A rapid method for estimating labile carbon and nitrogen pools in Mollisols under no-tillage

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SHORT COMMUNICATION

A rapid method for estimating labile carbon and nitrogen pools in Mollisols under no-tillage

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ABSTRACT

The objective of this study was to adapt the partial chemical digestion method for estimation of labile soil organic matter pools by evaluating the effect of different digestion times in Mollisols of the Argentine Pampas. The soils were sampled from nine agricultural fields under no-tillage at the 0–20 cm depth. A chemical method was performed through partial soil digestion with dilute sulphuric acid at 100°C on the basis of four digestion times: 1 (N_{d1}), 2 (N_{d2}), 4 (N_{d4}) and 6 (N_{d6}) hours. Soil organic carbon (C) and nitrogen (N) fractions were determined. The extracted organic N (N_d) ranged from 0.076 g kg^{-1} to 0.273 g kg^{-1}, with a mean of 0.154 g kg^{-1}. Statistically, the means for each digestion time indicated highly significant differences ($P = 0.008$). High correlations were found between N_d for different times and labile C and N fractions. However, the best fit prediction was observed between N_{d2} and soil total carbohydrates (CHt), with a high coefficient of determination ($R^2 = 0.94$). Partial chemical digestion for 2 h can be used as a rapid indicator to accurately predict CHt. Because of its speed and simplicity, this method may also be useful for rapid soil quality assessments.

Introduction

Over the last few decades, soil quality (SQ) definition and assessment techniques have become of major concern (Fernández et al. 2017). Furthermore, scientific information is essential for finding appropriate indicators that can accurately assess SQ (Duval et al. 2013). In this sense, soil organic matter (SOM) and its carbon (C) content, *i.e.* soil organic carbon (SOC), as well as its labile organic fractions are considered important SQ indicators (Duval et al. 2013). Besides, C and nitrogen (N) content in particulate organic matter (POM) and soil carbohydrates (CH) are regarded as the most sensitive SQ indicators of short-term changes (Duval et al. 2013, 2016), emphasizing the importance of measuring these labile organic fractions for SQ assessment. However, the methods for determining particulate organic C (POM-C) and N (POM-N) (Duval et al. 2013) and CH through a dilute acid and water hydrolysis (Puget et al. 1999) require a long process. Several different procedures have been described in the literature to characterize labile SOM pools; however there is a clear lack of uniformity in experimental conditions (Silveira et al. 2008). Furthermore, most techniques that measure labile SOM fractions are costly and hence are not performed by standard commercial soil testing laboratories (Culman et al. 2013). Modern soil testing laboratories strive for a short turnaround time and therefore need methods that require little time. Martínez & Galantini (2017) developed a new short-term method based on partial digestion of soil with 0.5 mol L^{-1} sulphuric

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acid (H_2SO_4). These authors found that potentially mineralizable N can be accurately estimated by performing this partial chemical digestion during 4 h. Given the close relationship between potential N mineralization and labile organic C- and N-fractions (St. Luce et al. 2011; Martínez et al. 2017), our hypothesis is that by modifying the digestion times of this chemical method it is possible to estimate different labile C- and N-pools through a short-term and low-cost procedure. The objective of this study was to adapt the partial chemical digestion method by evaluating the effect of different digestion times on estimation of labile SOM pools in Mollisols of the Argentine Pampas.

Material and methods

Experimental sites

The soils were sampled from nine no-tillage (NT) fields under wheat (*Triticum aestivum* L). The experimental design consisted of three completely randomized blocks. A composite soil sample was collected from N fertilization trials in wheat in the non-fertilized plots before crop seeding at 0–20 cm depth. Sites were located in the south-west of the Argentine Pampas. The predominant soils were Phaeozems (IUSS Working Group WRB 2015) developed from aeolian sediments (loess), with a wide-range variability in terms of depth, texture, SOC content and fertility conditions (Martínez & Galantini 2017).

