



Incorporation of bean plant residue in soil with different agricultural practices and its effect on the soil bacteria

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ABSTRACT

Agricultural practices, such as crop residue management and tillage, change biological, physical and chemical soil characteristics. The aim of this study was to investigate the effect of the application of bean plant residue (*Phaseolus vulgaris* L.), one of the major crops in Mexico, on the bacterial community structure in soil cultivated conventionally (conventional beds, CB) or under a conservation agriculture system (permanent beds, PB) and compared it to the effect of maize plant residue application. Soil samples were incubated aerobically at ambient temperature ($22 \pm 2^\circ\text{C}$) for 56 days. The bacterial community in the unamended PB soil was significantly different from that in the unamended CB soil, e.g. Acidobacteria, PB > CB, while Actinobacteria PB < CB. Considering no priming effect, 44% of the applied bean plant residue was mineralized in the PB soil after 56 days and 78% in CB soil, while mineral N increased by 7.9 mg kg^{-1} in both the PB and the CB soil. *Bacillus*, Micrococcaceae and *Streptomyces* were the first most abundant degraders of the bean plant residue. They continued to participate in the degradation of the bean plant residue and/or its metabolic products until day 7 (Micrococcaceae) or day 28 (*Bacillus* and *Streptomyces*). Actinobacteria and TM7, known copiotrophs, were the most abundant after harvest while Planctomycetes and Verrucomicrobia, known oligotrophs, during the growing season. It was found that agricultural practices had a significant effect on the bacterial community structure and bean plant residue was degraded primarily by phylotypes belonging to *Bacillus*, Micrococcaceae and *Streptomyces*. Further research is needed to improve our knowledge on how changes in microbial community structures alter plant nutrient cycling.

1. Introduction

Subtropical highlands of the world have become densely populated and intensively cultivated. They need to produce more from soils with declining fertility and with limited land that still can be used for cultivation. Agricultural sustainability problems resulting from soil erosion and fertility decline have arisen throughout these agro-ecological zones due to conventional practices based on mechanical soil disturbance, monoculture and removal of crop residues (Romero-Perezgrovas et al., 2014).

In the central highlands of Mexico, the international maize and wheat improvement center ("Centro de mejoramiento de maíz y trigo", CIMMYT) has started experiments to study sustainable agricultural systems that can provide higher and stable yields and at the same time are more robust

against drought and more profitable for the farmer (Govaerts et al., 2005). Conservation agriculture (CA) based on reduced tillage, retention of adequate amounts of crop residues and use of crop rotation has been proposed as a viable alternative for conventional practices (CP) which depends on intense tillage, maize monoculture and crop residue removal (Romero-Perezgrovas et al., 2014).

Crop residue retention is a key component in sustainable cropping systems (Omotayo and Chukwuka, 2009). Crop residue left on the soil surface prevents soil erosion, reduces soil temperature and evaporation, increases water infiltration, improves soil structure and provides essential plant nutrients when it is decomposed (Kong, 2014). Consequently it reduces the farmers' dependence on inorganic N fertilizers (Abera et al., 2013).

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Maize (*Zea mays* L.) is the main food crop in Mexico (Ranum et al., 2014) and common bean (*Phaseolus vulgaris* L.) an important provider of proteins (Broughton et al., 2003). As such, they are two of the most important basic agricultural products. Consequently, large amounts of maize and bean plant residues are available and are either left in the field or fed to livestock, but sometimes burnt (Mwangi et al., 2013). Decomposition rates and nutrient release of crop residue depend on its composition such as N concentration (Mwangi et al., 2013), C/N ratio (Mubarak et al., 2002), and hemi(cellulose) and lignin content (Fosu et al., 2004).

Bean residue has a high N content and it contains large amounts of easily decomposable organic material making it ideal to improve soil fertility. Application of bean plant residue to soil will change the bacterial community structure as the relative abundance of some groups will increase by the organic material (copiotrophs) while others will become less abundant (oligotrophs) affecting nutrient cycling in soil. We wanted to investigate how different agricultural practices, i.e. CP versus CA on beds affected (i) the bacterial community structure in soil; (ii) mineralization of amended bean plant residue; (iii) the bacterial community structure when the bean plant residue was applied and (iv) compare the results obtained in this study, i.e. soil sampled in January 2014 after harvest, with an earlier study when maize plant residue were applied to soil sampled in October 2012 during the growing season (Ramírez-Villanueva et al., 2015).

It has to be remembered that the experiments were done under controlled conditions so that fluctuations in soil moisture content, UV solarization and soil temperature, factors that are known to affect organic material decomposition, were kept constant. Follow up experiments, might want to investigate how combining changes in soil water content and soil temperature affect microbial community structures.

2. Materials and methods

2.1. Soil sampling site

The sampling site was located in El Batán (Texcoco) (19° 31' N, 98° 50' W, 2250 m.a.s.l.) situated to the north-east of Mexico City. The climate at the experimental station is Am (Tropical monsoon) according to the Köppen climate classification system (Kottek et al., 2006) with mean maximum and minimum temperature of 24 °C and 6 °C, respectively (1991–2009) and the average annual rainfall is 625 mm y⁻¹, with approximately 545 mm falling between May and October. Short, intense rain showers followed by dry spells typify the summer rainy season. The soil at the experimental site is classified as a Haplic Phaeozem (Calyic) in the world reference base system (IUSS Working Group WRB, 2006) and as a fine, mixed, thermic Cumulic Haplustoll in the USDA Soil Taxonomy system (Soil Survey Staff, 2003).

