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Is anaerobic mineralizable nitrogen suitable as a soil quality/health indicator?

¿Es adecuado el nitrógeno mineralizable en anaerobiosis como indicador de calidad/salud edáfica?
O azoto mineralizado em anaerobiose é um indicador adequado da qualidade/saúde do solo?

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ABSTRACT

Soil organic matter (SOM) and especially its labile fractions such as particulate organic matter (POM) are very sensitive to soil use and strongly influence soil ecosystem services. Particulate organic matter has been proposed as a soil quality/health indicator but its determination is tedious and time consuming (i.e. manhours). Anaerobic mineralizable nitrogen (AN) is closely related to the soil organic fraction and is very easily determined. Therefore, we proposed to evaluate AN as a soil quality/health indicator through the assessment of its relationship with SOM, POM, soil aggregate stability (AS), and maize (*Zea mays* L.) relative yield (RY) under different long term soil uses for cropping at Balcarce, Argentina (37º 45' 14" S, 58º 17' 52" W). Soil samples had been taken at two depths (0-5 and 5-20 cm) in the fall of 1998, 2000, 2003, 2006, 2009, and 2012 from a long term tillage system (TS, conventional (CT) and no-tillage (NT)) and nitrogen fertilization (NF, with and without nitrogen as fertilizer) experiment on a complex of Typic and Petrocalcic Argiudolls. Carbon contents in SOM (SOC), POM (POC) and AN were determined in all soil samples, whereas AS was determined in other soil samples taken in 2006, 2009 and 2012 from the arable layer (0-20 cm). Regardless of TS and NF, SOC, POC and AN decreased with time under cropping at both 5-20 and 0-20 cm. In the uppermost layer (0-5 cm) decreases of all three variables were observed only under CT. Anaerobically mineralized nitrogen variation related to SOC (R^2 0.59 - 0.78, $P < 0.05$) and especially POC ($R^2 = 0.80$-0.85, $P < 0.05$) variations. Likewise, changes in maize RY related better (R^2 0.92 and 0.95 ($P < 0.05$) for CT and NT, respectively) to variation in AN, than to SOC and POC variations. Besides, changes in the aggregate mean weight diameter (ΔMWD) related acceptably to AN at 0-20 cm ($R^2 = 0.67$, $P < 0.05$) and much better at 0-5 cm ($R^2 = 0.86$, $P < 0.05$). Both coefficients of determination were higher than those obtained relating ΔMWD to SOC or POC. Given the easiness of its determination, its sensitivity, and that it relates to the variation of different key soil parameters and crop behavior, AN could be proposed as an effective soil quality/health indicator. However, studies should be carried out taking into account a broader range of soil and management situations in order to validate the trends observed in this work.

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RESUMEN

La dinámica de la materia orgánica del suelo (SOM) y especialmente la de sus fracciones lábiles, tales como la materia orgánica particulada (POM), son muy sensibles al uso del suelo e influencian fuertemente sus servicios ecosistémicos. La POM ha sido propuesta como indicador de calidad/salud edáfica pero su determinación es tediosa y consumidora de tiempo (i.e. horas-hombre). El nitrógeno mineralizable en anaerobiosis (AN) está estrechamente relacionado con la fracción orgánica del suelo y es muy fácilmente determinado. Por lo tanto, proponemos evaluar el AN como indicador de calidad/salud edáfica a través de la evaluación de sus relaciones con SOM, POM, la estabilidad de los agregados (AS) y el rendimiento relativo de maíz (Zea mays L.*) (RY) bajo diferentes usos agrícolas de largo plazo en Balcarce, Argentina (37º 45' 14" S, 58º 17' 52" W). Las muestras de suelo fueron tomadas a dos profundidades (0-5 and 5-20 cm) en otoño de 1998, 2000, 2003, 2006, 2009 y 2012, de un ensayo de larga duración de sistemas de labranza (TS, labranza convencional (CT) y siembra directa (NT)) y fertilización nitrogenada (NF, con y sin fertilización nitrogenada) sobre un complejo de Argiudoles Típico y Petrocálcico. El contenido de carbono en la SOM (SOC) y en la POM (POC), y el AN fueron determinados en todas las muestras de suelo, mientras que la AS fue determinada en otras muestras de suelo tomadas en 2006, 2009 y 2012 de la capa arable (0-20 cm). Independientemente del TS y de la NF, SOC, POC y AN disminuyeron en el tiempo bajo cultivo a 5-20 cm y a 0-20 cm. En la capa más superficial (0-5 cm) las tres variables disminuyeron sólo bajo CT. La variación de AN se relacionó con la de SOC (R^2 = 0,59-0,78, $P < 0.05$) y especialmente la de POC (R^2 = 0,80-0,85, $P < 0.05$). Del mismo modo, la variación del RY de maíz se relacionó mejor (R^2 0,92 y 0,95 ($P < 0.05$) para CT y NT, respectivamente) con la de AN que con las de SOC y POC. Además, el cambio en el diámetro medio ponderado de los agregados (ΔMWD) a 0-20 cm se relacionó aceptablemente con AN (R^2 = 0,67, $P < 0.05$), pero la relación fue mucho mejor a 0-5 cm (R^2 = 0.86, $P < 0.05$). Ambos coeficientes de determinación fueron más altos que aquéllos obtenidos relacionando ΔMWD con SOC o POC. Dada la facilidad de determinación, su sensibilidad y que se relaciona con la variación de diferentes parámetros edáficos fundamentales y con el comportamiento del maíz, AN podría ser propuesto como un efectivo indicador de calidad/salud edáfica. Sin embargo, se deberían llevar a cabo más estudios ampliando el rango de situaciones de manejo a efectos de validar los resultados obtenidos en este trabajo.*