Soil and climate characteristics are reported in Table 1. The rainfall gradient indicates an udic soil moisture regime for continental sites (1, 3, 6, 8 and 9) and an ustic regime for sites next to the coast (2, 4, 5 and 7) (Martínez & Galantini 2017). The frequency and amount of precipitation is variable in all sites, the rainiest seasons being autumn (March-April) and spring (September-October). All soils had been under continuous NT for 10–15 years. This system was characterized by the absence of tillage with over 30% residues covering the soil surface in all fields. In general, a herbicide (1–2 L ha^{-1} of glyphosate) was applied for weed control and to start the chemical fallow. For all years and fields, winter wheat was the previous crop. Fertilizers were applied by farmers at a rate of 0–20 kg P ha^{-1} year^{-1} as diammonium phosphate (18–46-0) at crop seeding.

Table 1. Site characteristics and properties at 0–20 cm depth of the soils used in the experiment.

					Crop yield[b]	MAT[c]	MAR[d]	SOC	SON		Texture Sa	si	c
Soil	Location	Coordinates		Soil classification[a]	(kg ha^{-1})	(°C)	(mm)	(g kg^{-1})		pH	(g kg^{-1})		
1	Tornquist	38°04 62°05	57 'S 04 'W	Luvic Phaeozem	1523	14.5	695	22	1.7	7.0	414	380	206
2	Las Oscuras	38°49 61°37	07 'S 14 'W	Haplic Kastanozem	2616	15.0	533	15	1.3	7.4	567	399	34
3	Tornquist	37°58 62°09	12 'S 17 'W	Luvic Phaeozem	1542	14.3	550	19	1.6	6.7	368	404	228
4	Las Oscuras	38°47 61°39	15 'S 39 'W	Haplic Kastanozem	920	15.0	470	13	1.2	6.2	751	154	95
5	Cnel. Dorrego	38°36 61°02	34 'S 52 'W	Haplic Kastanozem	2823	14.7	648	20	1.5	6.2	466	321	214
6	Tornquist	38°10 62°01	15 'S 50 'W	Luvic Phaeozem	1032	14.5	777	18	1.4	6.4	541	284	176
7	Las Oscuras	38°47 61°38	52 'S 45 'W	Haplic Kastanozem	1946	15.0	624	16	1.2	6.0	615	177	208
8	Pigüé	37°34 62°09	26 'S 32 'W	Luvic Phaeozem	2768	13.8	1045	31	2.6	7.6	254	538	208
9	Saldungaray	38°12 61°46	'15 'S 06 'W	Haplic Kastanozem	1341	14.7	694	15	1.3	6.3	510	266	225

[a]World Reference Base for Soil Resources, IUSS Working Group WRB (2015). [b]Crop yield, wheat grain yield in non-fertilized plots during the sampled year; [c]MAT, mean annual temperature. [d]MAR, mean annual rainfall in the sampled site. SOC, soil organic carbon; SON, soil organic N; Sa, sand; si, silt; c, clay.

Chemical and physical analysis

The soil samples collected at 0–20 cm were air-dried, sieved (<2 mm) and chemically analyzed for the following parameters: SOC by dry combustion using a Leco automatic analyzer (Leco Corporation, St Joseph, MI), soil organic nitrogen (SON) by the micro-Kjeldahl method (Bremner et al. 1996), and pH in a soil-water suspension of 1:2.5. Soil texture was determined by the pipette method (Gee & Bauder 1979). The analytical data of the sampled sites are detailed in Table 1.

Partial soil digestion at different digestion times

The procedure was based on the micro-Kjeldahl method (Bremner et al. 1996) modified by Martínez & Galantini (2017). Briefly, 0.250 g of air-dried and sieved (<2 mm) soil was weighed in triplicate into test tubes. Then, 3 mL of 0.5 mol L^{-1} H_2SO_4 was added to the test tube. The difference with the micro-Kjeldahl method was that partial digestion was performed without catalysts due to the low digestion temperature. The suspensions were digested for 1, 2, 4 and 6 h at 100°C in a heating block. After digestion, the tube was allowed to cool. Then, 3 mL of 10 mol L^{-1} sodium hydroxide was added and a steam distillation was performed for 3 min using 5 mL of 2% boric acid indicator to collect the N released from the sample. An acid-base titration was then carried out with 0.01 mol L^{-1} H_2SO_4. The results of the extracted organic N (N_d) at the different times were named as N_{d1} (1 h), N_{d2} (2h), N_{d4} (4 h) and N_{d6} (6 h).

