Ciencia del Suelo
Revista científica de la Asociación Argentina de la Ciencia del Suelo

ANAEROBICALLY MINERALIZED NITROGEN WITHIN MACROAGGREGATES AS INDICATOR OF WHEAT NITROGEN NUTRITION

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ABSTRACT

This work aimed to evaluate the capacity of anaerobically mineralized nitrogen (AN) within large (AN_{LM}), small (AN_{SM}), and total macroaggregates (AN_{TM}) to predict grain yield, aboveground biomass, and total nitrogen (N) content of wheat (*Triticum aestivum* L.) plants as compared to AN in bulk soil (AN_{BS}). Eight fields with non-N-fertilized wheat on Mollisols of the southeastern Argentinean Pampas were studied. Soil AN_{BS}, AN_{LM}, AN_{SM}, and AN_{TM}, and wheat grain yield, aboveground biomass, and plant total N content were determined. The AN_{LM}, AN_{SM}, and AN_{TM} were positively related to grain yield, aboveground biomass, and plant total N content (R^2=0.34-0.65). The relationships between AN_{BS} and all those crop variables showed similar predictive capacity. Therefore, AN within macroaggregates was not a better indicator of soil nitrogen supply capacity than AN_{BS}.

Keywords: grain yield; aboveground biomass; total nitrogen.

NITRÓGENO MINERALIZADO EN ANAEROBIOSIS EN MACROAGREGADOS COMO INDICADOR DE LA NUTRICIÓN NITROGENADA DE TRIGO

RESUMEN

El objetivo de este trabajo fue evaluar la capacidad del nitrógeno mineralizado en anaerobiosis (AN) determinado dentro de macroagregados grandes (AN_{LM}), macroagregados chicos (AN_{SM}) y macroagregados totales (AN_{TM}) para predecir el rendimiento en grano, la biomasa aérea y el contenido de nitrógeno total en planta de trigo (*Triticum aestivum* L.) sin fertilización nitrogenada comparado con el AN determinado en la masa total del suelo (AN_{BS}). Fueron estudiados ocho lotes con trigo sin fertilización nitrogenada en Molisoles del sudeste de la Región Pampeana Argentina. El AN_{BS}, el AN_{LM}, el AN_{SM}, el AN_{TM}, el rendimiento en grano, la biomasa aérea y el contenido de nitrógeno total en planta fueron determinados. El AN_{LM}, el AN_{SM} y el AN_{TM} estuvieron relacionados con el rendimiento en grano, la biomasa aérea y el contenido de nitrógeno total en planta (R^2=0.34-0.65). Las relaciones entre el AN_{BS} y todas aquellas variables mostraron capacidades predictivas similares. Por lo tanto, el AN en macroagregados no fue un mejor indicador de la capacidad del suelo de suministrar nitrógeno que el AN_{BS}.

Palabras clave: rendimiento en grano; biomasa aérea; nitrógeno total.

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Recibido:
14-02-24

Recibido con revisiones:
12-05-24

Aceptado:
31-05-24

INTRODUCTION

Nitrogen (N) is the most influential nutrient for crop yield and quality (Echeverría & Sainz Rozas, 2014). Nitrogen fertilizer recommendations are generally based on bridging the gap between the grain yield of a non-N-fertilized crop and its grain yield potential with no N limitations under specific edaphoclimatic conditions (Correndo et al., 2021). Thus, the grain yield without N fertilization is determined by the soil N supply capacity (Reussi Calvo et al., 2013). In order to make sustainable decisions regarding N fertilization management, it is necessary to develop and use models for predicting the grain yield of unfertilized crops at its planting time or early growth stages (Archontoulis et al., 2020). However, such prediction is usually complex due to the large number of factors involved in the N dynamics (Archontoulis et al., 2020; Correndo et al., 2021). Along this line, N mineralization indicators are very important to help estimating the soil N availability, since they allow estimating the amount of N mineralized from the soil organic matter throughout the crop cycle (García & Reussi Calvo, 2014).

