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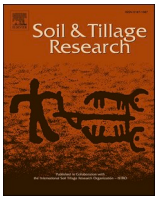

Quantifying short-term responsiveness and consistency of soil health parameters in row crop systems. Part 1: Developing a multivariate approach

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ABSTRACT

Quantifying soil health requires measuring different physical, chemical and biological soil properties, yet limits in time and resources often restrict the number of parameters that can be analyzed. The main objective of this research was to identify soil health parameters that showed measurable and consistent responses to reduced tillage and cover cropping over a short (2-year) study period. In September 2015, four treatments – reduced tillage with cover crops, reduced tillage without cover crops, conventional tillage with cover crops and conventional tillage without cover crops – were installed in five sites across Virginia. Sites were managed for corn or tobacco production. Soils were analyzed for 32 properties associated with soil health, and cash crop yields were also measured in September 2016 and September 2017. A multivariate approach was used to detect treatment differences and determine parameters driving those differences. We then developed two new indices to quantify the responsiveness and consistency of soil health parameters. The results showed that surface soil layers had more parameters with significant differences between treatments than subsurface layers. Tillage effects were observed within 0.5 years, which may be due to the lack of tillage history in 4 of the 5 sites. Cover crop effects appeared after 1.5 years, indicating that this practice can also induce changes in soil properties over relatively short periods. Soil aggregate stability, potassium, calcium, magnesium, boron and cash crop yield were the most responsive parameters to reduced tillage and cover crop practices, while aggregate stability also showed high consistency. These findings suggest that aggregate stability effectively indicated short-term changes in soil health within row cropping systems of Virginia.

1. Introduction

Intensive farming practices, such as tillage and residue removal, can negatively affect agroecosystems (Keesstra et al., 2016) and cause yield declines when implemented over long periods (Friedrich et al., 2012; Rusinamhodzi et al., 2011). Conservation agriculture was developed to address these concerns by balancing the productive (in terms of yield) and protective (in terms of environment) aspects of agriculture. Common conservation agriculture practices include reduced tillage, cover crops during fallow periods, and crop rotations (Friedrich et al., 2012; Reiter, 2020). As more producers move to adopt one or more of these practices, it is increasingly important to evaluate their effects on soil health, i.e., the capacity of soil to sustain plant and animal productivity and preserve agroecosystem functions (Doran and Zeiss, 2000).

Quantifying soil health typically involves measurement of a large set of biological, physical and chemical soil properties. For example, Stewart et al. (2018) identified 42 types of parameters that were used to assess effects of reduced tillage and cover cropping on soil health. However, funding and resource limitations means that it is often not possible to measure all or even most of these parameters. Thus, it is important to distinguish soil health parameters that reliably detect variations in soil health and convey information about soil functions and

Abbreviations: PR, Proportional Response; CP, Consistency Parameter.

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processes. In particular, there is a need to identify parameters that detectably and consistently respond to changes in management practices (Cardoso et al., 2013; Lazicki et al., 2021). This information can be especially important over seasonal to annual periods, which represent the typical time-scales over which farmers (1) plan and implement their management practices (e.g., tillage, residue management, planting of cover crops), and (2) sample for their soils for routine soil pH and nutrient analyses.

Different approaches have been used to evaluate the responsiveness of different indicators to conservation agriculture management practices, including using meta-analyses (Jian et al., 2020a), multiple univariate analyses (Jian et al., 2020b) and expert opinions to weigh scores representing various soil functions (Karlen et al., 1994). Meta-analyses have the advantage of identifying trends across many studies, but may not be useful when assessing individual operations. Opinion-based approaches may suffer from bias and can produce non-repeatable results (Andrews et al., 2002). Multiple univariate analyses (e.g., multiple analyses of variations, multiple regressions) can be hindered by problems of multicollinearity when dealing with large numbers of parameters; which makes the underlying assumptions of independence invalid.

Multivariate analyses were designed to counter shortcomings of these other methods (Brejda et al., 2000; Nosrati, 2013; Shukla et al., 2006; Zuber et al., 2017a). In a multivariate approach, analysis of multidimensional data (e.g., multiple soil parameters) occurs simultaneously. Overarching differences between data points can then be quantified and visualized (de Carvalho et al., 2018). Nonetheless, multivariate methods have never been used to quantify responsiveness and consistency of soil health parameters, even though they represent a set of statistically sound approaches to identify a smaller set of consistently responsive soil health indicators. Indeed, given the large number of indicators proposed for soil health assessment, multivariate approaches have high potential to identify parameters most useful for evaluating how changes in agriculture practices influence soil health.

In this study we sampled five sets of experimental plots across Virginia over a two-year period, and applied a multivariate analysis to evaluate the short-term responsiveness of different soil health indicators to reduced tillage and cover cropping. Our main study objective was to identify those parameters that consistently detected treatment differences for samples collected 1–2 times per year. As part of this process, we developed two new indices to evaluate the short-term responsiveness and consistency of indicators across sites. The results of this study should offer producers the ability to better monitor short-term changes in soil health due to management activities, and optimize these practices to improve soil health.

2. Methods and materials

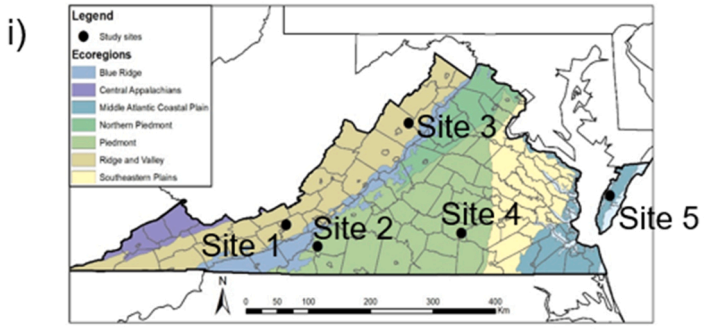
2.1. Description of sites

We performed this study at five sites located across Virginia (Fig. 1.i). The five sites were selected to cover a variety of eco-regions in the state of Virginia.

Soil series for each of the site were identified using the USDA-Natural Resources Conservation Services web soil survey (Table 1). Past management history was also investigated prior to the start of the experiment.

