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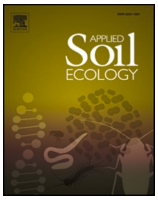

Short-term service crops affect the spatial organization of soil aggregates, microbial C—N biomass, and microbial activities in a degraded monoculture system

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ABSTRACT

Service crops are grown to provide ecosystem services, such as the ability to increase soil organic matter and fertility. Also, they reduce erosion processes, weed control, disease regulation, water purification, soil biodiversity, and physical restoration. The physical arrangement of elemental particles in soil aggregates controls many ecosystem functions such as soil stability and carbon sequestration. This study aimed to analyze the short-term effect of including different service crops on the soil aggregate dynamics in a degraded common bean monoculture system and how it influences the rhizospheric microbial activity, carbon, and nitrogen microbial biomass. Here, we measured soil water-stable aggregates**,** particulate and associated organic carbon, soil microbial biomass, microbial activity, service crop aerial biomass, and cash crop yield in bulk soils during the 2020/2021 and 2021/2022 agricultural cycles. Soil samples from depths of 0–10 cm from five management treatments (annual service crop/common bean) were analyzed under no-tillage: 1) Oat (O) = *Avena sativa*/common bean; 2) Wheat (W) = *Triticale*/common bean; 3) Vetch (V) = *Vicia villosa*/common bean; 4) Melilotus (Me) = *Melilotus alba*/common bean; 5) common bean monoculture (M) = common bean without service crop. Additionally, two controls were analyzed: 6) *Brachiaria* perennial (BP) = *Brachiaria brizantha* perennial; 7) Native vegetation (NV). Service crops significantly increased aggregate stability, mean weight diameter, particulate matter and associated organic carbon, promoting the formation of large macroaggregates (0.25–2 mm and > 2 mm). This led to an increase in carbon stocks. Microbial activity expressed as hydrolysis of fluorescein diacetate and acid phosphatase activity, increased in the largest fraction for all service crops. **Vicia** improved surface residues; on average all service crops **increased the** common bean yield by 107.25 %**.** In summary, Vicia represents the best alternative as a service crop to improve the quality and health of degraded monoculture soils.

1. Introduction

Soil organic carbon (SOC) supports essential ecosystem services and affects soil structure, aggregation, microbial processes, and their activities which improves soil fertility (Plaza et al., 2022). The protection of SOC is fundamental in fragile agroecosystems that were subjected to intensive production. Organic matter is widely recognized as a key factor in enhancing soil aggregation. Soil aggregates physically protect SOC against mineralization and oxidative processes limiting the access of decomposers, enzymes, and oxygen diffusion (Dash et al., 2019). Soil organic carbon (SOC) exhibits heterogeneous distribution across primary particles of varying sizes, giving rise to the formation of organo-mineral complexes that could potentially exhibit diverse responses to alterations in soil disturbance and management practices. The association of soil particles, their spatial arrangement and the abundance of organic matter partially decomposed, play a key role in the soil microbial dynamics because the micro-niches that are formed offer protection for microorganisms and promote microbial activity and microbial

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processes as a whole (Lagomarsino et al., 2009).

Soil degradation leads to changes in the basic physical and chemical properties of soils, moderates and decreases SOC, nitrogen (N), phosphorus (P) concentrations, and microbial activity (Bashagaluke et al., 2018). Soil degradation is an important agricultural and environmental problem in the north west region of Argentina (NWA). In this region, the high temperatures accelerate the mineralization processes of organic matter and negatively influence the soil fertility levels (Abán et al., 2021a; Pérez-Brandán et al., 2014). In addition, bean crops were traditionally managed as a monoculture with intensive tillage (especially plowing and tillage) for many decades (Pérez-Brandan et al., 2019). This has contributed to the progression of degradative processes and subsequently resulted in negative impacts on nutrient cycling, carbon sequestration, biodiversity, biotic homogenization (Abán et al., 2021a), organic matter, soil enzyme activities and microbial communities (Pérez-Brandan et al., 2017).

Soil management influences soil fertility, microbial processes, soil aggregates formation and stability through their impacts on destructive forces and aggregates formation processes (Six et al., 2004). Aggregate stability and aggregation formation are also influenced by microbiological activity and the types of microorganisms involved. The soil configuration affects numerous processes that are closely associated with soil function, as well as the diversity and activity of soil microorganisms(Havlicek and Mitchell, 2014). These microorganisms live in the ecological niches under the control of soil aggregates, which change significantly in size and shape, leading to a heterogeneous distribution of soil microorganisms in different-sized aggregates (Wilpiszeski et al., 2019). Therefore, a good distribution and spatial arrangement of macro and micro aggregates is important to sustain soil fertility improvements and agricultural productivity (Bronick and Lal, 2005). These improvements can be evaluated by diverse aggregation indices such as the mean weight diameter (MWD), the geometric mean diameter (GMD), and the distribution of particle size class. These indices vary according to the management practices established in soil showing if physical conditions are being improved or not (Mikha et al., 2024). Among the most employed management practices, the use of service crops is considered one of the most successful ones, promoting soil conservation and recovery, as well as protecting soil fertility by returning a high amount of carbon from crop residues to the soil (Büchi et al., 2018; Derpsch et al., 2010). These crops take the place of bare fallow periods in crop rotations and conclude prior to the planting of the next main crop (Abán et al., 2021b) leaving residues in the field. Service crops can also affect biological nitrogen fixation, biomass decomposition, nutrient cycling, formation and maintenance of soil aggregates and aggregate stability, thereby increasing carbon stocks (Lupwayi et al., 2017). Modifications in the soil after including service crops may result in significant shifts in soil function, fertility, physical restoration, and crop growth losses (Garbeva et al., 2004). There have been previous investigations on the effect of the use of service crops on soil aggregation, SOC and the implications for microbial activity in other regions of Argentina (Beltrán et al., 2018; Castiglioni and Kraemer, 2019; Fontana et al., 2024). However, little is known about the fate of macro aggregates, meso aggregates and micro aggregates when service crops are included in NWA, as well as their relative importance in terms of SOC stocks, microbial biomass, and microbial activities rates.

Several studies have confirmed that soil structure significantly impacts microbial communities and their biomass by providing habitats that influence substrate availability (Garcia-Franco et al., 2015; Gupta and Germida, 2015). Soil microbial biomass carbon (MBC) and microbial biomass N (MBN) are vital to regulate nutrient cycling and energy flow, although soil microorganisms comprise only a small proportion (1 %–5 %) of the total soil organic matter (Wilpiszeski et al., 2019). Aggregate size tends to positively correlate with the growth of microbial biomass (Helgason et al., 2010) mainly due to the heightened safeguarding of labile SOC substrates, as suggested by Chen et al. (2015). Also, soil enzymes play a substantial role in organic matter mineralization, transforming and modeling the aggregates in soils through a wide range of metabolic processes. Hence, it is plausible that specific microenvironments within and between aggregates play a role in shaping the composition and behavior of microbial communities by influencing the availability of organic matter and prevailing environmental conditions. However, the understanding of the relationships between microbial driven processes and their microbial community activities at the aggregate level remains incomplete.

Thus, the aims of this study were to 1) analyze the short-term effects of the inclusion of different service crops on the dynamics of soil macro, *meso* and micro aggregates in a degraded common bean monoculture system, 2) determine how the dynamics of macro, meso and micro aggregates influence the rhizospheric microbial biomass and activity in a degraded common bean monoculture system under different service crops, 3) assess the relationships between soil chemical, physical and microbiological parameters, service crop aerial biomass and common bean yield in response to the distribution of macro, meso and micro aggregates in a degraded common bean monoculture system. We hypothesized that i) regardless of the species, service crops have an overall positive effect on the distribution and dynamics of soil macro, meso and microaggregates compared to common bean monocultures (without service crops), ii) the microbial biomass and activity in the different soil aggregates fractions depend on the type and quality of the organic matter contribution of the different service crops, and iii) a higher proportion of macroaggregates contribute to better interrelationships among soil chemical, physical and microbiological parameters, crop aerial biomass and common bean yield.

