TOOL WORKSHEET NO.15

Fungal molecular biomass
estimated by quantifying ergosterol

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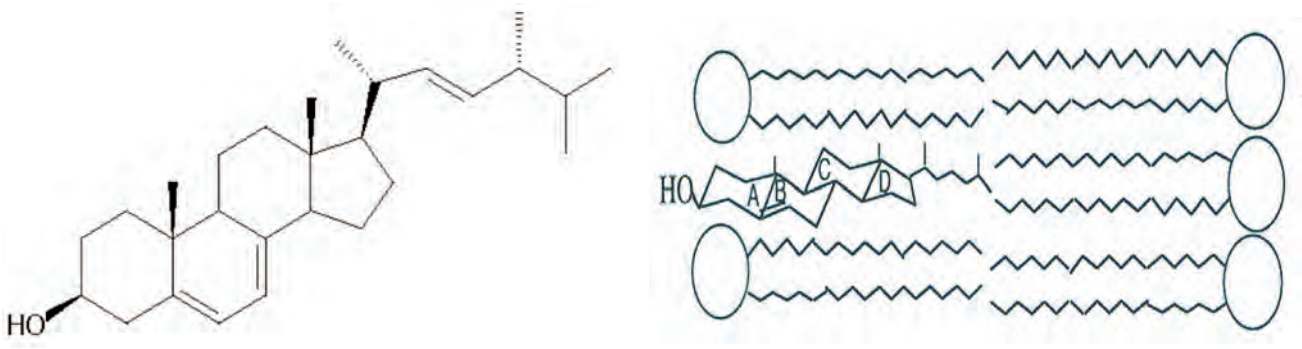
DESCRIPTION OF THE INDICATOR

Name of the indicator: Fungal molecular biomass estimated by quantifying ergosterol extracted from soil.

Ecological role of the organism under test: Fungi are dominant in number and mass in most soil ecosystems. They collect carbon necessary to their metabolism by symbiosis or **absorption** in their environment. Their filamentous growth allows them to create large networks within soils and therefore transport carbon compounds, nutrients and information over long distances. They are usually subdivided into three groups according to how they feed: **saprophytic fungi** (organic matter decomposers), symbiots (commensal or mutualistic) and parasites. With specific enzymatic processions (laccases, lignin peroxidases, cellulases...), fungi are the **main decomposers of organic matter held in soils**, including certain organic pollutants.

Products of degradation are mineralised, i.e. bioavailable for the nutrition of plants or transformed into humic compounds. Mostly held in membranes (also cytoplasm), the ergosterol molecule plays an essential role for fungal cells. It is the target molecule for a large number of antifungals.



Structure of ergosterol (ergosta-5,7,22-trien-3β-ol); its relative size versus phospholipids and their placement within the membrane

There are two types of ergosterol: free ergosterol within the fungal membrane and ergosteryl esters present in the cytoplasm. Depending on the type of extraction, the quantification of total ergosterol is possible.

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

- Anthropic impacts, related to tillage or presence of pollutants, which trigger a response of the total fungal biomass.
- Vegetation coverage type and age, which acts qualitatively and quantitatively on fungi.

DESCRIPTION OF THE METHOD

Reference standards and/or protocols

No reference standard or norm is currently available for this soil marker. However, as it is considered as a good marker for fungal presence, its quantification is standardised for animal feed (NF V18-112).

Sampling plan and method: For soil sampling, the NF X31-100 norm is a reference. The 0-15cm horizon is commonly used. In silty agricultural soils, the indicator is sensitive up to 30cm. The sampling strategy and the representativeness of the sample depend on the agronomic and/or environmental question at stake and on the scale of work. However, a minimum of 30g of soil is required when extracting.

Storage and pre-treatment of samples: Storage is not recommended: working on fresh soil is best. As a minimum, conduct the extraction if freezing is necessary.

Simplified description of the measurement method: Free ergosterol is obtained by simple percolation in a polar solvent. Cell lysis is achieved by physical disruption (abrasion by micron-scale glass debris). Extraction to quantify total ergosterol involves a microwave-assisted saponification reaction; more aggressive , we accept to obtain the totality of ergosterol held in the sample. Extracts are then separated and detected by HPLC/DAD before they are quantified.

Estimated time: Six hours are necessary for each 36 samples from extraction to results analysis.

Measured parameters: Measured fungal molecular biomasses are expressed in µg of ergosterol per g of dry soil. The measurement of sample humidity when weighing is required.

- Free ergosterol: Marker reflecting degrading fungal biomass.
- Ergosteryl esters: Marker reflecting viable fungal biomass.
- Ergosterol: Marker of the soil mycorrhizal potential.

INTERPRETATION OF RESULTS

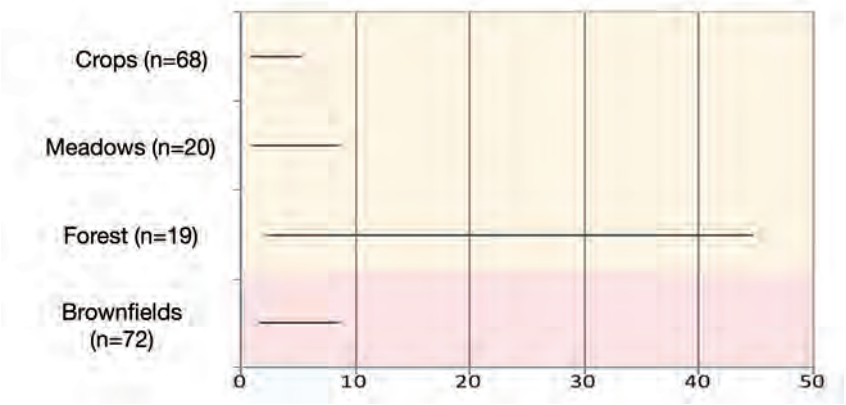
Need for a global reference system using a database

The BIO 2 program represents the first frame of reference on this indicator. The database of the BioSol laboratory capitalises data from this indicator, in particular on «haut-normand» soils.