2.2. Experimental design

Our experimental design had two factors: tillage and cover cropping. These factors led to four experimental treatments: (1) reduced tillage with winter cover crops (RT CC); (2) reduced tillage without winter cover crops (RT NC); (3) conventional tillage with winter cover crops (CT CC); and (4) conventional tillage without winter cover crops (CT



ii)

2014	2015			2016			2017
Sites	July	Sep	Oct	April	May	Sep	April
Site 1			Cover crop		Corn		Cover crop
			T	*#R	T	#T	#*
Site 2			Cover crop		Corn		Cover crop
			T	*#R	T	#T	#*
Site 3			Fallow		Corn		Cover crop
			T	*R	T	#T	#*
Site 4			Cover crop		Tobacco		Cover crop
			T	*#R	T	T	#*
Site 5			Cover crop		Corn		Cover crop
			T	*#R	T	T	#*

* Soil sample taken
\# Plant sample taken
R Glyphosate application
T Tillage done on the tilled plots

Fig. 1. (i) Map of Virginia showing site locations, and (ii) timeline of cover and cash crop plantings, soil and plant samplings, herbicide application, and tillage.

Table 1
Description of the experimental sites: Site number, location (city), coordinates, soil type (texture), site history and cash crop used for the study.

Site	Locations	Coordinates	Soil series (texture)	Past management	Cash crop for the study
1	Blacksburg, Virginia	37.207°N 80.486°W	Duffield-Ernest-Purdy undifferentiated group (fine loamy)	Long term no-till corn with winter cover crops	Corn [*Zea mays subsp. mays*]
2	Harrisonburg, Virginia	38.546°N 78.722°W	Frederick and Lodi complex (fine silt loam)	No-till corn/ cereal with 23 species cover crops with manure application	Corn [*Zea mays subsp. mays*]
3	Ferrum, Virginia	36.919°N 80.036°W	Bluemont-Spriggs-Redbrush complex (fine loamy)	Lightly grazed pasture in fescue	Corn [*Zea mays subsp. mays*]
4	Blackstone, Virginia	37.095°N 77.961°W	Appling and Durham (coarse sandy loam)	Non-grazed pasture with fescue	Tobacco [*Nicotiana tabacum*]
5	Painter, Virginia	37.591°N 75.821°W	Bojac (sandy loam)	Conventionally tilled potato [*Solanum tuberosum*]	Corn [*Zea mays subsp. mays*]

NC). In Sites 1, 2, and 3, we used a split-plot experimental design in which tillage was the whole plot factor and cover cropping was the subplot factor. Sites 4 and 5 had a complete randomized block design. Sites 1–4 had 8 physically replicated plots (two for each treatment), with two subplots within each main plot ($n = 4$). Site 5 had 16 physical plots ($n = 4$). Physically replicated plots had the following dimensions: 24 m x 30 m in Site 1; 6.1 m x 23 m in Site 2; 18 m x 36 m in Site 3; 19 m x 25 m in Site 4; and 3.0 m x 61 m in Site 5.

In September 2015 and 2016, we planted cover crop plots with a three-way mixture of winter barley [*Hordeum vulgare*], crimson clover [*Trifolium incarnatum*] and tillage radish [*Raphanus raphanistrum* subsp. *sativus*]. Note that Site 3 was not planted in cover crops until September 2016. Winter barley was planted at 129 kg ha^{-1}, clover was planted at 15 kg ha^{-1}, and radish was planted at 6 kg ha^{-1}. The cover crops were never fertilized, and were terminated using glyphosate in April 2016 and again in April 2017.

After cover crop termination, Sites 1–3 and 5 were planted with corn [*Zea mays subsp. mays*] each April, while Site 4 was planted in tobacco [*Nicotiana tabacum*]. The corn crops were fertilized at time of planting with 185 kg N ha^{-1} (all treatments) while the tobacco crops received 60 kg N ha^{-1} at time of planting (all treatments). Cash crops were harvested in September 2016 and 2017. Corn was harvested for silage in Sites 1, 2 and 3, meaning that most above-ground biomass was removed, and for grain in Site 5, meaning that crop residue was returned to the soil in all plots. All above-ground biomass was removed during harvest for the tobacco crops.

We performed disk tillage on the conventional tillage plots every September (after cash crop termination and prior to cover crop planting) and every April (after cover crop termination and prior to cash crop planting). For tillage operations, an offset disk harrow was set to a depth of approximately 15 cm, with a single pass used for each tillage event. Even though the disk was set at 15 cm, in the field we observed that the disk generally only disturbed the soil to a depth of 10 cm. For the reduced tillage treatments, we used no-till for Sites 1–3 and 5 and strip tillage for tobacco production in Site 4. Strip tillage occurred in April following cover crop termination.

2.3. Soil sampling

In April 2016 and April 2017, we collected unconsolidated soil samples from the surface (0–10 cm) and subsurface (10–20 cm) soil layers of the soil profile ($n = 4$ per layer and treatment). The only exception was Site 4, in which subsurface samples were not collected in April 2016. After collection we split the samples into two subsamples. One subsample was air-dried for determination of soil aggregate stability and soil chemical parameters, while the other was stored at 4 °C until being tested for soil biological parameters. Note that samples were collected prior to spring tillage, again with the exception of Site 4 in 2016, where tillage took place before sampling (Fig. 1.ii). We also collected volumetric core samples (7.62 cm diameter x 5.08 cm height) at Sites 1–3 to measure bulk density and porosity. These cores came from the middle of each studied layer (i.e., the surface sample came from 2 to 7 cm, and the subsurface sample came from 12 to 17 cm), so as to reduce edge effects in the cores and thereby best represent the studied depths. In total, we used 304 unconsolidated soil samples and 192 core samples to quantify soil health parameters.

2.4. Soil and crop parameters

We measured a total of 33 soil health parameters, including 14 microbiological parameters, 12 chemical parameters, 6 physical parameters and 1 crop-related parameter. Most properties were measured on both surface and subsurface samples, with the exceptions of soil respiration, field-saturated hydraulic conductivity, which were only measured at the soil surface, and wet aggregate stability, which was only measured on the 0–10 cm samples. Crop yield likewise had only one value per plot per year, which we matched to the surface samples in subsequent analyses.

2.4.1. Soil respiration (SR)

Soil respiration (SR) is measurement of CO_2 flux from the soil due to heterotrophic microbial respiration plus autotrophic root respiration. We measured SR using a LI-COR 8100 (LI-COR, Nebraska, USA). To perform the measurements, we installed a 20-cm diameter ring to a depth of approximately 3 cm into the soil. A 2-minute soil CO_2 flux measurement was then obtained from the LI-COR 8100. We collected 160 soil respiration measurements over the two-year study period.

