

Management history affects the ecosystem services from cover crop mixtures

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Abstract

Cover crop mixtures of functionally distinct species may increase multiple ecosystem services, depending on their establishment and composition. We assessed how unique management legacies, and resulting soil fertility properties, influenced the functions provided by a cover crop mixture. We used the Kellogg Biological Station's long-term Main Cropping Systems Experiment to test the legacy effects of four management systems (ranging from conventional to organic) on the production and function of crimson clover and cereal rye cover crops grown alone and in mixture, with a focus on their potential for improving agroecosystem N retention and supply. We applied bromide as a conservative tracer of solute flow in the soil profile to assess management legacy and cover crop treatment effects on potential anion leaching. A history of ecological nutrient management practices, including long-term cover crop use, increased soil fertility. Higher soil fertility, in turn, increased cover crop biomass and mixture evenness, and percent recovery of bromide, an estimate of soil anion retention. Mixture evenness was positively related to higher cover crop C/N ratios, N retention in corn biomass, and percent of clover N from fixation. In legacies with lower soil fertility, clover was more competitive than rye in the mixtures. Our results show that the benefits from cover crop mixtures depend on the management context into which they are adopted. In lower fertility soils with higher nutrient leaching potential, we suggest increasing crop functional diversity by including single species cover crops at different points within a crop rotation to build soil fertility. Once background soil fertility has been developed, farmers could include mixtures in their agroecosystems to maximize the functions provided by cover crops.

Keywords: cover crops, ecological nutrient management, functional diversity, leaching, legume nitrogen fixation, management history, nitrogen

1. Introduction

The global intensification of agriculture has decreased soil fertility and increased dependence on chemical fertilizers to ensure crop yields (Matson *et al.*, 1997). As a result, synthetic fertilizers are often applied in excess, leading to nutrient surpluses and associated nitrogen (N) and phosphorus (P) losses from fields (Carpenter *et al.*, 1998; Diaz and Rosenberg, 2008). In response, an ecological approach to nutrient management identifies practices that recouple carbon (C), N, and P cycles and reduce the need for external fertilizer inputs (Drinkwater and Snapp, 2007), which can restore soil fertility and substantially reduce nutrient losses (Syswerda *et al.*, 2012; Robertson *et al.*, 2014).

Cover crops, which are non-harvested crops planted in rotation with main crops, increase agroecosystem diversity to provide ecosystem services such as nutrient supply and retention (Schipanski *et al.*, 2014). Cover crops can increase net C inputs to soil, supporting increased microbial biomass and activity, and soil organic matter (SOM) content (McDaniel *et al.*, 2014; King and Blesh, 2018). The extended time of living plant cover, especially provided by overwintering cover crops, supports increased immobilization of nutrients through multiple mechanisms, including plant-microbe interactions that can better couple nutrient mineralization with crop assimilation (Drinkwater and Snapp, 2007).

Increasing functional diversity in crop rotations with mixtures of complementary cover crop species, such as grasses and legumes, can increase multiple ecosystem services at once (i.e., multifunctionality; Finney and Kaye, 2017; Blesh, 2018). Grasses have extensive root systems

that assimilate soil nutrients and reduce nitrate (NO_3^-) leaching (Tonitto *et al.*, 2006; Finney *et al.*, 2016). Grass-derived organic matter tends to have higher C/N ratios, which slows N mineralization rates (Parton *et al.*, 2007) to better couple N release with N demand of subsequent crops. Legumes, which supply new N to soil through biological N_2 fixation (BNF), can also decrease agroecosystem N surpluses and losses when they are managed to reduce synthetic N fertilizer inputs (Tonitto *et al.*, 2006; Blesh and Drinkwater, 2013). Given their complementary traits, legume-grass mixtures can support both nutrient supply and retention in agroecosystems (Hayden *et al.*, 2014; Finney and Kaye, 2017; Blesh *et al.*, 2019).

