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Seasonal Variation in Soil Bulk Density, Organic Nitrogen, Available Phosphorus, and pH

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USDA-ARS 48037 Tubbs Ranch Rd Adams, OR 97810 Scientists are being called on to measure and predict the effects of soil management and climate change on organic matter and other soil properties. The estimates and predictions generated from current conditions and short-term experiments will only be accurate if our measurement techniques produce data that represent actual soil properties during the period of prediction. Perhaps the least studied aspect of soil sampling is the possibility that the timing of samples might introduce large non-random errors. This study sampled replicated plots every month for 39 mo. Even after removing the measurement variation caused by surface soil bulk density fluctuations, variations in organic N, available P, and pH were always >10% of the mean. Much of the variation appeared to be temporally correlated in a seasonal cycle. In this experiment, with a 1-yr crop rotation, averaging 12 monthly samples allowed differences among soil treatments to be detected that were within 2 to 4% of the mean. When highly accurate estimates are desired, researchers need to consider combining multiple sample timings to overcome temporal variability.

Abbreviations: STL, seasonal decomposition of time series by loess.

ommon sampling methods are probably hampering the efforts of soil scientists in two aspects: measurement of soil constituents under a constantly changing soil surface elevation, and a lack of consideration for seasonal variation. Using the soil surface as the principle measuring point when its elevation fluctuates with time has a direct influence on the quantification of soil constituents under different soil management and weather conditions (Desaules, 2012; VandenBygaart and Angers, 2006). It also has a direct effect on laboratory measurement of constituent concentrations in any case where a vertical gradient exists. The second factor, temporal variation, adds to our uncertainty of measurement just like other forms of experimental error: spatial variability, differences between operators, handling and storage of samples, and instrument error.

We know very little about temporal variability because we rarely sample for it. In the cases where samples have been taken over time, the variability measured is usually substantial. A search for research reports where sites were sampled for organic C more than once in a single year resulted in soil C estimates from the same soil that differed from 18 to as much as 53% during a period of from 1 to 3 mo (Boerner et al., 2005; Dormaar et al., 1977; Scott et al., 1994; Stoyan et al., 2000). In a study where sampling was repeated multiple times across a period of 5 mo, repeated organic C estimates varied 4 to 20% within treatments (Campbell et al., 1999a, 1999b). As always, some of the variability measured would have been due to spatial variability or sample handling and analysis uncertainties, but it is

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likely that some soil constituents undergo seasonal cycles due to temperature, moisture, and other abiotic and biotic factors.

The objective of this research was to measure the variation in several soil properties with time to find out how the timing of soil samples might affect our ability to accurately measure long-term changes or meaningful differences between management treatments. An earlier study (Wuest, 2014) reported variations in soil organic C. This study investigated other soil properties measured at the same time.

MATERIALS AND METHODS

Three tillage treatments were compared in plots planted every fall with winter wheat (*Triticum aestivum* L.) near Pendleton, OR, on a Walla Walla silt loam (a coarse-silty, mixed, superactive, mesic Typic Haploxeroll). The 3.6- by 53-m plots were replicated in four randomized, complete blocks. Fertilizer, crop management, and crop rotation were identical. Fertilizer N at 100 kg ha⁻¹ and P at 20 kg ha⁻¹ were applied at planting time, which was mid-October. The average harvested grain yield was 3910 kg ha⁻¹. The three tillage treatments were (i) no-till, (ii) tillage to incorporate crop residues after harvest and before planting, and (iii) a novel treatment where surface residues were raked onto a tarp, the plots tilled the same as the above tillage treatment, and then the surface residues spread back onto the plot surface.

Plots were sampled mid-month in a transect, moving the sampling site 30 cm down a crop row each month. A different transect location was chosen each crop year. Soil cores were taken from the crop row, from halfway between rows, and from between the first two cores, and each core was divided into 5-cm increments to the 30-cm depth. The total core cross-sectional area was 51.17 cm² (4.66-cm-diameter cores, three per sample). Samples were weighed and spread to dry in an oven at 40°C within an hour of collection. The three cores from each plot were combined by depth increment. Analyses included water content (kg kg⁻¹ dry soil), total C and N by dry combustion, inorganic NO₃-N and NH₄-N (1 mol L⁻¹ KCl extraction, colorimetric detection), available PO₄-P (Olsen-P NaHCO₃ extraction, colorimetric detection), and pH (0.01 mol L⁻¹ CaCl₂). Dry mass and bulk density were determined for each sample. Inorganic NO3 and NH4 were subtracted from the total N to get organic N. For constituents where quantitative measurements were needed (P and organic N), cumulative amounts were calculated based on the sample area and mass of each 5-cm increment and then interpolated to equivalent mass-depths of 0 to 100 and 0 to 250 kg m⁻². These depths are approximately 0 to 7 and 0 to 20 cm. Soil bulk density and pH values were also interpolated to soil depths equivalent to 100 and 250 kg m⁻² (approximately 7 and 20 cm).

