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Anaerobically mineralized nitrogen within macroaggregates as a soil health indicator

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ABSTRACT

Anaerobically mineralized nitrogen (AN) in bulk soil (AN_{BS}) has been described as a soil health indicator. Considering that large macroaggregates (2000–8000 μm, MA) are more sensitive to management practices than the bulk soil (i.e. whole soil), AN within MA (AN_{MA}) would be a better soil health indicator than AN_{BS}. The aim of this study was to evaluate if AN_{MA} is a better indicator of: i) soil organic carbon (SOC) and particulate organic carbon (POC) in bulk soil (SOC_{BS} and POC_{BS}, respectively) and ii) aggregate stability (AS) than AN_{BS}. Soil samples were taken at 0–5 and 5–20 cm from 46 continuously cultivated plots (CC) and a reference plot for each CC (pseudo-pristine, PRIS). These soils, located in the Argentinean Pampas, were classified as Mollisols with contrasting surface textural classes. The AS, SOC_{BS}, POC_{BS}, AN_{BS}, SOC (SOC_{MA}), and POC (POC_{MA}) within MA and AN_{MA} were determined separately at 0–5 and 5–20 cm soil depths and estimated at the 0–20 cm layer. The AN_{MA} was a good indicator of SOC_{BS} (R^2 0.75, 0.48, and 0.61 at 0–5, 5–20 and 0–20 cm depths, respectively), POC_{BS} (R^2 0.66, 0.31, and 0.49, respectively), and AS (R^2 0.80, 0.68, and 0.76, respectively). The AN_{MA} performed similarly to predict SOC_{BS}, POC_{BS}, and AS as compared to AN_{BS}, because AN_{MA} was closely correlated to AN_{BS} (r 0.90 at 0–20 cm). Since AN_{MA} determination is more time-consuming than AN_{BS} determination, its use as a soil health indicator would not be convenient. Therefore, the use of AN_{BS} would be recommended over AN_{MA} as a variable to monitor soil health.

1. Introduction

Soil health deterioration (i.e. soil degradation) is being observed worldwide due to the change of land use and poor soil management and improper cropping practices (Doran, 2002). This degradation process implies a decrease in soil functioning capacity and a consequent decrease in the provision of ecosystem services to society (Powlson et al., 2011). Soil health indicators should be used to allow diagnosing and quantifying the rate and magnitude of the degradation process to help planning adequate management practices to improve soil health status. A suitable soil health indicator should comply with several characteristics: i) it must be sensitive to soil use changes, ii) it must be easily and economically measured, and iii) its changes should be associated with changes in other soil variables (Doran and Parkin, 1996).

Soil organic carbon (SOC) in bulk soil (i.e. whole soil) (SOC_{BS}) is related to several factors that define soil health (Lal, 2010). However, changes in SOC_{BS} content due to cultivation are generally slow and, consequently, do not reflect early changes in soil health (Domínguez et al., 2016). The separation of fractions of SOC_{BS} is very useful to study the carbon pools with different sensitivity to management practices (Six

Abbreviations: AN, anaerobically mineralizable nitrogen; AN_{BS}, AN in bulk soil; AN_{MA}, AN within MA; AS, aggregate stability; CC, plots under constinuos cultivation; CW, capillary wetting; MA, large macroaggregates (2000–8000 μm); $massMA_{FW}$, MA dry mass remnant after fast wetting; POC, particulate organic carbon; POC_{BS}, POC in bulk soil; POC_{MA}, POC within MA; PRIS, reference situation for each CC; SOC, soil organic carbon; SOC_{BS}, SOC in bulk soil; SOC_{MA}, SOC within MA.

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et al., 2002). For example, the fractionation of soil according to particle size allows isolating a labile fraction of SOC_{BS}, the particulate organic carbon (POC) in bulk soil (POC_{BS}). It has been reported that POC_{BS} is an early indicator of soil health changes (Cambardella and Elliot, 1992; Wander, 2004). However, POC_{BS} determination is laborious and time-consuming.

The SOC_{BS} and POC_{BS} are closely related to aggregate stability (AS) (García et al., 2020b) and, in turn, AS has a great influence on physical protection of SOC_{BS} and POC_{BS} (Six et al., 2002; Rasmussen et al., 2018; Lal, 2018). This is because organic carbon allocation within aggregates determines different degrees of accessibility to decomposers (Six et al., 2002; Lal, 2018). The fractionation of soil by aggregate size allows: i) isolating organic carbon fractions with different degree of stabilization and protection, and contrasting dynamics, and ii) characterizing the relationship of these fractions with aggregate size distribution (Six et al., 2002) and soil health.

It is known that soil AS is mainly defined by the stability of the large-macroaggregate (2000–8000 μm, MA) stability (García et al., 2020a). The preservation of MA stability has a big impact on soil health since it promotes the accumulation of SOC_{BS} (Lal, 2018) and, consequently, it improves soil physical health (García et al., 2020a). The MA stability depends, in turn, on their SOC and POC content (SOC_{MA} and POC_{MA}, respectively) (Six et al., 2004; Mandiola et al., 2011). Some authors (Novelli et al., 2017; Sarker et al., 2018; Sithole et al., 2019) reported that SOC_{MA} was more sensitive than SOC_{BS} to indicate changes produced by management practices. For Mollisols of the southeastern Argentinean Pampas, it has been reported that MA proportion, MA stability, and SOC_{MA} and POC_{MA} in the arable layer (0–20 cm) were sensitive to management practices (Mandiola et al., 2011; Roldán et al., 2012a, b). Thus, organic fractions within MA would be more sensitive than organic fractions in bulk soil as indicators of MA stability, AS, and soil health.

