

## Crop intensification influences water infiltration and microbial activity in agricultural soils from the southeast of the Argentinean Pampas

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**ABSTRACT.** Low crop diversification in highly productive areas has led to declines in total organic carbon (TOC) in soil, essential nutrients for plant's growth and microbial diversity/activity. This could have an impact on the movement of water in the soil profile and, consequently, on the production of crops. To address these challenges there is growing support for crops intensification, which involves increasing the number/variety of crops throughout the year. The purpose of this study was to assess crop influence of the intensification on the initial infiltration of water in the upper layer of the soil profile and the activity/abundance of soil microorganisms involved in the turnover of TOC and phosphorus (P). Three crop regimes were assessed in a long-term experiment established in the southeast of the Argentinean Pampas: without intensification (Monocrop: soybean), intensified (Cover crop: CC [oat]/soybean) and Rotation (CC [oat]/soybean-corn-wheat). Soil in the Monocrop regime exhibited the highest sorptivity values and a lower TOC, suggesting a higher initial rate of water entry into the profile, which could break down soil aggregates. Under rotation, the highest infiltration rate was recorded, which would guarantee more water flow into the profile. Intensified soils showed the highest total glomalin content and root colonization with arbuscular mycorrhizal fungi (AMF), which are known to contribute to plant nutrient uptake and growth and soil aggregate stability. *Trichoderma* abundance and their P-solubilizing capacity were also higher under Rotation, which could favor AMF activity. Correlation analysis revealed a significant positive correlation between sorptivity and glomalin under Rotation. Our study suggests that soils from the Argentinean south-eastern Humid Pampas under crop intensification promote soil water storage and maintenance of soil structure in the upper layers compared to Monocrop, which could be attributed—at least in part—to a greater microbiological activity and TOC content.

[Keywords: arbuscular mycorrhizal fungi, monocrop, cover crop, rotation, glomalin, water inflow]

**RESUMEN.** La intensificación de cultivos influye en la infiltración de agua y la actividad microbiana en suelos agrícolas del sudeste de la Pampa argentina. La escasa diversificación de cultivos en zonas muy productivas ha disminuido la calidad del suelo, impactando en el movimiento de agua del perfil de suelo y, consecuentemente, en la producción de cultivos. Por ello, la intensificación de cultivos (aumento del número/variedad de cultivos a lo largo del año) es una estrategia a considerar. Nuestro objetivo fue evaluar si la intensificación de cultivos impacta en la infiltración inicial del agua en las capas superficiales del perfil de suelo y en la actividad/abundancia de microorganismos edáficos que participan en el recambio de carbono orgánico total (TOC) y de fósforo (P). En un experimento de larga duración establecido en el sudeste de la Pampa argentina se evaluaron tres regímenes: sin intensificación (monocultivo de soja), intensificados (cultivo de cobertura: CC [avena]/soja) y rotación (CC [avena]/soja-maíz-trigo). El suelo bajo monocultivo exhibió mayor sortividad y menor TOC que los suelos bajo CC y rotación, sugiriendo una mayor tasa inicial de entrada de agua en el perfil y una potencial ruptura de los agregados. El suelo bajo rotación presentó una tasa de infiltración más alta, lo que garantizaría un mayor ingreso de agua en el perfil. Los suelos bajo intensificación mostraron mayor contenido de glomalina total y colonización de raíces por hongos micorrízicos arbusculares (AMF), que favorecen la nutrición y el crecimiento vegetal y la estabilidad de agregados del suelo. La abundancia de *Trichoderma* y su capacidad solubilizadora de P fueron mayores en rotación, lo que habría favorecido la actividad de los AMF. Suelos bajo rotación presentaron una correlación positiva entre sortividad y glomalina. Nuestros resultados sugieren que la intensificación de cultivos favorecería el almacenaje de agua en el suelo, manteniendo la estructura del horizonte superficial, en comparación con suelos bajo monocultivo, probablemente por una mayor actividad microbiológica y contenido de TOC.

[Palabras clave: hongos micorrízicos arbusculares, monocultivo, cultivo de cobertura, rotación, glomalina, flujo de agua]

## INTRODUCTION

In recent decades, Pampean agricultural systems have transitioned to a more simplified structure, with a pronounced focus on oil seed production in response to increasing pressures and demands of the market (Andrade et al. 2017; Wilson et al. 2020). This has threatened agricultural suitability, due, among others, to the high export of essential nutrients such as phosphorus (P) and the low incorporation of organic matter in the soil profile (Viglizzo et al. 2011; Barbieri et al. 2014). As a result, losses in edaphic biodiversity (Wall et al. 2019) and changes in soil structure would result in decreased water infiltration and storage capacity within the soil profile (Tourn et al. 2019; Behrends Kraemer et al. 2021). Crop intensification, which involves a greater variety and quantity of crops per unit of time and area (Caviglia and Andrade 2010), is a prevailing approach aimed at achieving sustainable agricultural management while increasing crop yields. Furthermore, incorporation of diverse crop rotations and cover crops before the main crop has been associated with improvements in soil hydrological characteristics (Rabot et al. 2018), potentially enhancing soil structural stability (Hosseini et al. 2021).

Water infiltration, storage and distribution in the soil profile are directly influenced by the characteristics of the upper soil layer (Soracco et al. 2019). Some research indicates that the initial rate of water inflow into the soil profile (sorptivity; herein after: Sp) (Karahan 2022) declines as the total organic carbon (TOC) content of the upper horizon increases (Vogelmann et al. 2017), while the infiltration rate increases due to increased aggregate stability and improved soil porosity (Blanco-Canqui and Benjamin 2013). In other words, lower Sp is associated with a higher organic content (Ruis et al. 2020), which could benefit water infiltration. In this scenario, agricultural systems with prevailing dominance of diverse and active roots, would change the soil water dynamics when compared to environments with less diverse root systems (Carminati et al. 2017). Moreover, this could be supported by the presence of elevated microbial activity, as laboratory studies have shown that it is correlated with reductions in Sp (Hallett and Young 1999). However, to our knowledge, field studies have not yet been able to demonstrate that.

Several edaphic root-associated microorganisms that are crucial for carbon

cycling, plant uptake of essential nutrients, soil stability, among others (Fierer 2017), are currently known as plant growth-promoting microorganisms (PGPM) (Tosi et al. 2020). For example, forms of soil P not available for plant uptake can be solubilized by some *Trichoderma* fungi and native bacteria and transformed into plant-available chemical forms (Bononi et al. 2020). Additionally, the external hyphae of arbuscular mycorrhizal fungi (AMF), which extends into the soil from the root cortical cells of colonized/host plants, facilitates the transport and uptake of the available P, other nutrients and water (Kobae 2019). Therefore, it is suggested that crop management strategies that increase the microbial P solubilization capacity of soils could also favor the development of AMF. This, in addition to improving crop growth, could improve the soil structure and consequently water flow in the profile. This is due, at least in part, to the fact that the external hyphae of AMF have also been reported to enable soil aggregation by entangling soil particles. Additionally, the AMF, along with other root-related microorganisms (i.e., bacteria) segregate extracellular polymeric substances (Morris et al. 2019; Holátko et al. 2021) that can contribute to maintaining soil aggregates. One example of these substances is glomalin, a glycoprotein whose structure is still not well understood. It has been proposed to be referred to as 'glomalin-related soil protein' (GRSP) because its extraction procedures can detect both the glycoprotein itself and a range of organic compounds, including those of various microorganisms (Rillig 2004). GRSP functions as a cementing and hydrophobic agent that binds soil particles and organic compounds in organo-mineral complexes (Hosseini 2021; Holátko et al. 2021). Although GRSP showed sensitivity as an indicator of increased crop intensification in the Argentine Pampas (Commatteo et al. 2023), its association with the dynamics of water flow in the soil profile is not known.

