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Introduction of Faba bean in crop rotation: Impacts on soil chemical and biological characteristics

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ABSTRACT

Agricultural practices such as crop rotation affect soil physical, chemical and biological properties. Legumes crop effect has been shown to provide several agro-ecological services as a cereal previous crop. The aim of the present field study was to estimate the middle term effects of introducing faba bean in crop rotation on the structure and function of soil microbial communities. Two experimental rotation systems were tested (i) Wheat-Beet-Faba Bean-Rape-Wheat (**Leg**$^{+}$) and (ii) Wheat-Flax-Wheat-Beet-Wheat (**Leg**$^{-}$). Soil samples were collected on tilled plots at 0–10 cm depth on July 2013 under wheat. Soil microbial biomass and soil enzymatic activities (β-glucosidase, cellulase, urease and arylamidase activities) were assessed. Soil microbial diversity was evaluated with two complementary approaches: Phospholipid fatty acid profiling (PLFA) and the metabolic capabilities of the microbial community (Biolog Ecoplates). Soil organic carbon and total nitrogen were significantly and respectively 1.5 and 1.3 times higher in faba bean's rotation compared to free faba bean rotation. Soil microbial biomass did not differ significantly between the two rotations. In general, **Leg**$^{+}$ rotation resulted in the greatest carbon mineralization and β-glucosidase and arylamidase activities. The analysis of Biolog data and PLFA profiling indicated that the rotation including faba bean has modified microbial populations and induced differences in the catabolic capability of soil microbial communities. Our results suggested that changing rotation crop by introducing faba bean two years before wheat modifies the surrounding habitat of microbial communities by providing available carbon and nitrogen as well as suitable soil pH. This new habitat could impact the structure of microbial communities and their functions. **Leg** $^{+}$ rotation seems to be a suitable practice promoting microbial activities in agricultural plots.

1. Introduction

In Europe, numerous innovative practices have emerged to reduce the impact of agriculture on climate and environment changes with a focus of scientists on the way crop rotation can be designed. Indeed, a well designed crop rotation can contribute to weed control and decrease in diseases and pest attacks (Bagayonko et al., 1992) leading to a reduction in the use of pesticides. Furthermore, plant species present in crop rotation influence the soil water holding capacity by reducing soil erosion (Kollas et al., 2015). Diversification of crop along rotations plays an important role in the amount and quality of organic matter entering the soil (Raphael et al., 2016). According to the species identity, the mineralization of preceding crop residues can release important quantity of nutrients, which maintain soil fertility for the following one and create suitable habitats for soil biota (Askegaard and Eriksen, 2007; Sauvadet et al., 2016). In this context, embedding grain legume in crop rotation has been shown to provide multiple environmental, agricultural and economical benefits. In fact, these plants have the ability to symbiotically fix atmospheric N_2 through their association with *Rhizobium* bacteria, leading to potential decrease in the use of inorganic N amendments and thus reduce fossil energy consumption for plant production (Hardarson et al., 1991; Lopez-Bellido et al., 2006; Turpin et al., 2002). Furthermore, some legumes have a greater ability to mobilize P from less labile P forms than cereals (Kamh et al., 1999; Nuruzzaman et al., 2005a, 2005b). Finally, legumes act as an important source of protein for human and livestock feed and improve gross margins of full crop rotation (Jensen et al., 2010; Khan et al., 2010; Kopke and Nemecek, 2010; Preissel et al., 2015). In the other hand,

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rotation including legumes at least for one time, can influence soil microbial communities directly (Bunemann et al., 2004; Voisin and Gastal, 2015) and indirectly through their effects on the quantity, the quality, and the distribution of soil organic matter in upper-soil horizons. Such systems tend to have higher microbial biomass and activities (Moore et al., 2000). Melero et al. (2012) also showed a greater amount of soil organic carbon, total N and β-glycosidase activity in legumes-wheat rotation under no tillage system than in continuous wheat and fallow-wheat rotation. Lupwayi et al. (1999) demonstrated that wheat plots preceded by legume crops had a higher microbial biomass and a lower qCO_2 than wheat proceeded by summer fallow. Moreover, the presence of legumes in crop rotation, through its implication in nitrogen fixation, promote the activity of nitrogenase enzyme and the release of hydrogen gas (H_2), which induces the multiplication of some specific microorganisms in soil (microorganisms which use H_2 as energy sources) (Dong et al., 2003). Nevertheless, if the beneficial effects of introducing legumes as wheat's previous crop have been clearly highlighted, a serious knowledge gap remains concerning its medium-term effects on soil characteristics (biotic and abiotic) during crop rotation *i.e.* are the benefits of legumes likely to be sustained beyondone crop? This lack of knowledge limits the agricultural community to diversify the technical solutions, such as identifying a panel of possibilities to built crop rotations around the presence of legumes, for the sustainable management of soil fertility.

