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Influence of a tropical grass (*Brachiaria brizantha* cv. Mulato) as cover crop on soil biochemical properties in a degraded agricultural soil

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ABSTRACT

The inclusion of tropical grass forage as a cover crop (CC) could be a useful tool to improve microbiological activity and, consequently, soil quality. The aim of this study was to evaluate the effect of *Brachiaria brizantha* cv. Mulato and maize (*Zea* mays) as CC on soil microbial communities and their contributions to a degraded common bean (*Phaseolus vulgaris* L.). monoculture system. Soil sampling was carried out in 2016 after six years of cumulative effect across different treatments: B. *brizantha*-B. *brizantha*-common bean (B2), *B. brizantha*-common bean (B1), maize-common bean (M) and common bean monoculture (control). B2 and B1 showed higher fluorescein diacetate hydrolysis (108.1% and 78.6%, respectively) and higher acid phosphatase activity (304.5% and 181.6%, respectively) compared with the control treatment. The metabolic efficiency was higher in treatments containing *B. brizantha* as CC, with a significantly lower metabolic quotient (respiration rate per unit microbial biomass carbon) in B2 (1.65) compared with the control (5.46). The B2 treatment also showed higher values of soil organic carbon, which was correlated with soil microbial activities. In contrast, qPCR analysis of microbial structure did not show significant differences in response to the evaluated treatments. Thus, fungal and bacterial abundance probably had less influence on the differentiation of treatments compared to microbial activity and soil chemical properties. In context of this research, the use of *B. brizantha* as CC increased soil fertility and generated a greater microbial metabolic efficiency. Our research demonstrates that *B. brizantha* cv. Mulato as CC is a suitable agricultural tool to restore soil biochemical properties.

1. Introduction

Microorganisms play an essential role in biogeochemical cycling promoting plant growth. The presence of a diverse and functional microbial community contributes to stress resistance and resilience in soils [1]. Therefore, the study of soil microbial communities is a useful measurement to assess the impact of land use change [2]. However, there is little information available about the impact of tropical forage grasses employed as cover crops (CC) on soil microbial diversity and activity in monoculture systems in valleys of northwest Argentina. This information could be used to address the urgent need to restore soil fertility and agroecosystem biodiversity in major agricultural areas. The subtropical valleys of northwest Argentina constitute a diverse, dynamic and productive territory, with congenial environment for production of a variety of crops due to presence of fertile soils and warm temperatures [3]. Subtropical agroecosystems, such as those in this region, are particularly susceptible to increased soil degradation and associated nutrient losses compared to temperate/cold regions because of the higher mineralization of organic matter [1]. Moreover, the natural vegetation was rapidly and extensively cleared for industrial agriculture in these valleys, with more than 60% of production based on tobacco or common bean monoculture [4]. These processes caused negative effects such as environmental resource degradation, fertility losses, a reduction of soil microbial diversity and low productivity [5]. Given the negative effects of predominance of monoculture in major agricultural regions of Argentina and in other parts of the world, it is important to study alternative agriculture strategies oriented towards producing high-yield crops without compromising natural resources

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and ecosystem services.

Employing certain plant species as CC in the fallow period represents a promising way to diversify agricultural systems [6]. In this regard, the use of *Brachiaria brizantha* cv. Mulato, a highly nutritious and palatable forage grass, could favor the activity and diversity of soil microorganisms due to the abundance, expansion and exploration of its roots [7]. *Brachiaria brizantha* cv. Mulato is a perennial and tiller grass, with vigorous stems reaching heights of 1.5–2 m, also characterized by its good growth rate and its deep root system. The inflorescence is a racemic panicle and crop establishment can be by sexual seed or in vegetative form, establishing quickly and the stolons rooted well. Moreover, this grass produces a high amount of stubble on the surface generating a high production of fodder in dry matter [8]. Grasses with deep root systems help pumping nutrients from the deeper layers to the surface soil horizons and their biomass extract the nutrients from the deeper layers, which are gradually released [9]. Therefore, the use of *B. brizantha* could prevent soil fertility loss under monoculture of tobacco or common bean in subtropical agricultural regions. In addition, recent studies have concluded that the use of *B. brizantha* as a CC contributed to improve the quality of chemical and physical attributes of soils [10]. We hypothesize that the use of *B. brizantha* cv. Mulato contributes to restore soil biochemical properties through an increase in fungal and bacterial abundance and microbial activity, increasing the availability of macronutrients. This effect would be higher when using *B. brizantha* as CC in comparison with the use of maize. Therefore, the objective of this research was to assess the effect of one and two cycles of *B. brizantha* cv. Mulato and maize (*Zea mays*) as CC on soil microbial activity and community composition (fungal and bacterial genes abundances) and chemical properties in a degraded common bean (*Phaseolus vulgaris* L.) monoculture system.