Potentially mineralizable N is considered the standard method to determine the nitrogen mineralization potential of a soil under laboratory conditions. However, it requires long incubation periods for its determination (>200 days), which restricts its use as a method for N fertility diagnosis for N fertilization recommendations in soil testing labs (Echeverría & Sainz Rozas, 2014). Consequently, numerous soil variables have been suggested as alternatives to potentially mineralizable N (Schomberg et al., 2009). One of such variables is the anaerobically mineralized N (AN) in the bulk soil (AN_{BS}), which involves a short (i.e., 7 days) anaerobic incubation period of soil samples (Keeney, 1982). Considering that AN_{BS} is strongly related to potentially mineralizable N, AN_{BS} has been proposed as a quick, simple, and precise alternative to estimate potentially mineralizable N (Schomberg et al., 2009). In this sense, it has been reported that AN_{BS} is positively associated with grain yields of non-N-fertilized corn (*Zea mays* L.) (Orcellet et al., 2017), wheat (*Triticum aestivum* L.) (Reussi Calvo et al., 2013), and barley (*Hordeum vulgare* L.) (Queirolo, 2018). The incorporation of AN_{BS} determined at 0-20 cm depth to the traditional methods for the N availability diagnosis has improved their capacity to predict grain yield of unfertilized crops and the response to N fertilization of wheat (Reussi Calvo et al., 2013) and corn (Orcellet et al., 2017). Such improvement allows a more precise and reliable determination of N fertilizer rates, leading to a more efficient and environmentally safer use of nitrogen fertilizers.

In Mollisols from temperate regions, macroaggregates (250-8000 µm) have a key role in the functioning of soil (García et al., 2021; Roldán et al., 2014). Since the concentration of organic fractions in soil macroaggregates is more sensitive to management practices than those determined in bulk soil (Roldán et al., 2014), AN within large (2000-8000 µm, AN_{LM}), small (250-2000 µm, AN_{SM}), and/or total macroaggregates (250-8000 µm, AN_{TM}) may be better indicators of soil N availability. Thus, AN_{LM}, AN_{SM}, and AN_{TM} could be more closely related than AN_{BS} to productive traits (grain yield, and aboveground biomass dry matter, and plant N accumulation) than AN_{BS}. The determination of AN within macroaggregates would allow a more precise N-fertilizer recommendation, reducing the economic and environmental impact of fertilization. The objective of this work was to evaluate the capacity of AN_{LM}, AN_{SM}, and AN_{TM} to predict grain yield, aboveground biomass, and plant total N plant content, as compared with AN_{BS} in non-N-fertilized wheat crops on Mollisols of the Southeastern Argentinean Pampas.

MATERIALS AND METHODS

In 2018, eight rainfed wheat fields of farms from the southeastern Argentinean Pampas (Figure 1) were selected to take soil and plant samples. Soils were Mollisols with surface textural classes typical of the region (i.e. loam, clay-loam, sandy-loam, and sandy-clay-loam, Rubio et al., 2019), and no evidence of erosion (slope<2%) or flooding. The climate in this area is classified as mesothermal subhumid-humid (according to the Thornthwaite classification) or as temperate humid without a 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). In each field, a 400 m^2 plot was delimited and georeferenced, and those plots were preserved from receiving N fertilization. Wheat was sown between June and July of 2018, using intermediate-growing-season varieties recommended for this region. Weeds, pests, and diseases control was done by the farmers, and the tillage system was no-tillage in all the fields.

Figure 1: Sampling sites (circles) throughout the southeastern Buenos Aires province at the Argentinean Pampas.
Figura 1: Sitios de muestreo (círculos) en el sudeste de la provincia de Buenos Aires en la Región Pampeana argentina.

At wheat sowing (June and July of 2018) composite soil samples (5 subsamples) were taken at 0-20 cm depth using a tubular sampler at field capacity to determine AN_{BS} in the unfertilized plots. The samples were dried at 50 °C until constant weight and ground to pass through a 2000-µm sieve removing all identifiable plant material. These samples were later used to determine AN_{BS}. Additional composite soil samples were taken at 0-20 cm depth with a shovel (5 subsamples from each plot) to determine AN_{LM}, AN_{SM}, and AN_{TM}. Upon extraction, aggregates 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. Macroaggregates separation was performed as described by Six et al. (1998). In detail, 100 g aliquot of dry aggregates were capillary re-wetted for 24 h until field capacity. Re-wetted aggregates were placed on the 2000-µm-mesh sieve and submerged in water for 5 min. After that, large macroaggregates and small macroaggregates were separated by sieving in water for 2-min (50 3-cm-run up and down oscillations) on a 2000 µm and 250 µm sieve, respectively. Aggregate fractions were back-washed from the sieve, allowed to flocculate for 24 h, and oven-dried at 50 °C until constant weight after removing the supernatant. Dry large and small macroaggregates were ground with mortar and pestle to pass through a 500-µm sieve. The AN_{BS}, AN_{LM}, and AN_{SM} were determined through short anaerobic incubation for 7 d at 40 °C (Keeney, 1982), and ammonium-N quantified by steam distillation (Keeney & Nelson, 1982). The AN_{BS} was expressed in mg ammonium-N per kg^{-1} bulk soil dry mass, whereas AN_{LM} and AN_{SM} were expressed in mg ammonium-N per kg^{-1} large and small macroaggregates dry mass, respectively. The AN_{TM} was calculated through the average of AN_{LM} and AN_{SM} weighted by the mass proportion of large and small macroaggregates masses, respectively. Additionally, soil samples were taken at 0-60 cm in each plot to determine N as nitrate through extraction with potassium sulfate and quantification by spectrophotometry (Keeney & Nelson, 1982).

