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Anaerobically mineralized nitrogen as a potential indicator of the activity and abundance of mycorrhizal fungi in Mollisols

Gisela V. García[1,2]✉; Fernanda Covacevich[1,3]; Silvina San Martino[2]; Nicolás Wyngaard[1,2]; Nahuel I. Reussi Calvo[1,2] & Guillermo A. Studdert[2]

[1] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).* [2] *Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata. Mar del Plata, Buenos Aires, Argentina.* [3] *Instituto de Investigaciones en Biodiversidad y Biotecnología-Fundación para las Investigaciones Biológicas Aplicadas.*

Abstract. Anaerobically mineralized nitrogen (AN) is a suitable soil health indicator. The AN is sensitive to soil use changes and is related to soil and particulate organic carbon and aggregate stability. This work aims to evaluate the relationship between AN and 1) easily extractable glomalin-related soil proteins; 2) abundance of arbuscular mycorrhizal fungi measured by the number of arbuscular mycorrhizal fungi spores, and 3) arbuscular mycorrhizal fungi activity (root colonization). Soil samples were taken at depths of 0-5 and 5-20 cm from cultivated and uncultivated plots throughout the southeastern province of Buenos Aires. Anaerobically mineralized nitrogen, soil organic carbon, particulate organic carbon, aggregate stability, easily extracted glomalin-related soil proteins and the logarithm of the number of arbuscular mycorrhizal fungi spores (log spores) at 0-5, 5-20 and 0-20 cm depths were determined. In wheat roots, the percentages of total infection and arbuscules at 0-20 cm were measured. At all depths, AN was positively correlated to easily extractable glomalin-related soil proteins (r=0.34-0.65), which is an indicator of arbuscular mycorrhizal fungi activity and abundance. Likewise, AN was positively related to log-spores (r=0.58-0.78), which is an indicator of arbuscular mycorrhizal fungi abundance. However, AN was not related to root colonization (the percentages of total infection and arbuscules) that manifests the activity of arbuscular mycorrhizal fungi at a specific moment. Thus, anaerobically mineralized nitrogen would be an indicator of mid- to long-term changes in arbuscular mycorrhizal fungi abundance and activity (easily extractable glomalin-related soil proteins and log-spores) resulting from soil use. Consequently, the AN would allow monitoring an important aspect of soil microbiological health associated with arbuscular mycorrhizal fungi. However, it is necessary to evaluate the relationships studied in this work in a wider range of soil situations.

[Keywords: soil health indicator, easily extractable glomalin-related soil proteins, spores of mycorrhizal fungi, soil organic carbon, particulate organic carbon, aggregate stability, colonized roots with mycorrhizal fungi]

Resumen. Nitrógeno mineralizado en anaerobiosis como un potencial indicador de la abundancia y la actividad de hongos micorrícicos en Molisoles. El nitrógeno mineralizado en anaerobiosis (NA) es un indicador de salud edáfica; es sensible a los cambios en el uso del suelo y se relaciona con el carbono orgánico total, el particulado y la estabilidad de agregados. Nuestro objetivo fue evaluar la relación entre el NA y 1) las proteínas fácilmente extraíbles relacionadas con la glomalina; 2) la abundancia de hongos micorrícicos arbusculares (número de esporas de hongos micorrícicos arbusculares), y 3) la actividad de hongos micorrícicos arbusculares (raíces colonizadas). Se muestreó el suelo a 0-5 y 5-20 cm en parcelas cultivadas y no cultivadas en el sudeste bonaerense. Se determinó el nitrógeno mineralizado en anaerobiosis , carbono orgánico total y particulado, estabilidad de agregados, proteínas fácilmente extraíbles relacionadas con la glomalina y el logaritmo del número de esporas de hongos micorrícicos arbusculares (log-spores) en 0-5, 5-20 y 0-20 cm. En raíces de trigo, se midió el porcentaje de infección total y de arbúsculos en 0-20 cm. En todas las profundidades, el NA correlacionó positivamente con proteínas fácilmente extraíbles relacionadas con la glomalina (r=0.34-0.65), indicadoras de la actividad y la abundancia de hongos micorrícicos arbusculares. El NA se relacionó positivamente con el log-spores (r=0.58-0.78), que indica la abundancia de hongos micorrícicos arbusculares. Sin embargo, el NA no se relacionó con la colonización de raíces (porcentaje de infección total y de arbúsculos), que manifiesta la actividad de hongos micorrícicos arbusculares en un momento dado. Así, el nitrógeno mineralizado en anaerobiosis indicaría la abundancia y la actividad de hongos micorrícicos arbusculares a mediano y largo plazo (proteínas fácilmente extraíbles relacionadas con la glomalina y log-spores) debido al uso del suelo. El NA permitiría monitorear un aspecto importante de la salud microbiológica del suelo asociada con hongos micorrícicos arbusculares. Sin embargo, es necesario evaluar las relaciones estudiadas en este trabajo en un rango más amplio de situaciones de suelo.