2. Materials and methods

2.1. Location and experimental design

The study was conducted at the Salta Agricultural Experimental Station of the National Institute of Agricultural Technology (EEA Salta–INTA), located at Cerrillos, Salta, in a subtropical area of Argentina (24°53′52.84S, 65°27′59.11W, 1420 m.a.s.l.) during the 2020/2021 and 2021/2022 agricultural cycles. Both campaigns began in late May or early June and ended with the common bean harvest in early/mid-May. The climate of the region is temperate with a mean annual precipitation of 900 mm concentrated in spring–summer, with little or no water deficit in January or February and a prolonged dry season in winter. The average temperatures are 23 °C in summer and 15 °C in winter (Volante and Paruelo, 2015). The dominant soil type of the region is Ustocrepte Udico (Soil Survey Staff - USDA, 1996) characterized as loam with 1.31 % organic matter (32 % sand, 44 % silt and 24 % clay), according to Vargas Gil (1990). The field in which the experiments were conducted is a degraded soil resulting of 50 years of monoculture of tobacco and common bean (intensive tillage includes several, ~20–30 soil inversions, per crop cycle, i.e., per year, and no-till for the last 10 years).

Plots, each measuring 5 m wide and 20 m long (distance between lines 0.23 m), were arranged in a complete randomized block design with three replicates. Five management treatments consisting of the incorporation of service crops before the main crop was established: 1) Oat (O) = *Avena sativa*/common bean (*Phaseolus vulgaris* L.); 2) Wheat (W) = *Triticale*/common bean; 3) Vetch (V) = *Vicia villosa*/common bean; 4) Melilotus (Me) = *Melilotus alba*/common bean; 5) common bean monoculture with chemical fallow during the winter. Additionally, two controls were analyzed 6) *Brachiaria* perennial = (BP) consisted of sowing *Brachiaria brizantha* and left to grow continuously (not killed), allowing its growth in a perennial manner to assess whether the prolonged; 7) Native vegetation (NV), which consisted in the natural and local vegetation as *Trema micrantha* (Afata colorada), *Celtis* L. (*Celtis pallida* var. Discolo, *Celtis tala, Celtis iguanea*) and (Novara, 2009) The annual service crops (O, W, V and Me) were sown in June 2020. Then, from November until the flowering stage of both growing cycles, all plots with crops were treated with glyphosate (48 % a.i., 3 L ha. In

January, at the beginning of the rainy season, the common bean was sown by seed drill in both growing cycles, using the no-till system managed using recommended production practices, including pesticide applications [Dimethoate 40 % p/v. EC (Perfekthion®, BASF, Mexico)] at a dose of 300 mL ha^{-1} and 2-metaxicarbamoil-bencimidazol (Nufarm 50 ®, Buenos Aires, Argentina at a dose of 400 mL^{-1}). Weeds were controlled using pre-emergent herbicide [imazethapyr 10.59 % (Pivot® H, BASF, Buenos Aires, Argentina)] and [S-Metolachlor: 96%p/v Syngenta (Dual Gold®, Buenos Aires, Argentina)] at doses of 400 mL ha^{-1} and 500 mL ha^{-1}, respectively. Thirty days after sowing, an herbicide was applied (fomesafen: 25 % p/v Syngenta [Flex®, Buenos Aires, Argentina)] at a dose of 500 mL ha^{-1}. Pesticide applications were made twice during the crop cycle. The first application was made 30 days after planting, and the second 15 days after the first application if the environmental conditions favored the development of diseases. No chemical fertilizers were used during the growth of the common bean. After the common bean reached physiological maturity, it was hand-harvested.

2.2. Soil sampling

Soil samples were collected at the common bean flowering stage (R5) in April 2022. For each replicate, all soil samples were collected from each plot using a shovel after the removal of surface residues by digging around the plant to a depth of 0–10 cm. Soil was pooled into a single composite sample (consisting of three subsamples), packed into polyethylene bags, and immediately transported to the laboratory. In total, 21 composite samples (7 treatments × 3 replicates) were collected. These samples were air-dried at 20 ± 2 °C for 24 h, gently broken apart and then divided in two subsamples: one subsample was sieved at 8 mm (for aggregate size proportions, carbon stocks, microbial activity, microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), and the second one was sieved at 2 mm (to remove roots, residues and stones), and stored at 4 °C for a general chemical and physical analysis (fertility range and particulate and associated organic carbon).

2.3. Soil chemical and physical analysis

For all size fractions, the soil organic carbon (SOC) was determined by wet oxidation following the Walkley and Black procedure (Allison and Black, 1965). Since these soils are free of carbonates, the total carbon content is equal to the SOC content. In addition, a general fertility analysis was determined: SOC, total nitrogen (TN) and extractable phosphorus (eP) were quantified using the micro-Kjeldahl method (Bremner, 1996) and Bray and Kurtz (1945) method, respectively. Soil pH and electrical conductivity (EC) were potentiometrically assessed in a soil/water suspension ratio of 1:2.5. Aggregate stability (AS) was estimated by following the method of micro-sieves (1–2 mm), according to Corvalán et al. (2000) and soil bulk density (BD) was measured by the core method described by Blake and Hartge (1986) using cores 3 cm in diameter, 10 cm in length, and 70.65 cm^3 in volume.

2.4. Aggregate size preparation

In order to separate soil water-sable aggregates into large macroaggregates (>2 mm in size), small macroaggregates (meso) (0.25–2 mm), microaggregates (0.053–0.25 mm), and silt/clay fractions (< 0.053 mm), the Six et al. (1999) method, with minor modifications, was applied. Briefly, 100 g dry weight of sieved field-moist soil subsamples (< 8 mm) were placed on the top of three stacked sieves (2 mm, 0.25 mm, and 0.053 mm in diameter) and gently immersed in water, pre-wetting for 10 min. Following this, the sieves were vertically shifted manually (with an amplitude of 3 cm) for 50 times within a 2-min interval. The aggregates obtained through the 2 mm, 0.25 mm, and 0.053 mm sieves were then moved to designated beakers. The aggregates retained on each sieve were collected, subjected to drying at 60 °C, and weighed for the computation of the respective fractions. In this study, four water-stable aggregate (WSA) fractions were obtained: 8–2 mm (> 2 mm), 0.25–2 mm, 0.053–0.25 mm, and < 0.053 mm. All fractions were stored for SOC analysis and the weight of oven-dried soil for each size class was expressed as a percentage of the total weight.

2.5. Aggregate stability calculations

Combining the different WSA fractions, the mean weight diameter (MWD; mm) and the geometric mean diameter (GMD; mm); the aggregate stability was calculated using the classical equation described below (Shirazi and Boersma, 1984; Van Bavel, 1950).

Soil means weight diameter and mean geometric diameter served as the key indicators to evaluate the aggregate stability, and were calculated as the following equations:

$$MWD = \sum\nolimits_{i=1}^{n} Wi\ xXi$$

Xi represents the average diameter of aggregate fraction 'i', while Wi denotes the mass proportion of this fraction (Kemper and Rosenau, 1986).

$$GMD = exp\left[\sum (Wi\ x\ lnXi) \Big/ \sum Wi\right],$$

where Xi refers to the average diameter of the ith-sized aggregates (mm), and Wi refers to the proportion of the ith-sized aggregates in bulk soil (as a percentage of weight).

2.6. Soil organic matter physical fractionation

In order to identify SOC pools that preferentially stabilize SOC in the medium term and elucidate possible SOC sequestration mechanisms in the field trail, the soil organic matter (OM) fractionations were evaluated at a depth of 0–10 cm. The method based on complete soil dispersion followed by wet sieving (Noellemeyer et al., 2006) adapted from Cambardella and Elliott (1994) was used. The soil suspension obtained was wet sieved through a 0.053-mm sieve for 3 min (Fritsch Analysette Spartan Vibratory 3). Soil fractions were oven-dried at 60 °C to constant weight. Dry weight of the fraction >0.053 mm (particulate organic carbon: POC) was recorded, and the weight difference with the original sample (50 g) was used to calculate weight of fraction <0.053 mm (associated organic carbon: AOC).