Studies in arid areas in central Argentina (Ontivero et al. 2023) revealed that changes in land use affect AMF diversity. In addition, studies at southeast of Pampas found that crop intensification may modify the diversity of soil total fungi and bacteria, and probably on *Trichoderma* and native AMF as well (Commatteo et al. 2019; Fernández-Gnecco et al. 2021). However, it is uncertain how these microbial communities interact with edaphic characteristics that might interfere with soil water dynamics in the profile, preventing soil

disaggregation. The objectives of this work were 1) to study Sp and overall infiltrations rates in soils under different regimes of crop intensification (monocrop, cover crop and crop rotations); 2) to analyze changes, if any, in microbial abundance/activity and TOC under different regimes of crop intensification, and 3) to evaluate the relationship between hydrological, microbial and chemical soil parameters under different regimes of crop intensification. We hypothesized that crops intensification by inclusion of cover crops and/or crop rotation as an alternative to monocrop influences the initial water infiltration in the soil profile due to changes on soil microbiota and TOC.

## MATERIALS AND METHODS

### *Experimental field and design*

The study was conducted on a long-term experiment located at the Agricultural Experimental Station of the National Institute of Agricultural Technology in Balcarce (E.E.A. INTA Balcarce), Buenos Aires province, Argentina (37°45' S - 58°18' W; 916 mm mean annual precipitation; 13.8 °C mean temperature; 138 m above the sea level). It was initiated in 2006 to evaluate different soybean cropping regimes under no-tillage. The soil is a Typical Argiudol (Soil Survey Staff 2014) with less than 2% slope and loamy textural class (23% clay, 36% silt and 41% sand) in the arable layer (0-20 cm). Soil chemical parameters during the study period were previously reported in Fernández-Gnecco et al. (2021).

The experimental design of the trial was a randomized complete block design with three replications. For this study, three cropping regimes (treatments) were evaluated: a) Monocrop (soybean, *Glycine max* L. Merr); b) Cover crop (*Avena bizantina* being the winter cover crop prior to soybean), and c) Rotation (rotation with wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and soybean with winter cover crop prior to soybean). In this study, regimes Rotation and Cover crop are considered intensified cropping systems. All cropping regimes are under no-tillage and without irrigation. The distribution of blocks and experimental plots were described in Commatteo et al. (2023).

Fertilization with P (triple superphosphate) and sulfur (calcium sulfate, 20% sulfur-16% calcium) was done on a supply/demand basis

for all crops except cover crop. Nitrogen (urea) was applied to corn and wheat as detailed by Martínez et al. (2020). Soybean seeds were inoculated pre-sowing with *Bradyrhizobium japonicum* E109 ( $2 \times 10^9$  bacteria kg/seed, inoculant Rizoliq®-Rizobacter). The herbicide, glyphosate, was used to control weeds and to stop the cover crop development in the respective cropping regimes, and these applications were consistent throughout the experiment. Post-harvest residues and/or terminated cover crop, as appropriate, remained on the soil surface after each harvest.

### *Water inflow into the soil*

The Sp (mm/ $\sqrt{\text{minute}}$ ) was estimated once during the fallow period (winter 2021) together with the hydraulic conductivity (K; mm/ $\sqrt{\text{minute}}$ ), based on the model of cumulative infiltration (F) (mm) as a function of time (t) (min) proposed by Philips (1957) (Equation 1).

$$F = Sp * \sqrt{t} + K * t \quad \text{Equation 1}$$

Field infiltration tests were conducted using the simple ring method with a high density of sampling points (arranged per plot in a grid and georeferenced, n=21). Briefly, a ring (20 cm diameter, 15 cm height) was introduced in the first 5 cm of the soil profile, over which a Mariotte bottle (filled with water) was inverted, and readings of the infiltrated water sheet were taken every 5 minutes for hour 1 (during the first 5 minutes, the readings were taken every minute for higher precision) from its outer face. Soil moisture was determined for all soil samples.

To address the spatial heterogeneity of the infiltration test, the scaling method was employed (Sharma et al. 1980; Chari et al. 2020). This approach involved analyzing each infiltration test based on the concept of similar means within a heterogeneous environment. The outcome of this analysis resulted in the extraction of scale factors, which allowed for the generation of new parameters values for the Philips model. As a result, a new scaled infiltration rate ( $F_{Ref}$ ) was obtained for each one of the tested points.

### *Soil and root samples*

Soil samples were collected after the infiltration data analysis, during the fallow period (winter 2021), at 0-15 cm depth. To

account for the spatial heterogeneity of the physical-hydrological variables (Soracco et al. 2019), 2 samples per plot were taken. The sampling locations within each plot were the highest and the lowest Sp georeferenced points. Each soil sample consisted of 5 cores (5 cm diameter, 15 cm depth) that were subsequently mixed to ensure homogeneity. Soybean roots were collected in summer, stage R1 (Fehr and Caviness 1977), and collection was performed as described for soil sampling in terms of subsamples and depth.

#### *Glomalin and total organic carbon*

Soil samples collected during fallow were also used for quantification of total glomalin-related soil proteins (T-GRSP). Samples from fallow were chosen for T-GRSP quantification, as reported by Commatteo et al. (2023) for this field experiment, to be the most sensitive season for detecting changes in crop intensification. Further, T-GRSP was extracted and quantified according to the methodology described by Wright et al. (2006), with modifications. Briefly, 0.4 mL of sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ) 100 mM (pH 9.0) was added to 0.5 g of previously dried soil. The suspension was homogenized, autoclaved (121 °C, 1 atm, 1 hour) and centrifuged (3000 rpm, 15 minutes). The concentration of T-GRSP was estimated spectrophotometrically (595 nm on Thermo Scientific TM Multiskan Sky) in microplates (96 wells - Deltalab) using Bradford's Reagent (Sigma-Aldrich®, Saint Louis, United States of America) as a standard. Absorbance readings were taken at each extraction cycle and stopped when the absorbance reached values between 0.04 and 0.09 (Commatteo et al. 2023).

TOC was determined in soil samples collected during fallow, according to the methodology of Nelson and Sommers (1982). Briefly, TOC quantification was performed from 0.25 g of soil, previously dried and grinded, by wet combustion with  $\text{K}_2\text{Cr}_2\text{O}_7$  (2 N) and  $\text{H}_2\text{SO}_4$ . The reaction temperature was held constant at 120 °C.

#### *Length of AMF external mycelia*

AMF root-external hyphae length (HL-AMF) was estimated in soil samples collected in winter fallow, according to the methodology of Hart and Reader (2002) with some modifications. Briefly, 10 g of soil were dispersed with sodium hexametaphosphate (3.6% w/v, 16 hours), centrifuged and

recovered on 37  $\mu\text{m}$  sieves. The collected material was stained with trypan blue in lactoglycerol, rinsed with deionized water and filtered on 25 mm diameter cross-linked nitrocellulose filters (Gamafil; Beccar, Buenos Aires, Argentina) of 0.22  $\mu\text{m}$  which were after placed on slides. Intersections of hyphae and filters lines were recorded, and the HL-AMF was calculated according to Newman (1966) considering the amount of soil used and the dilutions made.