Labile soil organic matter fractions

Soil fractionation by particle size

Soil fractionation by particle size was performed by wet sieving (Elliot & Cambardella 1991; Duval et al. 2013). Briefly, 50 g of soil previously air-dried and sieved (<2 mm) were dispersed in 120 mL glass containers and mixed with 100 mL of distilled water. Ten glass beads (5 mm diameter) were added to increase aggregate destruction and reduce potential problems created by sand (Elliott & Cambardella 1991). After dispersion, the soil suspension was sieved through two connected sieves (53 microns and 105 microns in diameter). The sieves were moved back and forth, and the soil retained in the top sieve was sprinkled with distilled water until the water in the bottom sieve was clear to the naked eye. The fractions obtained were i) a coarse particle size fraction (105–2000 microns) containing coarse sands and coarse POM, and ii) a medium particle size fraction (53–105 microns) containing more stable or fine POM, and fine and very fine sand; the fine particle size fraction (<53 microns) was discarded. Each particle size fraction was analyzed for C and N, and two fractions were obtained: i) particulate organic C and N in the coarse particle size fraction (cPOM-C and cPOM-N, respectively) and ii) particulate organic C and N in the medium particle size fraction (fPOM-C and fPOM-N, respectively). Carbon and N contents in POM were determined using the same methods as for SOC and SON, respectively.

Total and soluble carbohydrates

Total carbohydrates (CHt) were extracted by an acid hydrolysis as follows: 1 g of air-dried and sieved (<2 mm) soil was treated with 10 mL 0.5 mol L^{-1} H_2SO_4 and heated at 80°C for 24 h. In other sample, soluble carbohydrates (CHs) were extracted by suspending 1 g of air-dried and sieved (<2 mm) soil in 10 mL of distilled water and heating it at 80°C for 24 h. After extraction with hot water, H_2SO_4 was added to obtain a 0.5 mol L^{-1} concentration as in the dilute acid hydrolysis procedure, and immediately processed. After being extracted in each way, each suspension was centrifuged at 3081 g for 15 min (Puget et al. 1999). Carbohydrate contents of the extract were determined by spectrometry using the sulphuric-phenol spectrometric method with glucose as the standard (Dubois et al. 1956).

Statistical analyses

Normality and homoscedasticity for the different chemical digestion times were assessed by Bartlet's and Levene's tests. Descriptive statistics was performed for each time of chemical digestion. The means of each time were compared using a one-way analysis of variance (ANOVA), followed by Fisher's LSD test ($P < 0.05$). A Pearson´s correlation analysis was done to assess the relationships between N_d at the different times and soil organic fractions (including SOC and SON). All statistical analyses were performed using the INFOSTAT software (Di Rienzo et al. 2013).

Results and discussion

Soil organic N extracted at different digestion times

The N_d at different digestion times are detailed in Table 2. Extracted organic N ranged from 0.076 g kg^{-1} to 0.273 g kg^{-1}, with a mean value of 0.154 g kg^{-1} for all sites. Statistically, the means for each digestion time indicated highly significant differences ($P = 0.008$) (Table 2). However, no significant difference was found between N_{d1} and N_{d2}, N_{d2} and N_{d4}, N_{d4} and N_{d6}. These results show that longer digestion times partially increase the amount of N_d that is solubilized. However, solubilization increases up to 4 h of digestion, whereas it tends to stabilize after that time.

Variability in the coefficient of variation (CV) was small, from 24 to 32%, considering soil differences across sites (Table 1). The lowest CV was found in N_{d2}, whereas the N_{d4} time showed the highest CV across soils (Table 2). According to Pennock et al. (2007), this aspect is important in that the CV indicates the precision of the method. In this study the CV values for all digestion times may be classified as medium-high precision. Methods with low variability and repeatable results are essential to minimizing the burden of analysis and maximizing confidence in soil analysis interpretation (Morrow et al. 2016).