Statistics

Seasonal decomposition of time series by loess (STL) (Cleveland et al., 1990), as presented for R (R Development Core Team, 2012), was used to search for a seasonal compo-

nent among the monthly data of each treatment. This method searches for a repeating pattern on a 12-mo period by averaging data for each calendar month across years. (For other examples of this method, see Li et al. [2003] and Randerson et al. [1999].) These monthly averages are subtracted from the raw data. An overall trend is determined by smoothing the remaining data using locally weighted polynomial regression. Outliers are identified, their weighting factor reduced, and the process repeated. When the seasonal and long-term trend components converge on a stable solution, they are subtracted from the original data to produce the remaining, unexplained component for each month. The final output consists of four plots: the original data, the seasonal component, the long-term trend, and the remainder.

The STL procedure does not test the precision of the data. As a measure of the quality of the data set, we used a generalized linear mixed model (Littell et al., 2006) to test for significant differences between treatments and crop years (November–October).

RESULTS

A seasonal trend in soil organic C was discussed in detail by Wuest (2014). In this study, organic N, available P, pH, and soil bulk density were examined. Soil bulk density and pH were also examined in 5-cm increments.

Table 1 gives the mean and range for each measurement experiment-wide and by treatment. During the 39-mo period, organic N and pH varied from 9 to 18% of their respective means, with little difference in variance between the shallow and deeper soil depths. Available P variation ranged from 55 to 72% near the surface and from 35 to 71% from 0 to 250 kg m⁻². Soil bulk density varied from 19 to 33%, considering both depths together. In most cases, more than one-third of the range in monthly means could be assigned to a seasonal component; that is, the means of a particular calendar month tended to be greater or less than the overall average.

The measured time series data, averaged by treatment, are plotted in Fig. 1 and 2, the top graph in each set of four. The data mostly demonstrate gradual changes from month to month, but abrupt changes were not uncommon. Three years of data are not enough to establish well-defined seasonal trends, but it can be seen in Fig. 1 and 2 that the seasonal components (monthly deviations from the overall average) were correlated with time, with most of the change consisting of smooth increases or decreases across periods of several months. No fitting or smoothing is involved with the STL seasonal component, so the correlation between neighboring months was simply what resulted after computing the average of the three or four values for a month during the course of the 39-mo experiment. The three soil treatments follow similar seasonal trajectories, except in the case of organic N, where no-till has slightly greater maximums and lower minimums in both the shallow and deeper samples.

Abrupt changes in the seasonal cycle of pH and P near the surface occurred in November, immediately after the seeding and fertilizing operation, as one would expect. This was also the time

Table 1. Range of 39 monthly means of measurements of organic N, available P, bulk density, and pH as a percentage of the experiment-wide (n = 12) and treatment (n = 4) means. Soil depths are the top 100 kg m-2 (approximately 0-7 cm), and the top 250 kg m-2 (approximately 0-20 cm) for N and P. Bulk density and pH data were interpolated to locations in the soil profile at depths of 100 and 250 kg m-2. Also shown is the range for the seasonal component of the data determined by seasonal decomposition of time series by loess (STL) (Cleveland et al., 1990).

Treatment	Mean	Range	Seasonal range	Mean	Range	Seasonal range		
		0–100 kg m ^{–2} de	<u>pth</u>	<u>(</u>	0–250 kg m ^{–2} de	epth		
	$\mathrm{g}\;\mathrm{m}^{-2}$		- %	$\mathrm{g}\;\mathrm{m}^{-2}$		- %		
Organic N	118	18	8	272	13	6		
Tilled	118	11	3	272	9	2		
No-till	119	17	8	268	10	6		
Tilled, surface residue replaced	118	10	4	276	11	4		
Available P	2.5	68	30	5.6	71	20		
Tilled	2.3	72	30	5.2	42	17		
No-till	2.8	55	21	6.2	53	16		
Tilled, surface residue replaced	2.4	56	29	5.5	35	17		
		100 kg m ⁻² dep	<u>th</u>		250 kg m ⁻² dep	<u>oth</u>		
Bulk density	1.16	33	14	1.28	29	9		
Tilled	1.12	30	15	1.27	28	6		
No-till	1.21	22	9	1.30	27	8		
Tilled, surface residue replaced	1.14	30	13	1.25	19	7		
			- %					
рН	5.02	17	7	5.34	15	5		
Tilled	5.11	11	5	5.48	10	4		
No-till	4.91	10	5	5.20	10	6		
Tilled, surface residue replaced	5.05	15	7	5.35	12	4		

of year when the soil was re-wet by fall rain after becoming very dry during the summer.

