TOOL WORKSHEET **NO.12**

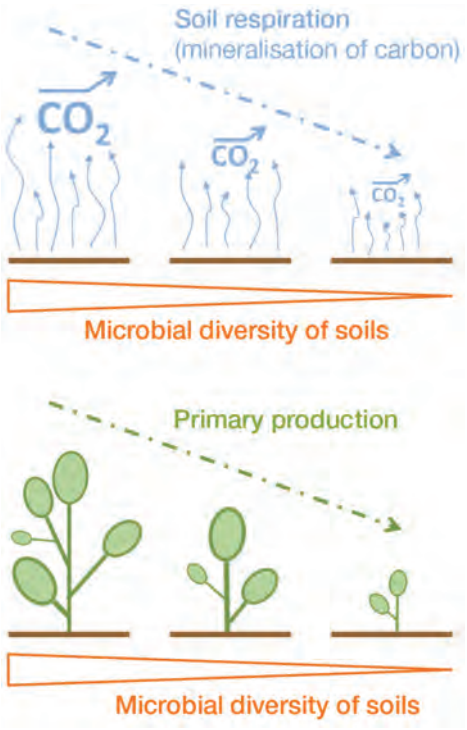
Microbial Taxonomic Diversity (pyrosequencing)

An indicator of ecological insurance for the prope biological functioning of soils

GenoSol Platform - INRA of Dijon

WHY MICROBIAL TAXONOMIC DIVERSITY?

Among the many soil organisms, **microorganisms are the most numerous and the most diversified. Human activities considerably impact soil microbial diversity.** Therefore, it has become crucial to understand **the functional significance of changes/losses of biodiversity in terms of soil stability and ability to maintain functions and services** for the development and well-being of society.



The concept of ecological insurance lies in this context. This concept foresees that **the risk and magnitude of the impact of a disruption on the functioning of ecosystems decrease when there is a high number of species** (Yachi et Loreau, 1999). Thus, **stronger biodiversity ensures the proper and sustainable functioning of a soil**. For example, the works of Maron et al. (in prep) and Mougel et al. (in prep) show that a decrease in soil microbial diversity leads to a **decrease in respiratory capacities** (carbon mineralisation) **and vegetation production** (health and growth of plants), and as a consequence, of **its biological fertility.**

Quantifying microbial diversity is possible by conducting taxonomic inventories. Inventories serve as indicators of the level of sustainability and performance/functioning of soil in terms of biological fertility, vegetation productivity, resistance to disruptions, degradation of pollutants, etc… They are also used **to identify specific populations involved** in various biological processes (symbiotic species, pathogens, pesticide degradants, etc…).

HOW ARE TAXONOMIC INVENTORIES CONDUCTED AND INTERPRETED?

Taxonomic inventory of present species is directly conducted from soil-extracted DNA. **Specific genes**, ribosomal genes, **found among all microorganisms, are targeted and amplified within this soil DNA**. Resulting products **are sequenced** using high-throughput next generation sequencer (454 technology Roche© pyrosequencer). These steps require **cutting-edge technical material and skills. Standardised protocol** is described in Terrat et al. (2012) and costs a few hundred euros (about 400€ but depending on resolution).

Data output of sequencers requires processing and analysis based on a set of **skills in bioinformatics, mathematics** and on significant **IT tools** (storage and calculation servers). Soil microorganism diversity can be expressed in terms of:

- **rarefaction curves**, which represent the cumulative number of newfound species for each sample. This representation allows for a quick and robust comparison of the levels of microbial diversity between different samples,
- biodiversity mathematical **indexes** capable of providing an estimation of the state of biodiversity and the pressures it is subjected to,
- **detailed species inventories and their relative abundance.**

Interpretation is based on the **comparison of different modalities** on a same site or by **positioning final values on the frame of interpretation** of soil microbial diversity provided by the GenoSol platform (MicroSol database© currently being finalised).

Richness and equitability indices: components of diversity

The equitability (value comprised between 0 and 1) of a community evaluates the level of regularity and distribution of the number of individuals for each species. The value is close to 1 when there is no dominant species. Conversely, it is close to 0 when the community is dominated by specific populations. This index is generally **lower in disrupted environments** that are dominated by the most adapted species.

