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## Ecological intensification with soil health practices demonstrates positive impacts on multiple soil properties: A large-scale farmer-led experiment

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#### Abstract

Improving soil health is critical to reversing trends of soil degradation and is of increasing interest to a range of stakeholders including policymakers, agricultural industry leaders, food companies, and farmers. Crop and soil management practices focused on ecological functions can be effective in restoring fundamental biological, chemical and physical soil properties. The call for ecological intensification of agricultural systems has the potential to improve soil health and input-use efficiency. In this study, we developed a framework to classify spatial and temporal ecological intensification with soil health practices: tillage, crop rotation, cover crop, organic amendment, and crop-livestock integration. We applied this framework in a statewide soil health project featuring collaboratively designed on-farm research. We found that ecological intensification affected all properties commonly used in soil health assessments, but the sensitivity of different practices to impact

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changes varied among the soil physical, chemical and biological properties. The use of cover crops had the greatest impact on driving changes in soil properties, in particular those closely related to organic matter and carbon (C) and nitrogen (N) dynamics. Soil-test biological activity and its association with soil-test predicted N release in cropping systems intensified with cover crop use was found to reduce predicted nutrient fertility needs substantially compared to less intensified systems. Evaluating the potential of existing agricultural systems to undergo ecological intensification at a farm scale provides insights about management options to enhance soil health, particularly in regards to nutrient cycling, biological activity, and input-use efficiency.

**Keywords:** Soil health practices, Soil properties, Ecological intensification, Soil management, On-farm study, Cover crop

**Abbreviations:** CCU, cover crop use; CDI, crop diversity index; YWSD, years without soil disturbance; OAI, organic amendment index; CL, crop-livestock integration; HSHT, Haney soil health tool; MLRA, Major Land Resource Area.

#### 1. Introduction

The dominant Midwestern U.S. agricultural production systems are highly specialized and input-dependent, most often focusing on high productivity while neglecting ecological processes such as nutrient and water cycling (Prokopy et al., 2020; Gliessman, 2014). These production systems not only contribute to the deterioration of fundamental properties of soils (Evans et al., 2020) but have also shown to be fragile and vulnerable to shocks such as extreme weather events and market fluctuations, as demonstrated by the COVID-19 global pandemic (Hart et al., 2020; NOAA, 2020a, 2020b; Westhoff et al., 2020). Incorporating principles of soil health - maximizing aboveground diversity, providing continuous roots and cover of the soil, and minimizing soil disturbance – is another approach to land management; utilizing these principles on agricultural lands is widely recognized as an opportunity to recouple ecological processes, improve the production capacity of agricultural lands, and reverse trends in soil degradation (Delgado et al., 2011). Ecological intensification is proposed as a related approach to land management that incorporates "ecological processes into soil and crop management strategies to enhance ecosystem service delivery and reduce external inputs" (Bender et al., 2016).

Enhancing ecological intensification spatially (e.g., diversified crop rotations) or temporally (e.g., long-standing cover cropping, no-tilling) has been found to provide beneficial effects on ecosystem processes (Bender et al., 2016). Also, incorporating principles of soil health provides farmers with strategies to maintain productivity while reducing negative environmental impacts. For example, cover crops can reduce off-site nutrient flow and increase infiltration (Basche et al., 2019; Taylor et al., 2016), diverse crop rotations can interrupt weed growth cycles lowering pesticide use (MacLaren et al., 2019), and prescribed grazing practices, such as adding legumes and avoiding overgrazing, can protect soil and water resources (Rakkar and Blanco-Canqui, 2018; DeLonge and Basche, 2018).

There is increasing interest in promoting soil health as a solution to many agri-environmental challenges from a diverse group of stakeholders, including policymakers, agricultural industry leaders, food companies, and farmers (Sherwood and Uphoff, 2000). Some of the recent movements and agro-environmental initiatives across the US supporting soil health include the creation of the Soil Health Institute (Soil Health Institute, 2018), the initiation of the Soil Health Division by the Natural Resources Conservation Service (NRCS), the formation of the "Healthy Soils - Thriving Farms Challenge Area" by the Foundation for Food and Agriculture Research (FFAR) as well as numerous other state, local and NGO soil health incentive programs (Karlen et al. 2019). Despite increasing enthusiasm for and attention toward soil health related practices, utilization of soil health practices are still low; for example it is estimated that < 30% of cropland acres in the U.S. utilize no-till management and 4–5% utilize cover crops (Seifert et al., 2018; USDA-NASS, 2019). Thus, this limited adoption rate raises opportunities for further advancement in research to understand the opportunity for management to improve key soil health related functions.

Emerging interest in soil health has increased the urgency to understand the potential benefits of farmers transitioning from conventional towards more ecologically-based production systems. This transition to soil health related practices can be identified in a continuum, ranging from annual crop systems with highly disturbed soils (i.e., intensive tillage, limited crop diversity) to perennially-based systems with less-disturbed soils (i.e., pasture, restored prairie) (Karlen et al., 2019). Further, farmers adopting conservation practices generally work in a whole-farm systems approach, fine-tuning and incorporating multiple elements of crop and soil management practices (Church et al., 2020). In addition, innovative farmers are interested in more systems-level experiments that incorporate multiple factors of management practices (e.g., crop rotation, cover crop, tillage methods, fertilizer application rate, time and placement) and the manner in which if one factor is implemented it influences the outcome from each of the other factors (Basche and Roesch-McNally, 2017). This suggests that analyzing soil properties in the traditional manner (i.e., individual measurement responses to single treatment factor) using small plot research are not optimal, because they have trouble evaluating more than two or three factors at once. Thus, new agronomic research methods are in need to scale up farmer's adoption of soil health practices.

Changes in physical, chemical, and biological soil properties are complex and variable. For example, soil aggregate stability, infiltration rates, and microbial indicators quantified by meta-analysis are shown to be very responsive (1-3 years for changes detection) to changes in cover crop and no-tillage adoption (Stewart et al., 2018). Other soil indicators, such as organic carbon accumulation, might require over five years to detect significant changes due to management interventions that reverse soil degradation (Angers & Eriksen-Hamel, 2008). In addition, the lack of studies jointly analyzing a range of agricultural management practices, particularly beyond small field-experiment scales, has hampered the development of a holistic approach to land management, which is important to intervene with protection and conservation strategies. To this point, a growing body of research examining current soil health assessments addresses one soil or crop practice (e.g., tillage, cover crop, organic production) at a time (de Paul Obade and Lal, 2016; Roper et al., 2017; Villamil et al. 2008; Xue et al., 2019; Zuber et al, 2017). Studies trying to differentiate among the effects of various soil management practices suggest results are inconclusive or site-specific (Roper et al., 2017; Chahal and Van Eerd. 2018; Morrow et al., 2016). However, the effect of management on soil health, which is crucial for multiple soil functions, are mostly derived from highly controlled experiments, which tend to be oversimplified in terms of system complexity (Whalen et al., 2003; Congreves et al., 2015; Alhameid et al., 2017; Nunes et al., 2018). Recent data analyses from on-farm studies considering multiple soil health practices concluded that crop diversity, tillage reduction, and the use of organic amendments are key practices for building soil health (Williams et al., 2020). However, there is a need to refine future studies by including a broader spectrum of management practices, particularly at the farm-scale.