[Palabras clave: indicador de salud edáfica, proteínas del suelo relacionadas a glomalina fácilmente extraíbles, esporas de hongos micorrícicos, carbono orgánico del suelo, carbono orgánico particulado, estabilidad de agregados, raíces colonizadas con hongos micorrícicos]

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✉ gisela_garcia@mdp.edu.ar
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Introduction

Soil health evaluation is important for diagnosing and quantifying the degree of soil degradation and to plan suitable management practices for sustainable agriculture and the preservation of ecosystem services. This evaluation is performed through soil health indicators, which are soil parameters that express changes produced by management practices; they are easy and cheap to determine, simple to interpret, and reflect changes in soil functioning (Bünemann et al. 2018). Soil organic carbon and particulate organic carbon are generally used as soil health indicators (Cambardella and Elliott 1992). However, neither of these two soil parameters fully complies with the requirements of a soil health indicator. Soil organic carbon is not sensitive enough to detect short to mid-term soil health changes (Domínguez et al. 2016). Contrarily, particulate organic carbon allows an early detection of soil health changes (Cambardella and Elliott 1992). However, particulate organic carbon quantification is complex and time-consuming, and therefore not suitable for routine analysis in soil testing laboratories.

Anaerobically mineralized nitrogen (AN) in a short incubation (i.e., 7 days) has been proposed as a suitable soil health indicator given that it 1) is easy and cheap to determine; 2) is simple to interpret; 3) expresses the changes produced by soil use in the short to mid term, and 4) is related to other soil properties that define soil health (Domínguez et al. 2016; García et al. 2020b). AN is a good indicator of soil biochemical health since it is strongly related to soil organic carbon, labile fractions of soil organic carbon such as the particulate organic carbon (Domínguez et al. 2016; García et al. 2020b) and the potentially mineralizable nitrogen (Reussi-Calvo et al. 2013) and sulfur (Carciochi et al. 2018). Likewise, AN is a good indicator of soil physical health, since it is closely associated with aggregate stability (García et al. 2020b). Thus, a single soil parameter, which is simple and inexpensive to determine, could be used to monitor soil biochemical and physical health status.

Among the properties associated with anaerobically mineralized nitrogen, aggregate stability is a physical property influenced by microorganisms that inhabit the soil, especially fungi. Arbuscular mycorrhizal fungi are obligate symbionts of the roots of 90% of terrestrial plants and are the most common fungi in cultivated soils (Covacevich and Vargas-Gil 2014). Arbuscular mycorrhizal fungi are one of the most important groups of microorganisms involved in aggregate formation and stabilization (Six et al. 2004), principally through two mechanisms. First, the arbuscular mycorrhizal fungi mycelium intertwines small aggregates forming aggregates of greater size and stability. Second, the arbuscular mycorrhizal fungi mycelium exudes a hydrophobic protein, glomalin, which acts as an adhesive agent to bond soil particles (Wright and Upadhyaya 1996; Chenu and Cosentino 2007). Similarly, glomalin increases soil organic matter hydrophobicity, contributing to dry aggregate resistance to rupture upon sudden moistening and, hence, helping to create more stable aggregates (Chenu and Cosentino 2007). Glomalin is isolated from the soil as a fraction known as total glomalin-related soil proteins. However, a fraction of total-glomalin-related soil proteins known as easily extractable glomalin-related soil proteins is more frequently measured due to its simplicity (Wright and Upadhyaya 1996). It has been reported that easily extractable glomalin-related soil proteins are sensitive to soil changes produced by their use and management (Liu et al. 2020). Close relationships have been demonstrated between easily extracted glomalin-related soil proteins and aggregate stability (Bedini et al. 2009; Fokom et al. 2012), and between easily extractable glomalin-related soil proteins and soil organic carbon (Nichols and Wright 2004; Thougnon-Islas et al. 2016). This last relationship suggests that the dynamics of soil organic carbon and easily extractable glomalin-related soil proteins accumulation and decomposition would be similar (Nichols and Wright 2004), or that arbuscular mycorrhizal fungi increase soil organic carbon content (Rillig et al. 2001; He et al. 2020). Likewise, it has been suggested that easily extractable glomalin-related soil proteins could be an indicator of nitrogen availability for plants (Hurisso et al. 2018). For these reasons, easily extractable glomalin-related soil proteins have been proposed as a soil health indicator (Fine et al. 2017; Šarapatka et al. 2019).

Since glomalin is produced by arbuscular mycorrhizal fungi spores and hyphal walls, easily extractable glomalin-related soil proteins are considered an indicator of arbuscular mycorrhizal fungi abundance and activity (Bedini et al. 2007). It was reported that arbuscular mycorrhizal fungi abundance

(spore number) and arbuscular mycorrhizal fungi activity (colonized roots) are positively correlated with easily extractable glomalin-related soil proteins and aggregate stability (Bedini et al. 2009; Fokom et al. 2012; Lozano-Sánchez et al. 2015). Considering that easily extractable glomalin-related soil proteins and arbuscular mycorrhizal fungi abundance and activity are related to aggregate stability and soil organic carbon content, and that aggregate stability and soil organic carbon are related to AN, a relationship between AN and easily extractable glomalin-related soil proteins and between AN and arbuscular mycorrhizal fungi abundance and activity could be expected. If that was the case, anaerobically mineralized nitrogen would allow monitoring not only the biochemical and physical health but also an aspect of soil microbiological health of the soil. The aim of this work is to evaluate the relationship of anaerobically mineralized nitrogen with easily extractable glomalin-related soil proteins, arbuscular mycorrhizal fungi abundance measured through arbuscular mycorrhizal fungi spores and arbuscular mycorrhizal fungi measured through root colonization.