RESUMO

A dinâmica da matéria orgânica do solo (SOM) e, em particular, a das suas frações lábeis, como a matéria orgânica particulada (POM) são muito sensíveis ao uso do solo e influenciam fortemente os ecossistemas. A POM tem sido proposta como um indicador da qualidade/saúde do solo, mas a sua determinação é complicada e demorada (i.e. horas-homem). O azoto mineralizado em anaerobiose (AN) está fortememente relacionado com a fração orgânica do solo e é muito simples de determinar. O objetivo deste trabalho é avaliar o AN como um indicador da qualidade/saúde do solo através do estudo das suas relações com a SOM, POM, a estabilidade dos agregados (AS), e a produção relativa do milho (Zea mays L.*) (RY), sob diferentes usos do solo a longo prazo, em Balcarce, Argentina (37 ° 45 '14' 'S, 58º 17' 52 " W). Analisaram-se amostras de solo a duas profundidades (0-5 e 5-20 cm) colhidas no Outono de 1998, 2000, 2003, 2006, 2009 e 2012 obtidas num ensaio de longa duração onde se estudaram sistemas de preparação do solo (TS, lavoura convencional (CT) e sementeira direta (NT)) e fertilização azotada(NF, com e sem adubo azotado) num solo Argiudol. O teor de carbono na SOM (SOC) e na POM (POC), e AN foram determinados em todas as amostras de solo, enquanto que a AS foi determinada noutras amostras obtidas em 2006, 2009 e 2012 a uma profundidade de 0-20 cm. Independentemente de TS e NF, de 5-20 cm e de 0-20 cm ocorreu uma diminuição de SOC, POC e AN ao longo período de cultivo. Na camada superficial (0-5 cm) as três variáveis diminuiram somente sob CT. AN relacionou-se com o SOC (R^2=0,59-0,78, $P < 0.05$) e com o POC (R^2 = 0,80-0,85, $P < 0.05$). Da mesma forma, o RY do milho correlacionou-se melhor com o AN (R^2 = 0,92 e 0,95 ($P < 0.05$), para CT e NT, respectivamente) que com o SOC e o POC. Além disso, a variação no diâmetro médio ponderado dos agregados (ΔMWD) a 0-20 cm relacionou-se com AN (R^2 = 0,67, $P < 0.05$), mas a relação foi muito mais evidente de 0-5 cm (R^2 = 0,86, $P < 0.05$). Os coeficientes de determinação entre ΔMWD e SOC ou POC foram inferiores aos obtidos com AN. Devido à facilidade na sua determinação, à sensibilidade, bem como à sua relação com diferentes parâmetros do solo e com o RY do milho, o AN poderia ser proposto como um indicador eficiente da qualidade/saúde do solo. Porém, devem ser realizados mais estudos para alargar a outras situações de manejo, a fim de validar os resultados obtidos neste trabalho.*

KEY WORDS
Particulate organic matter, aggregate stability, cropping systems, no-tillage, conventional tillage

PALABRAS CLAVE
Materia orgánica particulada, estabilidad de agregados, sistemas de cultivo, siembra directa, labranza convencional

PALAVRAS-CHAVE
Materia orgânica particulada, estabilidade de agregados, sistemas agrícolas, sementeira direta, lavoura

1. Introduction

Organic matter (SOM) content strongly influences soil functioning in the agroecosystem and on its ecosystem services. The properties and maintenance of the soil pore system which regulate water and air dynamics depend on SOM content and characteristics. They also determine the nutrient supply, activity and diversity of soil biota, and the capacity of a soil to resist disturbances and subsequently re-organize itself, all of which are characteristics that define soil quality/health (Weil and Magdoff 2004; Lal 2010). On the other hand, SOM is the most sensitive soil component to human intervention and therefore given the consequence of its variation on soil functioning, the goal of sustainable agriculture is closely linked to how SOM is managed (Lal 2010; Reicosky et al. 2011).

Changes of SOM associated with soil use can be seen mainly through changes in its labile fractions such as particulate organic matter (POM) (Fabrizzi et al. 2003; Six et al. 2004; Domínguez et al. 2009). Hence, POM content has been proposed as a sensitive soil quality/health indicator (Fabrizzi et al. 2003; Wander and Nissen 2004; Eiza et al. 2005) given its relationship with aggregate stability (AS) (Six et al. 2004; Agostini et al. 2012) and nutrient supply (e.g. nitrogen (N)) (Gregorich et al. 2006). However, even though POM determination is simple, the technique (Cambardella and Elliott 1992) is tedious and time consuming (i.e. manhours). Therefore, its use in commercial soil laboratories as a routine method is limited and expensive for producers (Diovisalvi et al. 2014). Potentially mineralizable N (PMN) is a fraction of soil organic N closely related to SOM labile fraction changes and is very affected by management (Liebig et al. 2004; Gregorich et al. 2006; Soon et al. 2007; Domínguez et al. 2009). Tillage increases mineralization and therefore, a decrease in PMN due to SOM labile fractions drop could be expected (Gregorich et al. 2006). On the contrary, the reduction in tillage intensity (e.g. under no-tillage, NT) may lead to an increase in PMN, but lower soil disturbance and the consequent less mineralization rate would produce N deficiency for crops (Domínguez et al. 2001, 2006). This increases the need of N fertilization (NF) and, therefore, of a precise and trustable diagnose of N availability that should take into account soil N mineralization capacity (Domínguez et al. 2006; Sainz-Rozas et al. 2008) and how it is managed. However, the determination of PMN requires very long aerobic incubations and cannot be performed to follow mid-term changes due to management (Echeverría et al. 2000).

Ammonium N produced during a short anaerobic incubation (anaerobic N, AN) has been proposed as a quick and precise estimator of PMN since there exists high correlation between them (Echeverría et al. 2000; Soon et al. 2007). Likewise, a close relationship of AN with organic carbon (C) in SOM (SOC) and, moreover, with organic C in POM (POC), has been reported (Fabrizzi et al. 2003; Domínguez et al. 2009). Anaerobic N is an easily determined soil parameter (Echeverría et al. 2000) and is very sensitive to changes produced by soil use for cropping (Fabrizzi et al. 2003; Liebig et al. 2004; Reussi-Calvo et al. 2014). The relationship of AN with the performance of crops like maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) has been demonstrated, and it has been proposed as a tool to diagnose N availability (Sainz-Rozas et al. 2008; Reussi-Calvo et al. 2013; Echeverría et al. 2015). It has also been demonstrated that AN does not change seasonally over the year between Fall and Spring (Studdert et al. 2015).

Given the simplicity of its determination, its sensitivity to mid- to long-term changes produced by soil use, and its relationship with some soil processes (i.e. N supply), AN has characteristics that coincide with those required to be used as soil quality/health indicator (Doran and Parkin 1996). On the other hand, since it is closely related to SOM dynamics, variations in some other soil processes and properties could also be related to AN changes due to soil use and this would improve its performance as soil quality/health indicator. The aim of this work was to evaluate the feasibility of using AN as soil quality/health indicator through the evaluation of: i) the long-term effect of continuous NF and use of contrasting tillage systems (TS) (conventional tillage (CT) and NT) on SOC, POC, and AN, ii) the relationship between SOC, POC and AN contents with maize yield and response to NF, and iii) the relationship between SOC, POC and AN contents and AS.

2. Materials and methods

2.1. Experimental site and experiment layout and design

The work was done with information from a TS and NF long-term experiment started in 1997 in the Unidad Integrada Balcarce's experimental field at Balcarce, Buenos Aires Province, Argentina (37° 45' 14'' S, 58° 17' 52'' W; 137 m over sea level). The climate is classified as mesothermal subhumid-humid (according to Thornthwaite classification) or as temperate humid without a dry season (according to Köppen classification) (A. Irigoyen, pers. comm. 2015). The median annual rainfall and the mean potential evapotranspiration and mean daily air temperature (1971-2010) are 937.8 mm, 946.1 mm and 13.9 °C, respectively (source: Unidad Integrada Balcarce's weather station (37° 45' 48'' S; 58° 17' 51'' W; 130 m over sea level, 1000 m away from the experimental site). The soil of the experimental site is a complex of Mar del Plata series (INTA 1979) (fine, mixed, thermic Typic Argiudoll, Soil Survey Staff 2014) and Balcarce series (INTA 1979) (fine, mixed, thermic Petrocalcic Argiudoll, Soil Survey Staff 2014) with less than 2% slope (very low erosion), high SOM content and loam surface texture. The petrocalcic horizon of the Balcarce series soil is below 0.7 m. The surface horizons of both series integrating the complex have almost the same characteristics (INTA 1979) and therefore they were considered as equal and sampled together. Its characteristics are shown in **Table 1**.