In this research, we developed a framework to classify spatial and temporal ecological intensification of management practices included in a series of farmer-led soil health experiments. By using the concept of ecological intensification, we described agronomic management practices (e.g., tillage, crop rotation, cover crop, organic amendment, and crop-livestock integration) based on their potential to promote crop growth and reduce soil disturbance (Caudle et al., 2013). We then employed this framework with a dataset of soil biological, chemical and physical properties from a statewide soil conservation program featuring collaboratively designed on-farm research to evaluate the impacts of management systems intended to improve soil health. Our research questions include:

- 1) What is the relationship between ecological intensification and soil properties?
- 2) How does ecological intensification influence physical, chemical, and biological soil properties?
- 3) What is the relationship between nutrient recommendation and savings and ecological intensification?

#### 2. Materials and methods

#### 2.1. Study sites and soil management systems description

This study was part of a state-wide partnership to monitor changes of soil properties through the adoption of conservation practices including reduced tillage, cover crops, diversified crop rotations, and crop-livestock integration. This collaborative project was launched in 2016 through a partnership between the University of Nebraska-Lincoln (UNL) On-Farm Research Network and the U.S. Department of Agriculture Natural Resources Conservation Service, and 17 farmer collaborators (Krupek et al., 2019a; Krupek et al., 2019b). In the first year of farm enrollment, NRCS field officer and UNL extension personnel worked with the farmer to select the field, the soil health management practice to be trialled, and to design the trial. Trials require at least an 8- hectare field (to obtain at least a 0.3-hectare minimum plot size), and the most common layout was an 8-strip or 12-strip format (n = 4 or n = 6 for each treatment). The treatment strips were designed in completely randomized blocks or alternated between treatments across the field. Farmers participating in the project compared at least two contrasting soil management practices for 5 years. The selection of treatment comparisons was based on research questions generated by the farmer based on their resource concern. Guidelines followed the "farmer-initiated" approach to research, which is commonly used in on-farm research programs (Thompson et al., 2019).

Ten on-farm study sites were included from the counties of Greeley, Howard, Merrick, Colfax, Otoe, Nemaha, Knox, Dodge, Stanton, and Seward in Nebraska (**Fig. 1**). Sites fall within five different Major Land Resource Area Map Unit (MLRA), which are geographically associated land resource units according to USDA-NRCS (1981) classification. On-farm sites were located in areas with a varied range of soil



**Fig. 1.** Map of on-farm trials located in five Major Land Resource Area (MLRA) in Nebraska. Farm locations are indicated by pin signs.



**Fig. 2.** Soil texture for each of the soils sampled in this analysis, displayed in a texture triangle.

textures (**Fig. 2**), classified predominantly as Mollisols and a few sites as Entisols and Alfisols (Supplementary Table S1) (Soil Survey Staff, 1999). Field history represented a range of soil health management practices in terms of cash crop diversity, cover crop use, soil disturbance, application of organic amendments, and mixed livestock and cropping enterprises. Such practices fall within the principles of soil health, which emphasizes reducing soil disturbance, extending periods of living roots in the soil, as well as maximizing crop and livestock diversity (USDA–NRCS, 2018).

The main cash crop species included in the on-farm experiments were corn (*Zea Mays* L.) and soybean (*Glycine* max (L.) Merr.). Different small grain crops were included in some the fields, such as wheat, triticale, millet, and oats, which functioned as either cash crops, as nongrazed cover crops (with seed harvested for income), or as forage crops grazed by cattle depending on the operation. In some fields where crop-livestock integration was not part of the main treatment comparison, cattle grazed corn and wheat residues during autumn to reduce high residue loads that can hinder planting and early seedling growth in subsequent crops. All fields were managed by the farmers according to best management practices, resulting in variation in cattle stocking rates and organic and chemical inputs used between rotations and over time. Cereal rye (*Secale cereale* L.) cover crop was commonly used in sites testing single species cover crops as a treatment. However, the majority of farmers used mixtures (5 + species) of cool-season small grain cereals, legumes, brassicas, and warm-season summer annual grasses based on NRCS cover crop guidelines (USDA-NRCS, 2011). Information regarding crop rotation, soil management practice comparison, organic amendment, tillage use, and crop-livestock integration was collected annually for each field through a research participation form (Supplementary Table S1).

# 2.2. Classification indexes for ecological intensification of soil health practices

The diversity of soil health practices applied at the on-farm experiments reflects a continuum of management from less to more ecologically intensified cropping systems as defined in Bender's et al. (2016) framework (Fig. 3). For each field-site treatment, we quantified the incremental changes, in space and time, of soil health practices such as crop diversity, frequency of mechanical soil disturbance, cover crop and organic amendment use, and crop-livestock integration proposed by Williams et al. (2020) and Tiemann et al. (2015). Across all sites, time of cover crop use varied from zero to up to twelve years and years without soil disturbance varied from zero to up to thirty years. Regarding the number of different plant species in a 5-year rotation, fields varied from two to eighteen. The crop diversity index (CDI) was calculated as a ratio between the number of different crop species used and the maximum number of crop species used across all field-site treatments considering a full cycle of crop rotation (5 years). The CDI included cash crops, cover crops, and forage crops. The cover crop use (CCU) and years without soil disturbance indexes (YWSD) were quantified based on the number of years farmers were cover cropping and no-tilling, respectively. Finally, we defined organic amendment use (OAI) and crop-livestock integration (CL) as the number of applications of organic amendment and frequency of livestock grazing crop residue and cover crops during the past five years. Each index (i.e., a proxy for either length of time or intensity of soil health management) was defined to represent a progression to a more ecologically intensified cropping system due to the adoption of soil health management



Fig. 3. Representation of the soil health practices used in the ecological intensification framework. Practices included in this analysis were no-till, crop rotation, organic amendment, crop-livestock integration, and cover crop. Spatial or temporal intensification of these practices could lead to changes in soil physical, chemical, and biological properties. Possible changes in soil physical properties are represented through porosity or compaction (distribution of soil aggregates) and water infiltration (depth of water in the soil profile). Possible changes in soil chemical properties are represented through the addition of nutrients represented in the soil coloration (higher organic matter in darker soil) and circles representing available carbon, nitrogen, phosphorus, and potassium. Possible changes in soil biological properties are represented through the addition of bacteria, fungi, and earthworms in the soil profile. Cover cropping was found to be the most impactful of the practices on soil properties in our analysis and is represented by darker soil color, lower compaction and larger soil aggregates, higher nutrient availability, and larger number of soil microbes. This could be a result of cover crops offering continuous living roots relative to the other practices and driving changes in water movement, organic matter, soil biological activity, and carbon and nitrogen dynamics as reported by this analysis. Sequence represents increases in relative importance of soil health practices averaged across all physical, chemical and biological properties included in the multiple linear regression analysis. Artwork by Lana Koepke Johnson.

practices (**Table 1**). Higher index values represent higher the number of different plant species in the rotation, greater the number of years with continuous living roots, the fewer number of years with tillage operation, increased number of organic amendment applications, and more frequent integration of livestock into cropping systems.