Materials and Methods

Soil samples were taken from 46 plots (400 m^2) in continuous cropping mostly for most of at least 20 years (cultivated plots, n=46) in the southeastern Buenos Aires province (Figure 1) (García et al. 2020b). Similarly, for each cultivated plot, a nearby uncultivated plot (<500 m away) with the same soil type was selected and sampled. These plots (n=34) were undisturbed for at least 20 years and present similar edaphic characteristics as the pristine soils of the region. The uncultivated plots were in landscaped areas around the houses or undisturbed spots among the cropping lots, mainly under grasses vegetation (e.g., *Lolium perenne* L., *Bromus unioloides* Kunth, *Festuca arundinacea* Schreb., *Dactylis glomerata* L. and *Phalaris tuberosa* L.). Sampled soils were Mollisols with different surface textural classes: loam, sandy-loam, sandy-clay-loam and clay-loam (Soil Survey Staff 2014); they did not show signs of erosion or flooding. The climate in the surveyed region is warm-temperate with no dry season and a warm summer according to the Köppen-Geiger classification (Kottek et al. 2006).

Soil sampling was carried out at field capacity with a 4.4 cm diameter tubular sampler and with a shovel between fall and winter of 2016 (i.e., 34 cultivated and 29 uncultivated plots) and 2018 (i.e., 12 cultivated and five uncultivated plots). A composite sample was taken from each plot at 0-5 (15 subsamples per sample) and 5-20 (5 subsamples per sample)

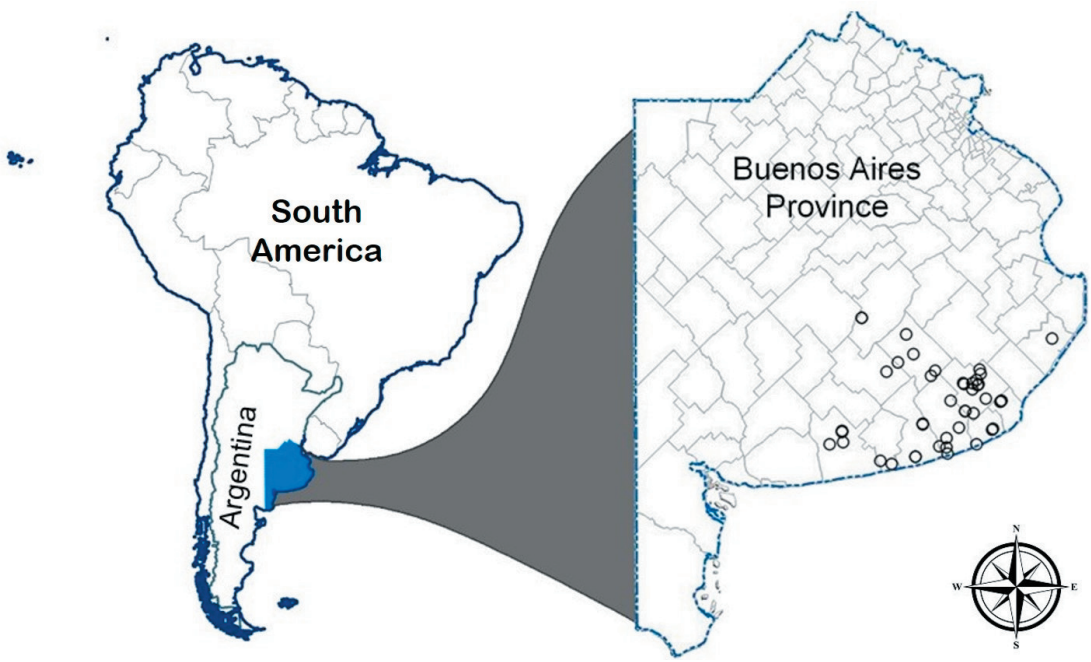

Figure 1. Sampled cultivated soil plots from the southeastern Buenos Aires province (n=46).
Figura 1. Parcelas de suelo cultivado muestreadas en el sudeste de la provincia de Buenos Aires (n=46).

cm depths with each tool (i.e., shovel and tubular sampler). The samples taken with the sampler were dried at 50 °C until constant weight and then ground to pass a 2000 μm sieve. These samples were used to determine particulate organic carbon, anaerobically mineralized nitrogen and arbuscular mycorrhizal fungi spore number (only in the 2018 samples). An aliquot of soil was re-ground to pass a 500 μm sieve to determine soil organic carbon and easily extractable glomalin-related soil proteins. The samples taken with the shovel were carefully handled to pass through an 8000 μm sieve, dried at 50 °C until constant weight and used to determine the stability of the aggregate. In addition, in spring of 2018, 11 cultivated plots that had been cropped with wheat (*Triticum aestivum* L.) were sampled at 0-20 cm depth with a soil core sampler to collect wheat roots when the plants were in the anthesis stage (Zadoks et al. 1974). Roots were separated from the soil, washed with water, and used to determine the mycorrhizal colonization degree.

The determinations and data of AN, soil organic carbon, particulate organic carbon and aggregate stability were previously informed by García et al. (2020b). Briefly, AN was determined through short anaerobic incubation for 7 days at 40 °C and steam distillation (Keeney 1982). Soil organic carbon was determined by wet combustion, maintaining the reaction temperature at 120 °C for 90 minutes (Nelson and Sommers 1982). The particulate and mineral-associated fractions were separated through fractionation by particle size according to Cambardella and Elliott (1992). In the <53 μm fraction, mineral-associated organic carbon was determined as previously described for soil organic carbon and particulate organic carbon was calculated as the difference between soil organic carbon and mineral-associated organic carbon (Cambardella and Elliott 1992). The aggregate stability was determined through the remnant 2000-8000 μm aggregates dry mass after sudden immersion in water for 5 minutes and sieving for 2 minutes (i.e., the mass of large macroaggregates) (García et al. 2020a).

