The fungal tinsels within the Christmas tree

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Plants harbor a vast diversity of microorganisms referred as microbiome (Fig. 1 A.). Constituent of that microbiome, fungal endophytes play an important role for their plant host. They are known for conferring resistance to biotic and abiotic stresses. While they have been found in every plant, their role remain largely uncharacterized. Here we focus on balsam fir to inspect the amplitude of their presence and the patterns of their distribution within a tree.

A single asymptomatic balsam fir tree (*Abies balsamea* [L.] Mill.) was sampled in the Forêt Montmorency. Branches were collected at six different heights from to opposite sectors. For each of the twelve branches, the needles, the buds, the bark, and the wood were considered as independent samples. Two tree-ring cores were also collected at five different heights; only the trunk-wood [TW] was considered for each core. The aerial system of the tree is then composed of 58 samples. For the root system, two fragments of about 1.2m were collected. Root tips ([RT] x10), portions of 0.5cm of diameter comprising root-bark ([RB5] x10) and root-wood ([RW5] x10), and portions of 1cm of diameter also comprising root-bark ([RB10] X10) and root-wood ([RW10] x10) were treated independently resulting with 50 samples for the root system.

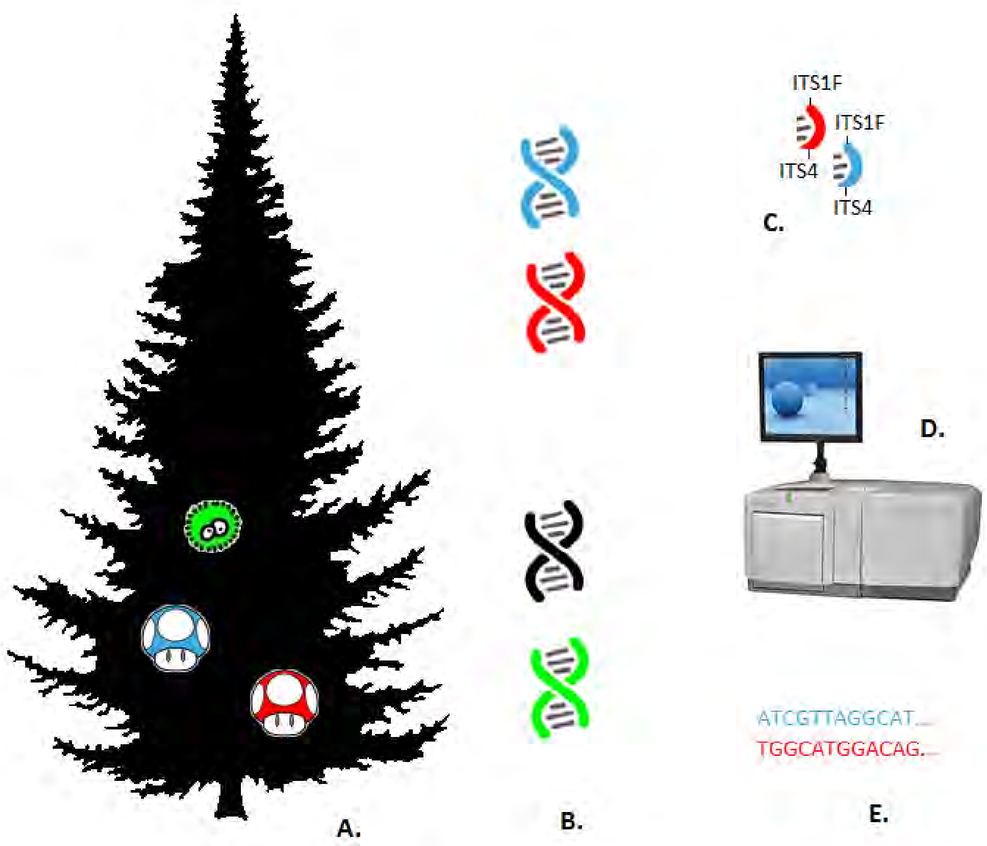

Fig.1 Principal steps of collecting the data, from sampling (A.) to sequenced amplicons (E.)

Whole DNA (of plant , fungal endophytes , and other microorganisms) was extracted after a superficial sterilization of the samples (Fig.1 B.). Using the fungal specific primer ITS1F coupled with the universal ITS4 primer, the molecular barcode of Fungi was amplified (Fig.1 C.). Those amplicons were then sequenced using the 454 Pyrosequencing technology (Fig.1 D.). The DNA sequences obtained (Fig.1 E.) were used to form molecular Operational Taxonomic Units (mOTUs) at a threshold of 95% of similarity. Those mOTUs correspond to "mathematical" species and are used to unveiled the structure of the distribution of the fungal endophytes.