Note that the remainders for many of the soil measurements show periods of distinctly time-correlated patterns. For example, in Fig. 1 the P measurements for both depth increments produce remainders that increment in a linear fashion from the second January through the second August. This indicates a deficiency in our STL model if we are trying to capture the entire pattern of what is happening with time. The STL method did not capture all of the time-correlated changes because it can only assign to season those effects that occur on exactly the same month every year. If we look at the P remainder from the third January through the third August, we see that the time-correlated change is downward instead of upward. The two periods are contradictory, meaning that these are 2 yr where, after identifying the monthly effects that are consistent in all 3 yr, and the overall smoothed trends, the remainders show some time correlation that is inconsistent from year to year. Given year-to-year variability in weather, it is not surprising to see inconsistencies, and it would take much more than 3 yr to produce a stable model of average annual soil dynamics. The goal of this study was to explore the magnitude of seasonal variation and how this might affect the accuracy of soil parameter estimates, so the residuals are not discussed further other than to say that our best guess as to the cause of year-to-year differences would be differences in rainfall patterns and that the estimates of the magnitude of seasonal effects may be conservative.

Because the samples were taken in 5-cm increments, we can also examine the measurements without adjustment to

equivalent mass-based depth. Figures 3 and 4 show pH and bulk density. Bulk density varied from about 0.6 to 1.2 g cm $^{-3}$ at the surface (Fig. 4, 0-5-cm plot, data graph). The second November to June period had a particularly dramatic drop in density, probably due to freeze-thaw conditions in these loess soils. A change in bulk density is necessarily accompanied by a change in surface elevation relative to soil horizons below the surface. For example, a decrease in surface bulk density means that the same mass of soil has a greater volume, and this expansion of volume must take place upward. A 20% decrease in the average bulk density in the top 30 cm would require roughly 6 cm of surface elevation rise. Because we sampled to the same distance from the soil surface, the samples were effectively less deep than under a denser surface condition. This effect is visible in the pH data, especially at the 20- to 25-cm depth (Fig. 3, 20-25 cm, data graph). Comparing the 13th and 16th months (the second November and second February), our oven-dry weight data show that the mass of soil recovered in February from 0 to 20, 0 to 25, and 0 to 30 cm was about 50 kg m^{-2} less than it had been in November. Therefore, part of the pH seasonal change seen in the measurements based on depth from the soil surface (Fig. 3) is because pH decreases as we move up in the soil profile, and our February samples were effectively shallower due to the greater elevation of the soil surface.

The influence of soil bulk density can also be measured by comparing its correlation with the standard depth method vs. the equivalent mass method. Linear regression of pH at 20 to 25 cm against bulk density at 0 to 5 cm produces an r^2 of 0.16 (p > F, 0.0001). When we interpolate pH to an equivalent mass

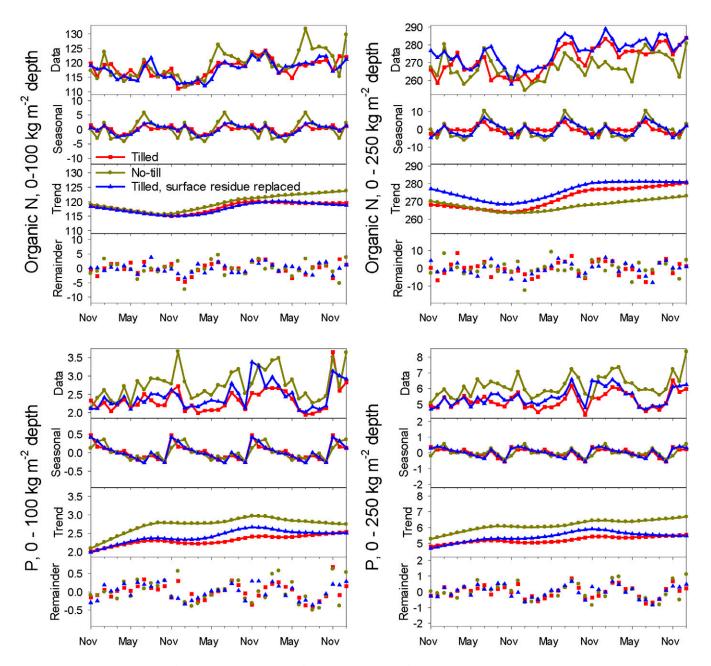


Fig. 1. Seasonal decomposition of time series by loess (STL) for soil samples taken for 39 consecutive months. Soil organic N in the top 100 kg m⁻² (approximately 0–7-cm depth), soil organic N in the top 250 kg m⁻² (approximately 0–20-cm depth), available P in the top 100 kg m⁻², and available P in the top 250 kg m⁻². All units are grams per square meter. In each analysis, the data (top graph), seasonal component (second graph), trend (third graph), and remainder (fourth graph) have equal range on the *y* axis. The replicated plots were planted annually with winter wheat under three soil management treatments: (i) no-till, (ii) tilled after harvest to 15 cm, incorporating surface residues into the tilled layer, and (iii) tilled as above, but surface residues raked aside and replaced on the soil surface after the soil was tilled.