2.4.2. Near-saturated hydraulic conductivity (K_n)

Near-saturated hydraulic conductivity (K_n) represents the ability of soil to intake water under realistic field conditions (Jian et al., 2021). We first measured infiltration rates using a minidisk tension infiltrometer (METER Group, Inc.; Pullman, USA), with the tension set to − 2 cm, and collected a minimum of 4 infiltration test per treatment per sampling time per location. We then calculated K_n from the measured infiltration rates using a two-term infiltration model (Zhang, 1997) with the capillary parameter (α) term estimated based on the measured soil texture for each site. We collected 160 K_n measurements in total.

2.4.3. Total soil carbon (TC), particulate organic matter (POMC), and mineral associated organic matter (MAOMC)

We quantified total soil carbon (TC) as the sum of two separately measured fractions: relatively labile particulate organic matter carbon (POMC) versus relatively slow-changing mineral-associated carbon (MAOMC). The fractionation was done using sodium hexametaphosphate (Na-HMP) shaking and a 53 μm sieve was used for POM separation. The percent carbon values were determined using a NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy). For details please refer to (Lucas et al., 2020), Bradford et al. (2008) and Strickland et al. (2010).

2.4.4. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

Total microbial biomass was estimated using a modified fumigation extraction method, where chloroform was used to lyse the cell membranes of microbes. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were then measured as a difference between C and N in chloroform-fumigated subsamples versus non-fumigated subsamples. The detailed description is presented in Fierer and Schimel (2002, 2003).

2.4.5. Substrate induced respiration (SIR)

The substrate induced respiration (SIR) technique is used to quantify active microbial biomass (Wardle and Ghani, 1995). Here we followed the procedures of Fierer and Schimel (2003). In our experiment, after a 1-hour pre-incubation with excess autolyzed substrate, we incubated soil slurries for 4 h at 20 °C. We then measured CO_2 using an infrared gas analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, NE, USA).

2.4.6. Mineralizable carbon (Cmin)

Mineralizable carbon represents the labile soil carbon pool, and specifically quantifies the rate at which organic matter in the soil will be broken down by the microbes in the soil at optimum temperature and moisture (Fierer et al., 2005). Here, we incubated soil samples in 50 ml tube for 60 days at 60% field capacity and at 20 °C. These samples were analyzed for respiration every week using the LI-7000 IRGA. One day before the measurement, the samples were taken out of the incubator and flushed using CO_2 free air and capped again to obtain accurate respiration rates. The detailed method can be found in Strickland et al. (2010).

2.4.7. Catabolic response profile (CRP) and microbial functional evenness (MFE)

The difference in substrate utilization between microbial communities in the soil is known as the catabolic response profile (CRP), which can be used to quantify the functional diversity of microbes. We quantified CRP following Degens and Harris (1997). Briefly, we used water versus five different organic substrates: glucose, glycine, oxalic acid, cellulose and chitin (Strickland et al., 2017). We then measured the CO_2 respiration resulting from each individual substrate using the LI-7000 IRGA.

The information from the six substrates was also combined to calculate microbial functional evenness (MFE) using the Simpson-Yule index: $MFE = 1 / \sum(p_i/t_i)^2$ where p_i is the respiration response due to a substrate and t_i is the total response due to all substrates used (Magurran, 1988; Schipper et al., 2001). Experimental treatments were compared based on respiration from each individual substrate and based on MFE.

2.4.8. Soil aggregate stability (AS_2mm, AS_0.25 mm, and AS_0.053 mm)

We analyzed soil aggregate stability using a modified wet sieve method (Kemper and Rosenau, 1986). Each sample was air-dried and gently sieved through a 4-mm sieve. Fifty grams of sample was then placed on top of nested sieves: 2, 0.25, and 0.053 mm. The sieve setup with the soil was lowered into the water and submerged for 5 min. After 5 min, we vertically oscillated the sieves 50 times by hand. The soil remaining in each sieve was dried, weighed, and corrected for pebble and sand content. Aggregate stability of each size fraction was quantified as a percentage of the initial sample weight corrected for moisture and pebble and sand content.

2.4.9. Bulk Density (BD)

Soil bulk density was quantified from soil cores by dividing the oven-dried soil mass by the volume of sampling rings (231.6 cm^3), following Blake and Hartge (1986). Bulk density was only measured in Sites 1, 2 and 3.

2.4.10. Porosity

The total amount of pore space (i.e., porosity) influences water and air movement in soil (Schoenholtz et al., 2000). We quantified porosity following Danielson and Sutherland (1986), specifically using the mass difference between saturated and oven-dried cores to determine volume of water and then dividing by the volume of the sampling rings. Porosity was only measured in Sites 1, 2 and 3.

2.4.11. pH

Soil pH (1:1 soil:H_2O by volume) was determined on a 3100 M benchtop pH meter (OHAUS, Inc., Parsippany, NJ, USA).

2.4.12. Elemental analysis

Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Manganese (Mn), Copper (Cu), Iron (Fe), Boron (B), and Aluminum (Al) were extracted using a Mehlich-1 extracting solution at a 1:5 vol ratio of soil: solution. The extracted samples were then analyzed using an ICP-AES (inductively coupled plasma atomic emission spectrometer; CirOS VISION model/Spectro Analytical Instruments/Kleve/Germany). For more details please refer to Maguire and Heckendorn (2005).

2.4.13. Cation Exchange Capacity (CEC)

Cation Exchange Capacity (CEC) is the capacity of soil to hold cations, including nutrients such as NH_4^+. We quantified CEC using a modified summation method (Sumner and Miller, 1996). Specifically, CEC was determined as the sum of non-acid generating cations (Ca^{2+}, Mg^{2+} and K^+), plus the acidity estimated from the Mehlich soil-buffer pH after conversion of all analytical results to meq 100 cm^{-3}. A detailed explanation can be found in Maguire and Heckendorn (2005).

2.4.14. Cash crop yield (Yield)

We analyzed cash crop yield using data from Sites 1, 2, 3 and 5 (i.e., the sites planted in corn). Cash crop yield in Sites 1, 2 and 3 was calculated as if corn was harvested as silage by cutting all plants from a 10 m stretch of two adjacent rows at a height of 15 cm. Two samples were collected from each plot. These samples were weighed in the field and then a subsample was collected and dried to a consistent weight at 50 °C to calculate dry sileage cash crop yield. Cash crop yield at Site 5 was calculated by harvesting and weighing all ears from the entire length of the two adjacent middle rows of each plot. Additional subsamples were taken to quantify moisture via a DICKEY-john GAC 2500 (DICKEY-john, Auburn, IL, USA) to correct grain to 15.5% moisture. We reported dry cash crop yield as mass per area.