Data analysis using several bioinformatics programs lead to the determination of 2,006 mOTUs in the single balsam fir sampled. Considering the species accumulation curve (Fig.2), that estimation seems pretty exhaustive of the total number of fungal species associated to this host.
Among the 2,006 mOTUs, 1,299 of them have an occurrence of at least 2 sequences and will be considered for the diversity and community composition analysis. The other 707 are singletons (=mOTUs represented by only 1 sequence) and are de facto specific to the sample they belong to.

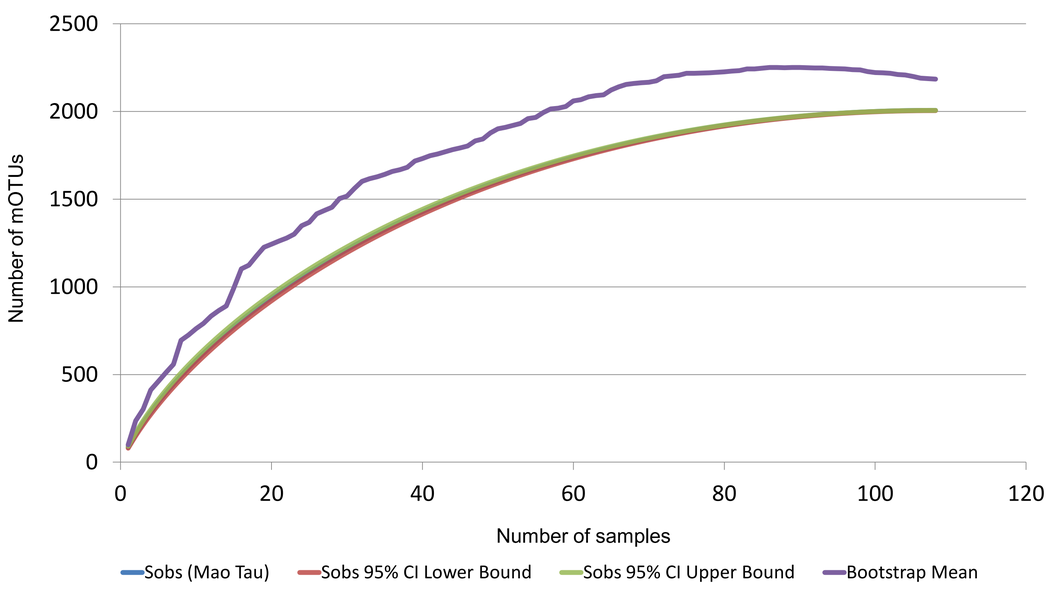

Fig.2 Species accumulation curves. Figure shows the number of fungal species (mOTUs) observed (Mao Tau; blue lines), lower and upper 95% confidence intervals (respectively red and green lines), and bootstrap estimate of richness (purple lines)

Diversity was modelised using Fisher's alpha, and the Kruskal-Wallis non-parametric statistical test established that the diversity was function of the system (p-value: $2.688e^{-11}$) and also of the tissues (p-value: $5.624e^{-10}$)

Similarity in the composition of communityies was assessed by ANOSIM with the Morisita-Horn's abundance index. The result was visualized using non-metric MultiDimensional Scaling (nMDS) (Fig.3). With the exception of the root-bark of the two different diameters (RB5 and RB10) which constitute a similar community, and the root-wood of the two diameters (RW5 and RW10) which also constitute one community, each one of the other types of samples is composed of a different community. With the exception of the Needles, communities belonging to the same system are more similar.

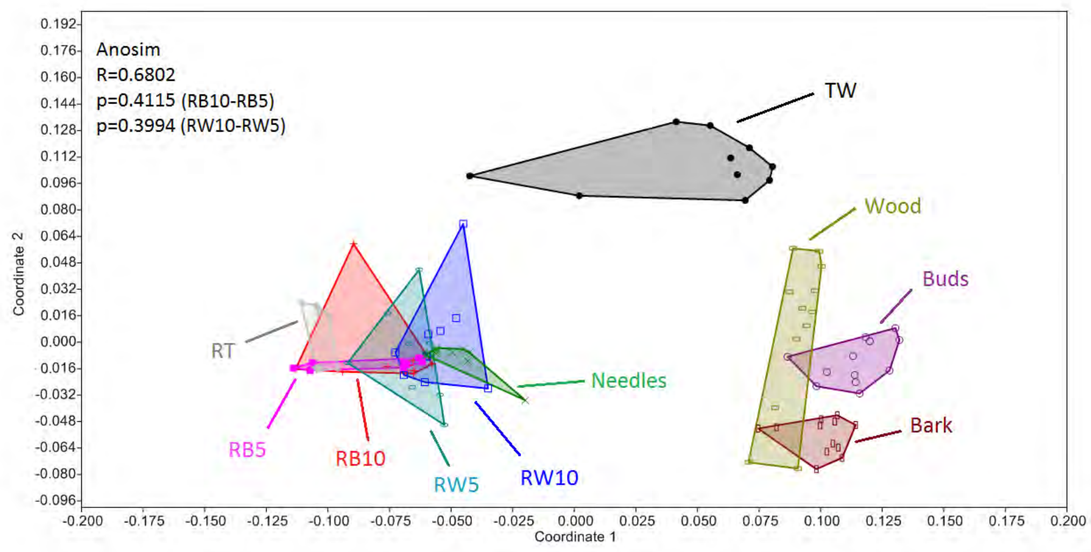

Fig.3 Community analysis of fungal communities. Results of nMDS using Morisita-Horn's index and relevant ANOSIM results. All singleton were excluded.

