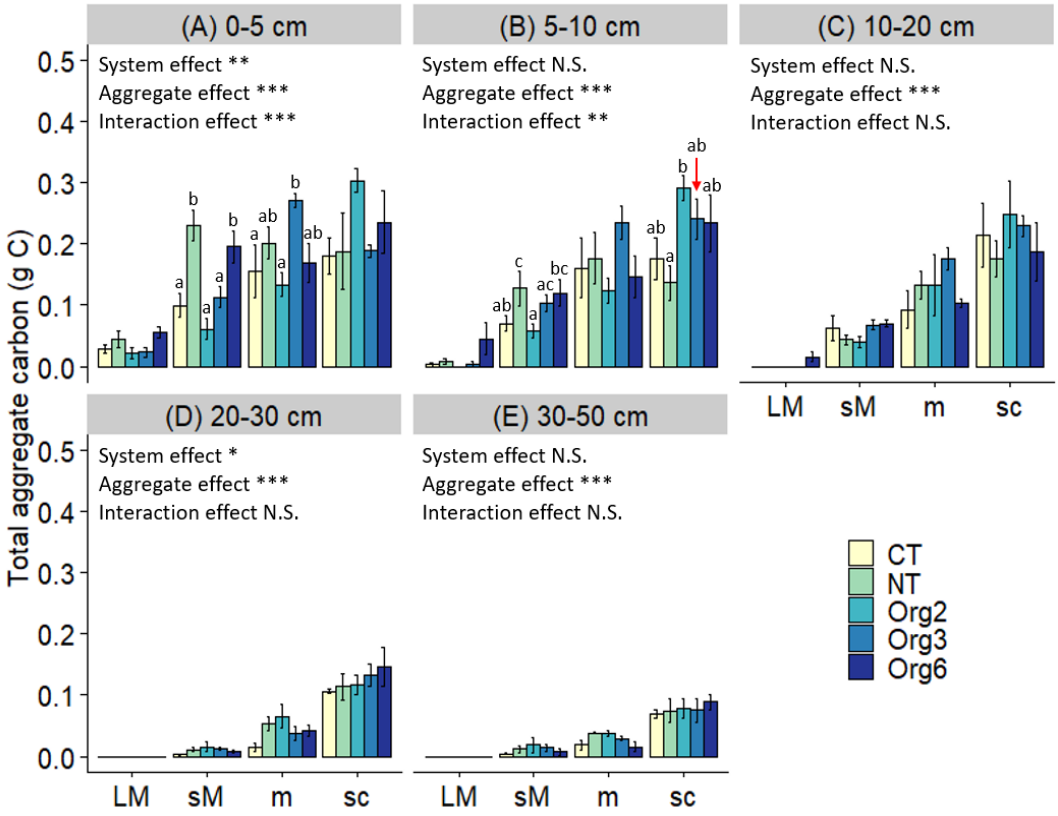

Fig. 2.6. Distribution of aggregate C (g C sand-free aggregate) among different aggregate size classes within cropping systems after 25 years at the Farming Systems Project (FSP) at the following depth increments: (A) 0-5 cm, (B) 5-10 cm, (C) 10-20 cm, (D) 20-30 cm, and (E) 30-50 cm depths. Where LM = large macroaggregate, sM = small macroaggregate, m = microaggregate, sc = silt and clay, CT = conventional chisel-till, NT = conventional no-till, Org2 = 2-year organic, Org3 = 3-year organic, and Org6 = 6-year organic. Error bars are the standard error of the mean. Different letters indicate statistically significant differences among cropping systems for the respective aggregate size class, at P < 0.05 in Tukey post hoc pairwise comparisons. Asterisks (*) indicate significant effects at *P<0.05, **P<0.01, ***P<0.001 in Tukey post hoc pairwise comparisons.

