Comparing Biological and Conventional Chemical Soil Tests in Long-Term Tillage, Rotation, N Rate Field Study

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The Haney Soil Health Tool is a suite of tests integrating chemical and biological factors to provide a sophisticated analysis of soil nutrient availability. This research was conducted to determine if Haney tests (including H^3A and water extracts, Solvita 24-h CO_2 evolution, and Soil Health Calculation [SHC]) can add value to conventional chemical soil testing methods and increase knowledge of the effects of tillage systems, crop rotation, and N rate on soil health and corn grain yield. We studied rainfed continuous corn (*Zea mays* L.) and corn following soybean [*Glycine max* (L.) Merr.] under three tillage systems and three N rates in a long-term field experiment in northeast Nebraska. The H^3A ammonium, H^3A inorganic N, and water-extractable total N tests detected more differences (significant F tests for main effects and interactions involving all treatments) than conventional NO_3–N analysis (which detected the interaction of rotation × N rate). Water-extractable organic C detected more differences than LOI. There was a three-way interaction of tillage × rotation × N rate for Solvita and SHC; high N and intensive tillage mostly corresponded to low Solvita and SHC values. We did not expect the treatments to affect P or K directly, but the tillage × rotation interaction was significant for Mehlich III P, tillage for H^3A organic P, and N rate for H^3A inorganic and total P. Correlation analysis confirmed linear relationships between many Haney and conventional soil tests, and we concluded via sensitivity ratio calculations that the tests offered similar precision.

Abbreviations: CC, continuous corn; CS, corn following soybean; K, potassium; LOI, loss on ignition; N, nitrogen; P, phosphorus; SHC, Soil Health Calculation; SR, sensitivity ratio.

Soil tests are used to develop crop fertility recommendations, so it is important that they accurately assess plant-available nutrients. Conventional chemical soil testing methods have changed little in recent decades, and while they are the first step in assessing a soil's ability to supply nutrients to a crop, they were not designed to measure biological processes. A soil assessment that can improve our ability to predict crop growth and inform supplemental management would be a welcome development.

The concept of soil health has recently generated much interest among growers and researchers, and refers to the capacity of a soil to function as a living system (USDA-NRCS, 2017). There is a need for soil assessments that directly help producers and managers measure and monitor their efforts to improve the soil properties they associate with soil health. Two recent tests, Solvita and the Haney Soil Health Test, are emerging soil test methods which attempt to meet this need; they integrate chemical and biological soil test data to quantify soil health (Haney et al., 2006; Haney et al., 2008b). For the purposes of this paper we will call them biological soil tests. The Haney and Solvita tests were developed to help overcome some of the limitations of conventional soil test procedures and add to

Core Ideas:

- **Haney N and P tests were correlated with conventional soil NO_3–N and Mehlich III P.**
- **Corn and soybean yields were generally not correlated with soil health indicators.**
- **Haney and conventional soil test methods had similar laboratory precision as determined by sensitivity ratio analysis.**
- **Haney Soil Health Tests generated additional information, particularly about organic C and N fractions, that is not available from conventional chemical soil tests.**

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the understanding of management effects and nutrient cycling in soils. Calibration research is ongoing to establish the extent to which biological tests can predict fertilizer needs on a range of soils (Franzluebbers, 2016, 2018). The information from the Haney tests can indicate if rapid N and P mineralization rates are likely, and therefore improve accuracy of fertilizer recommendations (Harmel and Haney, 2013). However, there is already a disconnect between extension recommendations and grower implementation of lower N rates.

The Haney Soil Health Test utilizes water and an organic acid extractant, H^3A (including citric acid, malic acid, and oxalic acid), which was designed to mimic plant root exudates to accurately measure plant available nutrients (Haney et al., 2006, 2010, 2017). The Solvita test measures the CO_2 respired over 24-h after rewetting a dried soil sample and has been proposed for use in quantifying microbial activity and mineralizable N and P (Haney et al., 2008a, 2008b, 2015). The Haney and Solvita tests are components of the Soil Health Tool/Soil Health Calculation (SHC), which was developed to help growers measure the effects of management on the health of their soils (Haney, 2015; Haney et al. , 2018).

Comparisons between long-term tillage studies utilizing the SHC are problematic because a previous version of the equation (Haney, 2015) was used in work published by Mitchell et al. (2017) and Roper et al. (2017), while an updated, authoritative SHC has recently become available (Haney et al., 2018). Previously a score above 7 was considered "good" (Haney, 2015), though the current recommendation is to focus on tracking the progress of individual soils over time because efficient crop production can be achieved even on soils with low SHC (Haney et al., 2018). More research is needed to guide interpretation of these tests, to standardize laboratory procedures and resolve issues with reproducibility of results (Sullivan and Granatstein, 2015). Expense may be a barrier to grower acceptance, as the Haney test package is more than double the cost of routine soil testing ($50 versus $19 per sample [2018 USD]) and in most cases many samples must be submitted to account for field spatial heterogeneity (Ward Laboratories, 2016).

The H^3A extract is well correlated with several of the established extractants (Haney et al., 2006, 2010). Our primary interest is not in these correlations, but with what the biological tests may add to our understanding of soils and whether we can use them to quantify the effects of management practices. The purpose of this research is to compare the results of conventional chemical soil tests with the biological tests to determine what additional information can be gained about the effect of long-term tillage, crop rotation, and N fertilizer rate field treatments on soils and grain yields.