#### *Mycorrhizal root colonization*

Soybean roots collected at stage R1 of crops were washed and stained according to the Phillips and Hayman (1970) methodology, but omitting phenol at the staining solution which consisted in lactic acid-glycerol (BioPack; Zárate, Argentina)-distilled water (1:1:1 in vol.), with trypan blue as staining reagent (0.05%; Biopack). Root colonization by AMF was quantified as frequency of colonization (CF-AMF) and frequency of arbuscules (AF-AMF) by microscopic observation (40X) (Mc Gonigle et al. 1990).

#### *Culturable microorganisms*

Colony forming units (CFU) were quantified in soil samples collected in fallow, through serial decimal dilutions in triplicate from 10 g of soil and 90 mL of sterile 0.3% NaCl. The culture solid media used were PDA (potato dextrose agar), NBRIIP (National Botanical Research Institute Phosphate Growth Medium) (Nautiyal 1999) and TSM (*Trichoderma* Selective Medium) (Elad and Chet 1983), without antibiotics, for the quantification of total mesophilic bacteria (TMB), P-solubilizing bacteria (PSB) and *Trichoderma*, respectively. CFU were quantified after growth for 48 hours at 37 °C, 5 days at 28 °C or 14 days at 28 °C, respectively, for each targeted microbial group.

#### *Phosphorus solubilizing capacity*

To evaluate the solubilization capacity of *Trichoderma*, 15 strains per crop regime (5 strains isolated per block, of each of the 3 analyzed cropping regimes) were randomly isolated from the  $10^{-2}$  dilution of *Trichoderma* CFU count plates with PDA medium. Thus, 45 strains were individually transferred to PDA medium and grown for 14 days at 28 °C. Subsequently, P solubilization capacity (PSC) in each medium was quantified for these strains according to Scervino et al. (2011).



Briefly, 1 mL of aqueous suspension with each of the *Trichoderma* strains ( $10^6$  conidia/mL) was inoculated into liquid NBRIP culture medium with  $\text{Ca}_3(\text{PO}_4)_2$  (5 g/L) as source of inorganic P. Cultures were maintained at 20 °C, in darkness and the supernatant (1 mL) was collected every 24 h for 7 days. Solubilized P was quantified per triplicate by the ascorbic acid method (Bray and Kurtz 1945). At the end of the experiment, the resulting mycelial dry weight (MDW) was harvested and quantified for each strain. The MDW and solubilized P were used to calculate the solubilization/mineralization capacity defined as the amount of P released per unit MDW (Della Monica et al. 2018).

#### Statistical analysis

For each variable, a linear model was fitted according to the effects of the blocks and the cropping regimes (treatments). Due to the asymmetry in the residuals in the Sp model, outliers were eliminated and the variable was transformed through the Box and Cox method and the transformation parameter was  $\lambda=0.3$  (Box and Cox 1964). Sp and parameters linked to AMF activity (T-GRSP and HL-AMF) were tested for the effect of crop regime by analysis of variance (ANOVA). When the crop regime was significant ( $\alpha=0.05$ ), parameter means for each crop regime were compared by Least Significant Difference (LSD) test ( $\alpha=0.05$ ). ANOVA and mean comparison test were omitted for PSC due to non-replicable isolated strains, making a statistical analysis of crop regimes differences unfeasible. To evaluate the relationships between the measured variables, a Pearson correlation analysis, as well as a hierarchical Cluster, were performed for each crop regime. Statistical analyses were conducted with the R-Studio 4.1.1 program (rstudio.com).

**Table 1.** Sorptivity (Sp), total organic carbon (TOC), total glomalin related soil proteins (T-GRSP) and external hyphae length of arbuscular mycorrhizal fungi (HL-AMF) in root associated soils from a long-term experiment conducted at Balcarce, Argentina, under different cropping regimes (Monocrop, Covercrop and Rotation). For each crop regime, the values correspond to the mean  $\pm$  standard error. For each parameter, means followed by similar letters indicate no significant difference between crop regime (LSD test,  $P<0.05$ ).

**Tabla 1.** Sorptividad (Sp), carbono orgánico total (TOC), proteínas del suelo relacionadas con glomalina total (T-GRSP) y longitud de hifas externas de hongos micorrízicos arbusculares (HL-AMF) en suelos asociados a raíces de un experimento de larga duración realizado en Balcarce, Argentina, bajo diferentes regímenes de cultivo (Monocultivo, Cultivo de cobertura y Rotación). Para cada régimen de cultivo, los valores corresponden a la media  $\pm$  error estándar. Para cada parámetro, las medias seguidas de letras similares indican que no hay diferencias significativas entre regímenes de cultivo (prueba LSD,  $P<0.05$ ).

Crop regime	Sp (cm/ $\sqrt{\text{minute}}$ )	TOC (g/kg soil)	T-GRSP (mg/g soil)	HL-AMF (m/g soil)
Monocrop	1.14 $\pm$ 0.1a	28.58 $\pm$ 0.8b	8.90 $\pm$ 0.4b	14.93 $\pm$ 2.3a
Cover crop	0.70 $\pm$ 0.1b	31.10 $\pm$ 0.9a	10.20 $\pm$ 0.2ab	9.78 $\pm$ 2.2a
Rotation	0.72 $\pm$ 0.2b	33.05 $\pm$ 1.6a	10.90 $\pm$ 0.2a	11.05 $\pm$ 1.3a