Table 1. Some soil surface horizon parameters at the beginning of the experiment (1997) (adapted from Domínguez et al. 2009)

Soil variablea		Soil layer		
		0-5 cm	5-20 cm	0-20 cm
Particle Size distribution (g kg^{-1})	Clay			23.1
	Silt			35.8
	Sand	-	-	41.1
Organic C (g kg^{-1})		33.9	33.1	33.3
Organic N (g kg^{-1})		2.52	2.58	2.57
C:N ratio		13.4	12,8	12,9
pH (1:2.5 in water)		5.6	5.3	5.4
CEC (cmol kg^{-1})		26.4	26.7	26.6
Na^+ (cmol kg^{-1})		0.3	0.3	0.3
K^+ (cmol kg^{-1})		2.6	1.7	1.9
Ca^{++} (cmol kg^{-1})		12.6	12.1	12.2
Mg^{++} (cmol kg^{-1})		1.9	1.7	1.8
Base saturation (%)		65.8	59.3	60.9

[a]CEC: cation exchange capacity.

The field where the experiment was laid out had been under a grass-based pasture (non-grazed) between 1993 and 1996. In 1996 a sunflower (*Helianthus annuus* L.) crop was planted under reduced tillage (disk tillage with tandem disk harrow). Since 1997, TS studied in this work were applied and the crop sequence maize – sunflower – wheat was started. The experimental design was a randomized complete block design with a split-plot treatment arrangement, three replications, and 5 * 40 m experimental units. Tillage systems (CT and NT) were assigned to main plots and NF (with (WN) and without (WON) N) was assigned to sub-plots. Conventional tillage comprised the use of moldboard plow, disk harrow and field cultivator with the least tillage operations necessary to an appropriate seedbed. Tillage operations to prepare the seedbed under CT or chemical treatments to control weeds under NT, were started no less than three months before the planting date of each crop. Nitrogen fertilizer (urea, 46-0-0) was surface broadcasted at six-leaf stage (V6) (Ritchie and Hanway 1982) for maize, six- to eight-leaf stages (V6-V8) (Schneiter and Miller 1981) for sunflower and at tillering (stage 30) (Zadoks et al. 1974) for wheat. Nitrogen fertilization rates were 0 kg N ha^{-1} for WON and at rates according to the crop (90 and 120 kg N ha^{-1} for sunflower and wheat, respectively, and 180 kg N ha^{-1} between

1997 and 2003 and 120 kg N ha^{-1}, later on, for maize) for WN. Nitrogen fertilizer was applied every year on the same sub-plot. Cultivars and crop management (planting rate, row spacing, weed control) were the best recommended for the region for each crop. All crops were fertilized with phosphorus as diammonium phosphate (18-0-0) according to soil analysis to avoid nutrient deficiency.

2.2. Soil sampling and analytical determinations

Composite soil samples (5 sub-samples per experimental unit) were taken in the fall of 1998, 2000, 2003, 2006, 2009 and 2012 at two depths (0-5 and 5-20 cm) with a 4.5 cm diameter tubular sampler. Soil core volume and moist weight of the whole sample were registered and water content determined (gravimetrically) on sample aliquots, to calculate bulk density (BD) (Agostini et al. 2014). Soil samples were dried at 30 °C until constant weight and ground to pass a 2 mm sieve and stored for later analysis. Recognizable crop residues and roots retained on the 2 mm sieve were eliminated. Whole soil samples were re-ground with mortar and analyzed for SOC by wet combustion with maintenance of the oxidation reaction temperature (120 °C) for 90 min (Schlichting et al. 1995). Particle size fractionation was performed (Cambardella and Elliott 1992) through wet-sieving of re-ground and dispersed soil samples through a 53 µm sieve. Soil organic C content was determined as described above on soil fraction that passed the sieve (associated with the mineral fraction) to determine organic C associated with silt and clay (AOC). Particulate organic C content was calculated by subtracting AOC from SOC (Cambardella and Elliott 1992). Results of organic C were expressed as concentration (g kg^{-1}) and as stock (Mg ha^{-1}). Bulk density, SOC, AOC and POC were also calculated for the 0-20 cm layer.

Soil AN was determined through a short anaerobic incubation (Keeney 1982; Echeverría et al. 2000) of 5 g of dry and ground (2 mm) soil in test tubes (150 * 16 mm). Tube volume was completed with deionized water. The tubes were hermetically capped providing all air bubbles were removed. Then they were incubated during 7 days at 40 °C. At the end of the incubation, 15.0 mL of 4 M KCl and 0.2-0.3 g of calcined MgO were added, and then, ammonium N (NH_4^+-N) was determined by steam micro-distillation (Keeney and Nelson 1982) directly on the resulting soil suspension (Echeverría et al. 2000). Anaerobic N was expressed in mg NH_4^+-N kg^{-1} and was also calculated for the 0-20 cm layer. The same analytical procedure was used to determine NH_4^+-N content of soil before incubation in order to subtract it from NH_4^+-N after incubation. Initial NH_4^+-N was always low and did not differ between management situations or time of storage (data not shown).

Besides, composite soil samples (5 sub-samples per experimental unit) were extracted with shovel only from the sub-plots WN at two depths (0-5 and 5-20 cm) in the fall of 2006, 2009 and 2012, for AS determination. Soil samples were sieved as taken (close to field capacity) through an 8 mm sieve and then oven dried (30 °C) until constant weight. Aggregate stability was determined as the change of mean weight diameter (ΔMWD) between dry sieving and wet sieving in water (De-Boodt et al. 1961). Briefly, dry samples were sieved through a 3-sieve nest (4.80, 3.36, and 2.00 mm sieves) and mean weight diameter calculated. A representative proportion of each dry fraction obtained up to complete 100 g was re-wetted capillary and sieved in water through a 6-sieve nest (4.80, 3.36, 2.00, 0.84, 0.50, and 0.30 mm sieves) for 30 min, and then oven-dried (105 °C) and weighed when dry. Mean weigh diameter was calculated. Results were expressed in mm of ΔMWD. Aggregate stability was also calculated for the 0-20 cm layer.

2.3. Maize grain yield and residue return estimation

Maize grain yield (crop planted in the years 2000, 2003, 2006, 2009, and 2012) was determined by collecting ears from 10 m^2 of the experimental units and threshing them with a stationary thresher. Grain moisture content was determined and yield corrected to 14.5% moisture. Grain yields were also expressed as relative yields (RY) to the average of the three highest grain yields of the corresponding growing season from the whole experiment regardless the TS.