2.7. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

Microbial biomass carbon (MBC) was estimated using the "chloroform-fumigation extraction" method (Vance, 1987), and calculated as:

$$\mathrm{MBC} = \mathrm{OcfOcnf/kEC}$$

where Ocf refers to organic carbon extracted from fumigated samples, Ocnf refers to carbon extracted from non-fumigated samples, and kEC refers to fumigation efficiency constant = 0.45.

Microbial biomass nitrogen (MBN) was determined by a modification of the fumigation–incubation method (Joergensen and Mueller, 1996). Liquid chloroform (1 mL) was added directly to 30 g moist soil samples, stirred and left for 20 h in sealed beakers in desiccators. Subsequently, the chloroform was eliminated with the aid of a vacuum pump. Both fumigated and non-fumigated samples were then subjected to a 10-day incubation period at 25 °C under field capacity conditions. Following the incubation, the samples were extracted using 2 M KCl and subjected to NH_4-N analysis via the Berthelot reaction. MBN was calculated as the disparity in N content between fumigated and non-fumigated samples, divided by a designated correction factor. The correction factor kN = 0.54 was utilized to compensate for the non-extractable portion of microbial nitrogen (Brookes et al., 1985).

2.8. Soil microbial activities

2.8.1. Hydrolysis of fluorescein diacetate activity

The overall microbial activity for each fraction was evaluated using fluorescein diacetate hydrolysis (FDA) activity, following a modified procedure, as described by Adam and Duncan (2001). Briefly, 2 g of soil sample and 15 mL of 60 mM potassium phosphate buffer pH 7.6 were mixed in a 50-mL conical flask. Substrate (FDA, 1000 μg mL^{-1}) was added to each sample to start the reaction. Flasks were incubated at 30 °C and 100 rpm for 20 min. After incubation, 15 mL of chloroform/methanol (2:1 *v*/v) was immediately added to stop the reaction. The contents of the conical flasks were then centrifuged at 2000 rpm for 5 min. Finally, the supernatant was filtered and measured at 490 nm on a spectrophotometer.

2.8.2. Dehydrogenase activity

Dehydrogenase activity (DHA) was measured following the approach outlined by García et al. (1997). For each fraction, soil samples (1 g) at 60 % of field capacity were exposed to 0.2 mL of 0.4 % INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water at 22 °C for 20 h in the dark. The resulting INTF (iodonitrotetrazolium formazan) was extracted using 10 mL of methanol through vigorous shaking for 1 min. and subsequent filtration using a Whatman No. 5 filter paper. Spectrophotometric measurements of INTF were conducted at 490 nm.

2.8.3. Acid phosphatase activity

The method described by Tabatabai and Bremner (1969) was employed to determine the activity of acid phosphatase (AP). In each fraction, 1 g of soil was combined with 4 mL of 0.1 M universal buffer (pH 6.5) and 1 mL of 25 mM *p*-nitrophenyl phosphate in a 50 mL conical flask, followed by incubation of 37 °C for 1 h. After the incubation, 4 mL of 0.5 M NaOH and 1 mL of 0.5 M $CaCl_2$ were introduced, and the absorbance of the supernatant was measured at 400 nm.

2.9. Common bean yield, aerial biomass and composition of service crop residues

Common bean yield was carried out at the end of May of each campaign. In late spring, aboveground biomass samples were taken from the service crops and the control perennial plant *Brachiaria*. In all cases the sampling was the same, cutting the plants 1 cm above the soil surface in three randomly quadrants (50 cm × 50 cm) per plot. Service crop samples were oven-dried at 62 °C for 48 h until constant weight and then weighed to calculate the dry matter of aerial biomass per unit area. A subsample was finely ground for nutrient analyses. The N and C concentrations of service crop biomass were then determined using LECO—TrueSpec® CN elemental analysis. The service crop biomass quality was expressed as the C to N ratio.

2.10. Statistical analysis

The effects of service crops, aggregate size distribution and their interactions on the microbial parameters were determined by the program InfoStat Professional version 2018 (Di Rienzo et al., 2011). An analysis of variance (ANOVA) was carried out and the Fisher's least significant difference (LSD) test was used to analyze any significant differences among treatments ($P \leq 0.05$). Before using the ANOVA, a normality test (Shapiro-Wilk test) was performed for all variables and homogeneity of the variances was examined by plotting residuals versus predicted values. A principal component analysis (PCA) was performed to determine the separation between treatments, and to identify the biochemical variables that most contributed to the separation of treatments. A second PCA was conducted to assess the relative effects of biochemical parameters (including MBC, MBN, DHA, AP, FDA, and SC) evaluated only in the different soil aggregate fractions. Pearson's correlation analysis was used for exploring the relationships of aggregate stability with the bio-chemical parameters using the R package "corrplot" version 0.84 (Wei et al., 2017), with the statistical significance set at $P \leq 0.01$.

3. Results

3.1. Soil chemical and physical analysis

Most soil chemical and physical properties were affected by the inclusion of service crops ((O) = *Avena sativa*; (W) = Triticale; (V) = *Vicia villosa*; (Me) = *Melilotus alba*) as seen in Table 1. Although not significant, soil organic carbon (SOC) and organic matter (OM) were lower under the common bean monoculture (M) than with annual service crops: oat (O), wheat (W), vetch (V) and melilotus (Me). Controls *Brachiaria perennial* (BP) and native vegetation (NV) had significantly more SOC (36 % and 164 %, respectively) and OM (43 % and 178 %, respectively) than M. Total nitrogen (Total N) did not differ between the service crops nor with M, however, total N was higher in controls BP and NV than M (100 % and 200 %, respectively). The only treatment that had higher levels of extractable phosphorus (eP) than the control M was NV. W and V had similar eP levels to M, while all other treatments had lower eP levels than M. Comparing the service crops, there was no significant difference in bulk density (BD) between them. The control NV had the lowest BD values, which were 29 % lower than M. Soil aggregate stability (AS) was 37 % higher (on average) in annual service crops than in M. However, there were no significant differences in AS between annual service crops. The controls BP and NV had 91 % and 104 % higher AS levels than M, respectively. Treatment V had a significantly higher water holding capacity (WHC) than M (18 %), but the highest WHC values were found in the controls BP (26 %) and NV (38 %).

3.2. Aggregates fractions, mean weight (MWD) and geometrical mean diameter (GMD)

At a depth of 0 to 10 cm, the percentages of soil water stable aggregate fractions varied between service crops (Fig. 1). In general, a higher proportion of macroaggregates larger than 0.25 mm were observed in all service crop treatments compared to M and with the other fractions of water-stable aggregates. The proportions of >2 mm aggregates were highest in the controls BP and NV and lower in M, while the fractions 0.25-2 mm were significantly higher in all treatments especially V with significant differences among annual service crops. In the fraction 0.053–0.25 mm the highest values were observed in W, Me, and M. The control NV presented the lowest values of the 0.053–0.25 mm fraction.

In general, all treatments exhibited higher values of MWD and ADG compared to the bean monoculture (Fig. 2). The highest MWD and GMD were observed in O and in the BP and NV controls.

3.3. Soil organic matter physical fractionation and carbon stocks

The following information pertains to different service crops The study found that the largest fractions of soil aggregates (> 2 mm and 0.25-2 mm) higher levels of carbon stocks, compared to monoculture of common bean (M), across all service crop treatments. However, the control native vegetation (NV) had the highest levels of carbon stock (C Stock) in all soil-size aggregate fractions (Fig. 3). M had the lowest C stocks, especially in the largest fractions. No differences were observed among service crop treatments for the aggregate sizes between 0.053 and 0.25 mm, except for O, which had the lowest levels. Finally, in the <0.053 mm fraction, Me had the highest and most significant values compared to the other service crops.