## RESULTS

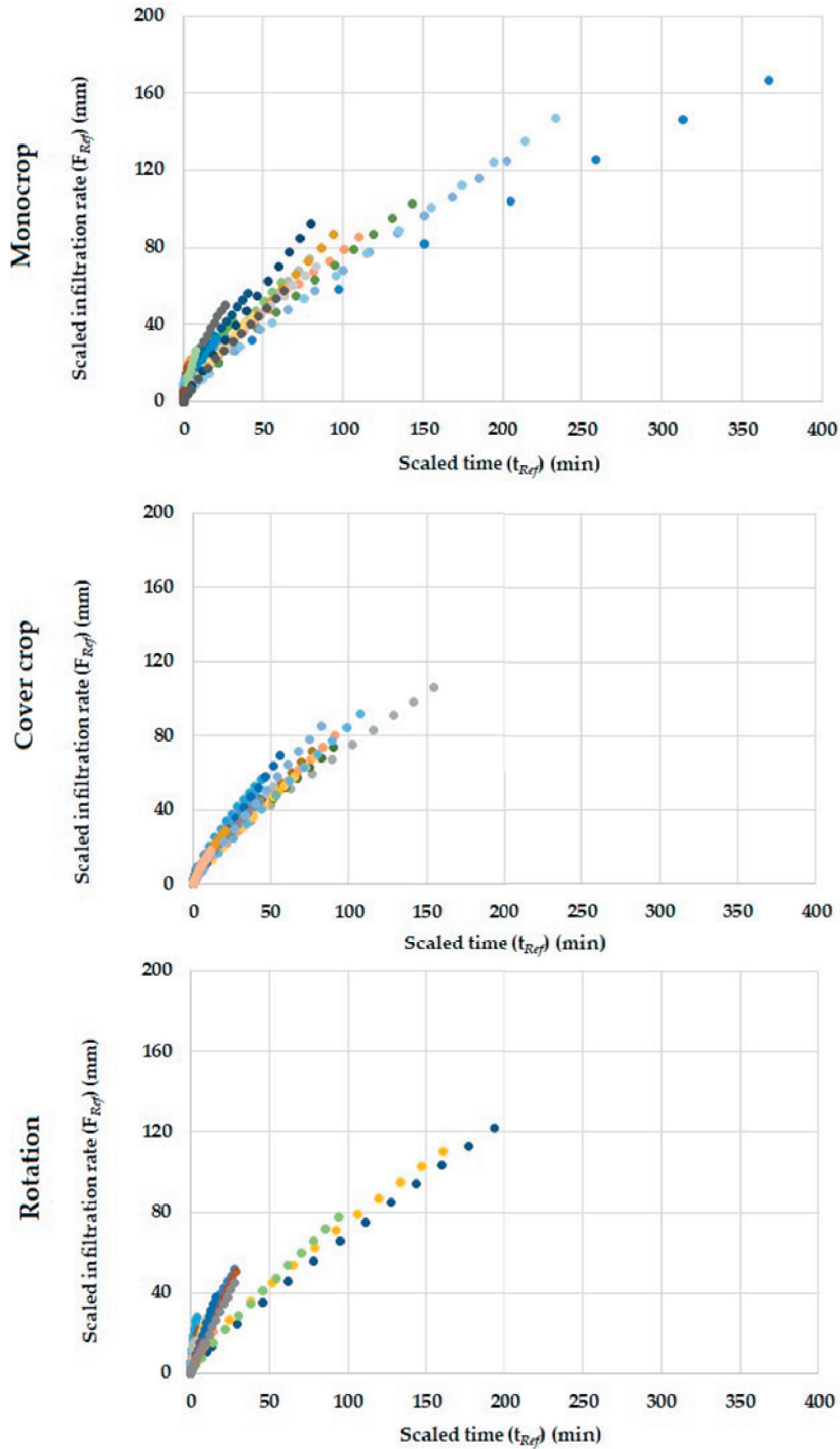
### Water inflow in soil profile

The scaling of infiltration rates showed a differential behavior between soil under the Monocrop regime compared to Cover crop and Rotation (Figure 1). Among the different cropping regimes, Monocrop exhibited the lowest infiltration rate. On average, Monocrop showed the  $F_{\text{Ref}}$  at 55 mm and after 50 minutes of scaled time. In contrast, the  $F_{\text{Ref}}$  values observed in Cover crop and Rotation were higher in less time with averages of 50 mm at 40 minutes and 40 mm in the first 25 minutes, respectively. Rotation demonstrated an overall higher infiltration rate in a less average scaled time, while Cover crop and Monocrop displayed greater variability, with infiltration tests taking longer scaled time to complete the infiltration tests.

The crop regime significantly influenced the rate of water entering the soil profile. Monocrop showed the highest Sp values and the lowest in Cover crop and Rotation (Table 1, Supplementary Material-Table S1).

### Organic carbon and Mycorrhizal activity

The content of TOC and T-GRSP were significantly affected by the crop regime ( $P<0.05$ ) (Table 1, Supplementary Material-Table S1). In soils under Rotation and Cover crop regimes, the TOC content was significantly higher ( $P<0.01$ ) when compared with Monocrop. Contrarily, Cover crop and Rotation soils exhibited the highest values of T-GRSP, although only the content recorded in Rotation was significantly different to Monocrop (Table 1). The length of external mycelia of AMF (HL-AMF) in soils did not significantly differ ( $P>0.05$ ) among



**Figure 1.** Scaled infiltration rate ( $F_{Ref}$ ) over scaled time ( $t_{Ref}$ ) in a long-term experiment conducted at Balcarce, Argentina, under different cropping regimes (Monocrop, Cover crop, Rotation).

**Figura 1.** Tasa de infiltración escalada ( $F_{Ref}$ ) a través del tiempo escalado ( $t_{Ref}$ ) en ensayo de larga duración en Balcarce, Argentina, bajo diferentes regímenes de cultivo (Monocultivo, Cultivo de Cobertura, Rotación).

three cropping regimes studied (Table 1, Supplementary Material-Table S1).

#### *Mycorrhizal colonization*

Typical structures such as external, inter- and intra-cellular hyphae, arbuscules and vesicles were detected in soybean roots colonized by AMF in all the cropping regimes evaluated (Figure 2). Both CF-AMF and AF-AMF exhibited significant differences between cropping regimes ( $P < 0.001$ ) with both parameters being higher in soybean roots from Rotation compared to Cover crop and Monocrop, which showed the lowest levels (Figure 3a).

#### *Abundance of culturable microorganisms*

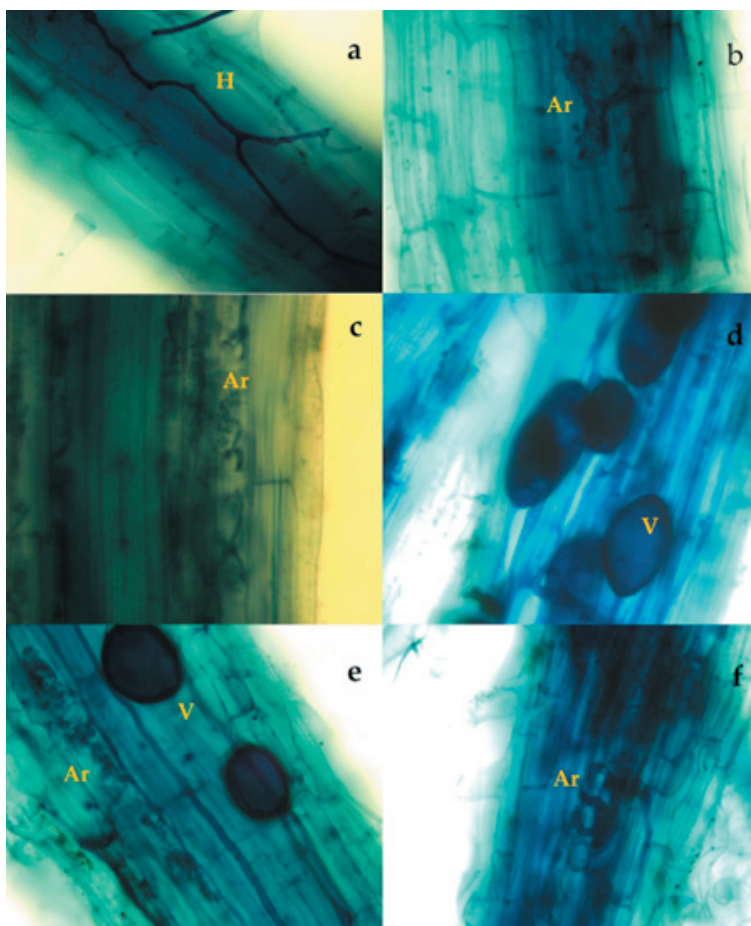
The abundance of total and P solubilizing bacteria (TMB and PSB, respectively) and *Trichoderma* soil fungi were significantly affected by the crop regime ( $P < 0.001$ ). Both PSB and *Trichoderma* abundance increased about 10-16% and 25%, respectively, in soils under Cover crop and Rotation as compared

to Monocrop. However, the abundance of TMB was significantly higher in Monocrop (about 4-5%) than other cropping regimes (Figure 3b).

