# A fungal community-based high throughput approach to determine ecological bio-indicators of Moroccan cork oak decline

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## Context and objectives

The cork oak (*Quercus suber*), an emblematic Mediterranean evergreen sclerophyllous tree, constitutes a major ecological resource and provides important financial support for Mediterranean populations, but suffers from various forms of decline, due to the worsening of environmental pressures.

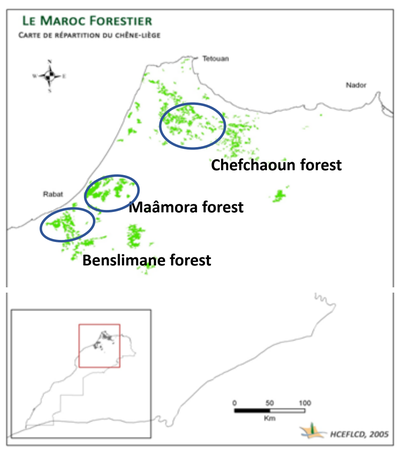
Cork oak-based ecosystem functioning is closely tied to two major biotic parameters, (i) the formation of symbioses between the cork oak and ectomycorrhizal fungi, and (ii) the interaction between plant cover (shrubs) and the cork oak.

The characterization of ecological bio-indicators based on this two biotic parameters is crucial to survey the health of ecosystems and to ensure a suitable response to environmental changes. Deciphering mycorrhizal community structure and diversity in cork oak ecosystems and assembly rules in relation to plant cover are major.

The current study aims at characterizing fungal networks in different Moroccan cork oak forests, characterized by degraded and non degraded stands, to determine multiple ecological bio-indicators.

## Study sites and sampling

Fieldwork was conducted in 3 cork oak forests characterized by degraded (D) and non-degraded (ND) stands. Three representative plants of cork oak ecosystems were selected for sampling, *ie. Quercus suber* (Q) , *Cistus salviifolius* (CS), and *Cistus monspeliensis* (CM).