Management decisions related to the diversity of crop functional types, tillage intensity, and levels of external input use interact to influence agroecosystem nutrient cycling and retention (Drinkwater and Snapp, 2007; Zimnicki *et al.*, 2020). Management systems affect soil fertility, including SOM stocks (Syswerda *et al.*, 2011) and microbial activity (Tiemann *et al.*, 2015). These soil conditions, in turn, influence the establishment and productivity of crops. For instance, nonlegume cover crops typically thrive in high fertility soils whereas legumes perform better than nonlegumes in nutrient-poor soils because they can fix N_2 (Brooker *et al.*, 2008; Blesh, 2018). In mixtures of both functional types, background soil fertility and interspecific competition—which depend on nutrient and resource availability—will determine the success of species in mixture (Brooker *et al.*, 2015) and thus the ecosystem services they provide (Grime, 1977). However, the interactive effects of management history, soil fertility, and cover crop functional diversity on ecosystem services are understudied.

To address this knowledge gap, we investigated the productivity, composition, and ecosystem services of a legume and grass cover crop grown alone and in a mixture across a management gradient. The Kellogg Biological Station (KBS) Long-term Ecological Research

(LTER) site has maintained four annual cropping systems (hereafter, management legacies) since 1988: conventional, no-till, reduced synthetic input, and certified organic (Robertson and Hamilton, 2015). Both the reduced-input and organic legacies have a long-term history of grass and legume cover crops at different points in rotation, however none of the legacies have a history of legume-grass cover crop mixtures.

We planted an experiment within this long-standing management gradient to determine: 1) how soil fertility properties that reflect distinct management histories affect cover crop biomass and composition, and 2) how differences in cover crop outcomes influence legume N supply and agroecosystem N retention. We hypothesized that the management legacies would lead to differences in cover crop establishment and productivity, such as increased legume biomass in the legacies with lower background soil fertility, and a resulting lower capacity for nutrient retention. In contrast, ecological nutrient management histories (i.e., reduced-input and organic) increase soil fertility (Grandy and Robertson, 2007; Syswerda *et al.*, 2011), which we expected to enhance complementary resource use and multifunctionality (i.e., nutrient retention and supply) in cover crops through increased mixture evenness.

2. Methods

2.1 Site description

Measurements were taken from June 2019 - October 2020 in the Main Cropping System Experiment (MCSE) of the KBS LTER site in southwest Michigan (42° 24' N, 85° 24' W; 288 m elevation). The mean annual air temperature at KBS is 9.2 °C ranging from a monthly mean of -4.1 °C in January to 21.9 °C in July. Rainfall averages 933 mm yr⁻¹. The MCSE sits on a glacial outwash plain with well drained loam, sandy loam, and sandy clay loam soils in the Kalamzaoo

and Oshtemo series which are mixed, mesic Typic Hapludalfs (Crum and Collins, 1995). The MCSE was established in 1988 to reflect a range of field-crop landscapes in the upper Midwest. Our experiment focused on four unique annual cropping systems with corn-soy-winter wheat rotations: 1) conventional, chisel plowed (“conventional”); 2) conventional, no-till (“no-till”); 3) reduced synthetic input, chisel plowed (“reduced-input”); and 4) biologically-based, certified organic (“organic”). The conventional and no-till legacies receive chemical inputs typical for farms in the Midwest. The reduced-input legacy receives one-third the amount of herbicide and fertilizer as the conventional and no-till legacies; the organic legacy receives no chemical inputs, compost, or manure, with legume cover crops as the sole N source. In the reduced-input and organic legacies, the winter wheat crop is followed by a red clover cover crop, while corn harvest is followed by a cereal rye cover crop. Each management system is replicated six times on 1 ha plots.

2.2 Baseline soil sampling and analysis

On June 11-12, 2019, we sampled soil in the standing wheat crop to determine baseline soil conditions prior to planting the experiment. In each plot, we composited 10 soil cores (2 cm diameter by 20 cm depth) for analysis. We estimated bulk density for the baseline soil sample by taking the fresh weight of the 10 soil cores and adjusting for soil moisture. We determined soil moisture gravimetrically by drying duplicate 20 g samples at 105 °C for 48 hours and weighing dry soil. Subsamples of ~100 g of sieved, dried soil were analyzed for pH, organic matter by loss on ignition, Bray-1 P, K⁺, Mg²⁺, Ca²⁺, and cation exchange capacity at the A & L Great Lakes Laboratories (Fort Wayne, Indiana, USA). We analyzed soil texture for each sample using the hydrometer method (Miller *et al.*, 2013)