The extraction of easily extractable glomalin-related soil proteins was carried out by autoclaving suspensions of soil and sodium citrate solution for 30 minutes at 121 °C and 0.1 MPa (Wright and Upadhyaya 1996). The concentration of easily extractable glomalin-related soil proteins in the extracts was quantified by spectrophotometry using the Bradford method (Bradford Reagent, Sigma-Aldrich, USA) (Sigma-Aldrich 2019). The extraction of arbuscular mycorrhizal fungi spores was carried out by wet-sieving (75 μm sieve) and centrifugation in sucrose (Covacevich and Consolo 2014). To determine the degree of root colonization with arbuscular mycorrhizal fungi, washed roots were immersed in a solution of potassium hydroxide (30 minutes at 90 °C) and stained with a trypan blue and lactoglycerol solution (15 minutes at 90 °C). Hyphae, vesicles and arbuscules were identified under a microscope. The degree of root colonization with arbuscular mycorrhizal fungi was expressed as the percentage of total infection (counting the number of hyphae, vesicles and arbuscules) and percentage of arbuscules (counting only arbuscules) (Brundrett 2008).

Quantified variables at depths of 0-5 and 5-20 cm were also expressed at the 0-20 cm layer as the weighted average by layer thickness. Descriptive statistics —such as minimum, maximum and mean values— were calculated. Variance analysis was performed to compare soil uses (cultivated and uncultivated soils) considering the effect of the site. Likewise, t-tests for paired samples were performed to compare 0-5 vs. 5-20 cm for each soil use. Pearson correlation coefficients were calculated to evaluate the relationships between variables. As the relationships between the number of arbuscular mycorrhizal fungi spores with other variables did not show linearity or constant variance, arbuscular mycorrhizal fungi spores number was transformed into decimal logarithm (log-spores). Statistical analyses were performed using R software (R Core Team 2018). A significance level of 0.05 was used.

Results

The magnitude of anaerobically mineralized nitrogen, soil organic carbon, particulate organic carbon and the mass of large macroaggregates was greater in uncultivated soils than in cultivated ones (Figure 2). Likewise, mean values of easily extractable glomalin-related soil proteins and log-spores were greater in uncultivated than in cultivated at each depth (Figure 2). The mean values of anaerobically mineralized nitrogen, soil organic carbon, particulate organic carbon and log spores were higher at 0-5 than at 5-20 cm in both evaluated soil uses. However, mean values of the mass of large macroaggregates and easily extractable glomalin-related soil proteins at 0-5 cm were greater than those at 5-20 cm, but only in uncultivated soils (Figure 2).

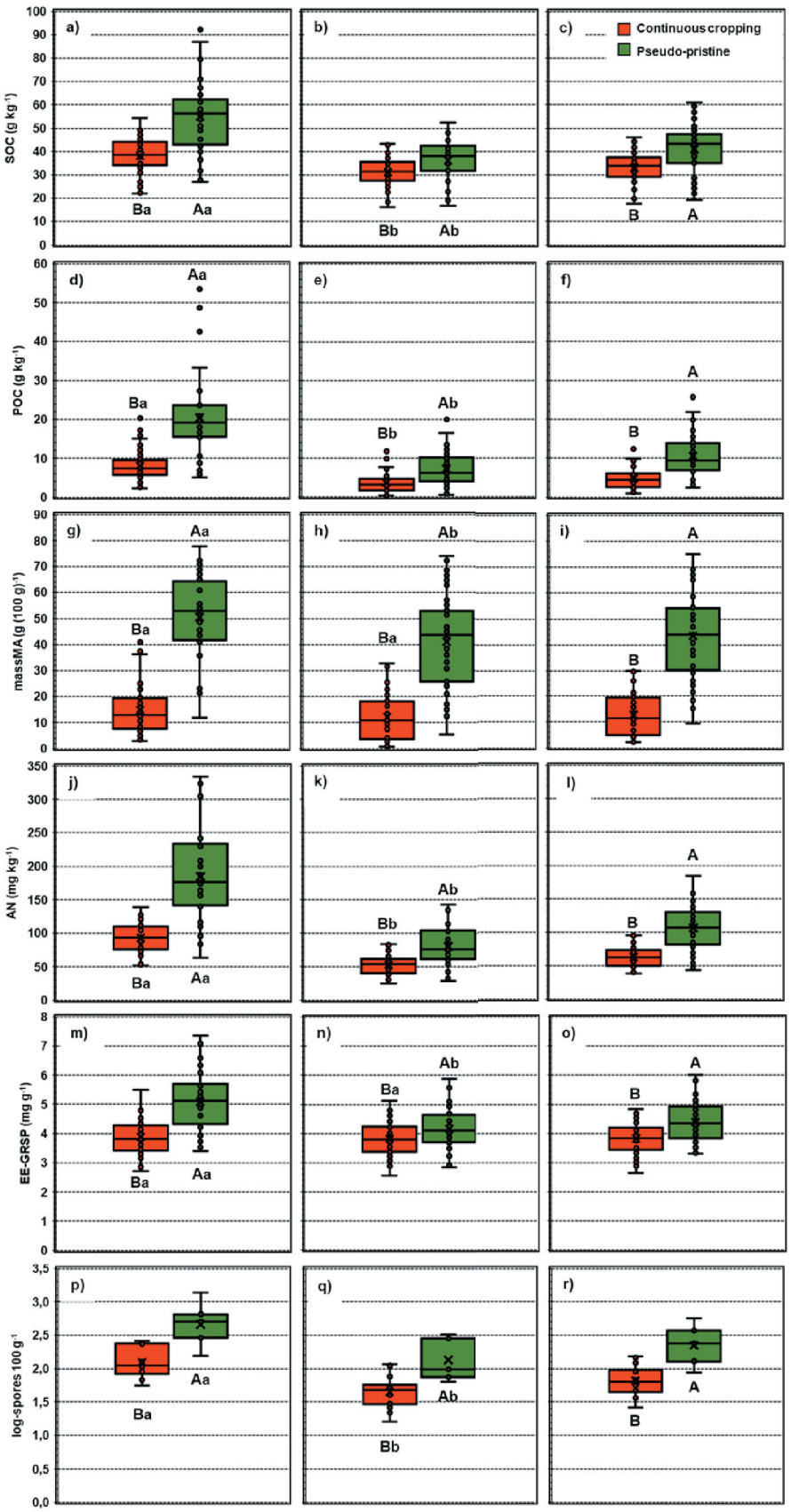
a)
b)
c)
Continuous cropping
Pseudo-pristine
SOC (g kg-1)
Ba
Aa
Bb
Ab
B
A
d)
e)
f)
POC (g kg-1)
g)
h)
i)
massMA (g (100 g)-1)
j)
k)
l)
AN (mg kg-1)
m)
n)
o)
EE-GRSP (mg g-1)
p)
q)
r)
log-spores 100 g-1

