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# Soil survey reveals a positive relationship between aggregate stability and anaerobically mineralizable nitrogen

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## ABSTRACT

Soil health status should be monitored to allow planning sustainable management, but indicators available do not encourage frequent soil health evaluation because of the complexity, time-consumption, and expensiveness of the methodologies. Aggregate stability (AS) is a good soil physical health indicator associated with soil (SOC) and particulate (POC) organic carbon but is difficult to monitor. Anaerobically mineralizable nitrogen (AN) has been proposed as soil health indicator because is cheap, simple, and safe to measure, is sensitive to soil-use changes, is also related to soil (SOC) and particulate (POC) organic carbon, and is frequently determined by farmers in Mollisols of the Southeastern Argentinean Pampas to support soil fertility diagnosis. We hypothesize that AN is positively related to and can be used as indicator of AS. Soil samples were taken at 0–5 and 5–20 cm depths from 46 sites throughout the southeastern Buenos Aires province, Argentinean Pampas. In each site, we sampled Mollisols under continuous cropping (CC) and others that had not been disturbed for many years (pseudo-pristine, PRIS). We determined texture, SOC, mineral-associated organic C, POC, AS and AN. We also calculated variable values for 0–20 cm. Soil organic carbon, POC, AN and AS were reduced by continuous cropping. Anaerobically mineralizable N was positively related to SOC ($R^2$ = 0.74, 0.46, and 0.62 at 0–5, 5–20, and 0–20 cm) and POC ($R^2$ = 0.73, 0.33, and 0.60, respectively). An important proportion of the total variability in AS was explained by SOC ($R^2$ = 0.77, 0.65, and 0.73 at 0–5, 5–20, and 0–20 cm, respectively), POC ($R^2$ = 0.75, 0.63, and 0.73, respectively), and AN ($R^2$ = 0.78, 0.69, and 0.81, respectively). The AS increased with the increase of SOC, POC, and AN at all three depths, with slopes that did not differ between CC and PRIS, but with intercepts that differed. Neither sand nor clay contents significantly contributed to explain the variations in AS as a function of SOC, POC, and AN. An independent validation of the regression model relating AS and AN at 0–20 cm was done and the output was very good (RPIQ (ratio of performance to interquartile distance) = 2.20). Results support our hypothesis because AN was positively related to AS. Consequently, AN would be a good indicator of AS, SOC, and POC. Based on our results, we consider that a simple and cheap soil analysis as AN can not only be used to diagnose soil fertility, but to monitor soil physical and biochemical health status.

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*Abbreviations:* AN, anaerobically mineralizable nitrogen; MAOC, mineral associated organic carbon; AS, aggregate stability; C, carbon; CC, plots under continuous cropping; CW, capillary wetting; ΔMWD, difference of mean weight diameter between CW and FW; MA, large macroaggregates (2000–8000 μm); $\text{massMA}_{FW}$, MA dry mass remnant after FW; $\text{MWD}_{CW}$, mean weight diameter after CW; $\text{MWD}_{FW}$, mean weight diameter after FW; N, nitrogen; $N_0$, potentially mineralizable N; POC, particulate organic carbon; POM, particulate organic matter; PRIS, reference situation for each CC; SHI, soil health indicator; SOC, soil organic carbon; SOM, soil organic matter; FW, fast wetting

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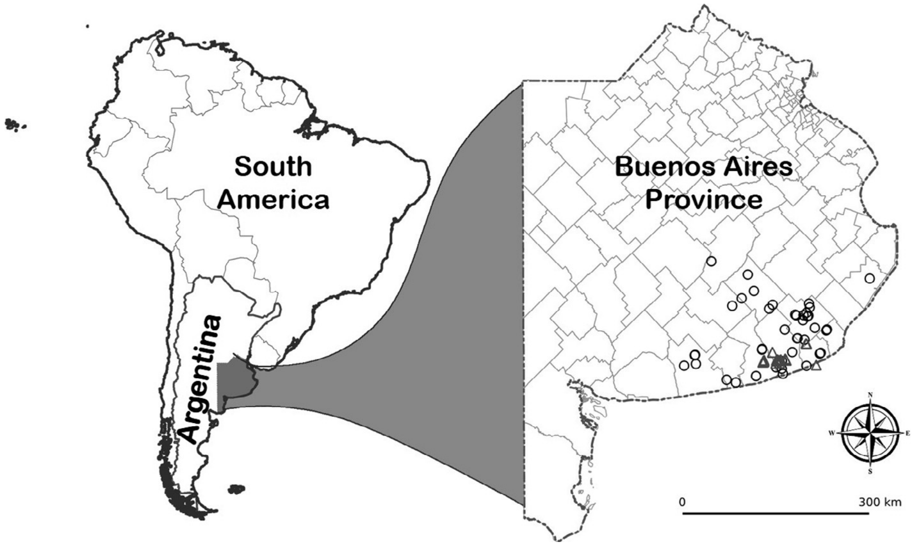

**Fig. 1.** Sampling sites throughout the southeastern Buenos Aires province at the Argentinean Pampas. ○: sampling sites to evaluate relationships among variables and regression model fitting (n = 46), △: sampling sites for model validation (n = 32). Only the location of continuous cropping (CC) plots is shown because the location of pseudo-pristine plots was very close to the CC plots (no more than 500 m away).

## 1. Introduction

Soil health is defined as the capacity of a soil to function in the agroecosystem. Therefore, a healthy soil should be able to sustain productivity, contributing to environmental quality, and improving human, animal, and plant health. Soil-use change, together with inadequate management practices, have led to a generalized gradual loss of soil health (i.e. degradation) (Doran, 2002). Therefore, it is necessary to monitor soil health status to diagnose and quantify the magnitude of soil degradation and to plan adequate management practices.

The evaluation of soil health is performed by monitoring parameters known as soil health indicators (SHI). A satisfactory SHI should be sensitive to management practices, easy to interpret, and its measurement should be simple, fast and cheap. Also, a SHI should be related to one or more soil functions and/or to other soil properties (Doran and Parkin, 1996). Soil organic matter (SOM) and particulate organic matter (POM) are generally used as SHI (Cambardella and Elliott, 1992; Wander, 2004). However, none of these two soil parameters fully comply with the requirements for a SHI. Soil organic matter is not sensitive enough to detect short to mid-term soil health changes (Domínguez et al., 2016). Contrarily, POM allows detecting early soil health changes (Cambardella and Elliott, 1992; Wander, 2004). However, POM quantification is complex and time-consuming and, therefore, unsuitable as a routine analysis in soil testing laboratories (Diovisalvi et al., 2014).

A recently proposed SHI, alternative to SOM and POM (Domínguez et al., 2016; García et al., 2016), is the nitrogen (N) mineralized along short anaerobic incubations (anaerobically mineralizable N, AN) (Keeney, 1982). Anaerobically mineralizable N is simple, fast, safe, and cheap to determine and its results are easily interpreted. Therefore, AN can be used and is being effectively performed as a routine analysis in soil testing laboratories of the Southeastern Argentinean Pampas (Reussi Calvo et al., 2018).