Fresh soil was sieved to 2 mm, and we extracted inorganic N ($\text{NO}_3^- + \text{NH}_4^+$) in triplicate with 2 M KCl and passed the extracts through a Fisherbrand Q2 filter. The amount of NO_3^- and NH_4^+ in each sample was analyzed colorimetrically on a discrete analyzer (AQ2; Seal Analytical, Mequon, WI). As an indicator of potential N availability from organic matter decomposition, we used triplicate subsamples of sieved, fresh soil for a 7-day anaerobic N mineralization incubation. We then extracted samples in 2 M KCl, passed extracts through a Fisherbrand Q2 filter, and analyzed them colorimetrically for NH_4^+ . Potentially mineralizable N (PMN) was calculated as the difference in the initial amount of NH_4^+ in the soil and the NH_4^+ released during the 7-day incubation (Drinkwater *et al.*, 1996).

We separated POM > 53 μm from triplicate 40-g subsamples of unsieved, air-dried soil, using a combined size and density method, to isolate POM into the free, light fraction (fPOM) and physically-protected or occluded POM (oPOM) fraction (Marriott and Wander, 2006). The subsamples were first gently shaken for 1 h in sodium polytungstate (1.7 g/cm^3), allowed to settle for 16 h, and fPOM floating on top of the solution was removed by aspiration. The remaining sample was shaken with 10% sodium hexametaphosphate to disperse soil aggregates and then rinsed through a 53- μm filter. The material larger than 53 μm was retained, and the oPOM was separated from sand by decanting. The C and N of both POM fractions were measured on an ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, California, USA). Total soil C and N were measured on dried, sieved soil by dry combustion on a Leco TruMac CN Analyzer.

2.3 Experimental design and crop management

We established the experiment in 12.2 x 12.2 m sections of the northern end of all six replicates of the four management legacies. In a randomized complete block design, we planted three cover crop treatments into plots of 3.1 x 12.2 m: a sole legume, crimson clover (*Trifolium incarnatum* L.) seeded at 16.8 kg ha⁻¹, a sole grass, cereal rye (*Secale cereal* L.) seeded at 100.9 kg ha⁻¹, and a cereal rye (50.4 kg ha⁻¹)-crimson clover (9.0 kg ha⁻¹) mixture, which were compared to a weedy fallow control. We planted the cover crops using a no-till drill, following wheat harvest on July 21, 2019, and terminated them before corn planting in spring 2020. Our design simulates a transition to cover cropping in the conventional and no-till legacies and a diversification of cover crop management in the reduced-input and organic legacies.

Corn was planted following cover crop termination in each legacy at a rate of 12,950 seeds ha⁻¹. In the conventional, no-till, and reduced-input legacies, Pioneer P0306Q variety corn seed was planted. Viking Organic Corn (O.84-95UP) Variety (A1025726) was planted in the organic legacy. At corn planting, herbicides and starter fertilizer was applied to the three legacies that receive chemical inputs. On May 23rd, 2020, Roundup Powermax (0.95 L ha⁻¹), Acuron (7.0 L ha⁻¹), and ammonium sulfate (3.8 kg ha⁻¹) were applied to the conventional and no-till legacies. On May 28th, 2020, Dual II Magnum (1.87 L ha⁻¹) was applied to the reduced-input legacy. On June 24th, 2020, urea-ammonium nitrate fertilizer (28% N at 79 kg N ha⁻¹) was applied to the corn in the rye and fallow treatments in the conventional and no-till legacies because they had not received legume N inputs. On June 25th, 2020, ammonium sulfate (3.8 kg ha⁻¹) and Roundup Powermax (0.95 kg ha⁻¹) were applied to the reduced-input legacy (Robertson and Simmons 2020).

2.4 Aboveground biomass sampling and analysis

Fall cover crop and weed biomass was sampled on October 24th, 2019. Spring biomass was sampled in the conventional legacy on May 4th, 2020, in the no-till legacy on May 5th, 2020, in the reduced-input legacy on May 12th, 2020, and in the organic legacy on May 26th, 2020. This later sampling date reflects the cropping system design at KBS, as the organic legacy typically has a later termination date for cover crops due to later planting of corn. We sampled aboveground biomass from one 0.25 m² quadrat placed randomly in each replicate plot avoiding edges. Shoot biomass was cut at the soil surface, separated by species, dried at 60 °C for 48 hours, weighed, and coarsely ground (< 2 mm) in a Wiley mill. We analyzed biomass for total C and N content by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI). Mixture evenness (excluding weeds) was calculated using the following equation:

$$\text{Evenness} = H'/H'_{\max}$$

where H' is the Shannon diversity index and $H'_{\max} = \ln(2)$.