#### *Phosphorus solubilizing capacity of Trichoderma*

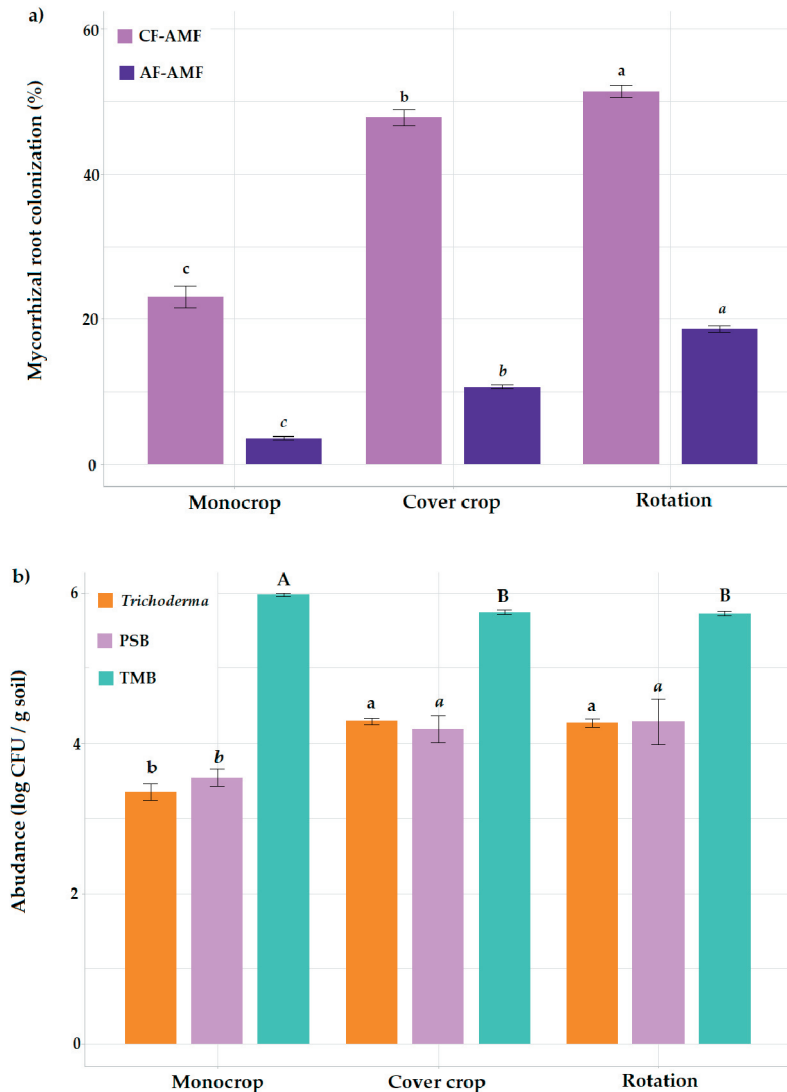
From the 45 *Trichoderma* strains evaluated in vitro, 32 solubilized P in liquid medium. The number of strains that solubilized P isolated from the intensified cropping regimes (Cover crop and Rotation) doubled those from Monocrop (Figure 4). Furthermore, the Rotation exhibited a greater number of strains with PSC compared to Cover crop. Strains with highest PSC from Cover crop and Rotation exceeded the PSC of the best strain from Monocrop by 13% and 57%, respectively.

Increases of ~50% of solubilized P were detected when the *Trichoderma* strain with the highest PSC, native to the system under Rotation (R.4B), was grown in vitro for 70 hours, compared to the strain with the highest PSC from Monoculture (M.17A) (Figure 4).



**Figure 2.** Images of soybean roots under a binocular microscope (50x) colonized by arbuscular mycorrhizal fungi (AMF) in root samples from a long-term experiment conducted at Balcarce, Argentina, under different cropping regimes (Monocrop, Cover crop and Rotation). a) external hypha (H) on root without internal colonization in root of Monocrop. b) Arbuscule (Ar) in root of Monocrop. c) *Paris* type arbuscules (Ar) in root of Cover crop. d) Vesicles (V) in root of Cover crop. e) *Paris* type arbuscules (Ar) and vesicles in root of Rotation. f) Arbuscules (Ar) *Arum* type arbuscules in root of Rotation.

**Figura 2.** Imágenes de raíces de soja bajo un microscopio binocular (50x) colonizadas por hongos micorrízicos arbusculares (AMF) en muestras de raíces de un experimento de larga duración realizado en Balcarce, Argentina, bajo diferentes regímenes de cultivo (Monocultivo, Cultivo de Cobertura y Rotación). a) Hifa externa (H) en raíz sin colonización interna en raíz de monocultivo. b) Arbúsculo (Ar) en raíz de monocultivo. c) Arbúsculos tipo *Paris* (Ar) en raíz de cultivo de cobertura. d) Vesículas (V) en raíz de cultivo de cobertura. e) Arbúsculos tipo *Paris* (Ar) y vesículas en raíz de rotación. f) Arbúsculos (Ar) tipo *Arum* en raíz de rotación.



**Figure 3.** a) Total colonization and arbuscules formed by arbuscular mycorrhizae fungi (CF-AMF and AF-AMF, respectively) in soybean roots. b) Colony forming units (CFU) of phosphorus solubilization bacteria (PSB), total mesophile bacteria (TMB) and *Trichoderma* fungi in soils associated to soybean roots grown at a long-term experiment conducted at Balcarce, Argentina, under different cropping regimes (Monocrop, Cover crop and Rotation). For each crop regime, values correspond to the mean  $\pm$  standard error. Similar letters indicate non-significant differences between cropping regimes (LSD test,  $P < 0.05$ ) ( $n=6$ ).

**Figura 3.** a) Colonización total y arbusculos (CF-AMF y FA-AMF, respectivamente) formados por hongos micorrícicos arbusculares en raíces de soja. b) Unidades formadoras de colonias (CFU) de bacterias solubilizadoras de fósforo (PSB), de bacterias mesófilas totales (TMB) y de hongos *Trichoderma* en suelos asociados a raíces de soja crecidas en un experimento a largo duración realizada en Balcarce, Argentina, bajo diferentes regímenes de cultivo (Monocultivo, Cultivo de cobertura y Rotación). Para cada régimen de cultivo, los valores corresponden a la media  $\pm$  error estándar. Letras similares indican diferencias no significativas entre regímenes de cultivo (prueba LSD,  $P < 0.05$ ) ( $n=6$ ).