Anaerobically mineralized nitrogen (AN) in bulk soil (AN_{BS}) is a suitable soil health indicator (Domínguez et al., 2016; García et al., 2016; Rivero et al., 2020; García et al., 2020b) since it complies with all expected soil health indicator characteristics.The AN_{BS} is simple, inexpensive, and safe to measure. Besides, AN_{BS} is sensitive to management practices (Soon et al., 2007; García et al., 2016) and it is closely associated with several soil properties, such as potentially mineralizable nitrogen (Wyngaard et al., 2018) and nitrogen availability for crops (Reussi Calvo et al., 2018), potentially mineralizable and available sulfur (Carciochi et al., 2018), SOC_{BS} and POC_{BS} (Domínguez et al., 2016; García et al., 2020b), and AS (Domínguez et al., 2016; García et al., 2020b; Rivero et al., 2020). Since SOC_{MA} and POC_{MA} have been reported as more sensitive than SOC_{BS} and POC_{BS}, respectively (Novelli et al., 2017; Sarker et al., 2018; Sithole et al., 2019), AN determined within MA (AN_{MA}) could be a more sensitive soil health indicator than AN_{BS}. Therefore, it is hypothesized that AN_{MA} is a better indicator of AS, SOC_{BS}, and POC_{BS} and, consequently, of soil health than AN_{BS}. The aim of this study was to evaluate the performance of AN_{MA} as an indicator of SOC_{BS}, POC_{BS}, SOC_{MA}, POC_{MA}, and AS as compared to AN_{BS}. If AN_{MA} was a more sensitive and precise soil health indicator than AN_{BS}, it would be a more useful and specific tool for monitoring soil health, despite the additional methodological steps involved in its determination.

2. Materials and methods

2.1. Site description and soil sampling

Soil sampling was carried out in Mollisols (Soil Survey Staff, 2014; Rubio et al., 2019) of 46 farms from the southeastern Buenos Aires Province, Argentina (Fig. 1). The climate in this area is classified as mesothermal subhumid-humid (according to the Thornthwaite classification) or as temperate humid without dry season (according to the Köpen classification). Median annual rainfall ranges from 759 mm (West) to 950 mm (East), and mean air temperature ranges from 14.1 °C (South) to 15.1 °C (North). The sampling sites were selected to represent surface textural classes typical of the studied region (Durán et al., 2011; Rubio et al., 2019), and did not show evidences of erosion (slope < 2%) nor flooding. In each site, the samples were taken from geo-referenced plots (400 m^2) representing both situations under continuous cultivation (CC), and undisturbed situations (pseudo-pristine, PRIS, no farther than 500 m from CC) as reference for each CC plot. The selected PRIS plots had been undisturbed for more than 20 years and were mainly under grasses (*Lolium perenne* L., *Bromus unioloides* Kunth, *Festuca arundinacea* Schreb., *Dactylis glomerata* L., *Phalaris tuberosa* L.). It was assumed that the PRIS soil condition was similar to that of the pristine soils of the region (Durán et al., 2011; Rubio et al., 2019). The selected CC plots had been under production of annual either summer or spring cash crops with no-tillage for the last 4 to 20 years. The number of CC plots (i.e. 46) was greater than the number of PRIS plots (i.e. 34), since in some sites one PRIS plot was the reference situation of more than one

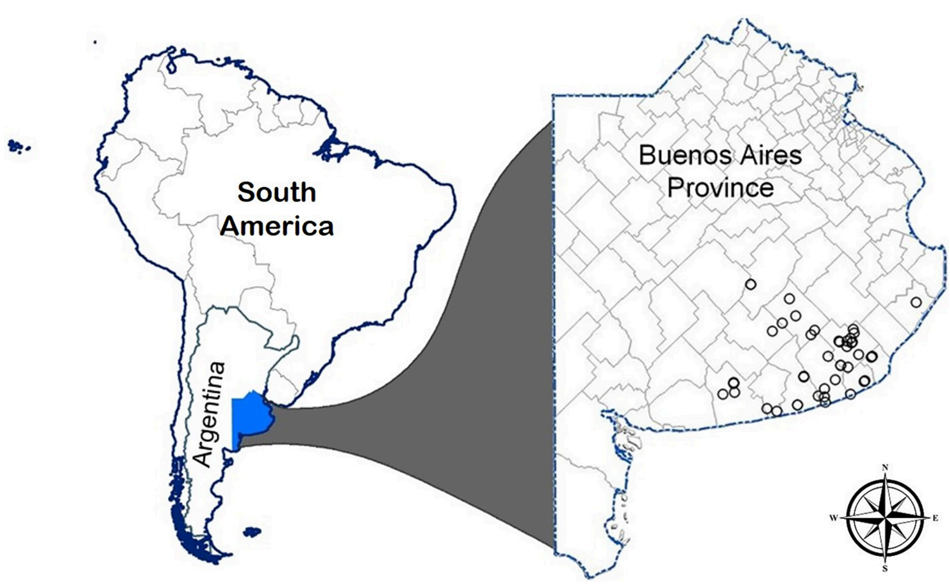

Fig. 1. Sampling sites throughout the southeastern Buenos Aires province at the Argentinean Pampas (n = 46). Only the location of continuosly cultivated (CC) plots is shown because the location of pseudo-pristine plots was very close to the CC plots (no more than 500 m away).

CC plot.

Composite soil samples to determine mineral particle size distribution, SOC_{BS}, POC_{BS}, and AN_{BS} were taken with a 4.4-cm-diameter tubular sampler at field capacity in the autumn–winter. Sampling depths were 0–5 cm (15 subsamples per sample) and 5–20 cm (5 subsamples per sample). Afterward, samples were dried at 50 °C until constant weight and then ground to pass through a 2000-µm sieve removing all identifiable plant material. Furthermore, to determine AS, SOC_{MA}, POC_{MA}, and AN_{MA}, composite samples from each plot were taken with a shovel (5 subsamples at 0–5 and 5–20 cm depth, respectively). Upon extraction (i.e. in moist condition), the aggregates of these latter samples were carefully and manually separated to pass through an 8000-µm sieve removing all identifiable plant material, and then dried at 50 °C until constant weight.