We estimated clover aboveground N fixation using the natural abundance method (Shearer and Kohl, 1986). Shoot biomass from the clover in monoculture and mixture, and rye in monoculture (the reference plant), were analyzed for $\delta^{15}\text{N}$ and total N content using a continuous flow Isotope Ratio Mass Spectrometer (UC Davis Stable Isotope Facility). The percent N derived from fixation was calculated using the following mixing model:

$$\%N \text{ from fixation} = 100 \times ((\delta^{15}\text{N}_{\text{ref}} - \delta^{15}\text{N}_{\text{legume}}) / (\delta^{15}\text{N}_{\text{ref}} - B))$$

where $\delta^{15}\text{N}_{\text{ref}}$ is the $\delta^{15}\text{N}$ signature of the reference plant (rye), $\delta^{15}\text{N}_{\text{legume}}$ is the $\delta^{15}\text{N}$ signature of the clover, and B is defined as the $\delta^{15}\text{N}$ signature of a legume when dependent solely on

atmospheric N₂. B values were determined by growing crimson clover in the greenhouse in a N-free medium according to methods in Blesh (2018).

We harvested corn with a Kincaid 8XP Plot Combine (Kincaid Manufacturing, Haven, KS) on October 21st, 2020, in the conventional and no-till legacies, and on October 28th in the reduced-input and organic legacies. To reduce edge effects, we sampled corn from the middle 8.5 m of all plots. We measured grain yield and moisture using the on-board Mirus Harvest Master computer software (Juniper Systems, Logan, UT) from the middle two rows (1.5m) of each treatment. From the combine bin, we collected a grain subsample for chemical analysis. Following harvest, we collected a representative grab sample of stover from the field for chemical analysis. Corn grain and stover were dried for 48 hours at 60° C and weighed. Corn grain was ground to the consistency of flour using a coffee grinder. Stover was ground using a Wiley mill. The ground stover and grain were analyzed for total C and N by dry combustion on a Leco TruMac CN Analyzer (Leco Corporation, St. Joseph, MI). We estimated total corn biomass from grain yield using a harvest index value of 50% (Bolinder *et al.*, 2007).

2.5 Bromide application and anion analysis

Bromide was applied as a conservative tracer of water flow through the soil profile, to better interpret measurements of soil NO₃⁻ concentration at depth following cover crop incorporation. On October 17th, 2019, we applied 0.037 M KBr in water to four of the six replicates of the MCSE (64 plots total), using a water transfer pump to spray KBr on a 9 m² subplot at a rate of 2.35 g Br⁻ m⁻². Due to restrictions during the beginning of the COVID-19 pandemic, we were unable to sample soil until the following summer, approximately two months following cover crop termination. Between July 6th - 10th, 2020, we collected a 7.5 cm diameter

soil core to 120 cm using a Geoprobe from each subplot, divided each core into three depth increments: 0 – 30 cm, 30 – 60 cm, and 60 – 120 cm, and calculated bulk density for each increment. We calculated bulk density of the 0 – 30 cm soil increments by sieving the entire section to 4 mm and separately weighing the sieved soil and the rocks/gravel bigger than 4 mm. We calculated bulk density for the other two increments (30 – 60 cm and 60 – 120 cm) for one randomly selected core per plot (16 total) with the same method we used for the top increment. We determined soil moisture gravimetrically by drying duplicate, 20 g subsamples of sieved soil from each depth increment at 105 °C for 48 hours, and then weighing dry soil.

Subsamples of sieved soil from each increment were extracted with 2 M KCl, passed through a Fisherbrand Q2 filter, and analyzed for soil inorganic N colorimetrically on a discrete analyzer (AQ2; Seal Analytical, Mequon, WI). Sieved soil samples were also extracted in deionized water and analyzed for bromide concentrations on a Dionex ICS-1000 Ion Chromatography system (ThermoFisher Scientific, Waltham, MA) with a detection limit of 0.02 mg L⁻¹.