Particulate organic carbon (POC) was higher in the service crops V (99 %), Me (89 %) than M. Associate organic carbon (AOC) was higher in the service crops, W (15 %), V (10 %) and Me (8 %) than M. The

Table 1
Mean values for soil chemical and physical parameters measured under different treatments in 0–10 cm depth.

Parameters	Treatments[a]						
	O	W	V	Me	M	BP	NV
SOC[b] (%)	1.5 ± 0.3 c	1.5 ± 0.07 c	1.6 ± 0.19 c	1.5 ± 0.07 c	1.4 ± 0.11 c	1.9 ± 0.57 b	3.7 ± 0.85 a
OM[c] (%)	2.6 ± 0.23 c	2.5 ± 0.12 c	2.8 ± 0.33 c	2.6 ± 0.12 c	2.3 ± 0.19 c	3.3 ± 0.99 b	6.4 ± 1.47 a
total N[d] (%)	0.1 ± 0.01 c	0.1 ± 0.01 c	0.1 ± 0.02 c	0.1 ± 0.01 c	0.1 ± 0.01 c	0.2 ± 0.04 b	0.3 ± 0.07 a
C/N	11.7 ± 0.5 a	11.0 ± 0 a	11.0 ± 0 a	11.0 ± 0.87 a	11.0 ± 0.87 a	11.7 ± 0.5 a	10.7 ± 1 a
eP[e] (ppm)	30.3 ± 7.47 c	33.3 ± 3.91 b	35.3 ± 9.18 b	28.0 ± 5.41 c	36.7 ± 2.18 b	26.0 ± 1.73 c	51.7 ± 5.07 a
pH (H_20)	7.4 ± 0.05 a	7.2 ± 0 b	7.2 ± 0.09 b	7.3 ± 0.05 b	7.2 ± 0.05 b	6.9 ± 0.22 c	7.5 ± 0.05 a
BD[f] (g/cm^3)	1.5 ± 0.09 a	1.46 ± 0.1 a	1.45 ± 0.17 a	1.47 ± 0.1 a	1.51 ± 0.15 a	1.54 ± 0.09 a	1.08 ± 0.17 b
AS[g] (%)	57.16 ± 3.56 b	43.11 ± 4.23 b	53.65 ± 4.6 b	51.75 ± 6.48 b	37.5 ± 3.7 c	71.75 ± 7.46 a	76.69 ± 4.4 a
WHC[h] (%)	36.67 ± 1.32 c	36.33 ± 1.8 c	38.33 ± 1.32 b	36.00 ± 1.5 c	32.33 ± 0.5 c	40.67 ± 8.05 b	44.67 ± 5.63 a
EC[i] (mmhos/cm)	0.5 ± 0.11 b	0.4 ± 0.02 b	0.5 ± 0.12 b	0.5 ± 0.09 b	0.4 ± 0.09 b	0.4 ± 0.05 b	1.0 ± 0.2 a

In each row, different lower-case letters indicate significant differences between treatments a $P \leq 0.05$ according to LSD's test.

[a] Treatments: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation.

[b] SOC: Soil organic carbon.

[c] OM: Organic matter.

[d] total N: Total nitrogen.

[e] eP: Extractable phosphorus.

[f] BD: Bulk density.

[g] AS: Soil aggregate stability.

[h] WHC: Water holding capacity.

[i] EC: Electrical conductivity.

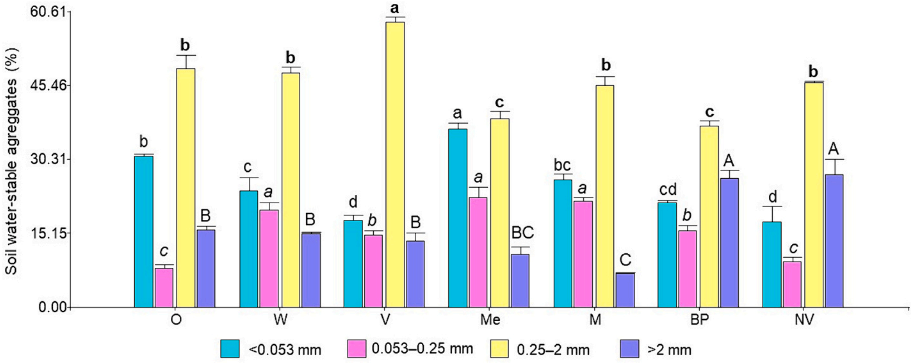

Fig. 1. Percentage of soil water-stable aggregate (%) for different soil fractions (< 0.053, 0.053–0.25, 0.25–2 and > 2 mm), measured under different treatments in 0–10 cm depth: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. Different lower-case letters indicate significant differences among treatments for the same aggregate fraction at $P \leq 0.05$ according to LSD's test. Error bars indicate standard error.

control NV had the highest POC and AOC than other treatments, while BP had 80 % and 25 %, respectively (Fig. 4).

3.4. *Carbon and nitrogen microbial biomass and soil microbial activities*

A higher microbial biomass carbon (MBC) content was observed in the larger fractions rather than in the smallest fractions of soil aggregates. The mean value of soil MBC varied between 160.21 μg C/g and 9.60 μg C/g in all soil aggregate fractions (Fig. 5a). In general, the >2 mm fractions exhibited the highest levels of MBC except for Me, which presented higher contents of MBC in the <0.053 mm and 0.053–0.25 mm fractions (although they also showed high MBC in the >2 mm fraction). MBC levels were generally low in the 0.25–2 mm fraction for all treatments, with M exhibiting a particularly pronounced decrease. The mean value of microbial biomass nitrogen (MBN) varied between 89.96 μg N/g and 3.20 μg N/g in all soil aggregate fractions (Fig. 5b). Although there was not a clear trend for the different fractions and treatments, the control NV showed the highest MBN values, particularly in the 0.053–0.25 mm and < 0.053 mm fractions, whereas the other treatments had slight differences among them.

In general, the enzyme levels decreased progressively from the >2 mm fractions of soil aggregates to the <0.053 mm fraction, although the AP showed a drastic decrease from the 0.25–2 mm fraction to the 0.053–0.25 and < 0.053 mm fractions (Table 2). The AP was significantly higher in the V and W treatments, mainly in the >2 mm and 0.25–2 mm fractions.

The FDA activity from each of the four aggregate-size fractions increased in V (particularly in the >2 mm fractions), whereas M presented the lowest value in all aggregate fractions. On the other hand, in general, NV presented the highest FDA values, and M the lowest. The DHA presented variable values between treatments and fraction size, although it showed the highest levels in the 0.25-2 mm fraction for all service crop treatments.

3.5. *Service crops dry aerial biomass, composition, and common bean yield*

The inclusion of service crops before the main crop significantly increased the yield of common bean. The yield was 136 % higher before Me, 122 % before V, 114 % before O, and 57 % before W, as compared to the yield before M (Table 3). Different service crops yielded different amounts of above-ground biomass, with V producing the highest