The AN has been associated with many soil functions and properties, like the potentially mineralizable N ($N_0$) (Echeverría et al., 2000; Schomberg et al., 2009; Wyngaard et al., 2018). Anaerobically mineralizable N has been shown to be the best $N_0$ estimator (Echeverría et al., 2000; Wyngaard et al., 2018) and the most sensitive to management practices (Soon et al., 2007). As a result of the close association between $N_0$ and AN, the latter is being used as a satisfactory indicator of soil N availability for crops such as wheat (*Triticum aestivum* L.) (Reussi Calvo et al., 2013, 2018) and corn (*Zea mays* L.) (Orcellet et al., 2017). Moreover, it has been recently demonstrated that AN can also be used to predict potentially mineralizable sulfur (Carciochi et al., 2018).

The AN is also positively related to soil (SOC) and particulate (POC) organic carbon (C) contents (Studdert et al., 2015; Domínguez et al., 2016). Consequently, the SOC and POC decrease (0–20 cm layer) caused by mid- to long-term cropping was accompanied by a decrease of AN (García et al., 2016). It has been also shown that AN does not show important seasonal changes (Studdert et al., 2015) nor changes in response to short term effects (e.g. amount and quality of preceding-crop residues (García et al., 2016)). The sensitivity of AN, and its relationship with SOC, POC, and N and sulfur availability position AN as an adequate potential SHI (García et al., 2016; Domínguez et al., 2016) and suggest that AN could be related to other soil properties associated with SOM dynamics.

Soil aggregate stability (AS) is a physical property key to soil functioning because it influences the soil pore system and, thus, soil water and air dynamics. The AS is associated with other soil properties such as bulk density, infiltration, and SOC content (Rabot et al., 2018). Consequently, AS influences erosion resistance, nutrient cycling, C sequestration, C dioxide ($CO_2$) emissions, root penetration and crop yields (Bronick and Lal, 2005; Rabot et al., 2018). Moreover, Aparicio and Costa (2007) postulated that AS is the soil physical parameter most sensitive to soil-use change. That is why AS is widely recognized as a physical SHI (Rabot et al., 2018).

Soil-use changes and the utilization of aggressive management practices lead to a decrease of AS and, consequently, to soil physical degradation (Bronick and Lal, 2005). Thus, it is necessary to monitor AS in order to evaluate soil physical health. However, the AS determination is complex and time-consuming and, hence, it is not adopted by soil testing laboratories. However, it is known that aggregate resistance to breakdown is related to SOM and, especially, to SOM labile fractions (Six et al., 1998, 2004). Therefore, determining and characterizing the relationship between AS and an easily measurable SOM labile fraction frequently checked by farmers, would facilitate monitoring and managing soil physical health. Given the close relationship between AN and SOC and POC (Domínguez et al., 2016; Studdert et al., 2017), AN could be a good indicator of AS. Therefore, we hypothesize that AN is positively related to AS in Mollisols of the Southeastern Argentinean Pampas. The aim of this work was to evaluate AN as an indicator of AS status, as compared with SOC and POC as indicators of AS.

## 2. Materials and methods

Forty-six sampling sites in farms throughout the southeastern Buenos Aires province at the Argentinean Pampas (Fig. 1) were selected. Soils at all sites were classified as Mollisols (Soil Survey Staff, 2014; Rubio et al., 2019). Sites were selected to represent the range of soil surface textures characteristics of the region (Durán et al., 2011; Rubio et al., 2019) and without signs of erosion (slope < 2%) or flooding. In each farm, one geo-referenced plot (400 $m^2$) under continuous cropping (CC), and one geo-referenced plot as reference situation for each CC (pseudo-pristine, PRIS, no more than 500 m away), were sampled. The CC plots had been under continuous cropping between 4 and 20 yr. On the other hand, PRIS plots had not been disturbed and were under grass vegetation for more than 20 yr, and it was assumed that their physicochemical properties were similar to those of pristine soils (i.e. grassland soils characteristic of the Southeastern Argentinean Pampas). In some farms more than one CC plot and only one PRIS plot were sampled since the PRIS represented the undisturbed situation for the CC selected.

In each CC and PRIS plots, composite soil samples were taken in the autumn–winter of 2016 (34 sites) and of 2018 (12 sites) at 0–5 (15 subsamples per composite sample per plot) and 5–20 cm depth (5 subsamples per composite sample per plot). These samples were taken at field capacity, using a 4.4-cm-diameter tubular soil core sampler, and dried in an oven with forced air circulation at 50 °C until constant weight. Then, the samples were ground to pass a 2000-μm-mesh sieve, and all identifiable plant materials were removed. This set of samples was used to determine texture, SOC, mineral-associated organic C (MAOC), POC, and AN.

Mineral particle size distribution was determined by the hydrometer method (Gee and Bauder, 1986). Sand, clay and silt contents were expressed in g $kg^{-1}$ dry soil mineral fraction. Soil organic C content was determined by colorimetry after wet combustion with potassium dichromate and sulfuric acid at 120 °C for 90 min (Schlichting et al., 1995). For the determination of POC and MAOC, a granulometric physical fractionation was performed following the procedure described by Cambardella and Elliott (1992). Briefly, 10 g of dry soil was dispersed with 30 mL of a 5 g $L^{-1}$ sodium hexametaphosphate solution and 16-h shaking in a rotative shaker. Then the suspension was passed through a 53-μm sieve with deionized water. The fraction < 53 μm was recovered to determine its C content (MAOC) as previously described for SOC. Particulate organic C content was calculated subtracting MAOC from SOC (Cambardella and Elliott, 1992). The results of SOC, POC, and MAOC were expressed in g C $kg^{-1}$ dry soil.

The AN was determined as described by Keeney (1982). Briefly, 5 g of soil was placed inside test tubes (150 * 16 mm) which were filled with deionized water, and hermetically capped ensuring that no air bubbles remained inside the tube. Then, the tubes were incubated for 7 d at 40 °C. At the end of the incubation, ammonium-N content was determined by steam distillation (Keeney and Nelson, 1982). Initial ammonium-N content was also determined in each sample before incubation. The AN value resulted from the difference between ammonium-N after and before incubation. The AN results were expressed in mg AN $kg^{-1}$ dry soil.

At the same time of sampling, other composite soil samples (5 subsamples per sample) were taken from each plot using a shovel at 0–5 and 5–20 cm depth. The soil in contact with the shovel was discarded. Upon extraction (i.e. in moist condition), the aggregates were carefully manually separated through their natural breakage lines to pass an 8000-μm-mesh sieve. Then, soil samples were dried in an oven with forced air circulation at 50 °C until constant weight. Aggregate fractionation by size (Fig. 2) was performed as described by Six et al. (1998). Briefly, dry aggregates were re-wetted through two different re-wetting procedures and then sequentially sieved to separate aggregates of different sizes. A 100-g aliquot of dry aggregates was capillary re-wetted for 24 h up to field capacity (capillary wetting, CW). Another 100-g aliquot of dry aggregates was submerged in water (fast wetting, FW). After re-wetting, soil aliquots were successively water-sieved on different mesh sieves (2000 μm, 250 μm, and 53 μm). The aggregates on top of each sieve were recovered by back-washing, and then dried and weighed. Four aggregate size fractions were obtained for both CW and FW: large macroaggregates (2000–8000 μm, MA), small macroaggregates (250–2000 μm), microaggregates (53–250 μm) and the fine fraction (< 53 μm). The latter was discarded, and its mass was calculated as the difference between the initial dry mass aliquot (100 g) and the sum of the dry masses of MA, small macroaggregates, and microaggregates.