In a California study, the Haney and Solvita indicators were able to differentiate among 15-yr tillage and cover crop treatments (Mitchell et al., 2017). A long-term field study in Michigan determined C mineralization (as estimated by Solvita 24-h CO_2 evolution) was influenced more by crop rotation than by management system, and was more highly correlated with grain yields than were other measures of labile C and N (Culman et al., 2013). Harmel and Haney (2013) suggested the Haney tests may more accurately estimate fertilizer requirements than conventional soil tests, but many of the differences were not statistically significant. In North Carolina, Roper et al. (2017) reported that biological tests did not adequately differentiate between long-term management systems, though tillage did affect WEON and total Haney N on two out of three soils, and soil health test results were not correlated with crop yields. The relationship of CO_2 flush with crop N uptake has already been established (Haney et al., 2001); recent research indicates that CO_2 flush is also strongly associated with N availability in the field (Franzluebbers, 2016, Franzluebbers et al., 2018). Additional work is still necessary for Solvita and other Haney indicators to predict biologically available N across a broader range of soils and management systems.

This research was conducted using soil samples from a long-term study in northeast Nebraska comparing different tillage regimes, crop rotations, and N application rates (Blanco-Canqui et al., 2014, Segal et al., 2017). We hypothesized that over 25+ years the extremes of the treatments (plow vs. no-till; high N vs. none; continuous corn [CC] vs. corn-soybean [CS]) should have produced measurable changes in soil health, including physical properties, organic matter, nutrient cycling, and microbial activity. We expected the biological soil tests to be more sensitive and to differentiate between management systems better than the conventional chemical test procedures. Articulating interactions where properties change will help us focus further research to understand the interaction effects of tillage system, crop rotation, and N rate. We did not expect difference in P or K since those variables were not part of the treatment set; these differences could inform management practices and help develop protocols for soil testing in the future.

MATERIALS AND METHODS

Site Description and Experimental Design

This study was conducted in an ongoing, long-term field experiment located at University of Nebraska—Lincoln's Haskell Agricultural Laboratory (HAL) near Concord, NE (42.38° N lat.; 96.98° W long.). The experiment was established in 1985 on mostly Coleridge silty clay loam (fine-silty, mixed, superactive, mesic Cumulic Haplustolls) with some amount of Baltic silty clay (fine, smectitic, calcareous, mesic Cumulic Vertic Endoaquolls) and is managed under rainfed conditions. Long-term average (1973–2016) yearly precipitation and seasonal evapotranspiration (ET) at the site are 702 ± 185 (standard deviation) mm and 881 ± 105 mm, respectively. Precipitation (year in total) and ET for 2013 growing season were 820 and 1291 mm, respectively.

Soil fertility is more than adequate for most nutrients [organic matter 0.4 g kg^{-1}; K 332 mg kg^{-1}; Mehlich-III P 58 mg kg^{-1}; CEC 25.5 cmol kg^{-1}; Zn 1.4 mg kg^{-1} (DTPA extraction)], but the pH is slightly acidic (5.8) with a buffer pH of 6.3. Phosphorus fertilizer was applied when necessary, based on University of Nebraska recommendations. The experiment is a split–split plot design where whole plot treatments were in a four-replication randomized complete block design. Whole plots were tillage treatment (moldboard plow [MP], reduced tillage [RT], and no tillage [NT]), sub-plots were crop rotation (CC and CS), and sub-sub-plots were N fertilizer rate (0, 40, 80, 120, and 160 kg N ha^{-1}) applied to corn as ammonium nitrate. For this research only three of the N levels were sampled (0, 80, and 160 kg N ha^{-1}; zero N, low N, and high N). Additional site management and treatment details are provided in Blanco-Canqui et al. (2014).

Measurements and Data Collection

Corn was harvested from the middle two rows of each plot using a plot combine (Almaco SPC40, Nevada, IA) on 10 Nov. 2013 and grain was adjusted to 155 g kg^{-1} moisture. All plant residues remained in place. Soil samples were collected in November 2013 using a 1.75-cm diameter probe (JMC Soil Sampler, Clements Associates, Inc.) to extract five soil cores from each plot, to a depth of 0.2 m. Cores from each plot were composited, air dried in paper bags, and stored at ambient temperature until laboratory analysis. Samples were sent to Ward Laboratories (Kearney, NE) for conventional chemical analysis in December 2013 and for Solvita and Haney Soil Health testing in May 2014.

For the conventional soil tests, K was extracted using ammonium acetate following the method of Warncke and Brown (1998); 1:1 and Sikora buffer pH were determined according to Watson and Brown (1998) and Sikora (2006), respectively; soil organic matter was determined by loss on ignition (LOI) following Combs and Nathan (1998); NO_3–N concentration was determined using potassium chloride extraction according to Gelderman and Beegle (1998); P concentration was determined using Mehlich-III extraction (Mehlich, 1984).

For the Haney Soil Health tests, samples were extracted with water and H^3A extractant as described by Haney et al. (2010) and Haney (2015). Water extracts were analyzed for water-extractable organic C (WEOC), water-extractable organic N (WEON), and total N (WEN); water and H^3A extracts were analyzed for NO_3–N, NH_4–N, and PO_4–P, and H^3A extracts were also analyzed using inductively coupled plasma optical emission spectrometry for Al, Fe, P, Ca, and K. For the Solvita test, dried and ground soil subsamples were rewetted via capillary action and incubated with a Solvita paddle in capped jars for 24 h at 25 °C, and then the paddles were removed and digitally analyzed for CO_2 concentration (Haney et al., 2008b, Haney, 2015). Values from the Haney and Solvita tests were used to determine SHC, according to the Soil Health Tool that was available at the time of lab analysis (measurements in mg kg^{-1}; Haney, 2015).

$$\text{SHC 2015} = (\text{Solvita CO}_2 \div \text{organic C/N}) + (\text{WEOC} \div 100) + (\text{WEON} \div 10) \qquad [1]$$