depth of 250 kg m⁻² (approximately 20 cm, Fig. 2), the regression against bulk density at 0 to 5 cm produces an r^2 of only 0.01 (p > F, 0.057). This decrease in correlation is because equivalent mass locates the sample in the same soil horizon regardless of volume changes above it or below it.

The STL analysis does not give us a statistical probability that differences actually exist among treatments or years. Table 2 contains the results from generalized linear model analysis. When months were grouped into three crop years, all four soil measurements showed significant treatment effects at both depths. In most cases, crop years differed also. Table 3 shows the same statistics for pH and bulk density at 5-cm-increment

depths. Note the interaction between crop year and treatment for the highly variable bulk density.

DISCUSSION

Thirty-nine consecutive soil samples demonstrated considerable temporal variability in all parameters measured (Fig. 1 and 2). Some of the variability appears to show the influence of fertilizer applied at planting time in the fall or the onset of fall rain (P and pH, for example). Near the surface, bulk density demonstrated a different response in different years. Most of the variability is temporally correlated, and about one-third to one-half appears to be a seasonal cycle. Additional temporal correla-

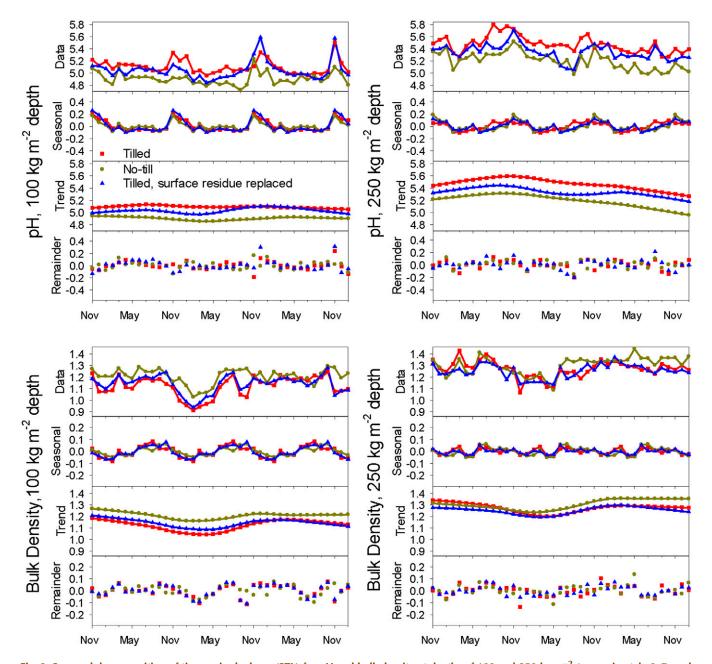


Fig. 2. Seasonal decomposition of time series by loess (STL) for pH and bulk density at depths of 100 and 250 kg m⁻² (approximately 0-7- and 0-20-cm depths, respectively). The replicated plots were planted annually with winter wheat under three soil management treatments: (i) no-till, (ii) tilled after harvest to 15 cm, incorporating surface residues into the tilled layer, and (iii) tilled as above, but surface residues raked aside and replaced on the soil surface after the soil was tilled.

tion appears in the remainder category of the STL computation but was not timed perfectly with calendar months and/or varies from 1 yr to the next. This is to be expected, because in this semi-arid environment rainfall is a key factor in the timing of plant growth and soil processes (Amato et al., 2013).

The total variability within tillage treatments during the 39-mo period ranged from 9 to 30% of the means for organic N, bulk density, and pH. These are of a similar magnitude to the variation found in the literature where more than one measurement was made in a single year (Boerner et al., 2005; Campbell et al., 1999a; Dormaar et al., 1977; Scott et al., 1994; Stoyan et al., 2000). This amount of variability is a serious problem to consider when attempting to compare soil properties in a single treatment

across a period of time because without adequate sampling to average out seasonal variation for each of the two sample points, it may not be possible to know how much of the difference between any two measurements to assign to long-term change vs. month-to-month fluctuation.