At wheat physiological maturity, aboveground biomass samples were randomly taken in each plot from three 9-m-long crop rows. Samples were dried at 60 °C until constant weight and weighed, and aboveground biomass was determined as the sum of the dry matter of leaves and stems, and grain yield. Then, spikes were separated from the rest of the plant (leaf and stem), threshed with a stationary thresher, and grains were weighted. Grain yield was expressed at 14% moisture. Both grains and leaves and stems were ground to pass through a 500-µm sieve to determine plant total N content by wet combustion followed by steam distillation (Bremner & Mulvaney, 1982). All crop variables (i.e., grain yield, aboveground biomass, and total N) were expressed as mass per area unit (i.e., kg ha^{-1}).

Water availability throughout the crop cycle was calculated as the sum of soil available water at sowing and rainfall during the crop growth cycle (i.e., from July to December). Available water in the soil at sowing was 150 mm considering that the soil profile was at field capacity (Carpaneto & Lanzavecchia, 2016). Rainfall records were obtained from records of weather stations located near the experiments (< 5 km). The association between variables was evaluated through Pearson correlation coefficients and simple linear regression and linear-plateau models. Statistical analyses were performed with R software (R Core Team, 2020). A significance level of 0.05 was used.

RESULTS

Minimum, mean, and maximum values of AN within macroaggregates (i.e. AN_{LM}, AN_{SM}, and AN_{TM}), AN_{BS}, N as nitrate, and crop variables are presented in Table 1. Available water during the crop cycle ranged between 400 and 845 mm, with a mean value of 587 mm. Nitrogen as nitrate was not correlated to AN_{LM}, AN_{SM}, AN_{TM}, AN_{BS}, and grain yield, but it was correlated to aboveground biomass (r=0.65) and plant total N (r=0.64). The AN_{BS} was related to grain yield (pseudo-R^2=0.67, Figure 2a) and aboveground biomass (pseudo-R^2=0.39, Figure 2b) through linear-plateau models. Grain yield increased with aboveground biomass up to an estimated value of 58.2 mg kg^{-1}, beyond which it stabilized at 6058 kg ha^{-1} (Figure 2a). The relationship between aboveground biomass and AN_{BS} showed a similar behavior showing an AN_{BS} threshold of 57.1 mg kg^{-1}, beyond which aboveground biomass stabilized at 13212.7 kg ha^{-1} (Figure 2b). The AN_{BS} was linear and positively related to plant total N (R^2=0.48, Figure 2c).

Table 1: Minimum, mean, and maximum values of anaerobically mineralized nitrogen (AN) in bulk soil (AN_{BS}), AN in large macroaggregates (2000-8000 µm, AN_{LM}), AN in small macroaggregates (250-2000 µm, AN_{SM}), AN in total macroaggregates (250-8000 µm, AN_{TM}), nitrogen as nitrate ($N\text{-}NO_3^-$), yield grain, aboveground biomass, and plant total nitrogen content of wheat without nitrogen fertilization.

Tabla 1: Valores mínimos, medios y máximos de nitrógeno mineralizado en anaerobiosis (AN) en la masa total del suelo (AN_{BS}), AN en macroagregados grandes (2000-8000 µm, AN_{LM}), AN en macroagregados chicos (250-2000 µm, AN_{SM}), AN en macroagregados totales (250-8000 µm, AN_{TM}), nitrógeno como nitrato ($N\text{-}NO_3^-$), rendimiento en grano, biomasa aérea y nitrógeno total en planta de trigo sin fertilización nitrogenada.

