TOOL WORKSHEET NO.11

Molecular Fingerprint of soil microbial communities

An indicator of the impact and stability of soil microbial life

GenoSol Platform - INRA of Dijon

WHY A FINGERPRINT OF MICROBIAL COMMUNITIES?

Soil microorganisms are the most diversified organisms at the scale of the planet. They have a short generation time, from tens of minutes to a day depending on the species and environmental conditions (temperature, nutrients, etc). This gives microbial communities a strong reactivity to habitat disruptions. In this regard, **Molecular Fingerprint**, which gives an image of the diversity **of soil microbial communities**, is an **early** (strong reactivity) and **sensitive** (strong adaptation of communities) indicator of the changes operated on soil's biological life in response to an anthropic or natural disruption of the environment. This method introduces the notions of **Resistance/Resiliency** of soil microbial communities.

Resistance/Resiliency of microbial communities = soil elasticity

In the same way it is necessary to apply specific force to stretch an elastic band, soil microbial communities can resist a disruption depending on its intensity and duration: it is their Resistance capacity.

Just as the elastic band, soil microbial communities can also move away from their equilibrium state in response to a disruption and return to equilibrium once the disruption is over: it is Resiliency. However, just as the elastic band can break by stretching it repeatedly or in excess, under constraints, microbial communities can definitively move away from this equilibrium state and lose their resiliency capacity.

METHOD OF CHARACTERISATION OF MICROBIAL COMMUNITIES: ARISA

Molecular analysis of the genetic structure **of communities relies on a method of genotyping of microbial communities (bacteria and fungi) called ARISA** (Automated Ribosomal Intergenic Spacer Analysis).

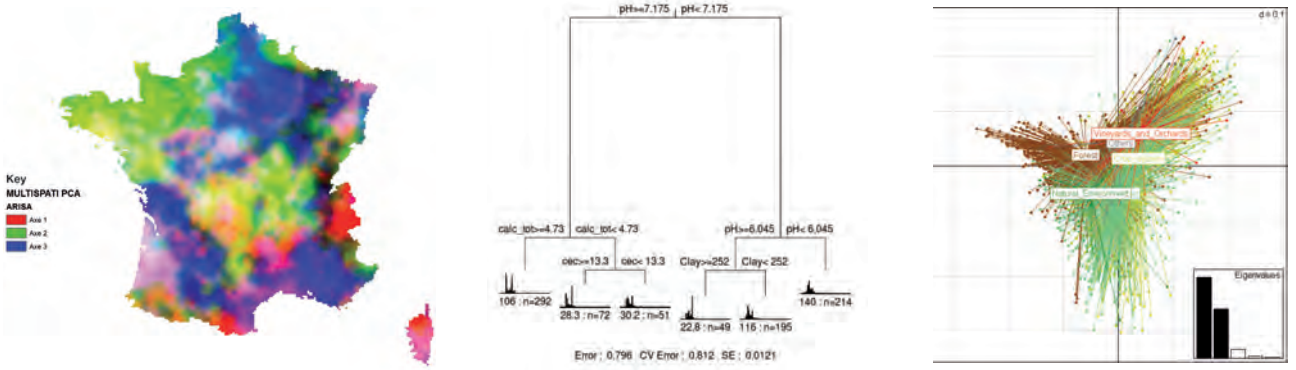
ARISA = the soil's barcode

ARISA results in a genetic barcode that is representative of the population structure of soil microbial communities. Each bar of the barcode refers to a population (or a group of populations) whose presence is more or less important depending on the intensity of the bar. Computer processing enables the analysis of data contained in the barcodes. Their comparison leads to the identification of modifications in the genetic structure of microbial communities.

