Effect of physico-chemical characteristics of agricultural soils on fungal biomass (FB) – Impact of copper pollution

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INTRODUCTION

Agricultural soils, and specifically cropped soils, constitute ecosystems highly disturbed by human activities. The agricultural management of soils has a great impact upon the functional process of soil microbial communities, particularly on fungal biomass (FB). In that context, our objective was to define the equilibrium status of the soil in order to estimate how pollutants can modify this reference status. For that purpose, we measured the variability of 40 physicochemical and biological quantitative descriptors in soils from cropped and meadow plots during three years, using field experiments and microcosms of undisturbed soils (for contaminated soils).

This study focuses on fungal community and the impact of copper contamination on this microbial compartment.

MATERIAL AND METHODS

Study sites

Field plots on silty soils are located in north-western France (Yvetot - Normandie). There are a permanent meadow (M) and a traditional crop (C) where the pedoclimatic conditions supported the development of an intensive agriculture. The climate is mild with an average rainfall of 800 to 900 mm and low seasonal ranges. The field plots characteristics are summarized in the Table 1.

Sampling

Fields sampling have been realized in order to observe temporal impact. The samples were collected in April, June, August and October from the layers 0-10 cm of each field plot. Each descriptor was measured at each date for both soils (200 samples).

We developed microcosms (Fig. 1) fitted with columns of undisturbed soil from the previous fields to assess the ecotoxicity of chemical pollutants and carried out the experiment for 2 months (60 samples, 3 dates). These microcosms were settled in the ground under external conditions (climate, temperature, moisture and pressure). Results of microcosm experiments: 45 columns of each agricultural soil (15 references, 15 inoculated with Cu to obtain 2 ppm content, 15 inoculated with Cu to obtain 200 ppm content). All samples of each situation were analyzed after 7, 21 and 60 days. All samples (fields and microcosms) were kept at 4°C one night until analyze, were sieved (2 mm) in the field moisture condition. All values are expressed on a dry weight basis (100°C, 24 h).

Determination of fungal biomass: 5 descriptors

The FB has been measured by using several approaches in this work. (i) genetic using real time PCR (18S rDNA) (Gangneux et al. 2004) (ii) chemical, by extraction and quantification of total ergosterol (Legras et al. 2004), free ergosterol (Gong et al. 2001) and specific PLFAs (C16:2w6,9, C16:2w8.9). 18S rDNA targets conserved sequences of DNA in the fungi kingdom. Free ergosterol has been extracted without saponification and seems to quantify the free molecules of ergosterol in soil. They are supposed to come from fungal cell membranes in degradation. Total ergosterol is obtained by Micro waves assisted Extraction and with saponification. This descriptor take into account the ergosterol molecules present in the form of ester within the viable membranes of the cells. Specific PLFAs are extracted by SPE columns and resulting FAMEs are especially for FB, using that unusual setup of microcosms - Total nitrogen and carbon, apparent density and pH are major variables in the behavior of the FB, - Agricultural practices constitute the main factor,

The ANalysis Of Variance of the whole data enable us to classify the different factors affecting FB:
- Whatever the approach considered (18S rDNA, ergosterol, PLFAs) very significant correlations (Table 2) have been found indicating that molecular and chemicals protocols are relevant to access FB. Moreover, the results show strong rank correlations (ergosterol vs C16:1, ergosterol vs 18S rDNA and linear correlation (ergosterol vs C18)).

RESULTS AND DISCUSSION

The analysis of all fields data shows that all the descriptors of fungal compartment are relevant to discriminate two contrasted agricultural soils. Fig. 3 indicates that the fungal biomass in the meadow is higher than in the crop whatever the descriptor (same results for free ergosterol and C16:2) throughout the experiments. In the same way, their spatial variations are less in crops than in meadows (strong fungal contents with a strong variability in the meadows, and less fungal contents in crops with more constant values).

At one point, this difference can be explained by having at the same time, the presence of grass, a moisture and a rhizosphere more significant in the meadows. Moreover, the crops had been plowed, they were drier, the plant cover and the rhizosphere were under development.

There is strong correlations, dot per plot, between physico-chemical variables and FB. It seems that C, N and the pH play a significant part in the contents of FB. Nevertheless, the temporal evolution of fungal community in the microcosms compared to the copper impact revealed that its impact is not significant (Fig. 4).

REFERENCES


Table 1. Means of physico-chemical characteristics of the soils from Yvetot. Permanent meadow and intensive crop.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Meadow</th>
<th>Crop</th>
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<tbody>
<tr>
<td>pH</td>
<td>5.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Temperature</td>
<td>14.5°</td>
<td>12.4°</td>
</tr>
<tr>
<td>Humidity</td>
<td>88.2%</td>
<td>80.7%</td>
</tr>
<tr>
<td>Moisture</td>
<td>14.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Clay</td>
<td>2.25</td>
<td>2.38</td>
</tr>
<tr>
<td>Organic matter</td>
<td>18.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Date</td>
<td>June</td>
<td>June</td>
</tr>
</tbody>
</table>

Table 2. Correlation coefficients (r) and p-value. (a) linear correlations (Pearson), (b) rank correlations (Spearman).

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>ergosterol</th>
<th>18S rDNA</th>
<th>C16:1w5</th>
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<tbody>
<tr>
<td>r</td>
<td>0.796*</td>
<td>0.607*</td>
<td>0.562*</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.000</td>
<td>&lt;0.000</td>
<td>&lt;0.000</td>
</tr>
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</table>

Figure 1: Microcosm setup – inoculation of copper at T0 – Before analysis

Figure 2: Total ergosterol contents in the M and C plots

Figure 3a: Total ergosterol contents in the M and C plots

Figure 3b: 18S rDNA contents in the M and C plots

Figure 3c: FAME of C16:1w5 contents in the M and C plots

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Figure 4 Contents of fungal biomass. (18S rDNA and (2) total ergosterol, in the field at T=0 days and microcosms (without, with 2ppm or 200ppm of copper) for the both agricultural practices at T=7 days and T=60days.

Figure 5: Impact of copper on the fungal biomass