Table 2.10. Type I ANOVA results from LME models testing the effect of cropping system on aggregate-associated C content for each aggregate size class within each of the five depth increments, followed by Tukey's honestly significant difference (HSD) test results. Significance was determined at $P < 0.05$.

Depth (cm)	Size class	*df*	Sum of Squares	Mean Square	*F*	*p-value*
0-5	LM	4	0.004	0.001	2.49	0.10
	sM	4	0.08	0.02	10.99	< 0.001
	m	4	0.05	0.01	3.78	0.03
	sc	4	0.04	0.01	1.69	0.22
5-10	LM	4	0.05	0.01	1.60	0.24
	sM	4	0.02	0.004	5.39	0.01
	m	4	0.03	0.01	1.38	0.30
	sc	4	0.06	0.01	3.59	0.04
10-20	LM	4	0.001	< 0.001	4.25	0.02
	sM	4	0.003	0.001	1.54	0.25
	m	4	0.02	0.004	1.31	0.32
	sc	4	0.01	0.003	0.47	0.76
20-30	LM	4	NA	NA	NA	NA
	sM	4	< 0.001	< 0.001	1.15	0.38
	m	4	0.004	0.001	2.05	0.15
	sc	4	0.003	< 0.001	0.56	0.70
30-50	LM	4	NA	NA	NA	NA
	sM	4	< 0.001	< 0.001	0.65	0.64
	m	4	0.002	< 0.001	4.02	0.03
	sc	4	< 0.001	< 0.001	0.34	0.85

Tukey HSD

0-5 cm - small macroaggregates

System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	0.13	0.03	< 0.001
Org2	CT	-0.04	0.03	0.69
Org3	CT	0.01	0.03	0.99
Org6	CT	0.10	0.03	0.01
Org2	NT	-0.17	0.03	< 0.001
Org3	NT	-0.12	0.03	< 0.001
Org6	NT	-0.03	0.03	0.78
Org3	Org2	0.05	0.03	0.41
Org6	Org2	0.13	0.03	< 0.001
Org6	Org3	0.08	0.03	0.05

Table 2.10. Continued

0-5 cm - microaggregates				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	0.05	0.04	0.75
Org2	CT	-0.02	0.04	0.98
Org3	CT	0.12	0.04	0.02
Org6	CT	0.01	0.04	1.00
Org2	NT	-0.07	0.04	0.40
Org3	NT	0.07	0.04	0.38
Org6	NT	-0.03	0.04	0.92
Org3	Org2	0.14	0.04	0.004
Org6	Org2	0.04	0.04	0.89
Org6	Org3	-0.10	0.04	0.07

5-10 cm - small macroaggregates				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	0.06	0.02	0.02
Org2	CT	-0.01	0.02	0.96
Org3	CT	0.03	0.02	0.36
Org6	CT	0.05	0.02	0.06
Org2	NT	-0.07	0.02	0.002
Org3	NT	-0.02	0.02	0.71
Org6	NT	-0.01	0.02	0.99
Org3	Org2	0.05	0.02	0.09
Org6	Org2	0.06	0.02	0.01
Org6	Org3	0.02	0.02	0.92

5-10 cm - silt & clay				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	-0.04	0.04	0.91
Org2	CT	0.12	0.04	0.07
Org3	CT	0.07	0.04	0.58
Org6	CT	0.06	0.04	0.69
Org2	NT	0.15	0.04	0.01
Org3	NT	0.10	0.04	0.14
Org6	NT	0.10	0.04	0.20
Org3	Org2	-0.05	0.04	0.80
Org6	Org2	-0.06	0.04	0.71
Org6	Org3	-0.01	0.04	1.00