From the results of the aggregate fractionation procedure, three indicators of AS were obtained: i) mean weight diameter (MWD) difference between CW and FW (ΔMWD) (Eq. (2)) as proposed by Six et al. (2000), ii) MWD after FW ($MWD_{FW}$) (Eq. (1)) as proposed by many authors (Chaplot and Cooper, 2015; Scott et al., 2017; Sarker et al., 2018; King et al., 2019), and iii) MA dry mass remnant after FW ($massMA_{FW}$) (García et al., 2020).

$$MWD_{VW} = \sum_{i=1}^{4} X_i W_i \tag{1}$$

$$\Delta MWD = MWD_{CW} - MWD_{VW} \tag{2}$$

In Equation (2), $MWD_{FW}$ was calculated according to Eq. (1) and MWD after CW ($MWD_{CW}$) was calculated as $MWD_{FW}$, but using the dry mass of the aggregate size fractions separated after CW. In Eq. (1), $i$ identifies each fraction separated after FW (i.e. 2000–8000 (1), 250–2000 (2), 53–250 (3), and < 53 (4) μm), $X_i$ is mean diameter of the $i$-th fraction calculated as the arithmetic mean of the mesh opening of the two successive sieves that define the $i$-th fraction, and $W_i$ is the mass proportion of the $i$-th fraction with respect to the original aliquot dry mass (100 g). The ΔMWD and $MWD_{FW}$ were expressed in mm, whereas $massMA_{FW}$ was expressed in g MA $(100\ g)^{-1}$ dry soil. Given 95% or more of the sand fraction presented a particle size below 250 μm (fine and very fine sands, Soil Survey Staff, 2014), the correction of MA and small macroaggregates dry masses by sand content indicated by Six et al. (2000) was not performed (Yamashita et al., 2006).

The values of all variables for the 0–20 cm layer were calculated by averaging the values corresponding to each sampled depth (0–5 and 5–20 cm) weighted by its thickness (i.e. 5 and 15 cm, respectively). Pearson correlation coefficients were used to analyze the association between variables. Multiple and simple linear regression models were fitted to evaluate the performance of AN as a predictor of AS, SOC, and POC. Likewise, the relationships between AS and SOC, and AS and POC, and the performance of SOC and POC as predictors of AS were also evaluated. Statistical analyses were performed with R (R Core Team, 2017). A significance level of 0.05 was used.

Validation of the regression models relating $massMA_{FW}$ with SOC and AN at 0–20 cm was performed with an independent data set from a soil survey required by a farmer association to evaluate soil health status in July 2018. Thirty-two CC and 21 PRIS plots (Fig. 1) with similar characteristics and surface texture within the range as the sampling sites described above, were sampled at 0–20 cm. The sampling protocol both with the tubular soil core sampler and with the shovel, was the same as indicated before. Samples were processed and analyzed for SOC, AN, and AS as described above. Plots of observed versus predicted values were made with a 1:1 line as a reference for comparison. Some statistical indicators of regression models performance to predict $massMA_{FW}$ were calculated: a) root mean square error of the prediction (RMSEP) (g $(100\ g)^{-1}$) (Fox, 1981), b) the ratio between RMSEP and the mean of the observed $massMA_{FW}$ (coefficient of variation of the prediction, CVP) (%) (Bellon-Maurel et al., 2010), c) the ratio between the standard deviation of the observed $massMA_{FW}$ and RMSEP (ratio of performance to deviation, RPD) (Bellon-Maurel et al., 2010), and d) the ratio between the difference between quartile 3 and quartile 1 of the observed $massMA_{FW}$ and RMSEP (ratio of performance to interquartile distance, RPIQ) (Bellon-Maurel et al., 2010). All statistical analyses were done with R version 3.5.2 (R Core Team, 2018) and with a significance level of 0.05.

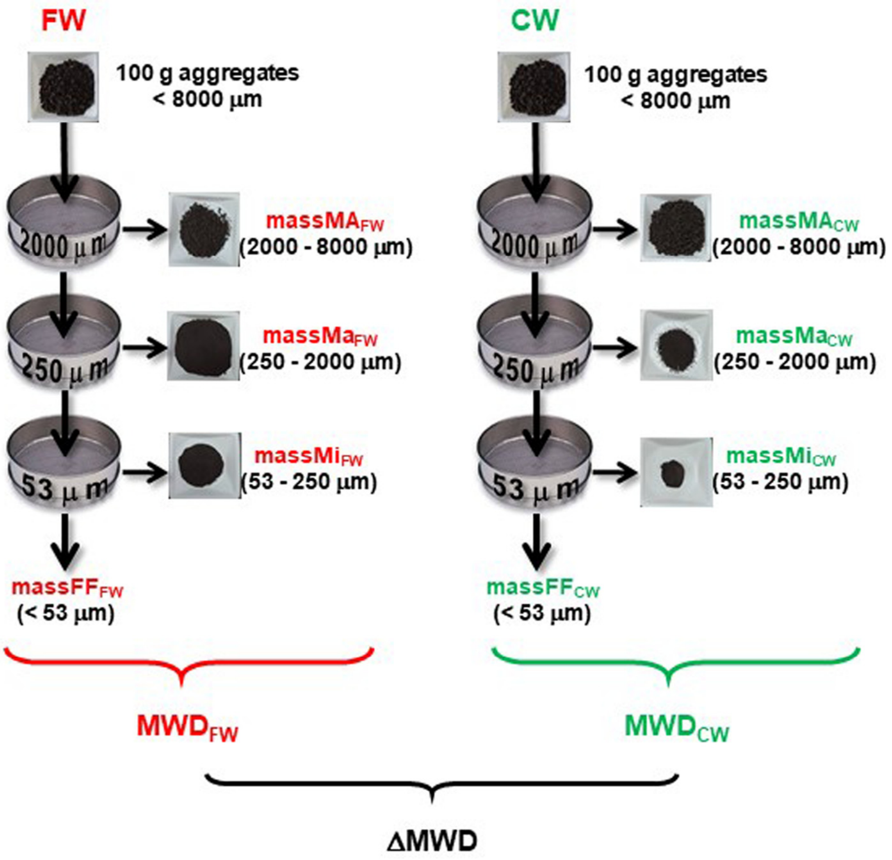

**Fig. 2.** Scheme of aggregate separation methodology. FW: fast wetting, CW: capillary wetting, MWD: mean weight diameter, ΔMWD: difference of MWD between CW and FW, massMA: 2000–8000 µm macroaggregate mass, massMa: 250–2000 µm macroaggregate mass, massMi: microaggregate mass, massFF: fine fraction mass.

## 3. Results and discussion

### *3.1. Texture*

The sand, clay and silt contents were similar at all three depths (Table 1) and were within the range described for southeastern Buenos Aires province soils (Durán et al., 2011). The textural classes corresponded to loam, sandy-loam, sandy-clay-loam and clay-loam soils (Soil Survey Staff, 2014). The finest textured soils (clay-loam) were located at the center of Buenos Aires province (Fig. 1), whereas the coarsest textured soils (sandy-loam) were mainly located close to the Atlantic Ocean shore (Fig. 1). This trend coincides with the particle size distribution of the parent material ("pampean" loess, coarser on the coast and finer in the center part of the province) (Durán et al., 2011).