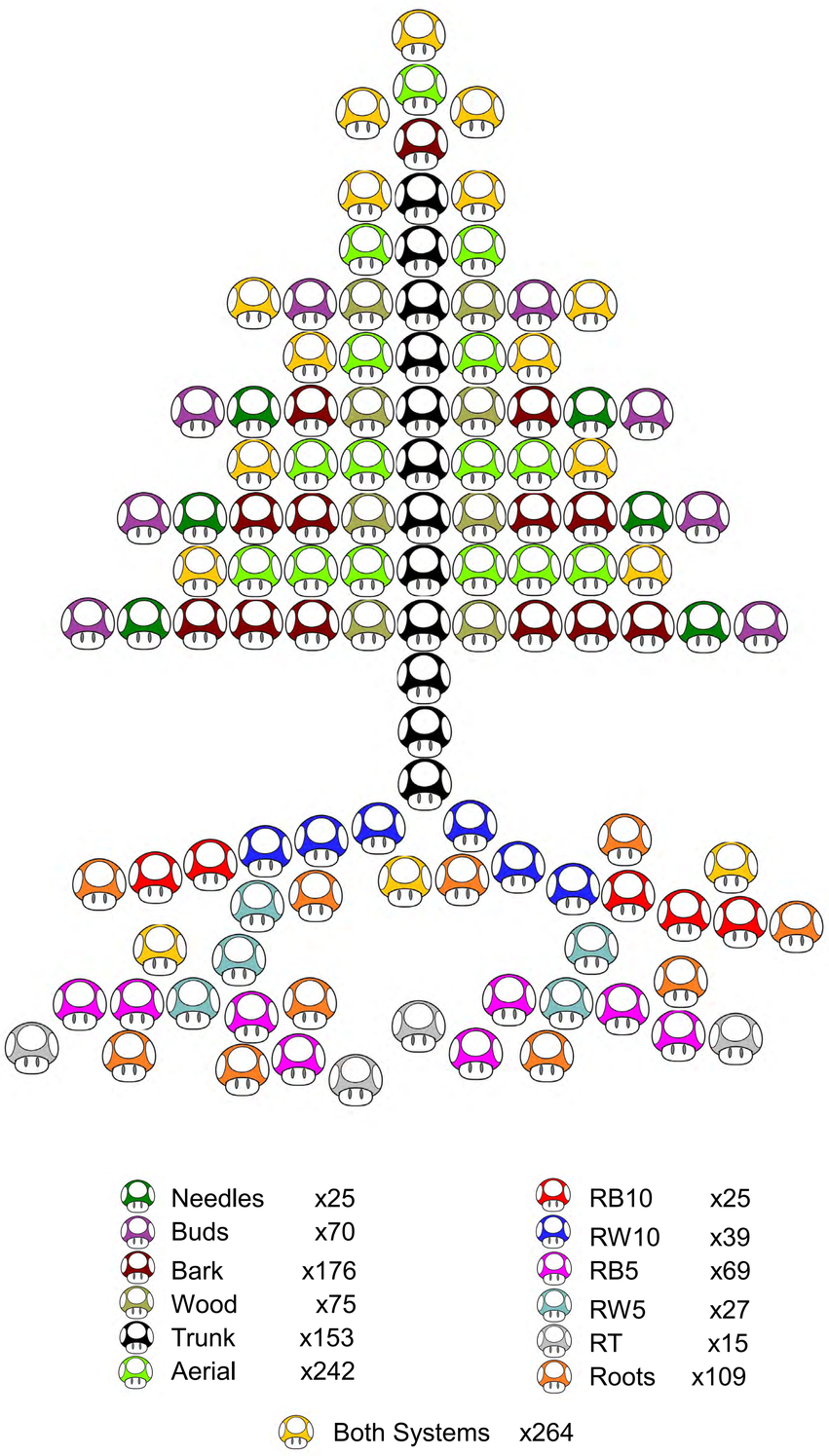

Fig.4 Distribution of the 1,299 non singleton mOTUs, illustrating both the specificity of systems and tissues using a square transformation.

• These results show the first estimation of the number of fungal species associated within a single tree. This value is of first importance for adapting sampling strategy to that richness and uncover the full diversity of fungal endophytes. A single *Abies balsamea* tree can harbor as many as 2,006 fungal species which show differences both in terms of diversity and community composition depending the system and the tissues considered **(Fig.4)**.