Table 2.10. Continued

10-20 cm - large macroaggregates				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	< 0.001	< 0.001	1.00
Org2	CT	< 0.001	< 0.001	1.00
Org3	CT	< 0.001	< 0.001	1.00
Org6	CT	0.02	< 0.001	0.01
Org2	NT	< 0.001	< 0.001	1.00
Org3	NT	< 0.001	< 0.001	1.00
Org6	NT	0.02	< 0.001	0.01
Org3	Org2	< 0.001	< 0.001	1.00
Org6	Org2	0.02	< 0.001	0.01
Org6	Org3	0.02	< 0.001	0.01

30-50 cm - microaggregates				
System1	System2	Mean Difference	Standard Error	*p-value*
NT	CT	0.02	0.007	0.06
Org2	CT	0.02	0.007	0.09
Org3	CT	0.01	0.007	0.63
Org6	CT	-0.003	0.007	0.99
Org2	NT	-0.001	0.007	1.00
Org3	NT	-0.01	0.007	0.71
Org6	NT	-0.02	0.007	0.02
Org3	Org2	-0.01	0.007	0.81
Org6	Org2	-0.02	0.007	0.03
Org6	Org3	-0.01	0.007	0.34

Discussion

Greater diversity of organic inputs and reduced tillage resulted in higher SOC stocks among cropping systems

The higher quantity and quality of C inputs from poultry litter and legume cover crops in Org3 (Fig. 2.1-A) likely resulted in the significantly larger SOC stocks found relative to CT at the 0-50 cm depth (Fig. 2.1-B). Greater diversity in C inputs likely leads to a smaller energy return on investment for microbes associated with its assimilation, thereby resulting in greater accrual and stabilization efficiency of C inputs in soil (Cotrufo et al., 2022; Lehmann et al., 2020). The lower SOC stocks found in CT compared to Org3 (Fig, 2.1-B) were likely a combined result of tillage and inefficient microbial processing of resistant (i.e., low quality) crop residues (Fig. 2.1-A). Tillage disturbs soil structure and aggregation, exposing once protected organic matter to higher O_2 levels, which increases mineralization rates, ultimately releasing more CO_2 into the atmosphere (Celik et al., 2011; Watts et al., 2010). These results are consistent with Marriott & Wander (2006), who found that organic management that included legumes and/or manure additions increased surface soil SOC concentrations by 14% compared to conventional management within nine farming system trials across the US, organic. Manure inputs such as poultry litter have been shown to significantly increase labile C stocks (POX-C) and microaggregates, leading to greater accumulation of microbial biomass C and stabilization of SOC (Zhang et al., 2021). Annual poultry litter applications in eastern Tennessee grain cropping systems increased SOC stocks at the 0-15 cm depth compared to a hairy vetch cover crop, winter wheat or fallow control (Bansal et al., 2021). This finding is likely due to the high-quality poultry litter stimulating biological processes that transform the labile C into microbial biomass C (Ren et al., 2019). Because of its low C:N ratio and partially degraded form, manure inputs are more easily assimilated by microbes, allowing for microbial by-products to be quickly protected within the soil matrix (Cotrufo et al., 2013). Without the addition of high-quality organic inputs such as legumes or manure to supplement C losses, SOC stocks in tilled systems are more likely to decline over time.

Surprisingly, after three years of no-till perennial forage management, Org6 did not have significantly higher SOC stocks compared to other systems (Fig. 2.1-B). This contrasts with previous studies that show perennial management is more effective at building SOC stocks compared to annual crop management, especially in the subsoil (below 20 cm), due to extensive rooting depth and rhizodeposition (Crews et al., 2018; Ledo et al., 2020; Peixoto et al., 2020). After a four-year study, Sprunger et al. (2019) found that established perennial grain crops increased labile C stocks (POX-C) and encouraged a more diverse microbial community compared to annual winter wheat. Compared to annual crops, perennial forages grown to maturity can grow deeper roots as well as produce root residues with much lower C:N ratios, stimulating biological activity in the soil profile (Ryan et al., 2018).