Note that in this study the monthly measurements were the means of four plots (and three cores each), so some spatial variability had already been averaged out. Increased sampling at one point in time cannot remove or reduce variation due to seasonal changes. Sampling at intervals during the crop year, which was a complete crop rotation in this case, did improve statistical precision. When averages from 12 monthly measurements were combined into crop years, treatment and crop year

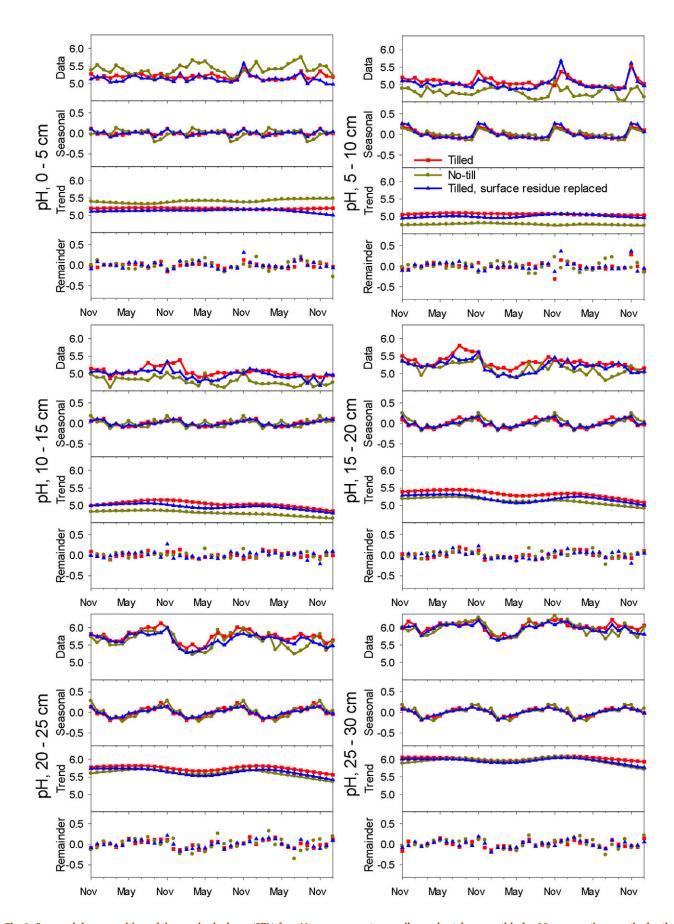


Fig. 3. Seasonal decomposition of time series by loess (STL) for pH measurements on soil samples taken monthly for 39 consecutive months for the 0- to 30-cm depth in 5-cm increments. Each set of four graphs has y axis scales of equal range. These data have not been corrected to equivalent soil mass, and this means that differences in effective sampling depth during the 39-mo period are likely.

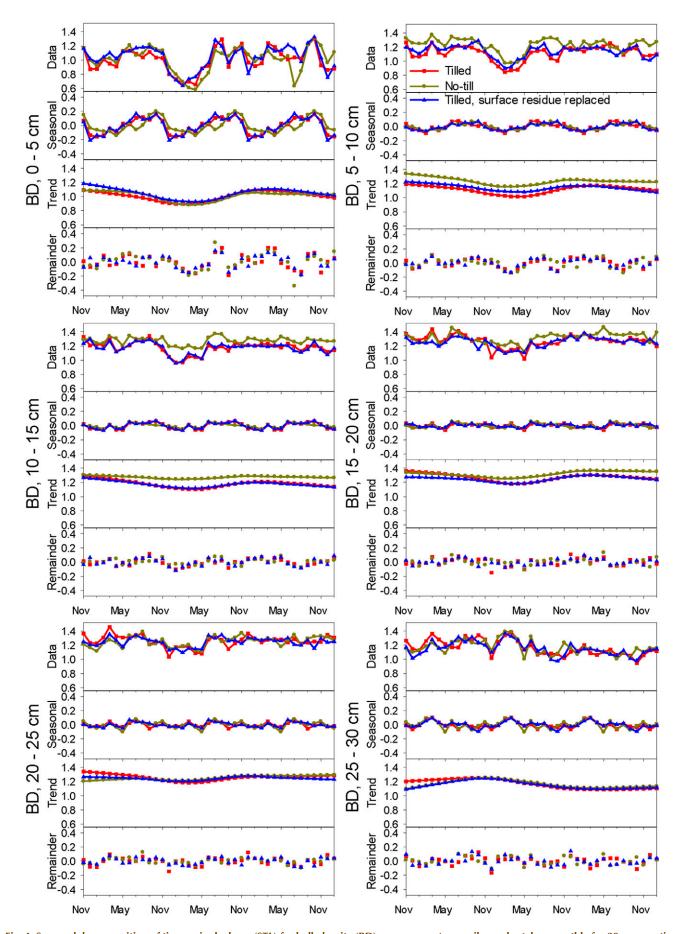


Fig. 4. Seasonal decomposition of time series by loess (STL) for bulk density (BD) measurements on soil samples taken monthly for 39 consecutive months for the 0- to 30-cm depth in 5-cm increments. Each set of four graphs has y axis scales of equal range.