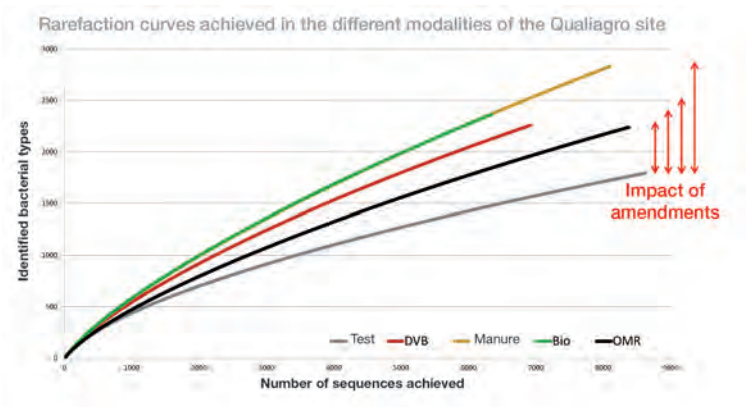
The richness refers to the number of different species within the community. A large number of species enables better ecological insurance. The most commonly used indexes are called Chaol and ACE.

The diversity indexes (Shannon or Simpsons for instance) provide a number that sums up the richness as well as the equitability of the studied community.

EXAMPLE OF APPLICATION FOR ORGANIC AMENDMENT ISSUES

The INRA Qualiagro experimental site of the ADEME BioII program was studied with the goal of **evaluating the impacts of organic amendments** (different composts (OMR, DVB, Bio) and manure) on soil bacterial diversity. Results achieved are compared to sites where there is no addition of organic amendments (Test). They show that **soil bacterial diversity is strongly impacted by these amendments**.

Rarefaction curves highlight **a systematic increase of the number of species regardless of the amendment.**

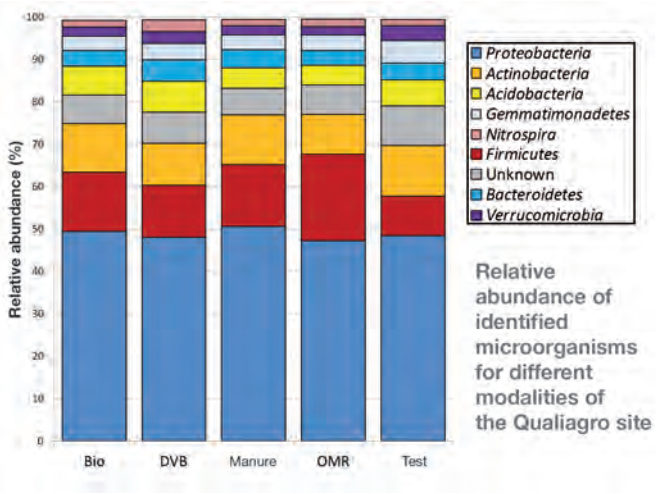


However, the magnitude of the increase of microbial richness **varies according to the type of organic amendment:** Bio = Manure > DVB > OMR > Test. **Organic additions therefore ensure an improvement of microbial diversity** but the nature of the amendment has to be adapted to the type of soil and cultivation technique to ensure optimal biological fertility.

		RICHNESS		DIVERSITY	
Modalities	Number of types detected (Richness)	Chao1	ACE	Shannon	Equitability
Bio	2365	6935	8339	6,72	0,86
DVB	2260	6043	6989	6,51	0,84
Manure	2832	8815	10147	6,76	0,85
OMR	2239	6071	6870	6,03	0,78
Test	1794	4381	4657	6,05	0,81

This is confirmed by indexes calculated for each modality on the same communities. For instance, the Manure and Bio amendments strongly increase diversity and equitability of various bacterial species.

As for the OMR amendment, it increases richness (2.239 detected types against 1.794 for the Test), but the Shannon index stays unchanged and equitability decreases. This reveals that **only certain types of microorganisms are influenced by the OMR amendment;** they become a majority and potentially disrupt the ecosystem.



Relative abundance of identified microorganisms for different modalities of the Qualiagro site

This imbalance is also shown by the advanced study of identified bacterial types. For instance, the OMR amendment significantly increases populations of Firmicutes (in red) in comparison with other modalities and populations identified. Besides, no pathogenic organism has been detected, no matter what amendment was used.

In a context of ecological insurance, a higher diversity of microbial species leads to a better stability of the biological functioning of soil; the so-called Bio and Manure additions are then considered as the most efficient among tested amendments, based on data of soil microorganism diversity collected.

INTERESTS AND LIMITS OF THE MOLECULAR BIOMASS INDICATOR

Interest:

- **Legitimate inventory of microbial populations**, which will, in the long term, allow to identify species responsible for key-functions ensuring the proper functioning of soil, but also to detect the presence of organisms of interest (symbiots) or pathogens,
- **Recent and constantly-evolving approach** (better resolution, rapidity, cost),
- **Cost/information ratio:** cost of several hundred euros for a characterisation much finer and informational than any other microbial indicator.

Limits :

- **Cutting-edge material and analysis environment** very costly (for example, for the Roche© sequencer: from 100 k€ to 1M€ depending on throughput),
- **Know-how and skills of cutting-edge molecular biology techniques,**
- **Frame of reference currently being finalised.**

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