MEASUREMENT AND INTERPRETATION PROTOCOL OF MOLECULAR FINGERPRINTS GENERATED BY ARISA

The **analysis procedure** is **standardised and tested within the GenoSol platform** and gave birth to many publications (ex: Ranjard et al., 2000a, 2000b, 2006; Lejon et al., 2007; Dequiedt et al., 2009a, 2009b; Pascault et al., 2010). It is conducted by a technician specialised in molecular biology and **directly based on microbial DNA** extracted from soil in accordance with a standardised procedure. The analysis is done at moderate flow rate and requires costly material: a thermal cycle apparatus (~ 8k€) and a Licor© automate sequencer (80 k€). **One to 2 week(s) is (are) required to achieve results**.

The interpretation of results is realised by **using mathematical tools** (ACP, co-inertia, distance or similarity calculation, variance partitioning, geostatistics, etc...). The **positioning of final results in relation to the interpretation frame of reference** of the GenoSol platform, but also the comparison of fingerprints achieved between a test and disrupted situations allow for the **diagnosis of the impact of different factors** (anthropic activity, pedo-climatic characteristics) **on the diversity of soil microbial communities**.

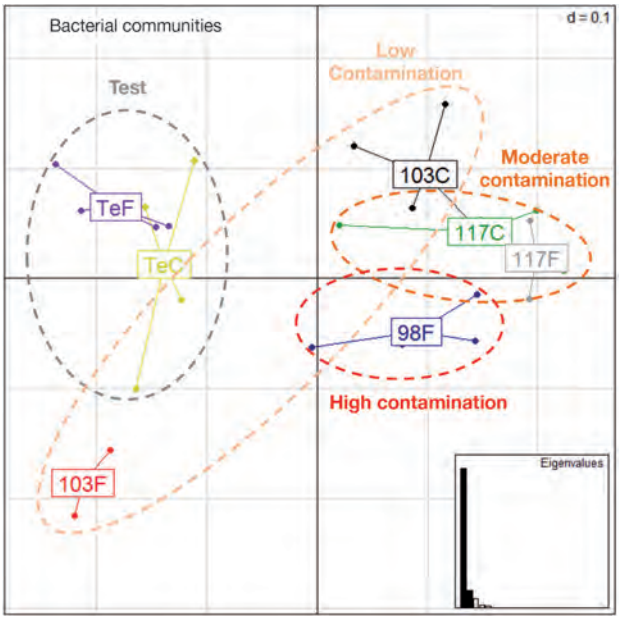


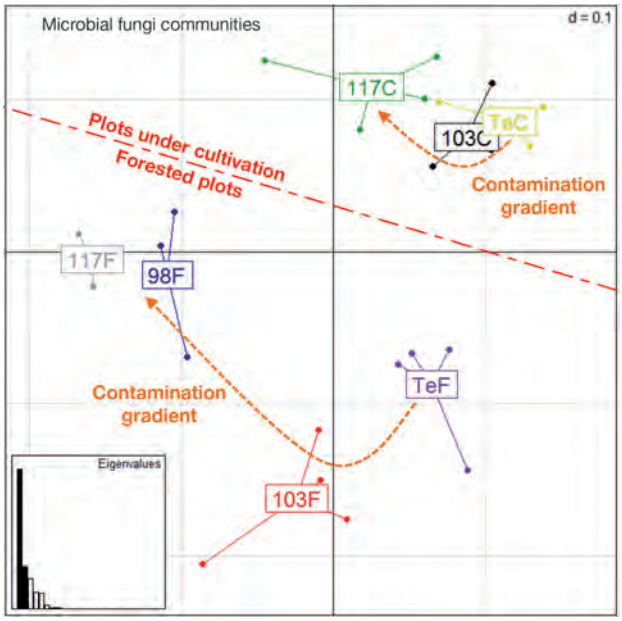
Interpretation frame of reference (MicroSol database©): spatial distribution of Molecular Fingerprints, regression tree and impact of soil uses at the scale of France

EXAMPLE OF APPLICATION TO A SITE CONTAMINATED BY HEAVY METALS

[mg.kg^{-1} MS]	Tests	Level of contamination		
		low	moderate	high
Plots under cultivation	TeC	103C	117C	-
Forested plots	TeF	103F	117F	98F

The Metaleurop site from the ADEME BioII program lies on the land of a former smelting plant in northern France and displays very high and even alarming levels of polymetallic contamination. The site consists of **7 plots under two soil uses** and 3 levels of contamination.