2.2. Soil fractionation and analysis

Soil mineral particle size (sand, silt, and clay) distribution was determined by the hydrometer method (Gee and Bauder, 1986) and soil textural classes were determined through the texture triangle (Soil Survey Staff, 2014). The MA stability was determined as an indicator of AS (García et al., 2020a) through the remnant MA dry mass after water sieving following fast wetting ($massMA_{FW}$) (Six et al., 1998). Briefly, 100 g of the processed dry sample (passed through 8000-µm sieve) were sieved through a 2000-µm sieve in water, moving the sieve up and down 3 cm with 50 repetitions for 2 min after sudden immersion of dry aggregates for 5 min. All free floating plant material was removed with a hand strainer before recovering the remnant MA. The remnant aggregates were recovered and dried at 50 °C until constant weight. The $massMA_{FW}$ was expressed in g MA (100 g)$^{-1}$ dry soil. Since all the evaluated soils showed more than 95% of the sand fraction lower than 250 µm (fine and very fine sands) (INTA, 1979), the correction of MA dry mass by sand content indicated by Six et al. (2000) was not performed as suggested by Yamashita et al. (2006). Another 100 g aliquot of the processed dry sample (passed through 8000-µm sieve) was used for MA separation for further SOC_{MA}, POC_{MA}, and AN_{MA} determination. In detail, after capillary wetting (CW) for 24 h until field capacity water content, the moistened aggregates were water-sieved through a 2000 µm sieve and processed as previously described (Six et al., 1998). Then, the dry MA after CW were grounded with mortar and pestle. The chemical determinations were performed on the MA remnant after CW since this wetting treatment allows aggregates to manifest their maximum possible stability at their present condition (Six et al., 1998). The SOC_{MA} and POC_{MA} after CW are indicators of the MA stability when facing more aggressive forces as those caused by fast wetting (Roldán et al., 2012a, b).

Soil organic carbon (either SOC_{BS} or SOC_{MA}) was determined by the wet combustion procedure, maintaining the reaction temperature at 120 °C for 90 min (Nelson and Sommers, 1982). The particulate fraction of both bulk soil and MA was separated according to Cambardella and Elliott (1992), after dispersion by shaking the soil sample for 16 h with a sodium hexametaphosphate solution. Organic carbon content was determined on the < 53 µm fraction (mineral-associated organic carbon either in bulk soil or within MA) as previously described for SOC. As proposed by Cambardella and Elliott (1992), either POC_{BS} or POC_{MA} were calculated by subtracting the mineral-associated organic carbon in bulk soil or in MA, from SOC_{BS} or SOC_{MA}, respectively. The results of SOC_{BS} and POC_{BS} were expressed in g carbon kg^{-1} bulk soil dry mass, whereas SOC_{MA} and POC_{MA} were expressed in g carbon kg^{-1} MA dry mass.

Anaerobically mineralized nitrogen (AN_{BS} and AN_{MA}) was determined through short anaerobic incubation for 7 d at 40 °C (Keeney, 1982). The ammonium-nitrogen concentration was quantified before and after the incubation period by steam distillation (Keeney and Nelson, 1982). The AN was calculated as the difference between the final and the initial ammonium-nitrogen. Anaerobically mineralized nitrogen in bulk soil was expressed in mg ammonium-nitrogen kg^{-1} bulk soil dry mass, whereas AN_{MA} was expressed in mg ammonium-nitrogen kg^{-1} MA dry mass.

2.3. Data analysis

All variables were also shown for the 0–20 cm layer through the average weighted by sampling layer thicknesses (i.e. 5 and 15 cm, respectively). To characterize the different variables, some descriptive measures were calculated. Addicionally, for each depth, analyses of variance were performed to compare mean values of all variables between soil uses (CC and PRIS), considering also the site effect. In these analyses, CC plots with the same reference PRIS plot were considered as coming from the same site. To compare depths (0–5 and 5–20 cm) for each soil use, t-tests for paired samples were used. The association between variables was evaluated through Pearson correlation coefficients and multiple and simple linear regression models. Diagnostic tools were used to check the assumptions. The statistical analyses were performed with R software (R Core Team, 2018). A significance level of 0.05 was used. The performance of AN_{MA} as a predictor of SOC_{BS}, POC_{BS}, and AS was compared to the performance of AN_{BS} as a predictor of SOC_{BS}, POC_{BS}, and AS, described by García et al. (2020b) for the same sampling sites.

3. Results and discussion

3.1. Soil texture

According to the soil mineral particl size distribution (Table 1), soil surface textural classes of the sampling sites were loam, sandy-loam, sandy-clay-loam, and clay-loam (Soil Survey Staff, 2014). The distribution of surface textural classes throughout the area under study responded to the particle size distribution ot the “pampean” loess (soil parent material) (Durán et al., 2011) given the finest textural class (i.e. clay loam) was described in the central part ot the Buenos Aires province, and the coarsest (i.e. sandy-loam) was found on the West of the study area and, besides, close to the ocean shore (Fig. 1).

3.2. Soil organic carbon within MA and POC_{MA} and their relationships with AS

Maximum, minimum, and mean SOC_{MA}, POC_{MA}, and $massMA_{FW}$ values are shown in Table 1. The PRIS showed greater ($P < 0.05$) SOC_{MA} and POC_{MA} mean values than CC (Table 1), indicating that both variables are sensitive to soil use. Previous studies had reported that SOC_{MA} and POC_{MA} were sensitive to soil use changes (Roldán et al., 2012a; King et al., 2019) and management practices (Six et al., 1998; Roldán et al., 2012b; Mandiola et al., 2011; Scott et al., 2017; Novelli et al., 2017; Sarker et al., 2018). Likewise, mean $massMA_{FW}$ values for PRIS were greater ($P < 0.05$) than those observed for CC, demonstrating that AS is also sensitive to soil use change, as previously described by Roldán et al. (2014), Scott et al. (2017), and King et al. (2019). When comparing between soil layers, SOC_{MA}, POC_{MA}, and $massMA_{FW}$ mean values showed stratification for both PRIS and CC, since they were greater ($P < 0.05$) at 0–5 than at 5–20 cm depth (Table 1). The same trends observed for SOC_{MA} and POC_{MA} were observed for SOC_{BS} and POC_{BS}, respectively (Table 1).

