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Fatima-Zahra Maghnia

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# THÈSE

## Pour obtenir le grade de Docteur

Délivré par l'**Université de Montpellier (Montpellier, France)** et l'**Université Sidi Mohamed Ben Abdellah (Fès, Maroc)**

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Présentée par **Fatima-Zahra MAGHNIA**

Décryptage des réseaux d'interactions plante-champignon pour une meilleure gestion des subéraies méditerranéennes

Soutenue le 19 juillet 2017 devant le jury composé de

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*« C'est une triste chose de songer que la Nature parle et que le genre humain n'écoute pas. »*

Victor Hugo

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## Résumé

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Le chêne-liège (*Quercus suber*) est une essence forestière d'une grande importance écologique et socio-économique pour les habitants de la Méditerranée. Cependant, au cours des dernières décennies, ces subéraies et particulièrement les subéraies marocaines ont été soumises à de fortes contraintes climatiques, environnementales et humaines entraînant une accélération des processus de dégradation. La conservation de ces écosystèmes est fortement dépendante de notre capacité à prédire les changements induits par ces différentes pressions ainsi que du développement d'approches durables pour leur réhabilitation. Dans ce contexte, l'identification d'indicateurs biologiques de l'état de santé des subéraies et l'intensification des processus de facilitation entre les plantes (arbres/arbustes) apparaissent comme des stratégies écologiques prometteuses. Le succès de ces approches est cependant assujéti à notre compréhension des interactions entre les communautés végétales et les champignons du sol, notamment les champignons mycorhiziens, éléments clés du fonctionnement des écosystèmes forestiers. Ce travail a visé le décryptage des réseaux fongiques, notamment mycorhiziens associés au chêne-liège et à la végétation du sous-bois dans trois subéraies marocaines (Maâmora, Benslimane, Chefchaoun) caractérisées par différents niveaux de dégradation. La diversité fongique associée aux racines du chêne-liège et à plusieurs plantes arbustives représentatives des subéraies (*Cistus salviifolius*, *Cistus monpeliensis* et *Lavandula stoechas*) a été étudiée en combinant les méthodes traditionnelles basées sur l'aspect morphologique des mycorhizes et les nouvelles technologies de séquençage haut-débit pour l'identification moléculaire des communautés fongiques.

Les résultats obtenus représentent la plus vaste enquête de la diversité fongique du sol, notamment mycorhizienne, au sein des subéraies marocaines. Différents niveaux de structuration des communautés de champignons du sol ont été révélés, en fonction de l'habitat, de l'espèce végétale et de l'état de dégradation. Une large gamme d'indicateurs fongiques de l'état de dégradation de la subéraie, en lien avec la plante hôte, ont pu être mise en évidence au sein des différents habitats, soulignant l'importance de plusieurs champignons ectomycorhiziens (notamment *Cenococcum*, *Russula*, *Terfezia* et *Tomentella*) mais aussi des champignons mycorhiziens éricoïdes (*Cladophialophora*, *Oidiodendron*) et à arbuscules (*Rhizophagus*, *Redeckera*, *Racocetra*, *Paraglomus*). Ce travail a permis d'établir une base de données majeure sur l'écologie des champignons du sol dans les subéraies marocaines, et de proposer un nouvel éclairage sur leur potentiel pour le suivi de l'état de santé des subéraies, ainsi que pour la mise en place de programmes de conservation adaptés tenant compte aussi des champignons associés. L'application des approches proposées à une plus large diversité d'écosystèmes forestiers devrait constituer un atout important pour une meilleure compréhension du fonctionnement biologique des écosystèmes forestiers et leur sauvegarde face à l'aggravation des pressions humaines et climatiques au niveau mondial.

**Mots clés :** subéraie, communautés, champignons mycorhiziens, indicateur biologique, dégradation, nouvelles technologies de séquençage à haut débit

## Abstract

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The Cork oak (*Quercus suber*) forests play an important role in terms of ecological services and socio-economic development for the Mediterranean populations. However, the cork oak forests, notably in the Southern Mediterranean basin are highly threatened by increasing human and climate pressures, which accelerates desertification. The conservation of this ecosystem is strongly dependent of our ability to predict the environmental changes induced by these pressures as well as to develop sustainable approach for their restoration. In this context, the identification of biological indicators of cork oak health and the intensification of plant-plant facilitation processes appear as promising ecological strategies. Their success is however subjected to our understanding of plant-fungal interactions, notably with fungal mycorrhiza, key factors of forest ecosystem functioning. The current work aimed at deciphering plant-fungal networks, notably mycorrhizal networks with cork oak and its understory shrub vegetation in three Moroccan cork oak habitats (Maâmora, Benslimane, Chefchaoun) characterized by different degradation levels. The root-fungal diversity associated to cork oak and major components of its understory shrub vegetation (*Cistus salviifolius*, *Cistus monpehiensis* et *Lavandula stoechas*) has been analysed by combining traditional methods based on morphological identification, and new generation high-throughput DNA sequencing methods to characterize communities at the molecular level.

The study represents the most extensive survey of soil fungal diversity, notably mycorrhizal diversity, in Moroccan cork oak ecosystems. Different fungal community structures were revealed, depending on habitat, plant host type, and degradation forest status. A wide range of fungal indicators of plant type × forest status has been identified, highlighting the importance of several ectomycorrhizal fungi (notably *Cenococcum*, *Russula*, *Terfezia* and *Tomentella*) as well as ericoid mycorrhizal fungi (*Cladophialophora*, *Oidiodendron*) and arbuscular mycorrhizal fungi (*Rhizophagus*, *Redeckera*, *Racocetra*, and *Paraglomus*). The current work provides an extensive database on the ecology of soil fungi related to the Moroccan cork oak forest, offers new insights into the potential of soil fungi for monitoring the health of the cork oak forest, and for the development of efficient conservation programs of this ecosystem by taking into account the soil fungal communities associated. The use of proposed approaches to a larger diversity of forest ecosystems are promising to better understand the biological functioning of forest ecosystem and their conservation in response to the worsening of worldwide human and climate pressures.

**Keywords:** cork oak forest, communities, mycorrhizal fungi, biological indicator, degradation, new generation DNA sequencing

تشكل غابات البلوط الفليني (السنديان الفليني) أحد أهم النظم الإيكولوجية ذات قيمة سوسيواقتصادية وإيكولوجية عالية بالنسبة لسكانة البحر الأبيض المتوسط. إلا أنه خلال العقود الأخيرة تعرضت هذه الغابات وخصوصاً بالمغرب إلى إكراهات وضغوطات مناخية وبيئية وبشرية شديدة أدت إلى تسريع وثيرة تدهورها.

إن الحفاظ على هذه النظم الإيكولوجية يعتمد بشكل كبير على مدى قدرتنا على التنبؤ بالتغيرات الناجمة عن مختلف الضغوطات والتحديات وعلى تطوير مناهج مستدامة من أجل إعادة تأهيل هذه النظم.

وفي هذا السياق، فإن تحديد المؤشرات البيولوجية الدالة على الحالة الصحية لغابات البلوط الفليني وتكثيف عمليات التسهيل ما بين النباتات (الأشجار / الشجيرات) تبدو كاستراتيجيات إيكولوجية واعدة.

ومع ذلك فإن نجاح هذه المقاربات أو المناهج مرتبط بفهمنا للتفاعلات ما بين المجتمعات النباتية وفطريات التربة، وبالأخص الفطريات الجذرية أو الفطريات التكافلية والتي تعد عناصر رئيسية لعمل النظم الإيكولوجية الغابوية.

ولهذا فإن الهدف من هذا العمل هو محاولة فك رموز الشبكات الفطرية، وخاصة الجذرية منها، شركاء البلوط الفليني وشجيرات غطائه النباتي بثلاث غابات البلوط الفليني بالمغرب والمتمثلة في غابة المعمورة، وغابة بنسليمان وغابة شفشاون والتي تتميز بمستويات تدهور مختلفة.

وفي هذا الصدد قمنا بدراسة تنوع الفطريات المرتبطة أو المتصلة بجذور البلوط الفليني وبالعديد من الشجيرات الممثلة لغطائه النباتي (قريضة مريرية الأوراق *Cistus salviifolius*، وقريضة متوسطة *Cistus monpeiliensis* وضرم مكور *Lavandula stoechas*) وذلك من خلال الجمع بين الطرق التقليدية (على أساس المظهر الخارجي للفطريات الجذرية) والتكنولوجيات الحديثة لتسلسل الحمض النووي ذات الإنتاجية العالية (تحديد جزيئي للمجتمعات الفطرية).

وتمثل النتائج المحصل عليها من خلال هذه الدراسة أكبر استطلاع لتنوع فطريات التربة وخاصة الفطريات الجذرية في غابات البلوط الفليني بالمغرب. ولقد تم الكشف عن مستويات مختلفة من الهياكل والبنىات للمجتمعات الفطرية للتربة وفقاً للمسكن البيئي أو الطبيعي ونوع النباتات وحدة التدهور مؤكدة بذلك أهمية عدة فطريات جذرية خارجية (خاصة سينوكوكو *Cenococcum* - روسولا *Russula* - الترفاس *Terfezia* - وتومنتيلا *Tomentella*). وكذلك فطريات جذرية إريكويدية (كلادوفيلوفورا *Cladophialophora* - وديوندرون *Oidiiodendron*) وفطريات جذرية داخلية (ريزوفاكوس *Rhizophagus* - ريديك *Redeckera* - راكوسيترا *Racocetra* - باراكلومس *Paraglomus*)

لقد ساهم هذا البحث في إنشاء قاعدة بيانات مهمة في مجال إيكولوجية فطريات التربة في غابات البلوط الفليني بالمغرب. وإعطاء رؤية جديدة لمؤهلاتها من خلال متابعة حالتها الصحية، وكذلك في وضع برامج ملائمة للحفاظ على هذه الأنظمة الإيكولوجية مع الأخذ بعين الاعتبار الفطريات المصاحبة لها.

إن تطبيق المقاربات المقترحة على نطاق أوسع من تنوع هذه الأنظمة الإيكولوجية الغابوية يجب أن يشكل ميزة أساسية من أجل فهم أفضل لعملها البيولوجي وحمايتها من تفاقم الضغوطات البشرية والمناخية على الصعيد العالمي.

**الكلمات المفتاحية:** غابات البلوط الفليني، مجتمعات، فطريات جذرية، مؤشرات بيولوجية، تدهور، تكنولوجيات حديثة لتسلسل الأحماض النووية ذات الإنتاجية العالية.



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## Liste des abréviations

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**ADN** : Acide DésoxyriboNucléique

**ADNr** : Acide Désoxyribo Nucléique ribosomal

**AM fungi** : Arbuscular Mycorrhizal fungi

**ANOVA** : Analysis of variance

**BSA** : Bovine serum albumin

**C** : Carbone

**Ca** : Calcium

**CEC** : Cation exchange capacity

**CIRAD** : Centre de coopération Internationale en Recherche Agronomique pour le Développement

**CM** : *Cistus monspeliensis*

**CS** : *Cistus salviifolius*

**CRF** : Centre de la Recherche Forestière

**D** : degraded

**Df** : degrees of freedom

**DNA** : Deoxyribonucleic acid

**dNTP** : désoxyriboNucléotide Tris-Phosphate

**Eco&Sols** : Ecologie fonctionnelle & biogéochimie des sols & des agro-systèmes

**EcM** : Ectomycorhize ou Ectomycorrhiza

**FAO** : Food and Agriculture Organization

**F. Model** : F value by permutation

**HCEFLCD** : Haut Commissariat des Eaux et Forêts et la Lutte Contre la Désertification

**IMBE** : Institut Méditerranéen de la Biodiversité et d'Ecologie marine et continentale

**IndVal.g** : corrected indicator value index

**IRD** : Institut de Recherche pour le Développement

**ITS** : Internal Transcribed Spacer ou espaceur intergénique transcrit

**IUCN** : International Union for Conservation of Nature

**K** : Potassium

**L** : *Lavandula stoechas*

**LSTM** : Laboratoire des Symbioses Tropicales et Méditerranéennes

**Mg**: Magnésium

**MS**: mean squares

**MT** morphotypes

**N** : Nitrogen ou Azote

**Na** : Sodium

**Necto** : nombre d'apex EcM total

**NCBI** : National Center for Biotechnology Information

**ND** : non-degraded

**NMDS** : nonmetric multi-dimensional scaling

**N<sub>MT</sub>** : nombre d'apex total d'un morphotype donné

**NS** : non-significant

**N<sub>total</sub>** : nombre d'apex mycorhizés et non mycorhizés.

**OTU** : Operational Taxonomic Unite

**P** : Phosphore

**PCR** : Polymerase Chain Reaction ou Réaction de Polymérisation en Chaîne

**PERMANOVA** : non-parametric permutational multivariate analysis of variance

**pH** : potentiel Hydrogène

**PR2 database** : Protist Ribosomal Reference database

**PVPP** : Polyvinylpolypyrrolidone

**p-value** : probability value

**Q** : *Quercus suber*

**R<sup>2</sup>** : partial R-squared

**r.g** : corrected Pearson's phi coefficient of association

**SOM** : soil organic matter

**SS** : sum of squares

**SYSTEM** : Fonctionnement et conduite des systèmes de culture tropicaux et méditerranéens

**Taq** : *Thermus aquaticus*

**UMR** : unité mixte de recherche

**UNITE** : Unified database for the DNA based fungal species

## Glossaire

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**Activité microbienne (ou Microbial activity)** : est un terme utilisé pour indiquer un ensemble d'activités enzymatiques effectuées par les microorganismes, alors que l'**activité biologique** reflète non seulement les activités microbiennes, mais aussi les activités d'autres organismes dans le sol, y compris les racines des plantes. Source : Nannipieri *et al.*, 2003.

**Conservation (écosystème)** : Mode de gestion de la biosphère ayant pour objectif de préserver sa capacité de répondre aux besoins des générations actuelles et futures (comprend la préservation, l'entretien, l'utilisation durable, le rétablissement et l'amélioration du milieu naturel). Source : IUCN/WWF/UNEP. 1981. Conservation Strategy

**Diversité biologique ou Biodiversité** : Variabilité des organismes vivants et des complexes écologiques dont ils font partie ; cela comprend la diversité au sein des espèces et entre espèces ainsi que celle des écosystèmes. Source : CDB, 1992

**Ecosystème** : un complexe dynamique de communautés végétales, animales et de micro-organismes et de leur environnement abiotique étroitement lié en une unité fonctionnelle. Source : CDB, 1992.

**Facilitation** : Situation où la présence d'une espèce bénéficie, via la création de conditions favorables, à l'installation, la vie ou la survie d'autres espèces. Celles-ci, plus exigeantes, n'auraient pas pu s'installer directement ou de développer correctement, sans ces modifications (Stachowics, 2001). On parle de facilitation (d'une espèce A envers une espèce B), lorsqu' au moins l'un des participants bénéficie de l'interaction, et qu'elle cause de dommages à aucune des deux espèces (Bastien & Gauberville, 2011). Source : <http://www.supagro.fr/>

**Habitat** : le lieu ou type de site dans lequel un organisme ou une population existe à l'état naturel. Source : CDB, 1992.

**Protocole de Nagoya** sur l'accès aux ressources génétiques et le partage juste et équitable des avantages découlant de leur utilisation relative à la Convention sur la diversité biologique a été adopté à la dixième réunion de la Conférence des Parties, le 29 octobre 2010, à Nagoya, au Japon, après six ans de négociations. Le Protocole fait progresser considérablement le troisième objectif de la Convention en assurant une plus grande certitude juridique et une transparence accrue pour les fournisseurs et les utilisateurs de ressources génétiques. Source: <https://www.cbd.int/abs/about/>

**Résilience (écologique)** : Le concept de résilience écologique fait référence à la capacité d'un écosystème à supporter diverses perturbations et adopter différentes stratégies pour recouvrer certaines de ses propriétés originelles (fonctions, structure, composition, etc.). Source : Peterson *et al.*, 1998 in Dia & Duponnois, 2013.

**Unité taxonomique opérationnelle moléculaire (en anglais Operational Taxonomic Unit OTU)** : représente un organisme (individu), un groupe taxonomique (par exemple une espèce ou à un genre), un groupe avec des relations évolutives non définies qui partage un ensemble de caractères observés. Dans le cas des analyses moléculaires, une OTU est un regroupement de séquences nucléiques basées par exemple sur leur similarité. Cette standardisation internationale permet de comparer des données moléculaires à l'échelle de populations ou de communautés mais elle n'a pas forcément une signification biologique. Différents types de regroupements sont actuellement proposés. Un seuil de 97% de similarité est couramment utilisé en microbiologie. Source : [http://www.drive5.com/usearch/manual/otu\\_definition.html](http://www.drive5.com/usearch/manual/otu_definition.html).

## Liste des publications

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Article 1:

**F.Z. Maghnia**, H. Sanguin, Y. Abbas, M. Verdinelli, B. Kerdouh, N. Elghachtouli, E. Lancellotti, S.E. Bakkali Yakhlef, R. Duponnois, 2017. Impact of cork oak management on the ectomycorrhizal fungal diversity associated with *Quercus suber* in the Mâamora forest (Morocco). *Comptes Rendus Biologies* (**accepté, en cours de publication**)

Article 2:

**F.Z. Maghnia**, Y. Abbas., F. Mahé, B. Kerdouh, E. Tournier, M. Ouajdi, Y. Prin, N. El Ghachtouli, S. Bakkali Yakhlef, R. Duponnois, H. Sanguin, 2017. Habitat- and soil-related drivers of the soil fungal community associated with *Quercus suber* in the Northern Moroccan forest. *PLoS One* (**en cours de révision**).

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**F.Z. Maghnia**, F. Mahé, S. Bakkali Yakhlef, B. Kerdouh, M. Ouajdi, Y. Prin, N. El Ghachtouli, R. Duponnois, Y. Abbas, H. Sanguin. A fungal community-based high throughput approach to determine ecological bio-indicators of Moroccan cork oak decline; 5<sup>th</sup> edition International ECOSUMMIT- Ecological Sustainability: Engineering Change, 29<sup>th</sup> August – 1<sup>st</sup> September 2016, Montpellier, France. *Communication affichée*.

**F.Z. Maghnia**, Y. Abbas, F. Mahé, B. Kerdouh, M. Ouajdi, Y. Prin, S. E. Bakkali Yahlef, N. El Ghachtouli, B. Kerdouh, R. Duponnois, H. Sanguin. Deciphering fungal bio-indicators of Moroccan cork oak forest functioning; Journée Jean Chevaugéon 2016, 11<sup>th</sup> Rencontres de Phytopathologie- Mycologie Aussois- 25-29 Janvier 2016. *Communication orale*.

**F.Z. maghnia**, Y. Abbas, B. Kerdouh, M. Ouajdi, E. Baudoin, N. El Ghachtouli, Y. prin, S.E. Bakkali Yakhlef, R. Duponnois, H. Sanguin. Effet de la dégradation sur les réseaux mycorrhiziens dans les subéraies naturelles au Maroc. 1er Workshop national de biodiversité, Faculté des Sciences et Techniques de Fès, 17 Décembre 2014. *Communication affichée*.

**F.Z. Maghnia**, Y. Abbas, B. Kerdouh, M. Ouajdi, E. Baudoin, N. El Ghachtouli, Y. Prin, S. Bakkali Yakhlef, R. Duponnois, H. Sanguin. Deciphering mycorrhizal networks in natural Moroccan cork oak stands related to forest decline; International Congress of Mycorrhizae: Mycorrhizal Symbiosis a Key Factor for Improving Plant Productivity and Ecosystems Restoration, 15<sup>th</sup>-17<sup>th</sup> October 2014, Marrakech- Morocco. *Communication affichée*.

**F.Z. Maghnia**, R. Duponnois, M. Verdinilli, E. Lancelloti, Y. Abbas, B. Kerdouh, N. El Ghachtouli, S. Bakkali Yakhlef : Contribution à l'étude de l'évolution spatio-temporelle des communautés de champignons ectomycorrhiziens du chêne-liège de la Maâmora ; Second edition of the international Congress : "Microbial Biotechnology for Development" (MICROBIOD 2) Marrakech, 02<sup>nd</sup> – 04<sup>th</sup> October 2012. *Communication orale*.



## Introduction générale

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Les écosystèmes forestiers sont une composante importante et indispensable pour la survie sur terre. D'une part, ils assurent d'importants services écologiques tels que (i) la conservation de la plus grande biodiversité terrestre du monde, (ii) le stockage du carbone, (iii) la protection des sols contre l'érosion et (iv) l'atténuation des processus de désertification et des phénomènes de changements climatiques. D'autre part, ils sont essentiels pour de nombreux secteurs économiques (par exemple l'agriculture, la sylviculture, l'écotourisme et l'énergie), et contribuent de manière significative au développement rural, à la sécurité alimentaire et la lutte contre la pauvreté. En effet, selon le rapport de la FAO publié en 2014 (FAO, 2014), des millions de personnes dans le monde dépendent des ressources alimentaires provenant des forêts, et environ un tiers des ressources énergétiques qui en découlent (le bois de feu étant la principale, voir la seule, source d'énergie pour ces personnes). De plus, les forêts offrent 13,2 millions d'emplois dans le secteur forestier structuré et au moins 40 millions dans le secteur informel. Elles offrent également des produits servant dans la construction de logement et d'abri pour environ 1,3 millions de personnes à travers le monde.

Au cours du temps, la superficie des forêts dans le monde a subi plusieurs phases de régression et d'extension [Adam (1997) cité par Blaser & Gregersen (2013)] à cause de phénomènes naturels. Mais depuis à peu près 3000 ans, la superficie forestière mondiale connaît une diminution continue, due principalement à l'activité humaine. Cette diminution ne cesse de s'accroître à cause de l'accroissement de la démographie, l'industrialisation, et le changement de mode de vie et de consommation de la société humaine à travers le globe. A titre d'exemple, 7 millions d'hectares de forêts tropicales par an ont été perdus entre 2000 et 2010 au profit de l'agriculture (FAO, 2016). Mais, la perte en superficie des forêts peut être aussi due à des phénomènes complexes de dégradation et/ou de mortalité des arbres (sécheresse, attaques parasitaires, feux, déficits de régénération naturelles et artificielles, programmes d'aménagement sylvicoles inappropriés, ...) (Battles & Fahey, 2000; Belghazi *et al.*, 2001; Benzyane *et al.*, 2002; Modrzyński, 2003; Laouina *et al.*, 2010; Camilo-Alves *et al.*, 2013; Sallé *et al.*, 2014; Cohen *et al.*, 2016; Herguido *et al.*, 2016).

L'accroissement de l'utilisation des terres et les changements climatiques constituent aujourd'hui les plus grandes menaces qui pèsent sur les forêts et sa biodiversité (Sala *et al.*, 2000; Blaser & Gregersen, 2013), même dans l'hypothèse du scénario le plus optimiste (Blaser & Gregersen, 2013). Dans le cas du bassin Méditerranéen, les écosystèmes terrestres hébergent une grande richesse en biodiversité avec un haut niveau d'endémisme (près de 25000 espèces dont presque la moitié sont endémiques) (Thompson *et al.*, 2005) ; troisième point chaud de diversité végétale au niveau mondial (Mittermeier *et al.*, 2004) avec 11 sites majeurs (Véla & Benhouhou, 2007). Les écosystèmes méditerranéens sont prédits comme les écosystèmes qui seront les plus affectés par les changements climatiques dans le futur en termes de perte de biodiversité (Sala *et al.*, 2000). En effet, une grande partie des espèces et de la richesse génétique qui font la particularité écologique des pays du bassin Méditerranéen encourent aujourd'hui un grand danger d'extinction, de migration vers d'autres zones climatiques plus adaptées, ou de substitution par des espèces plus résistantes.

De ce fait, depuis la première Conférence des Nations Unies sur l'environnement et le développement en 1992, qui a abouti à la convention sur la diversité biologique, de vastes programmes portant sur l'analyse et le recensement de la diversité biologique existante,

menacée ou en voie de disparition, se sont multipliées et ce pour différentes raisons ; d'une part pour une meilleure compréhension de son rôle dans le fonctionnement et la durabilité des écosystèmes, et d'autre part pour pouvoir faire face à la grande menace des changements globaux par l'adoption de stratégies plus adéquates, efficaces et respectueuses de l'environnement. Ces études se sont principalement focalisées sur les espèces animales terrestres et marines ainsi que sur les espèces végétales (Brooks *et al.*, 2002; Médail & Quézel, 2003; Cuttelod *et al.*, 2009). Le compartiment microbien a été largement sous-estimé alors qu'il représente une entité vivante particulièrement riche et complexe, et un support indispensable pour la croissance des végétaux. Les communautés microbiennes du sol ou les microorganismes telluriques sont par exemple d'une grande importance écologique dans le maintien du bon fonctionnement des écosystèmes terrestres et constituent un réservoir génétique important. Elles jouent un rôle majeur dans les grands cycles biogéochimiques (carbone, azote et phosphore), les processus de décomposition, de transformation et de transport de la matière organique, la mobilité et le transfert sol-plante des éléments nutritifs, ainsi que la détermination de la biodiversité végétale terrestre et la croissance de leurs hôtes. Les champignons du sol et notamment les champignons mycorrhiziens font parties des acteurs majeurs de cette communauté dans le fonctionnement des écosystèmes forestiers (Courty *et al.*, 2010; Uroz *et al.*, 2016; Baldrian, 2017)

Les champignons mycorrhiziens vivent en symbiose avec les racines de la majeure partie des espèces végétales. Ils favorisent ainsi la croissance des plantes en améliorant l'absorption de l'eau et des éléments minéraux grâce aux hyphes extramatriciels, et confèrent une meilleure résistance des plantes face à de nombreux stress comme les attaques de pathogènes ou le stress hydrique (Smith & Read, 2009). Dans les écosystèmes naturels, les plantes obtiennent par exemple jusqu'à 80 % de leurs exigences en azote et jusqu'à 90 % de celles en phosphore à partir des champignons mycorrhiziens (Hobbie & Hobbie, 2006; van der Heijden *et al.*, 2008). Ces communautés mycorrhiziennes sont cependant sensibles à de nombreuses perturbations comme le vieillissement de la plante-hôte, la destruction de certains biotopes, la pollution, l'urbanisation, et dans le cas plus particulier des forêts, aux pratiques sylvicoles (Baxter *et al.*, 1999; Byrd *et al.*, 2000; Nannipieri *et al.*, 2003; Lazaruk *et al.*, 2005; de Román & de Miguel, 2005; Azul *et al.*, 2009; Karpati *et al.*, 2011; Boudiaf *et al.*, 2013). Ces perturbations se manifestent souvent par des changements au niveau de l'abondance, la richesse, la diversité et la structure des communautés mycorrhiziennes.

Dans le présent travail de recherche, nous nous sommes intéressés à l'étude de cette composante majeure des écosystèmes forestiers, que sont les communautés fongiques du sol et plus particulièrement mycorrhizienne, et de leur lien avec la santé des subéraies (*Quercus suber*) marocaines en raison des menaces qui pèsent sur cet écosystème emblématique du bassin méditerranéen. En effet, comme de nombreuses forêts, les subéraies méditerranéennes (plus de 20,000 km<sup>2</sup> de superficie) sont fortement impactées par l'activité humaine et les changements climatiques (Acácio & Holmgren, 2014; Gauquelin *et al.*, 2016b). La dégradation des subéraies entraîne de fortes retombées négatives du point de vue écologique et socio-économique, particulièrement au Maroc, avec notamment une forte érosion des sols, une chute de la production du liège, une diminution de la taille et de la qualité des espaces de pâturage, une chute de la production de bois, de plantes aromatiques et médicinales, ainsi que de champignons comestibles. Certaines composantes écologiques majeures de la subéraie sont fortement altérées par les pressions humaines et climatiques, c'est-à-dire le couvert végétale, la densité des populations de chêne-liège (Costa *et al.*, 2010; Acácio & Holmgren, 2014; Ibáñez *et al.*, 2015b), mais aussi le compartiment microbien (Azul *et al.*, 2010; Costa *et al.*, 2013; Lancellotti & Franceschini, 2013; Bevivino *et al.*, 2014), aboutissant à une chute des taux de régénération du chêne-liège, aux vieillissements des populations, et *in fine* à leur mort

(Ajbilou *et al.*, 2006; Aafi, 2007; Curt *et al.*, 2009). Le couvert végétale des subéraies est caractérisé par une forte diversité d'espèces arbustives, notamment *Cistus monspeliensis*, *Cistus salviifolius*, *Pistacia lentiscus*, *Myrtus communis*, *Lavandula stoechas*, *Arbutus unedo* et *Erica arborea* (Carrión *et al.*, 2000; Aafi *et al.*, 2005; Boudiaf *et al.*, 2013), dont certaines ont un effet positif ou négatif sur la régénération et la survie du chêne-liège (Acácio *et al.*, 2007; Pérez-Devesa *et al.*, 2008; Curt *et al.*, 2009). La durabilité des subéraies est donc fortement liée aux interactions plante-plante se mettant en place au sein de l'écosystème. Plusieurs études ont montré que ces interactions plante-plante bénéfiques faisaient intervenir différentes composantes des communautés microbiennes du sol, notamment les champignons mycorrhiziens (Pugnaire, 2010; Montesinos-Navarro *et al.*, 2012a; Duponnois *et al.*, 2013).

Le projet de doctorat a pour hypothèse que les champignons du sol, et la structuration des réseaux fongiques établis avec le chêne-liège et le couvert arbustif sont les piliers de la santé des subéraies, et que leur suivi et optimisation est un paramètre clé pour améliorer notre capacité à prédire les futurs changements induits par les pressions humaines et climatiques, et développer à terme des approches durables basées sur l'utilisation de ces réseaux pour la réhabilitation de subéraies dégradées. Le décryptage des réseaux fongiques, et l'identification d'indicateurs biologiques de la santé des subéraies basées sur les interactions plante-champignon, sont les objectifs majeurs de ce doctorat.

Ce travail s'inscrit dans la lignée des politiques et des programmes forestiers nationaux, qui visent à lutter contre la dégradation des écosystèmes par une gestion durable des ressources et une conservation de la biodiversité naturelle.

Pour atteindre les objectifs fixés ci-dessus, trois types d'habitats au sein de la subéraie marocaine ont été choisis, les forêts de la Maâmora, Benslimane et Chefchaoun (**Figure 1**). Ces forêts présentent différents niveaux d'anthropisation et sont constituées de zones dégradées et non dégradées. Un plan d'échantillonnage du sol et de racines dans les différentes zones a été mis en place afin de caractériser les communautés fongiques (abondance, richesse, diversité et structure) associées aux chênes-liège et aux arbustes représentatifs des écosystèmes. La caractérisation des communautés fongiques a été effectuée en combinant les nouvelles technologies de séquençage haut-débit et les méthodes traditionnelles (basées sur l'aspect morphologique des champignons au niveau racinaire).

Ce mémoire de thèse sera présenté sous la forme de quatre chapitres :

Le premier chapitre est une synthèse bibliographique « **The plant-microbiome perspective of cork oak forest functioning** ». Il s'agit d'un état des lieux des connaissances sur le chêne-liège et le fonctionnement microbiologique des subéraies méditerranéennes. Cette synthèse fera l'objet d'une publication sous forme d'une monographie chez l'éditeur Nova.

Le second chapitre traite de la mise en relation des données abiotiques du sol avec la structure des communautés fongiques associée au chêne-liège dans les différents habitats de la subéraie marocaine (Maâmora, Benslimane, Chefchaoun). Ce chapitre fait l'objet d'une publication en cours de révision dans le journal PLoS One intitulée « **Habitat- and soil-related drivers of the soil fungal community associated with *Quercus suber* in the Northern Moroccan forest** »

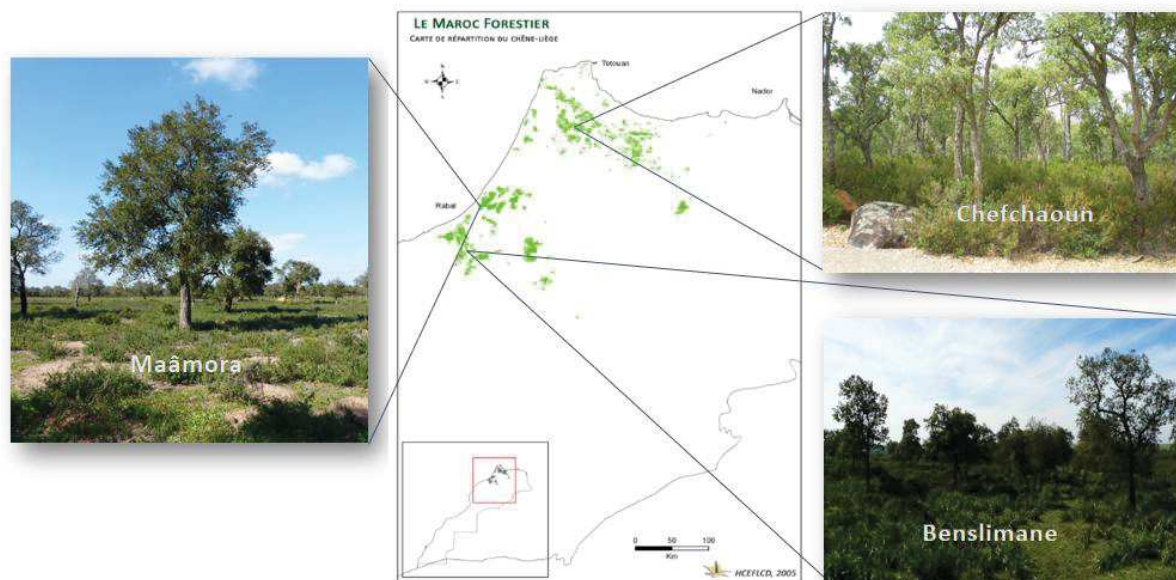
Le troisième chapitre traite de l'évaluation de l'impact des pressions humaines au cours de la période estivale et hivernale sur la diversité des champignons ectomycorhiziens associés au chêne-liège au sein de la subéraie de la Maâmora (Maroc). Ce chapitre fait l'objet d'une publication acceptée dans Comptes Rendus Biologies intitulée « **Impact of cork oak**

**management on the ectomycorrhizal fungal diversity associated with *Quercus suber* in the Mâamora forest (Morocco) ».**

Le quatrième chapitre est une étude plus large ciblant les champignons ectomycorhiziens, non-ectomycorhiziens et mycorhiziens à arbuscules associés au chêne-liège et à différentes espèces arbustives dans les différents habitats de la subéraie (Maâmora, Benslimane, Chefchaoun), et en fonction de l'état de l'habitat (dégradé et non-dégradé).

Dans l'ensemble des chapitres II, III et IV, la question des indicateurs biologiques du fonctionnement des subéraies est un point central.

Pour terminer, les conclusions de ce travail et différentes perspectives seront détaillées



**Figure 1.** Distribution du chêne-liège (en vert) au Maroc, avec la localisation des trois habitats sélectionnés pour l'étude.