Biomass production (below and above ground) was estimated for the all crops included in the sequence along the period between 1997-1998 and 2012-2013 growing seasons. Above ground biomass was estimated assuming average dry matter harvest indexes of 0.45, 0.35, and 0.45 for wheat, sunflower, and maize, respectively (Domínguez and Studdert 2006). Root biomass production and rhizodeposition at 20 cm depth were estimated according to the root:shoot ratios reported by Buyanovsky and Wagner (1997) and root distribution in the soil profile reported by Buyanovsky and Wagner (1986). Carbon content of plant tissue was assumed as 43% (Sánchez et al. 1996). The annual C return was calculated as the quotient between the accumulated below and above ground biomass C and the years of the experiment.

2.4. Statistical analyses

The statistical analysis employed linear mixed models in order to incorporate a variance-covariance model between observations of suitable form (Littell et al. 2006). Soil dependent variables were analyzed through a linear mixed model in which the effects of TS, NF, and Year were fixed and block effect was random. A repeated measure model was used to incorporate the correlations for the errors arising from measurements on the same experimental unit through the years. These analyses were done using the MIXED procedure of the Statistical Analysis System (SAS Institute 2004) and its RANDOM and REPEATED options (Littell et al. 2006). On the other hand, maize absolute and relative yields were also analyzed through a linear mixed model assuming the Year effect as random for absolute yield and as fixed for RY. Annual C return by crops was analyzed through a linear mixed model in which the effects of TS and NF were fixed and block effect was random, with the MIXED procedure (SAS Institute 2004; Littell et al. 2006). Significant effects were discussed and least square means for fixed effects were separated at the 0.05 significance level.

Simple linear regressions were run to evaluate the relationships among soil dependent variables and between each of them and Year. The relationships among soil variables and annual C return and between RY and response to NF and soil variables, were evaluated through linear simple regressions with the REG procedure (SAS Institute 2004).

3. Results and discussion

3.1. Bulk density

Interactions Year * TS * NF and Year * TS had significant effect on BD only in the 0-5 cm layer (**Table 2**). However, those interactions were not related to clear differential trends associated with treatment factors (data not shown). There were significant effects ($P < 0.05$, Table 2) of TS and Year on BD at all three layers. Bulk density was slightly higher under NT (1.24, 1.23, and 1.23 Mg m^{-3} for 0-5, 5-20 and 0-20 cm, respectively) than under CT (1.21, 1.19, and 1.20 Mg m^{-3} for 0-5, 5-20 and 0-20 cm, respectively). On the other hand, ranges of differences in BD among years were 0.03-0.09, 0.01-0.06, and 0.01-0.04 Mg m^{-3} for 0-5, 5-20 and 0-20 cm, respectively, with a general declining trend (decreases of 0.03, 0.02, and 0.03 Mg m^{-3} between 1998 and 2012 for 0-5, 5-20, and 0-20 cm, respectively). Due to the minor differences observed in BD, analyses of variance result patterns for SOC, and POC expressed in concentration and in stock (Table 2) were quite similar. This indicates that behavior of those variables were slightly affected by BD and therefore, only concentrations of SOC, and POC will be discussed.

3.2. Bulk soil, and particulate organic carbon and anaerobic nitrogen changes

The interaction Year * TS on SOC, POC and AN was significant ($P < 0.05$, **Table 2**) only at 0-5 cm. Soil organic C, POC (**Figure 1a**) and AN (**Figure 1d**) were, in general, significantly higher under NT than under CT. There was a significant ($P < 0.05$, Table 2) effect of Year on SOC, POC (**Figures 1b, c**) and AN (**Figures 1e, f**) at 5-20 and 0-20 cm. Soil organic C, POC, and AN concentrations decreased significantly

Table 2. Results of analyses of variance (*P* value) of fixed effects on bulk density, organic carbon fractions (concentration and stock) and anaerobic N

		Organic carbon				
Fixed effect	BD[a]	Concentration[a]		Stock[a]		AN[a]
		SOC	POC	SOC	POC	
0-5 cm[b]						
Tillage system (TS)	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
N fertilization (NF)	NS	0.011	0.038	0.012	0.043	NS
TS * NF	NS	NS	NS	NS	NS	NS
Year	<0.001	0.002	<0.001	<0.001	<0.001	0.002
Year * TS	<0.001	0.004	<0.001	NS	0.001	0.005
Year * NF	NS	NS	NS	NS	NS	NS
Year * TS * FN	0.002	NS	NS	NS	NS	NS
5-20 cm[b]						
Tillage system (TS)	0.043	NS	NS	NS	NS	NS
N fertilization (NF)	NS	0.010	NS	0.003	NS	NS
TS * NF	NS	NS	NS	NS	NS	NS
Year	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Year * TS	NS	NS	NS	NS	NS	NS
Year * NF	NS	NS	NS	NS	NS	NS
Year * TS * NF	NS	NS	NS	NS	NS	NS
0-20 cm[b]						
Tillage system (TS)	0.033	NS	NS	NS	0.040	0.002
N fertilization (NF)	NS	0.016	0.045	0.007	0.014	NS
TS * NF	NS	NS	NS	NS	NS	NS
Year	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Year * TS	NS	NS	NS	NS	NS	NS
Year * NF	NS	NS	NS	NS	NS	0.019
Year * TS * NF	NS	NS	NS	NS	NS	NS

[a]BD: bulk density; SOC: soil organic carbon; POC: particulate organic carbon; AN: anaerobic N.
[b]NS: not significant ($P > 0.05$).

($P < 0.05$) along the experiment regardless the TS, except under NT at 0-5 cm (**Figure 1**). **Table 3** shows the annual decrease (slopes of the linear regressions ($P < 0.01$) of the variables vs. Year) of SOC, POC and AN for all TS and NF combinations at 0-20 cm. It is worth pointing out that despite slopes not being significantly different, they tended to be lower under NT.

These results agree with very many other authors who reported SOC, POC (Puget and Lal 2005; Domínguez et al. 2009; Luo et al. 2010; Studdert et al. 2010), and AN (Diovisalvi et al. 2008; Domínguez et al. 2009; Studdert et al. 2010) stratification related to continuous use of NT respect to CT but without important differences between TS in the arable layer (0-20 cm). Our results showing decreases of SOC, POC and AN with time under cropping regardless of the TS also agree with other authors (Genovese et al. 2009; Studdert et al. 2010). In the arable layer (0-20 cm) SOC decreased 12% and POC decreased 60% along the experiment (**Figure 1c**). Particulate organic C represented 25% of SOC in 1998 and 12% of SOC in 2012. Likewise, AN decreased approximately 34% between 1998 and 2012. Therefore, both POC and AN are much more sensitive than SOC to show changes produced by soil use (Fabrizzi et al. 2003; Wander and Nissen 2004; Soon et al.