The present study was thus conducted to examine the persistence of faba bean impacts on wheat cultivation when faba bean was grown two years before wheat. We hypothesized that the crop rotation including faba bean could affect soil physicochemical properties and microbial communities by changing the diversity of these communities and their enzymes activities. To reach these objectives, we used a single-site agricultural assay designed for the evaluation of agronomic and ecological effects of faba bean position along crop rotation. It consisted in replicate comparisons of rotations with and without faba bean placed two years before a wheat culture within which soil was sampled for chemical analysis evaluation microbial communities diversity and soil functioning. We assessed both total organic carbon and nitrogen and the amount of active carbon. Microbial biomass was used to estimate the abundance of soil microorganisms and enquire us about modifications due to soil management. Molecular approach, using RNA and DNA co-extraction and real-time PCR, provided information about active bacterial and fungal community under the two studied rotation. Phospholipids fatty acid (PLFA) profiling were chosen to estimate the relative diversity of microbial community in sampled soil. Functions of microbial community were evaluated by enzymes measurement and soil carbon mineralization. The activities of enzymes involved in C and N cycle were studied, some of which have been previously shown to rapidly respond to soil perturbations and modifications of agricultural practice (Lebrun et al., 2012). Finally, the community-level physiological profile was employed to estimate bacterial functional diversity.

2. Materials and methods

2.1. Site, rotations and soil sampling

The study site was located in north western France and belongs to the Institute of Plant Arvalis in Rots (Calvados, Normandy) (N49°12′05.74", W 0°27′13.08"). The mean annual precipitation is 712 mm spread over 7 months from October to April and the mean annual temperature is 10.5 °C. The region has a maritime climate with low thermal amplitude during the year. The experimental site had a silt loam texture and was chosen to evaluate agronomic and ecological effects of the position of faba bean in crop rotation. The soil was classified as a Calcisol (FAO classification). The experimental design consisted in 8 plots (12 × 90 m) of two rotations with four replicates. Two rotation systems were examined with different position of leguminous in crop succession: (i) common rotation in Normandy area: Wheat-Flax-Wheat-Beet-Wheat (**Leg**$^-$), (ii) innovative rotation including legumes: Wheat-Beet-Faba Bean-Rape-Wheat (**Leg**$^+$). Sampling was performed in the final wheat crop of each rotation, corresponding to spring wheat. The spring wheat plots had been tilled in March and composite sample was collected in July, in the middle of the growth season, by combining five soil cores (0–10 cm) under spring wheat cover in order to homogenize rhizospheric properties.

2.2. Measurement of soil properties

Topsoil samples were air-dried and sieved at 2 mm for soil chemical and physical analyses. Soil pH in H_2O and in 1 M KCl was measured with a glass electrode in 1:2.5 suspension (NF ISO 10390, 2005), ΔpH ($\Delta pH = pH_{H2O} - pH_{KCl}$) was determined. For a given soil type, this index is positively correlated with base saturation (Baize, 2000). The cation exchange capacity (CEC) was determined using cobalt hexamine trichloride according to NF ISO 23470 (2007). Moisture content was recorded after drying at 105 °C for 48 h. Five grams of air dried soil were used to determine soil textural. Soil samples were firstly treated by HCl solution to remove soil carbonate then H_2O_2 33% was added to eliminate soil organic matter and finally hexametaphosphate solution was used to break up the soil particles. References concentration was done according to NFX-31-107. Particle size distributions (%) were measured by laser diffraction (Malvern Mastersizer 2000, UK) according to manufacture protocol. Total carbon (TC) and total nitrogen (TN) were quantified by a dry combustion method using an automatic analyzer (Flash2000 Thermo Scientific). The Organic Carbon (OC) was measured with TOC-Analyzer (Shimadzu TOC- SSM 5000). Permanganate Oxidizable Carbon (eq. Active carbon, POXC) was extracted from 2.5 g of air dried soil. Quantity of carbon oxidized by MnO_4 was measured as described by Culman et al. (2012) using spectrophotometer (Varian Cary 50 Scan) at 550 nm.

2.3. Structure of microbial community

2.3.1. Microbial biomass carbon

The microbial biomass carbon (MBC) in soil was determined by the chloroform fumigation-extraction method described by Vance et al. (1987) with some modifications. Briefly, sixty grams of field-moist soil were separated into two beakers. The first one was fumigated with ethanol-free chloroform for 24 h in dark conditions and the second was used as a control (same conditions without $CHCl_3$ fumigation). The extraction of Soluble Organic Carbon was performed from fumigated and non-fumigated soil samples with K_2SO_4 (0.5 M). In the extracts, carbon content was measured with a total organic C analyzer (Shimadzu TOC-V_{CSH}). MBC was calculated as the difference in C content in fumigated and non-fumigated sample without using a correction coefficient.

2.3.2. DNA/RNA co-extraction and quantitative reverse transcription qPCR

RNA and DNA were co-extracted from 2 g soil using the Total RNA isolation Kit® and the DNA Elution Accessory Kit® for Soil, respectively (MO BIO-USA). Extracts were stored at −80 °C for RNA and −20 °C for DNA until use. To remove contaminating DNA from RNA preparation, TURBO DNA-free™ kit (Applied Biosystems, France) was used and RNA extracts were further purified with the RNeasy® MinElute Cleanup kit (Qiagen Gmbh, Germany) according to the manufacturer's protocol using silica spin columns. Purified nucleic acid extracts were eluted in a final volume of 15 μL with DEPC-treated water and stored at −80 °C. The quality of RNA was assessed by Experion™ RNA StdSens Analysis Kit (BIO-RAD) according to manufacturer's recommendation (Laroche-Ajzenberg et al., 2012).

Purified dsDNA and RNA were quantified by fluorimetry. dsDNA quantification was operated by using the Fluorescent DNA quantitation Kit (Hoechst 33258, Biorad) and dsDNA extracts were stored at −20 °C (Gangneux et al., 2011). RNA was quantified using Quant-iT™