Nitrate and bromide content were expressed as a concentration and in g m⁻² using measured soil bulk density. Bromide retention in the soil was calculated as a percentage, by adding the areal concentration of bromide recovered in each subsection of the core and dividing that sum total by the amount of bromide that was applied to a 1 m² area (2.35 g). Mean bromide concentrations in all cores ranged from 0.17 mg kg⁻¹ to 2.17 mg kg⁻¹ (Figure S3).

2.6 Statistical analysis

All statistical analysis was performed in the R platform (R Core Team, 2021). We used linear mixed-effects models to determine the fixed effects of management legacy, cover crop

treatment, and their interaction on cover crop biomass, aboveground biomass N, corn yield and N assimilation, soil anion concentrations, percent recovery of bromide, and clover N derived from fixation using the *lme4* package (Bates et al., 2015). We used a simpler, linear mixed-effects model to assess the fixed effect of management legacy on baseline soil properties. In all models, replicate was treated as a random effect. When a mixed-effects model indicated a significant management legacy * cover crop treatment interaction, we used the *emmeans* package to conduct Tukey post-hoc comparisons to assess pairwise differences between cover crop treatments within each management legacy (Lenth *et al.*, 2020).

We also used linear mixed-effects models to test for correlations between variables in the data. Two outliers in the bromide tracer data that were greater than three standard deviations away from the variable mean were removed. In models with corn N or yield as a dependent variable, we included corn variety as a random effect because it differed in the organic legacy.

For all analyses, we checked that final models met assumptions of homoscedasticity and normality of residuals. Other packages used were *tidyverse*, *car*, *ggpubr*, *ggeffects*, and *cowplot* (Fox and Weisberg, 2018; Lüdtke, 2018; Wickham *et al.*, 2019; Kassambara, 2020; Mangioficio, 2020; Wilke, 2020).

3. Results

3.1 Baseline soil characteristics and corn yield

Based on several biological and chemical soil properties including SOM, fPOM and oPOM, nutrient content, and indicators of microbial activity (i.e., PMN), the reduced-input and organic legacies had higher fertility than the conventional and no-till legacies (Table S1). Cover crop treatment did not affect the yield or aboveground N assimilation of the following corn crop

(Table 1). However, these outcomes were strongly influenced by management legacy (Tables 2, S2, S3). Grain yields were highest in the no-till and reduced-input legacies and lowest in the organic legacy, which has a corn variety planted later than the other three management systems. Aboveground N assimilation by corn was lower in the organic legacy than the other three legacies (Table S2). Generally, corn yield was underestimated in our experimental plots, especially within the organic legacy (Table S3; Robertson and Snapp, 2019). At the whole plot scale, yields in the organic and conventional legacies were comparable (Table S3).

3.2 Cover crop productivity and quality

In the fall, aboveground cover crop and weed biomass ranged from 84 kg ha⁻¹ to 3440 kg ha⁻¹ (Figure S1). In the spring, aboveground cover crop and weed biomass ranged from 291 kg ha⁻¹ to 5013 kg ha⁻¹ (Figure 1A). The effects of cover crop treatment, management legacy, and their interaction on aboveground biomass in the spring were significant (Table 1). The clover treatment produced 2.4 times more biomass than the rye within the conventional management legacy and 5.8 times more biomass within the no-till legacy. The clover and rye-clover mixture treatments had comparable aboveground biomass in the conventional and no-till legacies. There were no treatment differences within the reduced-input legacy, but within the organic legacy the mixture produced 69% more biomass than rye. Overall, the organic legacy had significantly higher mean aboveground biomass than the other legacies because that cropping system allows for a longer cover crop growing period. There was similarly greater N in spring cover crop and weed aboveground biomass in the sole clover and rye-clover mixture treatments across all management legacies (Figure 1B).