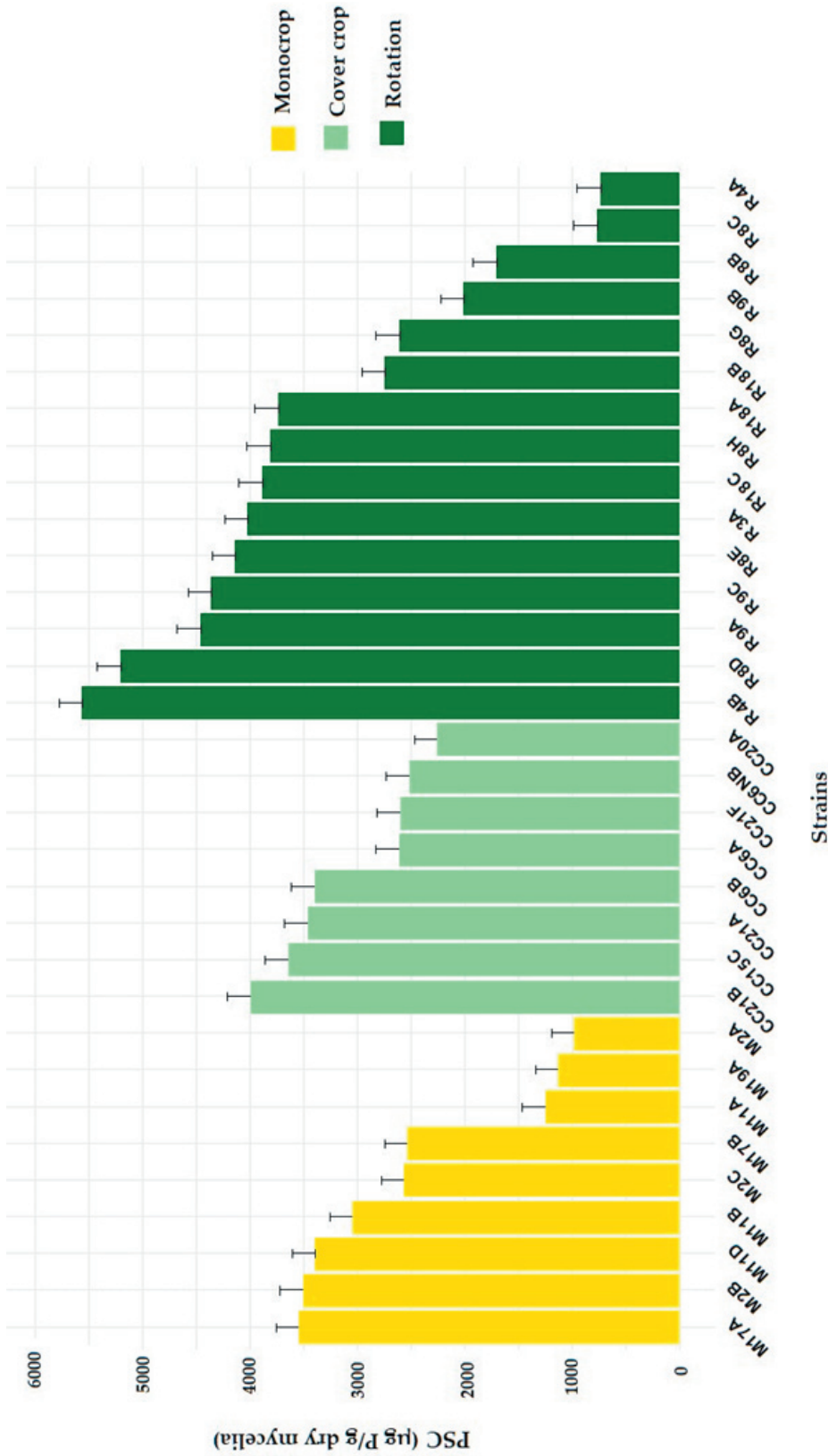
Although the strain with the highest PSC from Cover crop (CC.21B) also showed higher solubilization capacity compared to M-17A during the growth progress, the final PSC (at 70 hours of growth) was the same for both strains (Supplementary Material-Figure S1). Decreases of pH in the culture medium were, as expected, also detected during fungal

growth progress and as P concentration increased.

#### *Relationships between parameters*

Significant correlations were detected between microbiological and soil-measured parameters when data from all cropping





**Figure 4.** Soluble phosphorus (P) concentration and phosphorus solubilization capacity (PSC) of native P-solubilizing *Trichoderma* strains isolated from soil of a long-term experiment conducted at Balcarce, Argentina, under different cropping regimes (Monocrop [M], Cover crop [CC] and Rotation[R]). Values are mean of data  $\pm$  standard error (n=3, corresponding to three readings per strain).

**Figura 4.** Concentración de fósforo (P) soluble y capacidad de solubilización de fósforo (PSC) de cepas nativas de *Trichoderma* solubilizadoras de P aisladas del suelo de un experimento de larga duración instalado en Balcarce, Argentina, bajo diferentes regímenes de cultivo (monocultivo [M], cultivo de cobertura [CC] y rotación [R]). Los valores son las medias de los datos  $\pm$  error estándar (n=3, correspondiente a tres lecturas por cepa).

regimes were collectively analyzed (Figure 5a). The analysis revealed positive correlations between TOC and T-GRSP, as well as TOC and *Trichoderma* abundance. The T-GRSP showed a positive correlation with root mycorrhizal colonization. Although TOC exhibited positive correlations with root mycorrhizal colonization (both CF-AMF and AF-AMF), the relationship was negative with HL-AMF. The TMB were negatively correlated with soil bacteria and fungi abundance (PSB and *Trichoderma*, respectively) and also with root mycorrhizal colonization. Nevertheless, mycorrhizal colonization showed positive correlation with PSB. No significant correlations were found between Sp and the biological parameters.

The Pearson correlation analysis for each cropping regimes also revealed significant correlations among tested variables (Figure 5b). In Monocrop regime, positive correlations between *Trichoderma* and mycorrhizal colonization were detected. Furthermore, although the T-GRSP was positively associated with TOC, it was negatively associated with fungal abundance (AF-AMF and *Trichoderma* parameters). Within the Cover crop regime, only positive correlations were found between abundance/activity of microorganisms (*Trichoderma* with TMB and CF-AMF, PSB with AF-AMF and TMB with CF-AMF). In Rotation, positive correlations were found between Sp and T-GRSP and also between abundance/activity of microorganisms (*Trichoderma* with CF-AMF or TMB, TMB with CF-AMF and AF-AMF). However, a negative correlation between HL-AMF and T-GRSP was also identified.

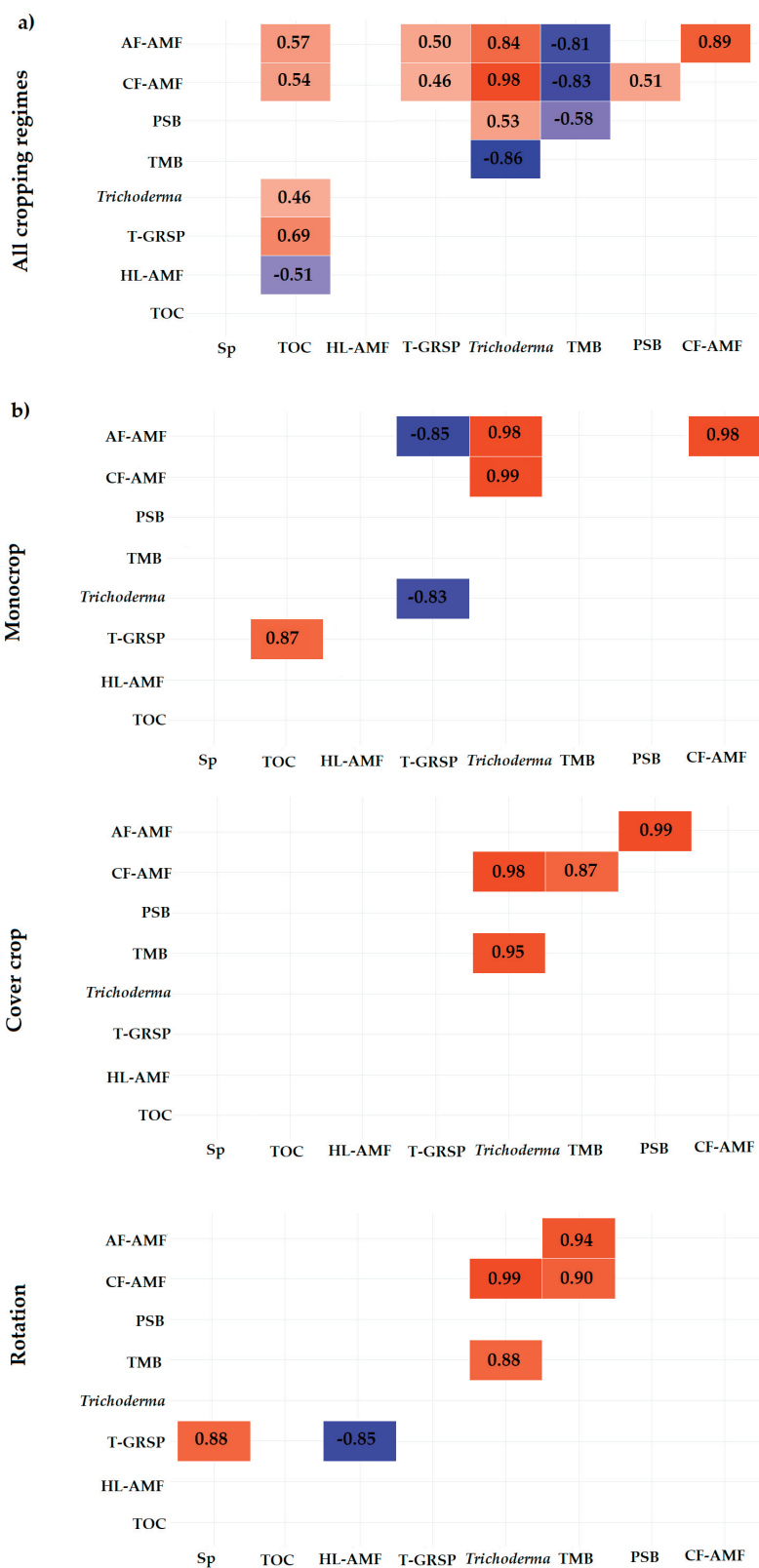
A hierarchical analysis using dendrograms revealed that Sp at Monocrop and Cover crop regimes was grouped separately from organic/biological variables, with shorter distances (dendrogram branches) in the Cover crop regime compared to Monocrop. In the Rotation regime, two primary clusters were found, showing shorter distances than Monocrop and Cover crop, indicating increased similarities between specific variables such as TOC, HL-AMF, Sp, T-GRSP, *Trichoderma* and TMB (Supplementary Material-Figure S2).