On the other hand, some studies have also shown that an increase in rhizodeposition can stimulate the mineralization of previously stable SOC, referred to as the rhizosphere priming effect (Dijkstra et al., 2013; Jilling et al., 2021). Jilling et al., 2021 reported that inputs of common root exudates (glucose and oxalic acid) led to the destabilization of mineral-associated organic matter through both biological and chemical pathways. The release of mineral-bound organic C can result in flushes of respiration and C loss from the soil system if not supplemented with additional C inputs (Zhang et al., 2016). Even though Org6 received higher quantities of C inputs over time largely due to perennial alfalfa rhizodeposition (Fig. 2.1-A), it did not have greater SOC stocks after 25 years of management than did any of the other systems (Fig. 2.1-B). This finding highlights the importance of C input quality, timing, and diversity for building SOC in agroecosystems (Kallenbach et al., 2015). The 3-year organic system received a continuous supply of diverse, high quality organic inputs throughout its rotation from more frequent poultry litter applications and cover cropping compared to Org6 and the other cropping systems (Fig 2.1-A; Table 2.1). High quality root inputs from alfalfa provided a continuous supply of C to the soil system over the three perennial years in Org6, however inputs throughout the crop rotation were not as variable compared to Org3. Carbon stabilization is greater when a variety of substrates are present at low concentrations, distributed throughout the soil profile in various forms of decomposition

(Lehmann et al., 2020). According to this line of thinking, the greater diversity of organic molecules in Org3 limits the ability of microorganisms to mineralize SOC, allowing C to persist in the soil longer.

SOC stocks are better maintained over time in extended rotations with perennial forages

After 25 years of continuous management, Org6 was the only system that maintained SOC stocks at the 0-50 cm depth (Fig. 2.1-C). Overall, these results were unexpected as we expected to find an increase in SOC stocks over time in systems with reduced tillage or greater quantities of diverse C inputs. King and Blesh (2018) found that the addition of annual cover crops or perennial crops into existing crop rotations increased SOC concentrations by 6.2% and 12.5%, respectively, after an average of 14 years across 27 different cropping systems. As stated earlier, prior to the initiation of our cropping systems experiment the site was under perennial forage management for at least 14 years, and then no-till corn production for three years before plot establishment. Long-term perennial forage management has been shown to sustain, and even increase, SOC stocks more efficiently compared to annual cropping (Ledo et al., 2020; Rui et al., 2022; Sprunger et al., 2019). Therefore, the initial SOC stocks at the experimental site were likely elevated because of continuous soil cover and above- and below-ground C inputs provided by perennial forage crops (Page et al., 2020; Paustian et al., 2016). The annual-perennial 6-year organic system was the most effective at maintaining baseline SOC stocks over time (Fig. 2.1-C), with three years of continuous perennial cover building up SOC in surface soils (Table 2.2; Fig. 2.2-A, B) due to no tillage and expected high rates of root biomass and rhizodeposition (Ledo et al., 2020). All other systems were also sampled at the end of their respective crop rotation (preceding corn), which consequently emphasized the positive effect of rotation complexity for minimizing SOC loss. For example, SOC change at the 0-20 cm depth showed a trend of seven times more loss in CT compared to Org6. The potential of an agroecosystem to accrue SOC is contingent on the initial conditions of the soil; soils with low baseline C concentrations can more quickly increase SOC stocks when C-building practices are implemented compared

to soils with high baseline concentrations (Arndt et al., 2022, Minasny et al., 2017). Converting these agroecosystems into grain crop rotations after 14+ years of perennial forage likely led to the overall downward trend in SOC stocks observed in this study. The ability of Org6 to minimize net SOC loss overtime compared to other systems suggests that management with minimal soil disturbance, greater rotation complexity, and greater inputs of labile C sources best sustains SOC stocks over time in grain production systems.