### *3.2. Soil organic C and POC and their relationships with AS*

In general, PRIS soils showed larger minimum, maximum, and mean values of SOC, POC, and MAOC (Table 1) than those observed for CC. This difference indicates that those three soil variables were sensitive to soil-use changes, being POC the most sensitive (Table 1). The PRIS situations showed high organic C content due to the great C inputs by aboveground and root biomass and reduced soil disturbance (Tisdall and Oades, 1982; Haynes et al, 1991). The continuous growth and recycling of the grass dense root systems are associated with greater microbial biomass and organic labile C fractions (Tisdall and Oades, 1982; Haynes et al., 1991; Haynes and Beare, 1997; McNally et al., 2015). Soil-use change from a pristine condition to an agricultural system generally produces a decrease in SOC, which is mainly expressed in labile fractions as POC (Studdert et al., 1997, 2017). This decrease in SOC and its fractions is due to the negative balance between C inputs and outputs in soils under cropping (Studdert and Echeverría, 2000). Thus, as SOC level decreases, soil loses its ability to properly perform its functions in the agroecosystem, and, therefore, soil health is reduced (Janzen, 2006).

The SOC, POC, and MAOC values were greater at the 0–5 cm layer than at the 5–20 and 0–20 cm layers at both CC and PRIS (Table 1). In the case of the PRIS, the greater accumulation of organic C near the soil surface is given by a greater amount of aboveground litter, root and microbial biomass (Franzluebbers and Stuedemann, 2009). Likewise, in CC the greater surface accumulation of organic C due to no-tillage is reflected in the stratification of soil organic fractions (Franzluebbers, 2002; Dolan et al., 2006; Blanco-Canqui et al., 2011). Approximately 80% of the CC plots had a history of more than 10 yr under no-tillage. The absence of soil disturbance under no-tillage promotes SOC protection mechanisms (Six et al., 2002) which, together with the presence of surface residues, lead to the stratification of organic C (Franzluebbers, 2002; Puget and Lal, 2005; Powlson et al., 2014).

As for SOC, POC, and MAOC, differences in the minimum, maximum and mean values between CC and PRIS were observed for all AS indicators (Table 1). The PRIS soils showed greater AS (i.e. lower ΔMWD and greater MWD$_{FW}$ and massMA$_{FW}$) than CC, indicating that AS was sensitive to soil-use change, as described by other authors (Roldán et al., 2014, Scott et al., 2017; King et al., 2019). When soil-use changes from PRIS to cropping, soil loses AS mainly due to the physical disturbance, the decrease of root activity and persistence, and the loss of organic C, among others (Cambardella and Elliott, 1993; Six et al., 1998; Domínguez et al., 2016). Likewise, it has been demonstrated that AS is either negatively or positively affected by tillage (Roldán et al., 2014; Sarker et al., 2018; Sithole et al., 2019) and cropping systems (Novelli et al., 2013) depending on what, how, and where they are performed.

**Table 1**
Maximum (Max), minimum (Min), and mean (Mean) values at three depths for: i) particle size distribution (sand, clay, and silt) at sampling sites ($n = 80$), ii) organic variables (soil organic carbon (SOC), particulate organic carbon (POC), mineral associated organic carbon (MAOC), and anaerobically mineralizable nitrogen (AN)) for plots under continuous cropping (CC, $n = 46$) and for pseudo-pristine (PRIS, $n = 34$), and iii) aggregate stability (AS) indicators (mean weight diameter (MWD) after fast wetting (FW) ($\mathrm{MWD}_{\mathrm{FW}}$), difference of MWD (ΔMWD), and 2000–8000 µm macroaggregate mass after FW ($\mathrm{massMA}_{\mathrm{FW}}$)) for CC ($n = 46$) and PRIS ($n = 34$).

| Variable | Use | Depth (cm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–5 | | | 5–20 | | | 0–20 | | |
| | | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min |
| *Texture* | | | | | | | | | | |
| Sand (g $\mathrm{kg}^{-1}$) | – | 684.2 | 428.5 | 265.2 | 699.5 | 431.5 | 258.9 | 695.7 | 430.7 | 264.7 |
| Clay (g $\mathrm{kg}^{-1}$) | – | 365.9 | 243.0 | 103.6 | 386.4 | 253.8 | 100.3 | 379.3 | 251.1 | 101.1 |
| Silt (g $\mathrm{kg}^{-1}$) | – | 487.9 | 328.6 | 157.7 | 472.7 | 314.8 | 147.9 | 472.7 | 318.2 | 150.4 |
| *Organic variables* | | | | | | | | | | |
| SOC (g $\mathrm{kg}^{-1}$) | PRIS | 92.9 | 55.0 | 26.9 | 52.5 | 36.2 | 16.7 | 61.1 | 40.9 | 19.3 |
| | CC | 54.2 | 38.2 | 22.0 | 43.4 | 31.1 | 16.2 | 46.1 | 32.9 | 17.7 |
| POC (g $\mathrm{kg}^{-1}$) | PRIS | 53.5 | 20.4 | 5.0 | 19.9 | 7.2 | 0.6 | 25.7 | 10.5 | 2.4 |
| | CC | 20.4 | 8.0 | 2.3 | 11.7 | 3.7 | 0.3 | 12.5 | 4.8 | 1.0 |
| MAOC (g $\mathrm{kg}^{-1}$) | PRIS | 47.6 | 34.7 | 19.6 | 42.1 | 29.0 | 15.3 | 42.5 | 30.4 | 16.8 |
| | CC | 40.9 | 30.2 | 18.4 | 37.4 | 27.4 | 15.6 | 38.1 | 28.1 | 16.3 |
| AN (mg $\mathrm{kg}^{-1}$) | PRIS | 334.5 | 184.6 | 63.1 | 142.6 | 80.2 | 29.1 | 184.6 | 106.3 | 42.8 |
| | CC | 138.4 | 93.1 | 51.7 | 84.1 | 52.8 | 25.0 | 95.4 | 62.9 | 38.3 |
| *Aggregate stability indicators* | | | | | | | | | | |
| ΔMWD (mm) | PRIS | 2.2 | 0.8 | 0.0 | 2.5 | 1.2 | 0.1 | 2.3 | 1.1 | 0.1 |
| | CC | 3.2 | 2.3 | 0.7 | 3.3 | 2.5 | 1.1 | 3.2 | 2.5 | 1.1 |
| $\mathrm{MWD}_{\mathrm{FW}}$ (mm) | PRIS | 4.1 | 2.9 | 1.1 | 3.9 | 2.5 | 0.9 | 4.0 | 2.6 | 1.1 |
| | CC | 2.5 | 1.4 | 0.7 | 2.2 | 1.3 | 0.6 | 2.1 | 1.3 | 0.7 |
| $\mathrm{massMA}_{\mathrm{FW}}$ (g(100 g)$^{-1}$) | PRIS | 77.7 | 50.5 | 11.9 | 74.0 | 41.1 | 5.4 | 74.9 | 43.4 | 9.4 |
| | CC | 41.0 | 14.9 | 2.9 | 32.7 | 12.0 | 0.5 | 30.0 | 12.7 | 2.1 |