The greater organic carbon content in the PRIS is attributable to the absence of disturbance and the greater carbon inputs (Tisdall and Oades, 1982). Soil use change from PRIS to CC generally produces a decrease of SOC and its labile fractions (Table 1) due to the increased carbon outputs and reduced carbon inputs (Studdert and Echeverría, 2000). Besides, for both soil uses, the aboveground litter (PRIS) and crop residues (CC) on the surface and the reduced soil disturbance (pseudo-pristine condition and no-tillage, respectively) led to the stratification of SOC (Franzluebbers and Stuedemann, 2009). These differences in carbon inputs/

Table 1
Maximum (Max), minimum (Min) and mean (Mean) values at three depths for sand, clay, and silt at sampling sites (n = 80), 2000–8000 μm macroaggregate (MA) mass after fast wetting ($massMA_{FW}$), soil organic carbon in bulk soil (SOC_{BS}) and within MA after capillary wetting (SOC_{MA}), particulate organic carbon in bulk soil (POC_{BS}) and within MA after capillary wetting (POC_{MA}), and anaerobically mineralized nitrogen in bulk soil (AN_{BS}) and within MA after capillary wetting (AN_{MA}) for two soil uses (SU): plots under continuous cultivation (CC, n = 46) and pseudo-pristine (PRIS, n = 34). Means followed by equal upper-case letters indicate not significant differences (P greater than 0.05) between PRIS and CC for each depth. Means followed by equal lower-case letters indicate not significant differences (P greater than 0.05) between 0–5 and 5–20 cm for each SU.

Variable	SUge	Depths (cm)								
		0–5			5–20			0–20		
		Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Sand (g kg^{-1})	–	684.2	265.2	428.5	699.5	258.9	431.5	695.7	264.7	430.7
Clay (g kg^{-1})	–	365.9	103.6	243.0	386.4	100.3	253.8	379.3	101.1	251.1
Silt (g kg^{-1})	–	487.9	157.7	328.6	472.7	147.9	314.8	472.7	150.4	318.2
$massMA_{FW}$ (g $(100\ g)^{-1}$)	PRIS	77.7	11.9	50.5 Aa	74.0	5.4	41.1 Ab	74.9	9.4	43.4 A
	CC	41.0	2.9	14.9 Ba	32.7	0.5	12.0 Ba	30.0	2.1	12.7B
SOC_{BS} (g kg^{-1})	PRIS	92.9	26.9	55.0 Aa	52.5	16.7	36.2 Ab	61.1	19.3	40.9 A
	CC	54.2	22.0	38.2 Ba	43.4	16.2	31.1 Bb	46.1	17.7	32.9B
POC_{BS} (g kg^{-1})	PRIS	53.5	5.0	20.4 Aa	19.9	0.6	7.2 Ab	25.7	2.4	10.5 A
	CC	20.4	2.3	8.0 Ba	11.7	0.3	3.7 Bb	12.5	1.0	4.8B
AN_{BS} (mg kg^{-1})	PRIS	334.5	63.1	184.6 Aa	142.6	29.1	80.2 Ab	184.6	42.8	106.3 A
	CC	138.4	51.7	93.1 Ba	84.1	25.0	52.8 Bb	95.4	38.3	62.9B
SOC_{MA} (g kg^{-1})	PRIS	120.7	31.4	57.6 Aa	61.7	24.4	39.2 Ab	60.7	28.1	43.8 A
	CC	63.3	24.9	40.0 Ba	43.6	19.6	32.9 Bb	46.9	20.9	34.7B
POC_{MA} (g kg^{-1})	PRIS	45.0	5.6	18.7 Aa	13.3	2.6	7.7 Ab	21.2	4.0	10.5 A
	CC	22.7	2.0	8.6 Ba	8.6	0.5	4.9 Bb	11.2	0.9	5.8B
AN_{MA} (mg kg^{-1})	PRIS	390.9	63.0	200.5 Aa	209.8	48.6	108.2 Ab	255.1	62.1	131.3 A
	CC	179.6	53.8	105.7 Ba	110.7	42.6	66.3 Bb	122.3	45.4	76.2B

outputs and stratification also explain why PRIS at the surface layer (0–5 cm) presented the greatest AS values (Six et al., 2004).

The correlations between $massMA_{FW}$ and SOC_{MA} and between $massMA_{FW}$ and POC_{MA} were positive at all three depths (Table 2, section A). Therefore, MA stability (i.e. $massMA_{FW}$), which defines AS (García et al., 2020a), is related to SOC_{MA} and POC_{MA} concentrations, confirming previous results (Mandiola et al., 2011; Roldán et al., 2012a; Scott et al., 2017). Linear statistical models to predict $massMA_{FW}$ from SOC_{MA} (Fig. 2a, b, c), showed that the positive slopes were statistically equal for both CC and PRIS, whereas the intercepts were different. The greater intercept (i.e. greater adjusted mean of AS) for PRIS than for CC can be attributed to the hydrophobicity and physical enmeshment of soil particles, caused by the greater abundance of roots and microbial activity (Chenu et al., 2000), and to the lack of disturbance in PRIS (Six et al., 2004). García et al. (2020b) reported similar results (i.e. equal slopes and different intercepts for CC and PRIS) when evaluating SOC_{BS} as a predictor of $massMA_{FW}$ at the same sampling sites. Neither clay nor sand contents showed a significant effect on the adjustment of $massMA_{FW}$ as a function of SOC_{MA} (data not shown) in coincidence with García et al. (2020b). For all depths, SOC_{MA} explained $massMA_{FW}$ variability ($P < 0.001$) and had slightly lower R^2 values (0.70, 0.62, and 0.68 at 0–5, 5–20, and 0–20 cm depths, respectively, Fig. 2a, b, c) than those reported by García et al. (2020b) between $massMA_{FW}$ and SOC_{BS} (0.77, 0.65, and 0.73 at 0–5, 5–20, and 0–20 cm depths, respectively, all with $P < 0.001$). Thus, contrary to the expectations, SOC_{MA} was not a better indicator of MA stability and, therefore, AS than SOC_{BS}.