When the most recent Soil Health Tool became available, we used the same laboratory test values to calculate updated SHC, as follows (measurements in mg kg^{-1}; Haney et al., 2018):

$$\text{SHC 2018} = (\text{Solvita CO}_2 \div 10) \times (\text{WEOC} \div 100) \times (\text{WEON} \div 10) \qquad [2]$$

Statistical Analysis and Calculations

For yield and soil test data, an analysis of variance (ANOVA) was used to evaluate main effects and interactions; calculations were made using the GLM procedure in SAS (SAS Institute, 2014) with a split–split-plot experimental design. The TEST statement was used to test the significance of the whole plots with ‘Error A’ (replication × tillage) and the sub-plots crop rotation and the interaction of crop rotation and tillage with ‘Error B’ (replication × tillage × rotation). LSMEANS are reported and LSD at 0.05 are reported for main effects. The LSD for tillage and rotation main effects were calculated using Error A and B, respectively.

We conducted a correlation analysis to determine relationships between pairs of analogous soil tests (for example, conventional NO_3–N and WEON). Sensitivity ratios (SR) were developed following Mandel (1984), Kull et al. (2003) and Otto-Hanson et al. (2009) for correlated pairs of analogous soil tests ($R^2 \geq 0.6$). The SR is used to compare test methods’ relative technical merit in their ability to measure a property. Assume a test method M measures a property Q where M is a function of Q, $M = f(Q)$. The sensitivity of method M in measuring Q is defined as $1/\sigma_{Q(M)}$, that is the inverse of the standard deviation of the property Q as measured by M. The larger this standard deviation, the more sensitive method M is for measuring property Q. It can be shown that the sensitivity of M can be expressed as $(dM/dQ)/\sigma_M$, where σ_M is the standard deviation of M. The sensitivity of another test method N to measure Q will yield $(dN/dQ)/\sigma_N$ similarly defined as with M. The SR for the two methods M and N is thus:

$$\text{SR}(M/N) = |dM/dN|/(\sigma_M/\sigma_N) \qquad [3]$$

where dM/dN is the relationship between the methods. If $\text{SR}(M/N) > 1$, method M is more sensitive in measuring property Q than method N and vice versa. We assumed a linear relationship between any two test methods and that dM/dN can be estimated by the slope of a linear regression of the means of M (dependent variable) regressed on the means of N (independent variable) (Kull et al., 2003; Mandel, 1984; Otto-Hanson et al., 2009). For each pair of test methods we tested, N was a conventional test and M was a Haney test which measured a similar soil parameter. We used root mean square errors from the ANOVA analyses to estimate standard deviations σ_M and σ_N. Sensitivity ratios are reported only for pairs of test methods with significant correlation ($R^2 \geq 0.6$) which was determined using PROC REG in SAS. To interpret the SR we used tests for H_0: SR ≤ 1 and H_a: SR ≥ 1 at 70 degrees of freedom, following Otto-Hanson et al. (2009), and calculated the minimum SR which would indicate superiority of test

Table 1. Analysis of variance showing main and interactive effects of tillage, crop rotation, and N rate on corn grain yields and soil properties at 0 to 0.20 m depth. WEN, water-extractable total N; WEON, water-extractable organic N; LOI, loss on ignition; WEOC, water-extractable organic C.

Main Effect and Interactions	Avg. corn grain yield 2004–2013†	2013 corn grain yield‡	Fall conventional NO_3–N§	WEN¶	WEON¶	H^3A nitrate¶	H^3A ammonium¶	H_3A inorganic N¶	Organic matter (LOI)	WEOC¶	Organic C/N¶
Tillage	NS#	*	NS	*	NS	NS	**	**	*	***	**
Rotation	***	***	NS	*	NS	NS	**	*	NS	***	NS
Rotation × Tillage	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
N Rate	***	***	***	***	***	***	***	***	NS	*	***
Tillage × N Rate	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	*
Rotation × N Rate	NS	NS	*	*	NS	NS	***	NS	NS	NS	NS
Rotation × Tillage × N Rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	9.7	7.1	25.0	14.3	10.7	31.3	34.6	27.8	9.4	6.6	13.6

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

† F-values average of annual ANOVA.

‡ 2013 was an atypical year following a severe drought in 2012.

§ Analysis using KCl extractant.

¶ Analysis conducted as part of the Haney Soil Health package.

NS, $P > 0.05$

method *M* to method *N* and the maximum SR which would indicate inferiority of test method *M* to method *N*. To reject the null hypothesis that the soil tests were equally sensitive, SR needed to be less than 0.67 or greater than 1.49. We can compare coefficients of variation (CV) for those measurements that share a common scale, but the interpretation of these measures is limited by a lack of appropriate statistical analysis (Kull et al., 2003).

RESULTS AND DISCUSSION

Grain Yields

The tillage and rotation study at HAL has become a valuable database regarding the effects of long-term agronomic management on grain yields and potential soil quality indicators (Blanco-Canqui et al., 2014; Segal et al., 2017). The ANOVA for 2013 and 10-yr corn grain yields are included to provide context to how this site is responding to the treatments (Table 1). There were consistent rotation (CS > CC) and N rate effects on grain yield, while tillage effects were more variable with significant yield differences in 3 yr of the 10-yr period (2004–2013; data not shown). No-till increased yields at HAL when seasonal precipitation was below normal (Shekhar and Shapiro, 2017). In addition to the yield effects, we hypothesized that the treatments in this long-term experiment should produce measurable changes in soil health, including physical properties, organic matter, nutrient cycling, and microbial activity.