2.4.15. Above-ground biomass of winter covers

Above-ground biomass of winter covers was quantified for all plots using a 0.3 m * 0.3 m quadrat that was randomly placed within the plots at the time of cover crop termination (i.e., in late April). Cover crop biomass and any weeds were cut at the soil surface, with the above-ground matter brought to the lab and weighed. After weighing, the sample was dried at 65 °C until a constant weight was achieved. Using these weights, we calculated dry biomass harvested per area. Two samples were collected in each plot. Note that biomass was not measured in April 2016 in Site 3 due to the lack of cover crops, while sampling in Site 4 occurred after tillage that year (Fig. 1), so cover crop biomass samples were only collected in the strip till plots. In April 2017, biomass samples were collected prior to tillage in all plots.

2.5. Statistical analyses

First, we used analysis of variance (ANOVA) with Tukey's HSD to compare total soil carbon in soil surface samples and cover crop biomass between the five sites. We also used unpaired-t-tests to compare above-ground biomass between cover and no cover plots. Second, we conducted a multivariate statistical analysis on the 33 quantified

parameters. Note that we used both particulate organic matter (POMC) and mineral associated organic matter (MAOMC) as separate indicators in our analysis, but did not include total carbon (TC) in the multivariate analysis because it was the sum of the other two fractions.

To perform the multivariate analysis, we subsetted the data by site (1−5), sampling depth (surface and subsurface), and sampling time (April 2016 and April 2017), and removed any missing values. In total we obtained 19 subsets of data, lacking only the subsurface depth for Site 4 in April 2016. For each subset, we used the Bray Curtis method for permutational multivariate analysis of variation (perMANOVA), and performed the analysis using the *Adonis* function in the *Vegan 2.5–2* package for R (Oksanen et al., 2018), which identified any overall differences between treatments. Whenever significant interaction effects were observed for the treatments ($p < 0.05$), we used the *Pairwise.Adonis* function as a post hoc test. The assumption of equal dispersion was checked by using the *betadisper* and *anova* functions in R. If any treatment differences were identified by perMANOVA, then we made non-metric multidimensional scaling (NMDS) plots to visualize those differences.

In the final step, we used the *Envfit* function in the package *Vegan 2.5–2* to identify which soil health parameters were associated with differences in perMANOVA. An alpha value of 0.05 was used to indicate statistical significance in this step. The NMDS plots showing treatment differences and the envfit detected parameters are presented in the supplemental material section.

All statistical analyses were performed using R Version 3.5.0 (R Development Core Team, 2018).

2.6. Proportional Response (PR) calculation

To quantify the responsiveness of the 33 soil health parameters to the imposed tillage and cover crop treatments, we developed a metric called Proportional Response (*PR*):

$$PR = N_{td}/N_{total} \quad (1)$$

where N_{td} represents summation of the number of instances that the parameter was associated with significant treatment differences ($P < 0.05$) in the *Envfit* analysis, and N_{total} represents the total number of times that the parameter was used in the perMANOVA analysis. Using this methodology, we corrected for any instances where a certain parameter was not measured or instances where subsurface samples could not be taken. Proportional response was calculated for each soil depth. This approach thus provided two scores (surface versus subsurface) for every soil health parameter, with scores based on responsiveness across all sites. The PR values ranged from 0 to 1, with PR = 1 indicating that the parameter was responsive to the treatments in all sites and at all times.

2.7. Consistency parameter (CP) calculation

We also evaluated the temporal consistency of soil parameters in terms of ranking the four treatments. First, we took the mean values for each parameter based on the combination of sampling date, sampling depth, site and treatment (i.e., every parameter had one mean value per treatment and site for each sampling date and depth). We then used the Spearman correlation (r_s) to determine rank correlations between parameter values in April 2016 and 2017. This analysis was done for each soil depth and each site. The mean of r_s for sites was then used to calculate the consistency parameter (CP) for each soil depth:

$$CP = \frac{1}{N}\sum_{j=1}^{N} r_{s,j} \quad (2)$$

where *j* indexes the sites and *N* is the total number of sites at which that the parameter was measured. *N* is equal to 5 for surface samples (total number of sites) and *N* is equal to 4 for subsurface samples (due to the missing subsurface sampling in Site 4 in 2016). Similarly, *N* is equal to 3 for parameters that were only measured in Sites 1–3 (i.e., bulk density, porosity). Note that *CP* can vary from − 1 to + 1. A *CP* value of + 1 indicates that the parameter showed identical treatment rankings between the two study years (2016 and 2017) at all five sites. A *CP* value of − 1 indicates that the parameter showed perfectly inversed treatment rankings between the two sets of study years at all five sites.

3. Results

3.1. Total soil carbon and cover crop biomass

The five sites had significant differences in total soil carbon concentrations in the surface soil samples (Fig. 2). Site 3 had significantly higher soil carbon than all other sites while Site 5 had significantly lower soil carbon than all other sites ($p < 0.05$).

Even though the same cover crop mixture was planted at the same seeding rate without fertilization, the resulting cover crop biomass differed between the five sites (Fig. 3). In April 2016, Site 5 had the highest cover crop biomass, followed by Site 2 ($p < 0.05$). In April 2017, Sites 2 and 5 had the highest dry cover crop biomass, whereas Sites 1 and 4 had the lowest cover crop biomass ($p < 0.05$). We also monitored volunteer above-ground biomass in the no cover crop control plots whenever weeds were substantially present (Supplemental information, Figure S.1). In 2016, only Site 5 had considerable above-ground biomass in the no cover crop plots, whereas in 2017, all the sites but Site 4 had considerable biomass on the no cover crop plots. Whenever quantified, no cover crop plots had significantly lower above ground biomass compared to cover crop plots ($p < 0.05$; Figure S.1).

3.2. PerMANOVA analysis to detect treatment differences based on soil health indicators

The perMANOVA analysis detected significant treatment differences in four of the five sites ($p < 0.05$; Fig. 4). During the first sampling event (April 2016), Sites 1 and 4 showed significant tillage effects in the surface soil, and Sites 2 and 3 showed significant tillage effects in the subsurface soil. During the second sampling event (April 2017), indicator differences due to tillage were detected in the surface soils at Sites 1 and 3, and in the subsurface soil at Site 1. An interaction effect between tillage and cover cropping was observed for the subsurface samples at Site 3 in April 2017. Cover crop treatments also caused significant differences in the surface soils during the second year at Site 2.