2. Materials and methods

2.1. Study site

A field trial was established in 2010 at the Salta Agricultural Experimental Station of the Instituto Nacional de Tecnología Agropecuaria (EEA-INTA), Cerrillos, Lerma Valley, Salta, Argentina (S 24º53′52.84´´ W 65º27′59.11´´, 1420 m. a.s.l.). The climate of the region is subtropical serrano with little or no water deficit in January and February. Mean annual precipitation is 900 mm, concentrated in spring-summer with a prolonged dry season in winter. The average temperature is 23 °C (74 °F) in summer and 15 °C (60 °F) in winter [4]. The soil texture was loam (32% sand, 44% silt, 24% clay) with 2.91% organic matter. Soil belong to Ustochrepts udic as per USDA Soil Taxonomy, Cerrillos series with A, AC and C horizons [11]. The soil in which the experiments were carried out is a degraded soil resulting from 50 years of monoculture of tobacco and common bean (intensive tillage includes several, approximately 20–30 plows to the soi). The experimental design followed a randomized complete block design with three replications. Each plot independently had a type of cover crop (*Brachiaria brizantha* cv Mulato, maize (*Zea mays*), and no CC) seeded with common bean as cash crop in summer with the experimental plots measuring 15 m wide and 50 m long with 12 rows of *B. brizantha* cv. Mulato or maize in each plot. *B. brizantha* was sown with seed drill (sowing machine) at a dose of 4–5 kg/ha and then it was cut with a mower. The stubble was left on the surface. On the ground with stubble, no agricultural work was done, no machinery entered. At the beginning of the rains, the common bean was planted with seed drill, so no tilling was done during the experiment. The four treatments were: a) *B. brizantha/B. brizantha*/common bean (B2); b) common bean/*B. brizantha*/common bean (B1); c) common bean/maize/common bean (M); d) common bean/fallow/common bean: common bean monoculture (control). Sown density was 25 seeds m^{-1} and row width was 52 cm, or 2-3 kg/ha, being seeds inoculated with *Rhizobium* spp (Rizofos Liq Soybean) at a dose of 140 ml/20 g seed. Common bean was managed using recommended production practices, including only one tillage before sowing and pesticide applications (Dimetoato 40% p/v. EC Basf at a dose of 300 ml ha^{-1} and Carbendazim 50 (2-metaxicarbamoilbencimidazol) Nufarm Limited). Weeds were controlled using pre-emergent herbicide Pivot® H Basf (imazetapir 10,59%) and Dual Gold® (S-metolacloro: 96%p/v Syngenta at a dose of 400 ml ha^{-1} and 500 ml ha^{-1}, respectively. Thirty days after sowing, a new herbicide was applied Flex® (fomesafen: 25% p/v) Syngenta at a dose of 500 ml ha^{-1}. No chemical fertilizers were used during the growth of the common bean crop.

2.2. Soil sampling

Soil samples were collected at common bean R1 stage (beginning of flowering: plants present an open flower in any internode of the main stem) in summer (February), during the 2016 crop cycle. For microbial activity analysis, sampling was done by taking soil from the roots of 10 plants in a linear meter, which constitutes 1 composite sample. In total, 6 composite samples of rhizospheric soil were collected per experimental unit from 0 to 10 cm layer [12]. Roots were gently shaken to remove loosely adhering soil, placed in plastic bags and processed immediately. For each of the biochemical parameters measured, triplicate measurements were performed. For chemical analysis, the same soil employed for microbial activity analysis was used and a subsample of 10 g from each sample was stored at -20 °C until molecular analysis. Soil samples were sieved at field moisture (2 mm), homogenized, air-dried and stored at 4 °C for further analysis.

2.3. Soil chemical properties

The soil pH and electrical conductivity (EC) were measured at soil-to-water ratio of 1:2.5. Total C was determined by wet oxidation following the Walkley and Black procedure [13]. Because these soils are free of carbonates [14], the total C content is equivalent to the soil organic C (SOC) content. Total N and extractable phosphorus (eP) were determined by micro-Kjeldhal method [15] and Bray-Kurtz method [16], respectively.

2.4. Soil microbial activities

Microbial activity was estimated by hydrolysis of fluorescein diacetate activity (FDA), according to Adam and Duncan [17]. Briefly, 2 g of soil and 15 ml of 60 mM potassium phosphate buffer pH 7.6 were placed in a 50-ml conical flask. Substrate (FDA, 1000 mg ml^{-1}) was added to start the reaction. The flasks were placed in an orbital incubator at 30 °C and 100 rpm for 20 min. Once removed from the incubator, 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 447 × *g* for 5 min. Finally, the supernatant was filtered and measured at 490 nm on a spectrophotometer.

Acid phosphatase (AP) was assayed using 1 g soil, 4 ml 0.1 M universal buffer (pH 6.5), and 1 ml 25 mM *p*-nitrophenyl phosphate [18]. After incubation at 37 ± 1 °C for 1 h, the enzyme reaction was stopped by adding 4 ml 0.5 M NaOH and 1 ml 0.5 M $CaCl_2$ to prevent the dispersion of humic substances. Absorbance was measured in the supernatant at 400 nm.

Dehydrogenase activity (DHA) was determined according to García et al. [19]. Briefly, 1 g of soil at 60% field capacity was 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 INTF (iodonitrotetrazolium formazan) formed was extracted with 10 ml of methanol by shaking vigorously for 1 min and filtering through a Whatman No. 5 filter paper. INTF was measured spectrophotometrically at 490 nm.