Table 2. Main effect of rotation on grain yields and soil H^3A inorganic N, significant at *P* < 0.05.

	Avg. corn grain yield 2004–2013†	2013 corn grain yield	H^3A inorganic N‡
Rotation main effect	Mg ha^{-1}	Mg ha^{-1}	mg kg^{-1} N
Continuous corn	8.0	9.7	27
Corn-soybean	9.9	11.2	22
LSD 0.05	–	0.67	4.6

† No *F* values since means are average of annual ANOVA.

‡ Analysis conducted as part of the Haney Soil Health package

Systems often experience a temporary phase of lower productivity immediately following conversion from conventional tillage and management to conservation tillage and/or organic management, which eventually resolves due either to improvements in soil fertility or management skills (Karlen et al., 2013b; Martini et al., 2004; Seufert et al., 2012). Long-term research on agronomic management provides insights beyond the initial implementation of alternative techniques. Recent technical improvements (mostly in weed control) have increased yields, so we used data from 10 yr prior to soil testing to avoid any transition effects and to better represent current production practices in our long-term yield data.

Long-term yield was affected by crop rotation with CS producing 24% more than CC (9.9 and 8.0 Mg kg^{-1}, respectively; Table 2), but not tillage, which was mostly consistent with research on established management systems in other regions. Corn yields were not affected by tillage at two of three sites in North Carolina, but at one site no-till was higher yielding (Roper et al., 2017). In San Joaquin Valley, CA, tillage did not affect yield of cotton (*Gossypium hirsutum* L.) and tomato (*Solanum lycopersicum* L.) (Mitchell et al., 2017). A long-term study in Iowa noted that CS rotation alleviated yield suppression experienced during the establishment phase of conservation tillage in CC; after establishment, CS yields were 17% higher than CC yields and tillage was not significant (Karlen et al., 2013b). In our study, 2013 corn yield was not correlated with soil parameters ($R^2 > 0.5$; results not shown).

Table 3. Main effect of N rate on grain yield and soil properties, significant at $P < 0.05$. §

N rate main effect	Avg. corn grain yield 2004–2013†	2013 corn grain yield	WEON‡	H^{3}A nitrate‡	H^{3}A inorganic N‡	WEOC‡	H^{3}A inorganic P‡	H^{3}A total P‡	pH
	Mg ha^{-1}	Mg ha^{-1}	mg kg^{-1} N	mg kg^{-1} N	mg kg^{-1} N	mg kg^{-1} C	mg kg^{-1} P	mg kg^{-1} P	1:1
0 kg ha^{-1} N	6.8	9.8	18	4	7	216	36	48	6.1
80 kg ha^{-1} N	9.0	10.8	21	17	21	222	29	41	5.7
160 kg ha^{-1} N	9.6	10.8	19	36	45	229	30	42	5.3
LSD 0.05	–	0.44	1.2	3.5	3.9	8.5	4.2	4.8	0.16

† No *F* values since means are average of annual ANOVA.

‡ Analysis conducted as part of the Haney Soil Health package

§ WEN, water-extractable total N; LOI, loss on ignition; WEON, water-extractable organic N; WEOC, water-extractable organic C.

Soil N Indicators

As expected, ANOVA results show the strong influence of N fertilizer rate on all the N indicators ($P \le 0.001$; Table 1). The main effect of N rate was the only significant effect for WEON and H^{3}A NO_3–N. Nitrogen rate was also significant for H^{3}A inorganic N, as well as the main effect of crop rotation (CC > CS; 27 and 22 mg kg^{-1} N; Table 2, 3). Tillage main effect was significant at $P \le 0.05$ for WEN and H^{3}A inorganic N; disk and no-till had higher values than plow for WEN (46, 48, and 41 mg kg^{-1}, respectively) and H^{3}A inorganic N (25, 27, and 20 mg kg^{-1}; Table 4).

The interaction effect of rotation × N rate was significant for conventional NO_3–N, WEN, and H^{3}A ammonium (CC > CS for low and high N rates; CC = CS for zero N; Table 5). This was expected due to nonzero N rates receiving N fertilizer every year for CC rather than every other year for CS but could also have been due to additional residue produced in fertilized CC. Tillage × N rate interaction was significant for H^{3}A ammonium, with tillage apparently counteracting the influence of N application (Table 6).

We expected to find lower values for the N indicators in the plowed system due to more rapid mineralization and decomposition of organic matter since residues were fully incorporated into the soil. Tillage was approaching significance ($P < 0.1$) for conventional NO_3–N and several Haney N indicators (data not shown). Research in Iowa also reported the effect of tillage at $P \le 0.1$ for conventional NO_3–N and potentially mineralizable N at 0 to 5 cm depth, with generally lower values for moldboard plow than other tillage systems (Karlen et al., 2013a, Karlen et al., 2013b). Mitchell and colleagues (2017) reported WEON was higher in a no-till system than with conventional tillage, but the effect was only significant at 0 to 5 cm depth (not 5–15 or 15–30 cm) and in the fall but not in the spring. It is possible that the lack of tillage effect on WEON in our study was due to sampling technique and that potential stratification between depths (i.e., elevated WEON in surface soil of no-till systems) may have been masked by mixing the entire 0- to 20-cm–depth soil cores. However, Roper et al. (2017) found WEON and total Haney N in two North

Table 4. Main effect of tillage on grain yield and soil properties, significant at $P < 0.05$. ‡

Tillage main effect	2013 corn grain yield	WEN†	H^{3}A inorganic N†	Organic matter (LOI)	H^{3}A organic P†
	Mg ha^{-1}	mg kg^{-1} N	mg kg^{-1} N	mg kg^{-1}	mg kg^{-1} P
Disk	10.8	46	25	41	13
No-till	10.1	48	27	42	13
Plow	10.5	41	20	38	11
LSD 0.05	0.40	4.5	3.1	3.4	1.7

†Analysis conducted as part of the Haney Soil Health package

‡ WEN, water-extractable total N; LOI, loss on ignition.