Table 2
Pearson correlation coefficients for: Section A: associations between 2000–8000 μm macroaggregates (MA) mass after fast wetting ($massMA_{FW}$) and variables within MA after capillary wetting (soil organic carbon (SOC) within MA (SOC_{MA}), particulate organic carbon (POC) within MA (POC_{MA}), or anaerobically mineralized nitrogen (AN) within MA (AN_{MA}); Section B: associations between AN_{MA} and organic carbon within MA (SOC_{MA} and POC_{MA}); Section C: associations between AN_{MA} and organic carbon in bulk soil (BS) (SOC in BS (SOC_{BS}) and POC in BS (POC_{BS}); Section D: associations between variables within MA and variables in BS (SOC_{MA} and SOC_{BS}, POC_{MA} and POC_{BS}, AN_{MA} and AN_{BS}) at three depths (0–5, 5–20 and 0–20 cm). n = 80. All correlations were significant ($P < 0.001$).

Depth (cm)	Pearson correlation coefficients		
	A – between $massMA_{FW}$ and variables in MA		
	SOC_{MA}	POC_{MA}	AN_{MA}
0–5	0.68	0.64	0.85
5–20	0.62	0.56	0.79
0–20	0.67	0.66	0.84
	B – between AN_{MA} and organic carbon in MA		
	SOC_{MA}	POC_{MA}	
0–5	0.81	0.70	
5–20	0.73	0.42	
0–20	0.79	0.59	
	C – between AN_{MA} and organic carbon in BS		
	SOC_{BS}	POC_{BS}	
0–5	0.87	0.81	
5–20	0.70	0.56	
0–20	0.79	0.70	
	D – between variables in MA and variables in BS		
	SOC_{MA} vs. SOC_{BS}	POC_{MA} vs. POC_{BS}	AN_{MA} vs. AN_{BS}
0–5	0.88	0.79	0.88
5–20	0.92	0.51	0.85
0–20	0.94	0.71	0.90

It has been reported that a great part of SOC_{BS} and POC_{BS} is located within MA (De Oliveira Ferreira et al., 2018; King et al., 2019; Rivero et al., 2020). Li et al. (2016) and King et al. (2019) informed that the main destination of carbon input into the soil is the aggregate size fraction that predominates within the soil mass. Thus, in soils where MA predominate, they are the main destination of carbon inputs into the soil (Li et al., 2016; King et al., 2019). Hence, the greater the proportion of MA, the greater the proportion of SOC_{BS} and POC_{BS} accumulated within them (King et al., 2019). In this study, the correlations between SOC_{BS} and SOC_{MA} (Table 2, section D) show that these two variables are highly and positively associated ($P < 0.001$), and this would explain the similarity of the relationships between $massMA_{FW}$ and SOC_{BS} (García et al., 2020b) and between $massMA_{FW}$ and SOC_{MA} (Fig. 2).

According to the previously described results, the associations between $massMA_{FW}$ and POC_{MA} (Fig. 2d, e, f) were expected to be similar to those between $massMA_{FW}$ and SOC_{MA} (Fig. 2a, b, c), SOC_{BS} or POC_{BS} (García et al., 2020b), which presented positive and equal slopes for CC and PRIS. However, in the current work, the statistical models to predict $massMA_{FW}$ from POC_{MA} (Fig. 2d, e, f), showed positive slopes for PRIS and negative slopes for CC at all three depths. Although POC_{BS} was