Treatment differences were observed slightly more often in the surface soils (5 instances where treatment differences were identified out of 10 total comparisons) than the subsurface soils (4 instances out of 9 total comparisons). Site 5 was the only site that did not show any treatment differences at any of the sampling events or for either of the soil layers. All of the detected treatment differences are presented in the Supplemental information (Figures S.2-S.10).

3.3. Proportional Response (PR) of soil heath indicators across sites and years

Surface soils had greater responsiveness to different management practices than subsurface soils, as indicated by a numerically higher average PR value (mean PR of surface soils = 0.25 versus mean PR of subsurface soils = 0.19; Fig. 5). In the surface soils, chemical parameters had highest responsiveness (mean PR = 0.34), followed by physical (mean PR = 0.23) and then biological (mean PR = 0.16) parameters. Out of 33 parameters measured in the surface soils, 13 parameters had PR values of 0.3 or higher, 11 parameters had PR values of 0.4 or higher, and only 6 parameters had PR values of 0.5 or higher (Fig. 5). Specifically, water-stable aggregates 2–4 mm in size (AS_2mm), several macro- and micro-nutrients (potassium, calcium, magnesium, boron),

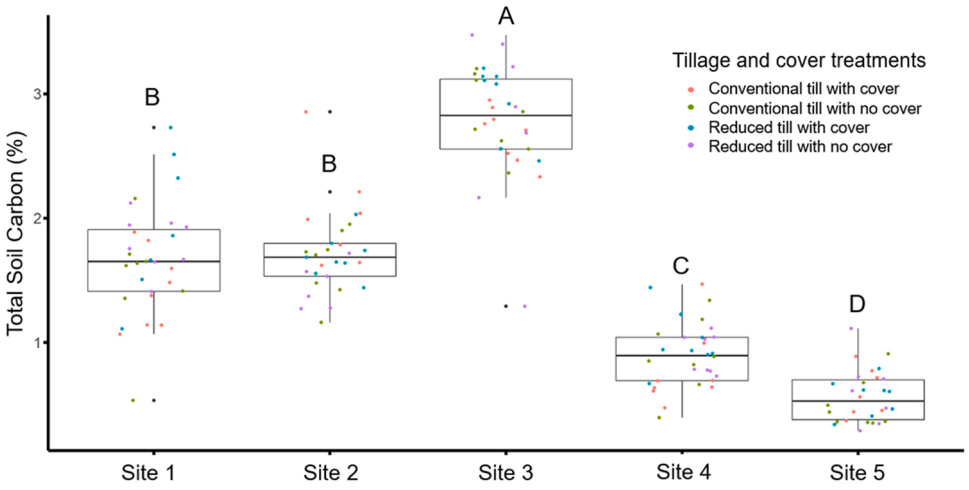


Fig. 2. Total soil carbon content for each site (surface soil). Different letters indicate significant differences between sites (ANOVA with Tukey's HSD; $p < 0.05$). Samples were collected in April 2016 and April 2017.

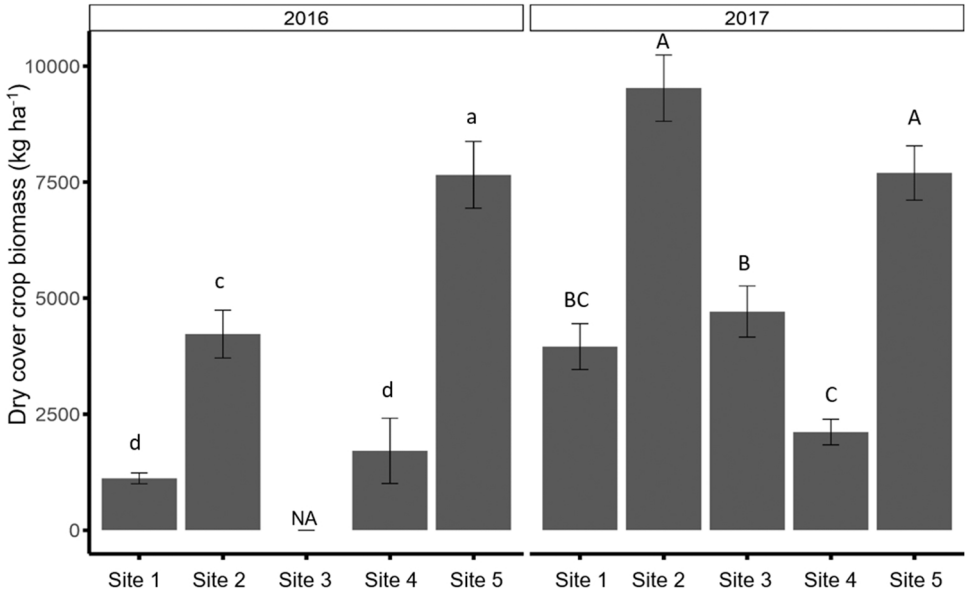


Fig. 3. Dry above ground cover crop biomass for five sites for April 2016 and April 2017. Sites associated with different lower-case letters are statistically different for 2016. Sites associated with different upper-case letters are statistically different for 2017 (ANOVA with Tukey's HSD; $p < 0.05$).

and yield all had PR values ≥ 0.5. Conversely, 3 parameters – catabolic response profile with water, catabolic response profile with chitin, and pH – had *PR* values = 0 in the surface soil samples, meaning that those parameters were never associated with treatment differences.

For the subsurface soils, 4 out of 27 parameters had *PR* values of 0.4 or higher, while 5 parameters had PR = 0. Specifically, calcium, magnesium, boron, and cation exchange capacity had PR ≥ 0.4. The catabolic response profile with glucose, catabolic response profile with oxalic acid, catabolic response profile with cellulose, and porosity all had PR = 0, as did microbial biomass carbon.

3.4. Consistency Parameter (CP) between treatments

In case of parameter consistency, the surface and subsurface soil samples both had mean CP values of 0.18 (Fig. 6). Out of the 33 parameters analyzed for the surface soils, 10 showed CP values ≥ 0.3, 6 had CP values ≥ 0.4, and 3 parameters (i.e., catabolic response profile with glucose, aggregate stability (2 – 4 mm), and manganese) had CP values ≥ 0.5. Soil manganese had the highest consistency of all parameters measured in the surface soils (CP = 0.64; Fig. 6). Soil respiration with chitin as the substrate had the lowest consistency, with CP = −0.36. In the surface samples, 9 out of 33 parameters had CP ≤ 0 (Fig. 6), indicating no or negative correlation between 2016 and 2017 soil samples.