**Table 2**
Pearson correlation coefficients for associations between soil organic carbon (SOC, g $\mathrm{kg}^{-1}$), particulate organic carbon (POC, g $\mathrm{kg}^{-1}$), or anaerobically mineralizable nitrogen (AN, mg $\mathrm{kg}^{-1}$) and aggregate stability (AS) indicators (mean weight diameter (MWD) after fast wetting (FW) ($\mathrm{MWD}_{\mathrm{FW}}$, mm), difference of MWD (ΔMWD, mm), and 2000–8000 µm macroaggregate mass after FW ($\mathrm{massMA}_{\mathrm{FW}}$, g (100 g)$^{-1}$)) at three depths. $n = 80$.

| Depth | Variable | AS indicators | | |
|---|---|---|---|---|
| | | ΔMWD | $\mathrm{MWD}_{\mathrm{FW}}$ | $\mathrm{massMA}_{\mathrm{FW}}$ |
| 0–5 cm | SOC | −0.62 | 0.76 | 0.75 |
| | POC | −0.69 | 0.76 | 0.77 |
| | AN | −0.72 | 0.83 | 0.83 |
| 5–20 cm | SOC | −0.40 | 0.61 | 0.58 |
| | POC | −0.60 | 0.64 | 0.64 |
| | AN | −0.61 | 0.74 | 0.74 |
| 0–20 cm | SOC | −0.51 | 0.70 | 0.68 |
| | POC | −0.70 | 0.75 | 0.75 |
| | AN | −0.72 | 0.86 | 0.85 |

The ΔMWD was negatively correlated with SOC and POC, whereas $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ were positively correlated with SOC and POC (Table 2). All AS indicators (ΔMWD, $\mathrm{MWD}_{\mathrm{FW}}$, and $\mathrm{massMA}_{\mathrm{FW}}$) presented a higher Pearson correlation coefficient with POC as compared to SOC. There was no significant relationship between ΔMWD and MAOC, whereas $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ were weakly associated with MAOC ($r < 0.50$, data not shown). Both $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ showed higher Pearson coefficients with SOC and POC than ΔMWD (Table 2). Also, the ΔMWD was not a good AS indicator for unstable soils (e.g. with high sand content) since these situations presented both low $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{MWD}_{\mathrm{CW}}$, and therefore, the ΔMWD value resulted similar to that of a stable soil (i.e. low ΔMWD). Thus, when using ΔMWD as an AS indicator, soils with a coarse texture and low SOC and POC content could show a low ΔMWD and, therefore, its AS wrongly interpreted as similar to that of a fine-textured soil with high-SOM-content (data not shown). Therefore, ΔMWD did not allow differentiating soils with very different textures and SOC and POC content.

On the other hand, $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ reflected more clearly the ability of the aggregates to resist stronger disruptive forces (i.e. FW). Hence, both parameters could be considered as better AS indicators than ΔMWD. King et al. (2019) reported increases in $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ with increases in SOC. In this study, both $\mathrm{MWD}_{\mathrm{FW}}$ and $\mathrm{massMA}_{\mathrm{FW}}$ were highly correlated with each other ($r = 0.99$) and had a similar association with SOC and POC (Table 2). However, $\mathrm{massMA}_{\mathrm{FW}}$ determination (only one sieving, Fig. 2) is easier than $\mathrm{MWD}_{\mathrm{FW}}$ determination (three sievings, Fig. 2). The observed associations between the AS indicators and SOC or POC (Table 2) are in accordance with those reported earlier (Six et al., 2002; 2004; Chaplot and Cooper, 2015; Domínguez et al., 2016; King et al., 2019), suggesting that the application of management practices that favor SOC or POC accumulation, would also increase AS (Six et al., 2004; Novelli et al., 2013; Roldán et al., 2014; Scott et al., 2017).

Fig. 3 shows the models describing the relationships between $\mathrm{massMA}_{\mathrm{FW}}$ as a function of SOC (Fig. 3a, b, c) or POC (Fig. 3d, e, f) for all three depths. The $\mathrm{massMA}_{\mathrm{FW}}$ increased with SOC (Fig. 3a-c) and POC (Fig. 3d-f), with the same slope for both CC and PRIS, but with different intercepts. Changes in $\mathrm{massMA}_{\mathrm{FW}}$ were explained as well by changes in SOC (Fig. 3a-c) and POC (Fig. 3d-f) at all three depths, with determination coefficients ranging between 0.63 and 0.77 (Fig. 3a-f). Consequently, even though aggregate ability to resist breakdown is determined by numerous other edaphic (i.e. texture, microbial activity) and vegetation characteristics (i.e. coverage, residue quality, root system), weather, and management practices (Six et al., 2004), AS is strongly related to SOC and POC content. However, since texture largely influences AS (Six et al., 2004; Plante et al., 2006), sand and clay content were introduced into the regression models developed to predict AS. Sand and clay content were strongly correlated to each other ($r = -0.72$, $-0.77$, and $-0.77$ at 0–5, 5–20, and 0–20 cm, respectively). Therefore, their introduction as AS predictors was evaluated one at a time. Neither sand nor clay content significantly contributed to better explain variations in $\mathrm{massMA}_{\mathrm{FW}}$ as a function of SOC and POC (data not shown). This could be due to the narrow range of sand and clay content of the studied soils (Table 1). Along the same line, King