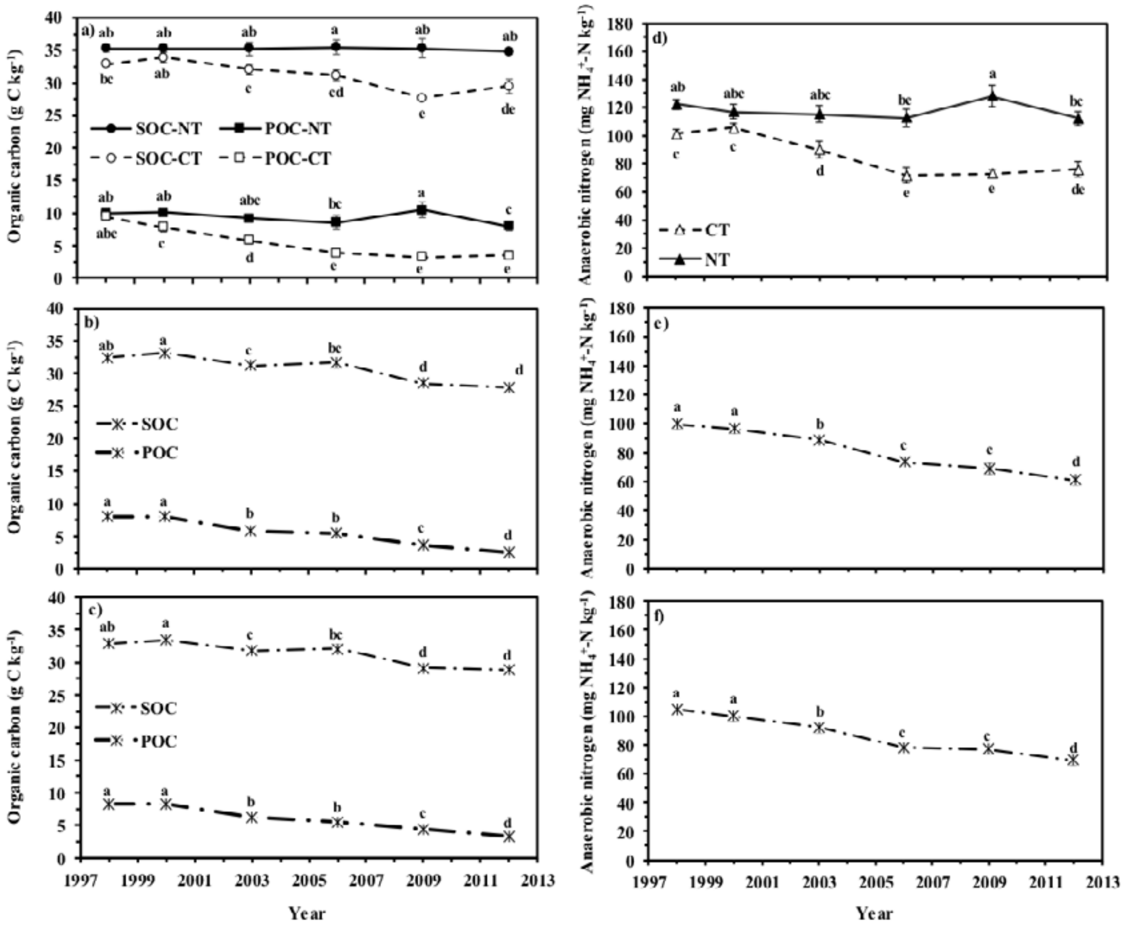

Figure 1. Soil (SOC) and particulate (POC) organic carbon, and anaerobic nitrogen (AN) at 0-5, 5-20, and 0-20 cm (a, b, and c, respectively for SOC and POC, and d, e, and f, respectively, for AN) as a function of time according to statistical analyses shown in **Table 2**. NT: no tillage, CT: conventional tillage. In each plot and for each dependent variable, equal letters indicate not significant differences (*P* > 0.05) among means. Vertical bars show standard error of the means.

Table 3. Slopes of the regression of bulk soil and particulate organic carbon and of anaerobic nitrogen at 0-20 cm vs. years of the experiment

Tillage system and nitrogen fertilization treatment		SOC[a,b]	POC[a,b]	AN[a,b]
		---------------- g C kg^{-1}yr^{-1} ----------------		mg NH_4^+-N kg^{-1} yr^{-1}
Conventional tillage	Without nitrogen	-0.38 a	-0.40 a	-3.04 a
	With nitrogen	-0.39 a	-0.46 a	-2.87 a
No tillage	Without nitrogen	-0.29 a	-0.32 a	-2.21 a
	With nitrogen	-0.26 a	-0.33 a	-2.15 a

[a]SOC: soil organic carbon; POC: particulate organic carbon; AN: anaerobic N.
[b]Mean values followed by the same letter are not significantly (*P* > 0.05) different within each column.

2007; Reussi-Calvo et al. 2014).

3.3. Relationships between soil and particulate organic carbon and anaerobic nitrogen

The similarity between SOC, POC, and AN changes in this high-SOM-content soil not exposed to erosion indicates a close relationship among them. Diovisalvi et al. (2014) had demonstrated the possibility of estimating POC through SOC content, especially for medium textured soils. Since AOC almost did not change throughout the experiment (data not shown), changes in SOC were mainly due to changes in the labile fraction (POC) which is demonstrated by the similarity between SOC and POC annual rates of decrease (**Table 3**). On the other hand, a close relationship has been shown between SOC, AN, POC and AN (Fabrizzi et al. 2003; Domínguez et al. 2009). **Figure 2** shows that in this experiment, AN strongly related to SOC and especially to POC contents both at 0-5, 5-20 and 0-20 cm depth.

Organic C dynamics in soils not exposed to erosion are mainly dependent on the balance between C gains through residues left by crops and losses through mineralization. When the former overcomes the latter, soil tends to sequester C and SOM content increases. On the contrary, when residue C returned is less than