**Sampling design**

3 plots/ site
3 cork oak/plot
2 shrubs/ cork oak

⇩

**144 root systems**
(+mycorrhizosphere soils)

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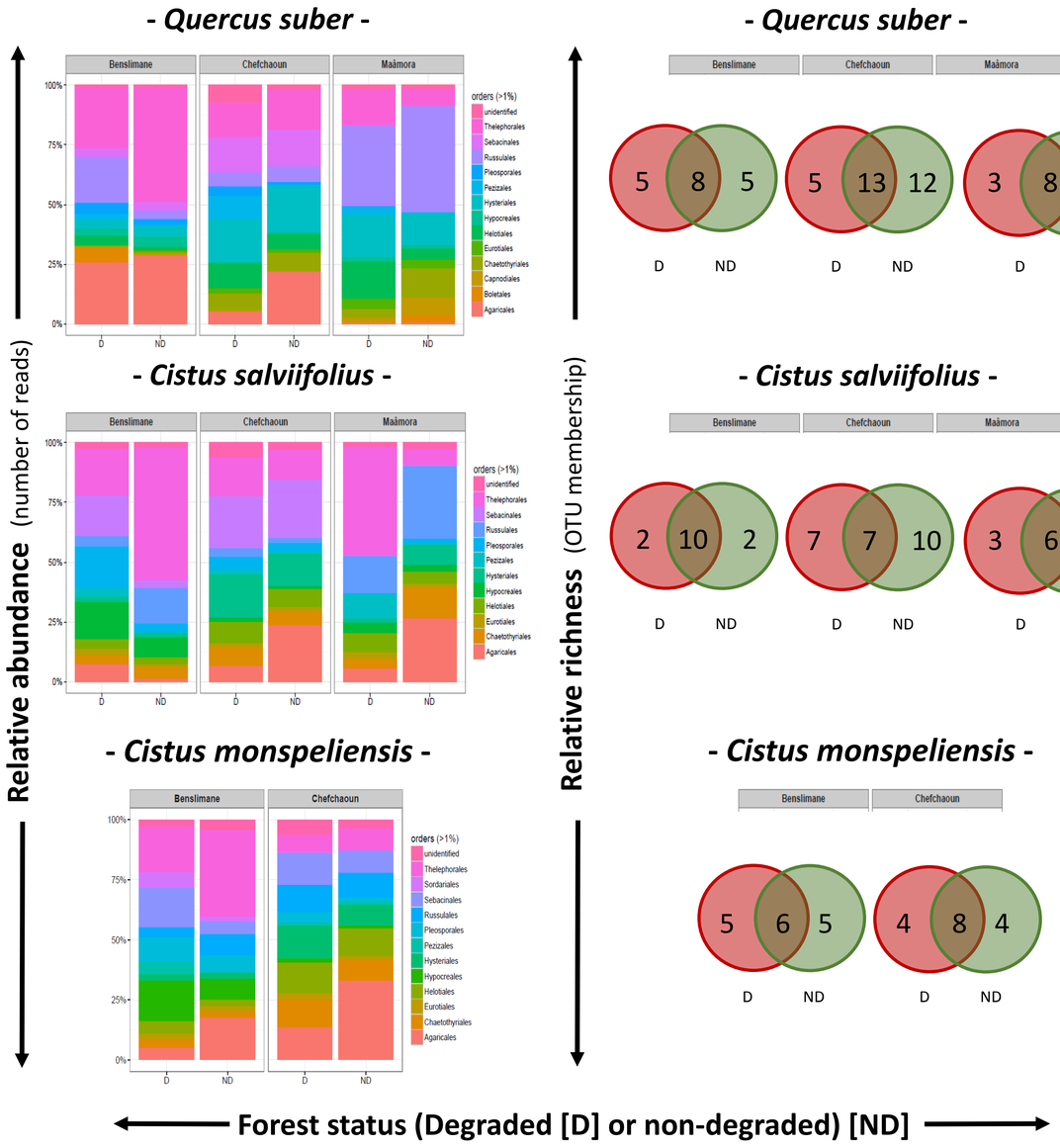
**Phenotypic** and **molecular** analysis of fungal community

## Fungal community analysis

Root-associated fungal community was assessed using two approaches, (i) morphological identification, and (ii) a high throughput ITS-based illumina sequencing

**Molecular** | **Phenotypic**




**Ecological drivers**

| | Richness | Diversity | Equitability | Structure |
|---|---|---|---|---|
| **Habitat** | *** | *** | *** | *** |
| **Status** | ns | ns | ns | *** |
| **Plant** | *** | ** * | *** | *** |
| **Habitat×Status** | ns | ns | ns | *** |
| **Habitat×Plant** | ns | * | * | *** |
| **Status×Plant** | ns | ns | ns | ns |

*Signif. codes: P < 0.001 '***' ; P < 0.01 '**' ; P < 0.05 '*'*

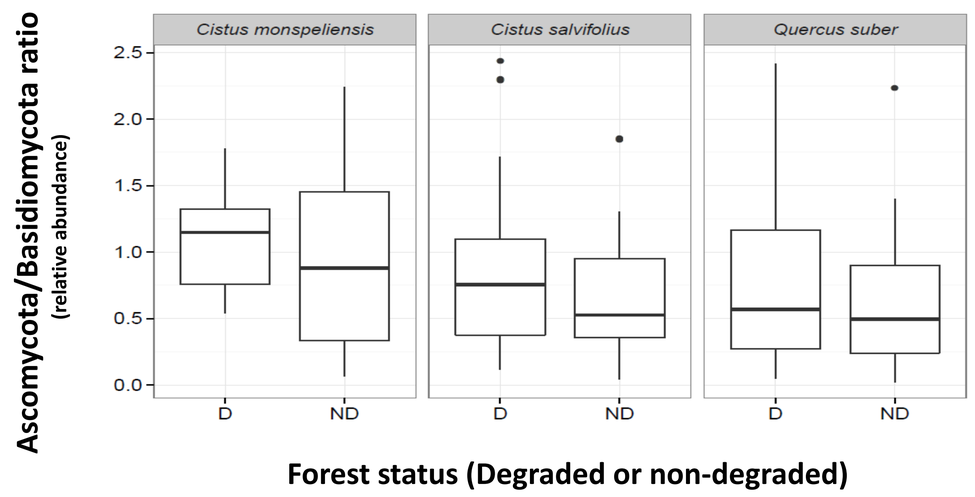
## Bio-indicator determination

Fungal-plant interaction network analyses were used to determine bio-indicators of Moroccan cork oak forests