Figure 2. Box and whisker diagram at 0-5 (a, d, g, j, m, n), 5-20 (b, e, h, k, n, q) and 0-20 (c, f, i, l, o, r) cm depths for soil (SOC) and particulate (POC) organic carbon, mass of large macroaggregate (massMA), anaerobically mineralized nitrogen (AN), easily extractable glomalin-related soil proteins (EE-GRSP) and the logarithm of arbuscular mycorrhizal fungi (AMF) spore number (log-spores) for two soil uses: cultivated (n=46 for SOC, POC, massMA, AN, and EE-GRSP and n=12 for log-spores) and uncultivated soils (n=34 for SOC, POC, massMA, AN, and EE-GRSP and n=5 for log-spores). Means followed by equal uppercase letters indicate no significant differences (P>0.05) between cultivated and uncultivated soils for each depth. Means followed by equal lowercase letters indicate no significant differences (P>0.05) between 0-5 and 5-20 cm for each soil use. Data of AN, SOC, POC, and massMA were taken from García et al. (2020b).

Figura 2. Diagramas de caja y bigotes en 0-5 (a, d, g, j, m, n), 5-20 (b, e, h, k, n, q) y 0-20 (c, f, i, l, o, r) cm de profundidad para carbono orgánico del suelo (SOC) y particulado (POC), masa de macroagregados grandes (massMA), nitrógeno mineralizado en anaerobiosis (AN), proteínas del suelo fácilmente extraíbles relacionadas con la glomalina (EE-GRSP) y logaritmo del número de esporas de hongos micorrícicos arbusculares (log-spores) para dos usos del suelo: suelos cultivados (n=46 para SOC, POC, massMA, AN y EE-GRSP y n=12 para log-spores) y no cultivados (n=34 para SOC, POC, massMA, AN y EE-GRSP y n=5 para log-spores). Los promedios seguidos por letras mayúsculas iguales indican diferencias no significativas (P>0.05) entre suelos cultivados y no cultivados para cada profundidad. Los promedios seguidos por letras minúsculas iguales indican diferencias no significativas (P>0.05) entre 0-5 y 5-20 cm para cada uso del suelo. Los datos de AN, SOC, POC y massMA fueron tomados de García et al. (2020b).

Table 1. Correlations matrix with Pearson correlation coefficients between pairs of variables at three depths: 0-5, 5-20, and 0-20 cm. AN: anaerobically mineralized nitrogen. SOC: soil organic carbon. POC: particulate organic carbon. massMA: large macroaggregates mass. EE-GRSP: easily extractable glomalin-related soil proteins. log-spores: logarithm of arbuscular mycorrhizal fungi spore number. *Correlations taken from García et al. (2020b). Cells are painted with different green color intensities according to the magnitude of the Pearson correlation coefficient, red cells indicate that variables are not correlated, and empty cells (-) indicate absence of data. Numbers in parentheses indicate quantity observations for each correlation.

Tabla 1. Matriz de correlaciones con coeficientes de correlación de Pearson entre pares de variables en tres profundidades: 0-5, 5-20 y 0-20 cm. AN: nitrógeno mineralizado en anaerobiosis. SOC: carbono orgánico del suelo. POC: carbono orgánico particulado. massMA: masa de macroagregados grandes. EE-GRSP: proteínas del suelo relacionadas con glomalina fácilmente extraíbles. log-spores: logaritmo del número de esporas de hongos formadores de micorrizas arbusculares. *Correlaciones tomadas de García et al. (2020b). Las celdas están pintadas con diferente intensidad de color verde de acuerdo con la magnitud del coeficiente de correlación de Pearson; las celdas rojizas indican que las variables no están correlacionadas y las celdas vacías (-) indican ausencia de información. Los números entre paréntesis indican la cantidad de observaciones involucradas en cada correlación.