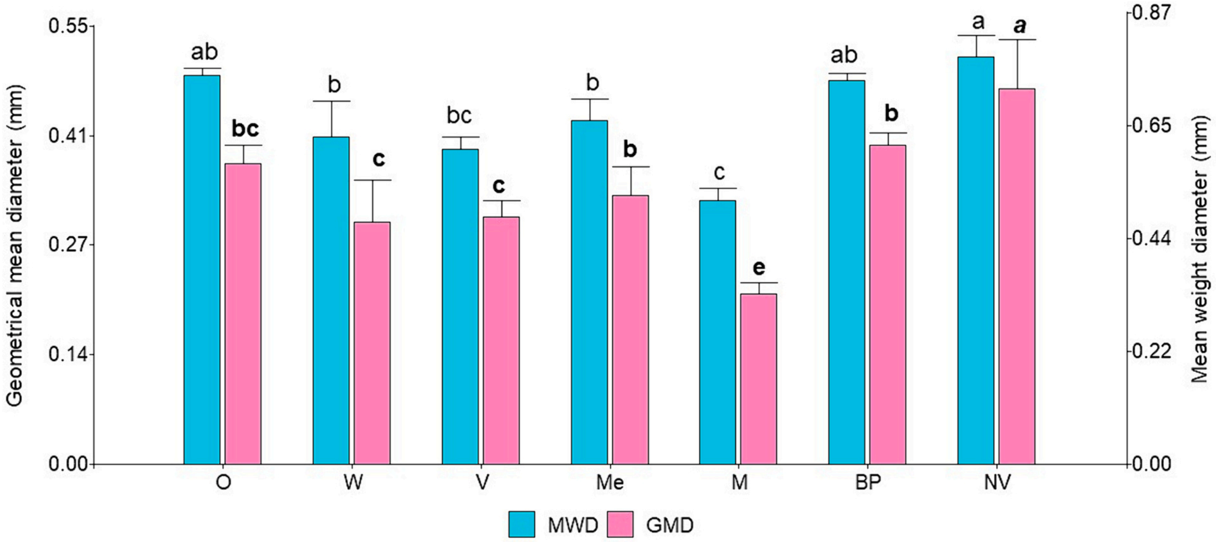

Fig. 2. Mean weight diameter (MWD) and geometrical mean diameter (GMD) of soil aggregates measured under different treatments in 0–10 cm depth: O = *Avena sativa*/common bean; W = *Triticale*/common bean; V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. Different lower-case letters indicate significant differences among treatments at $P \leq 0.05$ according to LSD's test. Error bars indicate standard error.

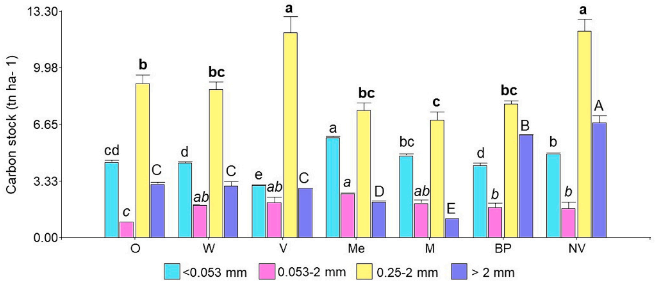

Fig. 3. Soil carbon stocks in different soil aggregate fractions (< 0.053, 0.053–0.25, 0.25–2 and > 2 mm) measured under different treatments in 0–10 cm depth: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. Different lower-case letters indicate significant differences among treatments for the same aggregate fraction at $P \leq 0.05$ according to LSD's test. Error bars indicate standard error.

biomass. However, no significant differences were found between O, W, and Me. The perennial (non-service crop) BP control produced the highest above-ground biomass, doubling the biomass production of Me and W. The carbon content of the aerial biomass was highest in Me, followed by O, W, and V, with BP control having the lowest carbon content values. The nitrogen content of the aerial biomass was higher in V and Me than in the other service crops. This resulted in high C/N ratios in the aerial biomass in O, intermediate high in W, and low in V and Me.

3.6. Relationship between variables

In all fractions of soil, the PCAs revealed a clear variation between the distribution of annual service crops, the controls BP, NV, and M treatment (Fig. 6). In the >2 mm soil aggregate fractions (Fig. 6a), the PCA explained 78 % of the total variance (PC1 and PC2 explained about 51 % and 27 %, respectively). Based on the PC1, NV showed a clear separation from the rest of the treatments, being allocated positively along PC1. MBC, MBN, DHA, FDA and CS were the variables that influenced the separation of annual service crops from the NV. In contrast, the common bean monoculture was allocated on the opposite side. The PC2 separated the annual service crops treatments from M. In 0.25–2 mm soil aggregate fractions (Fig. 6b), PC1 and 2 explained 44 % and 29 % of the total variance, respectively. The PC1 clearly separated M from the rest of the treatments. The variables that intervened in the separation of the treatments were: MBC, MBN, FDA and CS. Based on PC2, M separated from O and NV control. In 0.053–0.25 mm soil aggregate fractions (Fig. 6c), the PC1 explained 61 % and PC2 18 % of the variance. On PC1, V and NV control were clearly separated from the rest of the treatments, and were associated with MBN, CS, AP, and FDA variables. Based on PC 2, no clear separations between treatments were observed. In <0.053 mm soil aggregate fractions (Fig. 6d), the first two axes explained 65 % and 29 % of the variance, respectively. Based on the PC1, V and NV control showed a clear separation from the rest of the treatments, being allocated positively along PC1. The variables that influenced the separation of treatments were CS, AP, FDA and MBN.

A significant positive correlation ($P \leq 0.01$) among FDA activity with MWD, GMD, 2–0.25 mm fraction, WHC, EC, SOC, eP, and TN was observed (Fig. 7). There was also a significant positive correlation between AOC and POC with the MBN, FDA, MWD, GMD, WHC, EC, SOC, and TN. In addition, BD and DHA presented significant negative correlations with most variables used in this study. Finally, a significant positive correlation was found between common bean yield and AOC, POC, SOC, TN, AS, aggregates fractions, MWD, GMD, and MBN. In contrast, a significant negative correlation was observed between common bean yield and DHA and BD.

4. Discussion

Our short-term field trial conducted in a degraded soil due to tobacco and common bean monoculture systems of northwest Argentina revealed that annual service crops improved the physical rather than chemical properties of the bulk soil. In a previous study in a similar degraded monoculture system, the inclusion of service crops (legumes and gramineous) resulted in a significant improvement of the biochemical properties of the rhizospheric soil fertility, specifically in carbon and nitrogen contents along with an enhancement in soil fertility (Abán et al., 2021b). The inclusion of annual service crops in agricultural systems is sometimes unable to detect significant changes in soil chemical and physical properties in a few crop cycles. However, this general assumption depends on soil degradation.

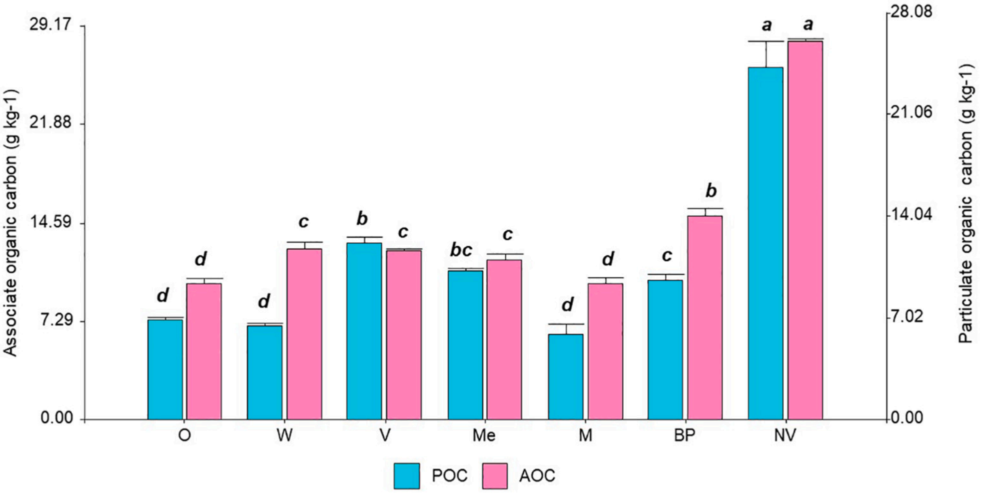

Fig. 4. Particulate organic carbon (POC) and associate organic carbon (AOC) measured under different treatments in 0–10 cm depth: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = Brachiaria perennial and NV: Native vegetation. Different lower-case letters indicate significant differences among treatments at $P \leq 0.05$ according to LSD's test. Error bars indicate standard error.