# **Chapitre I**

---

## **Synthèse bibliographique**

### **The Plant-Microbiome perspective of cork oak forest functioning**

## Résumé

Le chêne-liège (*Quercus suber* L.) est une composante emblématique de la région occidentale du bassin méditerranéen et joue un rôle majeur dans la formation du paysage. En effet, les subéraies ont toujours été très appréciées par la population méditerranéenne grâce à leur grande valeur écologique et socio-économique (protection des sols, production de liège, pâturage, production de champignons, plantes aromatiques et médicinales, écotourisme, etc.). Cependant, la durabilité des subéraies est particulièrement menacée ces dernières décennies par un phénomène complexe de dégradation touchant l'ensemble des espèces de chênes « *oak decline* ». Ce phénomène est dû à l'accroissement des perturbations d'origine anthropique et climatique (surexploitation, surpâturage, déforestation, rareté et irrégularité des précipitations, parasites, fréquence du feu, etc.) combinées à la chute de la régénération du chêne-liège. La conséquence est un profond bouleversement de composantes clés du fonctionnement de l'écosystème, notamment le microbiome du sol, et *in fine* la dégradation des subéraies. Le présent chapitre vise à donner un aperçu de l'écologie du chêne-liège, de statuer sur les causes du déclin et de dresser un bilan des connaissances sur la flore microbienne du sol des subéraies et des conséquences du déclin sur cette dernière. En perspective, des pistes de recherche sont proposées pour élaborer des stratégies écologiques utilisant les interactions plante-microbiome pour la gestion durable des forêts de chênes-lièges.



## Introduction

Forest ecosystems are vital to life on earth by providing major ecological (carbon sequestration, biodiversity conservation, protection of soils) and socio-economic (e.g. agriculture, eco-tourism, energy) services, but are highly threatened by global changes, notably in the Mediterranean basin (Allen *et al.*, 2010; Gauquelin *et al.*, 2016b). Mediterranean ecosystems, which are particularly rich in biodiversity (nearly 25,000 plant species with 60 % of endemics; (Thompson *et al.*, 2005)), may know the largest proportion of biodiversity change because of the influence of all the drivers (Sala *et al.*, 2000).

The cork oak (*Quercus suber* L.) forests are an emblematic component of the western region of the Mediterranean basin. Native Cork oak forests occupy 1.3 million ha in southern Europe (Portugal, Spain, France and Italy) and 0.9 million ha in North Africa (Morocco, Algeria and Tunisia) (Lancellotti & Franceschini, 2013). They play a major role in the landscape formation and are of great ecological and socio-economic value (soil protection, cork production, logging, grazing space, mushroom production, aromatic and medicinal plants, ecotourism, etc...) for Mediterranean populations (Varela, 2000; Aronson *et al.*, 2009). However, declining regeneration of cork oak was observed, notably in Moroccan cork oak populations (Ajbilou *et al.*, 2006). In recent decades, the sustainability of many cork oak ecosystems has been particularly endangered due the combined effects of oak decline and increasing human- (overexploitation of wood, overgrazing, deforestation ...) and climate-driven (scarcity of precipitations, frequency of fire ...) perturbations (Bakry & Abourouh, 1996; Acácio & Holmgren, 2014). Cork oak decline has multiple impacts at aboveground and belowground levels, strongly affecting resilience and productivity of cork oak forests. In addition to symptoms directly affecting cork oak itself, cork oak decline was shown to negatively impact key elements of ecosystem functioning, notably the soil microbiome (Lancellotti & Franceschini, 2013).

Indeed, plants have evolved in interaction with complex microbial assemblages (microbiome) colonizing the whole plant system (rhizosphere, endosphere, phyllosphere) (Turner *et al.*, 2013; Zarraindia *et al.*, 2015; Vandenkoornhuyse *et al.*, 2015). Deciphering plant microbiome, notably soil microbiome, and the better understanding of its role in plant functioning appears as a keystone of productivity and dynamic of natural ecosystems and agroecosystems (Philippot *et al.*, 2013; van der Heijden & Hartmann, 2016; Baldrian, 2017; Busby *et al.*, 2017).

The present review aims at giving an overview of soil microbiome characteristics (structure, diversity, functions) in Mediterranean cork oak ecosystems and the impact of cork oak degradation and decline. Finally, research avenues are proposed to develop ecological strategies using plant-microbiome interactions for the sustainable management of cork oak forests.

# I. Cork oak ecology

## 1. Cork oak physiology and genetic

Cork oak (*Quercus suber* L., Fagaceae) is a sclerophyllous tree occurring in the western Mediterranean Basin, selectively growing on acidic soils in hot parts of the humid and sub-humid Mediterranean areas (at least 450 mm mean annual rainfall and > 4-5 °C mean temperature for the coldest month) (Lumaret, 2005). Nevertheless, some cork oak populations are observed in the semi-arid zone in Tunisia and Morocco. This evergreen tree is a slow growing tree that can reach about 20 m in height and 250-300 years old, exceptionally 500 years, but due to exploitation of its bark, cork oak populations drastically are mostly 150 years old (Aronson *et al.*, 2009).

The origin of cork oak has been proposed as Middle-Eastern or central Mediterranean, with a subsequent westward colonization during the Tertiary Period (Lumaret, 2005). Refuge populations are suggested in Tunisia, Sardinia, Corsica, and Provence (Magri *et al.*, 2007). Its current distribution may be the result of episodic fires (Carrión *et al.*, 2000; Magri *et al.*, 2007). The chloroplast DNA (cpDNA) genetic analysis of Mediterranean cork oak populations revealed the existence of five haplotypes (Magri *et al.*, 2007) (**Figure I.1**) and the existence of events of multiple hybridization and genetic introgression between *Quercus ilex* and *Q. suber* (Lumaret, 2005).

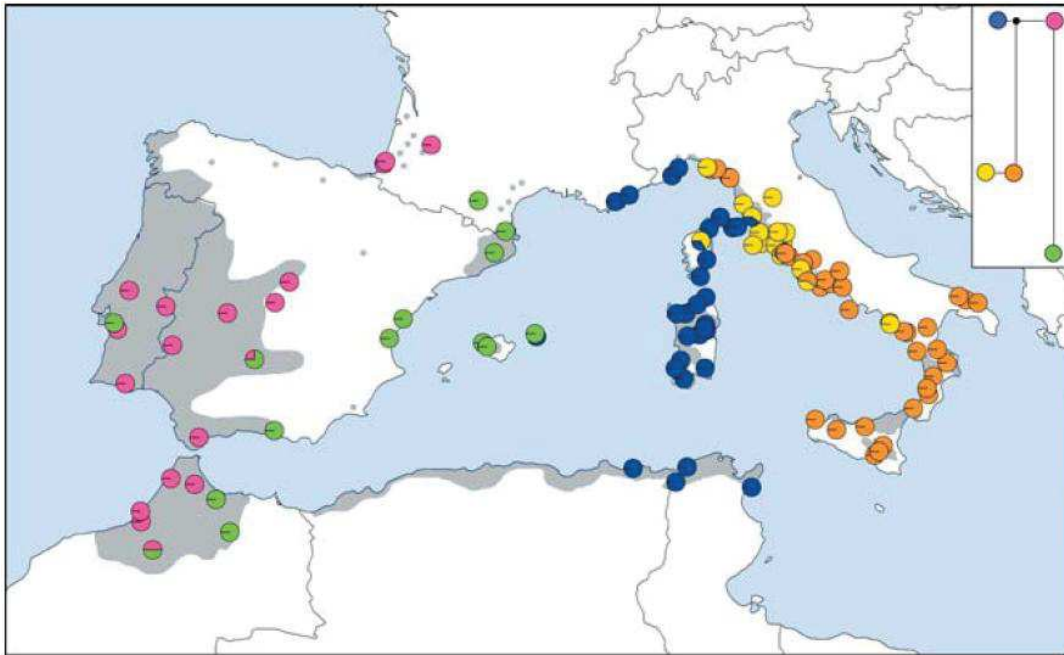
Cork oak populations exists on pure populations, probably due to human activities (Carrión *et al.*, 2000), or in mixed populations, notably with oak species and pines (Carrión *et al.*, 2000; Curt *et al.*, 2009). Cork oak ecosystems are characterized by a rich diversity of shrub species of ecological and economic added values, *e.g.* *Cistus monspeliensis*, *Cistus salviifolius*, *Pistacia lentiscus*, *Myrtus communis*, *Lavandula stoechas*, *Arbutus unedo*, *Erica arborea* (Carrión *et al.*, 2000; Aafi *et al.*, 2005; Boudiaf *et al.*, 2013).

## 2. Cork oak regeneration and decline

Cork oak has the most remarkable fecundity of all *Quercus* species and a high germination rate (Aafi, 2007), but poor regeneration rates are recorded in the Mediterranean basin (Aafi, 2007; Curt *et al.*, 2009; González-Rodríguez *et al.*, 2011). For instances, the analysis of a large set of *Q. suber* plots in Central Western Spain revealed that 62% were lacking any small seedlings and 96% did not have any large saplings (Plieninger *et al.*, 2010). Natural regeneration is directly linked to acorn dispersal and seedling establishment / growth (Pausas *et al.*, 2006). Physiology of tree has been shown as directly affecting regeneration rates (Aafi, 2007), but indirect factors can also strongly impact natural regeneration, notably land use, fire, forest understory and tree neighbors (Pons & Pausas, 2006; Pausas *et al.*, 2006; Aafi, 2007; Curt *et al.*, 2009; Plieninger *et al.*, 2010; Ibáñez *et al.*, 2015b).

The decrease of regeneration rates affecting cork oak is emphasize since the beginning of the 20<sup>th</sup> century by a larger multifactorial syndrome, named oak decline, with a worsening of severity during the 1980s (Camilo-Alves *et al.*, 2013). The symptoms are multiples and occur during a large range of time scales and with various intensity levels. Trees affected present mainly progressive necroses of bark and cambium, slime flux on the trunks and branches, crown thinning, epicormic shoots, reduction in diameter growth (Thomas *et al.*, 2002).





**Figure I.1.** Distribution of molecular lineages of *Quercus suber* populations in its modern natural area and phylogenetic reconstruction of the relationships among haplotypes. [from (Magri *et al.*, 2007)]

The consequences are higher tree mortality, reduced cork productivity, and a decrease in tree regeneration and density, leading to a decline of cork oak distribution area, as well as major changes in cork oak forest understory. A range of abiotic and biotic factors are implicated in the oak decline (Thomas *et al.*, 2002), and can be categorized as predisposing, contributing or inciting factors (Sallé *et al.*, 2014). The predisposing factors are acting constantly, mainly related to habitat characteristics and management strategies. Contributing or inciting factors are more limited in the time but intense. The main contributing and inciting factors are detailed below.

## 2.1 Drought and fires

Land use, pests and diseases are the most studied factors regarding cork oak decline, but because drought and fire episodes (frequency, intensity levels) lead to high levels of disturbances of cork oak ecosystem functioning in a short time scale, it has been assumed these abiotic factors strongly contributed to oak decline symptoms. In addition, the symptoms observed for trees affected by decline or drought are similar (Costa *et al.*, 2010).

Cork oak is able to adapt to drought conditions by reducing leaf water potential, specific leaf area, stomatal conductance, and by increasing leaf temperature and non-photochemical quenching (Ramírez-Valiente *et al.*, 2009; Grant *et al.*, 2010). Nevertheless, drought is considered as the main cause of enhanced tree mortality in oak forests, notably for cork oak (David *et al.*, 2007), and climate change analysis predicted drought as the main consequence in Mediterranean basin (Polade *et al.*, 2014). Severity or long period of drought was suggested as predisposal factors for decline of mature oaks (Costa *et al.*, 2010). Indirect impacts of drought on cork oak regeneration were also observed through the alteration of canopy neighbour trees and species relative abundance (Ibáñez *et al.*, 2015b), or by the use of plants with a root system unsuited to the sites generally characterised by insufficient water (Zine El Abidine *et al.*, 2016).

Fire is a major factor shaping Mediterranean forests (Tomaz *et al.*, 2013; San-Miguel-Ayanz *et al.*, 2013), with 600,000 ha burnt each year. Cork oak is considered as a resistant species to fire due notably to its thick and insulating bark (Pausas *et al.*, 2008). Nevertheless, cork oak management represents a major factor affecting resistance of *Q. Suber* to fire since trees with thin bark (young or recently debarked individuals) are particularly vulnerable to fire (Catry *et al.*, 2012). Fire was also shown to strongly impact cork oak forest understory and soil properties (abiotic characteristics and microbiota) (Buscardo *et al.*, 2010; Schaffhauser *et al.*, 2012; McLellan *et al.*, 2013). The characteristics of forest understory (e.g. composition, biomass and structure) related to fire history is directly linked to cork oak recruitment patterns (Curt *et al.*, 2009).

## 2.2 Pests and Pathogens

Oak decline is commonly studied through pests and pathogen perspectives because their interaction with oak is generally considered as the ultimate step before the death of trees. Two groups of organisms are implicated, i.e. insects and fungi (Thomas *et al.*, 2002; Tiberi *et al.*, 2016). Implication of bacterial pathogens or viruses is unclear (Thomas *et al.*, 2002). The insects affecting cork oak are categorized as defoliating and bark- and wood-boring insects. Depending of severity and frequency of defoliations, defoliating insects can be the cause of oak mortality or facilitate the attack of other pathogens (Tiberi *et al.*, 2016).

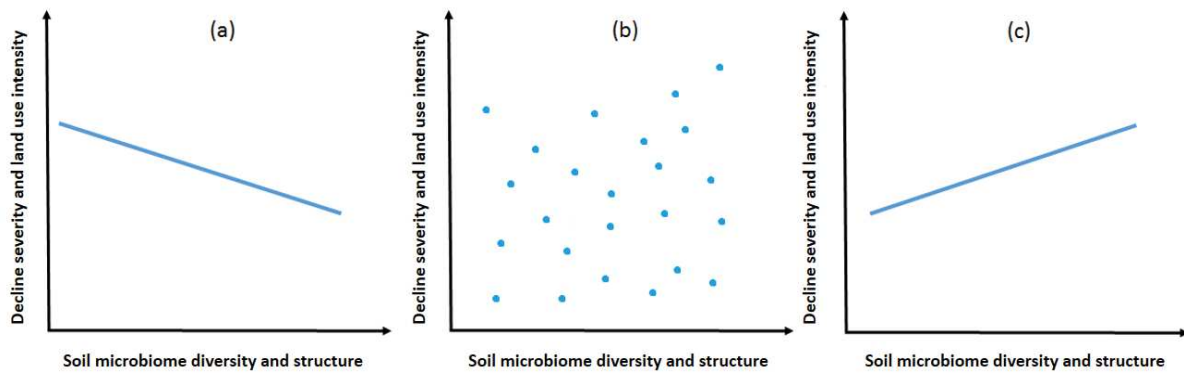
Moths represent the most abundant and harmful defoliating insects but difference in geographical distribution are observed depending of species (Tiberi *et al.*, 2016). The major species are *Lymantria dispar*, *Tortrix viridana*, *Euproctis chrysorrhoea*, *Malacosoma neustria*, *Periclista andrei*. The highest number of species was observed in Portugal (Tiberi *et al.*, 2016). The negative impact on oak species of bark- and wood-boring insects is major in all Europe (Sallé *et al.*, 2014). They particularly affect weakened trees and represent an important vector of phytopathogenic fungi (Tiberi *et al.*, 2016). The main species implicated in cork oak decline are xylophagous species belonging to Coleoptera order, i.e. *Platypus cylindrus*, *Cerambyx* spp., *Prinobius* spp. Two other species are more restricted to Iberian Peninsula, i.e. *Coroebus undatus* and *Coroebus florentinus* (Tiberi *et al.*, 2016).

Fungal pathogens implicated in cork oak decline can affect the whole tree, root, trunk, branches, and in a lesser extent leaves. *Phytophthora cinnamomi* represent the main root rot pathogen (Camilo-Alves *et al.*, 2013). Nevertheless, the detection at low and high frequency of *Pythium spiculum* and *Pythium sterillum* in decline stands, respectively, suggest a wider range of root rot pathogens implicated in oak decline (Romero *et al.*, 2007). Other pathogens causing the drying of branches, vascular necrosis and cankers on the trunks are also cited as main factors of cork oak decline, notably *Biscogniauxia mediterranea* (formerly *Hypoxylon mediterraneum*), *Botryosphaeria stevensii* and *Diplodia corticola* (anamorph of *B. stevensii*) (Elbadri & Abadie, 2000; Luque *et al.*, 2000; Henriques *et al.*, 2016).

The worsening of all decline factors due to global changes, and their interaction, may drastically and durably affect cork oak ecosystem functioning (Martín *et al.*, 2005; Acácio & Holmgren, 2014; Sallé *et al.*, 2014; Tiberi *et al.*, 2016; Hubbart *et al.*, 2016). The better characterization of drivers of cork oak ecosystem functioning is crucial to develop sustainable strategies to mitigate cork oak forest degradation and decline. However, the deciphering of cork oak ecosystem functioning mainly focuses on above-ground components and the mitigation of biotic (pathogens) and abiotic stresses, whereas below-ground functioning plays a crucial role in all terrestrial ecosystems (Wagg *et al.*, 2014), notably through soil microbiome diversity and functionalities (Nannipieri *et al.*, 2003; Rillig, 2004; Wardle, 2004; Marschner *et al.*, 2007; van der Heijden *et al.*, 2008; Bevivino *et al.*, 2014). The assessment of different scenarios regarding the impact of cork oak ecosystem disturbances on soil microbiome (**Figure I.2**) constitutes a key step for a better management and conservation of this emblematic Mediterranean ecosystem.

## **II. Impact of cork oak forest degradation and decline on soil microbiome**

Several studies reported the strong impact of cork oak degradation on soil microbiome, with a main interest about the role and diversity of soil ectomycorrhizal (EcM) fungal communities establishing a symbiosis with cork oaks (Azul *et al.*, 2009; Boudiaf *et al.*, 2013; Lancellotti & Franceschini, 2013). However, *Q. suber* and more generally *Quercus* spp. establish biotic interactions with a broader range of soil microorganisms, from arbuscular mycorrhizal (AM) fungi (Dickie *et al.*, 2001; Egerton-Warburton & Allen, 2001; Toju *et al.*, 2013a), ericoid mycorrhizal (ErM) fungi (Bergero *et al.*, 2000), root endophytic fungi (Toju *et al.*, 2013a,b), to soil bacteria (Marongiu *et al.*, 2006; Boudiaf *et al.*, 2013). An overview of the different soil microbiome compartments impacted by cork oak degradation and decline is detailed below.



**Figure I.2.** Hypothetical scenarios of interactions between soil microbiome (diversity and structure) and cork oak ecosystem status (decline severity and land use intensities). (a) negative correlation, (b) no significant correlation and, (c) positive correlation.

## 1. Soil mycorrhizal community

Mycorrhizal fungi constitute one of the most important microbial compartments for forest functioning (Courty *et al.*, 2010; Baldrian, 2017). They promote plant nutrition (N,P), notably in low nutrient soil, and improve the resistance to various abiotic stress (e.g. drought) and plant pathogens (Smith & Read, 2009).

EcM fungal surveys in Mediterranean cork oak ecosystems revealed a wide range of fungal genera, 37 belonging to Basidiomycota and 12 to Ascomycota (**Table I.1 and Table I.2**), with a high number of *Lactarius*, *Russula* and *Tricholoma* species. The majority of EcM fungal surveys detailed in **Table I.1 and I.2** were based either on fruitbodies or on fungal mantle structure covering the root surface, i.e. morphotypes (Azul *et al.*, 2009; Bakkali Yakhlef *et al.*, 2009; Lancellotti & Franceschini, 2012, 2013). These approaches can however lead to biases, (i) overestimations of fungal taxa with above-ground fruitbodies to the detriment of fungal taxa with inconspicuous structures or lacking sexual structures, and (ii) misinterpretations of fungal diversity due to incompleteness and variations of morphological criteria (Taylor & Alexander, 2005; Smith & Read, 2009). For instance, EcM fungi belonging to *Glomeraceae* (mostly *Cenococcum*), *Russulaceae* (mostly *Russula*) and *Thelephoraceae* (mostly *Tomentella*) were generally the most dominant (> 50% of EcM community) in cork oak ecosystems investigated in Portugal and Sardinia (Azul *et al.*, 2010; Buscardo *et al.*, 2010; Lancellotti & Franceschini, 2013). However, *Cenococcum* was absent from fruitbody surveys due to the lack of sexual structures. By contrast, *Pisolithus*, *Boletus* and *Lactarius* appeared as one of the most abundant EcM fungi in fruitbody surveys (Azul *et al.*, 2009; Bakkali Yakhlef *et al.*, 2009). Land-use intensity and decline were shown to negatively impact the richness and diversity of EcM fungal communities (Azul *et al.*, 2010; Buscardo *et al.*, 2010; Barrico *et al.*, 2010). However, the investment of EcM symbiosis (rate of colonization) was differentially impacted depending of forest status (decline and land use practices). Decline and fire did not significantly impact the EcM colonization rate of *Q. suber* (Buscardo *et al.*, 2010; Lancellotti & Franceschini, 2013), whereas plant invasion and land use intensity strongly affected it (Azul *et al.*, 2010; Boudiaf *et al.*, 2013). The differences of impacts on the EcM fungal communities regarding the type of disturbances are probably due to direct interactions of EcM fungal community with a various range of biotic and abiotic factors (Azul *et al.*, 2010; Lancellotti & Franceschini, 2013). For instance, differences in soil characteristics or plant cover types (due to a given disturbance) are known as major drivers of EcM diversity (Pena *et al.*, 2016).

The significance of AM fungi in cork oak ecosystem functioning is generally underestimated compared to those of EcM fungi since *Q. suber* is considered as an ectomycorrhizal tree. However, the presence of AM fungi in the juvenile states of trees and consequently their potential role in natural tree regeneration has been described for *Q. suber* (Boudiaf, 2012; Ibáñez *et al.*, 2015a) and other *Quercus* species (Dickie *et al.*, 2001; Egerton-Warburton & Allen, 2001). AM fungi belonging to the *Glomerales* order are the most abundant AM taxa observed in cork oak ecosystems (more than 95 % of AM fungal community), with a predominance of fungi affiliated to *Rhizophagus irregularis* (ex. *Glomus intraradices*) (Lumini *et al.*, 2010). This species is considered as generalist due to its worldwide distribution in various ecosystems and its ability to colonize a large range of hosts, but presents also “ruderal” characteristics (disturbance tolerant) (van der Heijden & Scheublin, 2007; Tisserant *et al.*, 2011). Land uses positively affected AM fungi community, probably because of the transition from an EcM vegetation (old growth cork oak woodland) to an AM fungal vegetation (pasture) (Lumini *et al.*, 2010).

**Table I.1.** ECM Basidiomycota fungi of Mediterranean cork oak ecosystems.

Family <sup>1</sup>	Genus	Species	Country	Habitat	References
Amanitaceae	<i>Amanita</i>	<i>A. battarae</i> , <i>A. boudieri</i> , <i>A. caesarea</i> , <i>A. citrina</i> , <i>A. crocea</i> , <i>A. curtipes</i> , <i>A. codinae</i> , <i>A. franchetii</i> , <i>A. gemmata</i> , <i>A. gilberti</i> , <i>A. lactea</i> , <i>A. muscaria</i> , <i>A. pantherina</i> , <i>A. phalloides</i> , <i>A. rubescens</i> , <i>A. spissa</i> , <i>A. vaginata</i> , Uncharacterized <i>Amanita</i> sp.	Morocco, Portugal, Spain, Italy	Natural forest, Managed forest	Ortega & Lorite, 2007; Azul <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Abourouh, 2011; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014
Agaricaceae	<i>Bovista</i> <sup>2</sup>	<i>B. aestivalis</i> , <i>B. plumbea</i>	Portugal	Managed forest	Azul <i>et al.</i> , 2009
Astraeaceae	<i>Astraeus</i>	<i>A. hygrometricus</i> , Uncharacterized <i>Astraeus</i> sp.	Portugal, Italy	Managed forest, Declined forest	Azul <i>et al.</i> , 2009; Barrico <i>et al.</i> , 2010; Lancellotti & Franceschini, 2013
Atheliaceae	<i>Byssocorticium</i>	<i>B. atrovirens</i> , Uncharacterized <i>Byssocorticium</i> sp.	Spain	Natural forest	Aponte <i>et al.</i> , 2010
Boletaceae	<i>Boletus</i>	<i>B. aereus</i> , <i>B. aestivalis</i> , <i>B. appendiculatus</i> , <i>B. calopus</i> , <i>B. edulis</i> , <i>B. fragrans</i> , <i>B. mamorensis</i> , <i>B. porosporus</i> , <i>B. reticulatus</i> , <i>B. satanas</i> , <i>B. subtomentosus</i> , Uncharacterized <i>Boletus</i> sp.	Morocco, Portugal, Spain, Italy	Natural forest, Managed forest	Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Abourouh, 2011; Orgiazzi <i>et al.</i> , 2012; Nounsi <i>et al.</i> , 2014
	<i>Xerocomus</i>	<i>X. armeniacus</i> , <i>X. chrysenteron</i> , <i>X. cisalpinus</i> , <i>X. ferrugineus</i> , <i>X. rubellus</i> , <i>X. subtomentosus</i>	Morocco, Portugal	Natural forest, Managed forest	Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Nounsi <i>et al.</i> , 2014

<sup>1</sup> Few other ECM fungi poorly affiliated with references were described in Lancellotti and Franceschini (2013) and Azul *et al.* (2009a)

<sup>2</sup> Hypothetical ectomycorrhizal status



Family <sup>1</sup>	Genus	Species	Country	Habitat	References
Cantharellaceae	<i>Cantharellus</i>	<i>C. cibarius</i> , <i>C. tubaeformis</i> , Uncharacterized <i>Cantharellus</i> sp.	Morocco, Spain	Natural forest	Ortega & Lorite, 2007; Nounsi <i>et al.</i> , 2014
	<i>Craterellus</i>	<i>C. cornucopioides</i> , Uncharacterized <i>Craterellus</i> sp.	Morocco, Spain	Natural forest	Ortega & Lorite, 2007; Abourouh, 2011
Clavulinaceae	<i>Clavulina</i>	<i>C. cinerea</i> , <i>C. cristata</i> , Uncharacterized <i>Clavulina</i> sp.	Italy, Spain	Managed forest, Natural forest	Aponte <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012
Cortinariaceae	<i>Alnicola</i>	Uncharacterized <i>Alnicola</i> sp.	Spain	Natural forest	Aponte <i>et al.</i> , 2010
	<i>Cortinarius</i>	<i>C. amoenolens</i> , <i>C. bicolor</i> , <i>C. caesiostramineus</i> , <i>C. casimiri</i> , <i>C. cedretorum</i> , <i>C. cephalixus</i> , <i>C. colus</i> , <i>C. cumatilis</i> , <i>C. decipiens</i> , <i>C. dionysae</i> , <i>C. elatior</i> , <i>C. evernius</i> , <i>C. gallurae</i> , <i>C. glaucopus</i> , <i>C. hinnuleus</i> , <i>C. holophaeus</i> , <i>C. infractus</i> , <i>C. incises</i> , <i>C. lividoochraceus</i> , <i>C. multiformis</i> , <i>C. nemorensis</i> , <i>C. paleaceus</i> , <i>C. purpurascens</i> , <i>C. rigidus</i> , <i>C. rubricosus</i> , <i>C. rufolivaceus</i> , <i>C. scobinaceus</i> , <i>C. scotoides</i> , <i>C. stillatitius</i> , <i>C. trivialis</i> , <i>C. torvus</i> , <i>C. umbrinolens</i> , <i>C. variicolor</i> , <i>C. venetus</i> , Uncharacterized <i>Cortinarius</i> sp.	Portugal, Morocco, Spain, Italy	Managed forest, Natural forest, Declined forest	Ortega & Lorite, 2007; Azul <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014
	<i>Hebeloma</i>	<i>H. ammophilum</i> , <i>H. cistophilum</i> , <i>H. crustuliniforme</i> , <i>H. pumilum</i> , <i>H. pusillum</i> , <i>H. sinapizans</i> , Uncharacterized <i>Hebeloma</i> sp.	Spain, Portugal, Morocco, Italy	Natural forest, Managed forest, Declined forest	Azul <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Lancellotti & Franceschini, 2012; Nounsi <i>et al.</i> , 2014; Haimed <i>et al.</i> , 2015
Entolomataceae	<i>Entoloma</i>	<i>E. lividum</i> , <i>E. politum</i> , <i>E. prunuloides</i> , <i>E. mougeotii</i> , <i>E. rusticoides</i> , Uncharacterized	Morocco, Italy,	Natural forest, Managed	Ortega & Lorite, 2007; Bakkali Yakhlef <i>et al.</i> , 2009; Orgiazzi <i>et al.</i> , 2012;

Family <sup>1</sup>	Genus	Species	Country	Habitat	References
		<i>Entoloma</i> sp.	Spain	forest	Nounsi <i>et al.</i> , 2014; Haimed <i>et al.</i> , 2015
Geastraceae	<i>Geastrum</i> <sup>2</sup>	<i>G. minimum</i> , <i>G. coronatum</i> , <i>G. floriforme</i>	Italy	Managed forest	Orgiazzi <i>et al.</i> , 2012
Gomphidiaceae	<i>Chroogomphus</i>	<i>C. rutilus</i>	Portugal	Managed forest	Barrico <i>et al.</i> , 2010
Gyroporaceae	<i>Gyroporus</i>	<i>G. castaneus</i> , <i>G. subalbellus</i>	Italy, Morocco	Managed forest, Natural forest	Orgiazzi <i>et al.</i> , 2012; Nounsi <i>et al.</i> , 2014
Helvellaceae	<i>Helvella</i>	<i>H. acetabulum</i> , <i>H. queletii</i>	Portugal	Managed forest	Azul <i>et al.</i> , 2009
Hygrophoraceae	<i>Hygrophorus</i>	<i>H. cossus</i> , <i>H. persoonii</i> , <i>H. arbustivus</i> , <i>H. chrysodon</i> , <i>H. latitabundus</i>	Italy, Morocco, Portugal	Managed forest, Natural forest	Barrico <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Nounsi <i>et al.</i> , 2014; Haimed <i>et al.</i> , 2015
Hymenogastraceae	<i>Hymenogaster</i>	<i>H. tener</i>	Italy	Managed forest	Orgiazzi <i>et al.</i> , 2012
Hysterangiaceae	<i>Hysterangium</i>	Uncharacterized <i>Hysterangium</i> sp.	Spain	Natural forest	Aponte <i>et al.</i> , 2010
Inocybaceae	<i>Inocybe</i>	<i>I. asterospora</i> , <i>I. cookie</i> , <i>I. eutheles</i> , <i>I. fastigiata</i> , <i>I. gausapata</i> , <i>I. hirtella</i> , <i>I. jacobi</i> , <i>I. maculata</i> , <i>I. rimoso</i> , <i>I. obscura</i> , <i>I. pyriodora</i> , Uncharacterized <i>Inocybe</i> sp.	Italy, Morocco, Portugal, Spain	Managed forest, Natural forest, Declined forest	Ortega & Lorite, 2007; Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Buscardo <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014
Paxillaceae	<i>Paxillus</i>	<i>P. involutus</i>	Portugal	Managed forest	Azul <i>et al.</i> , 2009
Peniophoraceae	<i>Peniophora</i>	Uncharacterized <i>Peniophora</i> sp.	Spain	Natural forest	Aponte <i>et al.</i> , 2010
Pisolithaceae	<i>Pisolithus</i>	<i>P. arhizus</i> , <i>P. tinctorius</i> , Uncharacterized	Morocco,	Natural forest,	Ortega & Lorite, 2007; Azul <i>et al.</i> , 2009;



Family <sup>1</sup>	Genus	Species	Country	Habitat	References
		<i>Pisolithus</i> sp.	Portugal, Spain	Managed forest	Bakkali Yakhlef <i>et al.</i> , 2009; Azul <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Bakkali Yakhlef <i>et al.</i> , 2011
Melanogastraceae	<i>Melanogaster</i>	<i>M. variegates</i>	Spain	Natural forest	Aponte <i>et al.</i> , 2010
Rhizopogonaceae	<i>Rhizopogon</i>	<i>R. luteolus</i> , <i>R. roseolus</i>	Portugal, Morocco	Managed forest, Natural forest	Azul <i>et al.</i> , 2009; Nounsi <i>et al.</i> , 2014
Russulaceae	<i>Lactarius</i>	<i>L. atlanticus</i> , <i>L. aurantiacus</i> , <i>L. azonites</i> , <i>L. bertillonii</i> , <i>L. chrysorrheus</i> , <i>L. decipiens</i> , <i>L. fulvissimus</i> , <i>L. glaucescens</i> , <i>L. hepaticus</i> , <i>L. kuehnerianus</i> , <i>L. pterosporus</i> , <i>L. quietus</i> , <i>L. romagnesii</i> , <i>L. rugatus</i> , <i>L. serifluus</i> , <i>L. vellereus</i> , <i>L. volemus</i> , <i>L. zonarius</i> , Uncharacterized <i>Lactarius</i> sp.	Morocco, Portugal, Spain, Italy	Natural forest, Managed forest, Declined forest	Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Azul <i>et al.</i> , 2010; Aponte <i>et al.</i> , 2010; Buscardo <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014; Haimed <i>et al.</i> , 2015
	<i>Gymnomyces</i>	<i>G. californicus</i> , <i>G. subfulvus</i> , Uncharacterized <i>Gymnomyces</i> sp.	Italy	Managed forest, Declined forest	Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013
	<i>Macowanites</i>	<i>M. ammophilus</i>	Spain	Natural forest	Aponte <i>et al.</i> , 2010
	<i>Russula</i>	<i>R. abietina</i> , <i>R. acrifolia</i> , <i>R. adusta</i> , <i>R. aeruginea</i> , <i>R. albonigra</i> , <i>R. alutacea</i> , <i>R. amethystina</i> , <i>R. amoena</i> , <i>R. amoenicolor</i> , <i>R. amoenolens</i> , <i>R. atropurpurea</i> , <i>R. aurata</i> , <i>R. betulina</i> , <i>R. carminipes</i> , <i>R. chamaeleontina</i> , <i>R. chlorides</i> , <i>R. cf. emetica</i> , <i>R. cyanoxantha</i> , <i>R. decipiens</i> , <i>R. delica</i> , <i>R. densifolia</i> , <i>R. emeticicolor</i> , <i>R. faustiana</i> , <i>R. fellea</i> , <i>R. foetens</i> , <i>R. fragilis</i> ,	Morocco, taly, Portugal, Spain	Natural forest, managed forest, Declined forest	Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Azul <i>et al.</i> , 2010; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Abourouh, 2011; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014; Haimed <i>et al.</i> , 2015

Family <sup>1</sup>	Genus	Species	Country	Habitat	References
Russulaceae		<i>R. fragrantissima</i> , <i>R. fuliginosa</i> , <i>R. graveolens</i> , <i>R. lepida</i> , <i>R. lepidicolor</i> , <i>R. lilacea</i> , <i>R. livescens</i> , <i>R. luteotacta</i> , <i>R. maculata</i> , <i>R. mairei</i> , <i>R. nigricans</i> , <i>R. pectinate</i> , <i>R. pectinatoides</i> , <i>R. persicina</i> , <i>R. praetervisa</i> , <i>R. prinophila</i> , <i>R. puellaris</i> , <i>R. ochroleuca</i> , <i>R. odorata</i> , <i>R. olivacea</i> , <i>R. straminea</i> , <i>R. raoultii</i> , <i>R. risigallina</i> , <i>R. romellii</i> , <i>R. rosacea</i> , <i>R. rubra</i> , <i>R. rubroalba</i> , <i>R. sororia</i> , <i>R. subfoetens</i> , <i>R. vesca</i> , <i>R. violacea</i> , <i>R. violeipes</i> , <i>R. vinosa</i> , <i>R. virescens</i> , <i>R. xerampelina</i> , Uncharacterized <i>Russula</i> sp.			
Sebacinaceae	<i>Sebacina</i>	<i>S. epigaea</i> , <i>S. helvelloides</i> , Uncharacterized <i>Sebacina</i> sp.	Italy, Spain	Managed forest, Declined forest, Natural forest	Aponte <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013
Sclerodermataceae	<i>Scleroderma</i>	<i>S. areolatum</i> , <i>S. bovista</i> , <i>S. citrinum</i> , <i>S. meridionale</i> , <i>S. polyhizum</i> , <i>S. vulgare</i> , <i>S. verrucosum</i> , Uncharacterized <i>Scleroderma</i> sp.	Portugal, Morocco, Italy	Managed forest, Natural forest	Azul <i>et al.</i> , 2009; Bakkali Yakhlef <i>et al.</i> , 2009; Azul <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Nounsi <i>et al.</i> , 2014
Suillaceae	<i>Suillus</i>	<i>S. bovinus</i>	Morocco	Natural forest	Haimed <i>et al.</i> , 2015
Thelephoraceae	<i>Thelephora</i>	<i>T. anthocephala</i> , <i>T. atra</i> , <i>T. penicillata</i> , <i>T. terrestris</i> , Uncharacterized <i>Thelephora</i> sp.	Portugal, Morocco, Italy, Spain	Managed forest, Natural forest	Castro <i>et al.</i> , 2006; Bakkali Yakhlef <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012