Labile organic fractions

The mean values of these labile organic fractions were 1.07; 1.39; 0.103 and 0.161 g kg^{-1} for cPOM-C, fPOM-C, cPOM-N and fPOM-N, respectively (Table 3). Considering POM-C as the sum of cPOM-C and fPOM-C, it represented about 15.6% of SOC, whereas POM-N (cPOM-N+fPOM-N) accounted for about 18.9% of SON. Total carbohydrates ranged from 2.09 to 3.41 g kg^{-1}, averaging 2.62 g kg^{-1}; CHs ranged from 0.22 to 0.44 g kg^{-1}, with an average of 0.29 g kg^{-1}. Total carbohydrates represented about 16.9% of SOC, and CHs about 1.8%. Labile organic fractions showed a low CV, from 7 to 28%. These results revealed that differences across sites are very few in terms of labile organic fractions because of a low CV. As expected, several significant correlations were found between soil organic fractions. The higher coefficients of correlation (r) were observed between SOC and SON ($r = 0.95$), SOC and CHt ($r = 0.90$), SON and CHs ($r = 0.89$), fPOM-C and fPOM-N ($r = 0.88$), and fPOM-N and CHs ($r = 0.89$) (data not shown). These close correlations between soil organic C- and N-fractions were consistent with other studies (St. Luce et al. 2011; Martínez et al. 2017).

Table 2. Mean values, standard deviation and range of values of extracted soil organic N at different digestion times.

	Mean	SD	minimum	maximum	CV
Digestion times (h)			g kg^{-1}		%
1	0.13 **c**	0.03	0.07	0.19	26
2	0.15 **bc**	0.03	0.09	0.22	24
4	0.17 **ab**	0.06	0.08	0.28	33
6	0.18 **a**	0.04	0.11	0.27	25

SD, standard deviation; CV, coefficient of variation of each digestion time for all sites. Different letters indicate significant differences among digestion times ($P < 0.05$).

Table 3. Labile organic matter fractions (mean± standard deviation) across sites at crop seeding in 0–20 cm soil layer.

	cPOM-C	fPOM-C	cPOM-N	fPOM-N	CHt	CHs
Soil			(g kg^{-1})			
1	1.54 ± 0.13	2.26 ± 0.14	0.126 ± 0.01	0.188 ± 0.01	3.08 ± 0.23	0.36 ± 0.04
2	1.20 ± 0.39	0.76 ± 0.14	0.083 ± 0.01	0.104 ± 0.03	2.13 ± 0.24	0.22 ± 0.02
3	0.83 ± 0.13	1.64 ± 0.41	0.096 ± 0.01	0.176 ± 0.01	2.68 ± 0.06	0.24 ± 0.03
4	0.73 ± 0.06	1.15 ± 0.81	0.122 ± 0.01	0.134 ± 0.02	2.33 ± 0.23	0.27 ± 0.01
5	1.34 ± 0.47	1.83 ± 0.93	0.106 ± 0.02	0.179 ± 0.01	2.29 ± 0.23	0.31 ± 0.03
6	1.65 ± 0.25	0.95 ± 0.08	0.152 ± 0.04	0.161 ± 0.01	2.97 ± 0.35	0.29 ± 0.05
7	0.62 ± 0.12	0.55 ± 0.03	0.052 ± 0.01	0.081 ± 0.004	2.09 ± 0.28	0.22 ± 0.01
8	1.01 ± 0.17	2.40 ± 0.89	0.102 ± 0.01	0.256 ± 0.01	3.41 ± 0.56	0.44 ± 0.07
9	0.70 ± 0.08	0.95 ± 0.06	0.087 ± 0.03	0.166 ± 0.01	2.57 ± 0.43	0.26 ± 0.01
mean	1.07	1.39	0.103	0.161	2.62	0.29
CV	19	28	15	7	18	25

cPOM-C, coarse particulate organic carbon (g kg^{-1}); cPOM-N, coarse particulate organic nitrogen (g kg^{-1}); fPOM-C, fine particulate organic carbon (g kg^{-1}); fPOM-N, fine particulate organic nitrogen (g kg^{-1}); CHt, total carbohydrates (g kg^{-1}); CHs, soluble carbohydrates (g kg^{-1}); CV, coefficient of variation of each organic fraction for all sites.

Table 4. Pearson´s correlation among extracted organic N at different digestion times and SOM fractions.