3.3 Cover crop evenness in mixtures

Cover crop community evenness for the rye-clover mixture was higher in management legacies that use fewer external inputs (Table 1; Figure 2A). Evenness was highest in the reduced-input (0.83 ± 0.11) and organic (0.82 ± 0.12) legacies and lowest in the no-till legacy (0.40 ± 0.03). Cover crop community evenness was positively correlated with oPOM ($r^2 = 0.65$, $p < 0.001$; Figure 2B) and PMN ($r^2 = 0.54$, $p < 0.001$; Figure 2C). Evenness was positively correlated with rye biomass N ($r^2 = 0.42$, $p < 0.001$; Figure 2D), negatively correlated with clover biomass N ($r^2 = 0.20$, $p = 0.054$; Figure 2E), and uncorrelated with total spring biomass N ($p = 0.603$). Greater evenness was positively correlated with a higher spring biomass C/N ($r^2 = 0.26$, $p = 0.022$). Finally, mixture evenness was positively related to N assimilation within the following corn crop ($r^2 = 0.10$, $p = 0.006$; Figure 2G).

3.4 Bromide recovery

While there was no impact of management legacy or cover crop treatment on total soil NO_3^- at any depth down to 120 cm (Figure S2; Tables 2, S2), using a bromide tracer as a proxy for potential anion movement in soil revealed differences among management legacies (Table 1). Nine months after application, we recovered significantly more bromide in the organic legacy than in the other three legacies (Figure 3A). Bromide recovery was positively associated with fPOM ($r^2 = 0.11$, $p < 0.001$; Figure 3B) and aboveground biomass sampled in the spring ($r^2 = 0.11$, $p = 0.007$; Figure 3C). Bromide recovery was not related to rye biomass ($p = 0.225$; Figure 3D), mixture evenness ($p = 0.580$; Figure 3E), or N assimilation in corn biomass ($p = 0.139$; Figure 3F). Bromide recovery had a weak but positive relationship with soil NO_3^- down to 120 cm ($r^2 = 0.08$, $p = 0.032$; Figure 3G).

3.5 Biological N fixation

Neither management legacy nor cover crop treatment had a significant effect on the percent of legume N derived from fixation (%Ndfa; Table 1; Figure 4A). However, management legacy significantly affected the amount of fixed N in clover biomass (Table 1; Figure 4B). Compared to the conventional legacy, clover in the reduced-input legacy supplied 44% less N from fixation [(due to its low in biomass in that legacy (Figure 1A)] while clover in the no-till and organic legacies provided 91% and 61% more N, respectively (Figure 4B). Percent Ndfa was positively correlated with PMN ($r^2 = 0.12$, $p = 0.037$; Figure 4C), but the total amount of N fixed by clover was negatively correlated with PMN ($r^2 = 0.09$, $p = 0.074$; Figure 4D). Fixed N in clover aboveground biomass was positively correlated with total clover biomass produced ($r^2 = 0.55$, $p < 0.001$; Figure 4E). Within mixtures, %Ndfa was positively related to rye biomass ($r^2 = 0.27$, $p = 0.015$; Figure 4F) and mixture evenness ($r^2 = 0.21$, $p = 0.034$; Figure 4G), suggesting that competition for N with rye increased N fixation rates. Finally, the total amount of fixed N in clover biomass was uncorrelated to N assimilation in corn biomass ($p = 0.306$; Figure 4H).

4. Discussion

To determine the effects of agroecosystem management history on ecosystem functions from cover crops, we measured background soil fertility, cover crop biomass and mixture evenness, and subsequent effects on N cycling processes, in four distinct management legacies from a long-term experiment. We applied the conservative solute bromide to determine how management legacy and overwintering cover crops interact to influence potential water flow and soil anion loss (including NO_3^-) below the root zone. We found that a history of ecological

nutrient management increased multiple soil fertility characteristics (e.g., SOM, POM, and PMN) compared to management legacies with lower crop diversity and higher external inputs. Soil fertility, in turn, influenced the establishment and growth of cover crops; there was greater overall biomass and mixture evenness in the organic and reduced-input legacies compared to the conventional and no-till legacies. Increased mixture evenness was positively related to higher cover crop C/N ratios, N retention in corn biomass, and clover N fixation rates, indicating that the potential benefits of cover crop mixtures on agroecosystem N cycling depend on the past management of the systems into which they are adopted.