Family <sup>1</sup>	Genus	Species	Country	Habitat	References
	<i>Tomentella</i>	<i>T. brevispina</i> , <i>T. ellisii</i> , <i>T. galzinii</i> , <i>T. stuposa</i> , <i>T. sublilacina</i> , <i>T. subtestacea</i> , <i>T. testaceogilva</i> , Uncharacterized <i>Tomentella</i> sp.	Portugal, Italy, Spain, Morocco	Managed forest, Declined forest, Natural forest	Azul <i>et al.</i> , 2009, 2010; Aponte <i>et al.</i> , 2010; Buscardo <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014
Tricholomataceae	<i>Laccaria</i>	<i>L. amethystine</i> , <i>L. laccata</i> , <i>L. pseudomontana</i> , Uncharacterized <i>Laccaria</i> sp.	Morocco, Portugal, Italy, Spain	Natural forest, Managed forest, Declined forest	Ortega & Lorite, 2007; Azul <i>et al.</i> , 2009, 2010; Buscardo <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013
	<i>Tricholoma</i>	<i>T. acerbum</i> , <i>T. albobrunneum</i> , <i>T. album</i> , <i>T. colossus</i> , <i>T. saponaceum</i> , <i>T. sculpturatum</i> , <i>T. sejunctum</i> , <i>T. sulphureum</i> , <i>T. sulphureum</i> , <i>T. terreum</i> , <i>T. ustale</i> , <i>T. ustaloides</i> , Uncharacterized <i>Tricholoma</i> sp.	Morocco, Portugal, Spain	Natural forest, Managed forest, Declined forest	Ortega & Lorite, 2007; Bakkali Yakhlef <i>et al.</i> , 2009; Azul <i>et al.</i> , 2010; Aponte <i>et al.</i> , 2010; Barrico <i>et al.</i> , 2010; Lancellotti & Franceschini, 2013; Nounsi <i>et al.</i> , 2014

**Table I.2.** ECM Ascomycota fungi of Mediterranean cork oak ecosystems

Family <sup>3</sup>	Genus	Species	Country	Type of site	References
Helotiales incertae sedis	<i>Cadophora</i>	Uncharacterized <i>Cadophora</i> sp.	Portugal	Natural forest	Buscardo <i>et al.</i> , 2010
Helvellaceae	<i>Helvella</i>	<i>H. lacunose</i>	Morocco	Natural forest	Nounsi <i>et al.</i> , 2014
Gloniaceae	<i>Cenococcum</i>	<i>C. geophilum</i> , <i>C. graniforme</i>	Italy, Portugal, Spain, Morocco	Managed forest, Declined forest, Natural forest	Abourouh, 1987; Azul <i>et al.</i> , 2009; Aponte <i>et al.</i> , 2010; Buscardo <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012; Lancellotti & Franceschini, 2013
Myxotrichaceae	<i>Oidiodendron</i>	<i>O. pilicola</i> , <i>O. chlamydosporicum</i> , <i>O. flavum</i>	Italy	Managed forest	Orgiazzi <i>et al.</i> , 2012
Pezizaceae	<i>Hydnobolites</i>	<i>H. cerebriformis</i>	Italy	Managed forest	Orgiazzi <i>et al.</i> , 2012
	<i>Peziza</i>	<i>P. badia</i> , Uncharacterized <i>Peziza</i> sp.	Portugal	Managed forest	Azul <i>et al.</i> , 2010
	<i>Terfezia</i>	<i>T. leonis</i>	Morocco	Natural forest	Abourouh, 2011
Pyronemataceae	<i>Genea</i>	Uncharacterized <i>Genea</i> sp.	Portugal	Managed forest	Azul <i>et al.</i> , 2009
	<i>Humaria</i>	<i>H. hemisphaerica</i> , Uncharacterized <i>Humaria</i> sp.	Italy, Spain	Managed forest, Natural forest	Aponte <i>et al.</i> , 2010; Orgiazzi <i>et al.</i> , 2012
	<i>Sowerbyella</i>	<i>S. rhenana</i>	Morocco	Natural forest	Bakkali Yakhlef <i>et al.</i> , 2009

<sup>3</sup> Few other ECM fungi poorly affiliated with references were described in Lancellotti and Franceschini (2013) and Aponte *et al.* (2010)

Family <sup>3</sup>	Genus	Species	Country	Type of site	References
Tuberaceae	<i>Tuber</i>	<i>T. borchii</i> , Uncharacterized <i>Tuber</i> sp.	Italy, Portugal, Spain, Morocco	Declined forest, Managed forest, Natural forest	Azul <i>et al.</i> , 2009, 2010; Aponte <i>et al.</i> , 2010; Lancellotti & Franceschini, 2013

<sup>3</sup> Few other ECM fungi poorly affiliated with references were described in Lancellotti and Franceschini (2013) and Aponte et al. (2010)

## 2. Soil bacterial community

The characterisation of bacterial communities in Mediterranean cork oak ecosystems has been poorly investigated compared to mycorrhizal communities. The predominance of Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes phyla have been reported (Lagomarsino *et al.*, 2011; Bevivino *et al.*, 2014), but the majority of studies focused on global response of soil microbial activity to land use practices (Marongiu *et al.*, 2006; Lagomarsino *et al.*, 2011; Costa *et al.*, 2013; Francioli *et al.*, 2014). A functional group was particularly investigated, the soil denitrifying bacteria. This group was shown as significantly affected by land use intensities, seasonal variations and soil characteristics (Pastorelli *et al.*, 2011). A parallel monitoring of vascular plant community and denitrifying bacteria revealed a similar trend regarding contrasting land uses (Bagella *et al.*, 2014).

The assessment of soil microbial functionalities demonstrated a correlation among microbial activity, functional biodiversity and land use intensities. Soils subjected to low land use intensities showed a higher stability of bacterial communities and microbial activities compare to strong land use intensities (Lagomarsino *et al.*, 2011; Bevivino *et al.*, 2014), but as for EcM community, land use impacts are dependant of seasonal variations (Costa *et al.*, 2013; Francioli *et al.*, 2014; Bevivino *et al.*, 2014). Biological invasion was also shown as a strong factor affecting soil microbial functional diversity in cork oak ecosystems (Boudiaf *et al.*, 2013), as well as *Q. suber* seedlings.

Overall, cork oak ecosystem degradation and decline deeply impact soil microbiome consequently. Different strategies were proposed to mitigate the effect of ecosystem degradation through soil microbiome management (microbial inoculation, microbial activity stimulation) but their durability in time is poorly investigated. The two most promising ecological strategies based on soil microbiome management are detailed below.

## III. Ecological strategies based on plant-microbiome interactions for the sustainable management of cork oak forests – perspectives

The forest ecosystem sustainability mainly depends on efficient natural tree regeneration and plant cover dynamic. These two ecological processes are strongly related to soil fertility (soil characteristics and soil microbiome efficiency), notably in the first stages of seedling growth (particularly sensitive to biotic and abiotic stresses). Soil fertility in cork oak ecosystems has been for example improved through the use of sewage sludge. The impact was the significant increase of cork oak litter decomposition by improving microbial biomass and activities, (Nèble *et al.*, 2007). Although, a strong stimulation of all microbial parameters was observed, the effects during time may be limited. Other promising alternatives directly implicating the plant-microbiome interactions are developed. These alternatives rely on two different ecological approaches aiming at (i) adapting a process to current ecosystems (the “reductionist” approach) or (ii) adapting ecosystems to fit a given process (the “holistic” approach) (Fester & Sawers, 2011). The former is based on microbial inoculations of plant beneficial microorganisms in soils or directly associated to seedlings transplanted from nurseries, and the latter on *in situ* plant-microbiome management by installing or managing a plant cover to promote plant beneficial microorganism abundance and activities directly on field.

## 1. The “reductionist” approach

The microbial inoculation consists in the introduction of a large amount of microorganisms in a given soil (generally in the vicinity of a targeted seedling). This ecological engineering process allows the production of seedlings growing faster and more resistant to biotic and abiotic stresses (Aronson *et al.*, 2009; Quiza *et al.*, 2015; Berruti *et al.*, 2016), which generally occur during seedling transplantations on field (afforestation programs). Significant differences in the efficiency of mycorrhizal inoculation depending of plant type was shown with higher efficiency on woody plants, non-fixing forbs and C<sub>4</sub> grasses (Hoeksema *et al.*, 2010). A wide range of plant parameters were shown to be improved on woody plants, e.g. the increase in leaf area, the shoot and root dry weights, N and P contents, a higher photosynthetic capacity, the water use efficiency and drought tolerance (Le Tacon, 1997; Mousain *et al.*, 1997; Ouahmane *et al.*, 2007; Duponnois *et al.*, 2007; Aronson *et al.*, 2009; Wang *et al.*, 2016). However, as for the majority of studies, the long-term impact of microbial inoculation is poorly assessed [rarely reaching the 2nd years after transplantation (Sousa *et al.*, 2014)], whereas various factors, notably fertilization rates, competition with indigene soil microbiome, plant cover diversity and soil characteristics, strongly affect the persistence and efficiency of microbial inocula (Hoeksema *et al.*, 2010; Johnson *et al.*, 2010; Verbruggen *et al.*, 2012; Berruti *et al.*, 2016).

Microbial inoculation in nursery mainly relies on two methods, (i) inoculation of exotic microorganisms, initially selected for their performance on a large range of plants, and (ii) inoculation of indigenous microorganisms selected on the field and for a local use. The first method is widely used for public research (controlled experiments) and by private companies (commercial inocula), particularly in the sector of edible mushroom production in forest (Le Tacon, 1997). The most famous example is the production of Truffles (Benucci *et al.*, 2012; Pereira *et al.*, 2013; Murat, 2015). The second method is based on the local adaptation theory of microorganisms (Johnson *et al.*, 2010), and thus the necessity to select microorganisms or a microbial community (soil community or artificial community) adapted to the environment where they will be inoculated. This method is very promising in comparison to controlled inoculations with exotic microorganisms (Requena *et al.*, 2001; Ouahmane *et al.*, 2007; Rowe *et al.*, 2007; Manaut *et al.*, 2015; Maltz & Treseder, 2015). The use of indigenous inocula is poorly adopted by the forest services (Sýkorová *et al.*, 2016), whereas it constitutes the most sustainable strategy at low cost (Emam, 2016). Nevertheless, this approach has to be developed in respect with new ecological directives (Nagoya protocol) and handles with cautious because of the risk of pathogenic agent dissemination (Fortin *et al.*, 2016).

The cork oak inoculation efficiency has been rarely assessed, but evidences were provided on the positive impact of EcM fungal inoculation on the growth and survival of *Q. suber* vitroplants and seedlings from nurseries to the field (Díez *et al.*, 2000; Aronson *et al.*, 2009; Sebastiana *et al.*, 2014). Several EcM fungi were tested, i.e. *Hebeloma sinapizans* (Branzanti & Zambonelli, 1990), *Paxillus involutus* (Branzanti & Zambonelli, 1990), *Pisolithus tinctorius* (Díez *et al.*, 2000; Sebastiana *et al.*, 2014) and *Pisolithus arrhizus* (Aronson *et al.*, 2009) [a well-known EcM fungal genus associated with *Q. suber* (Bakkali Yakhlef *et al.*, 2011)], *Scleroderma polyrhizum* (Díez *et al.*, 2000), and *Tuber albidum* (Branzanti & Zambonelli, 1990). However, no beneficial effect was observed for *H. sinapizans* and *P. involutus* (Branzanti & Zambonelli, 1990), except the contamination of seedlings by *Sphaerosporella brunnea* (a pioneer and opportunist ectomycorrhizal species).



## 2. The “holistic” approach

This approach rely on the plant facilitation process, which is a positive spatial interaction between seedlings of one species and sheltering adults of another species, leading to the enhancement of the establishment, growth, survival, and fitness of seedlings (Callaway, 1995, 1997). Facilitation plays an important role in the regulation of plant succession and community composition in stable non-successional communities (Brooker *et al.*, 2008). Therefore, facilitation could be adopted as a powerful ecological tool for i) ecosystem restoration, ii) maintaining a sustainable balance between functioning and production, and iii) conservation of natural resources and biodiversity, especially under higher disturbance conditions and climate change.

The plant-plant facilitation has different ways, (i) direct (e.g. favourable alteration of light, temperature, soil moisture, soil nutrients, soil oxygenation, substrate) and (ii) indirect (e.g. protection from herbivores, attraction of shared pollinators, root grafts, and beneficial changes in soil microbial communities) (Callaway, 1997). The balance between direct and indirect processes depends on the limiting factors and their strength. Where several limiting factors co-occur with similar strengths, indirect facilitation should be more common in plant communities. In contrast, when there is one dominant limiting factor (e.g. N-poor or low light conditions), indirect facilitation should be less important (Brooker *et al.*, 2008). Plant facilitation is described as a process occurring specifically between certain plant associations and notably under stress conditions (e.g. drought, low nutrient availability in soil) (Callaway, 1995, 1997; Carrillo-Garcia *et al.*, 1999; Gomez-Aparicio *et al.*, 2004; Liancourt *et al.*, 2005). The majority of plant facilitation processes in natural ecosystems were investigated in North-West Mediterranean basin (**Table I.3**).

Facilitation processes on different oak species were described (**Table I.3**), e.g. *Quercus agrifolia* with a range of shrubs (Callaway 1992), *Quercus ilex* with *Retama sphaerocarpa* (Cuesta *et al.*, 2010), *Q. ilex* with *Genista hirsuta* (Smit *et al.*, 2008), highlighting the complexity of plant facilitation processes at the ecosystem level (e.g. interaction specificity, direct and indirect interactions, impact of tree age and of climate conditions). It has been proposed that contrary to traditional silvicultural techniques, the conservation of shrubs was a key step for tree recruitment in Mediterranean forests and woodlands, and consequently for the success of afforestation and restoration programs (Gomez-Aparicio *et al.*, 2004; Smit *et al.*, 2008), with notably a shift from negative to positive interactions along an increasing aridity gradient from closed forests to gaps (Muhamed *et al.*, 2013).

The level of *Q. suber* seedling recruitment has been reported as highly influenced by the density and the type of shrublands. In fact, seedling survival was negatively correlated with the abundance of obligate seeders such as *Cistus salviifolius* (Pérez-Devesa *et al.* 2008) and *Cistus ladanifer* (Acácio *et al.* 2007), but positively correlated with the abundance of sprouting species such as *Erica arborea* (Curt *et al.* 2009; Pérez-Devesa *et al.* 2008). In addition, low density (less than 100 trees per ha) and disease status of cork oak forest was shown to negatively impact cork oak recruitment (Acácio *et al.* 2007; Ibáñez *et al.* 2015b). These studies confirmed the complexity of plant facilitation species that depends on the plant species in interaction and the habitat type, as well as on the level of disturbances.



**Table I.3.** Evaluation of plant facilitation processes, independently of the role of soil microbiome

Target plant	Nurse plant	Main funding	Country	References
<i>Quercus ilex</i> , <i>Quercus faginea</i> , <i>Prunus mahaleb</i> , <i>Sorbus torminalis</i> , <i>Fraxinus angustifolia</i>	<i>Cistus monspeliensis</i> , <i>Daphne gnidium</i> , <i>Junipenus oxycedrus</i> , <i>Pistacia lentiscus</i> , <i>Quercus faginea</i> , <i>Quercus ilex</i> , <i>Rosmarinus officinalis</i> , <i>Thymus mastichina</i> , <i>Teucrium poleum</i> , <i>Ulex parviflorus</i>	Nurse-based restoration increased the species richness and evenness of tall-shrubs and trees, as well as the life-form diversity	Spain	Rey <i>et al.</i> , 2009
<i>Pinus sylvestris</i> <i>Pinus nigra</i>	<i>Salvia lavandulifolia</i>	Shrubs enhanced the abiotic conditions of the microhabitats and improved the survival and growth of seedling especially under drought condition.  The use of shrubs as nurse plants for reforestation as an alternative technique offered both economic and ecological advantages.	Spain	Castro <i>et al.</i> , 2002
<i>Quercus pyrenaica</i>	<i>Salvia lavandulifolia</i>	A pioneer shrub increased the establishment of seedlings and offered a success of the <i>Q. pyrenaica</i> reforestation programs, and minimized the impact in the existing community.	Spain	Castro <i>et al.</i> , 2006

Target plant	Nurse plant	Main funding	Country	References
<i>Quercus ilex</i>	<i>Retama sphaerocarpa</i>	Shrubs acted as nurse plant by improving environmental conditions benefited smaller seedlings and reducing the mortality of smaller seedlings under drought conditions. <i>R. sphaerocarpa</i> indirectly facilitated the seedling establishment by suppressing the herb competition.	Spain	Cuesta <i>et al.</i> , 2010
<i>Quercus suber</i>	<i>Ulex</i> sp.	The creation of conservation zones in Mediterranean oak woodlands could promote oak regeneration especially in area with higher shrub diversity and richness.	Portugal	Dias <i>et al.</i> , 2016
	<i>Araucaria angustifolia</i>	<i>A. angustifolia</i> trees on grassland acted as nurse plants and promoted the colonization of the site by other forest species seedlings.	Brazil	Duarte <i>et al.</i> , 2006
<i>Podocarpus falcatus</i> , <i>Croton macrostachyus</i>	<i>Eucalyptus saligna</i> , <i>Eucalyptus globulu</i> , <i>Eucalyptus grandis</i> , <i>Cupressus lusitanica</i> , <i>Acacia</i> sp., <i>Pinus patula</i> , <i>Casuarina equisetifolia</i>	In tropical countries, the exotic trees could exert protective functions and had a nurse effect for the regeneration of indigenous plants. Thus, exotic tree plantations potentially may greatly improve physical and biological site conditions catalysing subsequent succession processes towards a natural forest.	Brazil, Australia, Ethiopia	(Feyera <i>et al.</i> , 2002
<i>Castanopsis hystrix</i> , <i>Michelia macclurei</i> , <i>Manglietia glauca</i>	<i>Acacia auriculiformis</i> , <i>Acacia mangium</i>	<i>Acacia</i> species acted as nurse plant for native understory plants by improving microclimate characteristics and physiological traits. The adaptation of the understory species to abiotic environmental	China	Yang <i>et al.</i> , 2009

Target plant	Nurse plant	Main funding	Country	References
		factors is species-specific and especially related to their shade tolerance.		
<i>Quercus suber</i>	<i>Erica arborea</i>	Cork oak recruited better than the other oaks in medium and high shrublands dominated by <i>Erica arborea</i> , which suggest possible facilitative effect.	France	Curt <i>et al.</i> , 2009
<i>Coryphanta durangensis</i> , <i>Echinocereus longisetus</i> , <i>Peniocereus greggii</i>	<i>Prosopis laevigata</i>	The spatial associations seemed to be important in maintaining the protection of cacti species. <i>Prosopis</i> appeared to be important for two cacti species ( <i>Coryphanta durangensis</i> and <i>Peniocereus greggii</i> ) and may promote biological diversity.	Mexico	Muro-Perez <i>et al.</i> , 2012
<i>Crataegus monogyna</i> , <i>Rhamnus alaternus</i> , <i>Retama sphaerocarpa</i> , <i>Quercus faginea</i> , <i>Quercus ilex</i> , <i>Quercus pyrenaica</i> , <i>Pinus halepensis</i> , <i>Pinus nigra</i> , <i>Pinus sylvestris</i> , <i>Acer opalus</i>	<i>Ulex parviflorus</i> , <i>Genista versicolor</i> , <i>Genista umbellata</i> , <i>Ononis aragonensis</i> , <i>Adenocarpus decorticans</i> , <i>Salvia lavandulifolia</i> , <i>Thymus mastichina</i> , <i>Thymus vulgaris</i> , <i>Rosmarinus officinalis</i> , <i>Santolina canescens</i> , <i>Artemisia campestris</i>	A pioneer shrub facilitated the establishment of woody (survival and growth), late-successional Mediterranean species and thus can positively affect reforestation success in many different ecological settings. However, there were differences in the magnitude of the interaction, depending on the seedling species planted as well as the nurse shrub species involved.	Spain	Gomez-Aparicio <i>et al.</i> , 2004

Target plant	Nurse plant	Main funding	Country	References
	<i>Prunus ramburii</i> , <i>Crataegus monogyna</i> , <i>Berberis hispanica</i> , <i>Cistus albidus</i> , <i>Cistus monspeliensis</i>			
<i>Acer opalus</i> , <i>Quercus ilex</i> , <i>Pinus nigra</i> , <i>Pinus sylvestris</i>	<i>Prunus ramburii</i> , <i>Crataegus granatensis</i> , <i>Berberis vulgaris</i>	Shrubs had positive effects on sapling survival by generating a favorable micro-environment crucial for saplings and providing a protection from abiotic stress (summer drought and winter frost) and protection from biotic stress (herbivory). However, this facilitative effect was depending on the climatic characteristics of the year, the herbivore pressure and the characteristics of the plant species interacting.	Spain	Gómez-Aparicio <i>et al.</i> , 2008
<i>Pinus clausa</i>	<i>Serenoa repens</i> , <i>Sabal etonia</i> , <i>Quercus myrtifolia</i> , <i>Quercus geminata</i> , <i>Quercus chapmanii</i> , <i>Lyonia ferruginea</i>	There was no apparent evidence of hardwoods and palmettos nurse plant effect on sand pine seedling distributions “microsite influences”, but biomechanics of seedling stems were greatly influenced by proximity to larger plants.	USA	Mattson & Putz, 2008
<i>Dipterocarpus alatus</i> , <i>Hopea odorata</i> , <i>Shorea roxburghii</i>	<i>Acacia mangium</i>	Dipterocarp species planted simultaneously with <i>A. mangium</i> showed better survival and growth than when planted alone. The findings suggest <i>A. mangium</i> is an effective nurse tree for dipterocarp seedlings by moderating the microclimate.	Thailand	Norisada <i>et al.</i> , 2005

Target plant	Nurse plant	Main funding	Country	References
<i>Quercus ilex</i>	<i>Genista hirsute</i>	Shrubs had a facilitative effect on <i>Quercus ilex</i> recruitment and survival, despite higher seed removal by rodents. Shading appears the crucial factor facilitating seedling survival.	Spain	Smit <i>et al.</i> , 2008
	<i>Coronilla juncea</i> , <i>Ephedra fragilis</i> , <i>Genista umbellata</i> , <i>Retama sphaerocarpa</i> , <i>Salsola oppositifolia</i>	Mid-successional shrubs were more adapted for restoration of arid shrublands than late-successional shrubs and trees.	Spain	Padilla <i>et al.</i> , 2009
	<i>Caragana korshinskii</i> (introduced shrub) <i>Stipa bungeana</i> (native shrub)	In semiarid grass slopes, the introduced shrubs increased soil resources heterogeneity that depends on soil types. Despite, rehabilitation with native grasses should be adopted to avoid any possible degradation caused by introduced shrubs.	China	Wei <i>et al.</i> , 2013
<i>Quercus pubescens</i> <i>Fagus sylvatica</i>	<i>Buxus sempervirens</i> , <i>Juniperus communis</i>	There existed some subtle differences among tree species in the relative importance of facilitation and competition. Shrubs and shade had direct facilitative effect on the establishment and the emergence of both tree species. Shade also indirectly facilitates <i>Fagus</i> survival by limiting herb competition. No indirect facilitation of <i>Quercus</i> survival was detected. These differences reflect variation in the tolerance of herb competition by seedlings of the two species. <i>Quercus</i> seedlings shown high tolerance of herb competition and allows regeneration over a wide area under each	France	Kunstler <i>et al.</i> , 2006

Target plant	Nurse plant	Main funding	Country	References
		shrub and some regeneration events in grasslands at low grazing intensity.		
<i>Quercus ilex</i> , <i>Quercus suber</i>	tree canopy	Tree canopy covered directly facilitated seedling survival of both <i>Quercus</i> species, mainly by ameliorating the abiotic factors (e.g. soil moisture and temperatures), and indirectly facilitated survival of <i>Q. suber</i> seedlings by negatively affecting the competing herb layer.	Portugal	Caldeira <i>et al.</i> , 2014
<i>Quercus robur</i> , <i>Quercus ilex</i> , <i>Quercus suber</i>	Shrubs	In costal dune forest, canopy and climate conditions have strong effects on interactions between understory shrubs and oak transplants. Competition was dominant in the forest plots of the wettest site and facilitation in the gap plots of the driest site of the studied forest. Oak survival without shrubs (but not with shrubs) was strongly related to Vapor Pressure Deficit values, which suggests that the positive effect of shrubs in the most stressful conditions was due to decreased atmospheric stress below their canopy.	France	Muhamed <i>et al.</i> , 2013
<i>Adenanthos barbiger</i> , <i>Dampiera linearis</i> , <i>Hibbertia commutata</i> , <i>Lechanaultia biloba</i> , <i>Lepidosperma squamatum</i> , <i>Loxocarya cinerea</i> ,	understorey plants	Reducing understorey competitive environment “herbivory (using plant guards) and other understorey plants” significantly increased survival, spread and height growth of the targeted species, and is essential for establishing long-term persistent plant populations in both newly restored and older restored sites.	Australia	Daws & Koch, 2015

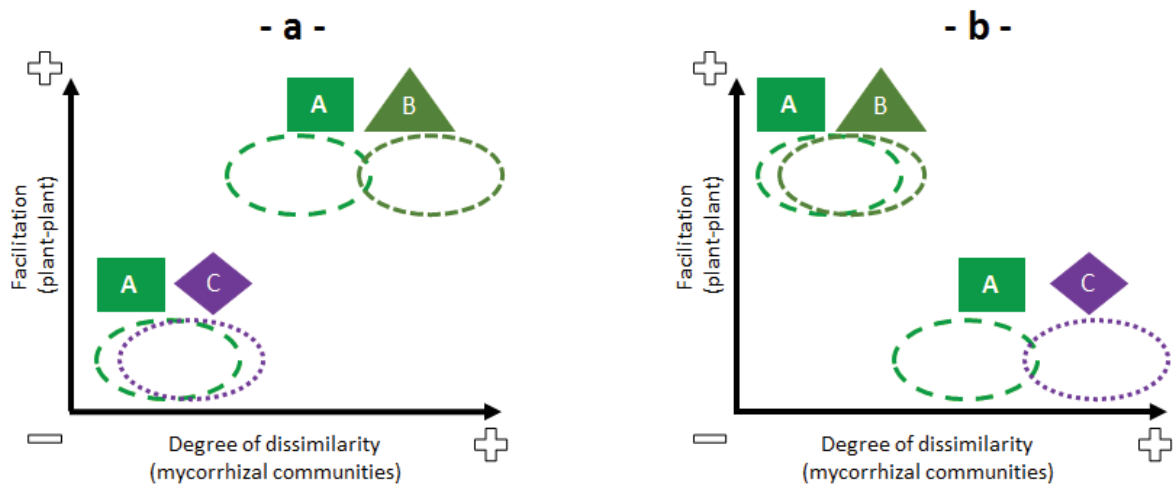
Target plant	Nurse plant	Main funding	Country	References
<i>Tetraria capillaris</i>				
<i>Quercus suber</i> , <i>Quercus canariensis</i> , <i>Olea europaea</i>	understorey plants	Under decline, neighborhood (species composition and <i>Q. suber</i> health status) strongly affect all the performance estimators, particularly seedling emergence and survival, and could be crucial for regeneration dynamics and suitability of oak Mediterranean forests.	Spain	Ibáñez <i>et al.</i> , 2015b



Although the crucial role of soil microbiome in ecosystem functioning is largely admitted, few studies attempted to characterize plant facilitation processes through a microbial point of view (**Table I.4**). The role of mycorrhizal fungi was the most assessed, notably due to their major positive impact on plant growth and nutrition. The establishment of common mycorrhizal networks between plants has been suggested as one of the key factor of facilitation processes in ecosystems (Courty et al. 2010; van der Heijden and Horton 2009). However, few studies investigated the significance of common mycorrhizal networks at the mycorrhizal community level (Montesinos-Navarro et al. 2012). Hence, it appears crucial to decipher in a large number of plant-plant interactions if plant facilitation processes occur among plants associated with mycorrhizal communities highly similar or highly dissimilar, in order to evaluate the abundance and diversity of potential common mycorrhizal networks that may explain or not the induction of facilitation processes in ecosystem (**Figure I.3**).

In Mediterranean ecosystems, Ouahmane et al. (unpublished work) showed a positive effect of different *Cistus* species on the growth of *Quercus rotundifolia* seedlings, notably through the increase of the soil mycorrhizal propagule number, contrary to previous studies demonstrating negative impacts of *Cistus* species on *Quercus* recruitment (Acácio et al. 2007; Pérez-Devesa et al. 2008). Other plants considered as pioneer in Mediterranean ecosystems such as *Lavandula* species showed a nurse plant effect on *Cupressus* and *Tetraclinis* species through the improvement of mycorrhizal soil infectivity (Abbas et al. 2013; Duponnois et al. 2011; Hafidi et al., 2013; Ouahmane et al., 2006a, 2006b). The only assessment of the role of mycorrhizal fungi in plant facilitation processes regarding *Q. suber* showed an AM and EcM fungal colonization partially affected by *Q. suber* decline (Ibáñez et al. 2015a). Surprisingly, *Q. suber* seedling survival and growth was more influenced by the intensity of AM mycorrhizal colonization than EcM mycorrhizal colonization (Ibáñez et al. 2015a).

Overall, cork oak ecosystem status, i.e. tree health, shrub cover and canopy characteristics, and their link with soil microbiome, notably the dynamic (abundance, colonization) and structure (richness, diversity) of both mycorrhizal communities (AM and EcM) constitute the pillars of the development of efficient ecological strategies based on plant facilitation processes. The development of studies aiming at determining the most suitable tree×shrub×mycorrhizal associations represent promising research avenues for cork oak ecosystem sustainability. However, the adoption of research outcomes to forest services is crucial to implement these strategies at large scales.



**Figure I.3.** Hypotheses regarding the implication of mycorrhizal community in plant facilitation [adapted from Montesinos-Navarro et al., (2012a)]. Symbols A, B, C indicate three different plants with their respective associated mycorrhizal community (circle). Mycorrhizal community associated with plant A is represented by dashed line, plant B by solid line, and plant C with dotted lines. a) positive interactions between plants (A and B) is due to high dissimilarity between mycorrhizal communities associated with each plant, and negative interaction between plants (A and C) is due to a low dissimilarity between mycorrhizal communities associated with each plant. b) positive interactions between plants (A and B) is due to a low dissimilarity between mycorrhizal communities associated with each plant, and negative interaction between plants (A and C) is due to a high dissimilarity between mycorrhizal communities associated with each plant. A low dissimilarity between mycorrhizal communities of each plant suggests a high abundance of common mycorrhizal networks between both plants.

**Table I.4.** Role of soil micorbiome in plant facilitation processes

Target plant	Nurse plant	Main funding	Country	References
<i>Cupressus arizonica</i>	<i>Lavandula multifida</i>	<i>Lavandula</i> species could act as a “nurse plant” by enhancing the mycorrhizal soil infectivity, even more if the soil is P deficient and stimulating the mycorrhizal colonization of <i>C. arizonica</i> .	Morocco	Ouahmane <i>et al.</i> , 2006b
<i>Cupressus atlantica</i>	<i>Lavandula stoechas</i> , <i>Lavandula dentata</i> , <i>Thymus satureioides</i>	<i>Lavandula</i> sp. and <i>T. satureioides</i> enhanced the growth of <i>C. atlantica</i> and facilitate their early establishment seedlings by increasing the AM fungal mycorrhizal soil infectivity and improving soil microbial characteristics.	Morocco	Ouahmane <i>et al.</i> , 2006a; Duponnois <i>et al.</i> , 2011; Hafidi <i>et al.</i> , 2013
<i>Quercus suber</i>	<i>Quercus suber</i>	Spatial distribution of the seedling-mycorrhiza association responded to the identity and health status of individual trees, and mycorrhizal infection did in turn affect seedling performace, tree decline might affect forest dynamics through changes in plant–soil biota feedbacks	Spain	Ibáñez <i>et al.</i> , 2015a
<i>Quercus rotundifolia</i>	<i>Cistus villosus</i> , <i>Cistus salviifolius</i> , <i>Cistus laurifolius</i> , <i>Cistus monspeliensis</i>	<i>Cistus</i> species could act as a “nurse plant” for reforestation programs by improving the growth and mycorrhizal colonization, and enhancing a microbial and biomass activity.	Morocco	Ouahmane et al. Unpublished work
<i>Salix reinii</i> , <i>Betula ermanii</i> , <i>Larix kaempferi</i> ,	<i>Salix reinii</i>	ECM fungi associated with established willow shrubs were essential in facilitating seedling establishment (growth and nitrogen content) of successional plant species in the early successional volcanic desert.	Japan	Nara & Hogetsu, 2004
<i>Uapaca bojeri</i>	<i>Leptolena bojeriana</i>	The use of ectotrophic early successional shrub species mitigated a negative effect of exotic tree species, facilitate the ectomycorrhizal infection and thus	Madagascar	Baohanta <i>et al.</i> , 2012

Target plant	Nurse plant	Main funding	Country	References
		establishment and growth of native tree species.		
	<i>Pistacia lentiscus</i> , <i>Rhamnus lycioides</i> , <i>Olea europaea</i> , <i>Retama sphaerocarpa</i> <i>Stipa tenacissima</i>	All plants tested maintain a high level of infective mycorrhizal propagules in soils constituting a potential source of inoculum for plant seedlings. The contribution of <i>S. tenacissima</i> in AM symbiosis and its potentiality as nurse plant was site dependent.	Spain	Azcon-Aguilar <i>et al.</i> , 2003
Different plant species	<i>Spartium junceum</i> L., <i>Anagyris foetida</i> L.	The management of indigenous shrub legume–rhizobium–AMF symbioses could aid restoration of anthropogenic soils without the use of ameliorants, by helping plant establishment and improving soil properties.  The inoculation with specific rhizobia increased autochthonous legume shrub productivity on the anthropogenic soil both in the short and in the long period.  The microbial inoculants (Rhizobia and Arbuscular mycorrhizal) in revegetation strategies strongly enhanced plant establishment and growth on the anthropogenic soil.	Italy	Cardinale <i>et al.</i> , 2010
<i>Pachycereus pringlei</i> , <i>Machaerocereus gummosus</i> , <i>Lemaireocereus</i>	<i>Prosopis articulate</i> , <i>Olneya tesota</i>	AM fungi contributed to the plant-soil system by helping to stabilization windborne soil that settles under dense plant canopies, enhancing the establishment of colonizer plants in bare soils od disturbed areas, and by influencing plant associations through differences in the mycotrophic status of the	Mexico	Carrillo-Garcia <i>et al.</i> , 1999

Target plant	Nurse plant	Main funding	Country	References
<i>thurberi</i> , <i>Agava datilyo</i>		associates.		
<i>Ceratonia siliqua</i>	<i>Retama monosperma</i> , <i>Cistus salviifolius</i> , <i>Pistacia lentiscus</i>	Shrubs enhanced the growth of seedling and could act as nurse plant because of their positive effects on the improvement of the mycorrhizogenic potential of soil.	Morocco	Manaut, 2015
<i>Phacelia secunda</i> , <i>Hordeum comosum</i> , <i>Taraxacum officinale</i>	<i>Laretia acaulis</i>	Indirect facilitation effect of a cushion nurse species on the survival and growth of both native and invasive species induced by root endophytic fungi	Chile	Molina-Montenegro <i>et al.</i> , 2015
Large number of species	Large number of species	In plant-AMF interaction networks, there is a nonrandom interaction pattern between plant and AMF communities. Closely related AMF (a group of fungal species within the genus <i>Glomus</i> ) tend to interact with the same set of plant species, but the tendency of plant species to interact with the same set of AMF OTUs is independent of their phylogenetic relatedness. Facilitation is more frequent among the most abundant plant species, this trend is significantly modulated by plant species' fungal-niche. There is a tendency of plant-plant facilitation specificity to occur among plant species that differ in their fungal-niche, resulting in stronger facilitation between pairs of plant species with different associated AMF. Thus, in this plant-AMF network there are few AMF ecological-generalists which interact with almost every plant species in the community.	Mexico	Montesinos-Navarro <i>et al.</i> , 2012a,b

Target plant	Nurse plant	Main funding	Country	References
<i>Tetraclinis articulata</i>	<i>Lavandula multifida</i>	In <i>Tetraclinis</i> stand, <i>Lavandula multifida</i> roots showed the highest AM fungal and its rhizospheric soil, rich on AMF spores, improved the <i>Tetraclinis</i> seedlings growth. This result suggest that <i>Lavandula</i> could be used as nurse plant in reforestation programs and its rhizospheric soil can be used as biofertilizer to inoculate nurseries for <i>T. articulata</i> production.	Morocco	Abbas <i>et al.</i> , 2013

## Chapitre II

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**Facteurs environnementaux influençant la diversité des champignons telluriques associés au chêne-liège (*Quercus suber*) dans la subéraie nord marocaine**



## Résumé

Le fonctionnement du chêne-liège au sein des subéraies est étroitement lié aux interactions avec les communautés fongiques du sol, notamment les champignons ectomycorhiziens (EcM), composante clé du fonctionnement du sol et de la strate végétale. Dans le but d'une meilleure compréhension des règles d'interactions arbre × champignon × sol au sein des subéraies, les principaux objectifs de ce chapitre sont (i) de caractériser de la manière la plus exhaustive possible la diversité moléculaire des champignons du sol, notamment EcM, associés aux racines du chêne-liège ; (ii) de déterminer les principaux facteurs environnementaux influençant la diversité fongique ; et (iii) d'évaluer les spécificités de la communauté fongique liées à l'habitat et aux facteurs environnementaux au sein de la subéraie marocaine.