Another unexpected result from this study was the lack of significant differences in net SOC change among cropping systems at the 20-50 cm depth, indicating that SOC response to cropping system management was limited to the 0-20 cm plow layer. I expected to find that higher levels of rhizodeposition and root biomass in Org6 would increase or minimize losses of SOC in the subsoil layer. However, these findings agree with many previous studies, indicating that SOC accumulation in the subsoil due to plant roots and root exudates is inconsistent and highly dependent on initial soil properties (Poffenbarger et al., 2020; Rumpel & Kögel-Knabner, 2011). After sampling to a depth of 1.2 m, Bell & Entz (2012) found that an alfalfa-annual crop rotation in Manitoba, Canada did not increase C concentrations in either the subsoil or total sample profile relative to an annual crop rotation after 18 years. However, a restored perennial grassland had greater increases in soil C after 18 years compared to both cropping systems (Bell & Entz, 2012). The length of time it takes for perennial plants to contribute significantly to subsoil SOC stocks may be longer than the three-year period of alfalfa forage production in Org6.

Cropping systems effect on SOC and POX-C concentrations are more evident in surface soils

Minimal soil disturbance in NT and Org6 likely led to higher total SOC and POX-C concentrations at the 0-5 cm depth compared to CT and Org2 (Fig. 2.2, 2.3). At the time of sampling, Org6 had not been tilled for three years, while NT had not been tilled for at least 28 years, allowing for organic matter to accumulate in the surface soil, inaccessible to further microbial degradation (Cavigelli et al., 2018; Wacker et al., 2022). However, these significant differences in POX-C concentrations were not found below 5 cm, which illustrates the effect

of tillage management on vertical C distribution in the soil profile (Fig. 2.3). Other studies have also shown that reducing tillage frequency causes a similar redistribution of SOC along the soil profile but does not necessarily increase total SOC stocks (King & Blesh, 2018).

Increased belowground C inputs from perennial alfalfa in combination with reduced soil disturbance in Org6 likely led to higher SOC concentrations at the 5-10 cm depth compared to Org2, NT and CT (Fig. 2.2-B). The extensive root system of perennial alfalfa, especially after 3 years of establishment, can provide high quality C inputs from root biomass and rhizodeposits (e.g., exudates, root hairs, sloughed off root cells; Peixoto et al., 2022). In a study on root biomass and shoot to root ratios of perennial forages in eastern Canada, Bolinder et al. (2002) found that total root biomass in in the topsoil layer (0-15 cm) made up 54% of total root biomass (0-45 cm) in the first, and 71% in the second year (Bolinder et al., 2002). Although root biomass and rhizodeposition were not quantified in our study, it is likely that the largest proportion of total root biomass from the perennial alfalfa in Org6 is concentrated in the topsoil depths, resulting in high SOC concentrations at 0-10 cm compared to other systems.

At the 5-10 and 10-20 cm depths, there were trends of higher SOC and POX-C concentrations in the organic compared to conventional systems (Fig. 2.2, 2.3). When comparing conventional to organic fertility management, findings from our study align with existing literature that claim long-term application of inorganic fertilizers (NPK) without supplemental organic amendments can lead to more C respiration than accumulation (Kaur et al., 2019; Menšík et al., 2018). Kallenbach et al. (2015) found that organic management led to higher SOC and MBC compared to conventional management due to more frequent additions of diverse, high quality C sources. Within vegetable production systems in central California, White et al. (2020) found that a combination of compost and frequent cover cropping increased both total SOC and POX-C in regularly tilled and intensively managed soils. The Org3 system had significantly higher SOC and POX-C concentrations at the 5-10 and 10-20 cm depths compared to CT and NT (Fig. 2.2-A, B, C; 2.3). A defining management factor in Org3 was more frequent poultry litter applications compared to the other organic