4.1 Cover crop composition and functions

Cover crop establishment and biomass largely followed our predictions regarding soil fertility. In the spring, clover dominated the rye-clover mixture biomass in the conventional and no-till legacies, while the reduced-input and organic legacies had more even mixture composition (Figure 1A). Rye was more competitive in mixture in the organic legacy, demonstrating that higher background soil fertility fosters nonlegume growth and increases mixture evenness (Brooker *et al.*, 2015), perhaps enhancing the complementary agroecosystem N cycling functions provided by legumes and nonlegumes. That mixture evenness was positively related to baseline soil fertility (Figures 2B and 2C) indicates that systems with a history of low external inputs create favorable conditions for even species expression in mixtures. In combination, low external inputs and cover crop mixtures could drive a positive feedback loop in which soil fertility maximizes the ecosystem services derived from mixtures by encouraging evenness, which further increases soil fertility.

Variation in cover crop mixture composition can reflect soil N availability, with low soil inorganic N concentrations favoring legumes in mixture (Baraibar *et al.*, 2020). Soil N availability also influences the functions provided by grasses and legumes in mixture. Blesh (2019) found lower rates of BNF by hairy vetch with higher levels of soil N availability from decomposition of SOM, and (White *et al.*, 2017) found that lower soil NO₃⁻ concentrations at cover crop planting increased legume growth and subsequent N supply without compromising N retention by nonlegumes in cover crop mixtures. Here, we found a positive relationship between PMN and %Ndfa (Figure 4C), which seems to disagree with these previous findings. However, we hypothesize that inorganic N fertilizer use in the management systems with lower fertility soils had a stronger impact on reducing BNF rates than background soil fertility. At the same time, higher fertility soils with higher PMN produced cover crop stands with less clover biomass (Figure 1A, Table 1), which corresponded with a lower amount of total N fixed (Figure 4E), supporting our expectations and reflecting the strong control that legume productivity exerts on total N supplied by N fixation.

The mixture treatment assimilated as much aboveground biomass N as the most productive monoculture, which was crimson clover (Figure 1B). Overall, rye biomass was low in the conventional and no-till legacies (Figure 1A) compared to rye biomass reported in other studies in the region (Finney and Kaye, 2016; Blesh *et al.*, 2019). Cereal rye is more commonly planted in the fall when temperatures are cooler. The summer planting date in our experiment may have constrained the growth and competitiveness of rye. Therefore, while our specific observation that higher evenness increased N retention in rye biomass (Figure 2D) may not apply in all contexts, we conclude that a cover crop mixture can provide high levels of N retention (Figure 1B) regardless of composition (Figures 2D and 2E).

Understanding how management history impacts cover crop establishment through changes in soil fertility is important because species composition and evenness determine the balance of functions provided by plant communities (Grime, 1977; Chapin *et al.*, 1998), including for cover crops (Finney and Kaye, 2017; Blesh *et al.*, 2019). In our study, higher evenness was associated with a higher cover crop C/N ratio (Figure 2F), greater N retention in the following corn crop (Figure 2G), and higher %Ndfa (Figure 4G), without negatively impacting yields of the following corn crop. Previous research has shown that crop yields are inversely related to the C/N ratio of the preceding cover crop mixture because N can be immobilized during the decomposition of high C/N organic matter (Hunter *et al.*, 2019). However, in our study, the C/N ratio of the cover crop mixture was mostly under 25 such that net N mineralization would have occurred. The mixture plots with the highest evenness had the highest C/N ratios (Figure 2F) suggesting that careful management and species selection for mixtures can create conditions that would slow N mineralization but still allow for overall N release to the subsequent crop (Figure 2G), especially if legumes are present (Figure 4G).

4.2 Management legacy and potential anion movement in the soil profile

The organic legacy had the greatest recovery of applied bromide, suggesting a lower potential for anion movement out of the soil, which may be due to the long-term effects of ecological management practices on soil fertility and hydrology. Winter cover crops can improve water infiltration and storage by altering soil physical properties, such as aggregate stability and plant available water, as well as biochemical properties, like SOM, but these effects often take several years to establish (Basche *et al.*, 2016; Beehler *et al.*, 2017; Rorick and Kladvko, 2017). However, in our study, cover crop biomass was positively associated with percent recovery of

bromide in soil after one overwintering season (Figure 3C). This may be due to increased water use and retention by vegetation, which would decrease flow out of the soil profile. While we did not directly test this hypothesis, soil moisture measured in the topsoil in July was significantly higher in the reduced input and organic legacies (Table S2).