	CDI <sup>a</sup>	CCU	YWSD	OAI	CL
Maximum	1	1	1	0.2	1
Minimum	0.11	0	0	0	0
Mean	0.40	0.35	0.41	0.04	0.23
Median	0.36	0.33	0.33	0	0

**Table 1.** Maximum, minimum, mean, and median for the crop diversity index (CDI), cover crop use (CCU), years without soil disturbance (YWSD), OAI (organic amendment index), and crop-livestock integration (CL).

a. Soil management index calculation of the *i*<sup>th</sup> field and the maximum measured value within our dataset:  $CDI = CDI_i / CDI_{max}$ ;  $CCU = CCU_i / CCU_{max}$ ;  $YWSD = YWSD_i / YWSD_{max}$ ;  $OAI = OAI_i / OAI_{max}$ ;  $CL = CL_i / CL_{max}$ 

#### 2.3. Soil sampling

Fields included in the analysis ranged from 10 to 40 ha with plot sizes ranging between 0.25 and 4.75 ha, depending on the farm field. All fields were sampled from a 0–15 cm depth in autumn 2019, two or three years after the initiation of the on-farm experiment (experiments initiated either in 2016 or in 2017). Samples from the same field were collected on the same day to avoid moisture or temperature fluctuations between sampling locations. Each sampling point was geolocated using a global positioning system (GPS).

Sampling points for soil properties analysis (conducted either in the field or in laboratory) were selected on a representative basis from the soil type, plot size, and replication (Supplementary Table S2). There was at least one sampling location within a replicate strip. To minimize spatial soil variation within a sampling location, a 6 m  $\times$  24 m area located at least 5 m away from the plot boundaries was designated for soil measurements (Supplementary Figure S1). Ten bulk soil samples, located at least 6 m apart from each other, were collected and composited for Haney test soil analysis, using soil sampler of a core diameter of 32 mm diameter model PN012, JMC Backsaver N-2 handle (JMC Soil samplers, Newton, IA, USA). In fields with a history of banded fertilizer application, soil cores were collected from a transect perpendicular to the row crop, and if banding fertilizer was not practiced samples were collected adjacent to the cash crop row or near the rooting structure (Franzen, 2017). A total of 148 individual soil composite samples from 0 to 15 cm depth were collected across 30 site-soil health management comparisons. Total sampling area was 267 ha. Soil samples were stored in sealed plastic bags and transported in an ice-filled cooler to the laboratory for refrigeration at 4 °C until being shipped for laboratory analysis.

#### 2.4. Assessment of soil properties

Soils were analyzed for 21 field and laboratory measurements of physical, chemical, and biological indicators. Field measurements included in the NRCS assessment protocol (USDA-NRCS, 2020) were soil temperature, soil porosity, and a measure of initial water infiltration using the method described by Smith (1999). Bulk density was determined using the core method (Blake & Hartge, 1986). Gravimetric soil moisture content was determined using methods described by Gardner (1986).

The remaining chemical and biological properties were analyzed using protocol from the Haney soil health test (HSHT), including soil respiration and nutrient testing (Zuber and Kladivko, 2018). Soil-test biological activity (e.g., soil respiration, flush of CO<sub>2</sub>) was determined from the flush of CO<sub>2</sub>-C following rewetting of dried soil with 24-h aerobic incubation at 50% water-filled pore space and 25 °C. For analysis, 40 g of soil samples in 0.25-L glass jars were wetted and CO<sub>2</sub>-C was determined by infrared gas analyzer of headspace (Franzluebbers, 2021). Soil organic matter content was analyzed by mass loss on ignition (LOI) at 500 °C for two hours and expressed as percent LOI (Nelson and Sommers, 1996). Nutrient testing for essential plant nutrients such as inorganic nitrogen (nitrate and ammonium), inorganic, organic, and total phosphorus relied on the H3A soil extractant, a weak acid containing organic plant root exudates typically associated with plant nutrient uptake from soil (Haney et al., 2006; Haney et al., 2010). HSHT also includes results of water-extractable organic C and N, measures of the pool of organic carbon and nitrogen readily available to the microbes. Water extractable C fractions are the most active SOC compounds comprised of mainly carbohydrate derived from plant roots, microorganisms, amino acids and humid substances (Kalbitz and Kaiser, 2008; Ćirić et al. 2016). Thus, these fractions can contribute to SOC changes due to management, being a suitable indicator for soil health assessment (Ghani et al. 2003). Other routine soil

measurements included total elemental potassium, calcium, aluminum, sulfur, manganese, magnesium, and sodium analysis using inductively coupled argon plasma (ICAP) atomic emission spectroscopy.

Soil health score, soil-test predicted nitrogen release, and nutrient recommendations for the subsequent cash crop were HSHT-based calculations included in this analysis. Soil health score was calculated based on values of soil-test biological activity, water-extractable organic C and N (Eq. (1)). Soil-test predicted N release is the total amount of N being released through microbial activity from the organic N pools. It is the product of water-extractable organic N and microbial-available C expressed in ppm (Eq. (2) and (3)). The soiltest predicted N release calculation is built on the assumptions that (i) water-extracted C and soil respiration represent the total potential food source and the potentially mineralizable C, the C accessible to microbes in 24-h incubation (including physically bound C active to microbes), respectively; (ii) soil microorganisms use a similar proportion of water-extracted C and water-extracted N, (iii) during the growing season N is released in the soil, on average, four times after significant precipitation and (iv) the soil-test predicted N release cannot exceed the water-extracted organic N (Haney interpretation guide, 2021). These HSHT calculations are evolving, but those presented here were used as of February 2020, by Ward Laboratories (Kearney, Nebraska).

$$SH_{score} = \frac{CO_2 - C}{10} + \frac{WEOC}{50} + \frac{WEON}{10}$$
 (1)

Soil – test predicted N release = 
$$WEON * MAC * 4$$
 (2)

$$MAC = \frac{CO_2 - C}{WEOC}$$
(3)

where  $CO_2$ -C is the soil-test biological activity, WEOC is water-extractable organic C, WEON is water-extractable organic N, and MAC is microbially active C.