	AN	SOC	POC	massMA	EE-GRSP	log-spores	% total infection	% arbuscules
0-5 cm								
AN	1	0.86* (80)	0.86*	0.83* (80)	0.65 (80)	0.78 (17)	-	-
SOC	< 0.01* (80)	1	0.92	0.75* (80)	0.77 (80)	0.79 (17)	-	-
POC	< 0.01* (80)	< 0.01 (80)	1	0.77* (80)	0.70 (80)	0.83 (17)	-	-
massMA	< 0.01* (80)	< 0.01* (80)	< 0.01* (80)	1	0.62 (80)	0.78 (17)	-	-
EE-GRSP	< 0.01 (80)	< 0.01 (80)	< 0.01 (80)	< 0.01 (80)	1	0.86 (17)	-	-
log-spores	< 0.01 (17)	< 0.01 (17)	< 0.01 (17)	< 0.01 (17)	< 0.01 (17)	1	-	-
% total infection	-	-	-	-	-	-	-	-
% arbuscules	-	-	-	-	-	-	-	-
5-20 cm								
AN	1	0.68* (80)	0.57* (80)	0.74* (80)	0.34 (80)	0.66 (17)	-	-
SOC	< 0.01* (80)	1	0.68 (80)	0.58* (80)	0.46 (80)	0.52 (17)	-	-
POC	< 0.01* (80)	< 0.01 (80)	1	0.64* (80)	0.33 (80)	0.59 (17)	-	-
massMA	< 0.01* (80)	< 0.01* (80)	< 0.01* (80)	1	0.14 (80)	0.55 (17)	-	-
EE-GRSP	< 0.01 (80)	< 0.01 (80)	< 0.01 (80)	0.22 (80)	1	0.25 (17)	-	-
log-spores	< 0.01 (17)	0.03 (17)	0.01 (17)	0.02 (17)	0.32 (17)	1	-	-
% total infection	-	-	-	-	-	-	-	-
% arbuscules	-	-	-	-	-	-	-	-
0-20 cm								
AN	1	0.79* (80)	0.78* (80)	0.85* (80)	0.47 (80)	0.77 (17)	-0.06 (11)	-0.02 (11)
SOC	< 0.01* (80)	1	0.80 (80)	0.68* (80)	0.60 (80)	0.69 (17)	-0.25 (11)	-0.54 (11)
POC	< 0.01* (80)	< 0.01 (80)	1	0.75* (80)	0.48 (80)	0.76 (17)	-0.54 (11)	-0.62 (11)
massMA	< 0.01* (80)	< 0.01* (80)	< 0.01* (80)	1	0.35 (80)	0.69 (17)	0.03 (11)	0.51 (11)
EE-GRSP	< 0.01 (80)	< 0.01 (80)	< 0.01 (80)	< 0.01 (80)	1	0.59 (17)	-0.21 (11)	-0.50 (11)
log-spores	< 0.01 (17)	< 0.01 (17)	< 0.01 (17)	< 0.01 (17)	0.01 (17)	1	-0.13 (11)	-0.14 (11)
% total infection	0.85 (11)	0.45 (11)	0.08 (11)	0.93 (11)	0.53 (11)	0.70 (11)	1	0.54 (11)
% arbuscules	0.96 (11)	0.09 (11)	0.40 (11)	0.11 (11)	0.12 (11)	0.68 (11)	0.08 (11)	1

The arbuscular mycorrhizal fungi structures (i.e., arbuscules, hyphae and vesicles) were present in all wheat root samples. Maximum, minimum and mean values were 78.2, 65.1 and 71.5, respectively, for the percentage of total infection and 65.7, 44.1 and 53.1, respectively, for the percentage of arbuscules.

Anaerobically mineralized nitrogen was positively correlated to the easily extractable glomalin-related soil proteins content at all depths (Table 1), and this correlation was greater at 0-5 cm depth than at 5-20 and 0-20 cm depths (Table 1). The AN was positive and closely correlated with log-spores at all three depths (Table 1). Anaerobically mineralized nitrogen was not related to the activity of arbuscular mycorrhizal fungi, measured by the percentage of total infection or arbuscules (Table 1).

Soil and particulate organic carbon were positively correlated with easily extractable glomalin-related soil proteins at all depths (Table 1). However, the correlation coefficients were higher at 0-5 cm than at 5-20 and 0-20 cm. The soil and particulate organic carbon were positively correlated to log-spores at all depths (Table 1), but not with the percentages of total infection and arbuscules (Table 1).

The mass of large macroaggregates was correlated with the content of easily extractable glomalin-related soil proteins at 0-5 and 0-20 cm, but not at 5-20 cm depths (Table 1). However, the relationship between the mass of large macroaggregates and easily extractable glomalin-related soil proteins at 0-20 cm was weak. The mass of large macroaggregates was positively and closely correlated with the logarithmic spores at all three depths (Table 1). The linear association was greater at 0-5 cm. Large macroaggregates was not correlated to the percentages of total infection and arbuscules (Table 1).

The easily extractable glomalin-related soil proteins content was positively correlated to log-spores at 0-5 and 0-20 cm depths. However, both variables were not linearly associated at 5-20 cm depth (Table 1). The log-spores was not correlated with the percentage of total infection or arbuscules (data not shown). The easily extractable glomalin-related soil proteins content was not related to the percentage of total infection or the percentage of arbuscules (Table 1).