Alternatively, this relationship may have been driven by the positive impact of the long-term use of ecological nutrient management practices on soil fertility within the organic legacy, which encouraged high cover crop productivity and evenness in the experiment. The strongest association we found between soil properties and anion movement was with fPOM (Figure 3B), a fraction of SOM that responds to management changes much more rapidly than total SOM (Marriott and Wander, 2006). Overall, the long-term effects of the four management systems on soil properties—rather than the short-term introduction of cover crops in the conventional and no-till legacies, and cover crop diversification in the reduced-input and organic legacy—account for the relationships we found.

We expected that high mixture evenness would decrease anion leaching potential in the soil, as previous research has indicated that grass-legume mixtures can reduce soil NO_3^- content as well as or better than sole grass cover crops (Tosti *et al.*, 2014), but this hypothesis was not supported. We did observe a positive relationship between bromide recovery and soil NO_3^- , indicating that bromide movement may have mirrored potential NO_3^- leaching below the root zone as intended (Schuh *et al.*, 1997; Ottman *et al.*, 2000). Syswerda *et al.* (2012) found that the organic legacy at KBS reduced NO_3^- leaching losses by 70% compared to the conventional legacy over an 11-year period, and that SOC was negatively correlated with leaching, suggesting that the patterns we found accurately reflect NO_3^- leaching potential at this site. In our study, mean estimates of bromide lost by leaching ranged from 15.68 kg ha⁻¹ in the organic legacy to

18.76 kg ha⁻¹ in the conventional legacy. These are estimates of maximum potential NO₃⁻ leaching because bromide does not undergo the same biologically mediated transformations as NO₃⁻. It thus does not reflect N that may be lost as a gas or assimilated into plants, microbial biomass, and SOM pools, but can provide a comparative indication of treatment differences in potential NO₃⁻ loss (Ottman *et al.*, 2000; Clay *et al.*, 2004).

4.3 Implications for agroecological management

Prior studies have found that synthetic fertilizer use and low crop diversity result in N surpluses (Gardner and Drinkwater, 2009; Blesh and Drinkwater, 2013), which correspond with larger N leaching losses compared to higher diversity systems with legume N sources (Syswerda *et al.*, 2012). During transitions to more diverse crop rotations, our results suggest that farmers should consider the legacy effects of past management when choosing cover crop functional types. Because the mixture did not improve corn yield or N assimilation in the lower fertility soil (Table 1), a sole grass cover crop may be desirable for improving agroecosystem nutrient retention during the early stages of diversification before adding legumes (and their associated higher seed costs) at different points within the rotation. Mixtures can improve ecosystem functions better than single-species cover crops in a variety of contexts (Finney and Kaye, 2017; Blesh, 2018; Reiss and Drinkwater, 2020), but due to the complex interactions between management history, soil fertility, and cover crop functions, the full benefits of mixtures may not be realized until later years. As demonstrated by the organic and reduced-input legacies, which had a history of grass and legume cover crops occurring at separate points in the crop rotation, increasing temporal diversity in cropping systems can build the soil fertility (Table 1) necessary for optimizing the evenness and functions of cover crop mixtures.

5. Conclusions

We tested how cover crop growth and mixture composition influenced agroecosystem N cycling within the context of distinct long-term management histories. Our results show that long-term use of ecological nutrient management practices increased soil fertility (e.g., POM content and microbial activity) compared to conventional management, which positively affected cover crop production and mixture evenness, reduced the potential for NO_3^- losses via leaching, increased N retention in cash crop biomass, and increased legume N derived from fixation. Although cover crop treatments did not have a strong effect on any of the measured N cycling processes in any of the legacies, we found that management legacies led to significant differences in cover crop establishment due to their background soil fertility. These findings indicate that soil fertility impacts cover crop productivity and mixture composition in ways that may enhance species interactions and ecosystem functions immediately after adoption, with the potential to evolve with sustained cover crop use. Future research should assess these interactions over time in different agricultural settings. Overall, this study provides valuable insight into the fundamental relationships that mediate the potential for managing nutrients with cover crop mixtures across historical management gradients.

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7. Data availability statement

Data and code supporting the conclusions of this article will be made available online upon acceptance of this publication.

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