Table 5. Two-way interaction of rotation and N rate on soil nitrate, water-extractable total N (WEN), and H^{3}A ammonium, significant at $P < 0.05$.

Rotation × N rate interaction		Fall conventional NO_3–N†	WEN‡	H^{3}A ammonium‡
		mg kg^{-1} N	mg kg^{-1} N	mg kg^{-1} N
Continuous corn	0 kg ha^{-1} N	4.7 ± 1.89§	25 ± 4.3	3.3 ± 1.21
Continuous corn	80 kg ha^{-1} N	25.1 ± 9.02	48 ± 11.3	4.6 ± 2.02
Continuous corn	160 kg ha^{-1} N	45.4 ± 7.81	71 ± 10.6	10.8 ± 4.54
Corn-soybean	0 kg ha^{-1} N	7.2 ± 2.65	24 ± 2.4	2.7 ± 0.57
Corn-soybean	80 kg ha^{-1} N	18.2 ± 4.08	38 ± 5.6	3.3 ± 1.20
Corn-soybean	160 kg ha^{-1} N	41.3 ± 9.03	62 ± 10.8	6.2 ± 2.94

† Analysis using KCl extractant.

‡ Analysis conducted as part of the Haney Soil Health package.

§ Treatment means ± 1 standard deviation.

Table 6. Two-way interaction of tillage and N rate on soil H^{3}A ammonium and organic C/N, significant at $P < 0.05$.

Tillage × N rate interaction		H^{3}A ammonium†	Organic C/N†
		mg kg^{-1} N	C/N
Disk	0 kg ha^{-1} N	2.4 ± 0.16‡	11.9 ± 1.19
Disk	80 kg ha^{-1} N	3.3 ± 1.17	10.6 ± 1.20
Disk	160 kg ha^{-1} N	9.6 ± 5.37	13.9 ± 4.04
No-till	0 kg ha^{-1} N	3.4 ± 0.93	13.2 ± 0.84
No-till	80 kg ha^{-1} N	5.5 ± 1.88	11.1 ± 0.93
No-till	160 kg ha^{-1} N	10.7 ± 2.95	13.9 ± 1.75
Plow	0 kg ha^{-1} N	3.1 ± 1.28	11.6 ± 1.63
Plow	80 kg ha^{-1} N	2.9 ± 0.92	9.9 ± 0.78
Plow	160 kg ha^{-1} N	5.2 ± 2.67	10.2 ± 0.72

† Analysis conducted as part of the Haney Soil Health package.

‡ Treatment means ± 1 standard deviation.

Table 7. Correlation and CV for conventional and Haney soil N test components. ¶

Soil N test	CV (%)	R^2 ‡ (linear regression vs. conventional NO_3–N)
Conventional NO_3–N	25.0	–
WEN †	14.3	0.956
WEON †	10.7	0.008
H^3A nitrate †	31.3	0.958
H^3A ammonium †	34.6	0.508
H^3A inorganic N †	27.8	0.937

† Analysis conducted as part of the Haney Soil Health package.

‡ Haney N test method means were each regressed on the mean of the conventional N test.

¶ WEN, water-extractable total N; WEON, water-extractable organic N

Carolina soils (sampled to 15 cm) increased with decreasing tillage intensity, with significant differences despite soil samples not having been separated by depth. We have not found any literature reporting on long-term field studies with which to compare our results for the H^3A N indicators.

We compared N indicators from the Haney suite of tests with conventional NO_3–N analysis and found WEN, H^3A NO_3–N, and H^3A inorganic N to be positively correlated ($R^2 > 0.9$; Table 7); for these tests, we conducted a SR analysis and determined that the correlated Haney N tests were no more or less sensitive than conventional NO_3–N (SR between 0.9 and 1.1; data not shown). Random variation associated with the means (CV) is not a robust statistical comparison, but still interesting to consider. The H^3A NO_3–N, ammonium, and inorganic N were equally or more variable than conventional NO_3–N (CV of 31.3, 34.6, 27.8, and 25.0%, respectively) while WEN and WEON were less variable (CV of 14.3 and 10.7, Table 7). The H^3A inorganic N and H^3A ammonium tests both detected more treatment differences (more significant effects) than conventional NO_3–N; H^3A inorganic N detected main effects of tillage, rotation, and N rate; H^3A ammonium detected the interactions of tillage*N rate and rotation*N rate; conventional NO_3–N detected rotation*N rate.

Table 9. Two-way interaction of tillage and rotation on soil water-extractable organic C (WEOC) and Mehlich III P, significant at $P < 0.05$.

Tillage × rotation interaction		WEOC†	Mehlich III P
		mg kg^{-1} C	mg kg^{-1} P
Disk	Continuous corn	241 ± 28.3‡	74 ± 28.7
Disk	Corn-soybean	213 ± 13.5	51 ± 19.9
No-till	Continuous corn	261 ± 21.9	74 ± 22.1
No-till	Corn-soybean	229 ± 14.6	61 ± 26.9
Plow	Continuous corn	196 ± 17.3	37 ± 9.9
Plow	Corn-soybean	195 ± 21.3	56 ± 28.5

† Analysis conducted as part of the Haney Soil Health package.

‡ Treatment means ± 1 standard deviation.