L'étude a été menée au sein de trois habitats représentatifs de la subéraie nord marocaine (Maâmora, Benslimane, Chefchaoun), en utilisant les nouvelles technologies de séquençage à haut débit. Les champignons appartenant aux ordres Thelephorales, Russulales, Agaricales et Hysteriales étaient prédominants. Une forte structuration des communautés de champignons associées au chêne-liège a été observée en fonction de l'habitat, et corrélée avec les propriétés du sol, notamment avec le pH, le rapport C:N ( $P = 0.0002$ ) et le phosphore assimilable ( $P = 0.0001$ ). Plus de 90 indicateurs fongiques ( $P < 0.01$ ) - soit de l'habitat et/ou d'une propriété du sol - ont pu être mis en évidence. Les résultats mettent en lumière l'importance écologique de plusieurs champignons EcM ubiquistes (*Tomentella*, *Russula*, *Cenococcum*), mais aussi de champignons potentiellement associés aux sclérotés ou des champignons ericoïdes (*Cladophialophora*, *Oidiodendron*) associés aux chênes-lièges. Ces travaux ont aussi permis de mettre l'accent sur la complexité de la variabilité fonctionnelle intraspécifique.

Le chapitre fait l'objet d'un article en cours de révision dans le journal PLoS ONE :

**F.Z. Maghnia**, Y. Abbas, F. Mahé, B. Kerdouh, E. Tournier, M. Ouadji, P. Tisseyre, Y. Prin, N. El Ghachtouli, S.E. Bakkali Yakhlef, R. Duponnois, H. Sanguin, 2017. Habitat- and soil-related drivers of the soil fungal community associated with *Quercus suber* in the Northern Moroccan forest.

**Mots clés** : Chêne-liège, communauté fongique du sol, champignon ectomycorhizien, ITS, Illumina MiSeq.

## Introduction

Forest ecosystems cover nearly 480,000 square kilometers in the Mediterranean basin, hosting a wide range of endemic species and constituting a major ecological and socio-economical resource (Blondel *et al.*, 2010). However, Mediterranean forests are highly threatened by global changes (Sala *et al.*, 2000; Allen *et al.*, 2010). *Quercus suber* L. (cork oak) is a major representative of Western Mediterranean forests (more than 20,000 square kilometers) despite its narrower geographical range compared with other oak species. It requires particular attention due to extensive human- and climate-driven negative impacts (bark exploitation, intensive agro-silvo-pastoral management, and oak decline) (Gauquelin *et al.*, 2016b).

Cork oak functioning is closely linked to interactions with soil fungi, notably ectomycorrhizal (EcM) fungi (Azul *et al.*, 2009; Sebastiana *et al.*, 2014). Soil fungi are one of the most diverse groups of organisms on Earth (Blackwell, 2011) colonizing a wide range of ecological niches (Tedersoo *et al.*, 2014). They play a central role in major ecological and biogeochemical processes (Courty *et al.*, 2010; Gessner *et al.*, 2010) and constitute a crucial component of forest ecosystem functioning. EcM fungi create a soil-tree continuum in forest ecosystems, increasing the mineral and water nutrition of host trees (Smith & Read, 2009). Despite their obvious ecological benefits, the potential of soil fungi has been largely underestimated in habitat conservation (Heilmann-Clausen *et al.*, 2015). The conservation of soil fungi themselves has also seldom been considered compared to other Eukaryotes (Heilmann-Clausen *et al.*, 2015) and initiatives are relatively recent (<http://iucn.ekoo.se/en/iucn/welcome>). In December 2016, 34 fungal species, mostly distributed in Europe, were included in the IUCN Red List of threatened species (<http://www.iucnredlist.org>).

The diversity and dynamic of the soil fungal community in cork oak ecosystems has mainly been investigated in the Northern Mediterranean basin (Azul *et al.*, 2009, 2010; Barrico *et al.*, 2010; Orgiazzi *et al.*, 2012; Lancellotti & Franceschini, 2013). Hitherto, estimations of soil fungal diversity in Southern cork oak forests have been almost exclusively based on fungal sporocarp surveys (Bakkali Yakhlef *et al.*, 2009), but this approach has been shown to provide a partial view of true below-ground soil fungal diversity, even for the EcM community (Baptista *et al.*, 2015). To address the challenges of cork oak forest conservation, soil fungal diversity in Southern cork oak ecosystems must be more extensively explored. A wide range of environmental factors are known to strongly affect soil fungal diversity. These factors have been shown to vary with the type of ecosystem, the spatial scale considered and the fungal taxa analyzed (Suz *et al.*, 2014; Tedersoo *et al.*, 2014; Creamer *et al.*, 2016). The identification of the major drivers for a given ecosystem is a key step towards its management and conservation. For instance, variations in phosphate (P) and nitrogen (N) soil content have been suggested as important drivers of intraspecific variability of *Pisolithus* spp. in the Maâmora habitat, the largest cork oak forest in Morocco (Bakkali Yakhlef *et al.*, 2011).

The main goals of the present study are (i) to characterize in depth the molecular diversity of the EcM-related fungal community (EcM fungi and associated soil fungal community) colonizing cork oak roots; (ii) to determine the main environmental drivers of fungal diversity; and (iii) to assess the habitat-related and environmental-related specificities of EcM-related fungal community in the Moroccan cork oak forest.

# I. Material and methods

## 1. Study site and sampling

The study was conducted in three habitats of the Moroccan cork oak forest, located in the Moroccan Northern Mountains known as “Chefchaoun” (35°15'5.14"N 005°30'6.68W, 1534 m elevation), and in the lowland bordering the Atlantic Ocean (North-west of Morocco) known as “Maâmora” (34°17'06.186"N 6°28'30.792"W, 27 m elevation) and “Benslimane” (33°41'9.85"N, 6°54'7.26"W; 326 m elevation). The three habitats are under a Mediterranean-type climate characterized by hot and dry summers and mild and wet winters. They are characterized by an abundant understory, notably *Cistus salviifolius*, *Lavandula stoechas*, and *Thymeleae lythroides* for Maâmora, and *Arbutus unedo* and *Pistacia lentiscus* for Benslimane, and *Erica arborea* and *Arbutus unedo* for Chefchaoun. Twenty-seven cork oak trees were sampled between February and June 2013. The sampling design was based on the selection of three plots per forest spaced 100 meters apart, each composed of three trees 20-30 meters away from any other. Roots with adherent soil were sampled under the crown of each tree and stored at +4°C. The roots were rinsed under tap water and observed under a binocular microscope to select root zones with ectomycorrhizal (EcM) fungi, dried and stored at -20°C. Soil physico-chemical parameters were measured at the LAMA Laboratory (Dakar, Senegal): pH, total nitrogen (N), total carbon (C), Carbon:Nitrogen ratio (C:N ratio), total and available phosphate (P), K<sup>+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, Cation exchange capacity (CEC).

## 2. DNA extraction, ITS amplification and Illumina Miseq sequencing

For each of the 27 cork oak tree samples, all root pieces selected were subjected to liquid nitrogen grinding for homogenization. The total DNA was extracted from a sub-sample (70 – 80 mg) using a FastPrep-24 homogenizer (MP biomedical Europe, Illkirch, France) and the FastDNA® SPIN kit (MP biomedical Europe) according to the manufacturer’s instructions. The quality of the DNA extracts was improved by adding 20-30 mg Polyvinylpolypyrrolidone (PVPP) during the first step of DNA extraction.

The Internal transcribed spacer ITS1 of the nuclear ribosomal acid RNA was amplified using the primers ITS1FI2 (5'-GAACCGCGGGARGGATCA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (Schmidt *et al.*, 2013). The amplification reaction was performed in a final volume of 25 µl with the primers ITS1FI2 and ITS2 (0.6 µM each), 2 µl of DNA extract, 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (2 units) and 1X Green GoTaq® Reaction Buffer (Promega, Charbonnières, France), with the following cycling conditions: 95°C for 15 min; 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s; a final elongation step at 72°C for 5 min. To increase richness recovery and to limit PCR biases, three PCR replicates per sample were pooled and purified using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Velizy-Villacoublay, France) following manufacturer’s guidelines. All amplicon products were subjected to paired-end Illumina MiSeq sequencing (2×300 bp) by Molecular Research LP (MR DNA, TX, USA).

### 3. Bioinformatic data processing

Paired Illumina MiSeq reads were assembled with *vsearch* v1.11.1 using the command *fastq\_mergepairs* and the option *fastq\_allowmergestagger*. Demultiplexing and primer clipping was performed with *cutadapt* v1.9, enforcing a full-length match for sample tags and allowing a 2/3-length partial match for forward and reverse primers. Only reads containing both primers were retained. For each trimmed read, the expected error was estimated with *vsearch*'s command *fastq\_filter* and the option *eeout*. Each sample was then dereplicated, i.e. strictly identical reads were merged, using *vsearch*'s command *derep\_fulllength*, and converted to FASTA format.

To prepare the clustering, the samples were pooled and submitted to another round of dereplication with *vsearch*. Files containing expected error estimations were also dereplicated to retain only the lowest expected error for each unique sequence. Clustering was performed with *swarm* v2.1.8, using a local threshold of one difference and the *fastidious* option. Molecular operational taxonomic unit (OTU) representative sequences were then searched for chimeras with *vsearch*'s command *uchime\_denovo*. In parallel, representative sequences received taxonomical assignments using the *stampa* pipeline (<https://github.com/frederic-mahe/stampa>) and a custom version of the fungal reference database UNITE v7 (<https://unite.ut.ee/>). In brief, the *stampa* pipeline requires the reference sequences to be trimmed with *cutadapt*, using the same primers as those used for the amplification of the environmental sequences. Using *vsearch*'s exact global pairwise comparisons, each environmental sequence is compared to all reference sequences and is assigned to the closest hit. In case of a tie, the environmental sequence is assigned to the last-common ancestor of the co-best hits. From the UNITE database containing only fungal sequences, 20 sequences were added to our custom reference database to identify the plants also amplified by our ITS primers.

Clustering results, expected error values, taxonomic assignments and chimera detection results were used to build a raw OTU table. Up to that point, reads that could not be merged, reads without tags or primers, reads shorter than 32 nucleotides and reads with uncalled bases ("N") were eliminated. To create the "cleaned" OTU table, additional filters were applied to retain: non-chimeric OTUs, OTUs with an expected error divided by length below 0.0002, OTUs containing more than 3 reads or, if less, seen in 2 samples, OTUs assigned to plant or fungi taxa with at least 80% similarity or, if not, containing more than 10,000 reads. Raw data are available under accession numbers the BioProject ID PRJNA378471 (<https://www.ncbi.nlm.nih.gov/bioproject>).

### 4. Statistics

Diversity (Shannon, inverse Simpson [1/D]), richness (number of MOTUs, Chao1) and evenness (Pielou) indexes were estimated and differences among cork oak habitats were assessed by non-parametric permutational multivariate analysis of variance (PERMANOVA), as implemented in the *perm.anova()* function from the R package *RVAideMemoire*.

Fungal community membership was assessed using venn diagram analysis with the R package *VennDiagram*. The differences in fungal community structure among the three habitats were

displayed with nonmetric multi-dimensional scaling (NMDS) implemented in the *metaMDS()* function. Significance in fungal community structure variation was also assessed using PERMANOVA in the *adonis()* function (McArdle & Anderson, 2001). Multivariate dispersion was estimated using the *betadisper()* function and *permutest()* because it can affect PERMANOVA results. Soil parameters were fitted to the NMDS using the *envfit()* function (9,999 permutations). Habitat and plots were categorical factors. All functions are available in the R package *vegan*. Correlation among soil parameters was assessed using the Pearson correlation coefficient, as implemented in the *cor.test()* function from the R package *vegan*.

The presence of fungal indicator species of a specific type of habitat (Maâmora, Benslimane, Chefchaoun) was determined using the corrected Pearson's phi coefficient of association ("r.g"; 9,999 permutations) implemented in the *multipatt()* function from the R package *indicspecies* (De Cáceres *et al.*, 2010). Fungal indicator species with respect to soil properties (pH, C:N ratio, and available P) were determined using the indicator value (IndVal) index, as implemented in *indicspecies'* *multipatt()*. Two different probabilities were calculated, *i.e.* A (specificity), representing the probability of a site to be defined by a given soil property, given that the species have been detected, and B (sensitivity) representing the probability of finding the species in different sites characterized by a given soil property. We considered as valid indicators the OTUs showing both A (specificity) and B (fidelity) superior to 0.8 and 0.6 respectively, as recommended in Suz *et al.* (2014).

## II. Results

### 1. Composition of EcM-related fungal community

A filtered dataset of 792,931 sequences were obtained from a raw dataset of 1,129,145 trimmed- and paired-sequences (see material and methods for more details). Sixty percent of sequences were affiliated to ITS sequences from plants (Streptophyta). Overall, a dataset of 315,597 fungal sequences (27 samples) was rarefied down to 3,447 sequences per sample to improve the robustness of fungal community comparison among the three habitats (Gihring *et al.*, 2012).

Analysis of taxonomic fungal community composition in the Moroccan cork oak forest detected 1,768 OTUs belonging to 4 known fungal phyla, 39 orders, 78 families, and 127 genera (**Table II.S1, see the annexe**). Certain fungal OTUs were affiliated to poorly characterized references (*e.g.* 30 OTUs with uncharacterized phylum, representing 149 sequences; 757 OTUs with uncharacterized genus, representing 28,559 sequences). The EcM-related fungal community was mostly composed of Basidiomycota and Ascomycota, 60% and 39%, respectively. However, a higher number of genera was found for Ascomycota compared to Basidiomycota, 94 and 31, respectively. The ten predominant fungal orders (89% of sequences) were Thelephorales (17,084 sequences), Russulales (16,275), Agaricales (14,017), Hysteriales (12,815), Sebaciniales (5,902), Chaetothyriales (5,622), Heliotales (4,796), Hypocreales (2,575), Capnodiales (1,895), and Eurotiales (1,690) (**Table II.S1, see the annexe**). The ten most abundant fungal genera (55% of sequences) were *Cenococcum* (12,459 sequences), *Tomentella* (11,609), *Russulla* (7,019), *Inocybe* (4,744), *Cortinarius* (4,568), *Cladophialophora* (3,046), *Hygrophorus* (2,643), *Lactarius* (2,196), *Sebacina* (1,667), and *Cryptosporiopsis* (1,317) (**Table II.S1, see the annexe**).



## 2. Habitat-related fungal indicators

No habitat-related impact (Maâmora, Benslimane, Chefchaoun) was detected on EcM-related fungal community richness, diversity and equitability (**Table II.1**), except for Chao1 estimation between Maâmora and Benslimane ( $P < 0.015$ ). By contrast, analysis of EcM-related fungal OTU distribution in ternary plots revealed strong fungal community patterns (abundance and membership) among habitats (**Figure II.1**). Russulales (40% of sequences) was the predominant fungal order in Maâmora, Thelephorales (41%) in Benslimane, and Hysteriales (20 % of sequences) and Agaricales (19%) in Chefchaoun (**Figure II.S1, see the annexe**). 65% of OTUs were habitat-specific (318 OTUs for Maâmora, 378 for Benslimane, 461 for Chefchaoun), but these accounted only 6 % of total sequences (**Figure II.2**). The most abundant taxonomic orders for each habitat (> 50% sequences) composed of habitat-specific OTUs were Trechisporales, Agaricales, Russulales in Maâmora, Thelephorales in Benslimane, and Agaricales, Cantharellales, Heliotales, Sebacinales in Chefchaoun (**Figure II.S1, see the annexe**). Meanwhile, 17 % of OTUs were shared among the three habitats, representing 84 % of total sequences (**Figure II.2**). However, strong differences were observed for shared OTUs among habitats in terms of relative abundance. For instance, few sequences belonging to Agaricales OTUs were present in Maâmora (**Figure II.S1, see the annexe**). A high variability in OTU abundance and distribution was also observed among habitats for the predominant genera (**Figure II.3; Figure II.S2, see the annexe**). *Cenococcum*, *Russula* and *Cladophialophora* were predominant in Maâmora and Chefchaoun, whereas *Tomentella*, *Inocybe* and *Cortinarius* were more predominant in Benslimane and Chefchaoun (**Figure II.3**).

Permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity matrix confirmed that cork-oak habitats are significant drivers of EcM-related fungal community structures (**Table II.2**). Non-significant multivariate dispersion of data was detected, emphasizing the robustness of PERMANOVA results (**Table II.2**). Pairwise PERMANOVA comparisons of cork oak habitats showed significant differences between EcM-related fungal communities (**Table II.3**). Indicator species analysis revealed 40 OTUs significantly associated with Maâmora, 21 to Benslimane and 49 to Chefchaoun (**Table II.S2, see the annexe**). The most significant OTUs (r.g > 0.5;  $P < 0.01$ ) (**Figure II.4**) belonged almost exclusively to Ascomycota, and were affiliated to Archaeorhizomycetaceae, Herpotrichiellaceae, Mycosphaerellaceae, Myxotrichaceae, Thrichocomaceae, Russulaceae and unidentified Sordariomycetes for Maâmora; unidentified Dothideomycetes and Sordariomycetes for Benslimane; Dermateaceae, Geoglossaceae, Gloniaceae, Herpotrichiellaceae, and unidentified Agaricomycetes, Dothideomycetes, Eurotiomycetes for Chefchaoun (**Table II.S1, see the annexe**).

**Table II.1.** Alpha diversity of EcM-related fungal communities associated to *Quercus suber* in Moroccan cork oak forests

	MOTUs number (Richness)	Chao1 (Richness)	Shannon's index (diversity)	Inverse Simpson's index (diversity)	Pielou's index (Evenness)
Maâmora	211±30 <sup>a 2</sup>	476±67 <sup>a</sup>	2.74±0.22 <sup>a</sup>	6.17±1.50 <sup>a</sup>	0.31±0.10 <sup>a</sup>
Benslimane	183±69 <sup>a</sup>	365±119 <sup>b</sup>	2.34±0.90 <sup>a</sup>	6.27±4.55 <sup>a</sup>	0.27±0.06 <sup>a</sup>
Chefchaoun	239±47 <sup>a</sup>	509±101 <sup>ab</sup>	2.97±0.55 <sup>a</sup>	8.42±6.52 <sup>a</sup>	0.34±0.02 <sup>a</sup>
Forest	NS <sup>1</sup>	*	NS	NS	NS

<sup>1</sup> Statistics were performed using PERMANOVA (Habitat type as factor). '\*\*\*' indicates  $P < 0,001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'NS'  $P > 0.05$ .

<sup>2</sup> Values indicate mean ± standard deviation

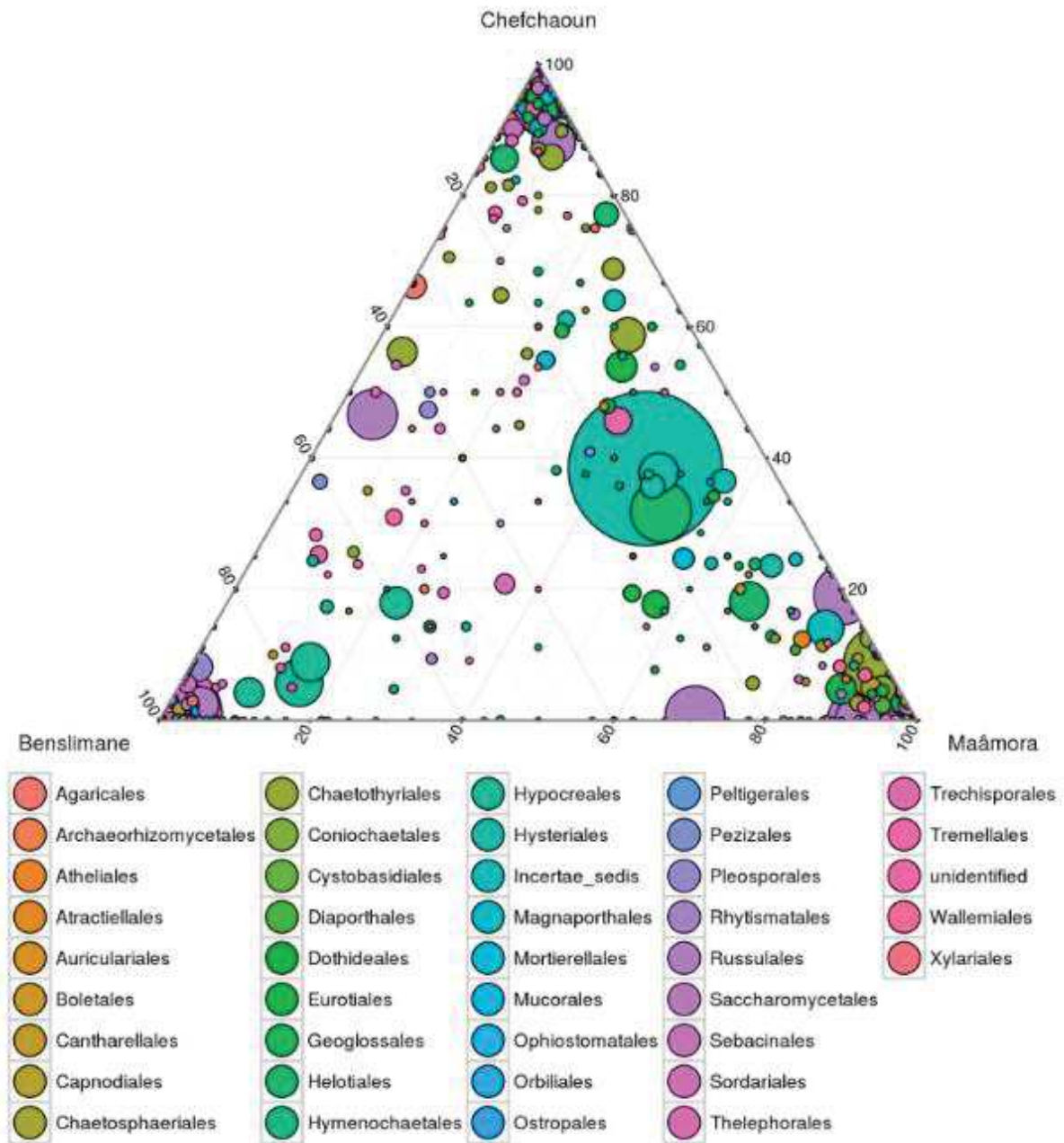
**Table II.2.** Impact of forest habitat on cork oak EcM-related fungal community structures

Model/Factors	Df	SS	MS	F. Model	R <sup>2</sup>	P value <sup>1</sup>
PERMANOVA						
Habitat	2	1.70	0.85	2.37	0.16	0.001
Residuals	24	8.66	0.36		0.83	
Total	26	10.36			1	
BETADISPER						
Habitat	2	0.03	0.02	2.91		0.077
Residuals	24	0.14	0.01			

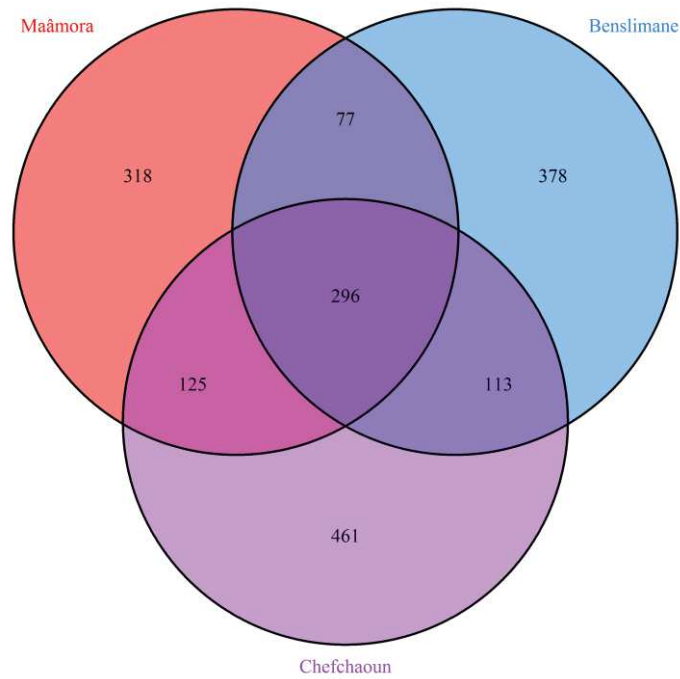
Df, degrees of freedom ; SS, sum of squares ; MS, mean squares; F.Model, F value by permutation

<sup>1</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (Iterations = 999)





**Figure II.1.** Distribution of fungal OTUs among habitats (Maâmora, Benslimane, Chefchaoun) in a ternary plot. The color indicates the taxonomic affiliation of OTUs at order level. The size of a dot represents the abundance of one OTU.



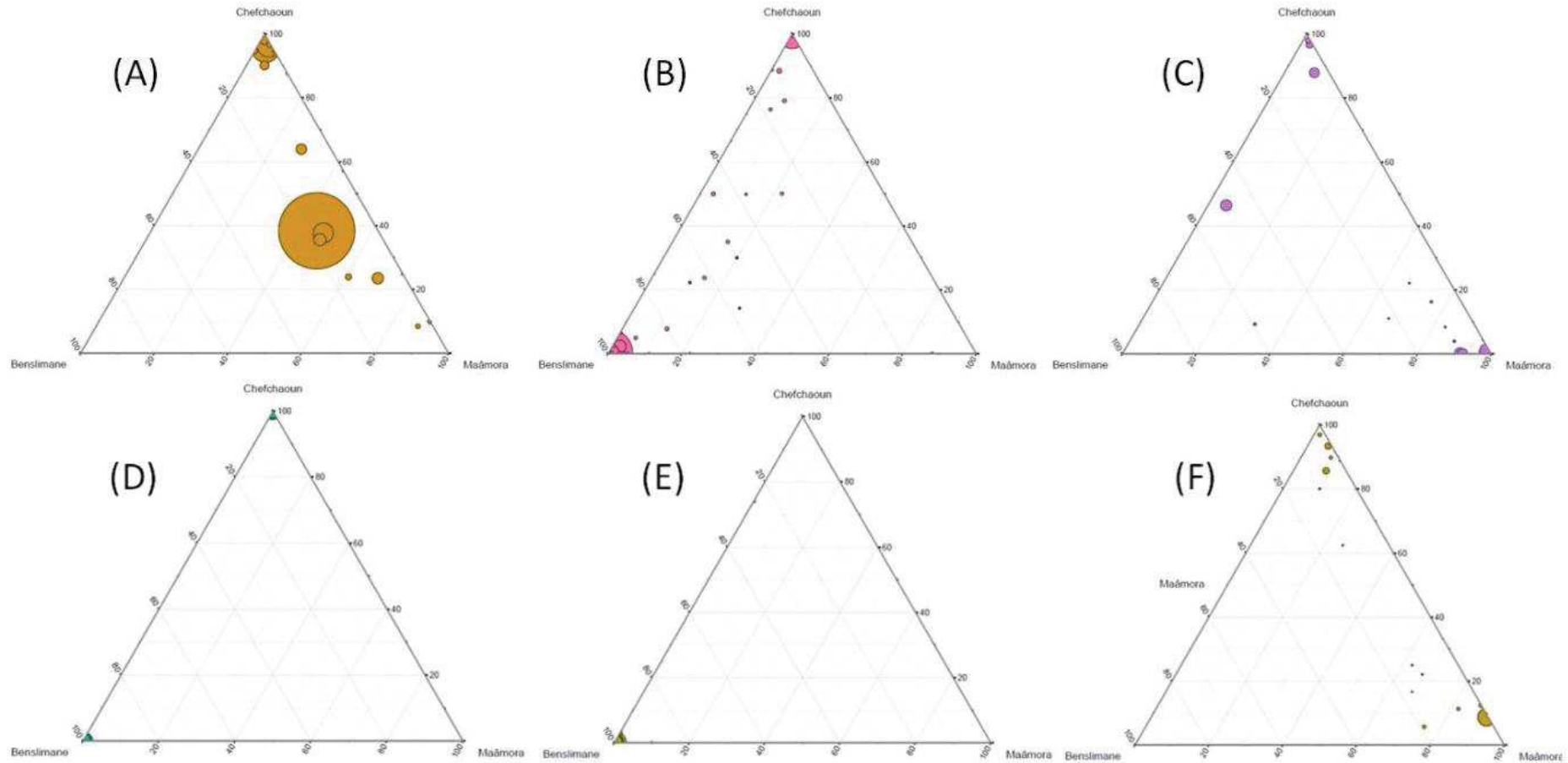
**Figure II.2.** Venn diagram representing shared and unique EcM-related fungal OTUs among habitats (Maâmora, Benslimane, Chefchaoun).

**Table II.3** Variation in EcM-related fungal community structures associated to *Quercus suber* among Moroccan cork oak forests

Model/Factors	Df	SS	MS	F. Model	R <sup>2</sup>	p-value <sup>1</sup>
Pairwise PERMANOVA						
Maâmora-Benslimane	1	0.96	0.96	2.66	0.14	0.001
Maâmora-Chefchaoun	1	0.81	0.81	2.42	0.13	0.001
Benslimane-Chefchaoun	1	0.79	0.79	0.11	0.11	0.002

Df, degrees of freedom; SS, sum of squares ; MS, mean squares; F.Model, F value by permutation

<sup>1</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (999 iterations).



**Figure II.3.** Distribution of fungal OTUs among habitats (Maãmora, Benslimane, Chefchaoun) at genus level in ternary plots. The six predominant genera are indicated, (A) *Cenococcum* (45 OTUs, 12,516 sequences), (B) *Tomentella* (140 OTUs, 11605 sequences), (C) *Russula* (64 OTUs, 7,027 sequences), (D) *Inocybe* (19 OTUs, 4734 sequences), (E) *Cortinarius* (39 OTUs, 4,569 sequences), (F) *Cladophialophora* (85 OTUs, 3,042 sequences). The size of a dot represents the abundance of one OTU.

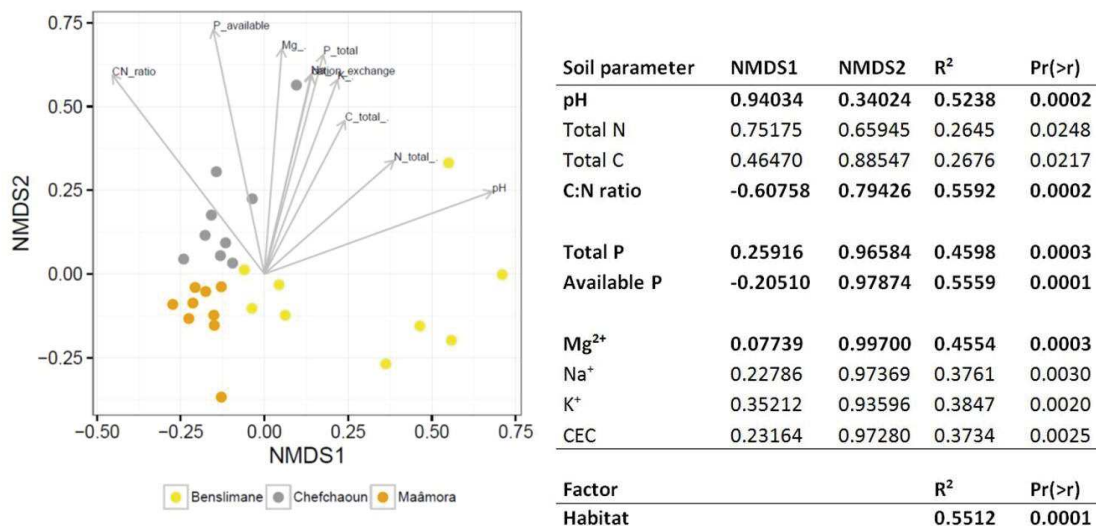
Taxonomic assignment	Fungal OTU (name)	Fungal abundance (number of sequences)	Habitat			Soil property		
			Maâmora	Benslimane	Chefchaoun	Soil acidity	SOM decomposition rate	Soil available P content
Archaeorhizomycetes	<i>Archaeorhizomyces</i>	236	360					
		493	271					
Dothideomycetes	Mycosphaerellaceae	75	1270					
	Capnodiales	292	92					
		32	1117					
		56	151					
	<i>Cenococcum</i>	362	79					
		471	91					
		1008	18					
	<i>Lophiostoma</i>	45	540					
	<i>Saccharicola</i>	28	294					
	Dothideomycetes	78	170					
	1190	13						
Eurotiomycetes		1137	17					
		33	2014					
		112	221					
	<i>Cladophialophora</i>	246	118					
		1194	10					
		1241	11					
		3363	5					
	Herpotrichiellaceae	1188	19					
	Chaetothyriales	492	31					
	<i>Aspergillus</i>	82	314					
<i>Paecilomyces</i>	19070	3						
Geoglossomycetes	<i>Sarcoleotia</i>	610	24					
	<i>Trichoglossum</i>	1664	81					
Lecanoromycetes	<i>Degelia</i>	703	28					
	<i>Cryptosporiopsis</i>	95	548					
	254	23						
Leotiomycetes	<i>Phialocephala</i>	545	3					
	453	54						
	<i>Oidiodendron</i>	105	472					
	999	19						
Pezizomycetes	Pezizales sp	551	5					
	<i>Ilyonectria mars-panacis</i>	69	311					
	759	27						
Sordariomycetes	Sordariales	928	5					
	1937	9						
	Sordariomycetes	366	54					
	407	57						
Agaricomycetes	<i>Hygrophorus</i>	51	2592					
	Agaricales	376	10					
	<i>Inocybe</i>	315						
	<i>Lactarius</i>	60	1260					
	<i>Russula</i>	72	37					
	Russulaceae	16	3230					
	<i>Sebacina</i>	705	197					
		4242	18					
	Sebacinales Group B	572	56					
		17	5448					
	780	70						
<i>Tomentella</i>	66	347						
	85	736						
	230	24						
	358	393						
	38	1024						
Thelephoraceae sp								
unidentified	Fungi sp	445	16					

**Figure II.4.** Major fungal indicators of habitat and soil property. Only fungal indicators with a significant association with at least one ecological condition (black box) are shown, i.e. fungal OTUs associated to a habitat with  $r.g > 0.5$  and  $P < 0.01$  and/or a soil property with  $A > 0.8$ ,  $B > 0.6$  and  $P < 0.01$ . For certain major fungal indicators, a low association (below the thresholds defined above) was also revealed (grey box). See Table II.4 and II.S3 for details. Taxonomic assignment is provided until the genus level; Except for the one with unidentified genus (higher taxonomic level is indicated).