Discussion

As expected, the effect of soil use over easily extractable glomalin-related soil proteins and log-spores was similar to that observed for AN, soil organic carbon, particulate organic carbon and the mass of large macroaggregates (Figure 2). Therefore, as previously reported by other authors, easily extractable glomalin-related soil proteins and log-spores were sensitive to changes in soil use. Both easily extractable glomalin-related soil proteins (Wright et al. 1996; Singh et al. 2016; Liu et al. 2020) and arbuscular mycorrhizal fungi spore number (Bedini et al. 2007; Fokom et al. 2012; Thougnon-Islas et al. 2014, 2016; Pagano et al. 2020) were reported to be higher under undisturbed conditions than in cultivated soils. Furthermore, both variables were described as sensitive to different management practices in different soils (Lozano-Sánchez et al. 2015; Liu et al. 2020). The uncultivated soils showed high organic carbon content (Figure 2) due to the great carbon inputs by aboveground and root biomass and reduced soil disturbance. The continuous growth and recycling of the grass-dense root systems are associated with greater microbial biomass and organic labile carbon fractions. The change in soil use from an uncultivated to a cultivated conditions generally produces a decrease in organic carbon due to the negative balance between carbon inputs and outputs, which is expressed mainly expressed in labile fractions as particulate organic carbon and anaerobically mineralized nitrogen (García et al. 2020b). Likewise, when soil use changes from uncultivated to cultivated, soil loses aggregate stability mainly due to the physical disturbance, the decrease of root activity and persistence and the loss of organic carbon, among others (García et al. 2020b). A greater easily extractable glomalin-related soil proteins content and log-spores in uncultivated than cultivated soils could be due to, firstly, the higher density and persistence of roots in uncultivated soils that lead to greater mycorrhizal colonization. Secondly, cultivated soils are associated with lower glomalin and arbuscular mycorrhizal fungi spore number because of 1) a greater decomposition of easily extractable glomalin-related soil proteins caused by aggregate rupture and subsequent exposition to microorganisms; 2) the disruption of the hyphal network, and 3) a less habitable environment for arbuscular mycorrhizal fungi due to compaction and/or the use of fertilizers

and pesticides (Singh et al. 2016). The fact that both easily extractable glomalin-related soil proteins and log-spores presented the same pattern as AN responding to change in soil use suggests that anaerobically mineralized nitrogen could be related to those variables and be used as an indicator of abundance and activity of arbuscular mycorrhizal fungi.

Easily extractable glomalin-related soil proteins were stratified throughout the soil profile, showing higher values at 0-5 than at 5-20 cm depth (Figure 2), although stratification was significant only in uncultivated soils (Figure 2). Likewise, the log-spores was stratified in both uncultivated and cultivated soils (Figure 2). Thus, the mean values of easily extractable glomalin-related soil proteins and log-spores showed the same behavior as anaerobically mineralized nitrogen, soil organic carbon and particulate organic carbon regarding stratification (Figure 2) (García et al. 2020b). Given that the uncultivated plots had not been disturbed for at least 20 years and cultivated plots had been under no-tillage for at least 10 years, the stratification can be attributed to a greater input of carbon on the surface, greater root growth and microbial biomass, and not to soil disturbance (Franzluebbers and Stuedemann 2009). The values of the percentage of total infection or arbuscules obtained in our study for roots grown in the field are greater than those reported by Covacevich et al. (2012) and Thougnon-Islas et al. (2014, 2016) for roots grown under controlled conditions in similar continuously cropped soils. Our results indicate that the evaluated situations presented a high arbuscular mycorrhizal fungi activity.

Relationships between anaerobically mineralized nitrogen and easily extractable glomalin-related soil proteins

Glomalin is a hydrophobic protein exuded by arbuscular mycorrhizal fungi, which acts as an indicator of the abundance and activity of arbuscular mycorrhizal fungi (Bedini et al. 2007; Chenu and Cosentino 2007). This protein contributes to soil organic matter (Nichols and Wright 2004) and nitrogen content (Rillig et al. 2001; Hurisso et al. 2018), and to aggregate formation and stabilization (Chenu and Cosentino 2007). In our study, AMN was positively correlated to easily extractable glomalin-related soil proteins at all depths (Table 1), indicating that AN could be an indicator of easily extractable glomalin-related soil proteins content and its contribution to soil health. Part of the nitrogen from easily extractable glomalin-related soil proteins could be mineralized during the anaerobic incubation performed to determine anaerobically mineralized nitrogen (Hurisso et al. 2018), explaining, in part, the relationship between anaerobically mineralized nitrogen and easily extractable glomalin-related soil proteins. Moreover, the relationship between both soil properties is expected considering that both anaerobically mineralized nitrogen and easily extractable glomalin-related soil proteins were, in turn, related to soil organic carbon, particulate organic carbon and the mass of large macroaggregate (Table 1). Relationships between easily extractable glomalin-related soil proteins and soil organic carbon (Singh et al. 2016; Thougnon-Islas et al. 2016; Fine et al. 2017; Nautiyal et al. 2019) and between easily extractable glomalin-related soil proteins and aggregate stability (Bedini et al. 2009; Fokom et al. 2012; Fine et al. 2017; Liu et al. 2020) have been reported for different soils. However, in this work, easily extractable glomalin-related soil proteins content was correlated to the mass of large macroaggregates at 0-5 and 0-20 cm depths, but not at 5-20 cm depth (Table 1). Likewise, the relationship between easily extractable glomalin-related soil proteins and the mass of large macroaggregates at 0-20 cm was weak, influenced by the absence of linear association at 5-20 cm (Table 1). It has been informed that the easily extractable glomalin-related soil proteins fraction does not contain only glomalin (Rossier et al. 2006; Hurisso et al. 2018). The method used in our study to quantify easily extractable glomalin-related soil proteins also measures other proteins that are not produced by arbuscular mycorrhizal fungi, are not necessarily hydrophobic and/or adhesive (Rossier et al. 2006; Hurisso et al. 2018), and if so, could not contribute to aggregate formation and stabilization. These results indicate that easily extractable glomalin-related soil proteins content alone does not always explain aggregate stability.

