

A quest for microbial indicators of the *Tuber melanosporum* truffle production using an environmental genomics approach

Alessandra Pontiroli¹, Hervé Sanguin¹, Marc Ducouso¹, Antoine Galiana¹, Christine Leroux¹, Odile Domergue², Daniel Mousain³, Robin Duponnois⁴, Yves Prin¹, Ezékiel Baudoin⁴

¹LSTM*-CIRAD, ²LSTM*-INRA, ³Société d'Horticulture et d'Histoire Naturelle de l'Hérault/INRA, ⁴LSTM*-IRD
*Laboratoire des Symbioses Tropicales et Méditerranéennes, MONTPELLIER, FRANCE - <http://www.mpl.ird.fr/lstm/>

Contacts: alessandra.pontiroli@cirad.fr; yves.prin@cirad.fr; Ezekiel.Baudoin@ird.fr

Introduction:

The « brûlé » -the area where the truffles tend to grow to a mature stage- is commonly characterized by a **drastic drop in the plant cover around those trees** whose root systems host *Tuber melanosporum*. Although « brûlés » are indicative of the mycelium network development of this symbiont, they are **not systematically associated with the production of ascocarps**.

To date, understanding how truffle grounds function has been puzzling task, especially with regard to the spatio-temporal dynamics of the colonization process, and the identification of factors influencing the intensity of the « brûlés » and their truffle yield. **The emphasis is put here on the microbial specificity of truffle grounds.**

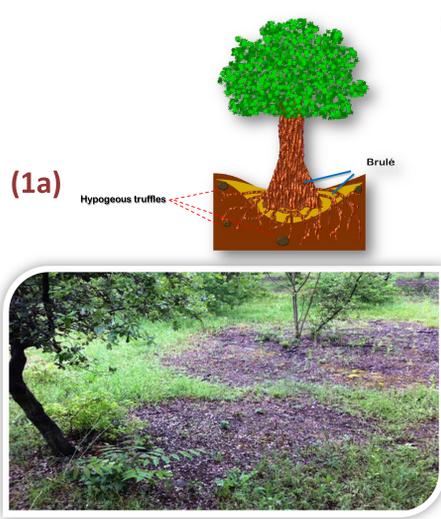


Fig. 1. (a) *Quercus ilex* in the Uzès orchard with the « Brûlé » phenomenon and (b) selected companion plants sampled for the *Glomeromycota* diversity analysis.

Site	Total Plants
Beaulieu (orchard)	21
<i>Sedum sediforme</i>	
<i>Dicanthium saccharoides</i>	
<i>Aphyllantes monspeliensis</i>	
Uzès (orchard)	19
<i>Medicago minima</i>	
<i>Plantago sp.</i>	
<i>Picris sp.</i>	
Tourbes (natural)	23
<i>Silene sp.</i>	
<i>Rubia peregrina</i>	
<i>Stipa bromoides</i>	
<i>Sedum sediforme</i>	
<i>Thymus vulgaris</i>	
<i>Asparagus acutifolius</i>	
Pézilla (natural)	21
<i>Sedum sediforme</i>	
<i>Ptycothis saxifraga</i>	
<i>Melilotus albus</i>	
<i>Ononis pusilla</i>	
<i>Ononis natrix</i>	
<i>Bituminaria bituminosa</i>	
<i>Smilax aspera</i>	
<i>Helichrysum stoechas</i>	
Graminées	

Aims:

- Are there bacterial and mycorrhizal (Glomeromycetes) markers specific to the productive status of the « brûlés »?
- Do the community structures of these microbial groups differ in relation to the productive status of the host tree (productive and non-productive « brûlés », «no-brûlés» trees)?

Material and Methods:

-**Two forest margins**, at Tourbes and Pézilla, and **two orchards**, at Beaulieu and Uzès, were sampled during November 2010 (ascocarp maturation stage) in the Languedoc-Roussillon region (France). These truffle grounds are composed of the **holm oak (*Quercus ilex*)** and are managed without soil tilling.

-Samples included the **soil (bacteria diversity)** and the **companion plants roots (Glomeromycetes diversity)**, both surrounding the oaks. Three oaks were sampled per productive status and per site for a total of **36 oaks**.

-Environmental DNA was extracted with the MoBio PowerSoil™ Kit for the 36 soil samples in triplicate and with FastDNA SPIN™ Kit for the 84 plant roots. Microbial diversity was studied by means of **454-pyrosequencing** with universal eubacteria primers targeting the V3-V4 region of the bacterial **16S DNA** and with specific primers targeting the arbuscular mycorrhizal **18S DNA gene**.



Fig. 2. Sampling sites

Results:

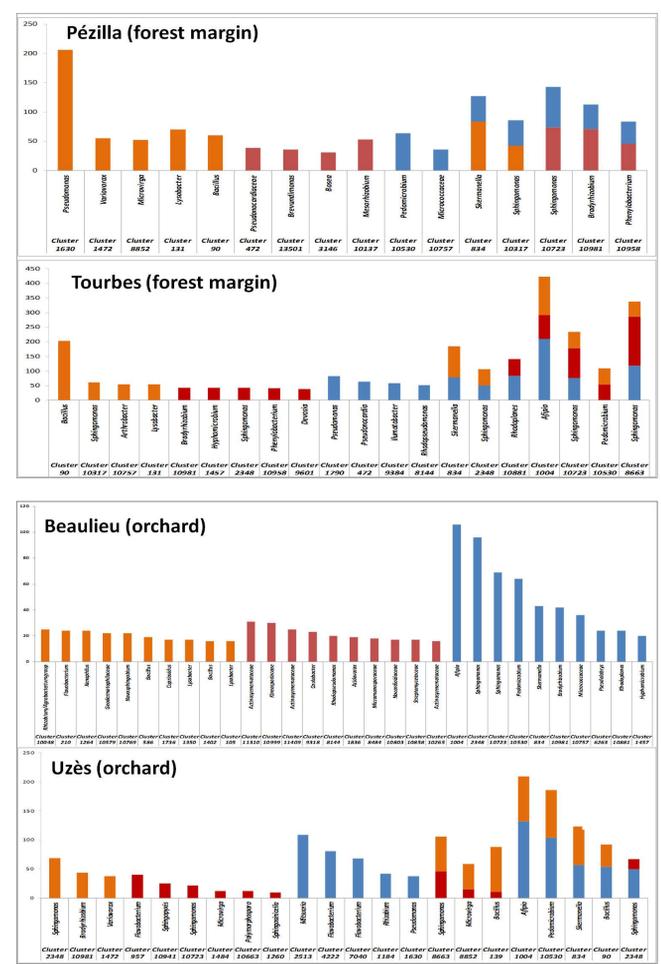


Fig. 3. Bacterial representative OTUs at the genus level after clustering at the 97% similarity cutoff and blasting to Silva db (ARB) the sequences amplified from the soil DNA.

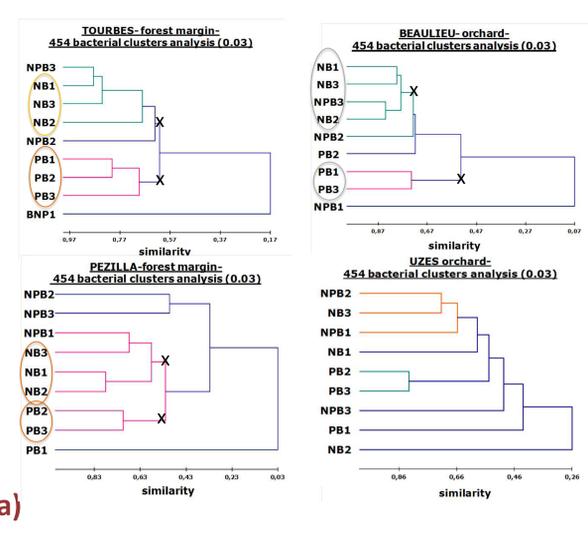
NB No Brûlé
PB Productive Brûlé
NPB Non-Productive Brûlé

Conclusions:

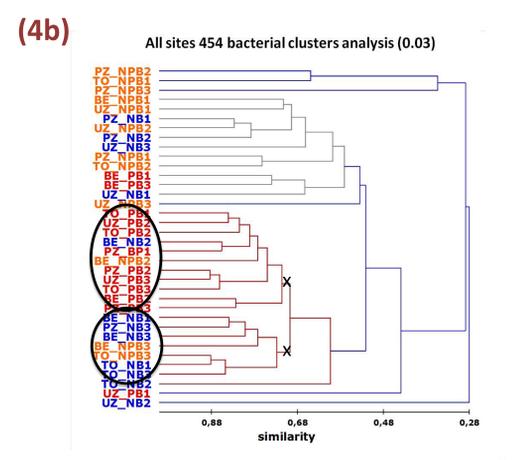
Preliminary statistical analyses of the **soil bacterial diversity** suggest a **certain level of specificity** in 3 out of 4 truffle grounds enabling the **distinction of the productive “brûlés” from the non-productive “no-brûlés”**. Further analysis is needed to confirm this interpretation and identify the bacterial taxa underlying these specificities. **In contrast, the diversity of the arbuscular mycorrhizal fungi** associated to the companion plants does not seem to be sensitive to the productive status of the oaks. Glomeromycota OTUs ranked by abundance matched to uncultured *Glomus* and *Rhizophagus* genera.

Globally, ongoing data generation and complementary statistical analysis will further allow us to relate sequence data with the productive status and the floral diversity of the studied ecosystems and to assess the interaction networks established between *Tuber melanosporum* and the microbial and floral compartments of these truffle grounds.

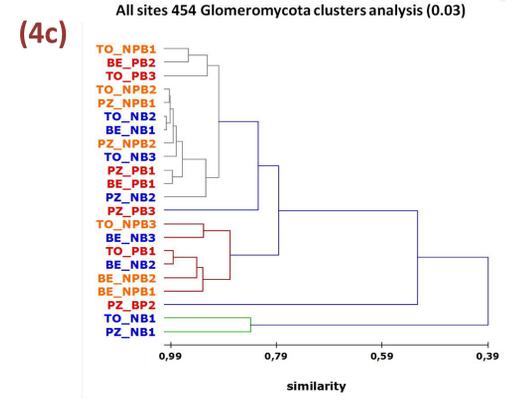
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(4a)



(4b)



(4c)

Fig. 4. Cluster analysis of the whole OTUs_{0.03} data set (a) evidenced a specific cluster of the 3/3 productive « brûlés » distinct from a cluster of the 3/3 « no-brûlés » only in the Tourbes truffle ground. In Beaulieu and Pézilla, clusters of 3/3 « no-brûlés » were also apparent, as well as clusters of 2/3 productive « brûlés ». Analyzing the 4 sites together (b), 9/12 productive « brûlés » clustered apart from 6/12 « no-brûlés ».

In contrast, (c) analysis of whole OTUs_{0.03} data set related to the Glomeromycetes failed to reveal specific groupings related to a given productive status as exemplified with the analysis performed on the 4 sites.