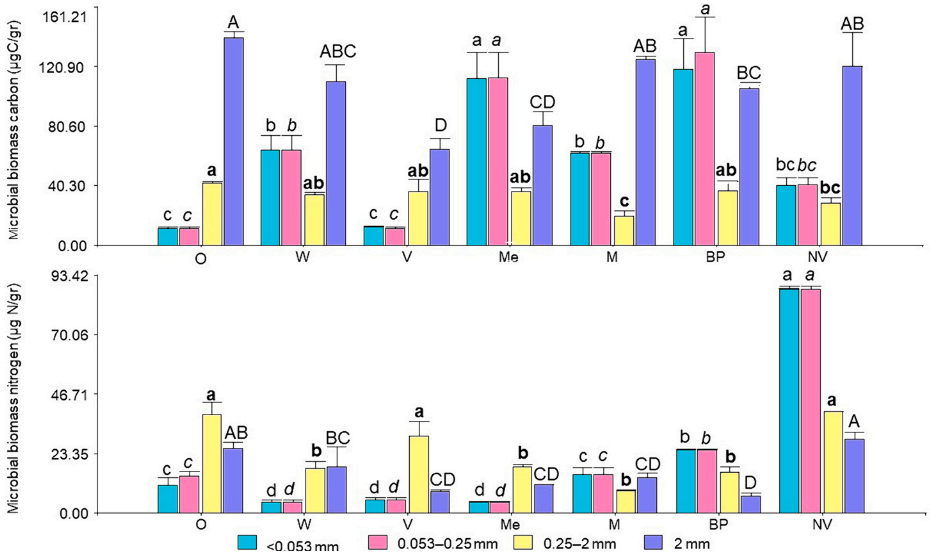

Fig. 5. a) Soil microbial biomass carbon (MBC) and b) soil microbial biomass nitrogen (MBN) in soil aggregate fractions (< 0.053, 0.053–0.25, 0.25–2 and > 2 mm) measured under different treatments in 0–10 cm depth: O = *Avena stiva*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. Different letters indicate significant differences among different treatments for the same aggregate fraction at $P \leq 0.05$ according to LSD's test. Error bars indicate standard error.

As expected, the positive effect of short-term service crops on physical soil properties was not enough to reach the aggregate stability and water-holding capacity of the BP or NV; however, annual service crops improved aggregate stability regarding M. The structural stability of soil aggregates corresponds to their resistance and ability to remain stable when exposed to exogenic forces facilitating decreases in erosion and improvements in carbon sequestration and fertility. On the contrary, bulk density presented the lowest values only in NV, although its levels decreased in W, V and Me compared to M, enhancing soil structure. Soil bulk density serves as a crucial indicator of soil compaction, exerting influence over a diverse set of soil physical attributes. Alterations in soil bulk density inversely affect total porosity, leading to consequential modifications in macro, *meso*, and micropores. Those changes can significantly impact the soil's water content and infiltration rate (Fuentes-Prior and Salvesen, 2004). Thus, one of the major findings of this study is that aggregate stability increased due to the inclusion of service crops (V, O, Me and W) during two growing cycles, in the fallow period when compared with the bare fallow (M). Water-stable soil aggregates (i.e., a group of primary soil particles that cohere to each other more strongly than to others surrounding it) can physically protect organic matter from rapid decomposition, inhibiting water loss and soil erosion. Another major finding of this study is that service crops were affected differently and positively in the formation of soil water-stable aggregate fractions, supporting the idea that soil aggregation is dynamic in nature. Regardless of the treatment, the largest fractions of soil aggregates (> 2 mm and 0.25-2 mm) were dominant in the aggregate composition. All service crops showed a higher proportion of water-stable soil aggregates of >2, and 0.25–2 mm fractions than M, although the highest values were recorded at NV followed by BP. Thus, short-term annual service crops improved soil structure suggesting that over the years it contributed to the disintegration of these fractions and increased the particulate and associated carbon content. On the other

Table 2
Enzymatic activity in soil aggregate fractions (< 0.053, 0.053–0.25, 0.25–2, and > 2 mm) measured under different treatments in 0–10 cm depth.

Enzymes[a]	Size fractions (mm)	Treatments[b]						
		O	W	V	Me	M	BP	NV
FDA (μg g^{-1} h^{-1} soil)	< 0.053	6.76 ± 0.52 b	7.08 ± 5.34 bc	12.62 ± 1.98 a	1.04 ± 0.20 cd	0.43 ± 0.71 d	3.02 ± 3.17 bcd	13.25 ± 1.52 a
	0.053–0.25	8.68 ± 0.61 b	8.06 ± 5.98 bc	14.86 ± 2.04 a	3.24 ± 0.13 cd	2.87 ± 0.67 d	5.62 ± 3.19 bcd	16.18 ± 1.49 a
	0.25–2	6.17 ± 0.12 d	13.60 ± 1.46 bc	20.38 ± 6.78 a	12.56 ± 1.53 c	8.94 ± 0.78 cd	8.72 ± 1.33 cd	17.76 ± 2.49 ab
	> 2	21.62 ± 0.39 c	19.47 ± 0.51 cde	27.06 ± 0.95 b	20.42 ± 2 cd	17.94 ± 0.08 e	19.02 ± 0.96 de	46.32 ± 2.72 a
DHA (μg g^{-1} h^{-1})	< 0.053	2.80 ± 1.29 d	4.34 ± 0.13 c	1.01 ± 0.4 e	7.47 ± 1.47 a	6.02 ± 0.12 b	3.65 ± 0.21 d	2.56 ± 0.02 de
	0.053–0.25	4.50 ± 1.33 d	6.14 ± 0.10 c	2.82 ± 0.38 e	10.98 ± 1.46 a	8.01 ± 0.15 b	4.56 ± 0.17 d	3.47 ± 0.01 de
	0.25–2	9.88 ± 4.25 a	11.74 ± 0.08 a	6.1 ± 1.26 b	5.63 ± 2.01 b	11.44 ± 0.07 a	3.66 ± 0.9 b	4.47 ± 0.2 b
	> 2	5.14 ± 0.03 cd	6.32 ± 0.5 b	4.96 ± 0.21 d	5.60 ± 0.09 c	4.62 ± 0.26 d	3.61 ± 0.14 e	8.14 ± 0.56 a
AP (μmol g^{-1} h^{-1})	< 0.053	0.87 ± 0.01 a	0.63 ± 0.37 ab	0.45 ± 0.09 a	0.34 ± 0.11 b	0.23 ± 0.03 b	0.67 ± 0.49 a	0.59 ± 0.11 ab
	0.053–0.25	0.94 ± 0 a	0.72 ± 0.41 ab	0.96 ± 0.11 a	0.47 ± 0.07 b	0.3 ± 0.05 b	0.96 ± 0.47 a	0.74 ± 0.05 ab
	0.25–2	0.59 ± 0.02 d	4.99 ± 0.39 ab	5.37 ± 2.49 a	5.19 ± 0 a	3.24 ± 0.39 c	3.44 ± 0.1 bc	2.74 ± 0 c
	> 2	9.62 ± 0.48 c	11.03 ± 0.7 b	12.49 ± 0.25 a	9.57 ± 0.2 c	7.50 ± 0.21 de	7.68 ± 0.4 d	6.77 ± 0.7 e

In each row, different lower-case letters indicate significant differences among different treatments for the same aggregate fraction a $P \leq 0.05$ according to LSD's test.

[a] FDA: Hydrolysis of fluorescein diacetate activity, DHA: dehydrogenase activity, AP: acid phosphatase.

[b] Treatments: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation.

Table 3
Mean values of service crops of dry aerial biomass (dry matter), common bean yield, composition, and organic carbon (C), nitrogen (N) content, and C:N ratio.