Nutrient recommendations from HSHT for the subsequent cash crop, expressed on a per-area basis, were calculated based on nutrient concentrations extracted from soil analysis and yield goals of 10.7 and 4.0 ton/ha for corn and wheat, respectively. Recommendations were developed based on calibrations from the University of Nebraska and Kansas State University (R. Ward, personal communications, November 4, 2020 and January 20, 2021). Yield goal values were selected to represent an average attainable yield for a typical farm in Nebraska considering both irrigated and rainfed systems (USDA-NASS, 2019). Finally, soil-test hypothetical N savings were calculated based on the difference (kg/ha) in N measured using the HSHT (using water-extractable C and N pools) and traditional soil testing using residual nitrate and considering an N price of \$0.91/ kg N.

#### 2.5. Statistical analysis

In order to examine the research questions of interest, we analyzed a dataset comprised of 148 observations (i.e., composite samples) collected from ten farms. Statistical analyses were performed using the R software version 4.0.4 (R Core Team, 2018). Because multiple soil properties were measured from the same on-farm sites, there may be correlations among the variables, errors, and responses. Therefore, the first step in the analysis was to perform Pearson correlation analysis and observe how the soil properties were correlated to each other (Supplementary Figure S2). Given the moderate to high correlation among variables, which supports the usefulness of a multivariate approach, the next step was to perform a principal component analysis (PCA) to analyze the variation in soil properties and ecological intensification indexes as affected by field location. This analysis was performed based on correlation matrix, rather than the covariance matrix, because the soil health indicators included in the data set have different units and variances (Supplementary Table S3). All individual variables were checked for normality, confirming approximate multivariate normality and suitable use of linear ordination methods. We also performed multiple linear regression to analyze the influence of ecological intensification of soil health practices (i.e., classification indexes) on soil properties according to the model: y = CDI + CCU + YWSD + OAI + CL. Variance inflation factor and collinearity diagnosis were performed to confirm the absence of a strong correlation among the management indexes used as predictors (Supplementary Tables S4 and S5). Residuals of all regression models were

checked for normality and homogeneity of variance. Log transformation of the dependent variable was used for infiltration and sulfur. Relative importance analysis was performed to understand the extent to which each soil health management index drives the prediction of soil properties. Relative importance (RI) was calculated and considering the R<sup>2</sup> contribution averaged over orderings among repressors (*lmq* metrics) and expressed as percentage, according to Chevan and Sutherland (1991). The explained multiple regression model variance was partitioned among the predictors to understand the role played by each soil health management index in the regression equation. The PCA, multiple regression models, and relative importance analysis were performed using the functions prcomp in the package stats, stepAIC in the package MASS, and relimp in the package relaimpo (Grömping and Lehrkamp, 2015), respectively. Simple linear regression was used to understand the relationship between the variables soil-test predicted N release and soil-test biological activity as well as corn and wheat N recommendation.

#### 3. Results

Soil physical, chemical and biological properties showed great variability across sites and ecological intensification with different soil health practices (Fig. 4). The first two principal components (PC) explained 35% of the total variability within the dataset. The first PC primarily described the variation due to physical versus biological and chemical soil properties. This is illustrated in Fig. 4 as the physical soil properties (e.g., bulk density, soil temperature, infiltration, volumetric water content) have low PC1 scores while biological and chemical properties (e.g., soil-test biological activity, soil health score, water extractable organic and total N) have high PC1 scores. Fields with higher intensification in crop diversity (CDI) were associated with greater infiltration and soil porosity, couple with reduced bulk density since the angle between CDI and these soil properties is either very small pointing towards the same direction (positive correlation) or in opposite direction (negative correlation) (Fig. 4). Likewise, higher intensification in cover crop use (CCU) was associated with greater water extractable organic C (WEOC), soil-test



**Fig. 4.** Biplot obtained from principal components analysis based on the correlation matrix, showing the two first principal components (explaining 21% and 14%, respectively). Each point represents samples collected in different fields (n = 148), loadings indicate soil properties and ecological intensification indexes. Descriptors: CDI = crop diversity index, CCU = cover crop use, OAI = organic amendment index, YWSD = years without soil disturbance, CL = crop-livestock integration, Vol. water content = volumetric water content, Inorg. N = inorganic nitrogen, Inorg. P = inorganic phosphorus, Org. P = organic phosphorus.

biological activity, SH score, calcium, magnesium and reduced bulk density. Intensification in organic amendment (OAI) was mainly associated with greater organic matter and soil-test biological activity. Fields with higher years without soil disturbance (YWSD) were associated with greater soil volumetric water content, coupled with reduced potassium, organic phosphorus and inorganic nitrogen. Fields intensified with crop-livestock integration (CL) were associated with greater soil temperature and reduced levels of potassium, organic P and inorganic N (Fig. 4). There was a clear separation between soil samples from the Central Nebraska Loess Hills and the rest of the MLRA. This is illustrated in Fig. 4, as the PC scores of Central Nebraska Loess Hills were primarily in the upper quadrants while the other MRLA data points were located mostly in the lower quadrants. The loading for PC2 indicate that organic matter, volumetric water content, potassium, organic P and inorganic N were helpful in separating the MRLA regions. PC1 was a contrast of high positive loadings of CDI, CCU, water extractable organic N, organic P, potassium and soil health score against the negative loading of bulk density (Supplementary Table S6). PC2 consisted of negative loadings of CCU, YWSD, and soil health score and high positive loading of potassium (Supplementary Table S6). Taken together, results from the principal component (PC) analysis provided evidence that the different experimental sites, located in different regions and MLRAs, were diverse with regard to soil properties and intensification in CDI, CCU, YWSD, OAI, and CL (Fig. 4). In addition, improvements in soil health through soil aggregation (reduced compaction and improved porosity and infiltration) and nutrient cycling, primarily organic C and N, were influenced by ecological intensification, particularly in CDI, CCU and OAI (Fig. 4, Supplementary Table S6).