In case of the subsurface soils, soil pH had the highest CP value, 0.75, while soil respiration with the glycine substrate had the lowest CP value, − 0.5. Five parameters had CP values greater than 0.5 (catabolic response profile with cellulose, microbial functional evenness, pH, magnesium and cation exchange capacity). In the subsurface samples, 9 out of 27 parameters had no or negative correlation with 2016 and 2017 samples (CP ≤ 0; Fig. 6).

4. Discussion

4.1. Soil health parameters responded to tillage treatments more quickly than cover crop treatments

Tillage caused a more rapid change in soil health parameters than

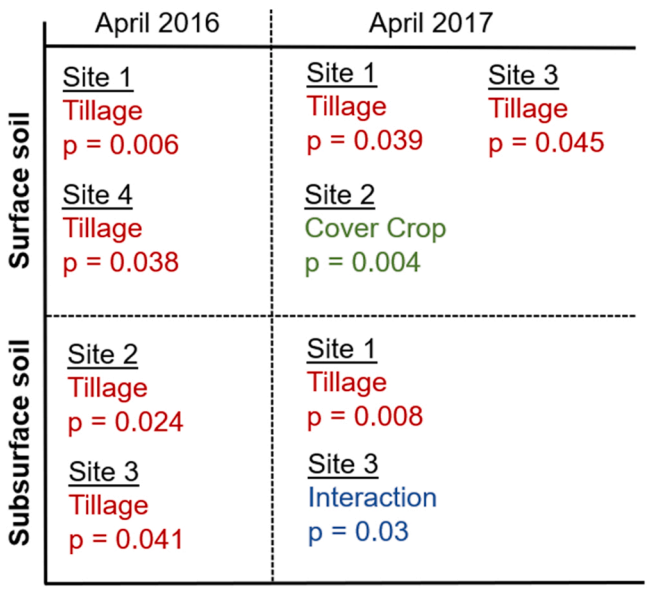


Fig. 4. Summary of the significant treatment differences detected using permutational multivariate analysis of variation (perMANOVA).

cover cropping. In the April 2016 sampling event, conducted ~7 months after the beginning of the study, reduced tillage treatments had significantly different soil health properties than conventional tillage treatments in four of the five sites (Fig. 4). In contrast, treatment differences related to cover cropping only started to appear during the April 2017 sampling, 1.5 years after the study started. These results emphasize that tillage induces an immediate and greater level of soil disturbance than cover cropping. Similar findings were previously shown by Raper et al. (2000), who also detected that soil physical properties changed more rapidly in response to tillage compared to cover cropping, and Gabriel and Quemada (2011), who only observed N benefits from leguminous cover crops after the second year of planting.

In the second year of the study, Site 2 demonstrated a significant cover crop effect on soil health parameters (Fig. 4; Figure S.8). This effect may have been driven, at least partly, by the fact that Site 2 had the highest dry cover crop biomass for that year (Fig. 3). This finding aligns with previous work suggesting soil health parameters may have greater response as cover crop biomass increases (Balkcom et al., 2007; Roldán et al., 2003). However, Site 5 had consistently high cover crop biomass for both years (Fig. 3), yet there were no cover crop or tillage effects detected in Site 5 (Fig. 4). The lack of parameter response in Site 5 might be partly related to its sandy loam soil texture, as previous work has suggested that changes in soil properties can be more difficult to detect in coarse-textured soils (Acosta-Martínez and Cotton, 2017). Site 5 also had the lowest soil carbon concentrations of the five sites (Fig. 2), likely due to a combination of coarse texture and previous tillage (Halpern et al., 2010). Soils with lower carbon concentrations tend to have poorer soil health (Wiesmeier et al., 2019), and can have lower efficiency when converting inputs like organic matter from cover crops to outputs such as soil ecosystem services (Kibblewhite et al., 2008). At the same time, coarse-textured soils tend to build soil organic carbon at relatively low rates when practices such as cover cropping and reduced tillage practices are used (Campbell et al., 1996; Jian et al., 2020a). It is likely that soils such as those of Site 5 may require multiple years of conservation agriculture to cause measurable changes in soil health properties.

4.2. *Soil physical and chemical properties were most responsive to agricultural management*

Soil chemical (mean PR = 0.34) and physical (mean PR = 0.23) parameters had greater responsiveness to differences in management practices compared to biological properties (mean PR = 0.16; Fig. 5). The responsiveness of physical parameters was primarily driven by aggregate stability of the 2–4 mm size fraction (AS_2mm), which was the only parameter that showed both responsiveness and consistency at least half of the time (i.e., PR and CP ≥ 0.5; Figs. 5 and 6). As a result, aggregation appears to be a good indicator for assessing short-term changes in soil health due to management. This result is consistent with a meta-analysis conducted by Stewart et al. (2018), along with