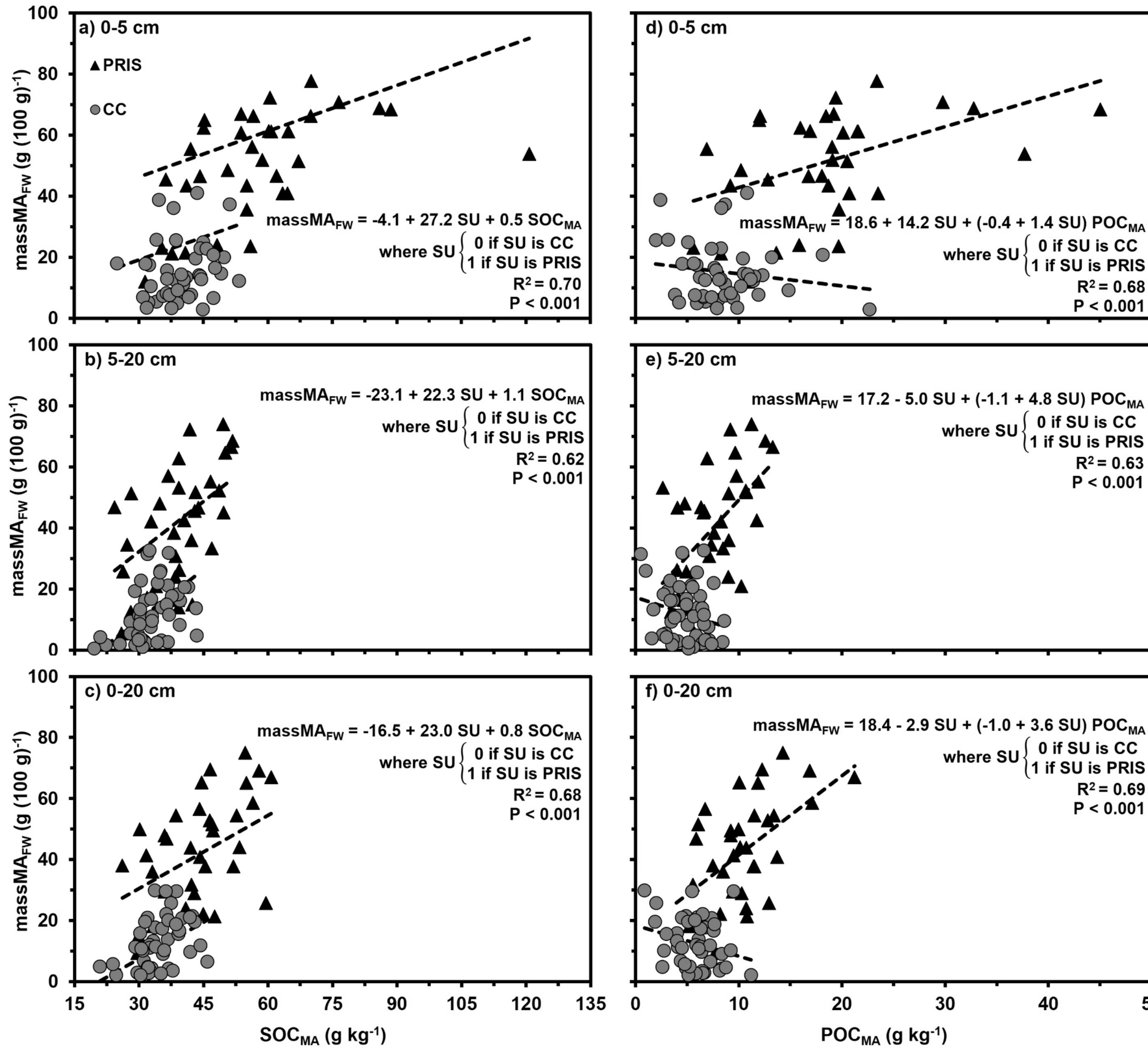

Fig. 2. Relationship between 2000–8000 μm macroaggregate (MA) mass after fast wetting ($massMA_{FW}$) and soil organic carbon within MA after capillary wetting (SOC_{MA}) (a, b, c) and particulate organic carbon within MA after capillary wetting (POC_{MA}) (d, e, f) for two soil uses at three depths. 0–5 (a, d), 5–20 (b, e), and 0–20 cm (c, f). n = 80. SU. soil use. CC: continuosly cultivated (n = 46). PRIS: pseudo-pristine (n = 34).

positively related to POC_{MA} (Table 2, section D), the Pearson correlation coefficients were lower than those for SOC_{MA} vs. SOC_{BS}, suggesting that part of POC_{BS} was not within MA. This observation can be explained by considering that, although POC_{BS} is accumulated mostly in MA, an important part of POC_{BS} is within free microaggregates (Six et al., 1998; Roldán et al., 2012a). Another possible explanation is that labile free compounds that were quantified when determining POC_{BS} and that were located mainly between aggregates could have been lost during water sieving. Thus, these compounds were quantified when determining POC_{BS}, but not when determining POC_{MA}. The PRIS showed more stable MA (greater $massMA_{FW}$, $P < 0.05$, Table 1, Fig. 2d, e, f) than CC and, therefore, POC_{MA} protection was greater in PRIS as compared to CC. Hence, during water sieving, the MA of PRIS would have lost less POC. On the contrary, the less stable MA of CC more likely lost more POC upon breaking off during sieving, resulting in the unexpected relationships between POC_{BS} and POC_{MA}. Pearson correlation coefficients between POC_{BS} and POC_{MA} for PRIS were 0.76, 0.49, and 0.71 (all with $P < 0.001$) at 0–5, 5–20, and 0–20 cm, respectively, whereas the correlations between POC_{BS} and POC_{MA} for CC were not significant. These results could also contribute to explain the differences caused by soil use in the relationships between $massMA_{FW}$ and POC_{MA} (Fig. 2d, e, f).

The relationships between $massMA_{FW}$ and SOC_{MA} (Table 2, Fig. 2a, b, c) show the importance of SOC_{MA} for the stabilization of MA and AS, and thus for soil organic matter physical protection. Given that soil AS is related to the capacity of the soil to store carbon (Six et al., 2002), the $massMA_{FW}$ is an indicator of the protection of labile organic carbon fractions (De Oliveira Ferreira et al., 2018). The MA play an important role in carbon protection and sequestration, since MA are formed by microaggregates that protect carbon within their structure against the attack by microorganisms (Six et al., 2002; Six and Paustian, 2014). The more stable the MA, the more protected the SOC and POC within them, promoting carbon sequestration (Six et al., 2002; Rasmussen et al., 2018). Thus, SOC_{MA} and POC_{MA} are good indicators of the magnitude of soil carbon sequestration (Six et al., 2002; Scott et al., 2017).

3.3. Anaerobically mineralized nitrogen within MA and its relationship with SOC, POC, and AS

The PRIS showed greater ($P < 0.05$) AN_{MA} mean values than CC (Table 1). Therefore, as expected, AN_{MA} was sensitive to soil use. Likewise, in the surface layer (0–5 cm depth), AN_{MA} was greater ($P < 0.05$) than at 5–20 cm depth (Table 1). Therefore, the effects of soil use and depth on AN_{MA} were similar to those found for AN_{BS} (Table 1).

The AN_{MA} showed positive linear association with SOC_{MA} and

POC_{MA} ($P < 0.001$, Table 2, section B). However, contrary to the results reported by Domínguez et al. (2016), AN_{MA} was better correlated to SOC_{MA} than to POC_{MA}. In accordance with these results, Gregorutti et al. (2013) did not observe a significant relationship between AN_{MA} and particulate organic nitrogen within MA. The lower correlation coefficients between AN_{MA} and POC_{MA} (Table 2, section B) could be attributed to the already discussed possible loss of POC within MA during water sieving due to the rupture of aggregates.

As observed for SOC_{MA} and POC_{MA}, AN_{MA} was positively correlated ($P < 0.001$) to SOC_{BS} and POC_{BS} (Table 2, section C). Simple linear regression models of SOC_{BS} and POC_{BS} as a function of AN_{MA} showed SOC_{BS} and POC_{BS} increase with AN_{MA} increases (Fig. 3), with statistically equal slopes and intercepts for both CC and PRIS. The relationships between SOC_{BS} and AN_{MA} (R^2 0.75, 0.48, and 0.61, $P < 0.001$, Fig. 3a, b, c) and between POC_{BS} and AN_{MA} (R^2 0.66, 0.31, and 0.49, $P < 0.001$, Fig. 3d, e, f) showed similar R^2 than those between SOC_{BS} and AN_{BS} (R^2 0.74, 0.46, and 0.62 at 0–5, 5–20, and 0–20 cm, respectively, $P < 0.001$) and between POC_{BS} and AN_{BS} (R^2 0.73, 0.33, and 0.60 at 0–5, 5–20, and 0–20 cm, respectively, $P < 0.001$) described by García et al. (2020b). Clay content slightly improved model adjustments of SOC_{BS} as a function of AN_{MA} (R^2 adjusted = 0.78, 0.60 and 0.70 at 0–5, 5–20 and 0–20 cm depths, respectively, $P < 0.001$). These models including clay content as a predictor variable had similar R^2 than the models to predict SOC_{BS} from AN_{BS} and clay content (0.79, 0.57, and 0.71 at 0–5, 5–20, and 0–20 cm, respectively, $P < 0.001$) reported by García et al. (2020b). However, the variations of POC_{BS} as a function of AN_{MA} were not better explained when including clay content in the model (data not shown), probably as a consequence of the narrow range of clay content of the studied soils (Table 1). Therefore, contrary to the hypothesis, AN_{MA} was not a better predictor of SOC_{BS} and POC_{BS} (Fig. 3) than AN_{BS} (García et al. 2020b).