To further explore our dataset and understand the PCA results, we performed multiple regression analysis to identify the combination of practices that perform best (i.e., most important factors leading to improvements in soil properties), when ecological intensification in various soil health practices is assessed simultaneously. This overcomes the constraints of previous research studies that usually evaluate only two to three management factor at once (such as no-till vs. conventional tillage, continuous corn vs. crop rotation or moderate vs. intensive vs. no grazing). We tested different random effect models to account for the three main sources of random variation in our study block, farm location and region (MLRA), but this did not significantly change the regression coefficient, slopes or the relationships between soil properties and ecological intensification indexes presented here (not shown). Additionally, we tested but did not add interactions to the model because interactions could produce results that were not grounded in biological processes or meaning, but rather a result of the varied indices across all experiments (not every index/soil health practice was included at every site). Thus, we considered it impractical to include all potential interactions knowing that the biological meaning of this inclusion was limited.

**Table 2** Average coefficients for the predictors of changes in soil properties based on multiple linear regression model y = CDI + CCU + YWSD + OAI + CL, as well as the R<sup>2</sup>, *i.e.*, the variance that explained by the regression model. Intercept is the intercept of the linear mixed-effect regression model; CDI, CCU, YWSD, OAI, CL are the ecological intensification indexes for crop diversity index, years of cover crop use, years without soil disturbance, organic amendment index, and crop and livestock integration.

		Soil ecological intensification index <sup>a</sup>					
Soil properties	Intercept	CDI	CCU	YWSD	OAI	CL	$R^2$
NRCS	Slope coefficie	ents					
Infiltration (mm/hour <sup>-1</sup> ) <sup>b</sup>	2.87***	1.08*	1.64***	0.27 <sup>ns</sup>	-4.44**	-0.07 <sup>ns</sup>	0.20***
Soil porosity (%)	55.27***	0.40 <sup>ns</sup>	2.85 <sup>ns</sup>	-1.90 <sup>ns</sup>	4.77 <sup>ns</sup>	-4.29***	0.19***
Soil temperature (°C)	3.07***	-0.48 <sup>ns</sup>	-1.60**	2.65***	7.97***	5.11***	0.79***
Volumetric water content (%)	20.21***	8.29***	-0.23 <sup>ns</sup>	10.30***	-12.14*	-3.44*	0.32***
Bulk density (g cm <sup>-3</sup> ) HSHT	1.21***	-0.04 <sup>ns</sup>	-0.09 <sup>ns</sup>	0.01 <sup>ns</sup>	-0.16 <sup>ns</sup>	0.13***	0.17***
Water-extractable Organic N (ppm)	12.96***	-5.16***	7.68***	-5.23***	-0.51 <sup>ns</sup>	-1.25 <sup>ns</sup>	0.62***
Water-extractable Organic C (ppm)	162.20***	-37.16*	45.04**	-46.38**	57.35 <sup>ns</sup>	9.74 <sup>ns</sup>	0.08**
Water-extractable Total N (ppm)	22.25***	-9.52***	19.59***	-5.60**	-8.29 <sup>ns</sup>	-5.81***	0.59***
Organic matter (% LOI)	3.07***	-0.50 <sup>ns</sup>	1.81***	-0.56 <sup>ns</sup>	3.43***	0.66**	0.27***
Soil-test biological activity (ppm CO2	38.27***	-4.84 <sup>ns</sup>	43.90***	-19.59*	88.34***	6.94 <sup>ns</sup>	0.22***
Inorganic N (ppm)	11.12***	-1.12 <sup>ns</sup>	8.01***	-1.65 <sup>ns</sup>	-12.51*	-5.79***	0.37***
Inorganic P (ppm)	13.53***	-5.22 <sup>ns</sup>	16.06***	-4.12 <sup>ns</sup>	45.56***	-9.80**	0.32***
Organic P (ppm)	5.76***	-1.41***	0.74 <sup>ns</sup>	-2.29***	3.55**	-1.43***	0.60***
Potassium (ppm)	115.35***	11.42 <sup>ns</sup>	-22.48 <sup>ns</sup>	-12.16 <sup>ns</sup>	-119.23*	-27.94*	0.13***
Calcium (ppm)	459.06***	-70.93*	7.89 <sup>ns</sup>	-59.63*	538.87***	167.92***	0.46***
Aluminum (ppm)	219.20***	-64.10***	61.33***	-54.47***	286.97***	-0.50 <sup>ns</sup>	0.46***
Sodium (ppm)	18.28***	1.70 <sup>ns</sup>	-2.41**	-3.77***	4.03 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.51***
Manganese (ppm)	3.05***	1.56 <sup>ns</sup>	-2.99***	7.51***	-15.64***	-0.01 <sup>ns</sup>	0.56***
Magnesium (ppm)	92.94***	-5.90 <sup>ns</sup>	66.60***	-48.91***	148.08***	18.81*	0.30***
Sulfur (ppm) <sup>b</sup>	1.79***	0.40 <sup>ns</sup>	-0.38*	-0.07 <sup>ns</sup>	3.73***	-0.91***	0.48***
Soil health score	8.10***	-1.98*	6.38***	-2.96***	9.48***	1.06 <sup>ns</sup>	0.31***

a. CDI, CCU, YWSD, OAI, CL are the crop diversity index, years of cover crop use, years without soil disturbance, organic amendment index, and crop and livestock integration.

b. Regression coefficients presented for infiltration and sulfur are based on relationship between the regression predictors and log transformed response variable. ns , \*, \*\*, \*\*\* indicates not significant and significant regression coefficients at p < 0.05, p < 0.01, and p < 0.001 respectively.

Results from multiple linear regression analysis showed that soil physical, chemical and biological properties were all significantly affected by site-specific ecological intensification, but the effects of ecological intensification on soil property improvements were practice-specific (**Table 2**). Soil properties were either positively or negatively related to different ecological intensification, and the regressions yielded multiple R<sup>2</sup> values between 0.07 and 0.79 (Table 2). For example, as intensification in CCU and OAI increased, soil water infiltration,

organic matter content, soil respiration (i.e., microbial activity), organic and inorganic P, and soil health score increased. Conversely, some soil health indicators were negatively correlated to intensification in soil management. For example, a high YWSD was associated with improvements in soil water infiltration but decreases in soil organic matter, water-extractable organic C and N, and soil respiration (Table 2). Further, analysis of regression equation coefficients showed that soil properties were influenced via different paths by intensification in soil health management. For example, CCU was the most important and only soil management practice identified by the multiple regression analysis that lead to increases in both organic (i.e., waterextractable organic and total N) and inorganic N (Table 2).