### 3. Drivers of fungal community structures

Soil characteristics in the Moroccan cork oak forest were investigated (Table II.S3, see the annexe) in order to identify the main abiotic soil parameters driving EcM-related fungal community structures. Correlation analysis between each of the soil parameters (Table II.4) revealed strong positive correlations among almost all of them, except for C:N ratio with the majority of other parameters (pH, Total N, Total C, Available P, Mg, and Na), available P with pH, and total N. Available P, Mg, K and CEC were the soil parameters the most strongly correlated ( $r > 0.75$ ) with others.

NMDS ordination confirmed the habitat effect on EcM-related fungal community structure (Figure II.5) with a significant correlation ( $R^2 = 0.5512$ ,  $P = 0.0001$ ). All ten soil parameters fitted in NMDS were also significantly correlated with the EcM-related fungal community structure (Figure II.5). The Maâmora habitat appeared as the most different in terms of soil characteristics compared to Benslimane and Chefchaoun habitats. The three most significant drivers of EcM-related fungal community structures were pH, C:N ratio ( $P = 0.0002$ ), and available P ( $P = 0.0001$ ). Analysis of fungal indicators with respect to these three main soil properties revealed the significant association (A [specificity]  $> 0.8$ ; B [sensitivity]  $> 0.6$ ;  $P < 0.05$ ) of 27 OTUs with soil acidity levels (pH), 28 OTUs with soil organic decomposition rates (C:N ratio), and 60 OTUs with soil available P contents (Table II.S4, see the annexe). As observed for the fungal indicators of habitat, a higher diversity of Ascomycota than Basidiomycota (only Agaricomycetes) was significantly associated with soil properties. Among the most significant OTUs ( $P < 0.01$ ), seven OTUs affiliated to *Cenococcum*, *Cladophialophora*, *Aspergillus*, *Cryptosporiopsis*, and *Ilyonectria*, were also indicators of a habitat (Figure II.4).



**Figure II.5.** Nonmetric multidimensional scaling analysis of OTU-based EcM-related fungal community structure in Moroccan cork oak habitats (Maâmora, Benslimane and Chefchaoun) and soil parameter fitting. The stress of the ordination is 0.1584. All significant soil parameters are shown by arrows (the length is proportional to the strength of the correlation). Factor fitting (Habitat) is indicated below the soil parameter. The most significant soil parameters and factor ( $P < 0.001$ ) are indicated in bold in the table.

**Table II.4.** Correlation coefficient between soil physical-chemical parameters among the Moroccan cork oak forest

	pH	Total N %	Total C %	C:N ratio	Total P mg / kg	Available P mg / kg	Mg_% meq %	Na_% meq %	K_% meq %	Cation Exchange Capacity meq %
Total N	0.769 <sup>***1,2</sup>									
Total C	0.702 <sup>***</sup>	0.965 <sup>***</sup>								
C:N ratio	-0.331 <sup>NS</sup>	-0.192 <sup>NS</sup>	0.067 <sup>NS</sup>							
Total P	0.625 <sup>***</sup>	0.557 <sup>**</sup>	0.700 <sup>**</sup>	0.429 <sup>*</sup>						
Available P	0.150 <sup>NS</sup>	0.277 <sup>NS</sup>	0.497 <sup>**</sup>	0.774 <sup>**</sup>	0.790 <sup>***</sup>					
Mg %	0.496 <sup>**</sup>	0.582 <sup>**</sup>	0.751 <sup>***</sup>	0.545 <sup>**</sup>	0.938 <sup>***</sup>	0.913 <sup>**</sup>				
Na %	0.530 <sup>**</sup>	0.572 <sup>**</sup>	0.652 <sup>***</sup>	0.225 <sup>NS</sup>	0.725 <sup>***</sup>	0.772 <sup>***</sup>	0.836 <sup>***</sup>			
K %	0.707 <sup>***</sup>	0.814 <sup>***</sup>	0.904 <sup>***</sup>	0.245 <sup>NS</sup>	0.895 <sup>***</sup>	0.712 <sup>***</sup>	0.931 <sup>***</sup>	0.814 <sup>***</sup>		
Cation Exchange Capacity	0.625 <sup>***</sup>	0.742 <sup>***</sup>	0.875 <sup>***</sup>	0.406 <sup>*</sup>	0.933 <sup>***</sup>	0.764 <sup>***</sup>	0.951 <sup>***</sup>	0.719 <sup>***</sup>	0.972 <sup>***</sup>	

<sup>1</sup> '\*\*\*' corresponds to  $P < 0.001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'NS'  $P > 0.05$ .

<sup>2</sup> Strong correlation coefficient (0.75) are indicated in red color.



### III. Discussion

Recent extreme droughts in Southern Mediterranean forests have increased tree mortality (Allen *et al.*, 2010). This negative impact has been exacerbated by increasing human pressure (e.g. deforestation, overharvesting) (Gauquelin *et al.*, 2016a). In this context, cork oak forests have been proposed as a major socio-ecological model for the Mediterranean basin (Gauquelin *et al.*, 2016b). The functioning of these ecosystems is highly dependent on interactions with soil fungi, notably EcM fungi, which are also at particular risk due to increasing human- and climate-driven negative impacts. Conservation strategies taking into account both cork oaks and soil fungi are thus crucial. However, soil fungi are rarely considered in conservation biology, despite being providers of major ecosystem services and indicators of sustainable ecosystem management (Heilmann-Clausen *et al.*, 2015).

An in-depth investigation of the molecular diversity of EcM-related fungal community associated with cork oak roots was conducted in the Moroccan cork oak forest. Remarkably, no *Quercus*-related fungal pathogen (Luque *et al.*, 2000; Romero *et al.*, 2007; Camilo-Alves *et al.*, 2013; Lancellotti & Franceschini, 2013; Corcobado *et al.*, 2015) was detected on cork oak roots sampled in the current work. Basidiomycota was the predominant EcM phylum, as previously observed in most oak forests (Smith *et al.*, 2007; Morris *et al.*, 2008; Aponte *et al.*, 2010; Suz *et al.*, 2014). Three main EcM families, Thelephoraceae, Russulaceae and Gloniaceae accounted for almost half of total EcM-related fungal abundance.

Similar observations were obtained at family level in different Mediterranean oak forests (Azul *et al.*, 2010; Corcobado *et al.*, 2015), whereas Cortinariaceae constituted one of the three predominant EcM families in Northern European oak forests (Courty *et al.*, 2008; Suz *et al.*, 2014), suggesting a potential Mediterranean oak-EcM association pattern. A clear difference between Mediterranean forests and temperate deciduous forests was observed for EcM fungal communities, but not for global soil fungal communities (Tedersoo *et al.*, 2014).

EcM surveys in Mediterranean oak forests were mainly based either on fruitbodies or on fungal mantle structure covering the root surface, i.e. morphotypes (Richard *et al.*, 2005; Azul *et al.*, 2010; Richard *et al.*, 2011; Lancellotti & Franceschini, 2013). The former can lead to overestimations of fungal taxa with above-ground fruitbodies (e.g. *Cortinarius*, *Lacaria*) to the detriment of fungal taxa lacking sexual structures (e.g. *Cenococcum*), or with inconspicuous structures (e.g. *Tomentella*), whereas the latter leads to misinterpretations of fungal diversity (variations in morphological characteristics, incompleteness of morphological criteria, lack of EcM species with known morphotypes) (Smith and Read, 2009; Taylor and Alexander, 2005). In addition, morphotype-based analysis is time-consuming and requires trained experts. *Cenococcum* and *Tomentella* are the two most abundant EcM genera associated with cork oaks in the current study. By contrast, *Pisolithus*, which has been largely studied because of the relative abundance of its fruitbodies in the Moroccan cork oak forest (Bakkali Yakhlef *et al.*, 2009) and its growth-promoting effect on *Quercus suber* (Sebastiania *et al.*, 2014), showed very low below-ground abundance (< 0.00001 % of total sequence number, not detected in the rarefied data set).

*Cenococcum* is one of the most common and abundant fungi in forest ecosystems (Trappe, 1962; LoBuglio, 1999). It represented all Gloniaceae detected in the present study (13 % of total sequences and 34 % of Ascomycota sequences). As observed in the Sardinian cork oak forest (Lancellotti & Franceschini, 2013), *Cenococcum* was detected in all root samples. However, over 40 different OTUs were unequally distributed in the three forest habitats, confirming reports of intraspecific diversity at local and large geographical scales (Jany *et al.*, 2002; Douhan *et al.*, 2007; Bahram *et al.*, 2011). The stronger predominance of *Cenococcum*



in Mediterranean forests may be linked to its resistance to drought conditions (Kerner *et al.*, 2012; Peter *et al.*, 2016) and thus also to precipitation rates (Aponte *et al.*, 2010).

Other Ascomycota (94 genera in this study), mostly endophytic and filamentous fungi were also detected with a relatively high abundance. For instance, *Cladophialophora* and *Oidiodendron* (10 % of Ascomycota sequences), which are potential parasites of *Cenococcum* sclerotia (Obase *et al.*, 2014), were present along with *Cenococcum*, though with no clear quantitative co-variation at habitat level (data not shown). An in-depth investigation of ecological interactions between sclerotia-associated fungi and *Cenococcum* may thus be important for a better understanding of fungal assembly rules affecting forest ecosystem functioning, particularly in Mediterranean drought scenarios. In addition, some members of *Oidiodendron* were described as ericoid mycorrhizal (ErM) fungi closely associated with EcM roots of *Quercus ilex* (Bergero *et al.*, 2000). *Cryptosporiopsis*, the third predominant Ascomycota detected in the current work, has also been described as containing ErM members (Leopold, 2016). Two principal ecological implications of EcM-ErM interactions in forest ecosystem functioning have been proposed by Bergero *et al.* (2000), (i) nutrient exchanges among EcM and ErM plants through hyphal links of shared mycorrhizal fungi, and (ii) EcM plants acting as an ErM fungal reservoir for the efficient land recolonization of ErM plants. The significance of the second hypothesis is of particular importance for cork oak ecosystems, strongly affected by fire events in the Mediterranean basin (Schaffhauser *et al.*, 2012).

*Archaeorhizomyces*, a genus of ubiquitous root endophyte fungi (Rosling *et al.*, 2011), was relatively abundant (> 3% of Ascomycota) in Moroccan cork oak roots. The presence of this genus in Mediterranean ecosystem surveys is rarely described and few data are available regarding their ecological role, but a continuum from root endophytic to free-living saprophytic life strategies is extremely likely (Rosling *et al.*, 2013).

The well-known oak-associated fungal taxa *Tuber* (Bonito *et al.*, 2010) represented a minor part of the EcM-related fungal community associated with cork oaks (0.4 % of total; 1% of Ascomycota), as observed in the Sardinian cork oak forest (Lancellotti and Franceschini, 2013). Sequences affiliated to the recently-characterized *Tuber cistophilum*, isolated from Spanish acidic argillaceous soils (Alvarado *et al.*, 2012), constituted 7 % of *Tuber* abundance. *Terfezia*, a genus of truffle-like fungi, which constitutes an important source of edible fungi for local Moroccan populations, but is mainly used for commercial export to the Middle East, was also detected, mainly *Terfezia pini*, but at low abundance (0.0005 % of total sequence number).

The predominant Basidiomycota belonged to Thelephorales, Russulales, and Agaricales, notably *Tomentella* > *Russula* > *Inocybe* > *Cortinarius*. *Tomentella* and *Russula*, as well as *Cenococcum*, were proposed as major actors of forest ecosystems under drought conditions (Azul *et al.*, 2010). Nevertheless, the role of *Tomentella* in cork oak functioning remains unclear since its abundance varies seasonally, and does not seem to be related to cork oak decline or to the presence of fungal pathogens (*Phytophthora cinnamomi*) (Corcobado *et al.*, 2015).

The predominance of *Russula* has been previously described in Northern Mediterranean cork oak ecosystems (Azul *et al.*, 2009, 2010; Corcobado *et al.*, 2015). *Russula* was described as one of the most important fungi in terms of species richness and productivity in the Portuguese cork oak forest (Azul *et al.*, 2009), but is highly sensitive to seasons, land use practices and cork oak decline (Azul *et al.*, 2009; Corcobado *et al.*, 2015). Several *Russula* species (*Russula heterophylla*, *Russula olivobrunnea*, *Russula violeipes*) were detected specifically in Morocco and not in other Mediterranean cork oak forests (Azul *et al.*, 2009, 2010; Barrico *et al.*, 2010; Lancellotti & Franceschini, 2013; Taudiere *et al.*, 2015). By

contrast, *Russula decipiens* was described in Corsica (Taudiere *et al.*, 2015), *Russula foetens* in Portugal (Azul *et al.*, 2009), and *Russula odorata* in Corsica and Sardinia (Orgiazzi *et al.*, 2012; Lancellotti & Franceschini, 2013; Taudiere *et al.*, 2015). Whereas *Russula* was the most abundant member of Russulaceae (43 %) in the present study, *Lactarius chrysorrheus* was the most represented Russulaceae species in the Sardinian cork oak forest (Lancellotti & Franceschini, 2013). *Lactarius chrysorrheus* is also widely distributed in Mediterranean oak forests (Richard *et al.*, 2005; Azul *et al.*, 2009, 2010; Aponte *et al.*, 2010; Barrico *et al.*, 2010), but absent in the present study. Seven other known species of *Lactarius* were detected, notably *L. quietus*, which is considered an oak specialist (Suz *et al.*, 2014). However, *L. quietus* distribution appears more related to Northern Europe oak ecosystems (Courty *et al.*, 2008; Suz *et al.*, 2014) than Mediterranean oak ecosystems (Richard *et al.*, 2005; Azul *et al.*, 2009).

Contrary to Thelephorales, Russulales, and Hysteriales, represented by one or two known fungal genera, Agaricales were highly diverse (*Amanita*, *Cortinarius*, *Entoloma*, *Hebeloma*, *Hygrocybe*, *Hygrophorus*, *Inocybe*, *Mycena*, *Omphalotus*, *Psathyrella*). *Inocybe* and *Cortinarius* represented more than 60% of Agaricales, confirming previous observations of Mediterranean oak ecosystems (Richard *et al.*, 2004, 2005; Aponte *et al.*, 2010; Barrico *et al.*, 2010; Orgiazzi *et al.*, 2012). As observed for *Russula*, a majority of *Inocybe* species (*Inocybe bresadolae*, *Inocybe glabripes*, *Inocybe posterula*, *Inocybe subporospora*) appeared specific to the Moroccan cork oak forest. Only *Inocybe asterospora* was described in the Portuguese cork oak forest (Azul *et al.*, 2009). By contrast, *Inocybe fibrosoides*, strictly associated with *Q. suber* in Corsica (Taudiere *et al.*, 2015), was not detected in the Moroccan cork oak forest. The majority of *Cortinarius* sequences could not be affiliated at the species level. Only *Cortinarius trivialis* was identified but at very low abundance. *Cortinarius trivialis* was described as particularly sensitive to land use in the Portuguese cork oak forest (Azul *et al.*, 2009). Surprisingly, *Laccaria* and *Tricholoma*, two ectomycorrhizal Agaricales observed at above and below-ground level in Mediterranean oak ecosystems (Richard *et al.*, 2004, 2005; Azul *et al.*, 2009; Barrico *et al.*, 2010; Orgiazzi *et al.*, 2012; Lancellotti & Franceschini, 2013) were not detected in the current work, strengthening the ecological specificity of belowground Moroccan cork oak ecosystems. These two fungal genera were however observed in the Moroccan cork oak forest at above-ground level, reflecting, as for *Pisolithus*, the discrepancy between above- and below-ground fungal abundance.

No fungal species listed in the official IUCN red list was found in the current work. However, the geographical distribution of fungal species listed concerned mainly North America and Europe, while Southern Mediterranean countries remain little explored. The only evidence of *Tricholoma acerbum* presence was reported in Morocco. Nevertheless, the Global Fungal Red List Initiative (<http://iucn.ekoo.se>), which aims to identify hundreds of threatened fungi at the very least, is still in progress, and efforts should be stepped up to take into account underexplored areas such as the Southern Mediterranean basin.

More than 90 fungal indicators ( $P < 0.01$ ) of habitat and/or soil property were determined, underlining the valuable use of fungi as below-ground indicators of forest status and environmental conditions (Cudlin *et al.*, 2007; Suz *et al.*, 2014). The high number of habitat-associated fungal indicator species for Maâmaora and Chefchaoun suggested their strong ecological specificity. Differences in human pressure levels may explain a part of these specificities since Maâmaora is highly disturbed (intensive cork exploitation, overgrazing, ageing of tree population) compared to Chefchaoun (lower human pressure, natural tree regeneration). Three *Cenococcum* OTUs out of 43 were significantly ( $r.g > 0.5$ ;  $P < 0.01$ ) associated with Chefchaoun (low disturbances). *Cenococcum* is recognized as a multi-stage fungus due to its worldwide predominance and stability in forest ecosystems, but the current

results evidenced an intraspecific *Cenococcum* ecological preference, potentially related to land use and succession stages, and not only to drought resistance. By contrast, *Russula* OTUs were significantly associated with Maâmora, and might thus be seen as indicators of higher levels of disturbances. A high sensitivity of *Russula* to land use practices was also observed in other cork oak ecosystems (Azul *et al.*, 2010; Orgiazzi *et al.*, 2012). *Russula* is generally considered as a late-successional fungal genus (Twieg *et al.*, 2007; Gao *et al.*, 2015b; Taudiere *et al.*, 2015), which may explain its high sensitivity to land use but also its reliability as an indicator of ecosystems characterized by the aging of cork oak populations (open forest with low regeneration rate). Nevertheless, ecological interpretations based on fungal guilds must be carefully made due to functional intraspecific variability (OTU level) for a given genus, as revealed in Gao *et al.* (2015) and in the present work. *Oidiodendron maius*, a potential ErM fungus (Rice & Currah, 2006; Kohler *et al.*, 2015), was also one of the most abundant indicator species significantly related to the Maâmora habitat. This observation strengthens the hypothesis that EcM plants can act as reservoirs of ErM fungi (Bergero *et al.*, 2000) in ecosystems characterized by strong Ericaceae layer degradation such as Maâmora. It raises the question: could other fungi, such as arbuscular mycorrhizal (AM) fungi, play a role in EcM plants? Evidence of AM fungal colonization of EcM plants and their functional role was demonstrated (Egerton-Warburton & Allen, 2001; Egerton-Warburton *et al.*, 2007; Toju *et al.*, 2013b). However, AM fungi generally constitute a minor part of the total fungal community in EcM-dominated ecosystems (Orgiazzi *et al.*, 2012). In the current work AM fungi were almost absent of raw data using the ITS-based approach, but the 18S-based approach using primers targeting AM fungi demonstrated their relevance to specifically addressing AM fungal community structures in EcM ecosystems (Lumini *et al.*, 2010; Bagella *et al.*, 2014).

A wide range of fungal indicators were determined regarding their preference for soil properties, notably pH, C:N ratio, and available P. Similarly, a wide range of soil characteristics, whether or not related to land use, was described as strongly affecting soil fungal communities in forest ecosystems (Suz *et al.*, 2014; Tedersoo *et al.*, 2014; Creamer *et al.*, 2016). Soil pH was demonstrated as a major driver on a global scale, strongly impacting fungal OTU richness and composition (Tedersoo *et al.*, 2014), whereas the impact of phosphorus and C:N ratio were taxa-specific (Tedersoo *et al.*, 2014). Although the soil pH range of the Moroccan cork oak forest is relatively narrow, a range of fungal indicator species was identified, notably Eurothiomycetes for strong acidity and Agaricomycetes for low acidity. Fungal indicator analysis regarding C:N ratio notably highlighted the association between *Tomentella* species and a high SOM decomposition rate. Some EcM fungi such as *Tomentella* are able to adopt a saprophytic lifestyle depending on environmental conditions (Churchland & Grayston, 2014), which may explain its role in soil C cycling as suggested by the current results. *Tomentella* species were also significantly associated with soil available P levels (mostly to low P soils). However, other fungal species appear strongly associated with high P soils, notably *Cenococcum*. By contrast, soils characterized by very high P levels showed unclear taxonomic signals with a wide range of fungal indicators showing a low association p-value, probably due to the absence of nutrient constraint. Certain fungal taxa related to EcM fungi (*i.e.* *Tomentella*, *Cenococcum*) and putative sclerotia-associated fungi (*Cladophialophora*) showed high functional intraspecific variability regarding their association with different ecological conditions. The significance of intraspecific variability in terms of functional diversity and ecosystem process has been only pointed out for some EcM fungi (e.g. *Tomentella*, *Russula*) (Gao *et al.*, 2015b), but its extent at community level remains a major issue in fungal ecology (Johnson *et al.*, 2012).

The present study sheds new light on the below-ground interactions taking place in the Moroccan cork oak forest, a severely threatened ecosystem. We evidenced a highly diverse soil fungi community, both in Ascomycota and Basidiomycota, strongly structured by habitat type and soil property. The results suggest a Southern Mediterranean fungal pattern, reinforcing the need to investigate the Southern Mediterranean fungal diversity more extensively. In addition, the strong geographical structure of *Q. suber* in the Mediterranean basin (Magri *et al.*, 2007) may also affect fungal diversity associated with cork oak, probably contributing to the differences between fungal community structures in Morocco, as compared to Sardinia and Corsica. Indeed, intra-specific plant host diversity has been described as a soil fungal diversity driver (Tedersoo *et al.*, 2016) but remains poorly investigated. The success of future conservation strategies thus depends on joint initiatives on a Mediterranean scale, associating plant population geneticists, botanists, naturalists, microbiologists and pedologists to picture in a broader framework the tremendous complexity of plant-fungal and fungal-fungal assembly rules in forest ecosystems (Toju *et al.*, 2013a; Clemmensen *et al.*, 2015; Taudiere *et al.*, 2015; Baptista *et al.*, 2015).

## **Chapitre III**

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**Impact du mode de gestion sur les  
communautés fongiques  
ectomycorhiziennes associées au chêne-liège**

## Résumé

La subéraie est un écosystème forestier occupant une place socio-économique et écologique majeure au Maroc. Cet écosystème est cependant fortement impacté par l'augmentation des pressions anthropiques et l'aggravation des conditions climatiques, entraînant une forte régression de sa superficie et une accélération des processus de désertification. L'étude a pour objet de caractériser l'impact du mode de gestion de la subéraie sur une composante majeure de son fonctionnement, la communauté de champignons ectomycorhiziens (EcMs) associée au chêne-liège, et de déterminer des bio-indicateurs EcMs relatifs aux perturbations. La communauté de champignons EcMs a été suivie au cours de la période estivale et hivernale au sein de deux sites de la subéraie de la Maâmora (Maroc), caractérisés soit par une exploitation ou non de la subéraie. Un impact significatif du mode de gestion de la subéraie sur la communauté de champignons EcMs a été mis en évidence, avec les différences les plus notables à la période estivale. Ces travaux ont permis de confirmer l'importance écologique probable de plusieurs groupes de champignons (e.g. *Cenococcum*) dans le maintien des fonctionnalités de la subéraie, mais aussi de l'assujettissement de certains champignons EcMs (*Pachyphloeus*, *Russula*, *Tomentella*) à une perturbation ou une saison, et par conséquent à l'état de la subéraie ou à des conditions climatiques, respectivement. La généralisation de ce type d'étude à l'ensemble de la subéraie méditerranéenne pourrait permettre l'établissement de modèles plus robustes pour prédire l'impact des changements globaux sur cet écosystème emblématique des régions méditerranéennes.

Le chapitre fait l'objet d'un article accepté dans le journal Comptes Rendus Biologies :

**F.Z. Maghnia**, H. Sanguin, Y. Abbas, M. Verdinelli, B. Kerdouh, N. El Ghachtouli, E. Lancellotti, S.E. Bakkali Yakhlef, R. Duponnois, 2017. Impact du mode de gestion de la subéraie de la Maâmora (Maroc) sur la diversité des champignons ectomycorhiziens associés à *Quercus suber*. Comptes Rendus Biologies

**Mots clés** : Bio-indicateurs, Champignons ectomycorhiziens, Chêne-liège, Gestion



## Introduction

Le chêne-liège est l'une des espèces forestières les plus importantes au Maroc, s'étendant sur une superficie de près de 350 000 ha, principalement dans les régions de la Maâmora, du Plateau central et du Rif (Aafi, 2007). La subéraie est très appréciée pour la diversité de ses produits (bois, liège, glands doux, miel, champignons, plantes médicinales, espace de pique-nique ...), et offre une importante protection des sols grâce au couvert végétal permanent, ce qui en fait un arbre d'une grande importance socio-économique et écologique. Cependant, le fonctionnement des subéraies est fortement menacé par l'aggravation des conditions climatiques (sécheresse) et l'accentuation des pressions anthropiques (surpâturage, surexploitation, ramassage du sous-bois par la population riveraine...) (Gauquelin *et al.*, 2016b), se traduisant par une chute de la productivité, une diminution de la régénération naturelle et une augmentation des attaques parasitaires (Lancellotti & Franceschini, 2013). La diminution du processus de régénération naturelle aboutit généralement à une baisse significative de la densité du peuplement.

Les champignons ectomycorhiziens (EcMs), paramètre clé du fonctionnement biologique des écosystèmes forestiers, peuvent être fortement impactés. Ces champignons jouent un rôle indispensable dans le développement des essences ectotrophes tel que *Quercus suber* (Smith & Read, 2009; Sebastiana *et al.*, 2014), et leur distribution dans le sol est généralement observée sous forme de mosaïque assujettie à la présence des espèces ectotrophes (Dickie & Reich, 2005). L'appauvrissement de l'abondance des propagules fongiques EcMs peut entraîner un ralentissement de la croissance des jeunes régénérations et leur fragilisation vis à vis de stress environnementaux. Cette richesse en propagules fongiques étant assujettie à la présence d'arbres adultes ectotrophes, toute modification de la structure du couvert forestier (e.g. densité, vieillissement) a des répercussions majeures sur la structure des communautés de champignons EcMs, et par conséquent sur les services écosystémiques auxquels ces communautés contribuent (Dickie & Reich, 2005). Plusieurs études portant sur l'inventaire des champignons EcMs ont été réalisées dans les écosystèmes forestiers au Maroc (Abourouh & Najim, 1998; Bakkali Yakhlef *et al.*, 2009; Nounsi *et al.*, 2014). Cependant, ces études ont été essentiellement basées sur la diversité des carpophores, reflétant une part infime de la diversité des champignons EcMs dans les sols forestiers. De plus, l'impact de la gestion des subéraies et donc du couvert forestier a été très peu pris en compte alors que ses variations (âge et phénologie des arbres, densité de peuplement, coupe à blanc, invasion biologique, ouverture de la canopée, pâturage, urbanisation, feu) affectent fortement la dynamique et la structure des communautés de champignons EcMs (Azul *et al.*, 2010; Buscardo *et al.*, 2010; Karpati *et al.*, 2011; Boudiaf *et al.*, 2013).

La présente étude a pour objectif de caractériser l'impact du mode de gestion de la subéraie sur les communautés de champignons ectomycorhiziens (EcMs) associées au chêne-liège, par comparaison de deux sites, (i) un site exploité soumis à différentes pressions anthropiques (récolte du chêne-liège, coupe du bois, pâturage) et (ii) un site protégé non soumis à des pressions anthropiques. Une des différences majeures observées au niveau du couvert forestier est la différence de densité du peuplement de chêne-liège avec une faible densité au niveau du site exploité et une forte densité au niveau du site protégé. Cette étude a été réalisée dans la subéraie de la Maâmora car elle constitue la subéraie marocaine la plus fortement impactée par les pressions anthropiques. L'analyse de la diversité des champignons EcMs par des approches morphologiques et moléculaires a été effectuée au cours de la période estivale et hivernale au niveau des racines de chênes-lièges.



# I. Matériel et Méthodes

## 1. Site d'étude et échantillonnage des champignons EcMs

La subéraie de la Maâmora, située au nord-ouest du Maroc, en bordure de l'océan Atlantique, a été sélectionnée comme site d'étude en raison de son importance écologique (Sites d'Intérêt Biologique et Ecologique (Haimed *et al.*, 2015)). Cette subéraie appartient à l'étage thermo-méditerranéen qui se caractérise par des étés chauds et secs et des hivers doux et humides. Les températures moyennes mensuelles sont de l'ordre de 12°C (Janvier) à 25°C (Juillet-Août) (Aafi, 2007). Les précipitations annuelles moyennes sont de l'ordre de 500 mm. Les sols de cette subéraie sont acides, constitués dans leur quasi-totalité par une couche de sable en surface reposant sur une assise marno-argileuse imperméable du Tortonien. La richesse en matière organique des sols est très variable selon les localités et dépend principalement de la présence ou de l'absence du couvert forestier (Aafi, 2007).

Au sein de cette subéraie, deux sites expérimentaux, distants de 21,6 Km, ont été définis. Le Site I (34 ° 12 '34 "N, 6 ° 35' 57" W) est un site non protégé en cours d'exploitation (récolte du chêne-liège, coup du bois et pâturage) présentant une faible densité d'arbres d'environ 50 arbres adultes à l'hectare. Aucune régénération n'a pu être observée. Le Site II (34 ° 1 '39 "N, 6 ° 32 '49" W) correspond à un site clôturé et protégé (sans exploitation ou pâturage) avec une forte densité d'arbres d'environ 290 arbres à l'hectare.

Des échantillons de racines de chêne-liège (avec sol adhérent) ont été collectés sur chaque site pendant l'été (Juillet 2010) et l'hiver suivant (Janvier 2011). Au niveau de chaque site, une surface d'étude a été définie (30 m de longueur et 7,5 m de largeur, soit 225 m<sup>2</sup>) dans laquelle 10 échantillons racinaires de chêne-liège, distant l'un de l'autre de 7,5 m, ont été prélevés selon la méthode préconisée par (Horton & Bruns, 1998), puis conservés à +4°C jusqu'à leur traitement. Les échantillons ont ensuite été lavés soigneusement puis observés sous la loupe binoculaire afin de réaliser une analyse morphologique des ectomycorhizes (Agerer, 1987) et déterminer les types de morphotypes (MTs) et leur abondance ( $N_{MT}/N_{total}$ ; N correspond au nombre d'apex EcMs). Le taux de mycorhization global par échantillon a été estimé selon  $N_{ecto}/N_{total}$ , avec  $N_{ecto}$  correspondant au nombre d'apex EcMs total et  $N_{total}$  au nombre d'apex mycorhizés et non mycorhizés. Au moins deux ectomycorhizes représentatives de chaque MT ont été séchés et conservés à -20°C pour identification moléculaire ultérieure.

## 2. Extraction d'ADN, amplification et séquençage de l'ITS1 de champignons EcMs

Pour chaque représentant de MT, l'ADN total a été extrait à partir du kit Dneasy Plant Mini Kit (Qiagen, France). Plusieurs modifications ont été apportées au protocole proposé par le fournisseur afin d'optimiser la qualité et le rendement d'extraction (ajout de 20-30 mg de Polyvinylpolypyrrolidon (PVPP) lors du broyage, préchauffage du tampon AE à +65°C).

Une étape d'amplification des espaceurs interne transcrit (ITS1 et ITS2) de l'ADN ribosomique (ADNr) a été effectuée en utilisant les amorces ITS1F et ITS4 (Gardes & Bruns, 1993) et le kit d'amplification MyTaq<sup>TM</sup> HS Red Mix (Bioline, France) en se référant aux recommandations du fournisseur. Les conditions d'amplifications ont été les suivantes : 94°C

(5min), suivie de 35 cycles de 94°C (30 s), 55°C (30s) et 72°C (1 min), et 72°C (7 min). Après purification des produits d'amplification par le kit Qiaquick PCR Purification kit (Qiagen, France) selon les recommandations du fournisseur, la région ITS1 a été séquencée au sein du Centre National de la Recherche Scientifique et Technique (CNRST) de Rabat, dans l'Unités d'Appui Technique à la Recherche Scientifique (UATRS).

### 3. Analyse phylogénétique des séquences ITS1 de champignons EcMs

Les séquences obtenues ont été comparées aux séquences déposées dans la base de données UNITE (<https://unite.ut.ee/>) en utilisant l'option massBLASter. Pour chaque séquence ITS1, la séquence de référence présentant le taux de similarité le plus élevé dans UNITE a été utilisée pour l'analyse phylogénétique. Cette dernière a été effectuée selon la procédure proposée dans phylogeny.fr (<http://www.phylogeny.fr/>) (Dereeper et al. 2008), option « One Click mode », basée sur la méthode statistique de maximum de vraisemblance du programme PhyML.