As observed for SOC_{MA} and POC_{MA}, AN_{MA} was positively correlated ($P < 0.001$) to $massMA_{FW}$ (Table 2, section A), indicating that MA stability and, consequently, AS, are related to AN_{MA}. as expected. Pearson correlation coefficients between AN_{MA} and $massMA_{FW}$ were greater than those observed between SOC_{MA} or POC_{MA} and $massMA_{FW}$ (Table 2, section A). Fig. 4 shows statistical models for $massMA_{FW}$ as a function of AN_{MA}. The $massMA_{FW}$ increased with increases of AN_{MA} for all three depths, with equal slopes for CC and PRIS, whereas CC sowed lower intercepts than PRIS. This coincides with the models for $massMA_{FW}$ as a function of SOC_{MA} (Fig. 2a, b, c), SOC_{BS}, $POC_{BS,}$ and AN_{BS} (García et al., 2020b). For each depth, the R^2 of $massMA_{FW}$ as a function of AN_{MA} ($P < 0.001$) (Fig. 4) was greater than the R^2 of $massMA_{FW}$ as a function of SOC_{MA} ($P < 0.001$, Fig. 2a, b, c). Likewise, the R^2 of $massMA_{FW}$ as a

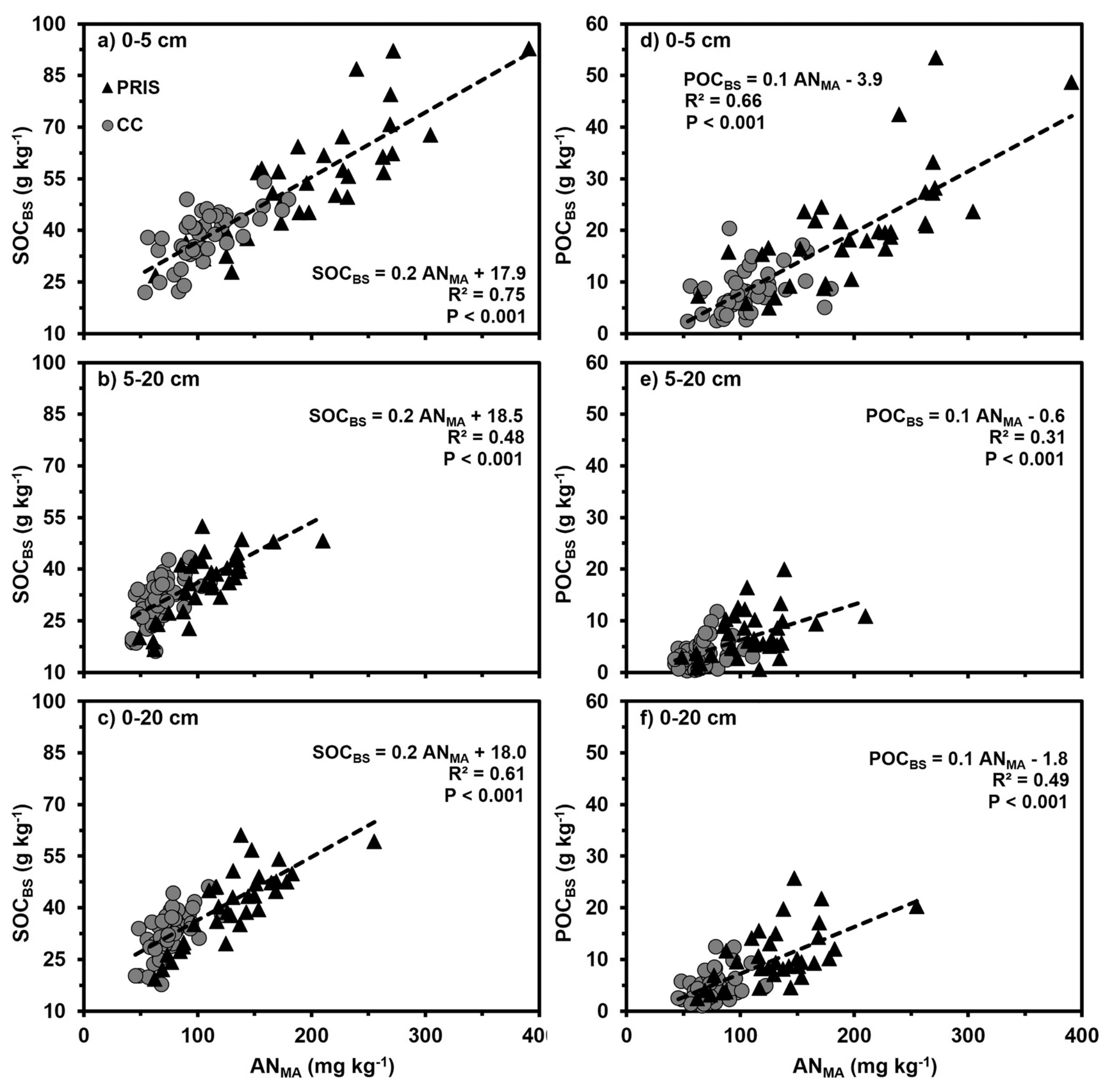

Fig. 3. Relationship between soil organic carbon in 2000–8000 μm macroaggregates (MA) after capillary wetting (SOC_{MA}) and anaerobically mineralized nitrogen within MA after capillary wetting (AN_{MA}) (a, b, c), and particulate organic carbon within MA after capillary wetting (POC_{MA}) and AN_{MA} (d, e, f), for two soil uses at three depths: 0–5 (a, d), 5–20 (b, e), and 0–20 cm (c, f). n = 80. CC: continuosly cultivated (n = 46). PRIS: pseudo-pristine (n = 34).