The contribution of management intensification to the different soil properties was quantified with relative importance analyses (Fig. 5). With respect to soil properties that are closely related to C and N dynamics such as soil respiration, organic matter, water-extractable organic C, N, total N, and soil health score, CCU explained 67, 68, 34, 39, 59, and 70% of the total variance, respectively (Fig. 5). The highest relative importance of CDI was observed for soil water infiltration and volumetric water content, with 77% and 23% respectively, CCU was observed for soil respiration and organic matter with 78 and 68%, respectively. Likewise, the highest relative contribution of YWSD was observed for manganese with 62%; OAI was observed for sulfur and aluminum with 57% and 46%, respectively; and CL was observed for bulk density and soil porosity, contributing to up to 59% of the variance explained. Averaged across all soil physical, chemical and biological properties, CCU was the most important and contributed 31% to the overall influence of all assessed properties, followed by YWSD, CL, OAI, and CDI (Fig. 5). Considering all the ecological intensification indexes used in this analysis, CCU is the only one that features continuous living cover and roots in the soil with cover cropping.

As a hypothetical estimate of soil N supply, soil-test predicted N release (mineralizable N considering a 24-h soil incubation) was highly associated with soil respiration (**Fig. 6**a). Further, our analysis showed that intensification in CCU resulted in greater soil-test biological activity and soil-test predicted N release (Fig. 6a). The results of the relationship between HSHT calculations of plant-available N show that organic N release was found to be negatively correlated to corn and





wheat N recommendations (Fig. 6b and c). Organic N release is an overall N credit the HSHT measures from the soil that the more conventional fertility tests utilizing only nitrate or ammonium do not account for. Because organic N release is the amount of N being released through microbial activity from organic N pool, this value typically increases as the soil system gets healthier. In our analysis, we found that in healthy and high functioning biologically active soils, particularly those with high intensification in CCU, this organic N release credit could be above 10 ppm and reduce N fertility needs substantially. On



**Fig. 6.** Relationship between soil-test biological activity and soil-test predicted nitrogen release (A), soil-test predicted nitrogen release and corn N recommendation (B), soil-test predicted nitrogen release and wheat N recommendation (C), and cover crop use index (CCU) and soil-test hypothetical N savings (D). Nutrient recommendation, variables calculated in the HSHT, considered a yield goal of 11.71 and 4.44 US ton/ha for corn and wheat respectively. N savings is the difference in the amount of N (kg/ha) measured between the Haney Test (HSHT) and traditional soil test using nitrate and considers a price of 0.91/kg N. N savings for a given CCU indicated by the same lower-case letter are not significantly different at p = 0.05 level. Circle size represents cover crop use (CCU) index, a higher CCU represents greater number of years of cover crop adoption.

the other hand, this credit can also be minimal and may not have an impact on the amount of fertilizer required in soils that are deemed as less healthy, for example, those with low intensification in CCU (Fig. 6b and c).

Finally, our results of soil-test hypothetical N savings showed that fields adopting cover crops for over eight years (CCU  $\ge$  0.66) could save on average \$44/ha in N application (Fig. 6d). Likewise, fields with low intensification in CCU (CCU < 0.66) could save less than intensified fields, on average \$32/ha (Fig. 6d). These values represent the potential amount (\$/ha) saved on N application based on the difference in the N results between HSHT (using organic N pools) and traditional soil test (using nitrate). Taken together, these results suggest that predicted N fertilizer recommendations could be reduced when organic N pools are considered as a way to capture a greater potential nutrient pool than standard soil testing. The addition of a cover crop was also found to enhance carbon inputs and facilitate biologically active N cycling. Farmers adopting intensified management practices to improve soil health, particularly related to long-term cover cropping, can lead to lower requirements for predicted N fertilizer input and higher savings when organic nutrient pools are considered in fertilizer recommendations.

#### 4. Discussion

# *4.1. Describing variation and association between ecological intensification and physical, chemical, and biological soil properties*

Our study included on-farm trials with a diversity of soil health management practices that are possible alternatives to the shift from conventional to more ecologically-based production systems in Midwestern U.S. (Fig. 3). Cropping systems have changed throughout the most recent decades in our study region; landscape complexity shifted from high diversity in the 1950 s and 1960 s, with corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench), alfalfa (*Medicago sativa* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and soybean (*Glycine* max [L.] Merr.) to maize-dominated systems comprising the current landscape (Hiller et al., 2009). However, farmers in our study region have shown increased interest and adoption of soil health management practices such the addition of cool-season cash crops, no-till, and cover crops in the last decades (Knowler and Bradshaw, 2007; Baumgart-Getz et al., 2012). The wide adoption of glyphosate-resistant crops contributed to the increase in no-till or reduced-till systems in Midwestern US (Givens et al., 2009). Additionally, over the last decade, intensification of the corn-soybean rotation has occurred through drilling multi-species mix cover crop after main crop harvest or interseeding cover crop prior to main crop harvest (Oliveira et al., 2019). There are, therefore some distinct differences in farmer's adoption timing for soil health practices between our study and studies carried out in other parts of the world. Low crop diversity in our study is represented by 1–2 different crops (primarily corn and soybeans) whereas a high crop diversity meant up to six different species within a five-year rotation. Cover crops on farms for over a decade represent a long amount of time for farms in eastern Nebraska. Farmers in our study region using no-till systems for two to three decades, particularly after the introduction of glyphosate-resistant crops in 1996 (Duke and Powles, 2008), is common.

This study was conducted using a dataset of soil biological, chemical and physical properties from a statewide soil conservation program featuring collaboratively designed on-farm research. We did not compare two to three management factors at once (no-till vs. conventional tillage, continuous corn vs. crop rotation or moderate vs. intensive vs. no grazing) as many previous traditional replicated field experiments using standard statistical designs. Instead, our proposed ecological intensification framework assessed various soil health practices simultaneously to identify the combination of practices that leads to improvements in soil properties. The on-farm design of the study and farmer-reported soil testing also made it possible to include evolving calculations linking soil biology with soil fertility, soil health and farming inputs. The results presented and discussed in the following sections are informative from a scientific perspective as it offers greater potential for enhancement of farmer's knowledge of the soil system and could be beneficial for making improvements in farm management decisions (Rhymes et al., 2021).

Agricultural management gradients representing incremental changes in soil health-promoting practices are often more difficult to evaluate (i.e., detect treatment differences) than those involving sharply contrasting practices. However, understanding soil processes and quantifying changes in these transitions to more ecologically-based production systems are critical steps to provide farmers information to support their management decisions and to quantify the benefits of these soil conservation practices. Research in soil health traditionally has focused only on one management practice at a time comparing highly contrasting treatments under controlled conditions (Rojas et al., 2016; de Paul Obade and Lal, 2016, Campbell et al., 1998). This approach of using traditional replicated field experiments often ignores the role of farmers' preference for management practices and/or implementation timelines and raises questions on less contrasting situations reflecting an agricultural management gradient towards ecologically-based farming practices. The PCA demonstrates the variables and soil processes that are more impactful in differentiating the transition toward ecologically intensified soil management practices (Fig. 4). The relatively low percentage of the variance explained by the first two PCs underscore the complexity of the system in this transition to utilizing more soil health related practices, with many possible feedback loops derived from soil function and processes. This also implies the existence of other factors that could affect soil properties in ecological intensification.