systems, and our results suggest that these higher manure inputs better promote both overall SOC and SOC stabilization in the topsoil, as POX-C is believed to represent a more processed, mineral-associated labile C pool compared to other metrics of labile C (Culman et al., 2012; Hurisso et al., 2016). Similar SOC results were observed in an earlier study at the FSP conducted by Spargo et al. (2011), who suggested that the higher frequency of poultry litter applications in Org3 resulted in higher SOC concentrations compared to CT after 13 years of management. Contradictory to our POX-C findings, Hurisso et al. (2016) found that POX-C pools in US agricultural soils were lower in systems with poultry litter applications or legume-grass cover crops, but that mineralizable C pools (short-term nutrient availability) increased from these practices. In agreement with our findings, many studies report that the partially degraded and bioavailable state of manure compounds allow them to be rapidly incorporated into soil organic matter, increasing stable, mineral-associated C pools (Hai et al., 2009; Maillard et al., 2015; Wen et al., 2019). In general, it is possible that other management or environmental factors apart from the use of manure alone could be affecting how this input is incorporated into the soil across studies, resulting in different SOC dynamics.

Management affected the mechanism of C-protection among aggregates

Trends in aggregate size distribution were closely related to aggregate C storage patterns across cropping systems. For example, systems with greater proportions of macroaggregates also had more C stored in macroaggregate fractions compared to other systems (Fig. 2.4, 2.5). Minimal soil disturbance in NT and the final three years of Org6 resulted in higher macroaggregate proportions and consequently, greater storage of C in macroaggregates in surface layers compared to other systems. The no-till management in these two systems at the time of sampling left soils and surface residues relatively undisturbed to allow for greater aggregate formation and C stabilization in topsoil (Devine et al., 2014; Green et al., 2005). Moreover, crop residues that are left on the soil surface decompose at slower rates than when incorporated in conventional tillage systems, leading to lower C mineralization rates (Mirsky et al., 2012; Thapa et al., 2022). Liu et al. (2021)

performed a meta-analysis of 89 studies evaluating the impact of tillage on aggregate formation and found that at least 6-years of no-till management significantly increased the proportion of water-stable large and small macroaggregates, as well as the SOC concentrations across all aggregate sizes in the topsoil (0-20 cm) compared to conventional tillage. They found that site-specific climatic conditions, experimental duration, and initial soil properties were the most significant factors affecting aggregation and aggregate-associated SOC (Liu et al., 2021). In our study, however, increased aggregation in NT and Org6 was only found at the 0-5 and 5-10 cm depths, indicating that in reduced or no-tillage systems soil aggregation and physically-protected SOC may decrease below the surface soil.

In addition to reduced soil disturbance, increased deposition of belowground C inputs in Org6 likely enhanced the amount of organic binding agents in the topsoil to better promote soil aggregation compared to other management factors (de la Reguera & Tully, 2021; Grandy & Robertson, 2007). Rhizodeposition releases into the rhizosphere low-molecular-weight organic compounds that enhance microbial activity and, in some cases, the formation of aggregates (Villarino et al., 2021). The presence of macroaggregates has been shown to positively correlate with soil stability and SOC occlusion, however macroaggregates are also found to be more sensitive to management changes in comparison to microaggregates and show faster turnover rates (Totsche et al., 2017). Disruption of soil aggregates can result in a flush of C loss from the agroecosystem, therefore long-term implementation of aggregate-building practices is crucial to achieve SOC accumulation in the topsoil.

Microaggregates stored the greatest amount of aggregate-associated C in Org3 surface soils compared to other systems and size fractions (Fig. 2.5-A). Microaggregates are associated with a more degraded portion of organic matter and are composed of mineral, organic, and biotic materials that form a strong complexation. Our results, therefore, indicate that frequent poultry litter applications and cover cropping with legumes can promote greater storage and stabilization of C in microaggregates (Bansal et al., 2021). The low C:N ratio of applied poultry litter and legumes likely induced the rapid assimilation of this organic material into compounds that bind organo-mineral associations (Zhang et al., 2021). These

