TOOL WORKSHEET NO.18

Fungal Molecular Biomass

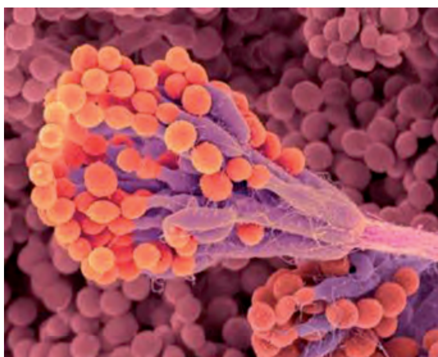
estimated by quantification of rDNA18S

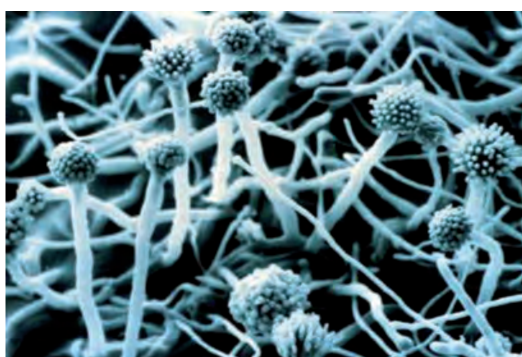
Christophe Gangneux, Esitpa / Unité Agri'Terr / Equipe BioSol
Contact : **cgangneux@esitpa.fr**

DESCRIPTION OF THE INDICATOR

Name of the indicator: Fungal molecular biomass estimated by quantification of ribosomal DNA specific to fungi.

Ecological role of the organism under test: Fungi comprise all eukaryotic heterotrophic thallophytes. They dominate numerically and in mass in most soil ecosystems. They harvest the amount of carbon necessary to their metabolism by symbiosis or absorption in their environment. Their filamentous growth enables them to create large networks within soils and to transport carbon compounds, nutrients and information over long distances.





They are usually subdivided into three groups in accordance with their diet: saprophyte fungi (organic matter decomposers), symbiots (commensal or mutualistic) and parasites. The specific enzymatic procession of fungi (laccase, peroxidase lignin, cellulose…) makes them dominant decomposers of organic matter held in soils, including organic pollutants. Products of degradation are mineralised, i.e. bioavailable for plant feed or transformed into humic compounds.

Type of indicator: Biomarker of effect and exposure. Fungal molecular biomass varies according to different types of influences:

- Anthropic impacts linked to tillage or presence of pollutants which cause a decrease in total fungal biomass.
- Type and age of vegetation settlement which act qualitatively and quantitatively on fungi.

DESCRIPTION OF THE SAMPLING METHOD

Reference standards and/or protocols

No reference standard or norm is currently available for this soil marker. The protocol has been published in Soil Biology & Biochemistry (Gangneux et al., 2011).

Sampling plan and method: Depending on the question asked and the scale of work (plot, landscape, territory...), the sampling plan is to be adapted. Generally speaking, sampling consists in collecting approximately 1.5 kg of soil using an auger on the surface horizon. These samplings have to be treated as quickly as possible but can exceptionally be preserved 2 days maximum between 4°C and 10°C before treatment.

Storage and pre-treatment of samples: Soil samples (about 1.5 kg) are sieved/homogenised to 2 mm. Total microbial DNA extraction is immediately conducted. Extracted DNAs can be conserved under -20°C for decades.

Stored DNA samples are diluted at 2 ng/µl prior to real time PCR analysis.

Simplified description of the measurement method: The amount of fungal DNA is estimated by real time PCR by using a couple of specific universal primers of Fungi according to Gangneux et al. (2011). A series of dilutions containing known amounts of genomic DNA of Fusarium graminearum is used as a standard for the quantification of DNA samples.

Estimated time: Eight hours are required by series of 45 samples from the dilutions to the analysis of results.

Measured parameters: Fungal molecular biomasses measured are expressed in µg of fungal DNA per g of dry soil.

INTERPRETATION OF RESULTS

Need for a global reference system using a database:
Scientific publications stating the quantification of fungal molecular biomass by real time PCR are increasing in number but remain rare. There is no frame of reference in the strict sense for this indicator today but threshold values and orders of magnitude are available in said publications, mostly for cultivated or forested plots.

Database availability/access:
There is no database for this biomarker.