Soil P and K Indicators

The conventional and Haney P tests' ANOVAs detected relatively few significant effects (Table 8). The interaction of rotation × tillage was significant for Mehlich III P with the highest (disk and no-till; both 74 mg kg^{-1} P) and lowest (plow; 37 mg kg^{-1}; Table 9) values in CC. Corn–soybean rotation averaged 56 mg kg^{-1} Mehlich III P across tillage systems. The main effect of N rate was significant for H^3A total P and inorganic P (zero N > low and high N), and H^3A organic P identified tillage main effects (plow < no-till and disk).

Karlen et al. (2013b) also found lower Mehlich-III P in moldboard plow (22 g kg^{-1}) than in chisel plow, disk, ridge till, and no-till systems (33 g kg^{-1} P on average). Differences in soil P response to tillage system and/or crop rotation could be linked to P removal via grain yields, but if this was a primary mechanism we would expect a stronger effect of N rate on soil P. This could be an artifact of the soil sampling depth relative to the tillage depth. Although some differences were significant within tillage × rotation interaction for conventional Mehlich III P, none of the treatments would have been yield limiting according to University of Nebraska Extension recommendations (Shapiro et al., 2008). A study in Minnesota by Kaiser et al. (2016) comparing Haney H^3A and conventional P tests determined similar critical values for H^3A, Mehlich-III, and Bray P1, suggesting the potential for Haney tests to be used to determine P fertilizer needs.

Table 8. Analysis of variance showing main and interactive effects of tillage, crop rotation, and N rate on soil properties at 0 to 0.20 m depth. SHC, soil health calculation.

Main Effect and Interactions	Solvita CO_2†	SHC 2015†	SHC 2018†	Mehlich III P	H^3A inorganic P†	H^3A total P†	H^3A organic P†	Conventional K	H^3A K†	pH
Tillage	NS ‡	NS	*	NS	NS	NS	*	NS	NS	NS
Rotation	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rotation × Tillage	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
N Rate	***	***	***	NS	**	*	NS	NS	NS	***
Tillage × N Rate	***	***	***	NS	NS	NS	NS	NS	NS	NS
Rotation × N Rate	**	*	*	NS	NS	NS	NS	NS	NS	NS
Rotation × Tillage × N Rate	**	**	*	NS	NS	NS	NS	NS	NS	NS
CV, %	12	8.3	16.8	19.5	22.8	18.6	13.4	17.2	16.4	4.79

* P ≤ 0.05

** P ≤ 0.01

*** P ≤ 0.001

† Analysis conducted as part of the Haney Soil Health package.

‡ NS, P > 0.05

Table 10. Correlation and CV for conventional and Haney soil P test components.

Soil P test	CV (%)	R^2 (linear regression vs. conventional Mehlich III P)‡
Mehlich III P	21.0	–
H^3A total P †	18.7	0.884
H^3A organic P †	13.2	0.483
H^3A inorganic P †	22.9	0.832

† Analysis conducted as part of the Haney Soil Health package.

‡ Haney P test method means were each regressed on the mean of the conventional P test.

Table 11. Correlation and CV for conventional and Haney soil K tests components.

Soil K test	CV (%)	R^2 (linear regression vs. conventional K)‡
Conventional K	17.2	–
H^3A K †	16.3	0.965

† Analysis conducted as part of the Haney Soil Health package.

‡ Haney K test mean was regressed on the conventional K test mean.

We compared Haney P indicators with conventional Mehlich III P and found H^3A total P and H^3A inorganic P to be positively correlated ($R^2 > 0.8$; Table 10); for these tests, we determined via SR analysis that the correlated Haney P tests were not more or less sensitive than Mehlich III (SR was 1.0 for each; data not shown). Mehlich III P, H^3A total P, and H^3A inorganic P were similarly variable (CVs were 21.0, 18.7, and 22.9%, respectively; Table 10).

Conventional and Haney (H^3A) K tests' ANOVAs were similar with no treatment effects or interactions significant at $p \leq 0.05$ (Table 8). Average H^3A K was 119 mg kg^{-1}. Conventional K was 330 mg kg^{-1}, which is more than adequate to support crop production (Shapiro et al., 2008). Conventional K was highly correlated with H^3A K ($R^2 = 0.97$). We calculated a sensitivity ratio of 0.84 which does not indicate a difference in sensitivity (Table 11). Conventional and H^3A K had similar CVs (17.2 and 16.3%).

Soil C Indicators

The conventional organic matter test was less similar to its Haney test counterparts (WEOC, organic C/N, Solvita CO_2, and SHC) than the analogous N tests. The LOI test only detected the main effect of tillage (plow < disk and no-till; 38, 41, and 42 mg kg^{-1}, respectively; Tables 1, 4). The main effect of N rate and the interaction of rotation*tillage were significant for WEOC. Continuous corn > CS for disk (241 and 213 mg kg^{-1}) and no-till (261 and 229 mg kg^{-1}); CC = CS for plow (196 and 195 mg kg^{-1}; Table 9). Tillage*N rate was significant for organic C/N. Within the interaction, organic C/N was lower in low N than zero N across tillage systems. In the plow system, organic C/N was the same for high and low N (10.1 on average); in the disk system, organic C/N was greater for high N than zero N (13.9 and 11.9, respectively); in the no-till system, organic C/N was the same for high and zero N (13.5 on average; Table 6). Organic C/N values were within good (13–15) to ideal (8–12) ranges for supporting microbial activity and nutrient cycling according to Haney (2015). We expected soil organic matter to be greater in CC than CS, because corn residue has higher C/N than soybean and presumably would be more persistent in the system. It was surprising, therefore, that rotation was not significant for LOI or organic C/N. Water-extractable organic C is likely a better measure of potential microbial activity than LOI or total C, since it represents the fraction of total organic matter that is immediately available to soil microbes (Mitchell et al., 2017).