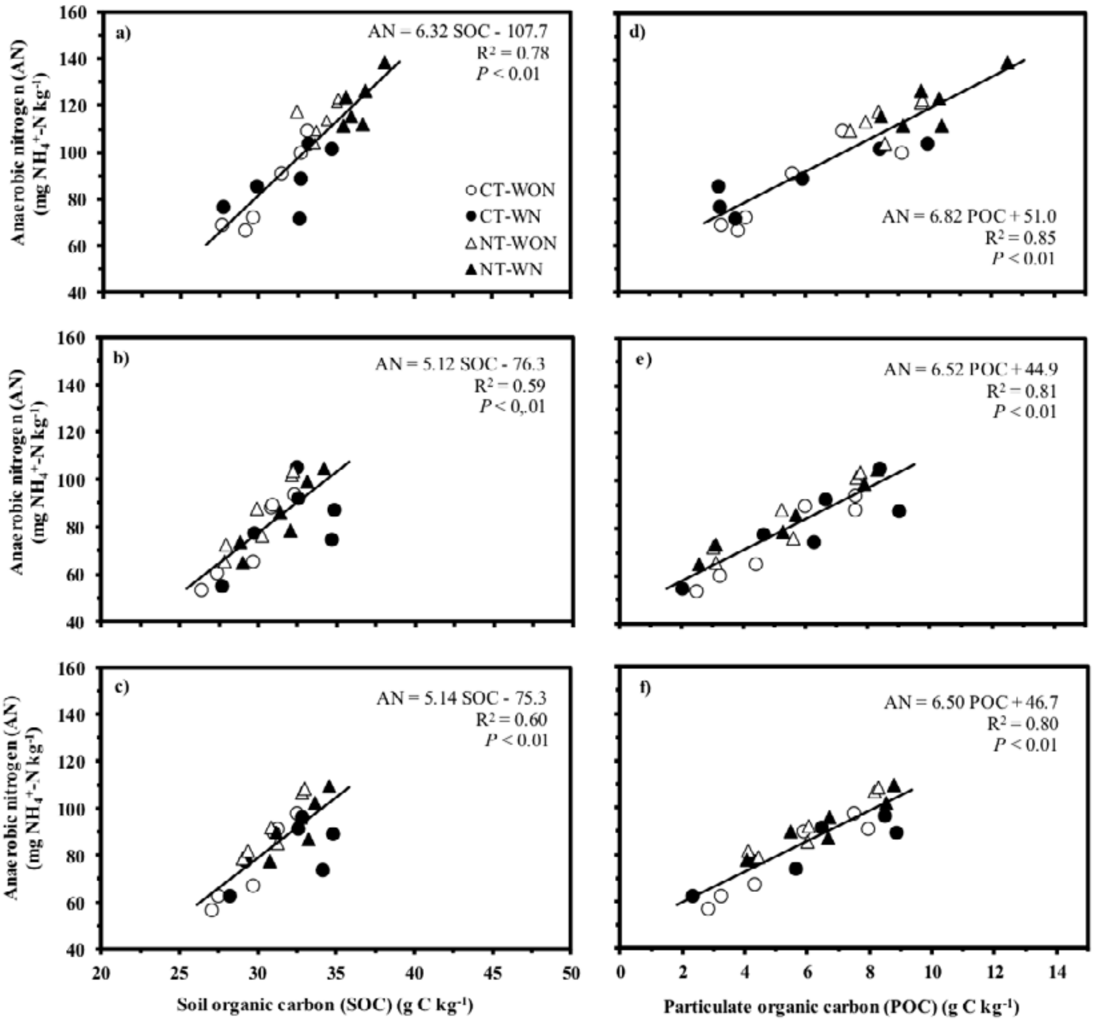

Figure 2. Relationship between anaerobic mineralizable nitrogen and soil organic carbon (a, b, c) and particulate organic carbon (d, e, f) at 0-5 (a,d), 5-20 (b,e), and 0-20 (c,f) cm. NT: no tillage, CT: conventional tillage, WON: without nitrogen fertilizer, WN: with nitrogen fertilizer.

the amount of C lost by C dioxide emission, SOM content decreases (Janzen 2006). Domínguez et al. (2009) showed that in soils like those studied in this experiment both under NT and under CT, SOC changes in the arable layer depended on the amount of C returned to the soil through crop residues. Those authors reported that regardless of the TS, it needed an average C input of approximately 6.0 Mg C ha^{-1} yr^{-1} in order to maintain a SOC content in the arable layer (0-20 cm) similar to that shown in Table 1. In this experiment, average annual C inputs were 3.39 ± 0.21 and 4.20 ± 0.10 Mg C ha^{-1} yr^{-1} for WON and WN, respectively, under CT, and 3.22 ± 0.13 and 4.47 ± 0.10 Mg C ha^{-1} yr^{-1} for WON and WN, respectively, under NT. There was neither significant ($P > 0.05$) TS * NF interaction nor effect of TS on the average annual C input, but N application did produce a significant ($P < 0.05$) increase. Anyway, C input was not enough to maintain a SOC content of 33.3 g C kg^{-1} (Table 1) and hence, according to Domínguez and Studdert (2006) and Domínguez et al. (2009), a decrease in SOC and POC throughout the experiment could have been expected regardless of TS and NF. Despite the higher C input due to N fertilization, it has been reported that continuous addition of N at high rates could lead to accelerated rates of residue decomposition and of organic soil C mineralization (Khan et al. 2007; Poirier et al. 2009; Casado-Murillo and Abril 2013; Melchiori et al. 2014). Conversely, higher C input does not always lead to an increased SOC content since this depends on the balance between increased input and higher C dioxide emission. In this experiment, the significantly higher C input associated with N addition led to a slight but in most cases significantly higher ($P < 0.05$, Table 2) SOC and POC (data not shown). Changes in SOC and POC associated with the balance between C input and C loss by mineralization were reflected in similar changes in AN (Figure 2).

3.4. Relationships of maize yield with soil and particulate organic carbon and anaerobic nitrogen

Soil organic matter plays a key role in the provision of many nutrients to crops. However, since N is the nutrient more demanded by plants and, on the other hand, is very mobile within the soil, careful management is fundamental for a sustainable agriculture (Echeverría and Sainz-Rozas 2015). Potentially mineralizable N can be estimated through AN (Echeverría et al. 2000) and, therefore, dynamics shown in Figure 1 (d, e, f) and Table 3 indicates the decrease of soil ability to supply N which would reflect in crop performance. There was no significant ($P > 0.05$) effect of the interaction TS * NF on absolute maize yields. However, grain yields were not significantly ($P > 0.05$) higher under CT than under NT (7.62 vs 7.15 Mg ha^{-1} for CT and NT, respectively) and were significantly ($P < 0.05$) higher for WN (8.82 Mg ha^{-1}) than for WON (5.94 Mg ha^{-1}). On the other hand, RY were significantly ($P < 0.05$) higher when N was applied (92.7 vs 62.9% for WN and WON, respectively) and were not different ($P > 0.05$) between TS (80.1 vs 77.5% for CT and NT, respectively). The effects of the interactions Year * TS, and Year * NF on RY were significant ($P < 0.05$). **Figure 3** shows RY along years for both TS (**Figure 3a**) and both levels of NF (**Figure 3b**). Despite the significant Year * TS interaction, differential trends in RY changes along years due to TS were not clear (**Figure 3a**). However, RY decreased ($P < 0.05$) over years under NT and kept relatively constant under CT. On the other hand, RY decreased sharply ($P < 0.05$) when N was not applied whereas they remained constant when N fertilizer was applied (**Figure 3b**). Since CT and WN led to the maintenance of a maize RY that was relatively constant with time and in the case of WN, close to the maximum, the main effect of soil management could be attributed basically to N supply dynamics.