## DISCUSSION

The goal of this study was to understand how intensified crop regimes (Cover crop and Rotation) influence the water flow in the profile and its association with changes in

organic soil C content and abundance/activity of plant growth promoting microorganisms (with a focus on AMF, *Trichoderma* and PSB), compared to a nonintensified regime (Monocrop). Higher levels of organic C and increased nutrient availability have been linked to crop intensification. In this context, the presence of organic compounds in the upper horizon would influence the rate/speed at which water enters the soil profile (Sp; Vogelmann et al. 2013). Variations in Sp are driven by alterations in soil (Ma et al. 2022) strongly related with organic compounds and edaphic biota content, which collectively define the cohesive forces of organomineral complexes and soil structure (Ramesh et al. 2019). For soils from the Pampas region, strong correlations have been reported between Sp and aggregate stability and TOC, respectively (Villareal et al. 2022).

In our study, cropping regimes with higher crop intensification (e.g., Rotation and Cover crop) showed the lowest Sp values. This finding suggests reduced pressure on organomineral complexes, which would otherwise cause rapid escape of air from the aggregates, leading to soil disaggregation (Moragoda et al. 2022). Interestingly, despite the lower Sp values in the soils under Rotation, the infiltration rates ( $F_{Ref}$ ) were reached in less time than in Monocrop (Figure 1). This could be due to an improvement in soil structure within cropping regimes characterized by a greater presence of active roots and microorganisms, which is consistent with previous findings in the area (Martinez et al. 2020; Fernández-Gnecco et al. 2021). Furthermore, a longer time for the infiltration rates to complete, as observed in Monocrop, may lead to increased surface runoff (Bharati et al. 2002; De la Vega et al. 2005). One contributing factor to the improved infiltration is the formation of a protective coating of soil aggregates, for example, by TOC (Franzluebbers 2002). This coating would be partly responsible for the hydrophobicity that favors gradual water infiltration. Furthermore, the TOC occluded within these aggregates is protected from these mechanical degradation forces, which favors the structural stability of the topsoil. In the current study, soils under crop intensification regimes showed increases in TOC content, as well as in abundance and activity of microorganisms (CF-AMF, *Trichoderma* and PSB) (Table 1, Figure 3). Although the impact of fungi and bacteria on TOC dynamics of TOC has been documented, the role of AMF in its stabilization is a subject of ongoing discussion (Morris et al. 2019).



**Figure 5.** Pearson's correlation between soil sorptivity (Sp), total organic carbon (TOC), length of arbuscular mycorrhizae fungi external hyphae (LH-AMF), total soil proteins related to glomalin (T-GRSP), abundance of *Trichoderma* fungi, of total mesophilic bacteria (TMB) and of phosphorus solubilizing bacteria (PSB) in soil, and frequency of total colonization and arbuscules formed by arbuscular mycorrhizae fungi (CF-AMF and AF-AMF, respectively) in soybean roots grown at a long-term experiment conducted at Balcarce, Argentina. Correlations for all tested cropping systems (a) and for data collected (b) at Monocrop, Cover crop and Rotation cropping systems (Pearson correlation-t test, only significant correlations are shown,  $P < 0.05$ ) ( $n = 6$ ).

**Figura 5.** Análisis de correlación de Pearson entre la sortividad del suelo (Sp), el carbono orgánico total (COT), la longitud de las hifas externas de los hongos micorrícicos arbusculares (LH-AMF), las proteínas totales del suelo relacionadas con la glomalina (T-GRSP), la abundancia de hongos *Trichoderma*, de bacterias mesófilas totales (TMB) y bacterias solubilizadoras de fósforo (PSB) en los suelos y la frecuencia de colonización total y arbusculos (CF-AMF y AF-AMF, respectivamente) formados por hongos micorrícicos arbusculares en raíces de soja crecidas en un experimento a larga duración realizado en Balcarce, Argentina. Correlaciones para todos los regímenes de cultivo evaluados (a), y para los datos colectados en los regímenes (b) monocultivo, cultivo de cobertura y rotación (prueba de correlación-t de Pearson; solo se muestran correlaciones significativas,  $P < 0.05$ ) ( $n = 6$ ).

The results of this study would contribute to this regard with significant positive correlations between TOC and mycorrhizal root colonization and soil content (Figure 5a). In addition to playing an active role in protecting TOC in aggregates, AMF external hyphae (in collaboration with roots) promote the redistribution and deposition of TOC in a larger soil volume (Frey 2019), leading to its spatial heterogeneity and depth stratification. Furthermore, in our study, the association of T-GRSP —known for its role in carbon sequestration— and the microbiota was reflected in the correlations and clustering patterns found (Figure 5, Supplementary Material-Figure S2).

Both TOC and T-GRSP have been reported to actively participate in the formation, binding and coating of the organo-mineral complexes (i.e., Rillig 2004). This, in turn, could support the gradual entry of water into the soil profile. Our study, while in contrast to previous findings showing the lack of correlation between T-GRSP and water infiltration (Feeny et al. 2004), reveals a positive correlation in the Rotation regime between Sp and T-GRSP (Figure 5b). To our knowledge, such strong and direct relationships have not been reported in the area of study, suggesting its importance for further investigations. However, we have no tools to justify the negative relationship between HL-AMF and T-GRSP found in the Rotation regime, which should also be the subject of future studies.