There was a significant three-way interaction for the Solvita CO_2 test (Table 8). Within the no-till system, CO_2 evolution was highest for CC at zero N, CS at zero N, and CS at low N (106, 99, and 98 mg kg^{-1} CO_2, respectively) and lowest for CC high N (51 mg kg^{-1} CO_2; Table 12). In the disk system, CC at high N had lower CO_2 evolution (51 mg kg^{-1} CO_2) than the other rotation × N rate combinations (85 mg kg^{-1} CO_2 on average). In the plow system (77 mg kg^{-1} CO_2 on average) there were no rotation or N rate effects. Differences in CO_2 evolution were more pronounced in no-till than in tilled systems; low Solvita values for CC receiving high N may have been due to high C/N residue accumulating yearly and remaining on the soil surface. Nitrogen immobilization could have limited the microbial activity in those treatments. Culman et al. (2013) reported a lack of management (tillage) system effect on Solvita CO_2 and suggested that treatment differences could

Table 12. Three-way interaction effect of tillage, crop rotation, and N rate on Solvita 24-h CO_2 flush, Soil Health Calculation (SHC) 2015, and SHC 2018, significant at $P < 0.05$.

Tillage × rotation × N rate interaction			Solvita CO_2†	SHC 2015†	SHC 2018†
			mg kg^{-1} CO_2–C	Index	index
Disk	Continuous Corn	0 kg ha^{-1} N	90 ± 3.2‡	12 ± 1.5	38 ± 11.0
Disk	Continuous Corn	80 kg ha^{-1} N	84 ± 17.1	12 ± 1.3	44 ± 11.9
Disk	Continuous Corn	160 kg ha^{-1} N	51 ± 6.2	8 ± 1.5	24 ± 9.2
Disk	Corn-Soy	0 kg ha^{-1} N	80 ± 23.2	11 ± 2.9	33 ± 16.0
Disk	Corn-Soy	80 kg ha^{-1} N	82 ± 14.1	12 ± 1.8	36 ± 10.8
Disk	Corn-Soy	160 kg ha^{-1} N	86 ± 26.3	11 ± 3.2	35 ± 18.2
No-till	Continuous Corn	0 kg ha^{-1} N	106 ± 6.5	12 ± 1.1	50 ± 13.4
No-till	Continuous Corn	80 kg ha^{-1} N	82 ± 17.7	12 ± 1.4	51 ± 13.8
No-till	Continuous Corn	160 kg ha^{-1} N	51 ± 14.4	8 ± 1.4	27 ± 10.9
No-till	Corn-Soy	0 kg ha^{-1} N	99 ± 10.1	12 ± 1.0	39 ± 6.1
No-till	Corn-Soy	80 kg ha^{-1} N	98 ± 9.0	13 ± 0.7	48 ± 7.9
No-till	Corn-Soy	160 kg ha^{-1} N	72 ± 15.2	9 ± 0.5	29 ± 5.9
Plow	Continuous Corn	0 kg ha^{-1} N	78 ± 6.4	10 ± 1.0	25 ± 2.1
Plow	Continuous Corn	80 kg ha^{-1} N	76 ± 10.5	12 ± 1.4	29 ± 5.6
Plow	Continuous Corn	160 kg ha^{-1} N	73 ± 7.3	11 ± 0.6	29 ± 3.6
Plow	Corn-Soy	0 kg ha^{-1} N	83 ± 12.2	11 ± 0.6	28 ± 8.0
Plow	Corn-Soy	80 kg ha^{-1} N	79 ± 10.3	12 ± 1.4	30 ± 9.2
Plow	Corn-Soy	160 kg ha^{-1} N	71 ± 13.6	11 ± 0.8	27 ± 5.8

† Analysis conducted as part of the Haney Soil Health package.

‡ Treatment means ± 1 standard deviation.

Table 13. Correlation and CV for conventional and Haney soil C test components. ¶

Soil C test	CV (%)	R^2 (linear regression vs. conventional organic matter LOI)‡
Organic matter (LOI)	9.2	–
WEOC †	6.6	0.290
Organic C/N †	13.3	0.103
Solvita CO_2 †	12.4	0.019
SHC 2015 †	8.3	0.030
SHC 2018 †	16.8	0.028

† Analysis conducted as part of the Haney Soil Health package.

‡ Haney C test method means were each regressed on the mean of the conventional LOI test.

¶ LOI, loss on ignition; WEOC, water-extractable organic C; SHC, soil health calculation.

have been masked by spatial heterogeneity in the field. The Solvita test has been used to predict crop N uptake and soil N availability (Franzluebbers, 2018; Franzluebbers et al., 2018), but there are concerns regarding cross-laboratory reproducibility of results (Sullivan and Granatstein, 2015; Wade et al., 2018). Standardized protocols would improve the robustness of CO_2 flush tests, as demonstrated by variations in mineralizable C resulting from procedural differences including water delivery method (Franzluebbers and Haney, 2018; Wade et al., 2018). Franzluebbers and Haney (2018) noted inhibition of C mineralization in coarse-textured soils rewetted by capillary action, but in fine-textured soils mineralizable C was not affected by rewetting method.

Organic matter by LOI was not correlated with Haney C indicators, Solvita CO_2, or SHC, which was expected because LOI measures total SOM while the Haney tests were developed to reflect only the biologically active portion (Haney et al., 2018; Table 13). Water-extractable organic C was less variable than LOI (CV of 6.6 and 9.2%; Table 13) while Solvita and organic C/N were more variable (CV of 12.4 and 13.3%). The Haney C indicators provided a much more complex and informative analysis than the conventional LOI test with rotation and N rate significant effects and interactions as well as tillage.

