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## Economic and Environmental Analysis of Maize Inoculation by Plant Growth Promoting Rhizobacteria in the French Rhône-Alpes Region<sup>★</sup>



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#### ABSTRACT

In the context of global agricultural challenges, plant growth-promoting rhizobacteria from the genus Azospirillum used to inoculate cereal seeds have attracted a lot of attention, mostly for their expected agronomic benefits. This study assesses the economic impact on farmers' profit from maize inoculation, along with the environmental impact in term of greenhouse gas emissions, in the Rhône-Alpes region of France. Yield-to-nitrogen relationships predicted by the STICS crop model were modified to simulate the effect of inoculation on maize yield, and four inoculation costs (0, 20, 40 and 60 euros/ha) were considered to assess the private economic effect of inoculation. The environmental impact was assessed using a Bouwman-type N<sub>2</sub>O emission function, modified to account for the inoculation effect on soil N<sub>2</sub>O production according to soil characteristics. Yield and N<sub>2</sub>O emission functions were then implemented in the AROPAj farm-type, supply side-oriented model. Several scenarios were considered and probability density functions were selected for the yield function parameters according to field trial results. Our results show that, mainly due to a decrease in fertilizer use rather than to an increase in yield, farming systems in the Rhône-Alpes region could benefit from maize inoculation if the inoculation cost is lower than €20/ha. Regardless of inoculation cost, maize inoculation might reduce the amount of synthetic fertilizer used on farmlands. However, the private economic benefits could be lower if maize is cultivated on soils with a high carbon content, where inoculation increases N2O emission. In these types of soils, greenhouse gas emissions at the regional level could increase by 2 to 5%.

#### 1. Introduction

In France, maize is the second largest crop after wheat, accounting for 25% of the country's cereal production (Agreste, 2016). In southern regions of France, irrigation and mineral nitrogen (N) fertilization are essential to meet high yield objectives. At the same time, farmers face major challenges if they are to achieve sustainable agriculture less dependent on chemical inputs. They are therefore further exploring the possibility of using biotic interactions to promote the performance of agroecosystems under lower chemical inputs (e.g. Barot et al., 2017).

In this context, plant growth promoting rhizobacteria (PGPR) have attracted a lot of attention over the past 40 years. These bacteria, naturally present in the soil, are able to sustainably enhance plant growth when associated with specific cash crops such as soybean, maize and wheat (Vacheron et al., 2013). PGPR from the genus *Azospirillum*, first described in 1925 by Beijerinck (1925), have been extensively

studied all over the world for their potential to improve crop growth. Their possible agronomic importance was noticed when Day and Döbereiner (1976) described their association with plants from various geographical origins. Pereg et al. (2016) show that the genus Azospirillum currently includes 12 species. Azospirillum lipoferum and Azospirillum brasilense are the two species most used for inoculating cereals.

With regard to maize inoculation, a number of field trials have shown that *Azospirillum* enhances root growth and increases leaf area and shoot dry matter in many cropping systems around the world (Marini et al., 2015; El Zemrany et al., 2006; Mehnaz and Lazarovits, 2006; Okon and Labandera-Gonzalez, 1994). However, the increased crop growth does not necessarily lead to increased crop yield. As a result, no economic analysis of the effect of inoculation by *Azospirillum* has been conducted so far. Moreover, no previous studies have addressed the possible environmental effect of such inoculation, in particular as regards greenhouse gas (GHG) emissions. In this context, we

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here investigate the effects that inoculation has on N fertilizer demand, farmers' economic profit and GHG emissions in a specific French region.

The first objective of this paper is to assess the economic impact of maize inoculation by Azospirillum based on realistic scenarios of its effect on yield. The main proxies used to assess the effect on farmers' profit are the possible influence of the PGPR on maize yield and its inoculation cost. The second objective of the study is to evaluate the environmental impact of inoculation on nitrous oxide (N2O) emissions from cropland soil. To better guide the development of scenarios and model parameterization, field trials with inoculated and non-inoculated maize were carried out at three sites in the Rhône-Alpes region of France, with measurements of both yield and potential soil N2O production for each site and treatment. For the economic and environmental analysis, we used a combination of the most recent version of the mixed-integer linear programming model AROPAj (Jayet et al., 2017) and outputs generated by the crop model STICS (Brisson et al., 2003). After generating multiple possible scenarios, we used a density probability function of the parameters of interest in the yield function, along with expert information on N2O emissions, to simulate the most likely outcomes of the economic and environmental effects of maize inoculation by Azospirillum.

## 2. Information Used to Guide Scenario Development and Model Parameterization

## 2.1. Inoculation Effect on Yield Reported in the Literature and in Field Trials

We used two types of information to guide the development of scenarios regarding inoculation effect on maize yield.

Firstly, we screened the results from previous studies reporting an inoculation effect. For most studies, the change in maize yield due to inoculation was not statistically significant or not consistent between situations (Müller et al., 2016; Marini et al., 2015; Sangoi et al., 2015; Rangel Lucio et al., 2014; Mehnaz et al., 2010). When an increase in yields was observed, it ranged from 3 to 25% (Dadnia, 2011; Faramarzi et al., 2012; Sharifi et al., 2011).