### 4. Analyses statistiques

Afin de caractériser l'impact de la densité de peuplement et de la saison sur la communauté de champignons EcMs, la richesse (nombre de MTs), la diversité (indice de Shannon) et la structure de la communauté de champignons EcMs, ainsi que le taux de mycorhization ont été estimés. Les analyses statistiques ont été effectuées par ANOVA à 2 facteurs suivie du test post hoc Tukey HSD (seuil de confiance de 95%) disponible dans le package *stats* du logiciel R (<https://www.r-project.org/>). Les données de taux de mycorhization ont été préalablement transformées par la fonction *arcsin*. L'abondance des champignons EcMs a été normalisée pour chaque morphotype par le nombre total d'apex EcM au sein d'un échantillon donné, puis transformée par la fonction *arcsin*. A partir de la matrice d'abondance normalisée, l'indice de Bray-Curtis a été calculé afin d'estimer la dissimilarité entre les communautés de champignons EcMs. La significativité de la dissimilarité en fonction du mode de gestion et de la période de prélèvement a été évaluée par PerMANOVA ( $P < 0.05$ ) en utilisant le package *vegan* [fonction *adonis*] disponible dans le logiciel R. La détermination de l'association de champignons EcMs avec un mode de gestion donné ou une période de prélèvement a été effectuée en utilisant le package *indicpecies* (De Cáceres & Legendre, 2009) disponible dans le logiciel R. L'index *IndVal.g* a été sélectionné afin d'estimer la significativité de l'association entre un ou plusieurs taxa EcMs [fonction *combinespecies*] et les modes de gestion (exploité et protégé) ou les saisons (hiver et été) [fonction *multipatt*]. Cette procédure permet de déterminer des valeurs d'association plus robustes qu'en considérant les taxa EcMs séparément (De Cáceres *et al.*, 2012). Deux types de probabilités ont été calculés, i.e. A (spécificité) qui représente la probabilité d'un échantillon d'appartenir à un groupe (i.e. exploité / protégé pour le mode de gestion ou hiver / été pour la saison), étant donné que le ou les taxa EcMs ont été détectés, et B (sensibilité) qui représente la probabilité de trouver le ou les taxa EcMs dans différents échantillons appartenant à un groupe donné.

## II. Résultats

### 1. Structure des communautés de champignons EcMs

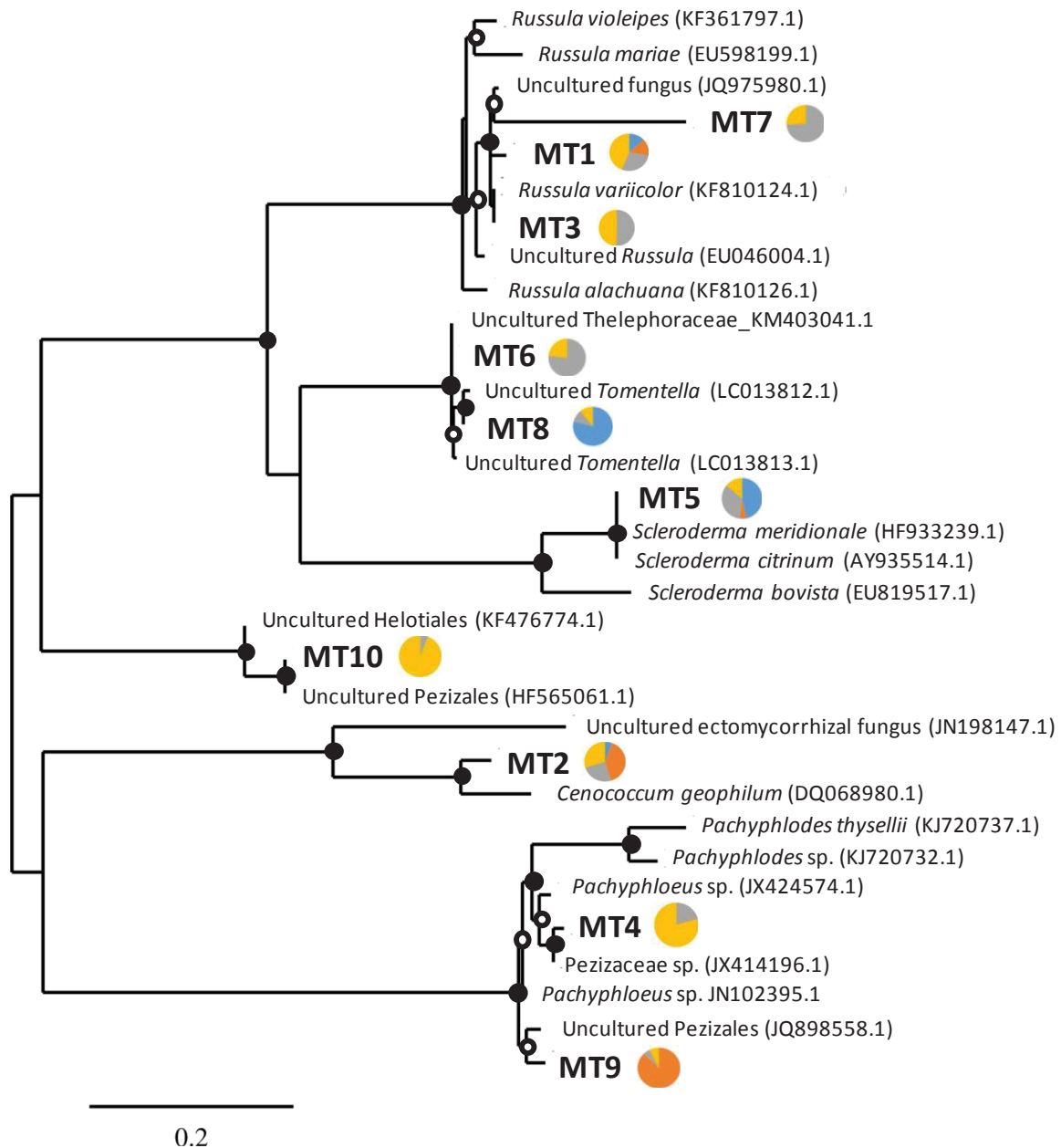
Un total de 7096 ectomycorhizes a été échantillonné. L'analyse morphologique des ectomycorhizes de chêne-liège au sein des deux sites d'études a révélé la présence de 11 morphotypes (MTs). L'amplification de la région ITS1 a été obtenue pour l'ensemble des représentants MTs, à l'exception du MT11. L'analyse phylogénétique des séquences ITS1 correspondantes a montré la présence de champignons EcMs appartenant aux Ascomycètes (6 MTs) et Basidiomycètes (4 MTs), affiliés à six ordres, i.e. Russulales, Telephorales, Boletales, Heliotales, Pezizales et Hysteriales (**Figure III.1**). Le morphotype MT10 qui était très proche phylogénétiquement d'une séquence de référence appelée « Uncultured Pezizales » (numéro d'accèsion HF565061.1) correspond à un clade appartenant à l'ordre des Héliotales et non des Pezizales.

La totalité des morphotypes a été détectée dans les deux sites, mais seulement cinq (MTs 1, 2, 5, 8 et 9) aux deux périodes d'échantillonnage (Figure 1). Six morphotypes (MTs 3, 4, 6, 7, et 10) ont été détectés seulement en hiver (Figure 1). Le MT11 qui n'a pu être identifié moléculairement a été détecté seulement en été dans la parcelle exploitée. Sur un total de 7096 ectomycorhizes échantillonnées, le MT2 affilié au genre *Cenococcum* représentait le MT prédominant, avec 45-73% de la communauté fongique (**Figure III.2**).

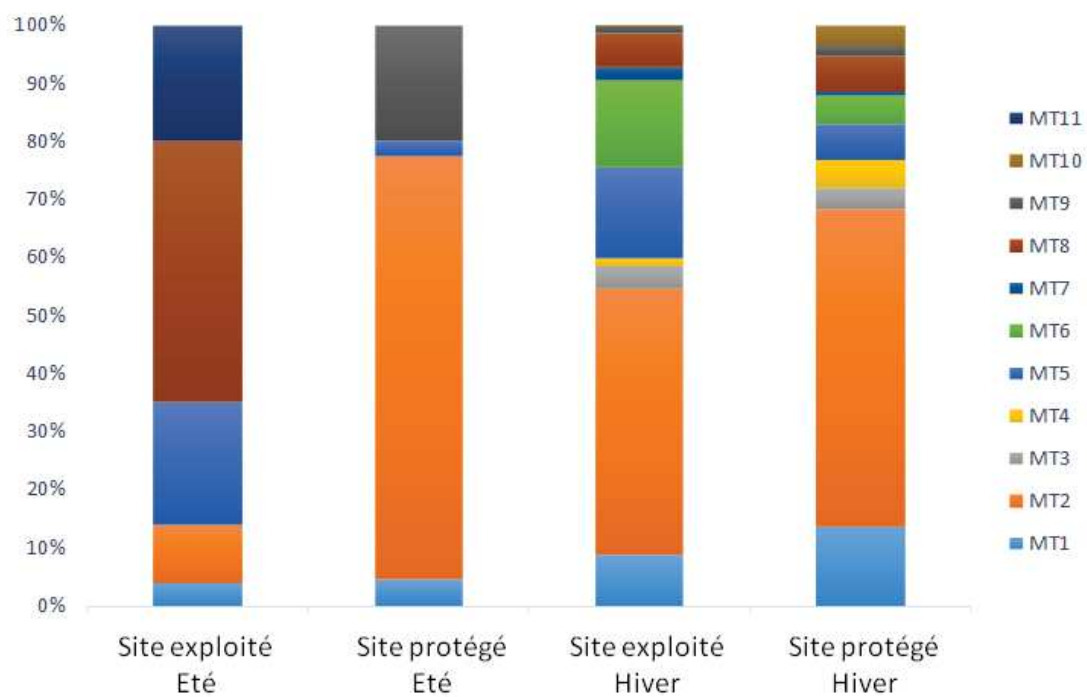
### 2. Impact du mode de gestion et de la saison sur les communautés de champignons EcMs

La richesse et la diversité de la communauté de champignons EcMs, ainsi que le taux de mycorhization ont été mesurés au niveau de l'ensemble des échantillons racinaires de chêne-liège. Un impact majeur de la période d'échantillonnage a été observé sur l'ensemble des paramètres mesurés, avec notamment des niveaux de richesse significativement plus forts en hiver comparé à l'été (**Tableau III.1**). Un impact beaucoup plus restreint du mode de gestion, significativement dépendant de la période d'échantillonnage, a été mis en évidence sur les niveaux de richesse et les taux de mycorhization en hiver et en été, respectivement (**Tableau III.1**).

De même que pour le taux de mycorhization, un impact significatif du mode de gestion sur la structure de la communauté EcMs a été seulement observé en été (**Tableau III.2**). Une très faible fréquence de *Cenococcum* était observée dans le site exploité comparé au site protégé, à contrario de *Tomentella* (**Figure III.2**). Une association significative de plusieurs taxa EcMs ou combinaisons de taxa EcMs avec un mode de gestion ou la période d'échantillonnage a été obtenue (**Tableau III.3**). Le plus grand nombre de taxa EcMs indicateurs (13) était associé à la période hivernale. Quatre taxa EcMs (*Russula*) ou combinaisons de taxa EcMs (*Russula* + *Pachyphloeus*, *Russula* + *Cenococcum*, *Tomentella* + *Pachyphloeus*, *Russula* + *Tomentella*) ont montré une forte spécificité ( $A > 0.8$ ) et sensibilité ( $B > 0.7$ ). La combinaison de taxa *Russula* + *Pachyphloeus* montrait la plus forte valeur d'association ( $\text{IndVal.g} = 0,894$ ), *Russula* seul présentant une valeur d'association plus faible ( $\text{IndVal.g} = 0,860$ ). Aucun taxa EcM n'a été significativement associé à la période estivale. Seulement huit taxa EcMs ou combinaisons de taxa EcMs étaient significativement associés à un mode de gestion, notamment *Tomentella* au site exploité, et *Pachyphloeus*, seul ou avec *Cenococcum*, au site protégé.



**Figure III.1.** Analyse phylogénétique basée par la méthode de maximum de vraisemblance des séquences ITS1 provenant de morphotypes d'ectomycorhizes de chêne-liège dans la subéraie de la Maâmora. Pour chaque morphotype est indiquée (à droite) son abondance relative dans les sites d'études pour la période estivale et hivernale, i.e. site exploité densité en été (bleu), site protégé en été (orange), site exploité en hiver (gris), site protégé en hiver (jaune).



**Figure III.2.** Fréquence des morphotypes EcMs associés au chêne-liège en fonction du mode de gestion (exploité ou protégé) et de la saison (été ou hiver).

**Tableau III.1** Impact du mode de gestion et de la période d'échantillonnage sur la richesse, diversité et taux de mycorhization des communautés de champignons EcMs associées au chêne-liège

	Nombre de MTs (Richesse) <sup>1</sup>	Shannon (diversité) <sup>2</sup>	Taux de mycorhization (%)
Gestion	Ns	ns	ns
Saison	***	***	*
Gestion × saison	**	*	***
site exploité - été	2,6 ± 1,4 a <sup>3</sup>	0,65 ± 0.42 ac	47,2 ± 19,7 a
site protégé – été	1,7 ± 0,7 a	0,41 ± 0.40 a	26,1 ± 10,9 b
site exploité - hiver	5,2 ± 2,5 b	0,98 ± 0.34 bc	20,9 ± 8,8 b
site protégé – hiver	7,4 ± 1,5 c	1,31 ± 0,25 b	34,4 ± 11,9 ab

<sup>1</sup> Les statistiques ont été effectuées en utilisant le test ANOVA 2-facteurs. '\*\*\*' indique  $P < 0,001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'ns'  $P > 0.05$ . Le test post-hoc Tukey's HSD (seuil de confiance de 95%) a été utilisé afin de comparer les différents sites. Les facteurs utilisés sont le mode de gestion (exploité ou protégé) et la saison d'échantillonnage (été et hiver). Les données dans une même colonne suivies d'une même lettre sont non significativement différentes selon le test Tukey HSD.

<sup>2</sup> L'indice de diversité shannon a été estimé à partir des données d'abondance de chaque morphotype après normalisation ( $N_{MT}/N_{total}$ ). N correspond au nombre d'apex EcM.

<sup>3</sup> Les valeurs indiquent la moyenne ± écart type de la moyenne.

**Table III.2.** Impact du mode de gestion de la subéraie (site exploité ou protégé) et de la période d'échantillonnage (été ou hiver) sur la structure des communautés de champignons EcMs (MT) associées au chêne-liège

Saison	Facteurs	Df	SS	MS	F. Model	R <sup>2</sup>	P-value <sup>1</sup>
Hiver-Eté	Gestion	1	1,3320	1,33197	10,4697	0,16628	0,001 ***
	Saison	1	1,0789	1,07888	8,4803	0,13468	0,001 ***
	Gestion × saison	1	1,0196	1,01962	8,0145	0,12729	0,001 ***
	Résidus	36	4,5800	0,12722		0,57175	
	Total	39	8,0105			1,00000	
Hiver	Gestion	1	0,1559	0,15590	1,3195	0,0683	0,28 <sup>ns</sup>
	Résidus	18	2,1267	0,11815		0,9317	
	Total	19	2,2826			1,00000	
Eté	Gestion	2	2,1957	2,19569	16,11	0,47229	0,001 ***
	Residuals	18	2,4533	0,13629		0,52771	
	Total	19	4,6490			1,00000	

<sup>2</sup> Les statistiques ont été effectuées en utilisant le test PerMANOVA ( $P < 0.05$ ). La dissimilarité entre les communautés de champignons EcMs a été mesurée en fonction de l'indice de Bray-Curtis calculé à partir de la matrice d'abondance des MTs. '\*\*\*' indique  $P < 0,001$ ; '\*\*'  $P < 0,01$ ; '\*'  $P < 0,05$ ; 'NS'  $P > 0,05$ .



**Table III.3.** Evaluation de la significativité d'association des champignons EcMs avec le mode de gestion ou la saison

Gestion / Saison	Taxa EcMs indicateurs <sup>1</sup>	A	B	IndVal.g	p-value <sup>2</sup>
Exploité	<b><i>Tomentella</i></b>	<b>0,8011</b>	<b>0,8500</b>	<b>0,825</b>	<b>0,002<sup>**</sup></b>
	<i>Tomentella</i> + <i>Scleroderma</i>	0,8395	0,5500	0,680	0,012 <sup>*</sup>
Protégé	<b><i>Pachyphloeus</i></b>	<b>0,8181</b>	<b>0,7500</b>	<b>0,783</b>	<b>0,049<sup>*</sup></b>
	<b><i>Cenococcum</i> + <i>Pachyphloeus</i></b>	<b>0,8109</b>	<b>0,7500</b>	<b>0,780</b>	<b>0,049<sup>*</sup></b>
	Heliales	0,8705	0,3000	0,511	0,049 <sup>*</sup>
	Heliales + <i>Cenococcum</i>	0,8705	0,3000	0,511	0,049 <sup>*</sup>
	<i>Russula</i> + Heliales	0,8691	0,3000	0,511	0,049 <sup>*</sup>
	<i>Scleroderma</i> + Heliales	0,9222	0,2500	0,480	0,049 <sup>*</sup>
Hiver	<b><i>Russula</i> + <i>Pachyphloeus</i></b>	<b>1,0000</b>	<b>0,8000</b>	<b>0,894</b>	<b>0,001<sup>***</sup></b>
	<b><i>Russula</i></b>	<b>0,8209</b>	<b>0,9000</b>	<b>0,860</b>	<b>0,001<sup>***</sup></b>
	<b><i>Russula</i> + <i>Cenococcum</i></b>	<b>0,8532</b>	<b>0,8500</b>	<b>0,852</b>	<b>0,001<sup>***</sup></b>
	<b><i>Tomentella</i> + <i>Pachyphloeus</i></b>	<b>1,0000</b>	<b>0,7000</b>	<b>0,837</b>	<b>0,001<sup>***</sup></b>
	<b><i>Russula</i> + <i>Tomentella</i></b>	<b>0,8547</b>	<b>0,7000</b>	<b>0,774</b>	<b>0,001<sup>***</sup></b>
	<i>Scleroderma</i> + <i>Pachyphloeus</i>	0,7886	0,6000	0,688	0,002 <sup>**</sup>
	<i>Russula</i> + <i>Scleroderma</i>	0,8188	0,5500	0,671	0,006 <sup>**</sup>
	Heliales	1,0000	0,4000	0,632	0,002 <sup>**</sup>
	<i>Russula</i> + Heliales	1,0000	0,4000	0,632	0,002 <sup>**</sup>
	Heliales + <i>Cenococcum</i>	1,0000	0,4000	0,632	0,002 <sup>**</sup>
	Heliales + <i>Pachyphloeus</i>	1,0000	0,4000	0,632	0,002 <sup>**</sup>
	<i>Tomentella</i> + Heliales	1,0000	0,3500	0,592	0,007 <sup>**</sup>
	<i>Scleroderma</i> + Heliales	1,0000	0,3000	0,548	0,013 <sup>*</sup>

<sup>1</sup> Les morphotypes (MTs) ont été regroupés par genre suite à l'assignation taxonomique détaillée dans la Figure 1. Les taxa EcMs ou combinaisons de taxa EcMs présents dans au moins 70% des échantillons d'un groupe donné (B > 0.7) sont indiqués en gras.

<sup>2</sup> Les statistiques ont été effectuées en utilisant la modèle *IndVal.g* (seuil de significativité à  $P < 0.05$ ). Les valeurs de  $P$ -value correspondent à la significativité pour chaque espèce indicatrice d'une condition donnée; '\*\*\*' indique  $P < 0,001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'NS'  $P > 0.05$ .

### III. Discussion

L'effet simultané des pressions croissantes de l'homme sur les écosystèmes forestiers méditerranéens et l'aggravation des conditions climatiques a entraîné un fort recul du couvert forestier, accentuant les phénomènes de désertification et de perte de biodiversité (Sala *et al.*, 2000; Allen *et al.*, 2010). La limitation de leur impact est fortement liée à notre capacité à prédire les changements en termes de fonctionnement. L'obtention de bio-indicateurs robustes apparaît comme une des clés pour la mise en place de stratégies de conservation adaptées. Dans le cas particulier des subéraies, les partenaires EcMs constituent des modèles de choix en raison de leur importance pour la croissance et nutrition du chêne-liège (Sebastiana *et al.* 2013).

Dans la présente étude, l'analyse de sites forestiers dans la subéraie de la Maâmora, protégés (sans exploitation du chêne-liège et de son couvert) ou non (récolte du chêne-liège, coupe du bois et pâturage) et caractérisés au niveau de leur couvert forestier par de fortes différences de densité de peuplement, a permis de mettre en évidence l'impact du mode de gestion de la subéraie en fonction de la saison sur la communauté de champignons EcMs.

La saison a été le paramètre environnemental affectant le plus fortement la communauté de champignons EcMs, confirmant de précédentes observations obtenues dans différents écosystèmes forestiers à base de chênes (de Román & de Miguel, 2005; Courty *et al.*, 2008; Richard *et al.*, 2011; Corcobado *et al.*, 2015), avec notamment l'augmentation de la richesse en hiver comparée à l'été (de Román & de Miguel, 2005; Courty *et al.*, 2008; Corcobado *et al.*, 2015; Gauquelin *et al.*, 2016a). Néanmoins, l'effet de la saison sur les communautés de champignons EcMs est variable suivant les années et les taxa EcMs (de Román & de Miguel, 2005; Courty *et al.*, 2008; Corcobado *et al.*, 2015; Gauquelin *et al.*, 2016b), limitant les comparaisons entre études, et soulignant la nécessité de mettre en place des suivis sur le long terme de parcelles forestières témoins (Gauquelin *et al.*, 2016b).

L'impact du mode de gestion sur la communauté de champignons EcMs était fortement dépendant de la saison, notamment sur la structure de la communauté EcMs et sur le taux de mycorhization des chênes lièges en été. Ces résultats renforcent l'hypothèse de l'action d'une communauté de champignons EcMs spécifique adaptée à des conditions hydriques défavorables en réponse à des perturbations (de Román & de Miguel, 2005; Azul *et al.*, 2010; Buscardo *et al.*, 2010), et soulignent l'importance d'un suivi de la communauté de champignons EcMs pendant des épisodes de sécheresses afin de mieux prédire les impacts des changements globaux (Azul *et al.*, 2010; Allen *et al.*, 2010; Richard *et al.*, 2011).

La recherche de champignons EcMs indicateurs d'une perturbation est un enjeu majeur pour améliorer la durabilité des subéraies méditerranéennes face aux changements globaux. La pertinence du ratio Ascomycètes/Basidiomycètes comme bio-indicateurs pour suivre l'impact de pratiques culturales sur la communauté fongique totale du sol a ainsi été évaluée (Orgiazzi *et al.*, 2012). Cependant, la réponse des communautés de champignons EcMs à des perturbations montre de grandes variabilités suivant les taxa (Richard *et al.*, 2011; Corcobado *et al.*, 2015; Pena *et al.*, 2016), limitant la pertinence d'indicateurs fongiques globaux. Dans la présente étude, quatre champignons EcMs (*Cenococcum*, *Pachyphloeus*, *Tomentella*, *Russula*) présentant des types d'exploration relativement peu développés (court ou par contact) (Agerer, 2001), ont été significativement associés, seuls ou en combinaisons, à un mode de gestion et/ou à une saison. Les champignons EcMs affiliés à *Scleroderma* et présentant un type d'exploration très développé ont quant à eux montré une faible association avec le mode de gestion ou la saison. Les champignons EcMs caractérisés par des types d'exploration peu

développés ont été principalement associés à des forêts présentant des densités racinaires élevées (Peay *et al.*, 2011).

*Cenococcum* est un des champignons EcMs les plus abondants parmi la communauté de champignons EcMs associée au chêne-liège, corroborant de précédentes observations dans les chênaies Méditerranéennes (Abourouh, 1991; Richard *et al.*, 2005; Azul *et al.*, 2010; Lancellotti & Franceschini, 2013). Son statut de généraliste dans le fonctionnement biologique des écosystèmes est souvent discuté en raison de son ubiquité (Peter *et al.*, 2016), mais aucun signal écologique robuste n'a été mis en évidence dans les subéraies (Azul *et al.*, 2010; Corcobado *et al.*, 2015). Dans la présente étude, *Cenococcum* seul n'a pu être associé à un mode de gestion ou une saison, mais sa coprésence avec d'autres champignons EcMs (*Pachyphloeus* ou *Russula*) apparaît indicatrice du site non perturbé présentant une forte densité d'arbre, ainsi qu'à la période hivernale. Ce résultat peut apparaître surprenant puisque *Cenococcum* a généralement été étudié en raison de ses propriétés de résistance à la chaleur et à la sécheresse, et de son rôle significatif dans la résistance des plantes aux contraintes hydriques (LoBuglio, 1999; di Pietro *et al.*, 2007). Ce paradoxe pourrait être dû à la forte diversité intraspécifique et donc potentiellement fonctionnelle de *Cenococcum* (Jany *et al.*, 2002; Douhan *et al.*, 2007; Bahram *et al.*, 2011) conduisant à un signal écologique complexe de *Cenococcum* dépendant de la diversité des individus au sein de la communauté de champignons EcMs.

A contrario, deux types de champignons EcMs, *Tomentella* et *Pachyphloeus*, étaient significativement associés au mode de gestion. Le genre *Pachyphloeus* appartenant aux Pezizales, est très peu documenté dans la littérature. Ce champignon hypogé est retrouvé dans les chênaies mais rarement détecté sur les racines (Smith *et al.*, 2007). Les Pezizales hypogés sont décrits comme prédominants parmi la composante fongique résistante du sol (spores et propagules) au sein des forêts matures (Tedersoo *et al.*, 2006), corroborant sa prédominance dans le site non perturbé. Le genre *Tomentella* (Thelephoraceae) est le genre le plus abondant dans les parcelles d'étude après *Cenococcum*. La prédominance des *Tomentella* a été mise en évidence dans différentes subéraies (Azul *et al.*, 2010; Lancellotti & Franceschini, 2013), avec de forte fluctuation géographique au sein de subéraies, mais aucun signal écologique spécifique n'avait été mis en évidence (Azul *et al.*, 2010).

Les variations saisonnières sont le facteur ayant impacté le plus largement la communauté de champignons EcMs, notamment *Russula*. Le potentiel rôle de *Russula* dans les subéraies avait été suggéré au regard du syndrome du déclin, entraînant entre autres une augmentation de la mortalité des chênes et une diminution de leur aire de distribution (Camilo-Alves *et al.*, 2013; Corcobado *et al.*, 2015), mais ses variations en fonction des saisons et donc des fluctuations de ressources en eau n'étaient pas très significatifs (Courty *et al.*, 2008). Son importance pour la résistance des chênes à des conditions de sécheresse avait été abordée (Azul *et al.*, 2010), mais cette dernière étude n'était basée que sur la forte abondance de *Russula* pendant la période estivale sans comparaison la période hivernale. *Tomentella* présentait quant à lui un lien plus évident avec les variations saisonnières et sa prédominance en hiver (Courty *et al.*, 2008). Dans la présente étude, le statut de *Tomentella* au regard de la saison n'était significatif que lorsque *Tomentella* était associé avec *Russula*.

Ces travaux confirment les impacts du mode de gestion sur la composante ectomycorhizienne associée au chêne-liège et leur potentiel accentuation suivant les conditions climatiques (saison). La saison estivale apparaît comme la période la plus critique en termes de changements de la communauté de champignons EcMs en réponse à une forte perturbation du couvert forestier (site exploité versus site protégé, faible densité de peuplement vs forte densité). Cette étude a aussi permis de préciser notre perception du rôle écologique de

champignons EcMs prédominants dans les subéraies (*Cenococcum*, *Tomentella* et *Russula*) ainsi que la mise en lumière de groupes sous-estimés (*Pachyphloeus*). Leur potentiel statut de bio-indicateurs d'une perturbation environnementale renforce la pertinence d'intégrer la diversité ectomycorhizienne dans le développement de modèles écologiques prédictifs afin de mieux évaluer et répondre à l'impact des changements globaux sur les subéraies méditerranéennes.

## **Chapitre IV**

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**Caractérisation d'indicateurs biologiques  
du fonctionnement des subéraies  
marocaines basée sur les interactions  
plante-champignon**

## Résumé

Les écosystèmes forestiers, notamment les subéraies, ont depuis longtemps été façonnés par les activités humaines. L'aggravation des pressions humaines combinée aux changements climatiques récents accentue les phénomènes de dégradation de certains écosystèmes forestiers, notamment la subéraie marocaine. Ces phénomènes de dégradation se traduisent par une altération de nombreuses composantes écologiques de la subéraie, comme les communautés fongiques du sol. Les champignons du sol, et particulièrement mycorhiziens, jouent un rôle important dans la résilience des peuplements forestiers par leur action sur les cycles biogéochimiques des nutriments mais aussi sur la dynamique de la strate végétale. Les champignons mycorhiziens sont notamment des acteurs majeurs de la mise en place des processus de facilitation entre plantes, processus clés dans l'établissement de la succession végétale et donc de la durabilité des écosystèmes forestiers. Les réseaux d'interactions des plantes avec leur communauté fongique apparaissent donc comme des indicateurs biologiques prometteurs pour suivre la santé des subéraies, ainsi que prédire les impacts futurs des pressions humaines et climatiques sur ces écosystèmes emblématiques du bassin méditerranéen. Ce dernier chapitre a pour objectif (i) la caractérisation de l'impact de la dégradation de la subéraie marocaine sur la communauté fongique du sol associée au chêne-liège et à différentes espèces arbustives représentatives de la subéraie (*Cistus salviifolius*, *Cistus monspeliensis*, *Lavandula stoechas*) ; (ii) l'identification de champignons indicateurs de la dégradation de la subéraie marocaine en fonction du type de plante auquel ils sont associés. Les trois habitats étudiés dans le chapitre II, Maâmora, Benslimane et Chefchaouen, ont été choisis comme modèle d'étude avec la définition pour chacun des habitats de zones dégradées et non dégradées. Les zones dégradées ont été sélectionnées selon différents critères, principalement, (i) une diminution de la densité du peuplement de chêne-liège et leur vigueur, (ii) la présence de clairières envahies par des plantes héliophiles remplaçant le sous-bois (e.g. palmier nain, asphodèles, cistes), et (iii) la présence de coupes anarchiques du bois. La caractérisation de la communauté fongique a été effectuée en utilisant les nouvelles technologies de séquençage à haut débit « Illumina MiSeq » et en ciblant soit la communauté fongique totale ou plus spécifiquement les champignons ectomycorhiziens et mycorhiziens à arbuscules. Les résultats obtenus ont permis de préciser l'impact de la dégradation de la subéraie marocaine sur la structure de la communauté fongique. Une large gamme d'indicateurs fongiques (421) spécifiques d'une ou plusieurs plantes et associée à un état de santé donné de la subéraie (dégradé ou non dégradé) ont pu être identifiée, dont plusieurs champignons ectomycorhiziens (*Terfezia*, *Tricholoma*, *Lactarius*, *Russula*, *Amanita*, *Tomentella*), mycorhiziens à arbuscules (*Paraglomus*, *Redeckera*, *Racocetra*) et non-mycorhiziens (*Cadophora*). Ces résultats soulignent la nécessité de prendre en compte les interactions plante-champignon dans le développement des stratégies de conservation des sols et écosystèmes forestiers, et mettent en exergue le potentiel de plusieurs taxa fongiques comme bio-indicateurs de la santé de la subéraie.

Le chapitre fera l'objet d'un article pour une soumission dans le journal *New Phytologist* :

**F.Z. Maghnia**, F. Mahé, P. Tisseyre, E. Tournier, B. Kerdouh, M. Ouadji, S.E. Bakkali Yakhlef, Y. Prin, N. El Ghachtouli, R. Duponnois, Y. Abbas, H. Sanguin, 2017. Deciphering bioindicators of the Moroccan using a plant-fungal network strategy.

**Mots clés** : Dégradation, subéraie, réseaux, champignons mycorhiziens, Illumina MiSeq.



## Introduction

Long-term land use practices have strongly shaped plant communities in Mediterranean forest ecosystems (Blondel *et al.*, 2010), and new risks emerge with climate change (Allen *et al.*, 2010). Cork oak (*Quercus suber*) forests, emblematic component of Western Mediterranean forests (more than 20,000 square kilometers), are particularly impacted by human management and climate changes (Acácio & Holmgren, 2014; Gauquelin *et al.*, 2016b). The consequences are an alteration of multiple ecological components of cork oak ecosystems, e.g. plant cover and tree density (Costa *et al.*, 2010; Acácio & Holmgren, 2014; Ibáñez *et al.*, 2015b), but also soil microbiome (Azul *et al.*, 2010; Costa *et al.*, 2013; Lancellotti & Franceschini, 2013; Bevivino *et al.*, 2014), leading to a global decrease of *Q. suber* regeneration rates in Mediterranean basin (Ajbilou *et al.*, 2006; Aafi, 2007; Curt *et al.*, 2009).

The status of *Q. suber* itself directly affects regeneration rates (Aafi, 2007; Acácio *et al.*, 2007), but indirect factors were shown to be strong drivers of natural regeneration, notably the characteristics of understory shrub vegetation (Pons & Pausas, 2006; Pausas *et al.*, 2006; Acácio *et al.*, 2007; Plieninger *et al.*, 2010; Ibáñez *et al.*, 2015b). Cork oak ecosystems are characterized by a rich diversity of understory shrub vegetation species, e.g. *Cistus monspeliensis*, *Cistus salviifolius*, *Pistacia lentiscus*, *Myrtus communis*, *Lavandula stoechas*, *Arbutus unedo* and *Erica arborea* (Carrión *et al.*, 2000; Aafi *et al.*, 2005; Boudiaf *et al.*, 2013) and shrub species-dependant impacts on *Q. suber* recruitment and survival were observed. For instance, *Cistus salviifolius* (Pérez-Devesa *et al.*, 2008) and *Cistus ladanifer* (Acácio *et al.*, 2007) were shown to negatively impact *Q. suber* seedling survival whereas *Erica arborea* was positively correlated (Acácio *et al.*, 2007; Pérez-Devesa *et al.*, 2008). Cork oak ecosystem sustainability is thus highly dependent of plant-plant interactions (facilitation and competition processes) taking place in cork oak ecosystems.

Beneficial plant-plant interactions were shown to be mediated through soil microbiome, notably mycorrhiza (Pugnaire, 2010; Montesinos-Navarro *et al.*, 2012a; Duponnois *et al.*, 2013). Soil microbiome plays indeed a central role in forest ecosystem functioning by improving nutrient cycling, plant nutrition and plant health (Uroz *et al.*, 2016; Baldrian, 2017; Lladó *et al.*, 2017), and fungi represent the most investigated soil microbiome compartments in forest ecosystems (Uroz *et al.*, 2016; Baldrian, 2017) highlighting the significance of mycorrhizal fungi in forest ecosystem functioning (Courty *et al.*, 2010; Davison *et al.*, 2011; Leopold, 2016). Consequently, soil fungi have been proposed as biological indicators for the development of policy directives aiming a better management and conservation of soils and forest ecosystems (Ritz *et al.*, 2009; Gao *et al.*, 2015a). The determination and use of suitable fungal indicators related to cork oak forest degradation may thus represent a major advance to monitor cork oak ecosystem health, to predict the impact of future human- and climate pressures and finally to improve the cork oak ecosystem sustainability. However, several fungal indicators were assessed in a limited range of environments or lacked of resolution (Ritz *et al.*, 2009), making extrapolations in various ecological conditions difficult.

The main goals of the present study are (i) to characterize in depth the molecular diversity of root-associated fungal community of *Quercus suber* and understory vegetation (*Cistus monspeliensis*, *Cistus salviifolius*, *Lavandula stoechas*) and (ii) to determine multiple plant-fungal indicators of cork oak forest degradation relying on fungal community diversity and structure data of different fungal guilds (Total fungal community, ectomycorrhizal (EcM) fungi, non-EcM fungi, and arbuscular mycorrhizal (AM) fungi).