Variable	n	Minimum	Mean	Maximum
AN_{BS} (mg kg^{-1})	8	44.4	61.5	95.0
AN_{LM} (mg kg^{-1})	8	48.2	72.0	109.4
AN_{SM} (mg kg^{-1})	8	53.9	72.3	111.7
AN_{TM} (mg kg^{-1})	8	49.4	72.1	109.9
$N\text{-}NO_3^-$ (mg kg^{-1})	8	4.6	9.7	23.1
Grain yield (kg ha^{-1})	24	2893.4	5217.6	7818.7
Aboveground biomass (kg ha^{-1})	24	7257.1	12084.7	16000.0
Total nitrogen (kg ha^{-1})	24	66.0	110.2	166.7

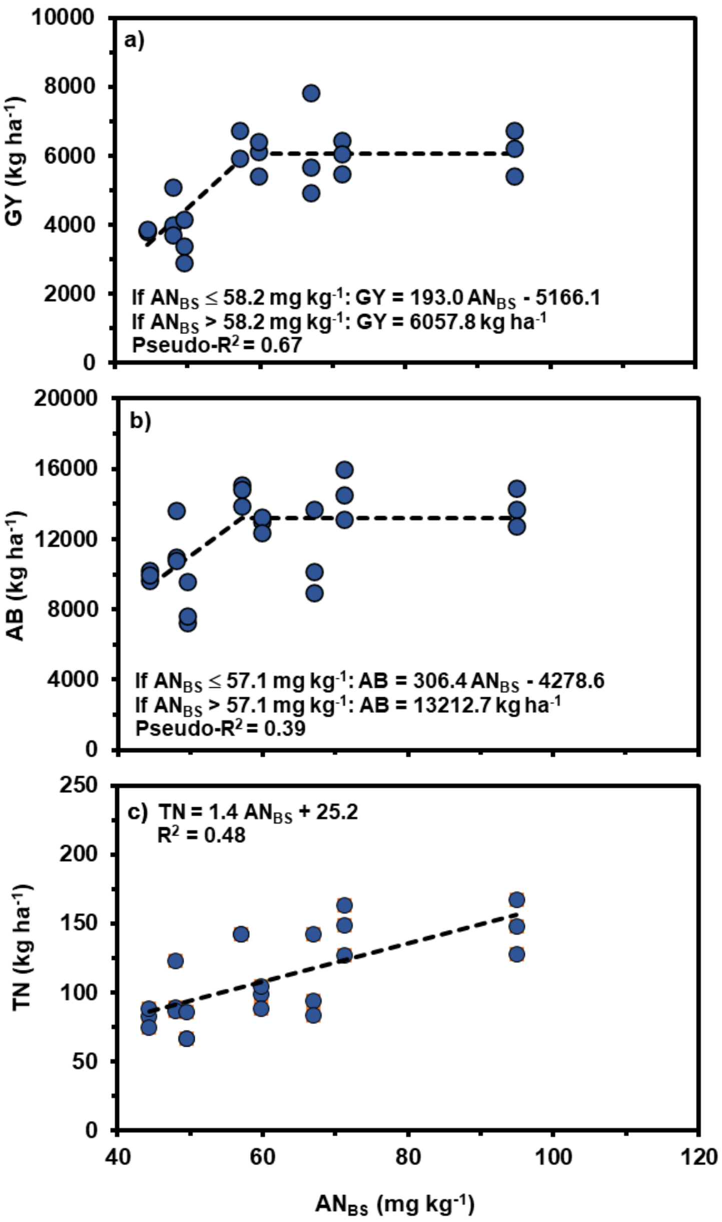

Figure 2: Relationships between anaerobically mineralized nitrogen (AN) in bulk soil (AN_{BS}) and yield grain (GY) (a), aboveground biomass (AB) (b), and total nitrogen in plant (TN) (c) of wheat without nitrogen fertilization (n = 24).

Figura 2: Relaciones entre el nitrógeno mineralizado en anaerobiosis (AN) en la masa total del suelo (AN_{BS}) y el rendimiento en grano (GY) (a), la biomasa aérea (AB) (b), y el nitrógeno total en planta (TN) (c) de trigo sin fertilización nitrogenada (n = 24).