Provided SOC, POC, and AN also decreased over time and that all three variables are closely related to the ability of soil to supply N to crops (Wander 2004; Quiroga and Studdert 2015), relationships could be established between them and RY. **Figure 4** shows the relationships between RY of WON treatments with SOC (**Figure 4a**), POC (**Figure 4b**) and AN (**Figure 4c**) at 0-20 cm under both TS. All relationships were highly significant ($P < 0.01$) both under CT and under NT, but slopes were significantly

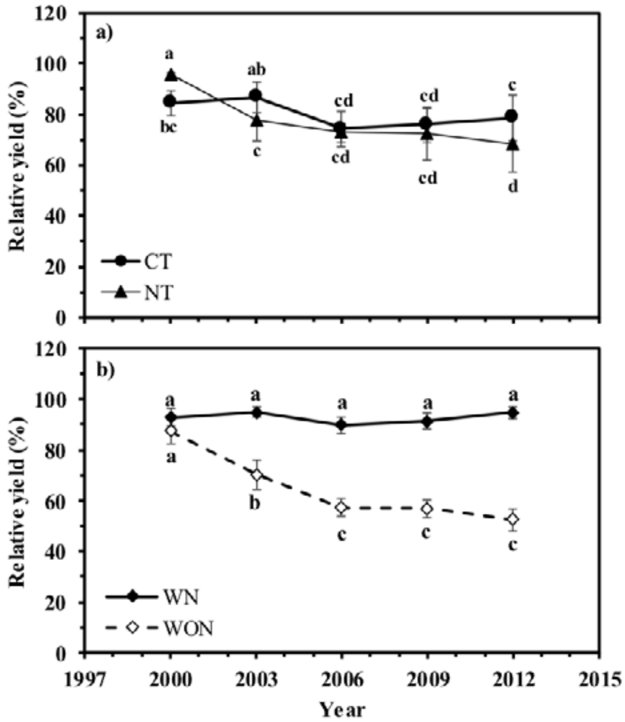

Figure 3. Relative yields of maize as a function of time under two tillage systems (a): NT: no tillage, CT: conventional tillage) and to levels of nitrogen fertilization (b): WON: without nitrogen fertilizer, WN: with nitrogen fertilizer). In each plot equal letters indicate not significant differences (*P* > 0.05) among means. Vertical bars show standard error of the means.

higher (*P* = 0.049, *P* = 0.027, and *P* = 0.001 for RY vs SOC, POC and AN, respectively) and intercepts were significantly lower (*P* = 0.041, *P* = 0.008, and *P* = 0.001 for RY vs SOC, POC and AN, respectively) under NT than under CT. Coefficients of determination for regressions RY vs SOC (**Figure 4a**) under CT and NT were lower than the R^2 of regressions RY vs POC (**Figure 4b**) and RY vs AN (**Figure 4c**). It is worth noting that when N fertilizer was applied RY ranged between 88.0 and 98.2% of maximum yield both under CT and NT and did not show a relationship with SOC, POC or AN (data not shown).

The close relationship observed between maize performance without N fertilization (i.e. depending on N supplied by soil) and SOM labile fractions and the difference observed between TS (**Figure 4**), reflect their sensitiveness to changes in soil. However, the simplicity of the determination of AN with respect to the determination of POC makes it much more feasible to be used to characterize N availability for crops. Previous works have described the relationship between maize (Calviño and Echeverría 2003; Sainz-Rozas et al. 2008) and wheat (Reussi-Calvo et al. 2013) yields and AN. This led to the proposition of AN as a complement to diagnose mineral N availability in soil for those crops (Sainz-Rozas et al. 2008; Reussi-Calvo et al. 2013; Echeverría et al. 2015). Nevertheless, all these works were done under NT and the results shown in Figure 4c indicate that when tillage is used (i.e. CT or other), N mineralization is promoted, and the increase of RY per unit of increase in AN is lower. Higher mineralization due to tillage increases N supply by soil even with low AN contents because of aggregate breakage and exposure of protected fractions (Six et al. 2004; Triplett and Dick 2008), and maize yields are greater than under NT (Domínguez et al. 2001). Therefore, TS to be used should be taken into account to adjust the coefficients proposed by Reussi-Calvo et al. (2013) and Echeverría et al. (2015) to better reflect the sensitiveness of AN to TS and therefore the difference in N supply according to tillage operations.

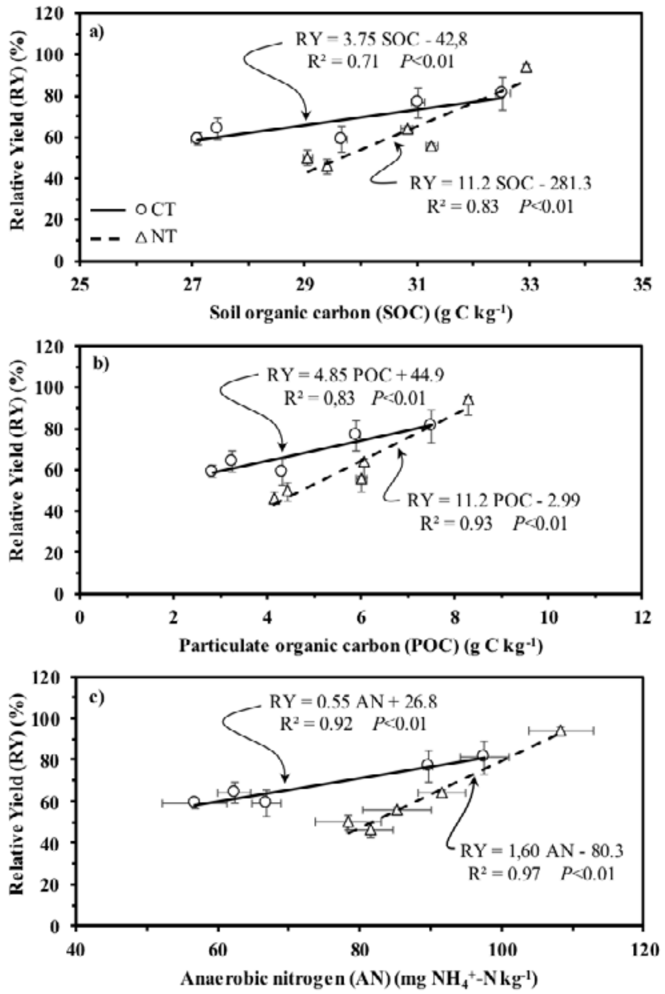

Figure 4. Relationship between relative yields of corn and soil (a) and particulate (b) organic carbon, and anaerobic mineralizable nitrogen (c) at 0-20 cm. NT: no tillage, CT: conventional tillage. Symbols represent the average of three replications. Vertical bars show standard error of the mean of relative yield and horizontal bars show standard error of the mean of the respective independent variable.