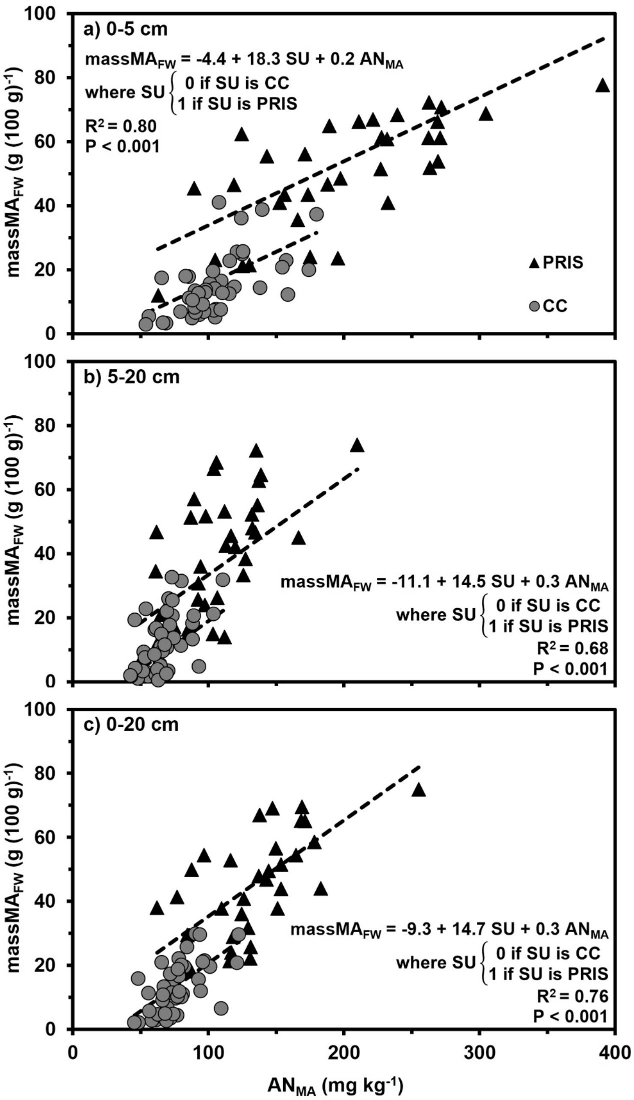

Fig. 4. Relationship between 2000–8000 µm macroaggregate (MA) mass after fast wetting (massMA$_{FW}$) and anaerobically mineralized nitrogen within MA after capillary wetting (AN$_{MA}$) for two soil uses at three depths: 0–5 (a), 5–20 (b), and 0–20 cm (c). n = 80. SU: soil use. CC: continuosly cultivated. PRIS: pseudo-pristine.

function of AN$_{MA}$ for each depth (Fig. 4) was similar to the R^2 of massMA$_{FW}$ as a function of AN$_{BS}$ (R^2 0.78, 0.69, and 0.81 at 0–5, 5–20, and 0–20 cm, respectively, $P < 0.001$) reported by García et al. (2020b). Sand and clay contents did not improve model adjustments of massMA$_{FW}$ as a function of AN$_{MA}$ (data not shown).

In summary, contrary to the hypothesis, AN$_{MA}$ was not a better indicator of SOC$_{BS}$, POC$_{BS}$ (Fig. 3), or massMA$_{FW}$ (Fig. 4) than AN$_{BS}$ (García et al., 2020b). The concentration of AN$_{MA}$ was strongly and positively correlated ($P < 0.001$) to AN$_{BS}$ concentration at all depths (Table 2, section D). Similar results were reported by Rivero et al. (2020) for a Mollisol with loam surface texture. The close relationship observed between AN$_{MA}$ and AN$_{BS}$ (Table 2, section D) indicates that for the range of soils studied in this work, the changes in AN$_{BS}$ associated with soil use and management were in line with the changes in AN$_{MA}$. This observation could be explained by the fact that a great part of the AN measured in the bulk soil (AN$_{BS}$) is located within MA (AN$_{MA}$) (Gregorutti et al., 2013; Rivero et al., 2020). Therefore, changes in AN$_{MA}$ directly reflect changes in AN$_{BS}$.

3.4. Scopes and limitations of AN$_{MA}$ as soil health indicator

Soil health evaluation is crucial for a sustainable soil management (Doran, 2002). Determining the health status of a soil is essential to diagnose and quantify its degree of degradation and to plan sustainable management practices. Some authors suggest the use of indexes or sets of variables to evaluate soil health (Drobnik et al., 2018). However, the use of a single variable to characterize the soil health status would be more suitable, practical, and economical for consultants and farmers to frequently monitor their soils.

It has been demonstrated that AN$_{MA}$ was as sensitive as AN$_{BS}$ to describe changes in AS, SOC$_{BS}$, and POC$_{BS}$ due to soil use and management. Therefore, AN$_{MA}$ is a suitable soil health indicator, but not better than AN$_{BS}$ (García et al., 2020b). Besides, AN$_{MA}$ determination requires special care for soil sampling and sample processing, and more methodological steps (i.e. MA separation and AN determination) as compared to AN$_{BS}$. These aspects discourage the use of AN$_{MA}$ as soil health indicator. However, even though this study was carried out in a wide region, further research is needed to confirm its results. Future studies should be focused on evaluating the performance of AN$_{MA}$ as a soil health indicator in a wider range of soil types, land uses and management situations.

4. Conclusion

The AN$_{MA}$ was positively related to SOC$_{BS}$, POC$_{BS}$, and AS, and, therefore, it could be used as a suitable soil health indicator. However, AN$_{MA}$ showed a similar performance as a predictor of SOC$_{BS}$, POC$_{BS}$, and AS than AN$_{BS}$. Besides, since AN$_{MA}$ determination is more laborious and time-consuming than AN$_{BS}$ determination, it is concluded that the use of AN$_{MA}$ as a soil health indicator is not convenient. Therefore, the use of AN$_{BS}$ is recommended over AN$_{MA}$ as a variable to monitor soil health status.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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