TOOL WORKSHEET NO.14

Potential metabolic diversity

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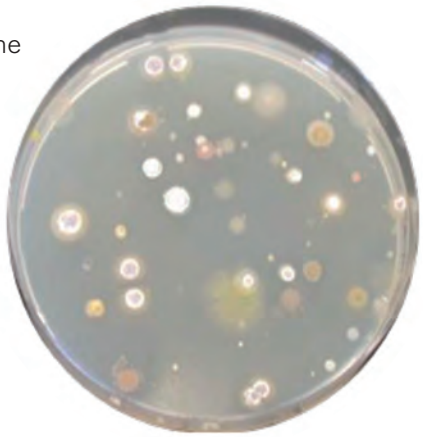
DESCRIPTION OF THE INDICATOR

Name of the indicator: Bacterial level physiological profile (PMD) is mesured by substrate richness (R: number of positive wells) and global metabolic activity of the layer (AWCD).

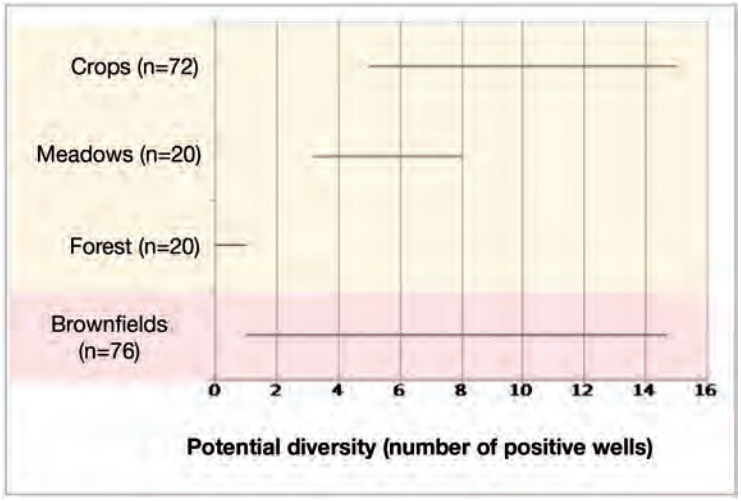
Ecological role of the organism under test: Bacteria are found in the organic fraction of soil. There are on average 10^9 bacteria per gram of dry soil, of which 0.1 to 10% are cultivable. Bacteria are involved, just like fungi and protozoa, in the functioning and the provision of soil ecosystemic services. The large variety of bacterial species ensures a diversity of functions, which allows them to occupy all ecological niches of the soil. They can also, under a variety of habitat conditions, use organic substrates held in or brought to soil, and even use some xenobiotics products.

Type of indicator: Biomarkers of effect and exposure.



Culture of bacteria on nutrient agar

DESCRIPTION OF THE METHOD



The functional diversity of cultivable bacteria, presented in this example by functional richness (R), shows large variations of this parameter mainly observed in brownfields and crops.

This assessment is probably linked to the strong heterogeneities of modalities (sites).

Variation range of functional richness on sites of the Bioindicateur2 program

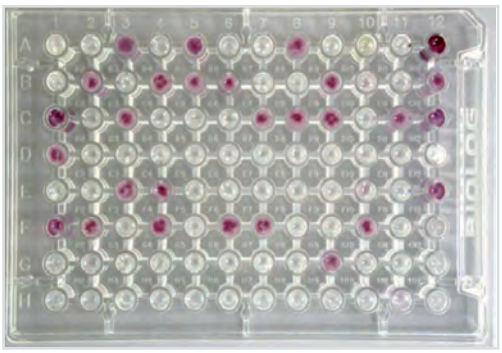
Reference standards and/or protocols

The Biolog method was adapted for the study of environmental bacteria and standardised by Calbrix et al, (2005) [1] for soil bacteria.

1 Calbrix R, Laval K., Barray S., 2005. Analysis of the potential functional diversity of the bacterial community in soil : a reproducible procedure using sole-carbon-source utilisation profiles. European Journal of Soil Biology 41:11-20.

Sampling plan and method: For soil sampling, the 0-10cm depth is commonly used. Spring and autumn are the recommended soil sampling periods for the measurement of this biomarker. Generally, sampling consists in collecting approximately 500g of soil using an auger on the soil horizon.

Storage and pre-treatment of samples: Samples are sieved/homogenised to 2 mm. Storage is not recommended: working on fresh soil is best.



Biolog plate

Simplified description of the measurement method: The Biolog system uses microplates (Eco-Plate) of 96 wells ready to use with 31 carbon substrates from 6 different classes (amines, carbohydrates, complex sources of carbon, carboxylic acids, amino acids, carbon phosphates). The metabolic activity of cultivable bacteria relies on their capacity to metabolise a specific number of carbon substrates. This consumption is revealed by a colour indicator of aerobic microbial development (tetrazolium salts). The intensity of this purple colour is proportional to substrate consumption. It is measured spectrophotometrically at 590nm (with the microplate reader and Microlog™ System Release 4.0 software) after 48-hour incubation at 20°C in the dark.

Estimated time: A full day and a half is needed to conduct the experiments on 28 soil samples.