CCU, CDI, and OAI were the indexes featuring temporal intensification in continuous living cover and roots in the soil, spatial diversification in quantity and quality of the crop residue, and addition of external organic inputs over time, respectively. Physical soil properties such as infiltration and bulk density were strongly related to CCU, CDI, and OAI (Fig. 4). The soil-test biological activity, organic matter, and water-extractable organic C and N were also indicators associated with these indexes and very important for soil biological activity and C and N dynamics. These results are consistent with previous findings including meta-analyses documenting increases in soil carbon, microbial biomass, and organic matter dynamics in response to cover crops and crop rotation (McDaniel et al., 2014a, 2014b; Poeplau and Don, 2015). Improvements in water cycling with the adoption of cover crops, which maximize soil cover and period with roots in the system, was also found in a meta-analysis evaluating infiltration rates with different soil health related practices (Basche et al., 2019). Another important finding in our analysis is the negative loading of high bulk density being displayed in the opposite direction of the indexes CCU, CDI, and OAI (Fig. 4). Low bulk density with increased intensification with cover crop, crop diversity and organic amendment is possibly attributed to the high organic matter and the presence of continuous roots in soils, with reduced disturbance and maximized periods without roots (Rojas et al., 2016).

As previously described by Zuber et al. (2017) and McDaniel et al. (2014a), the influence of crop rotation on soil health varies according to the specific crop species selected in the rotation. The high association between CCU and CDI and chemical properties such as sodium, calcium, magnesium, organic C, and N are most likely associated with the quantity and quality of the crop residue and the fertilizer management program adopted depending on the species in the rotation (Fig. 4). The observed association between OAI and soil properties such as soil porosity, organic matter, water-extractable organic C and N was associated with improved soil structure and nutrient retention (Fig. 4). As observed in previous findings, manure application increases aggregate stability and retention of applied nitrogen (Gardner and Drinkwater, 2009; Jiao et al., 2006; Wortmann and Shapiro, 2008). In addition, the high C and N loads in the PC was also observed by Rojas et al. (2016), where total organic C and N were found to be the most sensitive chemical soil properties when using multivariate statistical techniques in deforested areas for agricultural use. Organic C and N are related to multiple soil properties such as soil texture, pH, cationic exchange capacity, soil aggregation, nutrient storage and supply, being critical in multiple soil processes and commonly used as a soil health indicator (Reynolds et al. 2002; Govaerts et al., 2006).

The YWSD index describes the temporal soil disturbance through tillage operations. Regular soil disturbance caused by tillage practices causes direct changes on soil structure and pore space, which alter soil hydrologic properties (Pires et al., 2017; Kay and VandenBygaart, 2002). For example, improvements in aggregate stability, saturated hydraulic conductivity, and available water capacity have been quantified by meta-analyses in response to conservation tillage practices such as no-till, ridge-till, and mulch-till (Li et al., 2019). We found that volumetric water content, soil temperature, and organic matter were strongly related to YWSD, suggesting a change in soil structure and organic C dynamics with intensification in YWSD (Fig. 4). Recent study using X-ray computer tomography, a cutting-edge technology to access soil pore space, found that conventional tillage reduces near-surface (0–5 cm) soil organic matter by increasing pore anisotropy

(i.e., degree of dissimilarity in orientation) and total macroporosity. Conversely, in the same study, no-tillage increased near-surface (0–5 cm) soil organic matter by increasing soil aggregate stability and pore connectivity (Guo et al., 2020). Macropores play a role in water infiltration and drainage (Ferro et al., 2013). The pattern observed in the PCA suggests a combination of near (0–5 cm) and below (>5 cm) surface effects of low YWSD on soil organic matter and hydrological properties once soil natural permeability is altered by mechanically disturbed fields (Parra et al., 2011; Sanzano et al., 2005).

The multiple regression analysis takes a different approach in evaluating how incremental changes of the practices - crop diversity, avoidance of mechanical soil disturbance, use of cover crop, application of organic amendments, and crop-livestock integration - impact on soil properties (Table 2). Our results show that the effects of these different management practices do not always follow the same trend in terms of their impacts on soil health, indicating that combining the effects into a single index may not be appropriate to understand its effects on soil properties. In addition, as highlighted by Williams et al (2020) in their approach, knowledge of the interaction between soil health management and soil properties is lost when focusing on a single soil management composite index.

By studying multiple soil management practices and not integrating the practices into a single index via the multiple linear regression models, our results show slightly different patterns than other studies based on long-term plot experiments with highly contrasting treatments. For example, YWSD was negatively related to soiltest biological activity (CO<sub>2</sub>-C), organic matter, total N, and waterextractable organic C and N, indicating that the longer the years without soil disturbance by tillage practices, the lower the values for these soil properties related to organic matter and C and N dynamics (Table 2). In contrast to our findings, studies across soil textural classes found that no or reduced tillage increase near-surface (0-5 cm) stocks of organic C and N and respired CO<sub>2</sub>- C (soil respiration) in the long term (>20 years) (Mikha & Rice, 2004; Hermle et al., 2008; Kaiser et al., 2014). These reported near-surface increases were associated with a great amount of crop residue in the soil surface, improved physical protection of OM against microbial decomposition due to occlusion in aggregates, increase OM mineralization, and greater microbial abundance under no-tillage systems (Balota et al., 2004; Kaiser et al., 2014).

The most likely explanation for the contradictory results between our findings and the literature is related to the sampling depth adopted in our soil health assessment (0–15 cm depth), which may have caused a dilution effect for C and N on the surface and sub-surface under long term reduced soil disturbance. Also, our approach did not differentiate between tillage types (e.g., mouldboard ploughing, chisel ploughing, disc harrowing) or accounted for tillage depths (e.g., various forms of reduced tillage). As opposed to near-surface, greater CO<sub>2</sub>-C emissions and labile organic C and N pools were observed under conventional tillage for sub-surface soil (5-25 cm). This corroborates our findings and can be explained by the transfer and redistribution of fresh plant residues from the soil surface to greater soil depths under conventional tillage and also by the percolation of dissolved OM from the surface into the sub-surface soils (Kaiser et al. 2014). Thus, tillage effects on soil functions related to organic matter dynamics are soil depth-specific (Kaiser et al., 2014; Blanco-Canqui et al., 2021), suggesting the importance of standardized sampling depths when considering multiple soil properties and the need for deeper soil sampling to fully understand the impact of soil disturbance on soil organic C dynamics.