Table 2. Generalized linear model type-3 test of fixed effects for 36 of the 39 mo of organic N, available P, pH, and bulk density data, divided into three 12-mo crop years. Replication (four blocks) and month within year were designated as random effects. Treatment codes: T = tilled, N = no-till, R = tilled, with surface residues removed and replaced after tillage. These means can be compared with 12-mo segments of the appropriate trend graphs in Fig. 1 and 2.

Organic N				Availab			рН		Bulk density			
<u>0</u> –100 kg m ⁻² soil depth												
<u>Effe</u>	Effect $p > F$ Effect		p > F	p > F Effect		p > F	<u>Effect</u>		p > F			
Treatment	(Trt)	0.0064 Trt		< 0.0001	Trt		< 0.0001	Trt	< 0.0001			
Crop year	(Cyr)	0.0005	Cyr		0.2488	Cyr		0.1928	Cyr		0.0005	
$Trt \times Cyr$		0.1406	$Trt \times Cyr$		0.2268	$Trt \times Cyr$		0.0085	$Trt \times Cyr$		< 0.0001	
<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	
		$\mathrm{g}\;\mathrm{m}^{-2}$			$\mathrm{g}~\mathrm{m}^{-2}$						${ m g~cm^{-3}}$	
Ν	3	123 A†	Ν	3	2.81 A	I	1	5.12 A	Ν	1	1.24 A	
R	3	120 BA	Ν	2	2.79 BA	I	2	5.10 A	Ν	3	1.21 BA	
T	3	120 BA	Ν	1	2.57 BAC	R	3 5.08 BA		I	3	1.19 BA	
Ν	2	118 BC	R	3	2.54 BC	I	3	5.07 BA	R	3	1.18 BA	
T	1	117 BC	R	2	2.35 C	R	1	5.05 BAC	R	1	1.18 BA	
Ν	1	117 BC	T	3	2.35 C	R	2	4.98 BC	Ν	2	1.17 BA	
R	1	117 BC	R	1	2.31 C	Ν	1	4.94 DC	I	1	1.15 BC	
T	2	116 BC	T	1	2.25 C	Ν	3	4.94 DC	R	2	1.08 C	
R	2	116 C	T	2	2.25 C	Ν	2	4.84 D	I	2	1.04 D	
			(N 1	, R 1), (N			(R 1, N	1)	(N 1, R 1)			
					<u>0–250 kg n</u>	n ⁻² soil dept	<u>h</u>					
Effect $p > F$		<u>Effe</u>	<u>ct</u>	p > F	<u>Effe</u>	ect	p > F	<u>Effe</u>	<u>ect</u>	p > F		
Trt		< 0.0001	Trt		< 0.0001	Trt		< 0.0001	Trt		< 0.0001	
Cyr		0.0003	Cyr		0.198	Cyr		0.0183	Cyr		< 0.0001	
$Trt \times Cyr$		0.0329	$Trt \times Cyr$		0.7223	$Trt \times Cyr$		0.0956	$Trt \times Cyr$		0.0012	
<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	
		$\mathrm{g}\;\mathrm{m}^{-2}$			$\mathrm{g}\;\mathrm{m}^{-2}$						${ m g~cm^{-3}}$	
R	3	282 A	Ν	3	6.28 A	I	1 5.54 A		Ν	3	1.35 A	
T	3	278 BA	Ν	2	6.08 BA	I	2 5.52 A		I	1	1.32 BA	
R	2	272 BC	Ν	1	5.86 BAC	R	1	5.40 BA	I	3	1.30 BAC	
R	1	272 BC	R	3	5.62 BDC	I	3	5.39 BA	Ν	1	1.29 BAC	
Ν	3	271 DC	R	2	5.38 DC	R	2	5.33 B	R	3	1.29 BC	
T	2	268 DC	T	3	5.32 DC	R	3	5.33 B	R	1	1.26 DC	
Ν	1	267 DC	R	1	5.19 D	Ν	1	5.28 B	Ν	2	1.25 DC	
T	1	266 DC	T	1	5.11 D	Ν	2	5.24 BC	R	2	1.20 D	
Ν	2	264 D	T	2	5.09 D	Ν	3	5.11 C	I	2	1.20 D	
	(R 1, N	N 1), (R 1, T 1)				(I 1	, R 1), (R	1, N 1)	(N 3, I 3), (N 2,I 2)			

[†] Estimates followed by different letters are significantly different at p < 0.05, with the experiment-wide error rate protected at p < 0.05 by the Simulate method (Littell et al., 2006).

differences of 2 to 4% of the mean were statistically significant (Table 2).