Necessary supplementary information (ex: climate, use, type of soil…):
The pedological context exerts strong influence on fungal biomass and especially the content in organic matter, pH, soil texture, type of vegetation in place, as well as the type of tillage or organic amendments for agricultural plots. Climatic and seasonal conditions also have a prime influence on the biomarker. In a nutshell, it is preferred to conduct samplings in the spring, long after a frosting period and tillage, even superficial (≥ 6 months) or a provision of organic matter.

EXAMPLE OF APPLICATION

Metaleurop site: Soil use.

The Metaleurop site presents two types of uses of soils: 3 cultivated plots and 4 forested plots. Furthermore, these plots, situated near the operation site of a former lead foundry, present gradients of contamination by metallic trace elements (ETM), as shown by the figure below:

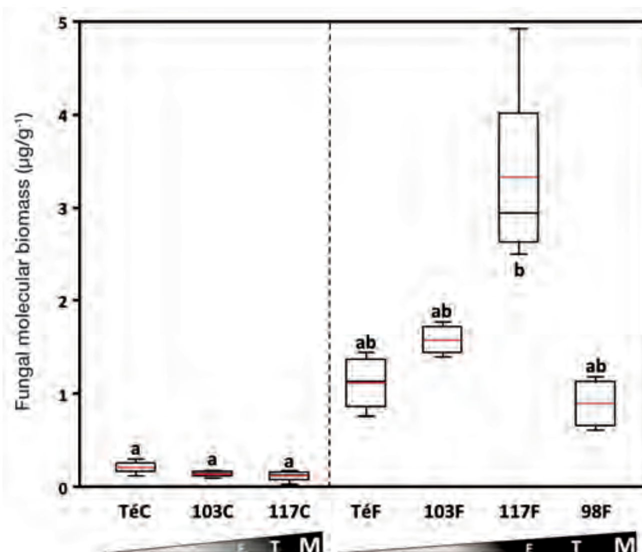
TéC & TéF

103C & 103F

117C & 117F

98F

The quantification of fungal molecular biomass for these plots showed the following results.



Fungal molecular biomasses measured by quantitative PCR targeting the fungi-specific sequences of rDNA 18S (Gangneux et al., 2011). Each box plot shows minimum and maximum values (⊥, ⊤), the first and third quartiles ( ), the median (—) and the average (—) measured for each plot. The gradients of soil pollution (◢) by metallic trace elements (ETM) are represented by type of soil occupation [cultivated plots (x…xC) or forested plots (x…xF)]. Distinctive letters (a, ab, b) indicate a significant difference to the $p<0.05$ threshold (Kruskal-Wallis test).

Results clearly highlight the differences in sets of practices:

The plots of the MetalEurop site show three ranges of values (TéC-103C-117C; TéF 103F-98F; 117F) distributed across 3 significantly different statistical classes. The key to differentiate between the modalities of this site does not present any link to the more or less important presence of metallic pollutants (zinc, lead, cadmium) if we refer to the established gradient of pollution (98F>>117F=117C>103F>103C>TéF=TéC). However, accordingly to data from bibliography (Plassart et al., 2008; Gangneux et al., 2011), cultivated plots display the lowest fungal biomasses on this site compared to values achieved on forested plots. From a statistical point of view, the 117F plot shows the most important fungal biomass, significantly different from those measured in all the other plots, and especially the forested plots. The recent conversion (2001) of this plot to a "forest" could explain this fungal abundance because Trap et al, (2001) have shown a transitory increase of fungal biomass in superficial horizons 15 years after the planting of a forest before a return to equilibrium 50 years later.

INTERESTS AND LIMITS OF THE INDICATOR

- \+ Integrates all factors modulating soil fungal biomass.
- \+ High sensitivity to changes in cultivation practices and types of soil occupation (tillage, mode of occupation).
- – The frame of reference of fungal biomass in contrasted pedoclimatic contexts is being built but still lacks universality because of a scarce amount of data.
- – A decrease of fungal biomass is generally the result of a combination of factors.
- – The quantification is global and does not allow for the detection of the emergence of depletion of fungal sub-communities in charge of potentially crucial functions.

Unité Agri'Terr, BioSol team's goal are (1) the understanding of determinisms in the structure of bacterial and fungal communities, (2) the connections between community structures and expression of needs in situ, and (3) the adaptative strategies of communities under different anthropic constraints. Our work aims at contributing to innovation in the fields of agriculture and environment.

CONTACT

http://www.esitpa.org/recherche/
igattin@esitpa.org

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