Measured parameters:

The measurements of resulting optical densities (OD) make it possible to calculate:

- The functional richness (R) defined by the number of positive wells (OD well OD initial $>$ 0.25)
- The AWCD (Average Well Colour Development), which represents the average metabolic activity of soil cultivable bacteria ($\sum$ [OD well - OD initial)/31 (total number of substrates)])

Concurrently to the measurement of Metabolic Activity, bacterial counts are conducted in order to determine the abundance of cultivable bacteria in soils and ensure the homogeneity of bacterial density inoculated in each well of the microplate. The result as Colory Forming Units UFC/g of dry soil.

INTERPRETATION OF RESULTS

Use of a frame of reference

There are many scientific publications on the functional diversity of soil bacteria. Orders of magnitude in AWCD and R are available in these publications, especially for metallic pollutions, use and occupation of soils.

Database availability/access

Laboratories using the Biolog method have their own databases with specific threshold values related to experimental conditions. Given the variability of protocols, it is important to address the same laboratory.

Necessary supplementary information

Pedo-climatic variables have a strong influence on the functional diversity of bacteria, especially soil texture, pH, content in organic matter of soil, organic amendments and metallic pollutions. Soil use and tenure also impact this biomarker.

EXAMPLE OF APPLICATION

QualiAgro site: Organic amendments of agricultural soils

On this site, 5 organic treatments are studies: Cattle Manure (FUM), Compost from Residual Household Waste (OMR), Compost from Biowaste (BIO), Compost from Green Waste and Sewage Sludge (DVB), Control with no organic addition (Control).

Modalities	Control	OMR	FUM	BIO	DVB
Content in organic matter (g/kg)	17,8 ± 1,8	21,5 ± 5,2	24,4 ± 1,4	25,4 ± 6,1	26,4 ± 3,0

Table 1: contents in organic matter present in treatments

The effect of adding organic residual products on cultivable bacteria and their metabolic potential activity is presented on figure 1.

The diversity of data on the 4 blocks of each modality does not show statistically significant differences. However, the observed trends depict an increase of functional richness in amended modalities. It seems that the average metabolic activity of cultivable bacteria is not influenced in the same manner depending on the nature of organic amendments, which is commonly discussed in literature.

Samplings may have been conducted a little early for the season (mid-March) for this type of indicator.

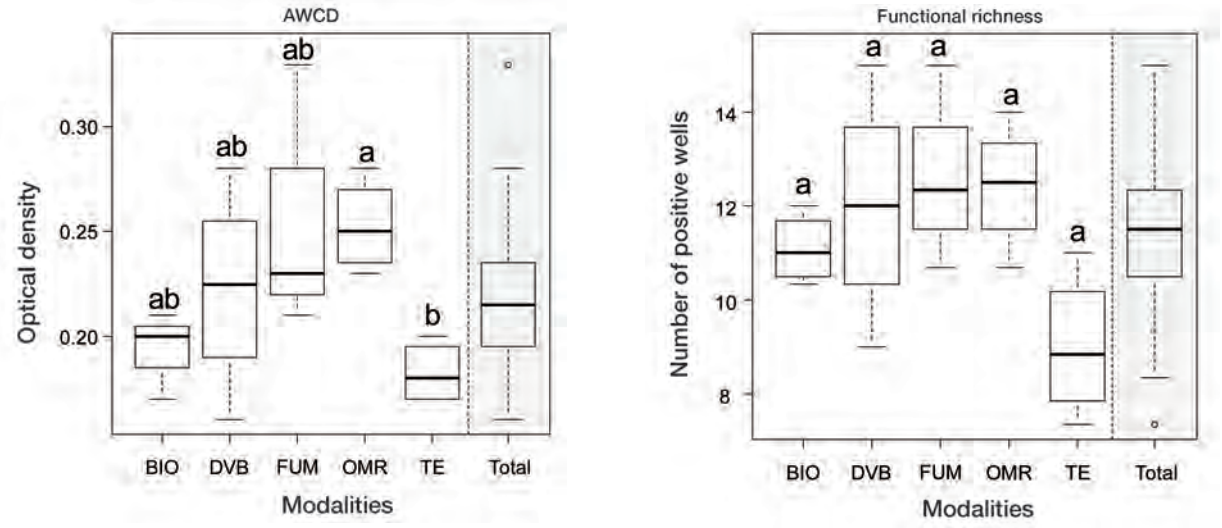


Figure 1: Abundance, metabolic activity (AWCD) and functional richness - Qualiagro site. Different letters show a significant difference to the $p<0.05$ threshold.

INTERESTS AND LIMITS OF THE INDICATOR

Advantages

- PMD enables to highlight space-time changes within a microbial community in a variety of environments (soil, water, sediments, biofilm…).
- PMD often makes it possible to observe the effects linked to modifications in land use and content, to metallic and organic contaminations, and to physic-chemical changes in soils.
- Detailed analysis of the level of consumption of carbon substrates brings precise information on Metabolic Activity

Limits :

- Carbon substrates potentially consumed by bacteria are not all representative of those held in soil (Tween 40 and 80 for example).
- Results achieved rapidly imply a significant volume of data to format and process. The time spent on the mission will depend on the expected level of expertise.
- The method reflects the potential functional diversity of culturable and not in-situ bacteria. Besides, it favours fast-growing microorganisms.

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.

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