3.5. Relationships of aggregate stability with soil and particulate organic carbon and anaerobic nitrogen

Aggregate stability has been reported as the most sensitive soil physical property to soil management (Aparicio and Costa 2007) and that in loamy soils it is closely related to the soil organic fraction, especially its labile part (Six et al. 2004). Eiza et al. (2006) and Agostini et al. (2012) demonstrated that the relationship between AS and POC was stronger than the relationship between AS and SOC. Eiza et al. (2006) showed that those relationships were verifiable mostly when soil was not or was slightly disturbed (i.e. under pasture or NT), but when soil was disturbed (i.e. under CT) relationships were very poor or did not exist. Intra-aggregate POC has an important role in aggregate formation and re-cycling. When soil is disturbed by tillage, intra-aggregate POC is reduced, aggregate re-cycling interrupted and formation of stable aggregates affected (Six et al. 2004). Therefore, labile organic fractions content, especially within aggregates, would show the ability of soil to form and maintain stable aggregation. Table 4 shows ΔMWD under CT and NT at 0-5, 5-20, and 0-20 cm. There was no significant ($P > 0.05$) Year * TS interaction effect nor Year effect on AS. However, a trend towards a decrease of AS (higher ΔMWD) throughout the experiment, can be seen at all three depths. On the other hand,

Table 4. Mean weight diameter change at 0-5, 5-20, and 0-20 cm under two tillage systems. Mean values followed by the same letter are not significantly (*P* > 0.05) different for each depth

Depth	Tillage system[a]	Year			
		2006	2009	2012	Mean
cm		---------- mm ----------			
0-5	CT	0.94	1.07	1.14	**1.05 a**
	NT	0.48	0.31	0.56	**0.45 b**
	Mean	**0.71 a**	**0.69 a**	**0.85 a**	
5-20	CT	1.06	1.26	1.25	**1.19 a**
	NT	0.50	0.43	0.68	**0.54 b**
	Mean	**0.78 a**	**0.84 a**	**0.96 a**	
0-20	CT	0.91	1.21	1.22	**1.11 a**
	NT	0.49	0.40	0.65	**0.52 b**
	Mean	**0.70 a**	**0.81 a**	**0.94 a**	

[a]CT: conventional tillage; NT: no-tillage.

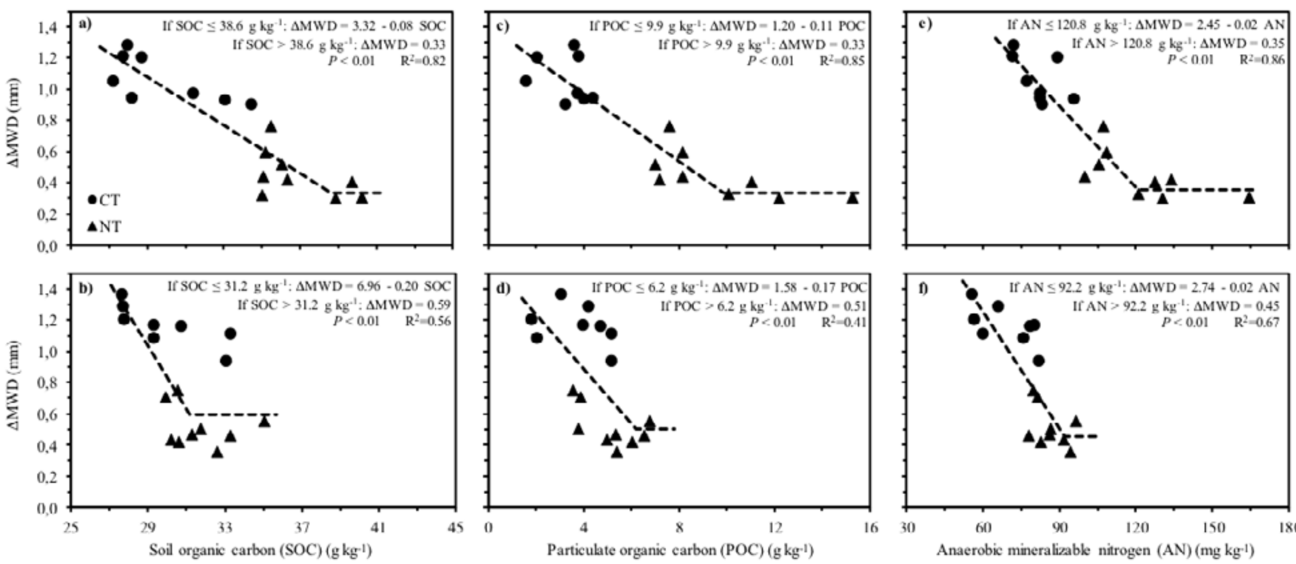

Figure 5. Relationship between change of mean weigh diameter (ΔMWD) and soil (a, b) and particulate (c, d) organic carbon, and anaerobic mineralizable nitrogen (e, f) at 0-5 (a, c, d) and 0-20 (b, d, f) cm. NT: no tillage, CT: conventional tillage.

under NT, AS was consistently significantly (*P* < 0.05) higher (lower ΔMWD) than under CT. This agrees with previous research (Six et al. 2004; Eiza et al. 2006; Agostini et al. 2012) and given the differences and trends observed for both TS in SOC, POM and AN contents, relationships between them and AS could be evaluated.

Figure 5 shows the highly significant (*P* < 0.01) relationships between AS and SOC (**Figures 5a, b**), POC (**Figures 5c, d**), and AN (**Figures 5e, f**) at 0-5 and 0-20 cm, respectively. There were no significant (*P* > 0.05) relationships between AS and any of the other variables at 5-20 cm (data not shown). Changes in SOC, POC and AN explained much more of the variability of AS in the uppermost layer than in the whole arable layer. At the 0-5 cm layer both POC and AN explained more variability of AS than SOC, but at the 0-20 cm layer the variation in AN explained more AS variability than the other two variables. The recorded variability in AS was not as high as expected for a wider range of situations. The first year with AS data was nine years after the beginning of the experiment. According to Domínguez et al. (2008) the AS index in the arable layer (0-20 cm) dropped sharply after the start of cropping under CT and in the ninth year it had reached the lowest level. On the other hand, those authors showed that under NT the decrease in AS index was not as steep as under CT but in the ninth year of cropping after a pasture AS index was higher than under CT but much lower than at the beginning. In this study, the range of AS values was not as high as could have been desirable to evaluate the relationship with soil organic fractions. However, differences between TS and even trends among Years were reflected by AN content better than either SOC or POC, especially in the uppermost layer.

4. Conclusions

Nitrogen mineralized during a short anaerobic incubation (AN) has been demonstrated to be sensitive to soil management under different tillage systems and nitrogen fertilization practices. Its variation responds to changes in soil and particulate organic carbon, and relates to unfertilized maize yield and can be associated with changes in aggregate stability as a consequence of soil use for cropping. Its determination is simple, and relatively rapid. Despite this study being carried out with data from a limited number of situations, the results encourage the use of AN as soil quality/health indicator since it presents most of the conditions required for a soil variable to be used as a soil quality/health indicator.

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