The factorial plans of the co-inertia analysis of bacterial and fungal communities' Molecular Fingerprints in the Metaleurop site plots show **the relevance of Molecular Fingerprints as an indicator:**

- of **the impact of metallic contamination on the biological quality of soils:** test plots host communities different from contaminated soils,
- of **the impact of the level of contamination:** fungi and bacteria communities are structured differently according to the intensity of contamination; they evolve gradually (bacteria) or by increments (fungi) depending on the importance of contamination,
- to **rank parameters influencing communities:** metallic contamination > soil use, for bacteria; soil use > metallic contamination, for fungi.

INTERESTS AND LIMITS OF INDICATORS OF SOIL MICROBIAL COMMUNITIES GENETIC STRUCTURES

Interests:

- **Strong sensitivity to all types of disruptions:** changes in crop routings, practices (tillage, organic amendments, etc...), contaminations (metallic, HAP, etc...),
- **Standardised acquisition and interpretation techniques,** tested and applicable at moderate flow rate (approximately 1 week for analytical procedure) at affordable cost (about 100 €),
- Possibility of **comparison of different modalities to identify the most impacting situations** for the microbial component,
- Frame of reference (MicroSol database©) built (for bacteria and still ongoing for fungi communities) by the GenoSol platform allowing for the **interpretation of results in relation to variations observed by pedo-climatic type.**

Limits:

- **Need for a range of specific and expensive materials (technical equipment),**
- **Analysed effects can be masked by pedological variability.**

- *Dequiedt S, Lelièvre M, Jolivet C, Saby NPA, Martin M, Thioulouse J, Maron PA, Mougel C, Chemidlin Prévost-Bouré N, Arrouays D, Lemanceau P, Ranjard L., 2009a -. ECOMIC-RMQS : biogéographie microbienne à l'échelle de la France - Etat d'avancement et premiers résultats. Etude et Gestion des Sols, 16 : pp 219-233.*
- *Dequiedt S, Thioulouse J, Jolivet C, Saby NPA, Lelievre M, Maron PA, Martin MP, Chemidlin-Prévost-Bouré N, Toutain B, Arrouays D, Lemanceau P, Ranjard L., 2009b - Biogeographical patterns of soil bacterial communities. Environmental Microbiology Report, 1 : pp 251-255.*
- *Lejon D.P.H., Sebastia J., Lamy I., Chaussod R., Ranjard L., 2007 - Relationships between soil organic status and microbial community density and genetic structure in two agricultural soils submitted to various types of organic management. Microbial Ecology, 53 : pp 650-663.*
- *Pascault N., Cécillon L., Mathieu O., Hénault C., Sarr A., Lévêque J., Farcy P., Ranjard L., Maron P.A., 2010 - In situ dynamics of microbial communities during decomposition of wheat, rape and alfalfa residues. Microb. Ecology, 60 : pp 816-828.*
- *Ranjard L., Nazaret S., Gourbiere F., Thioulouse J., Linet P., and Richaume A., 2000a - A soil microscale study to reveal the heterogeneity of Hg (II) impact on indigenous bacteria by quantification of adapted phenotypes and analysis of community DNA fingerprints. FEMS Microbiol. Ecol., 31 : pp 107–115.*
- *Ranjard, L., Poly, F., Combrisson, J., Richaume, A., Gourbiere, F., Thioulouse, J., and Nazaret, S., 2000b Heterogeneous cell density and genetic structure of bacterial pools associated with various soil microenvironments as determined by enumeration and DNA fingerprinting approach (risa). Microb. Ecol., 39 : pp 263–272.*
- *Ranjard L., Echairi A., Nowak V., Lejon D.P.H., Nouaïm R. & Chaussod R., 2006 - Field and microcosm experiments to evaluate the effects of agricultural copper treatment on the density and genetic structure of microbial communities in two different soils. FEMS Microbiology Ecology, 58 : pp 303–315.*

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