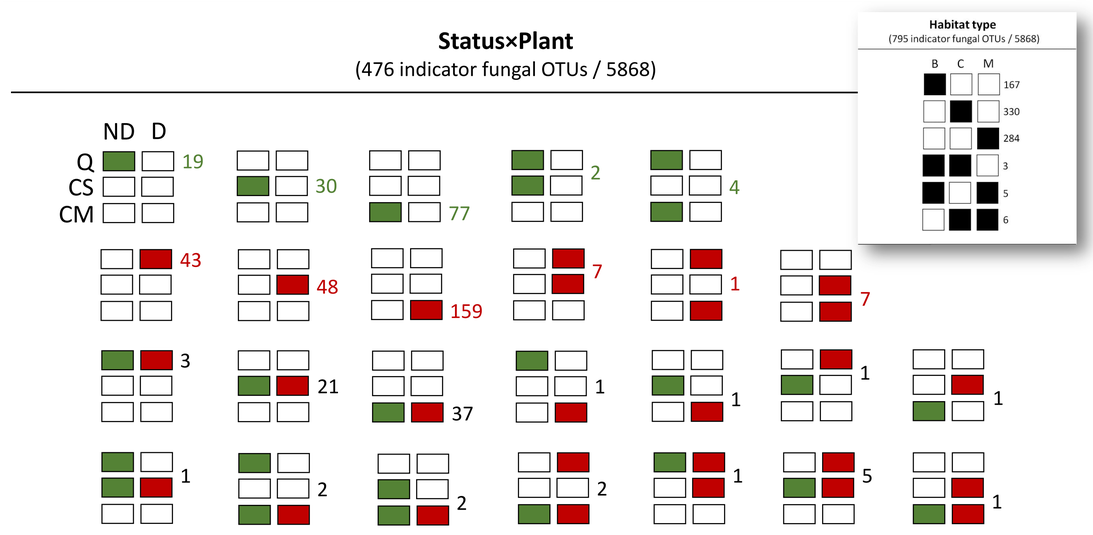
**Are phylum-based ratios good bio-indicators ?**

**D > ND**

(*P*=0.01592; all plants + all habitats)




**Are there ecological indicator species in the fungal community ?**




The presence of indicator fungal species regarding the habitat and the status×plant was determined using the corrected Pearson's phi coefficient of association ("r.g") and the corrected indicator value index ("IndVal.g"species), respectively (R package indicspecies [De Caceres et al., 2010]).

## Results and discussion

- This study represent **the most extensive** characterization of fungal diversity in cork oak ecosystems.
- Fungal community in Moroccan cork oak forest were dominated by Ectomycorrhizal fungi groups belonging to ***Russulales***, ***Thelephorales***, ***Agaricales***, ***Sebacinales***, ***Hysteriales***.
- No impact of forest status was observed on fungal richness and diversity.
- Fungal community structure was **significantly impacted by habitat** (forests), **status** (degraded or non degraded), and **host plant** (*Q. suber, C. salviifolius, C. monspeliensis*), but no interaction was observed between status and plant
- The **Ascomycota / Basidiomycota ratio** appeared as a promising fungal bio-indicator to monitor cork oak forest health, but highly dependant of number and type of samples, highlighting the **need to develop multi-plant-based and geographically-based approaches**.
- A wide range of **indicator fungal OTUs** was determine at the **habitat** and **status×plant levels.** More than 376 OTUs were specific to both a forest status and a plant type. 61 OTUs were specific to a plant type, independently of forest status.
- Results strengthened the importance of fungal community in ecosystem functioning and the need to integrate fungal bio-indicator based monitoring as innovative guidelines for a better management of cork oak forests ecosystems

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