Soil Health Calculations

Analysis of variance was similar for SHC 2015 and SHC 2018 with a significant three-way treatment interaction of rotation × tillage × N rate for both (Tables 8, 12). The major differences between SHC 2015 and 2018 are the omission of organic C/N from the updated equation, and the shift from addition of CO_2, WEOC, and WEON components to multiplication. Solvita is the main driver of SHC with almost identical ANOVA results and treatment effects. Values for the SHC 2018 differ from the 2015 version in magnitude, with greater numerical separation between means for SHC 2018, though overall p value for the three-way interaction was lower for SHC 2015 (p = 0.0018 compared with 0.0489 for SHC 2018). Since treatment effects were mostly the same for the two calculations, we will focus on results for the updated SHC 2018. Within the three-way interaction, N rate was significant for disk CC (SHC 2018 = 44, 38, and 24 for zero, low, and high N rates, respectively), no-till CC (SHC 2018 = 50, 51, and 27), and no-till CS (SHC 2018 = 39, 48, and 29), but not for disk CS (SHC 2018 = 35 on average), plow CC, or plow CS (SHC 2018 = 28 for both; Table 12). There was a general trend of lower SHC 2018 at high N in the no-till and disk systems, but it appears that intensive tillage overrode the N rate effect since N rate was not significant in the plowed system. It is unclear whether the rotation differences indicate a meaningful trend.

We are unaware of other long-term studies utilizing SHC 2018 with which to compare our results. Mitchell et al. (2017) reported SHC 2015 of 6.9 for conventional tillage system (plowed and cultivated) and 9.0 for no-till in 0- to 5-cm depth. We applied the updated equation to their reported values for Solvita CO_2 evolution, WEOC, and WEON, and calculated SHC 2018 of 12.1 for conventional tillage and 24.9 for no-till. We also calculated SHC 2018 for the Haney test values reported by Roper et al. (2017) which, compared to the previous SHC calculations, amplified the contrasts between management systems and soils (Table 14).

Table 14. 24-hour CO_2 evolution (Solvita CO_2), water-extractable organic C and N (WEOC, WEON), and soil health calculations (SHC) for long-term tillage experiments from the present study and the literature. Letters indicate groupings for statistical differences at P < 0.05.

Location (soil)	Tillage system	Solvita CO_2†	WEOC†	WEON†	SHC 2015†	SHC 2018†
		mg kg^{-1} CO_2–C	mg kg^{-1} C	mg kg^{-1} N	index	index
Northeast NE (silty clay loam) ‡	disk	79	227 b	19	11	35 ab
	no-till	85	245 a	20	11	41 a
	plow	77	196 c	19	11	28 b
San Joaquin Valley, CA (clay loam)§	conventional till	23	254	20	7	12
	no-till	40	281	22	9	25
Piedmont area, NC (sandy loam)¶	no-till	124 a	154	16 a	16 a	31
	chisel plow/disk	52 b	101	10 ab	8 b	5
	moldboard plow/disk	34 b	85	9 b	5 b	3
Mountain area, NC (silt loam)¶	no-till	80	315	26	12	66
	conventional till	48	237	16	7	18

† Components of the Haney Soil Health Tool (Haney, 2015; Haney et al., 2018)

‡ 0 to 20 cm depth

§ 0 to 5 cm depth (Mitchell et al., 2017)

¶ 0 to 15 cm depth (Roper et al., 2017)

Haney et al. (2018) expected but did not find decreasing SHC with increasing N rate (average was 172.5 kg N ha^{-1}), but the management systems they surveyed would not have had N rates applied consistently over time. In our study the low N rate increased SHC while high N decreased SHC relative to zero N, suggesting a nonlinear relationship between N rate and SHC. The 2015 Soil Health Tool publication stated that SHC of 7 or higher indicates a "good" score (Haney, 2015). Haney et al. (2018) do not propose a universal favorable score but emphasize the importance of monitoring over time with the goal of improving SHC on individual soils. Our data support the shift away from using a set threshold to indicate a favorable score, since the most intensive tillage and highest N treatments still scored SHC 2015 above 7 (Table 12).

CONCLUSIONS

The Haney and conventional soil tests detected some differences among management systems but results for both suites of tests indicate statistical significance that may not translate to agronomic significance. We expected that the management systems with the most favorable soil health indicators would also be the highest yielding, but yields were not correlated with soil parameters. We speculate that results might have been more straightforward on soils with less inherent fertility. Crop rotation influenced corn grain yields both in 2013 and over the 10-yr period but did not have clear effects on SHC; tillage system influenced SHC but not long-term average grain yields. Fertilizer-N rate was highly significant for yields and for many soil parameters.

Use of SR to compare soil test methods is a novel application; previously, SR analysis has been used to compare disease screening methods (Kull et al., 2003; Otto-Hanson et al., 2009). It is possible that differences in sensitivity between the tests may have been apparent with a different experimental design, a range of soils, or with a larger sample size. The Haney tests added some interesting information to the conventional/routine soil test panel, especially in the organic C, N, and P fractions, and they discerned among some long-term management factors. These components can be useful in predicting N and P release. Many of the Haney soil nutrient tests were correlated with their analogous conventional tests, but our SR analysis did not indicate superiority of one method over another in terms of precision. Additional research on the impacts of management on soil parameters is needed, particularly where soil differences do not translate to yield response. The Haney Soil Health Test would be especially useful on fields where crop production history is unknown or to quantify the effects of a change in management such as cover cropping to replace fallow periods in an annual system.

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