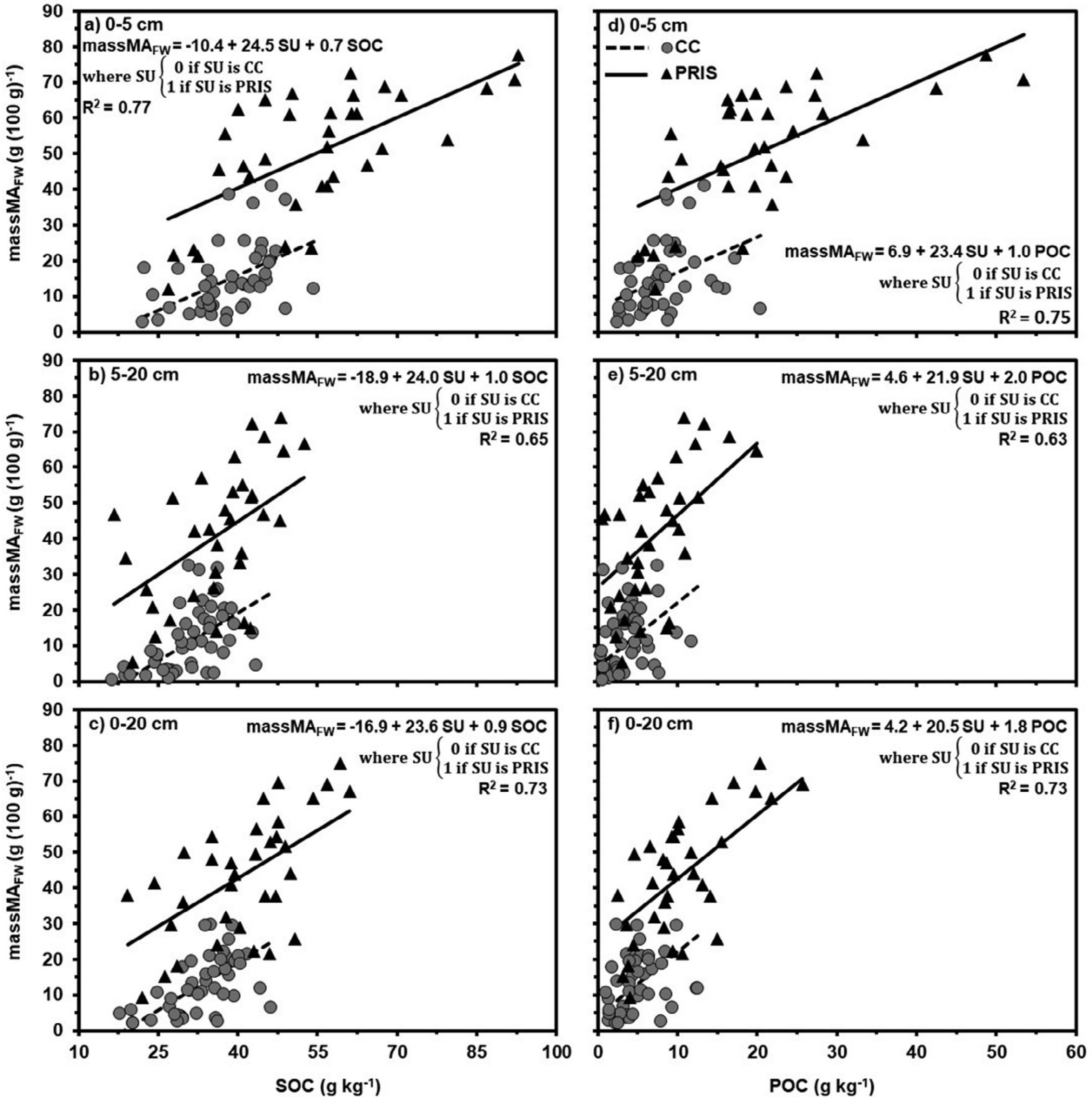

**Fig. 3.** Relationship between 2000 and 8000 µm macroaggregate mass after fast wetting (massMA$_{FW}$) and soil organic carbon (SOC) (a, b, c) and particulate organic carbon (POC) (d, e, f) for two soil uses at three depths: 0–5 (a, d), 5–20 (b, e), and 0–20 cm (c, f). n = 80. $P < 0.05$. SU: soil use. CC: continuous cropping (n = 46). PRIS: pseudo-pristine (n = 34).

et al. (2019), studying different soil orders reported that clay content did not explain massMA$_{WV}$ changes.

The relationships shown in Fig. 3 under CC indicate that management practices that lead to an increase of SOC or POC content, would increase AS at the same rate as PRIS. However, the different intercepts between CC and PRIS regression lines (Fig. 3) denote that at the same SOC or POC content, AS is lower for CC than for PRIS. This difference between soil uses can be a consequence of other factors, alternative to texture, SOC and POC that affect AS. Biochemical properties of organic fractions determined by vegetation, root exudates, and the related soil microbial activity could confer attributes such as hydrophobicity, that would increase AS for PRIS (Chenu et al., 2000). Likewise, the absence of disturbance (Six et al., 2004) and the greater abundance of roots (Six et al., 2004; Rashid et al., 2013; Erktan et al., 2016) in PRIS, would increase AS due to the physical and microbiological effects of roots. The greater colonization and persistence of roots increase the rhizosphere (Haynes and Francis, 1993), the production of microbial binding substances (i.e glomalin, polysaccharides, among others), and soil particles enmeshment by fungi and actinomyces (Chenu and Cosentino, 2007). On a long-term study on a loam soil, Tourn et al. (2019) studied how AS was modified when long-term conventional tillage management (moldboard plow for 18 yr) was shifted to continuous no-tillage or pasture (20 yr). Those authors observed that no-tillage (20 yr) was not able to increase AS up to the level of continuous grass-based not-grazed pasture (similar to PRIS). Moreover, five years of cropping under no-tillage after a pasture led to a sharp decrease of AS, whereas two years of pasture after no-tillage cropping increased AS, but not up to the level of continuous grass-based not-grazed pasture.

### *3.3. Anaerobically mineralizable nitrogen and its relationship with AS*

As observed for SOC and POC, AN showed greater maximum, minimum, and mean values in PRIS than in CC (Table 1), indicating that AN was also sensitive to soil-use change. This observation is in agreement with those from García et al. (2016) and Domínguez et al. (2016) who reported that AN was sensitive to soil-use change and to cropping systems. Those authors reported that, as for SOC and POC, AN decreased with cropping years. As observed for SOC and POC, AN also showed greater values in the uppermost layer (0–5 cm) than in the underlying layer (5–20 and 0–20 cm) (Table 1). Similar results had