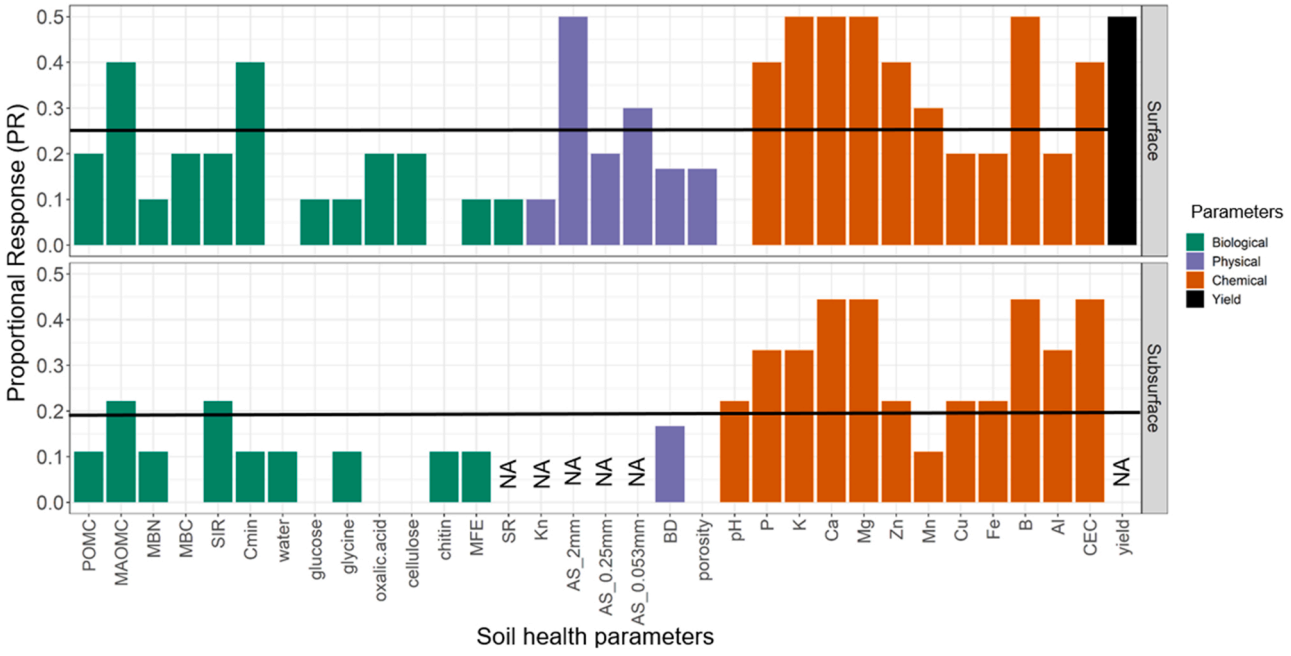


Fig. 5. Proportional Response (PR) values for each soil health parameter for both surface and subsurface soil samples. Black horizontal lines represent the mean PR for each soil layer. NA (Not applicable) represents the parameters that were not applicable or were not measured for subsurface samples. PR values are out of a possible maximum of 1.

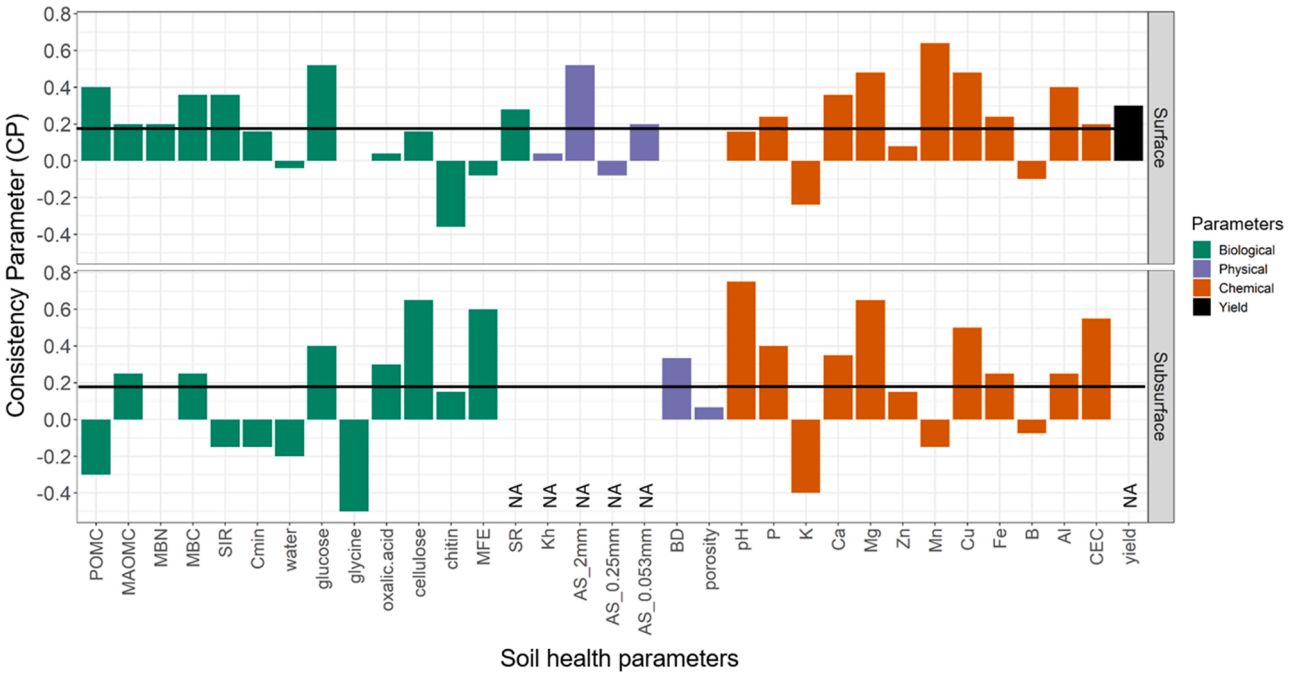


Fig. 6. Consistency Parameter (CP) for all measured soil health parameters, separated between surface and subsurface soil samples. Black horizontal lines represent the mean CP for each soil layer. NA (Not applicable) represents the parameters that were not applicable or not measured for the subsurface samples. CP values can range from − 1 to + 1.

findings of other studies on tillage (Bhattacharyya et al., 2012; Roldán et al., 2003; Schwen et al., 2011) and cover crops (Hubbard et al., 2013; Ramos et al., 2010). Previous work has also indicated that aggregate stability forms the basis for a wide range of soil functions (An et al., 2010), including increased carbon stabilization (Kong et al., 2005), higher water infiltration (Franzluebbers, 2002) and greater resistance to erosion (Barthes and Roose, 2002). Therefore, aggregate stability appears to be a useful indicator for its sensitivity and for its relations to important soil functions (Allen et al., 2011).

Responsiveness of soil chemical properties was more evenly distributed, with 7 out of 12 parameters found to have *PR* values ≥ 0.4. These responsive indicators included nutrients such as phosphorous, potassium, calcium, magnesium, and boron, along with cation exchange capacity of the subsurface soils. Many of the nutrients also had positive CP values, including phosphorous (P), calcium (Ca), and magnesium (Mg), as did pH and cation exchange capacity (CEC), indicating some consistency in the ranking of the different experimental treatments between sampling years. Previous studies have also noted high responsiveness of macro-nutrients (i.e., P, K, Ca, Mg) and CEC to tillage (Lienhard et al., 2013; Lou et al., 2012; Zuber et al., 2015, 2017a) and cover cropping (Dabney et al., 2001; Nascente et al., 2015; Sharma et al., 2018) practices. One reason that the macro-nutrients may have shown greater treatment effects than other chemical parameters may be due to higher demands imposed by the cover crops (Hossain et al., 2017). At the same time, 4 of the 5 sites were previously in no-till or continuous sod, which has been associated with nutrient concentration near the soil surface (Houx Iii et al., 2011; Norton, 2020). It is therefore probable that disk tillage caused nutrients to become mixed to greater depths in the tilled plots. As a result, the differences in chemical properties that we measured in this study could due to the physical soil mixing and hence may not be linked to any real changes in soil function. Longer-term studies under such conditions may thus be needed to determine if soil chemical properties are suitable indicators for informing farm management decisions. Otherwise, careful consideration should be given to the history of a site before using nutrient concentrations as indicators of soil health.