Dividing the data into a seasonal pattern (based on a periodicity of 12 mo), a smoothed trend, and the remaining variation resulted in a substantial amount of the variation being classified as a repeating seasonal variation. After subtracting the seasonal and smoothed trend from the original data, in some places the remainder showed a strong temporal correlation. These temporal correlations in the remainder are likely to result from actual physical and biological processes. If there was a more biologically based method of timing the seasonal cycle, like growing degree days or a combination of moisture and growing degree days, we should be able to separate more of the true seasonal dynamics from the long-term trend and random error.

Graphing pH and bulk density in the six sampled increments of depth from the soil surface at the time of sampling (Fig. 3 and 4) shows how variation in the soil bulk density at the soil surface influences the effective sampling depth and the resulting pH measurement. A researcher needs to decide whether distance from the soil surface at the time of sampling is more important than sampling the same amount of soil and in the same soil horizon as previous or future samples to which the measurements might be compared.

Seasonal trends for organic N in the top 250 kg m $^{-2}$ are very similar between the three soil treatments. When measuring just the top 100 kg m $^{-2}$, the no-till trend remains very similar, but the tilled treatments have slightly smaller peaks than they did for the deeper sample. This seems to indicate that seasonal organic N processes occur very near the surface in no-till, but

[‡] These pairs are also significantly different.

Table 3. Generalized linear model type-3 test of fixed effects for pH and soil bulk density at 5-cm increments. Thirty-six of the 39 mo were divided into three crop years. Replication (4 blocks) and month within season were designated as random effects. Treatment codes: T = tilled, N = no-till, R = tilled with surface residues removed and replaced after tillage.