biologically-produced hydrophobic compounds cement organic and mineral fractions into water-stable microaggregates (Rabbi et al., 2020). After 70 years of mineral fertilizer and/or cattle manure inputs in an agricultural research plot in western Nebraska, Mikha et al. (2015) found more aggregate formation in plots that received manure, or both manure and mineral fertilizers, compared to only mineral fertilizers or a no fertilizer control. Like our study, they found greater quantities of aggregate-associated C in microaggregates compared to macroaggregates due to a larger presence of microaggregates in the soil (Mikha et al., 2015). Regular tillage in Org3 may have limited macroaggregate formation, increasing the proportion of microaggregates and silt and clay in the bulk soil. The greater quantity and quality of organic inputs in Org3 likely made up for some C loss that occurred due to tillage, allowing for mineral-associated C fractions to be stored in microaggregates.

Fewer C inputs in Org2 compared to the other organic systems likely led to the limited aggregate formation and greater proportion of silt and clay fractions at the 0-5 and 5-10 cm depths (Fig. 2.4-A, B). Even with the relatively poor soil structure in Org2, total aggregate-associated C was similar among all organic systems across depths (Table 2.3). This result contradicts the aggregate hierarchy theory, which claims that aggregate-C content increases with increasing aggregate size (Tisdall & Oades, 1982). While investigating aggregate-C distribution across different clay-type soils, Six et al. (2000) found that aggregate-C in 1:1 clay-dominated soils did not follow the aggregate hierarchy theory as closely as in 2:1 clay-dominated soils. Ultisol soils at the experimental site are dominated by 1:1 clays, primarily in the form of kaolinite or Al- and Fe- oxides (Weil & Brady, 2017; Chen et al., 2014). Compared to 2:1 type clays, 1:1 type clays have a small specific surface area, providing fewer binding sites for organic materials in the soil matrix (Kome et al., 2019; Weil & Brady, 2017). However, degraded organic matter fractions can bind to 1:1 clay types through electrostatic interactions (Oades & Waters, 1991; Six et al., 2000). Further research is needed to understand how clay mineralogy may impact the relative effect of long-term management practices on aggregate formation and C stabilization mechanisms.

Conclusion

Within the Farming Systems Project, the most effective practices for supporting SOC stocks were (1) greater diversity of high-quality inputs, and (2) reduced tillage frequency. Under conventional management, no tillage promoted soil aggregation at the soil surface, which in turn increased SOC and labile C stocks compared to a tilled system. The increased frequency of poultry litter inputs and cover cropping in Org3, or reduced tillage and greater belowground inputs from perennial forage in Org6, were the most effective practices to maintain SOC in organic systems. Both reduced tillage and greater input diversity increased soil aggregation across systems, although the proportions of macroaggregates and macroaggregate-associated C compared to microaggregates were higher in systems with reduced- or no tillage (Org6, NT). Agricultural practices with the greatest potential to support SOC stocks in grain systems over a 25-year period involve continuous living soil cover through perennial forages or cover crops, and regular inputs of high-quality organic residues such as poultry litter or legumes. The adoption of one or several of these C-building practices shows potential in helping growers improve the soil structure and fertility of their grain cropping systems while simultaneously encouraging soil C sequestration. However, in our case, long-term perennial forage production prior to plot establishment resulted in high baseline SOC stocks at the FSP and SOC stocks have declined over time in all systems with only annual grain crops in rotation. This finding emphasizes the C-building potential of annual-perennial management over annual crop management with respect to soil C sequestration in the Mid-Atlantic US. Further research is needed within the FSP to determine whether trends in SOC losses across systems will continue or if SOC change is reaching a plateau, and if so, what future SOC dynamics will look like across systems. Evaluating SOC quantity and quality across all crop rotation entry phases for each system may also provide additional insight into how SOC dynamics vary throughout crop rotations, and whether differences in SOC change among systems is more pronounced when all rotation phases are pooled.

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Chapter 1

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Chapter 2

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