Only two soil biological parameters – mineral-associated organic matter carbon (MAOMC) and carbon mineralization – were found to have high responsiveness to the tillage and cover crop treatments (i.e., PR = 0.4). Likewise, those parameters had some consistency in how they ranked between treatments year to year, with CP $\geq$ 0.2. These results are consistent with several recent studies, which showed good responsiveness in those two properties (Culman et al., 2013; Jilling et al., 2020; Morrow et al., 2016). However, a number of other studies have identified soil biological parameters such as microbial biomass (Cardoso et al., 2013), substrate induced respiration (Stone et al., 2016), and soil respiration and soil enzymes (Nunes et al., 2020) as the fastest responding metrics. Islam and Weil (2000) also found that microbial properties like microbial biomass and active carbon were sensitive to management over seasonal to annual timescales.

This discrepancy in the sensitivity of biological properties might be related to the higher spatial variability of soil biological parameters, including active SOM pools, compared to physicochemical properties (Baldrian, 2014; Morrow et al., 2016). At the same time, other studies have also identified high temporal variation of soil biological properties as a reason for the absence of treatment differences (Zuber et al., 2017b). Sampling time can be a more important factor for biological parameters like microbial biomass and activity than tillage or fertilization (Shi et al., 2013). Therefore, accurate representation of management effects on many soils biological properties may require collecting a relatively large number of soil samples coupled with seasonal sampling.

4.3. Despite limitations, study generated new insights for quantifying soil health

In this study we tested 33 parameters for responsiveness and consistency, representing a fairly large and comprehensive dataset. Indeed, the mean number of parameters tested per soil health study was recently determined to be between 3 and 4 (Stewart et al., 2018), an order of magnitude fewer parameters. Nonetheless, due to limitations in time and resources, our study omitted a large number of other soil health parameters like soil enzymes, CO_2 burst tests, and microbial community analysis. Our study was also limited in duration, with only two measurements points in time for all samples (April 2016 and April 2017).

The annual sampling frequency was by design, as we chose our sampling design to identify indicators that are sensitive enough to detect changes in management on annual timescales and that also have consistency across years. We note that this sampling frequency reflects the timescale over which producers typically implement and modify practices (e.g., cover crops are typically planted once per year in row crop systems in temperate climates). Nonetheless, additional years of data collection would be necessary to evaluate and quantify long-term responsiveness and consistency of soil health parameters.

Regardless of these limitations, our study generated several results that may be particularly useful for farmers and other researchers. One, as discussed above, we observed that cover crop biomass can influence soil health parameters, but the magnitude of this effect may depend on soil type and previous management history. We therefore recommend that information of previous land use and practices should be collected as part of any routine soil health assessment. Two, we observed that most soil biological properties did not consistently detect differences between cover crop and tillage treatments on an annual basis. Therefore, proper soil sampling schemes should be developed that can account for the variability in soil biological properties, at least when working in similar cropping systems, soil types, and climates as represented in this study. Three, aggregate stability had both high responsiveness and consistency, meaning that indicator may be one of the most useful for detecting immediate effects of conservation agriculture practices on soil health. Fourth, we developed two new indices (PR and CP) that can be used by others to evaluate the responsiveness and consistency of soil health indicators in other systems and contexts. While our study used a somewhat complex multivariate analysis to parameterize those indices, the underlying principles can be used in conjunction with more common and straightforward analyses (e.g., analysis of variation). Five, cash crop yield showed relatively high responsiveness (PR = 0.5) and some year-to-year consistency across sites (CP = 0.3), thus indicating that cover cropping and tillage changes can influence farm productivity.

Finally, we note that the multivariate analysis used in this study was useful for identifying differences in treatments, but did not indicate the direction of any effects. Therefore, a companion paper (Part 2) will investigate the direction and magnitude of responses for parameters identified in this part as showing evidence of consistency and responsiveness. This type of hierarchical approach, i.e., using a multivariate analysis to first identify consistently responsive soil health parameters, followed by in-depth analysis of variations in those parameters between treatments or through time, may also be useful for interpreting other datasets or even may be included in larger meta-analyses. Given the expense and effort associated with many proposed indicators, it is important to conduct such rigorous assessments when deciding and recommending which parameters farmers and regulators should measure.

5. Conclusion

The ability to objectively evaluate both existing and new agronomic practices is essential to a sustainable food supply. In this study, we measured 33 soil health indicators in treatments representing two conservation practices, reduced tillage and cover cropping, compared to conventional tillage and no-cover crop controls. Using these data, we developed two new metrics that quantify the short-term responsiveness (PR) and consistency (CP) of each indicator to these conservation practices. Our analysis showed that only six parameters were responsive half of the time (i.e., PR $\geq$ 0.5) to tillage and cover crop treatments: wet aggregate stability of the 2–4 mm size fraction, soil potassium, soil calcium, magnesium, boron and cash crop yield. On top of being responsive, aggregate stability showed consistency in the ordering of values between experimental treatments through time, with CP values $>$ 0.5. Cash crop yield also had moderate consistency (CP = 0.3). The latter result suggests that conservation management can influence farm productivity in this region.

Here we note that our study installed similar treatments at five different sites, with underlying variations in soil type, climate, and cropping systems. We had a total of 10 site-years, thus providing rigorous backing to the indicators identified here as having appropriate sensitivity and consistency. However, we also found that the management history of the sites may have influenced results. Four of the five sites were previously managed under no-till row crops or continuous sod, which may have caused nutrient stratification that was then altered in the tillage treatments. The fifth site, which had been intensively cultivated prior to the study, did not show any tillage-related effects. These contrasting behaviors emphasize that disturbance is much more rapid and pronounced when soil is converted from no-till to tillage, compared to relatively slow recovery when soil transitions from tillage into no-till. Such factors are important to consider when using soil health indicators to inform management practices.

This study provides a framework by which practitioners can identify and monitor consistently responsive soil health parameters. This information can then be used by farmers and agencies when prioritizing the type and frequency of measurements they collect. By only analyzing indicators that are appropriate for their cropping system, and that consistently detect seasonal-to-annual changes in soil health, these people can save both time and money. This information can also encourage greater adoption of practices that enhance soil health, since producers will be able to have more direct evidence of the effectiveness of these approaches.

Declaration of 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2022.105354.

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