0–5 cm			5–10 cm 10–15			5 cm	m 15–20 cm			20–25 cm			25–30 cm				
<u>рН</u>																	
<u>Effe</u>	ect	p > F	<u>Ef</u>	<u>fect</u>	p > F	<u>Eff</u>	fect	p > F	<u>Ef</u>	ect	p > F	<u>Ef</u>	fect	p > F	<u>Ef</u>	<u>fect</u>	p > F
Treatmen	t (Trt)	< 0.0001	Trt		< 0.0001	Trt		< 0.0001	Trt		< 0.0001	Trt		< 0.0001	Tt		0.0123
Crop yea	r (Cyr)	0.3399	Cyr		0.8961	Cyr		0.052	Cyr		0.0045	Cyr		0.0561	Cyr		0.3785
$Trt \times Cyr$		0.0034	Trt \times Cyr 0.0742			$\text{Trt} \times\\$	Cyr	0.2397 Trt \times Cyr			0.0068 Trt \times Cyr		0.1099 Trt × Cyr		0.4636		
<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	Cyr	Estimate	<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	Cyr	Estimate	<u>Trt</u>	Cyr	Estimate
Ν	3	5.47 At	T	1	5.09 A	Τ	1	5.10 A	Τ	1	5.46 A	Т	1	5.84 A	T	3	6.07 A
Ν	2	5.41 A	T	2	5.09 A	Τ	2	5.08 A	R	1	5.32 BA	Т	3	5.78 BA	T	1	6.06 A
Ν	1	5.34 A	R	3	5.04 A	R	1	5.05 BA	Τ	3	5.3 BA	R	1	5.75 BAC	R	1	6.01 A
T	1	5.22 B	T	3	5.03 A	Τ	3	5.00 BA	Т	2	5.28 BA	Ν	1	5.72 BAC	R	3	6.01 A
R	3	5.19 B	R	1	5.01 A	R	3	4.95 BAC	R	3	5.25 BC	R	3	5.69 BAC	Ν	1	6.00 A
T	3	5.18 B	R	2	4.97 A	R	2	4.94 BC	Ν	1	5.25 BCD	Т	2	5.67 BAC	Ν	3	6.00 A
T	2	5.18 B	Ν	1	4.78 B	Ν	1	4.85 DC	Ν	2	5.11 CD	Ν	3	5.59 BC	Ν	2	5.97 A
R	1	5.14 B	Ν	3	4.78 B	Ν	2	4.78 D	R	2	5.08 CD	Ν	2	5.58 BC	Т	2	5.96 A
R	2	5.12 B	Ν	2	4.77 B	Ν	3	4.73 D	Ν	3	5.07 D	R	2	5.54 C	R	2	5.92 A
				(T 2,R	2)‡					(T 1	,R 1)		(T	1,N 1), (T 3	3,N 3),	(T 2,R	2)
								<u>Bulk den</u>	sity								
<u>Effe</u>	<u>ect</u>	p > F	<u>Ef</u>	<u>fect</u>	p > F	<u>Eff</u>	<u>fect</u>	p > F	<u>Ef</u>	ect	p > F	<u>Ef</u>	fect	p > F	Ef	fect	p > F
Trt		0.0062	Trt		< 0.0001	Trt		< 0.0001	Trt		< 0.0001	Trt		0.5786	Trt		0.4663
Cyr		0.011	Cyr		0.0003	Cyr		0.0011	Cyr		< 0.0001	Cyr		0.0216	Cyr		0.0069
Trt x Cyr		0.0581	Trt × 0	Cyr	< 0.0001	Trt ×	Cyr	< 0.0001	Trt ×	Cyr	0.0014	Trt ×	Cyr	0.0012	Trt ×	Cyr	0.047
<u>Trt</u>	Cyr	Estimate	<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	<u>Cyr</u>	Estimate	<u>Trt</u>	<u>Ćyr</u>	Estimate	<u>Trt</u>	<u>Cyr</u>	<u>Estimate</u>	<u>Trt</u>	<u>Cyr</u>	Estimate
		${\rm g~cm^{-3}}$			${\rm g~cm^{-3}}$			${\rm g~cm^{-3}}$			${\rm g~cm^{-3}}$			${\rm g~cm^{-3}}$			${ m g~cm^{-3}}$
R	3	1.12 A	Ν	1	1.29 A	Ν	1	1.29 A	Ν	3	1.36 A	Τ	1	1.31 A	Т	1	1.24 A
R	1	1.10 BA	Ν	3	1.22 BA	Ν	3	1.28 BA	Τ	1	1.33 BA	Ν	3	1.28 BA	Ν	2	1.20 BA
Т	3	1.10 BA	R	1	1.20 B	Ν	2	1.25 BAC	Ν	1	1.31 BAC	Τ	3	1.27 BAC	R	1	1.19 BA
Ν	1	1.08 BAC	Τ	3	1.18 B	Τ	1	1.24 BAC	Τ	3	1.31 BC	R	1	1.26 BAC	Ν	1	1.19 BA
Ν	3	1.04 BAC	R	3	1.17 BC	R	1	1.23 BC	R	3	1.30 BC	R	3	1.25 BAC	Т	2	1.18 BA
T	1	1.03 BAC	Ν	2	1.17 BC	Т	3	1.20 C	Ν	2	1.27 BC	Ν	1	1.24 BAC	R	2	1.18 BA
R	2	0.93 BAC	Т	1	1.16 BC	R	3	1.19 DC	R	1	1.26 DC	Ν	2	1.22 BAC	Ν	3	1.11 B
Т	2	0.91 BC	R	2	1.07 C	R	2	1.11 ED	R	2	1.19 ED	R	2	1.21 BC	R	3	1.09 B
Ν	2	0.89 C	Т	2	1.01 D	Т	2	1.10 E	Т	2	1.18 E	Т	2	1.18 C	Т	3	1.08 B
(N 3,R 3), (N 2,R 2)											(T 1	,N 1)					
(1 1/1 1) (1 1/1 1) (1 1/1 1) (1 1/1 1) (1 1/1 1) (1 1/1 1) (1 1/1 1)											1 .1						

[†] Estimates followed by different letters are significantly different at p < 0.05, with the experiment-wide error rate protected at p < 0.05 by the Simulate method (Littell et al., 2006).

very similar processes occur throughout a greater depth for tilled soil. The 3-yr trend indicates that no-till is causing a segregation of surface and deeper soil organic N accumulation with time, as would be expected for this recently established no-till treatment (Needelman et al., 1999). The seasonal peaks occur at the mid-July sampling. This was very close to harvest, and may reflect fine aboveground crop residues. The same seasonal peak timing occurred for organic C but only under no-till (Wuest, 2014).

CONCLUSIONS

The results presented here show that a variety of soil measurements undergo substantial seasonal fluctuation. The amount of temporal correlation remaining in the data after removing monthly and experiment-long trends prevents this source of error from being overcome by simply matching calendar dates in this study. These data did not include crop rotations of differ-

ent length or multiple species, which would be expected to add even more seasonal variance in soil measurements. It is recommended that researchers consider multiple samples over an annual or crop rotation cycle to improve measurement accuracy. At the least, research projects requiring accurate estimates or comparisons should take several samples on a seasonal basis to learn something about the stability of the measurement being made.

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[‡] These pairs are also significantly different.

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