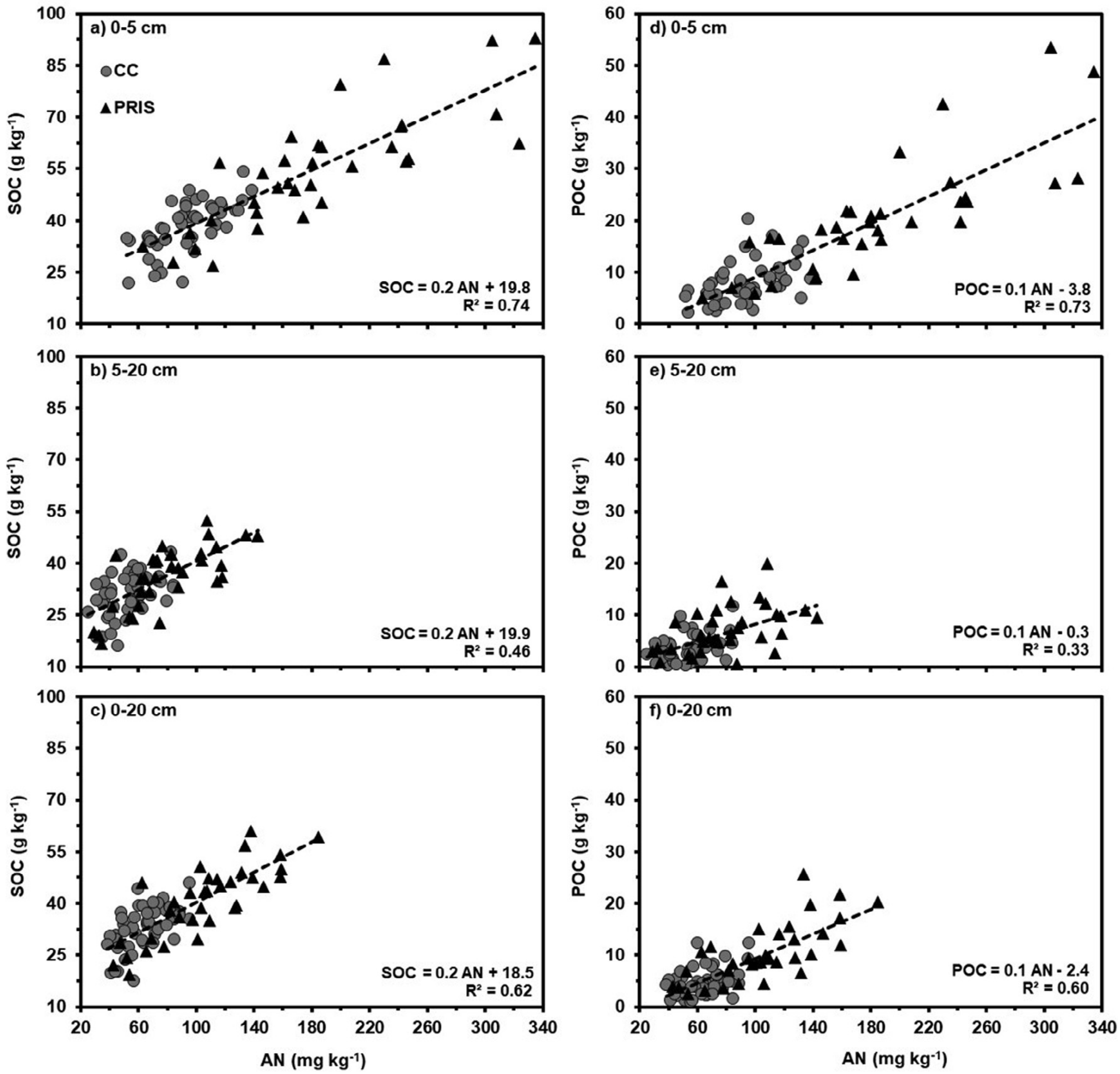

**Fig. 4.** Relationship between soil organic carbon (SOC) (a, b, c) and particulate organic carbon (POC) (d, e, f), and anaerobically mineralizable nitrogen (AN) for two soil uses at three depths: 0–5 (a, d), 5–20 (b, e), and 0–20 cm (c, f). n = 80. $P < 0.05$. CC: continuous cropping (n = 46). PRIS: pseudo-pristine (n = 34).

been reported by Domínguez et al. (2016) and García et al. (2016) for soils under no-tillage cropping or under pasture.

Anaerobically mineralizable N was positively correlated with SOC (r = 0.86, 0.68, and 0.79 at 0–5, 5–20, and 0–20 cm, respectively), POC (r = 0.86, 0.57, and 0.78, respectively), and, to a lesser extent, with MAOC (r = 0.62, 0.53, and 0.56, respectively). Fig. 4 shows the simple linear regression models for predicting SOC and POC as a function of AN at all three depths. As expected, SOC and POC increased with the increase of AN (Fig. 4). It is worth remarking that the relationships between SOC or POC and AN did not differ based on soil use (CC or PRIS). These trends coincide with results from previous studies carried out in the southeastern Buenos Aires province, evaluating a narrower range of soil situations (Domínguez et al., 2016; Studdert et al., 2017). However, contrary to the results of this study (Fig. 4), Domínguez et al. (2016) and Studdert et al. (2017) obtained greater determination coefficients for models relating POC with AN, than for models relating SOC with AN, at all three depths.

The determination coefficients of the regression models between AN and SOC or POC were greater in the uppermost layer (0–5 cm) than in the other, as previously described by Domínguez et al. (2016) and Studdert et al. (2017) (Fig. 4a, d). This difference was probably a consequence of the greater range of values for each variable at 0–5 cm than at 5–20 and 0–20 cm. Therefore, these results validate those from previous studies, but on a wider range of soil textures and management practices. The introduction of sand and clay content together with AN as a predictor of SOC and POC was evaluated. Clay content slightly improved the adjusted determination coefficients of all the models to predict SOC from AN ($R^2$ = 0.79, 0.57, and 0.71 at 0–5, 5–20, and 0–20 cm, respectively) but did not improve the $R^2$ of the models of POC as a function of AN.

Anaerobically mineralizable N was positively related to SOC and POC (Fig. 4) and, these last two variables were, in turn, positively related to AS (Fig. 3). Thus, AN could be associated with AS as proposed by Domínguez et al. (2016). For the environmental conditions considered in this study, AN was negatively correlated with ΔMWD, and positively correlated with $MWD_{FW}$ and $massMA_{FW}$ as AS indicators (Table 2). As for SOC and POC, AN had a similar association with $MWD_{FW}$ and $massMA_{FW}$ (Table 2).

The relationship between $massMA_{FW}$ and AN was linear with a positive but not different slope between CC and PRIS at all three depths (Fig. 5). However,as for SOC and POC (Fig. 3), the intercept for CC was significantly lower than for PRIS. Likewise, the addition of clay or sand content to the models describing the relationship between $massMA_{FW}$ and AN did not increase their adjusted $R^2$ (data not shown). Even though it would be important to take into account multiple factors to explain AS changes (Six et al., 2004), the variation in AN explained an

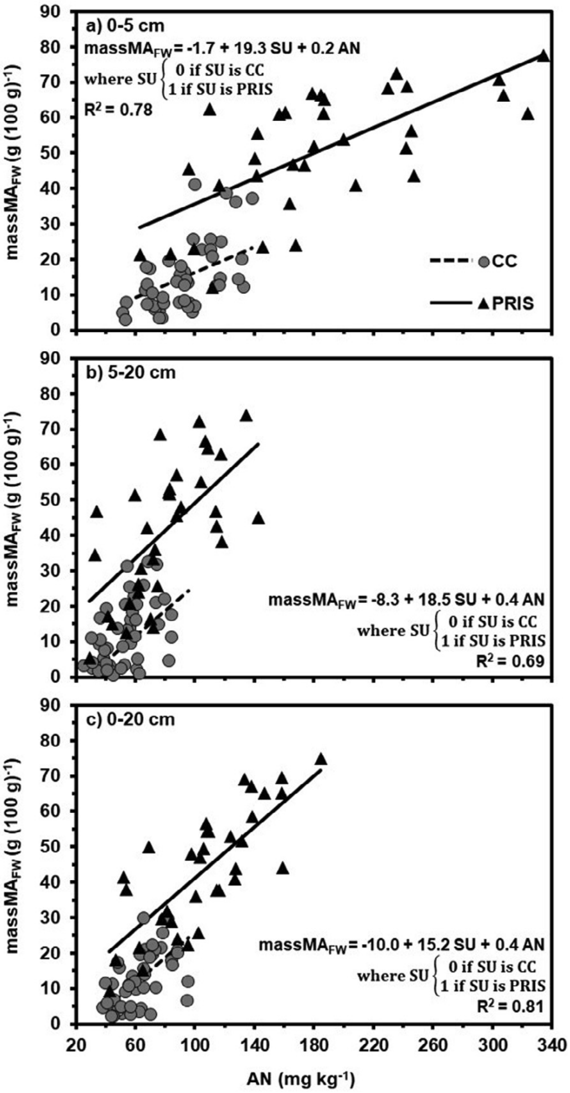

**Fig. 5.** Relationship between 2000 and 8000 μm macroaggregate mass after fast wetting (massMA$_{FW}$) and anaerobically mineralizable nitrogen (AN) for two soil uses at three depths: 0–5 (a), 5–20 (b), and 0–20 cm (c). n = 80. $P < 0.05$. SU: soil use. CC: continuous cropping (n = 46). PRIS: pseudo-pristine (n = 34).

important part of AS variability at 0–5, 5–20 and 0–20 cm depth ($R^2$ ranging from 0.69 to 0.81, Fig. 5).