Relationships between anaerobically mineralized nitrogen and arbuscular mycorrhizal fungi spores

A linear association between anaerobically mineralized nitrogen and the log-spores was observed at all three depths (Table 1). These relationships suggest that AN could be used as an indicator of arbuscular mycorrhizal fungi abundance. The arbuscular mycorrhizal fungi

spores (>75 μm) are part of the particulate organic matter (>53 μm) (Nichols and Wright 2004). Likewise, arbuscular mycorrhizal fungi spores produce glomalin (Bedini et al. 2007) and, given that they are the most persistent arbuscular mycorrhizal fungi structures, they would have an average lifetime similar to glomalin (Wright and Upadhyaya 1998; Jamiołkowska et al. 2017). The greater the spore number, the greater the arbuscular mycorrhizal fungi abundance, the greater the content of glomalin, hyphae and carbon that would contribute to a greater aggregate stability (Miller and Jastrow 2000). Consequently, log-spores were closely related to soil organic carbon, particulate organic carbon, easily extractable glomalin-related soil proteins and the mass of large macroaggregates (Table 1), which are soil properties that were also associated with anaerobically mineralized nitrogen (Table 1) (Domínguez et al. 2016; García et al. 2020b, 2021; Rivero et al. 2020).

Relationships between anaerobically mineralized nitrogen and root colonization of arbuscular mycorrhizal fungi

Contrary to our expectations, anaerobically mineralized nitrogen was not related to the arbuscular mycorrhizal fungi activity measured through the percentage of total infection or arbuscules. Root colonization with arbuscular mycorrhizal fungi reflects the activity of arbuscular mycorrhizal fungi in response to the environmental conditions that regulate the colonization with arbuscular mycorrhizal fungi propagules at a specific time (Jamiołkowska et al. 2017). However, easily extractable glomalin-related soil proteins and log-spores, which are variables that were associated with AN (Table 1), manifest changes in the abundance and activity of arbuscular mycorrhizal fungi caused by soil use and/or management in the mid- to long term. Along this line, the percentage of total infection or arbuscules were not related to easily extractable glomalin-related soil proteins and log-spores (Table 1). The easily extractable glomalin-related soil proteins (Wright and Upadhyaya 1996, 1998) are very stable hydrophobic proteins. Exposure to optimal conditions for decomposition showed that glomalin remains constant in time and has a long lifetime (from 6 to 92 years) under different conditions, which is an indication of a low recycling rate (Rillig et al. 2001). Therefore, easily extractable glomalin-related soil proteins are not as sensitive as arbuscular mycorrhizal fungi's activity to short-term soil environmental changes. Similarly, the lack of relationship between log-spores and the percentage of total infection or arbuscules could be attributed to reduced germination of spores of arbuscular mycorrhizal fungi due to dormancy or unfavorable environmental conditions (Jamiołkowska et al. 2017) and/or the colonization produced by other propagules of arbuscular mycorrhizal fungi different from spores (Thougnon-Islas et al. 2014). Arbuscular mycorrhizal fungi propagules are not only spores but also hyphal fragments and infected roots that represent soil arbuscular mycorrhizal fungi potential inoculum (Bethlenfalvay and Linderman 1992; Covacevich et al. 2006). It is important to mention that the narrow range of edaphic, climatic and management situations evaluated in our study could not have allowed a clear characterization of the relationships between the percentage of total infection or arbuscules and other variables.

Conclusion

The anaerobically mineralized nitrogen is an easy-, safe- and cheap-to-measure soil parameter that allows monitoring soil biochemical and physical soil health in the short to mid-term because of its relationships with soil organic carbon, particulate organic carbon and aggregate stability, among other soil properties. Besides, AN is routinely used by farmers and consultants from the studied area to diagnose soil fertility. In this study, we demonstrated that anaerobically mineralized nitrogen was also positively related to easily extractable glomalin-related soil proteins content which is a proposed indicator of arbuscular mycorrhizal fungi activity and abundance. Likewise, AN was positively related to log-spores which is an indicator of arbuscular mycorrhizal fungi abundance. However, anaerobically mineralized nitrogen was not related to root colonization, which describes the arbuscular mycorrhizal fungi activity at a given moment.

Thus, anaerobically mineralized nitrogen could be used as an indicator of mid- to long-term changes in the arbuscular mycorrhizal fungi abundance and activity caused by soil use and/or management. Our results show that anaerobically mineralized nitrogen can be used as a soil health indicator that also helps to monitor an aspect of soil microbiological health associated with arbuscular mycorrhizal fungi that is not usually monitored. However, it is necessary to evaluate the relationships

studied in this work for a broader range of soil situations (i.e., more edapho-climatic and management conditions) and the number of observations. This would allow performing statistical analyses with an integrative approach to the soil ecosystem using structural equation models to deepen the knowledge of the causes of the correlations between anaerobically mineralized nitrogen and arbuscular mycorrhizal fungi activity and abundance.

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