# I. Material and methods

## 1. Study site and sampling

The study was conducted in three habitats of the Moroccan cork oak forest, located in the Moroccan Northern Mountains known as “Chefchaoun” (35°15'5.14"N 005°30'6.68W, 1534 m elevation), and in the lowland bordering the Atlantic Ocean (North-West of Morocco) known as “Maâmora” (34°17'06.186"N 6°28'30.792"W, 27 m elevation) and “Benslimane” (33°41'9.85"N, 6°54'7.26"W; 326 m elevation). The three habitats are under a Mediterranean-type climate characterized by hot and dry summers and mild and wet winters. They are characterized by an abundant understory, notably *Cistus salviifolius*, *Lavandula stoechas*, and *Thymeleae lathyroides* for Maâmora, and *Arbutus unedo*, and *Pistacia lentiscus* for Benslimane, and *Erica arborea* and *Arbutus unedo* for Chefchaoun. The sampling design was based on the selection of six plots per habitat spaced 100 meters apart, three plots in a degraded (D) area and three in a non-degraded (ND) area. For each plot, three trees (*Q. suber*) at least 20-30 meters apart were sampled, as well as understory shrub vegetation (*Cistus monspeliensis*, *Cistus salviifolius*, *Lavandula stoechas*) associated with each tree. Overall 144 cork oak trees and understory shrubs were sampled between February and June 2013 (*C. monspeliensis* has not been found in for Maâmora). For each plant, roots with soil were collected and stored at +4°C. The roots were rinsed under tap water to remove the non-adherent soil, dried and stored at -20°C. For ectomycorrhizal plants (*Q. suber* and *Cistus* spp.) roots were observed under a binocular microscope to select root zones rich in ectomycorrhiza (EcM) before drying. The product of this sampling process resulted in a fungal community including fungal endophytes, EcM fungi and fungi in adherent soil. This fungal community is hereafter named “total root-associated fungal community”. Soil physico-chemical parameters were measured at the LAMA Laboratory (Dakar, Senegal): pH, total nitrogen (N), total carbon (C), Carbon:Nitrogen ratio (C:N ratio), total and available phosphate (P), potassium cation ( $K^+$ ), magnesium cation ( $Mg^{2+}$ ), sodium ions ( $Na^+$ ), Cation exchange capacity (CEC). The habitats are managed by the High Commission for Water and Forests and Combatting Desertification. The permissions for root and soil sampling were provided by the Forestry Research Centre of Rabat (Morocco).

## 2. DNA extraction, PCR amplification and Illumina MiSeq sequencing

For each plant sample, all root pieces and the adherent soil were subjected to liquid nitrogen grinding for homogenization. The total DNA was extracted from a sub-sample (70 – 80 mg) using a FastPrep-24 homogenizer (MP biomedical Europe, Illkirch, France) and the FastDNA® SPIN kit (MP biomedical Europe) according to the manufacturer’s instructions. The purity of DNA extracts was improved by adding 20-30 mg Polyvinylpolypyrrolidone (PVPP) during the first step of DNA extraction in order to limit the presence of PCR inhibitors.

Two approaches were used to assess either the total root-associated fungal community (EcM and non-Ecm fungi) or more specifically the AM fungal community. For the total root-associated fungal community, the internal transcribed spacer ITS1 of the nuclear ribosomal RNA was amplified using the primers ITS1F12 and ITS2 (Schmidt *et al.*, 2013). For the AM fungal community, the 18S rRNA gene was amplified using the primers NS31 and AML2

(Simon *et al.*, 1992; Lee *et al.*, 2008). The amplification reaction was performed in a final volume of 25 µl with the corresponding primers (0.6 µM each), 2 µl of DNA extract, 200 µM of each dNTP, 200 ng/ml BSA, GoTaq® DNA Polymerase (2 units) and 1X Green GoTaq® Reaction Buffer (Promega, Charbonnières, France). The following cycling conditions were applied for (i) the total root-associated fungal community: 95°C for 15 min; 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 30 s; a final elongation step at 72°C for 5 min, and (ii) the AM fungal community: 94°C for 5 min; 30 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 80 s; a final elongation step at 72°C for 5 min. To increase richness recovery and to limit PCR biases, three to four PCR replicates per sample were pooled and purified using illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare Life Sciences, Velizy-Villacoublay, France) following manufacturer's guidelines. All amplicon products were subjected to paired-end Illumina MiSeq sequencing (2×300 bp) by Molecular Research LP (MR DNA, TX, USA).

### 3. Bioinformatic data processing

Paired Illumina MiSeq reads from the ITS dataset were assembled with vsearch v1.11.1 using the command `fastq_mergepairs` and the option `fastq_allowmergestagger`. For the 18S dataset, only reads corresponding to NS31 strands were considered because pairing was not possible due to the amplicon length. Demultiplexing and primer clipping was performed with `cutadapt` v1.9, enforcing a full-length match for sample tags and allowing a 2/3-length partial match for forward and reverse primers. For the ITS dataset, only reads containing both primers were retained. For each trimmed read, the expected error was estimated with vsearch's command `fastq_filter` and the option `eeout`. Each sample was then dereplicated, i.e. strictly identical reads were merged, using vsearch's command `derep_fulllength`, and converted to FASTA format.

To prepare the clustering, the samples were pooled and submitted to another round of dereplication with vsearch. Files containing expected error estimations were also dereplicated to retain only the lowest expected error for each unique sequence. Clustering was performed with `swarm` v2.1.8, using a local threshold of one difference and the `fastidious` option. Molecular operational taxonomic unit (OTU) representative sequences were then searched for chimeras with vsearch's command `uchime_denovo`. In parallel, representative sequences received taxonomical assignments using the `stampa` pipeline (<https://github.com/frederic-mahe/stampa>) and either a custom version of the fungal reference database UNITE v7 (<https://unite.ut.ee/>; (Kõljalg *et al.*, 2013)) for the ITS sequences or the PR2 database (gb203) for the 18S sequences. In brief, the `stampa` pipeline requires the reference sequences to be trimmed with `cutadapt`, using the same primers as those used for the amplification of the environmental sequences. Using vsearch's exact global pairwise comparisons, each environmental sequence is compared to all reference sequences and is assigned to the closest hit. In case of a tie, the environmental sequence is assigned to the last-common ancestor of the co-best hits. To improve the ITS taxonomic assignment from the fungal UNITE database, 20 sequences were added to identify the plants also amplified by our ITS primers. In addition, an OTU was considered to be ectomycorrhizal (EcM) if its closest reference belonged to known EcM lineages. In case of an OTU matching both EcM and non-EcM references, the latter was not considered as EcM.

For the 18S taxonomic assignment, the PR2 database was used to identify Glomeromycota sequences and exclude the others, but because the PR2 database is not relevant regarding lower taxonomic level for Glomeromycota, a reference sequence for each OTU was selected for taxonomic assignment with the 18S database from (Krüger *et al.*, 2012) using the *k*-

*nearest neighbor consensus* method (Wang *et al.*, 2007) implemented in Mothur software (*Classify.seqs* command). Clustering results, expected error values, taxonomic assignments and chimera detection results were used to build a raw OTU table. Up to that point, reads that could not be merged (not for 18S), reads without tags or primers, reads shorter than 32 nucleotides and reads with uncalled bases ("N") were eliminated. To create the "cleaned" OTU table, additional filters were applied to retain: non-chimeric OTUs, OTUs with an expected error divided by length below 0.0002, OTUs containing more than 3 reads or, if less, seen in two samples, OTUs assigned to plant or fungi taxa with at least 80% similarity or, if not, containing more than 10,000 reads. Raw data are available under the BioPproject ID PRJNA378471 (<https://www.ncbi.nlm.nih.gov/bioproject>).

## 4. Statistics

Diversity (Shannon, inverse Simpson [1/D]), richness (number of MOTUs, Chao1) and evenness (Pielou) indexes were estimated using the R package *vegan* and differences among cork oak habitats (Maâmora, Benslimane, Chefachaoun) and status (D, degraded and ND, non-degraded) were assessed by non-parametric permutational multivariate analysis of variance (PERMANOVA), as implemented in the *perm.anova()* function from the R package *RVAideMemoire*.

Fungal community structures combining the OTU distribution and the taxonomic information were visualized through barcharts, volcano plots and boxplots using the R package *ggplot2*. Differences in fungal community structure among cork oak habitats (Maâmora, Benslimane, Chefachaoun) and status (D and ND) was assessed using PERMANOVA in the *adonis()* function (McArdle & Anderson, 2001). Significance of differences between D and ND forest area regarding Ascomycota / Basidiomycota ratios were estimated using Mann-Whitney-Wilcoxon tests.

Fungal indicator OTUs associated with one or several plant types and a forest status (D and ND) were determined using the corrected indicator value index (IndVal.g), as implemented in *indicspecies'* *multipatt()*. Two different probabilities were calculated, *i.e.* A (specificity), representing the probability of a sample to be defined by a given plant × status interaction, given that the species have been detected, and B (sensitivity) representing the probability of finding the species in different samples characterized by a given plant × status interaction. Indicators OTUs showing both A (specificity) and B (fidelity) superior to 0.8 and 0.5 respectively, were considered as highly significant.

## II. Results

Two fungal datasets of 1,962,639 ITS sequences and 5,646,731 18S sequences were obtained from raw datasets (see material and methods for more details). The ITS and 18S datasets were rarefied down to 3,457 and 1,101 sequences per sample, respectively, to improve the robustness of fungal community comparisons among the Moroccan cork oak habitats.

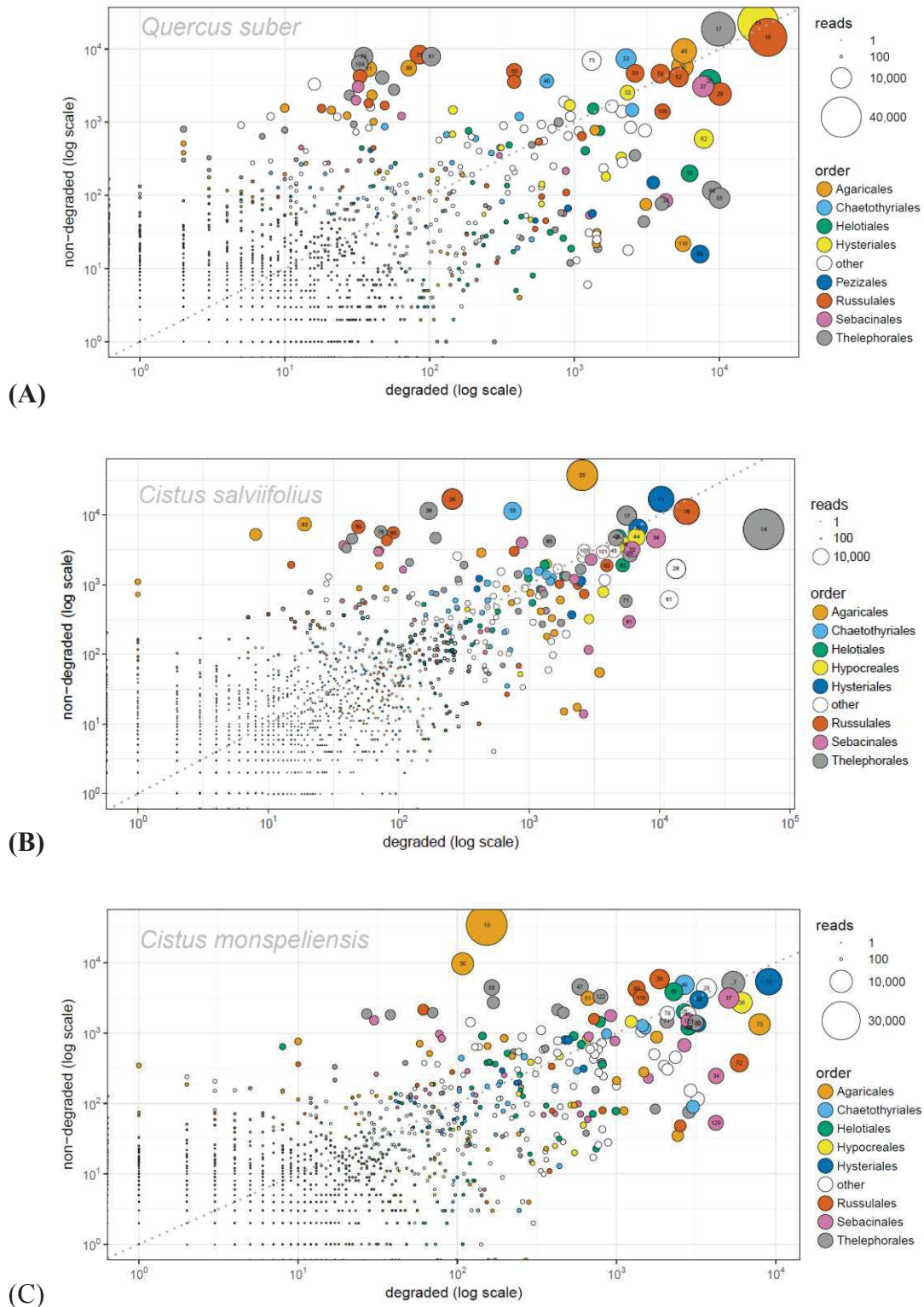
### 1. Fungal communities associated with cork oak and its understory vegetation

The total root-associated (ITS) fungal community (EcM and non-EcM) of *Q. suber* and understory shrubs (*C. salviifolius*, *C. monspeliensis*) was composed of 4,837 OTUs belonging to five known fungal phyla, 48 orders, 109 families, and 207 genera (**Table IV.S1, see the annexe**). Basidiomycota and Ascomycota represented 99% of total fungal abundance, Glomeromycota accounting for only 0.003%. The ten most dominant fungal orders were Thelephorales (20% of total fungal abundance), Russulales (12%), Agaricales (11%), Hysteriales (10%), Sebaciales (9%), Heliotales (8%), Chaetothyriales (6%), Pleosporales (4%), Hypocreales (4%), Pezizales (3%) (**Table IV.S1, see the annexe**). Differences in the most dominant orders were however observed among plants, i.e. Russulales > Thelephorales > Hysteriales > Agaricales for *Q. suber* (**Figure IV.1A**); Thelephorales > Russulales > Sebaciales > Agaricales for *C. salviifolius* (**Figure IV.1B**); Thelephorales > Sebaciales > Agaricales > Heliotales for *C. monspeliensis* (**Figure IV.1C**). The ten most dominant fungal genera were *Tomentella* (11%), *Cenococcum* (10%), *Russula* (6%), *Sebacina* (4%), *Ilyonectria* (2%), *Cortinarius* (2%), *Cladophialophora* (2%), *Inocybe* (2%), *Cryptosporiopsis* (2%), *Saccharicola* (1%), but more than 38 % of ITS sequences were not affiliated at the genus level (**Table IV.S1, see the annexe**). *Tomentella*, *Cenococcum* and *Russula* were also the most dominant genera considering each plant separately.

Fungal community richness, diversity, equitability and structure were significantly different among the habitat and plant types (**Table IV.1, Table IV.2**), but the differences among the plant types were dependent of habitats.

Although a low abundance of AM fungal community constituted the total root-associated fungal community of *Q. suber* and both *Cistus* species, an approach targeting specifically the AM fungi was applied because of their significance in ecosystem functioning, even for ectomycorrhizal plants (notably seedling establishment). The AM (18S) fungal community of *Q. suber* and understory shrubs (*C. salviifolius*, *C. monspeliensis* and *L. stoechas*) was composed of 1,750 OTUs belonging to ten known families, and 19 genera (**Table IV.S2, see the annexe**). Five families accounted for more than 90% of total AM fungal abundance, i.e. Glomeraceae (70%), Diversisporaceae (7%), Gigasporaceae (6%), Archaeosporaceae (6%) and Claroideoglomeraceae (5%). The five most dominant fungal genera were *Rhizophagus* (42%) (mostly *Rhizophagus clarus*, *Rhizophagus intraradices*, *Rhizophagus irregularis*), *Archaeospora* (6%), *Claroideoglosum* (5%), *Gigaspora* (5%), *Acaulospora* (4%). Twenty-one percent of OTUs were affiliated to references with uncertain position in Glomeraceae.





**Figure IV.1.** Distribution of root-associated (ITS) fungal OTUs of (A) *Quercus suber*, (B) *Cistus salviifolius* and (C) *Cistus monspeliensis* between non-degraded and degraded forest areas. All habitats (Maâmora, Benslimane, Chefchaoun) combined and rarefied by status (D vs ND). The color code indicates the taxonomic affiliation of OTUs at the order level. A dot represents an OTU, with the size of the dot representing the number of reads in the OTU.

**Table IV.1.** Richness, diversity and equitability of total root-associated fungal communities in the Moroccan cork oak forest

	MOTUs number (Richness)	Chao1 (Richness)	Shannon's index (diversity)	Inverse Simpson's index (diversity)	Pielou's index (Evenness)
Habitat	**	ns	***	***	***
Plant	***	**	***	***	**
Status	Ns	ns	Ns	ns	ns

<sup>1</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (999 iterations). The habitats (Maâmora, Benslimane, Chefchaoun), plants (*Q. suber*, *C. salviifolius*, *C. monspeliensis*) and status (degraded or non-degraded area) were used as factors. '\*\*\*' indicates  $P < 0,001$ ; '\*\*'  $P < 0,01$ ; '\*'  $P < 0,05$ ; 'ns'  $P > 0,05$ .

**Table IV.2.** Differences in the total root-associated fungal community structures<sup>1</sup> among habitats, plants and status

Model/Factors	Df	SS	MS	F. Model	R <sup>2</sup>	p-value <sup>2</sup>
PERMANOVA						
Habitat	2	6.004	3.002	8.649	0.108	0.001 ***
Plant	2	2.121	1.061	3.056	0.038	0.001 ***
Status	1	0.718	0.718	2.067	0.013	0.001 ***
Habitat : status	2	1.447	0.724	2.085	0.026	0.001 ***
Habitat : plant	3	1.864	0.621	1.790	0.033	0.001 ***
Plant : status	2	0.593	0.296	0.854	0.011	0.870 ns

Df, degrees of freedom ; SS, sum of squares ; MS, mean squares; F.Model, F value by permutation

<sup>1</sup> Permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis dissimilarity matrix

<sup>2</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (999 iterations). The habitats (Maâmora, Benslimane, Chefchaoun), plants (*Q. suber*, *C. salviifolius*, *C. monspeliensis*) and status (degraded or non-degraded area) were used as factors. '\*\*\*' indicates  $P < 0,001$ ; '\*\*'  $P < 0,01$ ; '\*'  $P < 0,05$ ; 'ns'  $P > 0,05$ .

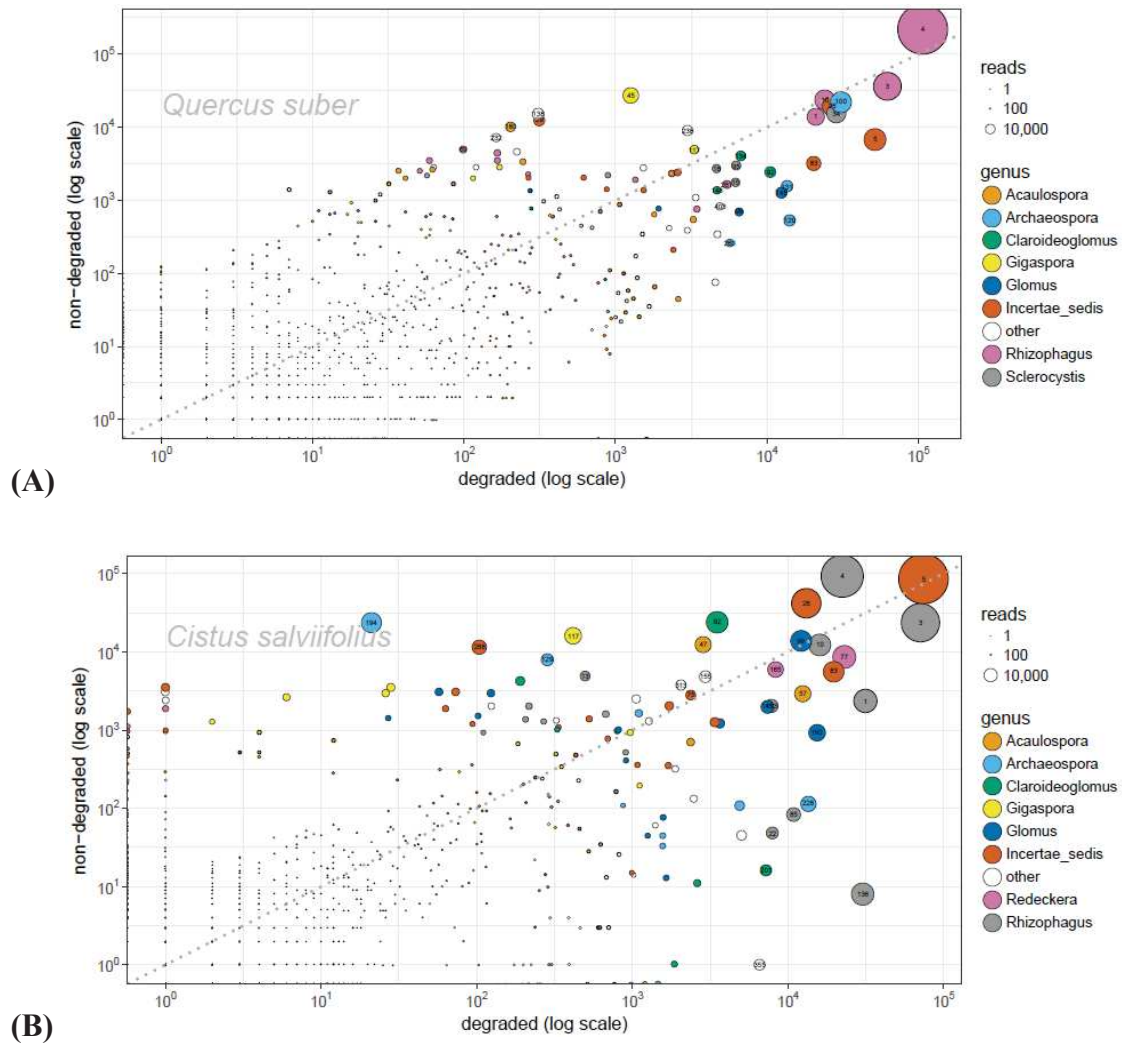
*Rhizophagus* and uncharacterized Glomeraceae strongly dominated the AM community of each plant but major differences among plants were observed considering the other predominant genera, i.e. *Archaeospora* and *Sclerocystis* for *Q. suber* (**Figure 2A**); *Archaeospora*, *Glomus*, and *Sclerocystis* for *C. salviifolius* (**Figure 2B**); *Claroideoglomus*, *Diversispora*, and *Redeckera* for *C. monspeliensis* (**Figure 2C**); *Acaulospora*, *Gigaspora*, and *Archaeospora* for *L. stoechas* (**Figure 2D**). The AM fungal community richness, diversity, equitability were significantly different among the plant types (**Table 3**). By contrast, both the habitat and plant types significantly affected the AM fungal community structure, but differentially regarding the type of habitat (**Table 4**).

## 2. Impact of cork oak degradation on fungal communities

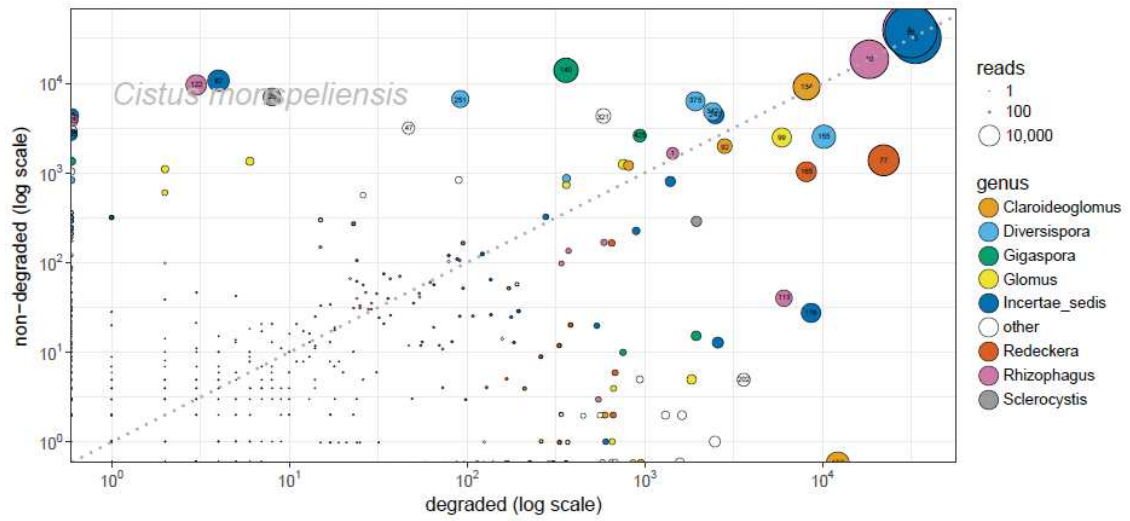
Cork oak degradation significantly impacted the total root-associated fungal community structures (**Table IV.2**), but not their richness, diversity, or equitability (**Table IV.1**). The impact of plant type (*Q. suber*, *C. salviifolius*, *C. monspeliensis* and *L. stoechas*) and forest status (degraded and non-degraded) on the total root-associated fungal community structures was significantly dependent on the type of habitats (Maâmora, Chefchaoun, Benslimane) (**Table IV.2**). By contrast, no global impact of cork oak degradation was observed on the AM fungal community structures (**Table IV.4**), neither on their richness, diversity, or equitability (**Table IV.3**). However, low but significant interactions were observed among the types of habitat and plant, and the forest status regarding the AM fungal community structures (**Table IV.4**).

The comparison of fungal OTU distribution regarding the forest status revealed major differences among plant types. For the total root-associated fungal community of *Q. suber* (**Figure IV.1A**), differences between non-degraded and degraded forest areas were mainly due to the predominance of OTUs affiliated to Telephorales, Russulales and Agaricales, and OTUs affiliated to Telephorales, Agaricales and Pezizales, respectively. For *C. salviifolius* (**Figure IV.1B**), a similar trend was observed regarding the non-degraded forest areas whereas the degraded forest areas were characterized by the predominance of Agaricales and Sebaciniales OTUs, but with low abundant OTUs in both cases. For *C. monspeliensis* (**Figure IV.1C**), two Agaricales OTUs were highly predominant in the non-degraded forest areas, whereas no clear signal was observed for the degraded areas. For the AM fungal community, the major differences in OTU distribution were observed for the two *Cistus* species (**Figure IV.2B and IV.2C**). *Cistus salviifolius* was mainly characterized by the predominance of OTUs affiliated to *Archaeospora*, *Gigaspora*, and uncharacterized Glomeraceae spp. in non-degraded forest areas and to *Archaeospora*, *Rhizophagus*, and *Claroideoglomus* in degraded forest areas (**Figure IV.2B**). *Cistus monspeliensis* was mainly characterized by the predominance of OTUs affiliated to *Gigaspora*, *Diversispora*, *Sclerocystis*, *Rhizophagus* and uncharacterized Glomeraceae spp. in non-degraded forest areas and *Claroideoglomus*, *Rhizophagus Redeckera*, *Gigaspora* and uncharacterized Glomeraceae spp. in degraded forest areas (**Figure IV.2C**).

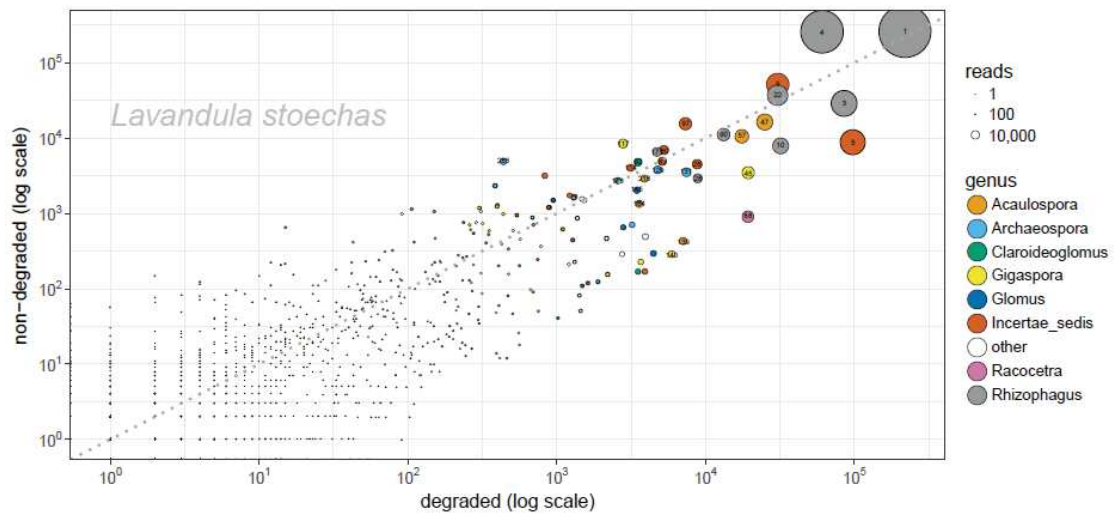




**Figure IV.2.** Distribution of AM (18S) fungal OTUs of (A) *Quercus suber*, (B) *Cistus salviifolius*, (C) *Cistus monspeliensis* and (D) *Lavandula stoechas* between non-degraded and degraded forest areas. All habitats (Maâmora, Benslimane, Chefchaoun) combined and rarefied by status (D vs ND). The color code indicates the taxonomic affiliation of OTUs at the order level. A dot represents an OTU, with the size of the dot representing the number of reads in the OTU.



(C)



(D)

Figure IV.2. Continued

**Table IV.3.** Richness, diversity and equitability of AM fungal communities in the Moroccan cork oak forest

	MOTUs number (Richness)	Chao1 (Richness)	Shannon's index (diversity)	Inverse Simpson's index (diversity)	Pielou's index (Evenness)
Habitat	ns	ns	ns	ns	ns
Plant	***	***	*	*	*
Status	ns	ns	ns	ns	ns

<sup>1</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (999 iterations). The habitats (Maâmora, Benslimane, Chefchaoun), plants (*Q. suber*, *C. salviifolius*, *C. monspeliensis*, *L. stoechas*) and status (degraded or non-degraded area) were used as factors. '\*\*\*' indicates  $P < 0,001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'ns'  $P > 0.05$ .

**Table IV.4.** Differences in the AM fungal community structures<sup>1</sup> among habitats, plants and status

Model/Factors	Df	SS	MS	F. Model	R <sup>2</sup>	p-value <sup>2</sup>
PERMANOVA						
Habitat	2	3.800	1.900	6.601	0.071	0.001 ***
Status	1	0.420	0.420	1.459	0.008	0.130 ns
Plant	3	5.432	1.811	6.290	0.101	0.001 ***
Habitat : status	2	1.285	0.642	2.232	0.024	0.001 ***
Habitat : plant	5	4.320	0.864	3.001	0.080	0.001 ***
Status : plant	3	1.225	0.408	1.419	0.023	0.039 *
Habita : status : plant	5	1.945	0.389	1.352	0.036	0.029 *

Df, degrees of freedom ; SS, sum of squares ; MS, mean squares; F.Model, F value by permutation

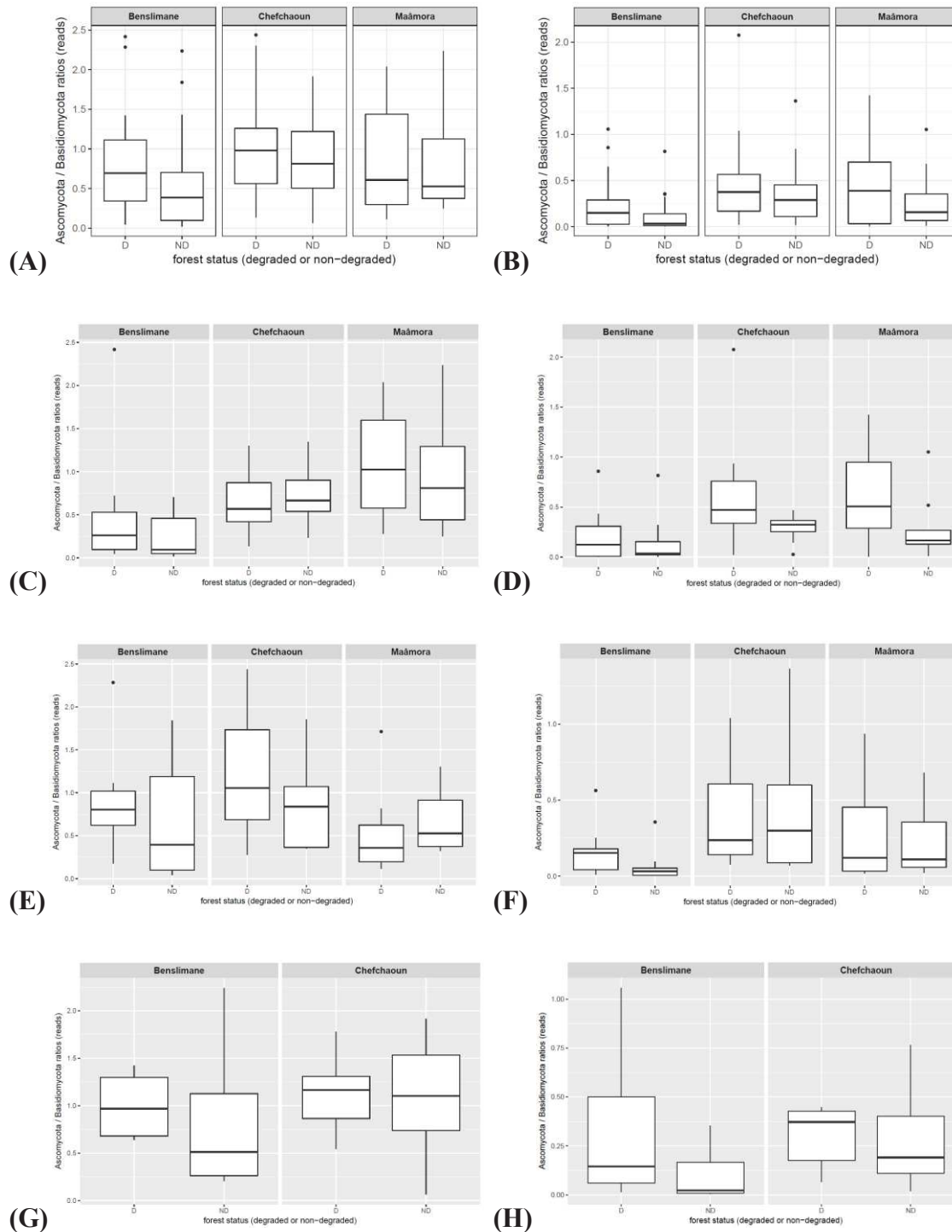
<sup>1</sup> Permutational multivariate analysis of variance (PERMANOVA) based on a Bray-Curtis dissimilarity matrix

<sup>2</sup> The significance of multivariate analysis of variance and dispersion was assessed with permutational test (999 iterations). The habitats (Maâmora, Benslimane, Chefchaoun), plants (*Q. suber*, *C. salviifolius*, *C. monspeliensis*) and status (degraded or non-degraded area) were used as factors. '\*\*\*' indicates  $P < 0,001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'ns'  $P > 0.05$ .

### 3. Identification of plant×status-based fungal indicators

Two main approaches, i.e. at the phylum level or OTU levels, were applied to characterize fungal indicators of cork degradation taking into account the specificity of plant-fungal interactions. The phylum-based approach consisted in the monitoring of Ascomycota / Basidiomycota ratio for the total root-associated fungal community in degraded and non-degraded forest areas. The degradation of the Moroccan cork oak forest tended to increase the Ascomycota / Basidiomycota ratio in all the habitats (Benslimane, Chefchaoun, Maâmora) (**Figure IV.3**), notably when EcM OTUs were considered rather than all OTUs constituting the total root-associated fungal community. The most significant differences were observed either when all plants (*Q. suber*, *C. salviifolius*, *C. monspeliensis*) were considered in Benslimane or when all plants were considered in all habitats together (**Figure IV.3A and IV.3B**, **Table IV.5**), and to a lesser extent either with *C. salviifolius* in Benslimane (**Figure IV.3E**, **Table IV.5**), or with *C. monspeliensis* considering all habitats together (**Figure IV.3H**, **Table IV.5**).