Necessary supplementary information

Climatic and seasonal conditions have a dominating influence on this biomarker. It is preferable to conduct samplings in spring far from a frost episode or in autumn far from a drought period.

The physic-chemical context is to be taken into consideration, especially content in organic matter, pH, and soil texture. Land use and tenure as well as tillage in agricultural plots and/or load capacity in meadows can also have a strong influence.



Total ergosterol under the ADEME Bio II program

Studies at the scale of the BioSol frame of reference (GESSOL I and II; ADEME BIO I and II) have shown the predominance of **local parameters** (soil use, soil cover, soil types, practices...) over more global parameters (location, climate...) on the variation range of Fungal Molecular Biomass.

EXAMPLE OF APPLICATION

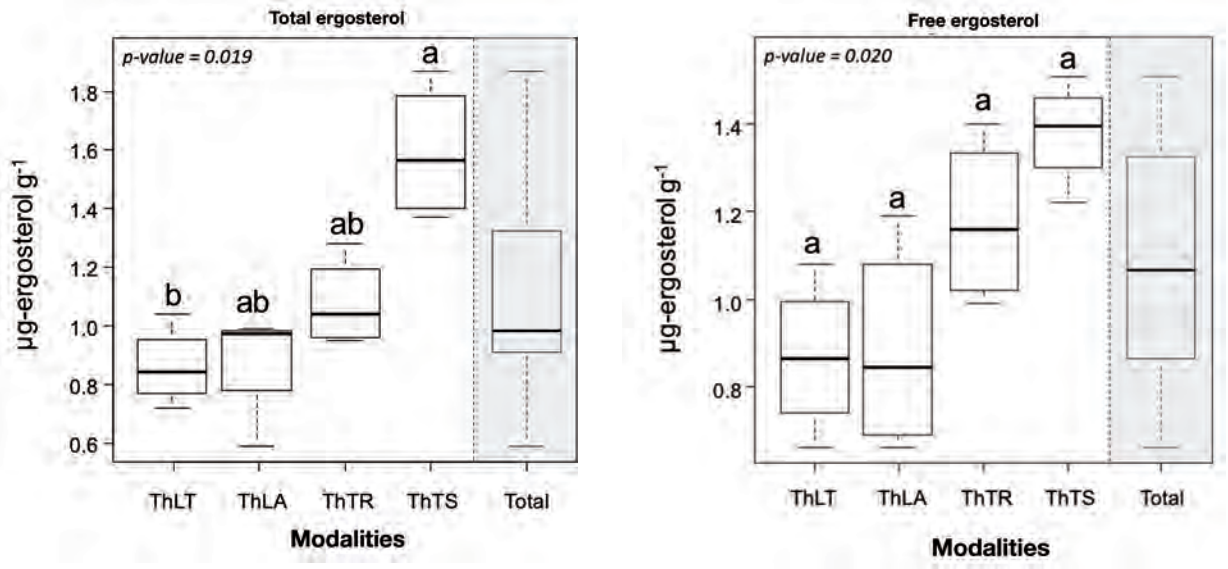
Thil site: 4 tillage modalities.

The goal of the experimental Thil site is to compare the effect of 4 tillage techniques in Organic Agriculture. Implemented in 2005, plough is characterised by turning over the layer of the laboured soil: ThLT (Traditional Plough 30cm), ThLA (Agronomic Plough 18 cm). Conversely, limited tillage does not involve turning over this layer: ThTR (limited tillage 15 cm with a toothed tool), ThTS (superficial tillage at 7 cm with a toothed tool).

On the Thil site, significant differences are observed depending on the intensity of tillage. Low quantities are measured in the plot undergoing traditional plough. Agronomic plough and limited tillage show intermediary situations; superficial tillage offers the largest quantities of ergosterol.

Logically, the less soils are tilled, the more fungal communities are abundant, on the studied horizon.

However, the "free ergosterol" marker does not enable us to distinguish tillage intensity even if a trend is observed. The choice of marker and sensitivity are therefore most important. This marker responds preferably to an **immediate stress** and a degradation of fungal cells. The quantities of fungal biomass estimated by "total ergosterol" are representative of tillage practices; they can reflect the behaviour of the fungal community in the longer term.



Fungal biomass measured through total ergosterol and free ergosterol on the Thil site. Different letters indicate a significant difference from the $p<0.05$ threshold.

INTERESTS AND LIMITS OF THE INDICATOR

Strong points:

The "ergosterol" marker integrates **all factors** modulating soil fungal biomass; it brings information on the **dynamics of fungal communities**. Finally, it allows for the **qualification of the effect** of agricultural practices, organic or metallic pollutants (all other things being equal).

Limits of the tool:

There is no **frame of reference** for soil fungal biomass estimated by this indicator at the international level.

The evolution of fungal biomass is generally multifactorial.

The amount of ergosterol differs depending on fungal species.

Unité Agri'Terr, équipe BioSol a comme objectif (1) la compréhension des déterminismes de la structure des communautés bactériennes et fongiques, (2) les relations entre structure des communautés et l'expression des fonctions *in situ*, et (3) les stratégies adaptatives des communautés sous différentes contraintes anthropiques. Les finalités de ces travaux contribuent à l'innovation dans les domaines de l'agriculture et de l'environnement

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