The AN_{LM}, AN_{SM}, and AN_{TM} were positive and closely correlated to AN_{BS} (r of 0.87, 0.81, and 0.87, respectively). The AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and/or AN_{TM}) were related to grain yield (Figure 3a, d, g, respectively), aboveground biomass (Figure 3b, e, h, respectively), and plant total N (Figure 3c, f, i, respectively) through different models, depending on the analyzed variables. The AN_{LM}, AN_{SM}, and AN_{TM} were related to grain yield (Figure 3a, d, g, respectively) and aboveground biomass (Figure 3b, e, h, respectively) through linear-plateau models. Grain yield and aboveground biomass increased with the increase of AN within macroaggregates up to estimated values between 64.7 and 69.0 mg kg^{-1}, respectively, beyond which grain yield and aboveground biomass stabilized at average values of 5713 and 13330 kg ha^{-1}, respectively. The AN within macroaggregates was related to plant total N (Figure 3c, f, i) through linear models. Model fittings of the relationships between AN_{LM}, AN_{SM}, and AN_{TM} and grain yield (Figure 3a, d, g, respectively, pseudo-R^2 of 0.52, 0.38, and 0.50, respectively) and plant total N (Figure 3c, f, i, respectively, R^2 of 0.46, 0.34, and

0.44, respectively) were lower than those between AN_{BS} and grain yield (Figure 2a, pseudo-R^2 of 0.67) and plant total N (Figure 2c, R^2 of 0.48), respectively. Contrarily, higher pseudo-R^2 were found between AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and AN_{TM}) and aboveground biomass (Figure 3b, e, h, pseudo-R^2 of 0.62, 0.65, and 0.64, respectively) than between AN_{BS} and aboveground biomass (Figure 2b, R^2 of 0.39)

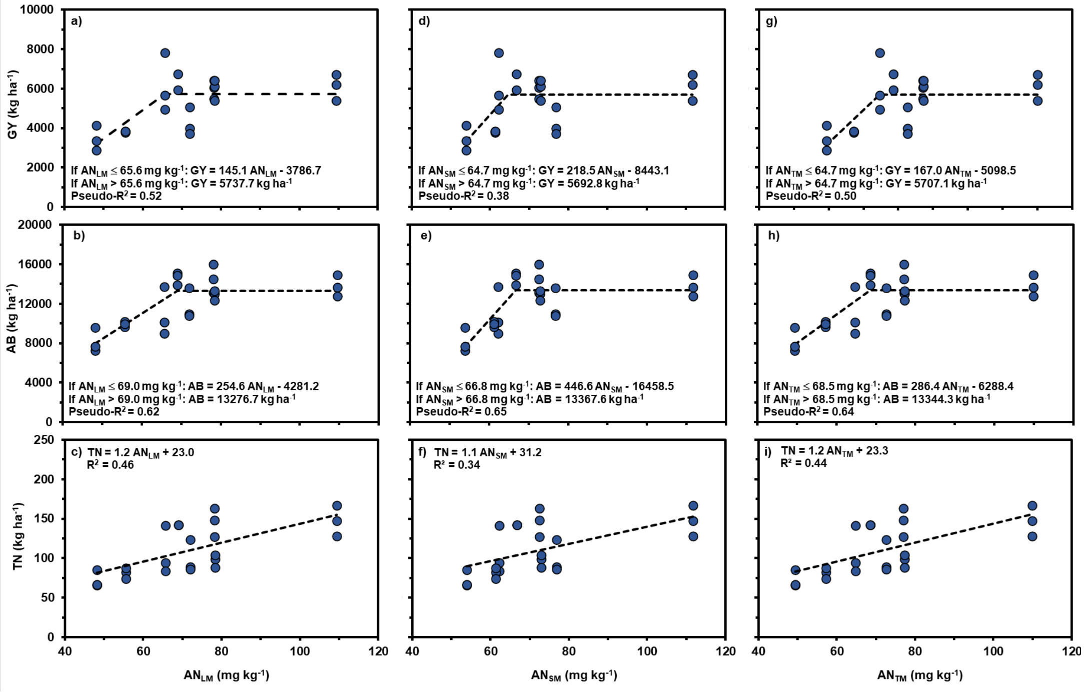

Figure 3: Relations between anaerobically mineralized nitrogen (AN) in large macroaggregates (2000-8000 µm) (AN_{LM}) and yield grain (GY) (a), aboveground biomass (AB) (b), and total nitrogen in plant (TN) (c), AN in small macroaggregates (250-2000 µm) (AN_{SM}) and GY (d), AB (e), and TN (f), AN in total macroaggregates (250-2000 µm) (AN_{TM}) and GY (g), AB (h), and TN (i) of wheat without nitrogen fertilization (n = 24).

Figura 3: Relaciones entre el nitrógeno mineralizado en anaerobiosis (AN) en macroagregados grandes (2000-8000 µm) (AN_{LM}) y el rendimiento en grano (GY) (a), la biomasa aérea (AB) (b), y el nitrógeno total en planta (TN) (c), el AN en macroagregados chicos (250-2000 µm) (AN_{SM}) y el GY (d), la AB (e), y el TN (f), el AN en macroagregados totales (250-2000 µm) (AN_{TM}) y el GY (g), la AB (h), y el TN (i) de trigo sin fertilización nitrogenada (n = 24).