Parameters	Treatments[a]						
	O	W	V	Me	M	BP	NV
Dry aerial biomass (kg ha^{-1})	5948.40 ± 2185.41 bc	4815.60 ± 1516.58 c	6683.73 ± 456.06 b	4111.80 ± 338.85 c	fallow	8800 ± 4.04 a	n. d.[b]
Common bean yield (kg ha^{-1})	4365.38 ± 725.44 a	3192.31 ± 226.73 b	4512.82 ± 1237.71 a	4801.28 ± 1343.72 a	2032.88 ± 162.14 c	n.d.	n.d.
C (%)	40.69 ± 0.18 b	39.57 ± 0.23 cd	39.82 ± 0.51 c	42.32 ± 0.1 a	fallow	39.23 ± 0.45 d	n.d.
N (%)	0.90 ± 0.16 b	1.24 ± 0.04 b	3.66 ± 0.39 a	3.46 ± 0.2 a	fallow	1.19 ± 0.13 b	n.d.
C/N ratio	46.11 ± 8.93 a	32.01 ± 0.92 b	10.95 ± 1.17 c	12.25 ± 0.73 c	fallow	33.15 ± 3.63 b	n.d.

[a] Treatments: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. In each row, different lower-case letters indicate significant differences among different treatments at $P \leq 0.05$ according to LSD's test.

[b] n.d.: undetermined.

hand, fraction 2–0.25 seems to be more sensitive to treatments with legumes (V and Me) showing values closer to the undisturbed controls, NV and BP. Likewise, the 2–0.25 mm fraction shows greater positive correlations with SOC, OM, TN and AS, and negative correlations with BD, which suggests an important functional role of this fraction.

Changes in soil aggregate stability were easily detected by variations in soil mean weight diameter (MWD) and geometric mean diameter (GMD), which could be considered sensitive indicators to assess aggregate stability (Galantini and Suñer, 2008; Mao et al., 2021). Specifically, if the MWD and GMD are higher, the stability of the soil aggregates is stronger, as demonstrated in this study where annual service crops increased the stability of the soil aggregates by an average of 37 % compared to M. In M, the low levels of soil MWD and GMD were driven by the dynamics of the aggregates, in particular by the disintegration of fractions of >2 mm into fractions of <0.25 mm. Additionally, the decrease in the amount and cover of residues, driven by eluviation from rainfall, might have further caused the disintegration of coarse macroaggregates in the soil, which induced the transition from coarse macroaggregates to microaggregates in the soil, supporting hypothesis I. O and Me, as service crops, increased, in similar proportion, the <0.053 mm fractions compared to M, suggesting an increase in organic carbon associated with the mineral fraction (clay and silt) that allows improvements in the stability of soil aggregates and persistent soil organic carbon that in turn indicate carbon sequestration (Gulde et al., 2008). Moreover, our hypothesis i) is also supported by the general increase of POC and AOC in service crops regarding M. The residues of the legumes like V and Me had low C:N ratio (Table 3), which indicates that the stubble is easily combined into coarse (>2 mm) macroaggregates during the waste decomposition process, which, in turn, promotes the formation of coarse macroaggregates compared to M, whereas the residues of grasses like BP and W (Table 3) had high C:N ratio, leading to a slow decomposition of residues and stubble, which led to a higher content of AOC regarding to M. These results indicated that the quality and quantity of the stubble is determinant to define increases or decreases of these fractions (Nicolardot et al., 2001; Parwada and Van Tol, 2019). Also, as a core component of aggregates, POC is involved in the turnover and formation of aggregates, and it is protected by aggregates (Six et al., 2000). The stabilization of POC is greatest when the aggregate stability is high (Six et al., 1998) as demonstrated in control BP and in the service crops: Me and V. These results suggest that continuous residues and stubble inputs can effectively contribute to soil carbon accumulation and carbon sequestration in the 0–10 cm surface soil (Deng et al., 2014) and that soil aggregation plays a pivotal role in the process of soil carbon accumulation, operating through three fundamental mechanisms: physical (entrapment of POC in macro- and micro-aggregates), chemical (adsorption of POC), and biochemical (recalcitrance and condensation) protection in aggregates (Hao et al., 2023).

Microorganisms maintain soil functions such as nutrient cycling, including carbon sequestration, and plant-soil feedback. This condition makes it important to study the dynamics of soil enzymatic activities and the carbon and nitrogen biomass content in soil aggregates under improved management practices. In agreement with other studies (Gupta and Germida, 2015; Li and Yang, 2019; Zhao et al., 2014), we observed that soil MBC and MBN were not uniformly distributed in soil

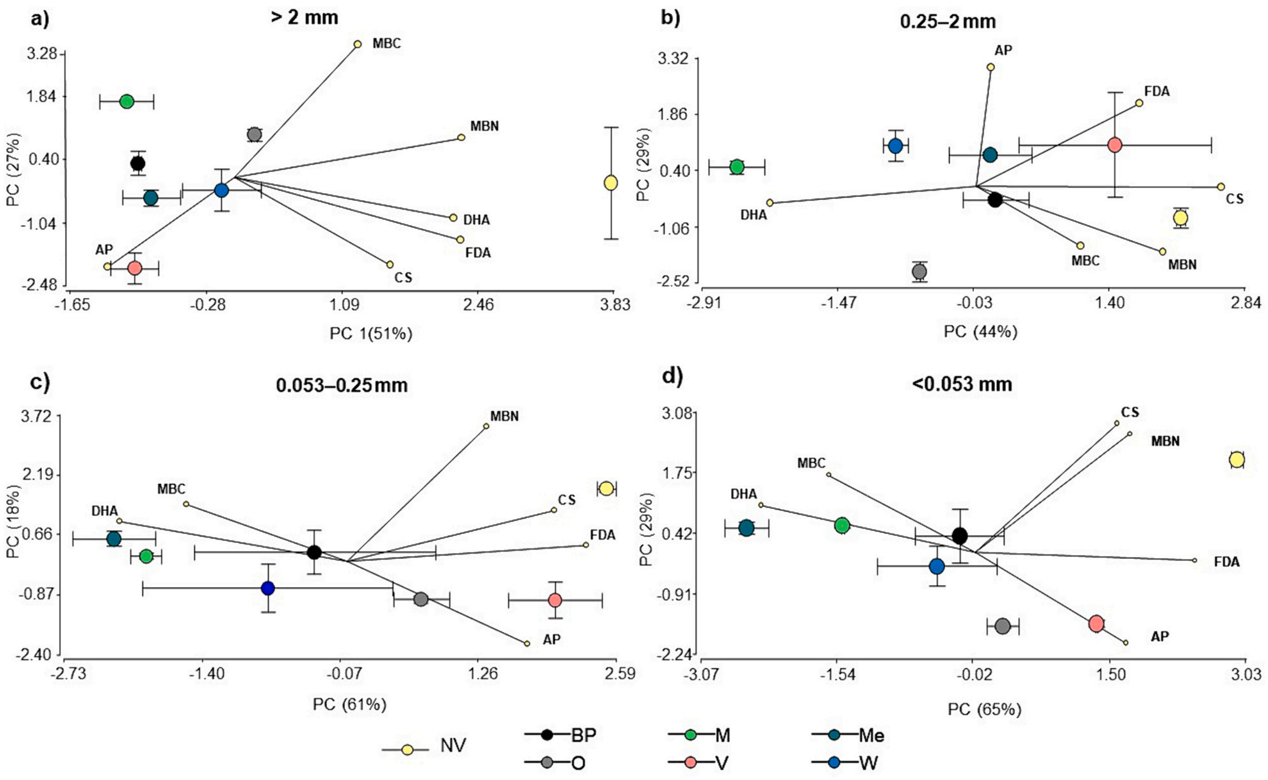

Fig. 6. Principal component analysis (PCA) showing the relationships between specific biological parameters (MBC, MBN, FDA, DHA, AP, CS) in each soil aggregate fractions: a) > 2 mm, b) 0.25–2 mm, c) 0.053–0.25 and d) < 0.053, under different treatments: O = *Avena sativa*/common bean; W = *Triticale*/common bean V = *Vicia villosa*/common bean; Me = *Melilotus alba*/common bean; M = common bean monoculture; BP = *Brachiaria* perennial and NV: Native vegetation. MBC (Microbial biomass carbon), MBN (Microbial biomass nitrogen), FDA (Hydrolysis of fluorescein diacetate activity), DHA (Dehydrogenase activity), AP (Acid phosphatase activity) and CS (Carbon Stock).