Soil used in this study was collected from a rain-fed field experiment in January 2014. Two treatments were used in this study with crops cultivated on raised beds. The first treatment combined conventional practices (CB treatment), i.e. crop residue removed, maize-wheat crop rotation and furrows reshaped after harvest without disturbing the soil on top of the beds. The second treatment implied soil under conservation agriculture system, i.e. permanent beds with crop residue management and crop rotation. More details of the experimental site and management, e.g. seeding and fertilizer application, can be found in Govaerts et al. (2008) and Ramírez-Villanueva et al. (2015).

The soil was cultivated with maize in the growing season before sampling. The 0–20 cm deep layer was sampled 20 times from two plots (6 m × 20 m) with a 2 cm auger. Soil from each plot was pooled so that two soil samples were obtained from each of the two treatments. The sampling procedure is schematized in Fig. S1.

2.2. Cultivation of the bean plants

Bean seeds were surface-sterilized with 1.5% (v/v) sodium

hypochlorite for 12 min and washed thoroughly with sterile distilled water. Seeds were germinated on 0.8% agar-water plates to induce etiolation and incubated in the dark at 28 °C for 48 h. The bean seedlings with roots of approximately 2 cm were placed on sterilized soil in the PVC growth chamber (10 L cylinder) and moistened with a sterile water and nutritive Steiner solution (1961). After 20 days, the whole bean plants (roots and shoots) were harvested, air-dried, finely grinded by a mortar and pestle, and characterized. The bean residues were analysed according to the Van Soest method (Van Soest, 1963; Van Soest and Wine, 1967). Details of the hot extraction with neutral detergent solution and determination of the plant characteristics can be found in Ruiz-Valdiviezo et al. (2010).

The C concentration of the bean plant residue was 353 g kg⁻¹ and the N concentration 28 g kg⁻¹. The lignin concentration of the bean plant residue was 55 g kg⁻¹, the (hemi)cellulose 164 g kg⁻¹, the soluble fraction (the whole material minus the neutral detergent fiber) 607 g kg⁻¹, polyphenol concentration 28 g kg⁻¹ and the ash content 201 g kg⁻¹.

2.3. Experimental design and aerobic incubation

The collected soil was taken to the laboratory, passed through a 5 mm sieve and characterized. The soil samples were analyzed for total carbon (C) and nitrogen (N), electrolytic conductivity (EC), pH, particle size distribution and water holding capacity (WHC) as described by Aguilar-Chávez et al. (2012).

The rest of the soil was adjusted to 40% WHC with distilled water and pre-incubated for a week at ambient temperature (22 ± 2 °C). The soil samples were separately (< 10 kg) placed in the 70-L drums containing a 1-L jar with 1 mol L⁻¹ NaOH to trap evolved CO₂ and a 5-L jar with distilled water to avoid desiccation during storage.

Fourteen sub-samples of 25 g soil from each plot (n = 2) and treatment (n = 2) were added separately to 120 mL glass flasks. Seven sub-samples were amended with 95 mg bean residue and seven were left unamended. The amount of residue applied was such that 2 g C kg⁻¹ was applied to soil.

Each flask was placed in a 1-L glass jar containing a 25 mL flask with 20 mL 0.5 mol L⁻¹ NaOH to capture the evolved CO₂ and a 25 mL flask filled with distilled water to avoid desiccation of the soil during incubation, and closed air-tight. All the samples were incubated at ambient temperature (22 ± 2 °C). After 0, 1, 3, 7, 14, 28 and 56 days, the jars were opened, the 25 mL flask with 0.5 mol L⁻¹ NaOH taken out and analyzed for CO₂ (Jenkinson and Powlson, 1976). The soil was removed from the flask, 6 g was extracted for DNA as described below, while the rest was extracted for mineral N (NH₄⁺, NO₂⁻ and NO₃⁻) with 100 mL 0.5 mol L⁻¹ K₂SO₄. The K₂SO₄ extract was then analyzed on a San Plus System—SKALAR automatic analyzer (Skalar, Breda, the Netherlands) (Mulvaney, 1996).

2.4. DNA extraction and PCR amplification of bacterial 16S rRNA genes

The soil samples (each 0.5 g) were washed with 0.15 mol L⁻¹ sodium pyrophosphate and 0.15 mol L⁻¹ phosphate buffer pH 8 to remove the humic and fulvic acids (Ceja-Navarro et al., 2010). Three different techniques were then used to extract the DNA from the washed soil. Each technique was used to extract four times 0.5 g soil (a total 2 g soil) and pooled. As such, a total of 6 g soil was extracted for DNA per plot and 12 g soil per treatment. The first method used was based on the technique described by Valenzuela-Encinas et al. (2008) and consisted in a chemical and thermal shock for cell lysis. The second method was developed by Sambrook and Russell (2001) and cells were enzymatically lysed, while the third method used a detergent solution and mechanic disruption for cell lysis as described by Hoffman and Winston (1987).

Details of the primers used, and amplification, PCR and purification of the extracted DNA are given in Navarro-Noya et al. (2013) and

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