Driver et al. (2005) suggested that about 80% of glomalin produced by AMF is contained in the external mycelium and spores, primarily entering the soil via hyphal release rather than secretion. However, Zhou et al. (2020) observed variations in external hyphal production among different species of fungal AM. Although our study did not reveal differences in external mycelium length between intensification of crops —possibly due to methodological limitations—, variations in the contents of T-GRSP may be due to differences in native AMF species within each crop regime. Preliminary studies by Commatteo et al. (2019) suggested changes in AMF diversity associated with crop intensification in the same area studied as our research. Future studies should explore the identity of AMF in each cropping regime and their relationship with the content of T-GRSP and external mycelium in the soil.

In addition to the importance of defining crop management strategies that enhance water flow in the soil profile, it is also important to

consider those that favor sufficient nutrient availability. In our study, the intensified regimes —and in particular, the regime under Rotation— showed the highest abundance of PSB and fungi with PSC, in comparison with Monocrop (Figure 5). This skill becomes particularly important when considering that most current agricultural practices lead to a reduction in available P levels in the soils of the Humid Pampas (Sainz Rozas et al. 2012). In this sense, the higher abundance of PSB, as well as of PSC of *Trichoderma* in regimes with crop intensification (detected particularly in Rotation and to a lesser extent in Cover crop), could have both ecological and productive implications. The higher activity and presence of AMF recorded in the regimes under crop intensification (Table 1, Figure 3a) could favor the transport of available P from the soil matrix near to the roots (Etesami et al. 2021), probably benefiting the uptake of this nutrient as previously stated. Thus, the positive and significant correlations found between *Trichoderma* abundance and CF-AMF in Rotation (Figure 5b) may lead to increased availability of P in the root-associated soil.

Sustainable agricultural practices ought to be included in the planning of an agricultural system whose aim is to preserve/improve soil quality. They should be based on the assessment of the productive system using soil quality indicators. This study's findings suggest that —in contrast to Monocrop— agricultural management practices that enhance the activity/abundance of root-associated microorganisms (Rotation and Cover crop) may have beneficial impacts on organic carbon levels, on the availability of essential nutrients as P and on the potential stability of the soil structure. Fostering a sustainable soil ecosystem could encourage not only crop yield, but soil water infiltration and upward storage capacity.

## CONCLUSIONS

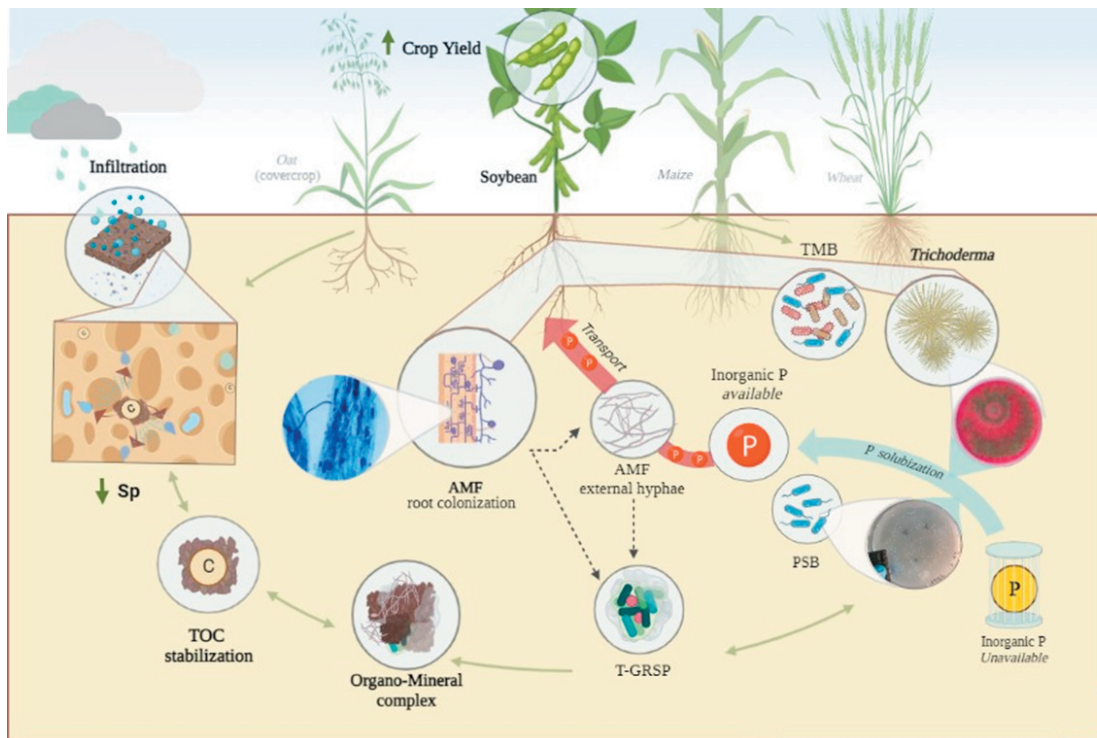
Findings of this study suggest a link between higher content of soil organic compounds in more intensified cropping regimes, resulting in a gradual initial water infiltration, mainly related with the content of total glomalin related soil proteins. Furthermore, crop intensification resulted in higher abundance/activity of microorganisms —mainly related to phosphorus solubilization—, which would favor arbuscular mycorrhizal activity (i.e., highest release of glomalin), aggregate stability and, consequently, water flow. In this sense,



cropping regimes such as Rotation revealed overall higher infiltration rates into the soil profile. However, given the heterogeneity observed within the studied systems, both at the plot level and between plots, future microcosm studies could be a useful tool for a clearer identification of the effects of the variables studied.

In conclusion, this study advances our understanding of potential interactions between microbial abundance and activity, and water dynamics in the upper soil horizon (Figure 6). The proposed model illustrates the parameters sensitivity of the assessed as soil indicators under different cropping systems.

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**Figure 6.** Proposed model of the possible contributions of beneficial microorganisms (AMF, *Trichoderma*, PSB and TMB), their activity (LH-AMF, T-GRSP, P solubilization) and organic compounds (TOC) modulating the initial stages of water infiltration (Sp) and fertility in the root-associated soil of the upper-horizon. Brightly colored plant: representation of the soybean crop. Plants in color with transparency: representation of crops that can be incorporated in crop intensification systems. AMF: Arbuscular mycorrhizal fungi. PSB: Phosphorus solubilizing bacteria. TMB: Total mesophilic bacteria. LH-AMF: AMF external hyphal length. T-GRSP: Total glomalin-related soil proteins. TOC: Organic carbon total. Sp: Sorptivity. Scheme created with BioRender.com. Photos: F. Covacevich and G. A. Fernández-Gnecco.

**Figura 6.** Modelo propuesto de las posibles contribuciones de microorganismos benéficos (AMF, *Trichoderma*, PSB y TMB), su actividad (LH-AMF, T-GRSP, T-GRSP, solubilización de P) y compuestos orgánicos (TOC) modulando las etapas iniciales de infiltración de agua (Sp) y fertilidad del suelo del horizonte superior asociado a las raíces. Planta en colores vivos: representación del cultivo de soja. Plantas en color con transparencia: representación de cultivos que pueden ser incorporados en sistemas de intensificación de cultivos. AMF: Hongos micorrícicos arbusculares. PSB: Bacterias solubilizadoras de fósforo. TMB: Bacterias mesófilas totales. LH-AMF: Longitud de hifas externas de AMF. T-GRSP: Proteínas del suelo totales relacionadas con la glomalina. TOC: Carbono orgánico total. Sp: Sortividad. Esquema creado con BioRender.com. Fotos: F. Covacevich y G. A. Fernández-Gnecco.

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