aggregates, which exhibited negative, positive, or uncorrelated relationship with the aggregate size and were distributed differentially according to the service crops. Nevertheless, control BP and Me showed an increase in MBC within the <0.053 and 0.053–0.25 mm fractions, whereas the other treatments did so in the big fractions. The small fractions are related to the highest AOC and soil aggregate stability, which could be associated with more metabolized forms of carbon and nitrogen, favoring the growth of certain microbial groups. On the other hand, the other treatments (including M) presented the highest MBC in the largest fractions (> 2 mm), suggesting that these treatments could be conducive to novel carbon accumulation, with POC being significantly and positively correlated with MBC in surface soil (Ashraf et al., 2020). It was not possible to establish a clear relationship between the MBN and soil aggregate fractions, but an increase in the intermediate fractions was observed in all service crops. NV registered the highest values in the smallest fractions. Such results might be attributed to the slow decomposition of organic matter because of its high C:N ratio associated with the larger fractions and the necessary time this requires to achieve visible changes. This situation could provide more pore spaces for microbial growth and reproduction, thus facilitating the increases in microbial biomass. The prevailing hypothesis posits a close association between organic matter content and the spatial distribution of soil microbial biomass within soil aggregates. However, the findings of our study do not substantiate this proposition. Our research suggests that the correlation between organic matter content and the dispersion of microbial biomass may not hold in the specific context under examination. This inference is drawn from the observed marginal variations in organic matter content within the top 10 cm of soil across diverse service crops, as compared to a monoculture reference. Our results highlight the importance of BP as a potential restoration treatment, emphasizing the role of the plant material and the substantial residue deposition as soil organic matter. Previous research provides substantial evidence supporting the multitude of beneficial effects of BP on soil aggregation, as observed in this trial (Zhao et al., 2014) (Abán et al., 2023, Abán et al., 2021b; Pérez-Brandan et al., 2017, Pérez-Brandan et al., 2016). This result may also be linked to the presence of a higher abundance of the main microbial functional groups (bacteria, fungi and mycorrhizas) that are stimulated by BP (Abán et al., 2021a; Pérez-Brandan et al., 2019, Pérez-Brandan et al., 2017).

In line with other studies, our results showed that FDA and AP content increased in macroaggregates in all service crops compared to M, with a greater increase observed in V, across all fractions, which was consistent with hypothesis ii). These results may suggest that mineral-associated organic matter is protected by these mineral associations (smaller than 50–53 μm) that are difficult for microorganisms to utilize, especially since vetches have lower C:N ratios than grasses, which would facilitate residue decomposition and increase soil microorganism activity (Lavallee et al., 2020). Therefore, AOC in most aggregate size fractions increased slightly after service crop implantation. Consequently, the increase in AOC was mainly attributed to the increase in POC after crop planting. According to Burke et al. (2011), the activities of soil enzymes produced by microorganisms and plants are closely linked to the movement of energy and nutrients in the soil. The introduction of service crops can lead to notable enhancements in soil aggregate stability and cohesiveness, as demonstrated by the significant and positive correlation ($P < 0.05$) between MDW and enzymatic activity (except for DHA) which is by hypothesis iii).

Beyond the positive effect of annual service crops on physical, chemical, and microbiological properties, they also increased common bean yield compared with M. Particularly, the greatest yield increases occurred when legumes were used as service crops. Even though many factors affect yield generation, it is generally reported that higher cash

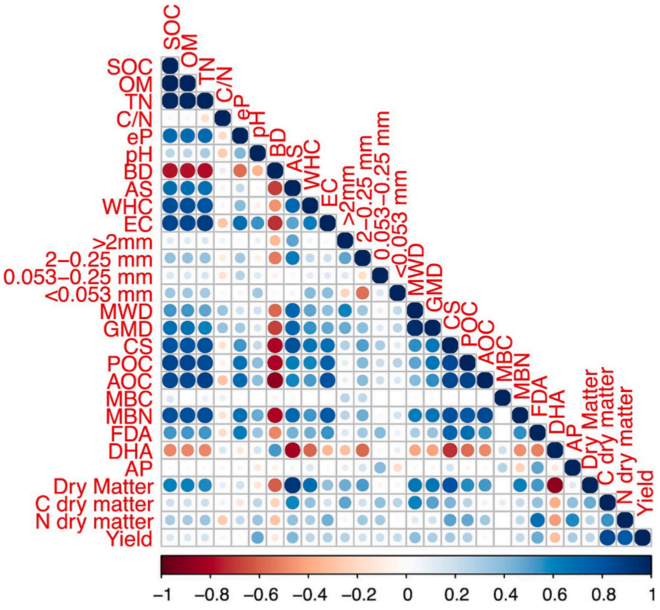

Fig. 7. Heat map showing the pairwise Pearson's correlation (P value ≤ 0.01) among chemical, physical, and microbiological parameters, dry matter of service crops, and common bean yield. Blue and red colors indicate positive and negative correlations, respectively. The color intensity and the circle size are proportional to the correlation coefficients, with bigger and darker circles representing higher correlations. Blank squares denote insignificant correlations (P value >0.01). SOC (Soil organic carbon), OM (organic matter), TN (total nitrogen), eP (Extractable phosphorus), AS (aggregate stability), BD (Bulk density), AS (aggregate stability), WHC (Water holding capacity), EC (Electrical conductivity), MWD (Mean weight diameter), GMD (Geometric mean diameter), CS (Carbon stock), POC (Organic particulate carbon), AOC (Associate organic carbon), MBC (Microbial biomass carbon), MBN (Microbial biomass nitrogen), FDA (Hydrolysis of fluorescein diacetate activity), DHA (Dehydrogenase activity), AP (Acid phosphatase activity), Dry matter, C dry matter (carbon dry matter), N dry matter (nitrogen dry matter), Yield (common bean yield). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

crops yield is observed under service crop treatments in most of the studies (Garba et al., 2023; Raut et al., 2020; Sanyal et al., 2023). Cropping systems with service crops provided a helpful tool for maintaining yields while maximizing the soil chemical and physical properties (Abán et al., 2021a). This could be related to the low C:N relationship of their aerial biomass. A lower C:N ratio in plant residues is conducive to decomposition (Nicolardot et al., 2001; Parwada and Van Tol, 2018), thus serving as a soil carbon and nutrients (N, P) source.

5. Conclusions

The use of annual cover crops in a short-term experiment led to improvements in the physical properties of degraded soil under common bean monoculture, rather than chemical properties. The inclusion of service crops (V, O, Me, and W) during two growing cycles has been found to increase aggregate stability and the formation of soil water-stable aggregates fractions. This contributes to a greater amount of macroaggregates being dominant in the composition of soil aggregates. The study demonstrates that the inclusion service crops had a positive effect on aggregate stability, with legumes (V and Me) contributing to the increased amount of POC and AOC in soil. The mean weight diameter (MWD) and geometric mean diameter (GMD) of soil were used as sensitive indicators to assess aggregate stability shifts. *Brachiaria* as a control, introduced a large amount of stubble into the soil, resulting in an increase in macroaggregate fractions, as well as elevated microbial activity over the years of implantation. In contrast, enzymatic activities were more sensitive to changes and increased their contents in macroaggregates in all service crops, compared to monoculture. Our findings emphasize the importance of incorporating service crops, particularly legumes, which positively influenced common bean yields.

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CRediT authorship contribution statement

Antonella Ducci: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carla Abán:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Jorgelina Huidobro:** Methodology, Investigation. **Silvina Vargas-Gil:** Writing – review & editing, Supervision. **Martin Acreche:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Carolina Pérez-Brandan:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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