DISCUSSION

The mean value of grain yield without N fertilization recorded at this study was greater (Table 1) than the average wheat grain yield at the studied area during the same year (4614 kg ha^{-1}) and at the previous 15 years (4240 kg ha^{-1}) (MAGyP, 2020). No water limitations were observed during the crop growing season since water availability exceeded crop water demand (i.e. 385 mm, Abbate, 2017) in all experiments.

Given that AN within macroaggregates was related to crop variables (Figure 3), our results suggest that AN_{LM}, AN_{SM}, and AN_{TM} are indicators of wheat performance and, specifically, of the soil N availability during the growing season, as AN_{BS} is. In general, the relationships between AN within macroaggregates and crop variables (Figure 3) presented a similar behavior as the relations between AN_{BS} and crop variables (Figure 2). This could be due to the close relations between AN_{BS} and AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and/or AN_{TM}). Similar relations between AN_{BS} and AN within macroaggregates were reported by other authors in Mollisols (García et al., 2021; Gregorutti et al., 2014; Rivero et al., 2020) and Vertisols (Gregorutti et al., 2014). These results indicate that the largest proportion of organic nitrogen that is quantified through AN_{BS} is found in macroaggregates (García et al., 2021; Gregorutti et al., 2014; Rivero et al., 2020).

The AN_{BS} and AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and/or AN_{TM}) explained reasonably well the grain yield variability (pseudo-R^2 between 0.38 and 0.67, Figure 2a, 3a, e, i) and the aboveground biomass variability (pseudo-R^2 between 0.39 and 0.65, Figure 2b, 3b, f, j). For the southeastern Argentinean Pampas soils, other authors reported positive linear relations between AN_{BS} and grain yield in unfertilized barley (Queirolo, 2018) and wheat (Reussi Calvo et al., 2013) crops, in which AN_{BS} explained 45% and 41% of grain yield variability, respectively.

The associations between AN_{BS}, AN_{LM}, AN_{SM}, and AN_{TM} with total nitrogen (Figure 2c) confirm that AN expresses the soil nitrogen supply capacity (Reussi Calvo et al., 2013). The AN_{BS} and AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and/or AN_{TM}) explained as much variability of plant total N (34-48%, Figure 2c, 3c, g, k) as reported by Reussi Calvo et al. (2013) for wheat.

Although no differences were observed between AN within macroaggregates (i.e., AN_{LM}, AN_{SM}, and/or AN_{TM}) and AN_{BS} in their ability to predict wheat grain yield (lower pseudo-R^2, Figure 3a, d, g vs Figure 2a) and plant total N content (Figure 3c, f, i vs Figure 2c), AN within macroaggregates was a better indicator of aboveground biomass (Figure 3b, e, h vs Figure 2b). Previous studies have reported that AN_{LM} and AN_{TM} were not better indicators of soil properties (i.e. total and particulate organic carbon, and aggregate stability) than AN_{BS} (García et al., 2021; Rivero et al., 2020).

CONCLUSION

Under the conditions of this study, AN_{LM}, AN_{SM}, and AN_{TM} were not better indicators of grain yield and plant total N than AN_{BS}. For this reason and considering that grain yield was better explained by AN_{BS} than by AN within large, small and/or total macroaggregates, the determination of AN_{BS} would be enough to estimate the capacity of soils to supply N. This allows to predict wheat N nutrition, allowing precise N-fertilizer recommendations. Hence, it would not be necessary to use a more complex procedure to determine AN within macroaggregates, which additionally demands more careful sampling and conditioning of soil samples to avoid aggregate disturbance, and the separation of macroaggregates through water sieving after capillary wetting.

ACKNOWLEDGMENTS

Part of the results presented in this work corresponds to the Doctoral Dissertation of the senior author at the Programa de Posgrado en Ciencias Agrarias, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina. This work was supported by the Universidad Nacional de Mar del Plata (AGR653/21 and AGR689/22), the Fondo para la Investigación Científica y Tecnológica (PICT-2021-I-INVI-00619), and the Instituto Nacional de Tecnología Agropecuaria (PNCyO 1127032), Argentina. The authors want to express their deep appreciation to the farmers who allowed soil sampling in their fields and data collection and use for this study.

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