Our results show that regression models considering multiple practices could explain as much as 79% of the variation in our data (Table 2). Our results are consistent with findings reported by Williams et al. (2020) using on-farm data from outside the USA and considering a range of soil health-building practices. Some of the remaining variations in our dataset could be a result of other factors such as climate (precipitation and mean annual temperature), dry mass aboveground plant residue retained, or other soil management that were not included in the analysis.

## *4.2. Cover crop effects on biological properties and nutrient use efficiency*

Despite variation in sensitivity of how ecological intensification affected soil properties, cover crop use (CCU) was found to be the most impactful soil health practice, particularly on properties that are

closely related to organic matter and C and N dynamics (Fig. 5). The identification of soil management practices that not only improve crop yield but also enhance ecosystem efficiency is critical for the determination of soil health (Arshad and Martin, 2002; Lal, 2013). Practices that promote continuous living roots into the soil, such as the use of cover crops, can help to capture nitrogen in the soil and reduce nitrate leaching in ground and surface water. This response is attributed to mechanisms such as a reduction in water drainage volume, reduction in nitrate concentration in the leachate, and microbial immobilization from C inputs (Quemada et al., 2013; Valkama et al., 2015; Thapa et al., 2018). Improvements in soil aggregation upon adoption of soil health practices may also decrease soil compaction and water saturation (e.g., anaerobic soil conditions), reducing the potential for N losses following intense precipitation or irrigation events. A recent meta-analysis found that continuous living roots in the system with the use of cover crops improve soil structure and enhance water cycling through increased water infiltration rates (Basche et al., 2019). Thus, the observed improved soil infiltration, organic matter content, water-extractable organic C and N and soil health score in fields with incremental additions of continuous soil cover with the use of cover crop corroborate with findings from previous studies (Table 2).

There is growing interest in the U.S. Midwest in the implementation of conservation practices to reduce nutrient losses from farmland and improve fertilizer management of high-input demanding crops such as corn and wheat (García et al., 2016). Because soil biological properties are often overlooked in traditional nutrient recommendations, we analyzed not only data on soil physical, chemical and biological properties, but also the plant-available nutrient and fertilizer rate recommendation portions of the HSHT for sites with incremental changes in cover crop use over time (Fig. 6). A unique aspect of the HSHT nutrient recommendation, particularly for N, is the subtraction of the plant available N from the expected yield (Yost et al., 2018). This credit accounts not only for the residual inorganic N (nitrate and ammonium), commonly available in traditional soil fertility tests, but also estimates of mineralizable N during a 24-h aerobic incubation, an additional credit, termed organic N release, that traditional fertility tests do not account for.

In our analysis, greater organic nitrogen credits (organic N release) were obtained from fields with higher intensification in CCU, which lowered the requirements for fertilizer inputs for both corn and wheat (Fig. 6). A recent study evaluating the HSHT for corn N recommendations across eight Midwest states found that the plant-available N portion of the HSHT recommendation accounted for up to 49% of the variation in economically optimum N rate (EONR) and could potentially be used, with other factors, to better estimate EONR for corn in the Midwest (Yost et al., 2018). Similarly, a study including 111 fields adopting minimum tillage, multi-species cover cropping, and amendment with animal manures as soil health practices found a strong association between both HSHT variables, soil-test biological activity, and N mineralization, and corn EONR (Franzluebbers, 2020). Another recent large-scale study using data from multiple N rate trials across central and eastern Corn Belt found that biological indicators of soil health (e.g., permanganate oxidizable C, soil protein, and mineralizable C) accounted for approximately 20% of N fertilizer effects (Wade et al., 2020). Although understanding site-specific effects of cover cropping on EONR for corn and wheat needs further experimental work (cover crop decomposition experiments, for example), and HSHT fertilizer recommendations need further testing and calibration which was beyond the scope of our study, our results suggest the importance of accounting for soil biological activity and its association with nutrient credits as indicators of soil health to increase profit and reduce environmental impacts. Taken together, these recent efforts in understating soil biology to fine-tune nutrient fertilizer recommendations along with the results from our study suggest that soil health-promoting practices can provide a greater supply of N which can be used to reduce nutrient fertilizer costs and improve system input-use efficiency.

#### 4.3. Limitations of the framework and uncertainties

Due to the distribution of sites, farmer-selected management practices and protocol analysis (HSHT) to study, there were some limitations with our data and analytic approach. First, ecological intensification via the use of organic amendment was not well represented in our data – we only considered a 5-year frequency of organic amendment in the cropping system. The quantity and type of organic amendments were also not included because of uncertainties regarding the exact composition or the amount applied. Second, the data is essentially agroecosystem-focused, and extrapolation of our results to natural ecosystems may not be possible. For example, organic matter and infiltration changes might be more difficult to detect in natural ecosystems than in agroecosystems. However, the effect of intensification of soil health related practices on soil properties should be robust regardless of ecosystem types, which share the same soil formation mechanisms. Third, HSHT analytical procedures use unique soil extractants to measure microbial-available C and N pools, which require further data calibration for comparisons to traditional soil test labs. Additionally, the measured C and N pools are constantly replenished and rapidly changed by plant root exudates and dead microbial cells. In this paper, we focused on trends in the comparison of different soil health management systems over absolute values when interpreting HSHT results. Despite these limitations, the novelty in this effort is to account for the often overlooked role that field management history plays when analyzing data from participatory, on-farm research (Supplementary Table S1). This allowed us to propose a classification framework of ecological intensification that considers multiple soil health management practices.

#### **5.** Conclusions

This study was conducted on working farmlands, allowing us to consider a large variation in soil management decisions and field (e.g., crop sequence and species selection, avoidance of mechanical disturbance, application of organic amendment, elimination of fallow periods, and crop-livestock integration) in the dataset. The intensified cropping systems included in our study are possible alternatives to the conventional farming practices (input-intensive, maize-dominated rotations with limited diversity) in Midwestern U.S. (Fig. 3). Overall, our results indicate that (i) ecological intensification affected all properties commonly used in soil health assessments, but the sensitivity of the impacts of management varied among the physical, chemical and biological soil properties; (ii) the feature of continuous living cover and roots in the soil was reflected by the variable CCU index – the frequency of cover crop use in the rotation system. Relative importance showed that intensification in CCU was the most important management factor influencing changes in soil properties; and (iii) soil-test hypothetical N credits in cropping systems intensified with cover crop use can reduce nutrient fertility needs substantially as opposed to less intensified systems, in which organic nutrient credits are minimal and may not have an impact on the amount of N fertilizer required. The data presented here demonstrate the importance of understanding how ecologically based intensification of agricultural systems affects soil properties. Reported findings are informative and beneficial for promoting soil health management practices, better-informing farmers about management strategies that foster healthier soils and represent steps forward in land stewardship.

**Competing Interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data** Supplementary data is attached to the archive record for this article.

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