Minimum, maximum, and mean values of massMA$_{FW}$, SOC, and AN of the independent data set are shown in Table 3. All ranges were within those shown in Table 1 for massMA$_{FW}$, SOC, and AN at 0–20 cm of the model-fitting data set. The independent validation of the regression models presented in Fig. 3c and Fig. 5c showed that SOC (Fig. 6a) and AN (Fig. 6b) adequately predicted massMA$_{FW}$. For both models, observed values were in relatively close agreement to predicted ones, but with more dispersion around the 1:1 line in the case of SOC (Fig. 6a) and with a relative and slight underestimation in the case of AN (Fig. 6b). On the other hand, RMSEP were 9.10 g and 8.08 g (100 g)$^{-1}$ when SOC and AN were the predictor variables, respectively, which were very close to the square roots of the mean square errors of the regressions at the building process (Fig. 3c and 5c), whose values were 10.27 and 8.69 g (100 g)$^{-1}$, respectively. Coefficients of variation of the prediction were relatively high (34.4 and 32.2%, respectively). Although there is not agreement in the literature, some authors like Chang et al. (2001) considered that RPD between 1.4 and 2.0 meant an acceptable predictive model performance and that RPD above 2.00 indicated excellent predictive performance. The RPIQ is usually considered a better indicator of model predictive performance (Bellon-Maurel et al., 2010). Values above 2 would indicate good model performance. In this case, the RPD were 1.81 and 1.99 and the RPIQ were 2.00 and 2.20 for the predictions done with SOC and AN as predictor variables, respectively. Then, all these statistical indicators show that SOC and AN could be used to satisfactorily predict massMA$_{FW}$ as an indicator of AS status through the models shown in Fig. 3c and 5c, respectively.

**Table 3**
Minimum, mean, and maximum values of soil organic carbon (SOC) content, anaerobically mineralizable nitrogen (AN), and 2000–8000 μm macroaggregate remnant dry mass after fast wetting (massMA$_{FW}$) at 0–20 cm for plots under continuous cropping (CC, n = 32) and pseudo-pristine plots (PRIS, n = 21) sampled in July 2018.

| Variable | Use | Minimum | Mean | Maximum |
|---|---|---|---|---|
| SOC (g kg$^{-1}$) | PRIS | 23.2 | 38.3 | 49.5 |
| | CC | 26.5 | 32.8 | 40.6 |
| AN (mg kg$^{-1}$) | PRIS | 37.7 | 86.8 | 156.8 |
| | CC | 35.2 | 58.1 | 84.4 |
| massMA$_{FW}$ (g(100 g)$^{-1}$) | PRIS | 22.5 | 41.0 | 68.6 |
| | CC | 3.3 | 15.9 | 28.6 |

Hence, AN (Figs. 5 and 6b) resulted as good indicator of AS as SOC (Fig. 3a-c and 6a) and POC (Fig. 3d-f). Thus, a simple statistical model with AN as the sole predictor variable would allow having a reliable and acceptably precise indication of soil AS status (Fig. 5). Since AS is related to some other properties that define soil physical health (like porosity, bulk density, and infiltration, among others, Rabot et al., 2018), AN would be a good soil physical health indicator. Further research should be focused on validating the fitted models for a wider range of soil uses and management situations, and on defining AN threshold compromising soil functioning.

## 4. Conclusion

The results of this study support the hypothesis because AN was positively related to AS in Mollisols of the southeastern Argentinean Pampas with loam textures (loam, sandy-loam, sandy-clay-loam, and clay-loam), and under different soil uses. Likewise, AN also related positively to SOC and POC. Thus, AN would be a good indicator of AS, and also of SOC and POC contents. So, AN determination could be used as indicator of soil physical and biochemical health status.

The relationship between AS and AN, improves AN performance as SHI, making it a very suitable and versatile SHI that facilitates monitoring the soil health status. Frequent AN determination would contribute to planning management practices to avoid compromising soil functioning in the agroecosystem.

## CRediT authorship contribution statement

**Gisela V. García:** Writing - original draft, Investigation, Conceptualization, Methodology, Formal analysis, Resources. **Nicolás Wyngaard:** Writing - review & editing, Investigation. **Nahuel I. Reussi Calvo:** Writing - review & editing, Investigation. **Silvina San Martino:** Formal analysis, Writing - review & editing. **Fernanda Covacevich:** Writing - review & editing. **Guillermo A. Studdert:** Writing - review & editing, Supervision, Conceptualization, Investigation, Project administration.

## 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.

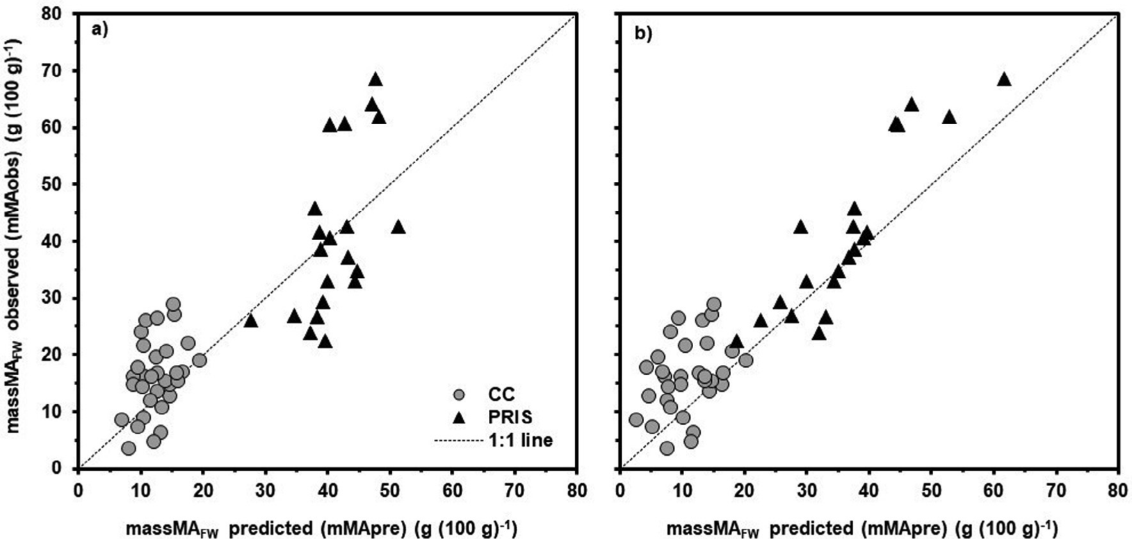

**Fig. 6.** Plot of observed 2000–8000 µm macroaggregate mass after fast wetting (massMA$_{FW}$) at 0–20 cm versus predicted massMA$_{FW}$ with a) soil organic carbon (SOC) (Equation in Fig. 3c) and b) anaerobically mineralizable nitrogen (AN) (Equation in Fig. 5c) at 0–20 cm as predictor variables for two soil uses: CC: continuous cropping (n = 32). PRIS: pseudo-pristine (n = 21).

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