Secondly, field experiments were conducted in the Isère department of the Rhône-Alpes region. Three experimental sites located along a *catena* within the Isère-Porte-des-Alpes territory, southeast France, were monitored in two consecutive years: a site in a valley (V site; 45°37′ N, 5°16′ E), a site on a slope (S site; 45°56′ N, 5°33′ E), and a site on a plateau (P site; 45°28′ N, 5°14′ E). Site characteristics are presented in Table 1. The experiment was set up as a randomized block design with five blocks and treatments randomly assigned to one plot in each block. Maize (*Zea mays*, cv. Seiddi) seeds were inoculated with *Azospirillum lipoferum* CRT1 previously isolated from the rhizosphere of maize field-grown in France (Fages and Mulard, 1988). The inoculum load targeted was 10<sup>6</sup> CFU (colony-forming unit) added per seed for inoculated plants (I), coated in a commercial peat-based Azo-GreenTM formulation (Agrauxine, Beaucouzé, France). Coated, but non-inoculated, seeds (NI) were used as controls.

For inoculated and non-inoculated situations, four N rates were applied in 2014 to assess the effect of N combined with inoculation on maize yield (Florio et al., 2017). The N rates applied were 40, 80, and 120 units of N per hectare, and no N application was used as the

control. In 2015, only two N rates were chosen (0 and 60 U of N/ha). The maximal amounts of N fertilizer were chosen to be close to optimal N availability, taking into account the N fertilizer recovery efficiency by maize the previous year.

As in previous studies, the effect of inoculation in these field trials was not clear and consistent. At S site, maize yield on NI plots ranged from 96 kg/ha without fertilization to 110 kg/ha with 120 N-units. Hence, *Azospirillum* tended to have the desired effect in site S, with an increased yield of around +8 kg/ha at any N-rate. However, at P site, the PGPR did not have any significant effect on maize yield. Moreover, at V site, NI maize had a higher yield at all N rates than I maize (i.e. -6 kg/ha for I as compared to NI plots).

Therefore, neither previous reports nor the results of these field trials provide clear and consistent patterns of inoculation effect on the yield of maize, which could be used to assess the economic impact of the inoculation practice. We thus decided to create a range of randomized realistic scenarios of the possible effect of inoculation on yield using the dose (N)-response function generated by the STICS crop model (Section 4.1).

## 2.2. Inoculation Effect on Potential $N_2O$ Production Reported from Field Trials

On the plots studied at each of the three experimental sites in the Isère region, we also measured the potential CO2 and N2O production from soil through respiration and denitrification, respectively (no further information is available in the literature). However, no consistent pattern of inoculation effect on potential CO2 production could be detected (Bérard et al., personal communication, January 2017). Therefore, a possible inoculation effect on soil CO2 emission was not considered in this study. Each year, six soil samples were collected on each plot at the 6- and 12-leaf stage. The six soil samples were pooled, sieved using 2-mm mesh size, and stored at +4 °C a few days before activity measurements. Potential N2O production rate was measured in line with Patra et al. (2005) using fresh soil sub-samples (10 g dry weight equivalent soil). It was determined as the linear rate of production of N2O during short-term (8 h) incubation under anaerobic conditions using a gas chromatograph (µGC R3000, Santa Clara, CA, USA). Activity measurements were carried out at the AME platform (Microbial Ecology UMR1418, Lyon).

Our results (Florio et al., 2017) show that the inoculation effect varied greatly between sites, but was consistent between sampling times: potential N2O production was stimulated by inoculation up to > 80% at S site, but was slightly decreased by inoculation down to - 8% at P site. In fact, soil carbon (C) availability is known to be a major factor driving denitrification in cropping systems (Attard et al., 2011). In this context, we observed that the inoculation effect on the potential  $N_2O$  production rate was affected by soil organic C and to a lesser extent by nitrate content (Florio et al., 2017). In particular, the higher the soil C content, the greater the increase in potential N2O production by inoculation, due to the stimulating effect of maize root functioning on denitrifier abundance (Florio et al., 2017). Conversely, the lower the soil C content, the greater the decrease in potential N<sub>2</sub>O production by inoculation, likely explained by increased competition between roots and denitrifiers for nitrate. These results were used to elaborate the scenarios for the possible effects of inoculation of soil N2O

**Table 1**Soil characteristics of the experimental study sites.

Site name	Soil type (WRB)	Altitude (m asl)	Texture			pН	Organic matter content (%)	Total N content (g N kg <sup>-1</sup> )
			Clay (%)	Loam (%)	Sand (%)			
S	Fluvic Cambisol	279	34.7	38.3	26.9	7.05	5.43	3.4
V	Calcisol (siltic)	217	10.3	74.1	15.6	8.16	4.46	3.1
P	Luvisol (skeletic)	535	14.2	42.9	42.9	7.25	3.7	1.6

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