	N_{d1}	N_{d2}	N_{d4}	N_{d6}
Soil organic fraction		coefficient of correlation (r)		
SOC	0.59	0.73*	0.57	0.75*
SON	0.58	0.75*	0.56	0.76*
cPOM-C	0.17	0.31	0.37	0.29
fPOM-C	0.53	0.80**	0.82**	0.81**
cPOM-N	0.31	0.41	0.24	0.22
fPOM-N	0.80**	0.88**	0.87**	0.90***
CHt	0.81**	0.97***	0.83**	0.86**
CHs	0.64	0.92***	0.72*	0.85**

*,**,*** significant correlation at 0.05; 0.01 and 0.001 probability levels, respectively. $N_{d1, 2, 4, 6}$ extracted organic N for 1, 2, 4 and 6 h of digestion time. See abbreviations in Table 3.

Relationships between N_d and soil organic fractions

Several significant correlations were found ($P < 0.05$) between N_d at different times and soil organic fractions (Table 4). However, the best fit predictions ($P < 0.001$) were seen for N_{d2}-CHt ($R^2 = 0.94$), N_{d2}-CHs ($R^2 = 0.84$) and N_{d6}-fPOM-N ($R^2 = 0.81$) (data not shown). Despite the correlations among some organic fractions (Table 4), the highly significant associations showed that N_d at different digestion times helps to accurate estimate some organic fractions, thus indicating that partial digestion at each time extracted different C- and N-pools.

For N_{d2}, the extracted pools would be more closely related to the more soluble C- fractions (CH). Coincidentally, Ghani et al. (2003) reported that when CH is extracted other pools of labile nutrients are also extracted along with C. On the other hand, N_{d6} would be more closely related to the particulate N-fraction with lower lability. Martínez & Galantini (2017) established close relations between N_{d4} and POM-N (cPOM-N+fPOM-N). However, when considering POM as two fractions of different lability (cPOM-N and fPOM-N), as proposed by Duval et al. (2013), the highest estimation of N_{d6} was achieved with fPOM-N. It is hence suggested that a CH increase would also indicate an increase of other labile organic pools of nutrients such as N (Sá Pereira et al. 2017). This could explain the relationships of N_d with CHt, CHs and fPOM-N found in this study. Most of these measurements have been actively promoted as key indicators of SQ, according to several authors (Puget et al. 1999; Duval et al. 2013, 2016). In the present study, the results indicated that N_{d2} was a reliable estimator of CHt due to the high R^2 obtained. It is

important to note that N_{d2} is a tool to achieving a high precision method. Despite that the repeatability of results was not reported in this study, a useful way to compare reproducibility between any two methods is to use regression analysis, because the R^2 is a measure of relative reproducibility (Pribyl 2010). The high R^2 obtained in the relationship between N_{d2} and CHt, may indicate that partial chemical digestion for 2 h can be a reproducible methodology. This leads to the conclusion that a 2 h partial digestion with an acid concentration of 0.5 mol L^{-1} can be used to accurately estimate CHt concentration under these soil conditions. Soil CHt include poly-saccharides (extracted soluble CH and hemicellulose) of plant and microbial origin (Ghani et al. 2003), and they are easily degraded by microorganisms (Silveira et al. 2008). In addition, CH content increases under NT as a result of crop residue accumulation on the surface (Duval et al. 2013). For this reason, it is important to evaluate CHt when assessing the quality of soils under these conditions. Moreover, partial chemical digestion method would optimize SQ assessment in the short-term at a low cost in Mollisols under NT. Furthermore, N_{d2} and N_{d6} allowed a reasonably accurate prediction of CHs ($R^2 = 0.84$) and fPOM-N ($R^2 = 0.81$) (Table 4), respectively. In both cases, the use of N_{d2} and N_{d6} will save time and cost in estimating these indicators, but with lesser accuracy.

Conclusion

This study demonstrates that partial chemical digestion for 2 h can provide meaningful estimates of CHt in Mollisols under similar conditions. This technique is easy to perform, gives repeatable results, and may be suitable for use by any laboratory on a routine basis. However, its effectiveness for other soils and tillage conditions has yet to be evaluated.

Disclosure statement

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