The OTU-based approach consisted in the determination of fungal indicator OTUs regarding a plant × status interaction matrix. A wide range of fungal indicator OTUs (358 out of 4,837 OTUs from the total root-associated fungal community) were associated with a given forest area status (degraded or non-degraded) regarding one to three plant types (**Figure IV.4**). In addition, 282 fungal indicator OTUs were associated with both forest status and one to two plant types (plant-specific OTUs) (**Figure IV.4**). The rest of fungal indicator OTUs provided non-informative biological signals. The most significant fungal indicator OTUs associated with the degraded status (Indval.g index;  $A > 0.8$ ;  $B > 0.5$ ;  $P < 0.01$ ) belonged mainly to Ascomycota, notably *Terfezia pini* (Pezizomycetes) (**Figure IV.5**). Non-degraded forest status was mainly associated with Basidiomycota, notably *Lactarius* sp. and *Tricholoma columbetta* (Agaromycetes) (**Figure IV.5**). For the AM fungal community, 144 fungal indicator OTUs out of 1,751 OTUs were identified. Sixty three fungal indicator OTUs were associated with a given forest area status (degraded or non-degraded) regarding one to three plant types (**Figure IV.6**), and 38 were associated with both forest status and one to three plant types (plant-specific OTUs) (**Figure IV.6**). The rest of fungal indicator OTUs provided non-informative biological signals. Only three fungal indicators OTUs were highly significantly (Indval.g index;  $A > 0.8$ ;  $B > 0.5$ ;  $P < 0.01$ ) associated with a non-degraded status (*Paraglomus brasilianum*) or a degraded status (*Redeckera fulva* and *Racocetra castanea*) (**Figure IV.6**).



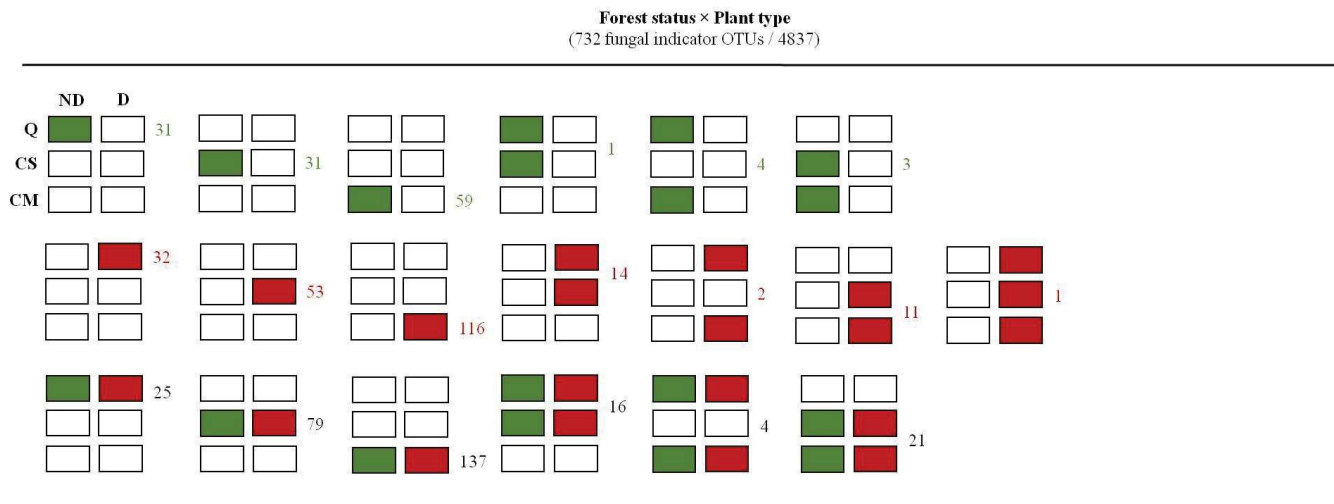
**Figure IV.3.** Distribution of Ascomycota / Basidiomycota ratio in the Moroccan cork oak forest according the type of habitat (Benslimane, Chefchaoun, Maâmora) and the forest status (D, degraded; ND, non-degraded) and the plant type. (A-B), all plants; (C-D), *Quercus suber*; (E-F), *Cistus salvifolius*; (G-H), *Cistus monspeliensis*. The ratios were estimated using the abundance of all OTUs from the total root-associated fungal community (A-C-E-G) or only EcM OTUs (B-D-F-H).

**Table IV.5.** Differences in the Ascomycota / Basidiomycota ratio in the Moroccan cork oak forest

	Benslimane		Chefchaoun		Maâmora		All habitats	
	D!=ND <sup>1</sup>	D > ND	D!=ND	D>ND	D!=ND	D>ND	D!=ND	D>ND
All plants <sup>all fungi, 2</sup>	0.048*	0.024*	ns	ns	ns	ns	0.032*	0.016*
All plants <sup>EcM</sup>	0.079 <sup>#</sup>	0.039*	ns	0.099 <sup>#</sup>	ns	ns	0.026*	0.013*
Q <sup>all fungi</sup>	ns	ns	ns	ns	ns	ns	ns	0.096 <sup>#</sup>
Q <sup>EcM</sup>	ns	ns	ns	ns	ns	ns	ns	ns
CS <sup>all fungi</sup>	0.093 <sup>#</sup>	0.047*	ns	ns	ns	ns	ns	ns
CS <sup>EcM</sup>	ns	0.095 <sup>#</sup>	ns	ns	ns	ns	ns	ns
CM <sup>all fungi</sup>	ns	ns	ns	ns	ns	ns	ns	ns
CM <sup>EcM</sup>	ns	0.060 <sup>#</sup>	ns	ns	-	-	0.068 <sup>#</sup>	0.034*

<sup>1</sup> Statistics were performed using Mann-Whitney-Wilcoxon tests (corrected for small dataset) based on bilateral (D != ND) and unilateral (D > ND) tests. D and ND indicate degraded and non-degraded forest area, respectively. ‘\*\*\*’ indicates  $P < 0.001$ ; ‘\*\*’  $P < 0.01$ ; ‘\*’  $P < 0.05$ ; ‘#’  $P < 0.1$ ; ‘ns’;  $P > 0.1$ .

<sup>2</sup> All plants indicate Q + CS + CM; Q, *Quercus suber*; CS, *Cistus salvifolius*; CM, *Cistus monspeliensis*. The ratios were estimated using the abundance of all OTUs from the total root-associated fungal community (named<sup>all fungi</sup>) or only EcM OTUs (named<sup>EcM</sup>).



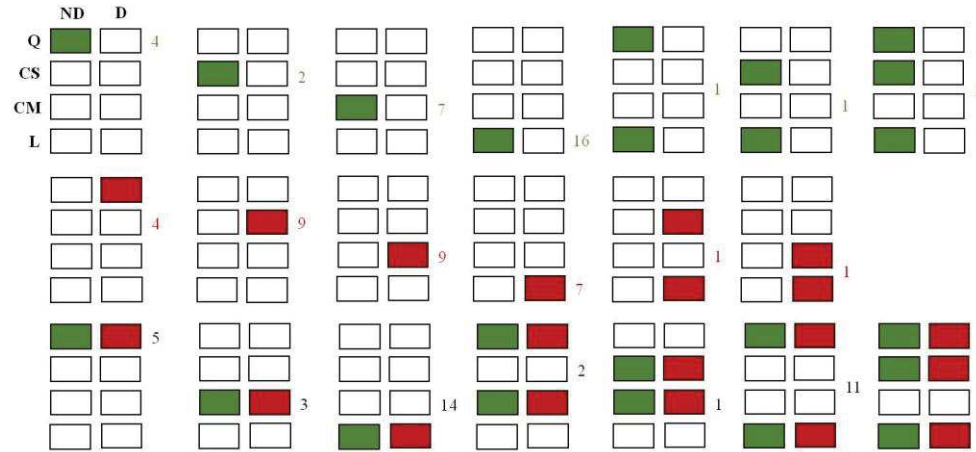
**Figure IV.4.** Fungal indicator OTUs from the total root-associated fungal community associated with plant types (Q, *Q. suber*; CS, *C. salviifolius*; CM, *C. monspeliensis*) and forest status (D, degraded; ND, non-degraded). Only fungal indicators associated with one to three plants and a given forest status or associated with both forest status and one to three plants are represented. Statistics were determined using the corrected indicator value index (“IndVal.g”) (R package indicpecies [De Caceres et al., 2010]). Fungal indicator OTUs associated with a degraded status are represented in red and a non-degraded status in green. The number at right of a plant × status matrix indicates the number of fungal indicator OTUs for the given matrix.



Taxonomic assignment	Fungal OTU (name)	Fungal abundance (number of sequences)	Plant type			Forest status	
			<i>Quercus suber</i>	<i>Cistus salviifolius</i>	<i>Cistus monspeliensis</i>	non-degraded	degraded
unidentified	Ascomycota sp	284	561				
		61	2245				
Pezizomycetes	<i>Terfezia pini</i>	410	644				
	Pyronemataceae sp	363	410				
Sordariomycetes	Nectriaceae sp	361	306				
Leotiomycetes	<i>Cadophora</i> sp	394	221				
		64	2278				
	Thelephoraceae sp	81	1844				
		214	509				
	<i>Tomentella</i> sp	464	304				
		210	504				
Agaricomycetes	<i>Tricholoma columbetta</i>	19	2834				
	<i>Lactarius</i> sp	60	2521				
	<i>Helvellosebacina</i> sp	165	1059				
	<i>Russula</i> sp	217	592				
	<i>Amanita torrendii</i>	180	1006				
	Agaricales sp	371	175				

**Figure IV.5.** Taxonomic affiliation of major fungal indicators OTUs from the total root-associated fungal community associated with plant types and forest status. Statistics were determined using the corrected indicator value index (“IndVal.g”) (R package indicpecies [De Caceres et al., 2010]). Only highly significant fungal indicators ( $A > 0.8$ ,  $B > 0.5$  and  $P < 0.01$ ) associated with one to three plants and a given forest status are indicated (black box). The taxonomic assignment is provided until species level, except for those with unidentified species (where the higher taxonomic level is indicated) (see Table IV.S1 for more details).

Forest status × Plant type  
(144 fungal indicator OTUs / 1751)



**Figure IV.6.** Fungal indicator OTUs from the AM fungal community associated with one to four plant types (Q, *Q. suber*; CS, *C. salviifolius*; CM, *C. monspeliensis*; L, *L. stoechas*) and forest status (D, degraded; ND, non-degraded). Only fungal indicators associated with one to four plants and a given forest status or associated with both forest status and one to four plants are represented. Statistics were determined using the corrected indicator value index (“IndVal.g”) (R package indicpecies [De Caceres et al., 2010]). Fungal indicator OTUs associated with a degraded status are represented in red and a non-degraded status in green. The number at right of a plant × status matrix indicates the number of fungal indicator OTUs for the given matrix.

Taxonomic assignment	Fungal OTU (name)	Fungal abundance (number of sequences)	Plant type				Forest status	
			<i>Quercus suber</i>	<i>Cistus salvifolius</i>	<i>Cistus monspeliensis</i>	<i>Lavandula stoechas</i>	non-degraded	degraded
Paraglomeraceae	<i>Paraglomus brasilianum</i>	138	409					
Diversisporaceae	<i>Redeckera fulva</i>	2825	11					
Gigasporaceae	<i>Racocetra castanea</i>	88	726					

**Figure IV.7.** Taxonomic affiliation of major fungal indicators OTUs from the AM fungal community associated with plant types and forest status. Statistics were determined using the corrected indicator value index (“IndVal.g”) (R package indicpecies [De Caceres et al., 2010]). Only highly significant fungal indicators ( $A > 0.8$ ,  $B > 0.5$  and  $P < 0.01$ ) associated with one to three plants and a given forest status are indicated (black box). The taxonomic assignment is provided until species level, except for those with unidentified species (where the higher taxonomic level is indicated) (see Table IV.S2 for more details).

### III. Discussion

The monitoring of ecosystem health and functioning through biodiversity analysis has become a key issue for the development of efficient environmental directives. However, the determination of reliable biological indicators allowing the assessment of environmental changes and providing early warning signals of changes remains a challenging task. Due to their importance in ecosystem functioning, soil fungi have been proposed as pertinent biological indicators of soils and notably of forest ecosystems (Parmasto, 2001; Ritz *et al.*, 2009; Gao *et al.*, 2015a; Mair *et al.*, 2017). The current work focused on the determination of plant-fungal indicators related to cork oak forest degradation, a Mediterranean forest ecosystem highly threatened by human- and climate pressures (Acácio & Holmgren, 2014; Gauquelin *et al.*, 2016b).

Ascomycota / Basidiomycota ratios were previously used as fungal indicators to evaluate land-use impacts in Mediterranean landscapes, showing an increase of this ratio with the increase of land-use intensity (Orgiazzi *et al.*, 2012). These results are strongly in accordance with current results that demonstrated a significant increase of the Ascomycota / Basidiomycota ratio with cork oak ecosystem degradation. It is likely that the increase in the Ascomycota / Basidiomycota ratio may be linked to the decrease in EcM fungi, mostly belonging to Basidiomycota, associated with the decline tree-understory vegetation in degraded forest areas. These observations were however poorly supported at the habitat level (except for Benslimane), highlighting the necessity to increase the sampling effort for that indicator ratio in particular to better address the ecological issues of forest degradation. Remarkably, a higher significance of the Ascomycota / Basidiomycota ratio was observed when only EcM fungi were considered, probably due to the analysis of ectomycorrhizal plants. Several EcM ascomycota, notably truffle fungi (*Tuber*, *Terfezia*), are described as pioneer or early-successional species because of their tolerance to disturbances and consequently their ability to colonize disturbed areas (Peay *et al.*, 2011; Zambonelli & Bonito, 2012), and hence contributed to the increase of the Ascomycota / Basidiomycota in degraded forest areas. Low tree density, one of the criteria defining the degradation status in the current work, was also shown to favour early-successional species (Dickie & Reich, 2005; Peay *et al.*, 2011).

The determination of fungal indicators at the OTU levels confirmed the significant association of early-successional Ascomycota species (notably *Terfezia*) with a degraded forest status, and late-successional Basidiomycota (*Tricholoma* and *Lactarius*) with a non-degraded forest status. However, well-known late-successional fungi, i.e. *Russula* and *Amanita* (Last *et al.*, 1983), were surprisingly associated with the degraded status whereas *Tomentella*, considered as early-successional fungi, was associated to non-degraded status. These observations confirmed precedent results pointing out the limitations of interpretations based on ecological colonization patterns since they not refer to life history strategies, competition and intraspecific variability (Twieg *et al.*, 2007; Gao *et al.*, 2015b). In addition, it is likely that this inconsistency may be due to belowground history, i.e. the persistence of certain fungal species following a disturbance, rather than the specific colonization properties of other fungal species adapted to new ecological conditions (Twieg *et al.*, 2007). However, *Tomentella* has been also described as an excellent competitor in mature forest (Taylor & Bruns, 1999), which may be consistent with current results since *Tomentella* indicator OTUs colonized specifically pioneer understory vegetation (*Cistus* spp.) and not *Quercus* in the non-degraded habitats. Remarkably, a wood-decaying fungus, *Cadophora* (Travadon *et al.*, 2015), has been identified as one of the most significant fungal indicator associated with the degraded forest status. Wood-decaying fungi were proposed as major fungal indicator species due to their

association with natural old-forest (Lonsdale *et al.*, 2008), and recently used in climate change models (Mair *et al.*, 2017).

Because of the relative importance of AM symbiosis for EcM plant functioning (Egerton-Warburton & Allen, 2001; Egerton-Warburton *et al.*, 2007; Toju *et al.*, 2013b), AM fungal community associated with cork oak and its understory vegetation was assessed. Current results have confirmed the low percentage of AM fungal community in the total fungal community in EcM-dominated ecosystems (Orgiazzi *et al.*, 2012). Consequently, a lower number of AM fungal indicator OTUs associated with forest status were identified in comparison to the one with EcM fungi. AM fungal communities in the Moroccan cork oak forest was mainly composed of Glomerales, which is in accordance with previous investigations in Italian cork oak ecosystems (Lumini *et al.*, 2010). However, a higher diversity of AM fungal taxa was unraveled in the current study, with notably the detection of Paraglomerales. In Italian cork oak ecosystems, Paraglomerales were only detected in pasture (dominated by pasture or grass species with a low tree density) (Lumini *et al.*, 2010). AM fungal community was mainly impacted by the habitat and plant types, and weakly by the forest status, contrary to the survey in Italian cork oak ecosystems (Lumini *et al.*, 2010). However, the disturbance intensity in this latter study was much higher, i.e. from cork oak formation to tilled vineyard, which may explain the discrepancy in AM fungal community responses. Members of Diversisporales (*Redeckera* and *Racocetra*) were one of the most significant indicators of cork oak degraded status. Interestingly, Diversisporaceae were proposed as global indicator of disturbed habitats (Moora *et al.*, 2014). Nevertheless, the latter study was based on a disturbance scale ranging from primeval forest to intensive agricultural field, weakening the robustness of the comparison. The identification of AM fungal indicators of land-use intensity or ecosystem health is rather difficult and dependant of the diversity of land-use compared (Bouffaud *et al.*, 2016). The lack of biological signal of AM fungi regarding land-use intensity may be partly due to the strong impact of local soil characteristics, climatic effects and the nature of land-use (Thomson *et al.*, 2015).

Current results give evidences of the reliability of fungi, notably mycorrhiza, as biological indicators of cork oak degradation status, but also raise the fact that a unified framework for forest degradation status has to be made in order to develop efficient wide forest monitoring based on fungal indicator biodiversity and address the future challenge of forest conservation (Egli, 2011; Gao *et al.*, 2015a).

## Conclusion générale et Perspectives

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La conservation des écosystèmes forestiers est fortement menacée par les pressions humaines et climatiques croissantes. Les conséquences sont une accélération des processus de désertification, notamment dans les écosystèmes forestiers du sud du bassin méditerranéen. La subéraie marocaine est un exemple emblématique de la dégradation des écosystèmes forestiers (Gauquelin *et al.*, 2016a,b). Face à l'urgence, il est crucial de développer des stratégies de conservation adaptées et représentatives des écosystèmes dans leur ensemble, mais aussi de pouvoir prédire les changements à court et long terme qui seront induits par les pressions actuelles ou par de nouveaux modes de gestion. Un des enjeux majeurs pour atteindre ces différents objectifs sont l'amélioration de nos connaissances sur les composantes écologiques majeures à la base du fonctionnement des écosystèmes forestiers, et l'identification d'indicateurs permettant de suivre l'état de santé de ces écosystèmes (Ritz *et al.*, 2009; Gao *et al.*, 2015a).

C'est au carrefour de ces enjeux que s'est placé le présent travail, avec pour objectif la mise en lumière des communautés fongiques du sol, notamment des champignons mycorhiziens, et des interactions avec la strate végétale comme éléments clés pour la mise en place de nouvelles stratégies de conservation et de gestion de la subéraie marocaine.

Le présent travail représente une des études les plus complètes sur la diversité des champignons du sol au sein d'une subéraie, soulignant l'originalité des communautés fongiques de la subéraie marocaine au regard de celles identifiées dans les subérais européennes et plus largement dans les chênaies européennes (**chapitre II**). Les résultats démontrent la forte variabilité spatiale (liée aux types d'habitat) des communautés fongiques du sol associées au chêne-liège et à la strate arbustive des subérais (**chapitres II et IV**), s'expliquant notamment par la variabilité des caractéristiques physico-chimique du sol (**chapitre II**).

Le décryptage des réseaux fongiques associés au chêne-liège ainsi qu'à la strate arbustive de la subéraie (**chapitre II, III, IV**) a permis de déterminer une large gamme d'indicateurs fongiques potentiels du type d'habitat (**chapitre II**), du type de plante hôte (**chapitre IV**) et de l'état de santé de la subéraie (**chapitres III et IV**). Le lien entre plusieurs indicateurs avec leur fonction écologique (symbiotique, saprophytique, ...) et leur plante hôte (**chapitre III**) démontre la nécessité d'adopter des approches multicritères pour déterminer des indicateurs biologiques performants en adéquation avec la problématique écologique abordée.

L'importance écologique de plusieurs taxa fongiques dans la durabilité des subérais a ainsi pu être précisée ou démontrée (**chapitre II, III et IV**), principalement des champignons (i) ectomycorhiziens affiliés à *Amanita*, *Cenococcum*, *Lactarius*, *Pachyphloeus*, *Terfezia*, *Tomentella*, *Tricholoma* et *Russula*, (ii) ericoïdes affiliés à *Oidiodendron*, (iii) mycorhiziens à arbuscules affiliés à *Rhizophagus*, *Paraglomus*, *Redeckera* et *Racocetra*, mais aussi (iv) saprophytes affiliés à *Archaeorhizomyces*, *Cadophora* et *Cladophialophora*.

Néanmoins, une variabilité interspécifique plus ou moins forte au sein de différents taxa (**chapitre II, III, IV**) complexifie la mise en place de règles générales au niveau du genre, soulignant l'importance de développer des approches à différentes échelles, par exemple plus large avec la prise en compte du ratio Ascomycota / Basidiomycota (**chapitre IV**), ou plus précise avec la mise en place de suivi au niveau de l'individu (Jany *et al.*, 2002; Douhan & Rizzo, 2005; Beiler *et al.*, 2009; Ehinger *et al.*, 2012).

La liste d'indicateurs fongiques obtenue dans le cadre du présent travail constitue une base de données inédite pour mettre en place des recherches sur l'ensemble des subérais à l'échelle

méditerranéenne afin d'améliorer leur conservation. Les retombées sont aussi majeures pour la conservation des écosystèmes forestiers puisque ce travail propose des approches transposables à une grande diversité d'écosystèmes. De plus, les résultats mettent en exergue un signal biologique plus significatif des cortèges fongiques associés au couvert arbustif (*Cistus salviifolius*, *Cistus monspeliensis*, et *Lavandula stoechas*) que ceux associés au chêne-liège au regard de l'état de santé de la subéraie, démontrant la pertinence de l'approche développée dans le présent travail sur la prise en compte des interactions plante-champignon.

Les résultats prometteurs obtenus quant au décryptage des interactions plante-champignon au sein de la subéraie marocaine renforce la nécessité de valoriser ces interactions pour améliorer la conservation et la gestion de la subéraie. Cette valorisation est très peu mise en œuvre dans les programmes de réhabilitation des écosystèmes (**chapitre I**), alors que le rôle de des interactions plante-champignon dans les processus de facilitation plante-plante a été démontré dans plusieurs études (**chapitre I**), ces processus étant fortement impliqués dans la régénération des chênes-liège au sein des subéraies (Acácio *et al.*, 2007; Pérez-Devesa *et al.*, 2008; Curt *et al.*, 2009; Ibáñez *et al.*, 2015b).

La valorisation des interactions chêne-liège × champignons × couvert arbustif est donc un enjeu majeur pour la mise en place de stratégies en ingénierie écologique performantes afin de faire face aux problèmes de dégradation de la subéraie marocaine. Le suivi de différents indicateurs fongiques identifiés dans ce travail au sein d'expérimentations consistant dans l'évaluation de la survie et la régénération du chêne-liège, en fonction de différents couverts arbustifs installés dans des zones dégradées de la subéraie, pourrait alors servir de guide pour évaluer la performance des différents couverts. En parallèle, des systèmes plus simplifiés pourraient être mise en place avec l'utilisation comme inoculum de différents champignons mycorhiziens natifs révélés comme indicateurs de la bonne santé de la subéraie afin d'évaluer leur impact sur la régénération, survie et croissance de plants de chênes-liège. Ces deux approches complémentaires pourraient constituer le futur des programmes de réhabilitation de la subéraie marocaine et plus largement de nombreux écosystèmes forestiers



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## **Annexe**

L'annexe regroupe l'ensemble des tableaux et figures incluse comme matériel supplémentaire dans les articles qui constituent les chapitres II et IV.

En raison de la grande taille des tableaux Table II.S1, Table IV.S1 et Table IV.S2, seules les 50 OTUs les plus abondantes seront représentées. De même, seuls les 20 genres les plus abondants sont représentés dans la Figure II.S2

Listes des tableaux et figures par ordre d'apparition

Table II.S1, Table II.S2, Table II.S3, Table II.S4, Figure II.S1, Figure II.S2, Table IV.S1 et Table IV.S2

**Table II.S1.** Taxonomic affiliation of fungal OTUs associated with cork oak in the Moroccan habitats<sup>1</sup>

OTU <sup>2</sup>	reads	phylum	class	order	family	genus	species
11	9014	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	<i>Cenococcum</i>	<i>Cenococcum geophilum</i>
17 <sup>C:N</sup>	<b>5448</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Thelephorales</b>	<b>Thelephoraceae</b>	<b><i>Tomentella</i></b>	<b><i>Tomentella atramentaria</i></b>
16 <sup>C:N</sup>	<b>3230</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Russulales</b>	<b>Russulaceae</b>	<b>unidentified</b>	<b>Russulaceae sp</b>
51 <sup>pH</sup>	<b>2592</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Agaricales</b>	<b>Hygrophoraceae</b>	<b><i>Hygrophorus</i></b>	<b><i>Hygrophorus cossus</i></b>
49	2454	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	<i>Cortinarius</i>	<i>Cortinarius</i> sp
33 <sup>MA, C:N</sup>	<b>2014</b>	<b>Ascomycota</b>	<b>Eurotiomycetes</b>	<b>Chaetothyriales</b>	<b>Herpotrichiellaceae</b>	<b><i>Cladophialophora</i></b>	<b><i>Cladophialophora</i> sp</b>
79	1886	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	<i>Tomentella</i>	<i>Tomentella</i> sp
30	1843	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	<i>Inocybe</i>	<i>Inocybe subporospora</i>
81	1744	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
58	1594	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula</i> sp
26	1393	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp
98	1389	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	<i>Cortinarius</i>	<i>Cortinarius</i> sp
37	1386	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Sebacinaceae sp
25	1326	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula violeipes</i>
93	1315	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp
75 <sup>MA</sup>	<b>1270</b>	<b>Ascomycota</b>	<b>Dothideomycetes</b>	<b>Capnodiales</b>	<b>Mycosphaerellaceae</b>	<b>unidentified</b>	<b>unidentified</b>
60 <sup>C:N</sup>	<b>1260</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Russulales</b>	<b>Russulaceae</b>	<b><i>Lactarius</i></b>	<b><i>Lactarius</i> sp</b>
211	1244	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	<i>Inocybe</i>	<i>Inocybe posterula</i>
32 <sup>CH</sup>	<b>1117</b>	<b>Ascomycota</b>	<b>Dothideomycetes</b>	<b>Hysteriales</b>	<b>Gloniaceae</b>	<b><i>Cenococcum</i></b>	<b><i>Cenococcum geophilum</i></b>
104	1080	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
160	1041	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Sebacinaceae sp
38 <sup>C:N</sup>	<b>1024</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Thelephorales</b>	<b>Thelephoraceae</b>	<b>unidentified</b>	<b>Thelephoraceae sp</b>
315	995	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	<i>Inocybe</i>	<i>Inocybe</i> sp
138	967	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp
296	954	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	Sebacina	Sebacina sp
52	943	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula</i> sp
29	898	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula</i> sp
76	865	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	<i>Tomentella</i>	<i>Tomentella</i> sp src857
87	819	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	<i>Trichoderma</i>	<i>Trichoderma erinaceum</i>
124	744	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	<i>Cenococcum</i>	<i>Cenococcum geophilum</i>
85 <sup>pH</sup>	<b>736</b>	<b>Basidiomycota</b>	<b>Agaricomycetes</b>	<b>Thelephorales</b>	<b>Thelephoraceae</b>	<b><i>Tomentella</i></b>	<b><i>Tomentella</i> sp</b>
115	736	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Sebacinaceae sp
132	716	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula</i> sp
55	714	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
164	711	Basidiomycota	Agaricomycetes	Boletales	Boletaceae	<i>Boletus</i>	<i>Boletus subtomentosus</i>
100	687	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	<i>Russula</i>	<i>Russula odorata</i>

Table II.S1. Continued<sup>1</sup>

OTU <sup>2</sup>	reads	phylum	class	order	family	genus	species
276	678	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Entoloma	Entoloma sordidulum
46	578	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	unidentified	Herpotrichiellaceae sp
139	565	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	<i>Cenococcum</i>	Cenococcum geophilum
270	560	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Lactarius	Lactarius quietus
<b>95<sup>CH, C:N</sup></b>	<b>548</b>	<b>Ascomycota</b>	<b>Leotiomycetes</b>	<b>Helotiales</b>	<b>Dermateaceae</b>	<b><i>Cryptosporiopsis</i></b>	<b>Cryptosporiopsis brunnea</b>
<b>45<sup>pH</sup></b>	<b>540</b>	<b>Ascomycota</b>	<b>Dothideomycetes</b>	<b>Pleosporales</b>	<b>Lophiostomataceae</b>	<b><i>Lophiostoma</i></b>	<b><i>Lophiostoma cf cynaroidis</i> A33</b>
88	538	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp
36	530	Ascomycota	Sordariomycetes	Hypocreales	Incertae_sedis	<i>Ilyonectria</i>	<i>Ilyonectria estremocensis</i>
283	506	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe asterospora
195	504	Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Archaeorhizomyces	Archaeorhizomyces sp
<b>105<sup>MA</sup></b>	<b>472</b>	<b>Ascomycota</b>	<b>Leotiomycetes</b>	<b>Incertae_sedis</b>	<b>Myxotrichaceae</b>	<b><i>Oidiodendron</i></b>	<b>Oidiodendron maius</b>
268	469	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp
108	446	Ascomycota	Eurotiomycetes	Chaetothyriales	unidentified	unidentified	Chaetothyriales sp
92	427	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp

<sup>1</sup> only the 50 most dominant OTUs out of 1768 are shown

<sup>2</sup> all significant fungal indicator species are in bold (see Table II.4 and II.S4 for details)

**Table II.S2.** Fungal indicators of habitat (Maâmora, Benslimane and Chefchaoun)

Fungal OTU (Taxonomic assignment)	Maâmora <sup>1</sup> (40)	Benslimane (21)	Chefchaoun (49)
<b>72-58 (<i>Russula</i> sp), 246 (<i>Cladophialophora</i> sp), 366-407-141 (<i>Sordariomycetes</i> sp),</b>	*** <sup>2</sup>		
<b>75 (<i>Mycosphaerellaceae</i> sp), 105 (<i>Oidiodendron maius</i>),</b> 16-138 ( <i>Russulaceae</i> sp), 46-97 ( <i>Herpotrichiellaceae</i> sp), 243 ( <i>Capnodiales</i> sp)			
<b>33-1241 (<i>Cladophialophora</i> sp), 1937 (<i>Chaetosphaeriales</i> sp),</b>	**		
<b>82 (<i>Aspergillus amstelodami</i>), 236 (<i>Archaeorhizomyces</i> sp), 999 (<i>Oidiodendron</i> sp),</b> 593 ( <i>Herpotrichiellaceae</i> sp), 1674 ( <i>Fungi</i> sp), 403 ( <i>Valsaceae</i> sp), 550 ( <i>Rasamsonia</i> sp), 359 ( <i>Cryptosporiopsis</i> sp)			
2013 ( <i>Sordariomycetes</i> sp), 2073 ( <i>Hansfordia</i> sp), 1064 ( <i>Penicillium adametzii</i> ),	*		
277-242-92 ( <i>Russulaceae</i> sp), 198 ( <i>Chaetothyriales</i> sp), 1577 ( <i>Archaeorhizomyces</i> sp), 106 ( <i>Penicillium nodositatum</i> ), 656 ( <i>Dothideomycetes</i> sp), 453 ( <i>Oidiodendron griseum</i> ), 351 ( <i>Cladophialophora</i> sp), 405-1188 ( <i>Herpotrichiellaceae</i> sp), 784 ( <i>Lactarius</i> sp), 318 ( <i>Trechispora</i> sp)			
<b>759-69 (<i>Ilyonectria mors-panacis</i>), 1190 (<i>Dothideomycetes</i> sp),</b> 17 ( <i>Tomentella atramentaria</i> ), 66 ( <i>Tomentella</i> sp)		**	
67-358 ( <i>Tomentella</i> sp), 928 ( <i>Sordariales</i> sp), 187-582 ( <i>Ascomycota</i> sp),		*	
949 ( <i>Ilyonectria mors-panacis</i> ), 36 ( <i>Ilyonectria estremocensis</i> ), 1114 ( <i>Mortierella amoeboidea</i> ), 184-98-989 ( <i>Cortinarius</i> sp), 536-390 ( <i>Tomentella atramentaria</i> ), 28 ( <i>Saccharicola</i> sp), 38 ( <i>Thelephoraceae</i> sp), 315 ( <i>Inocybe</i> sp)			

**Table II.S2.** Continued

Fungal OTU (Taxonomic assignment)	Maâmora <sup>1</sup> (40)	Benslimane (21)	Chefchaoun (49)
<b>32-362 (<i>Cenococcum geophilum</i>), 292 (Capnodiales sp), 112 (<i>Cladophialophora</i> sp), 492 (Chaetothyriales sp), 78 (Dothideomycetes sp), 572 (Sebacinales Group B sp), 95 (<i>Cryptosporiopsis brunnea</i>)</b>			***
<b>1194 (<i>Cladophialophora</i> sp), 56 (<i>Cenococcum geophilum</i>), 610 (<i>Sarcoleotia globosa</i>), 254-59-413 (<i>Cryptosporiopsis brunnea</i>), 1137 (<i>Cladophialophora chaetospira</i>), 51 (<i>Hygrophorus cossus</i>), 298 (<i>Tuber</i> sp), 369 (Helotiales sp)</b>			**
2349-271 (Helotiales sp), 3714-2720 ( <i>Cryptosporiopsis brunnea</i> ), 127 (Sebacinaceae sp), 551 (Pezizales sp), 1197-3556-526-646 (Chaetothyriales sp), 704 ( <i>Degelia plumbea</i> ), 3266-1008-467-471 ( <i>Cenococcum geophilum</i> ), 177 ( <i>Penicillium restrictum</i> ), 2844 (Saccharomycetales sp), 1677 ( <i>Hygrophorus cossus</i> ), 1633 ( <i>Penicillium malmesburiense</i> ), 321-445 (Fungi sp), 100 ( <i>Russula odorata</i> ), 596 ( <i>Humicola nigrescens</i> ), 2866 (Pleosporales sp), 741 (Sebacinales Group B sp), 372 ( <i>Sarcoleotia globosa</i> ), 493 ( <i>Archaeorhizomyces</i> sp), 1487 (Leotiomyces sp), 444 ( <i>Cladophialophora</i> sp), 171-37 (Sebacinaceae sp)			*

<sup>1</sup> The corrected Pearson's phi coefficient of association ("r.g") was used as model to determine indicator OTUs. The total number of indicator OTUs is indicated between brackets. For each line, OTU are sorted by decreasing r.g values.

<sup>2</sup> '\*\*\*' corresponds to  $P < 0.001$ ; '\*\*'  $P < 0.01$ ; '\*'  $P < 0.05$ ; 'NS'  $P > 0.05$ . OTUs with a r.g > 0.5 and  $P < 0.01$  are indicated in bold.



**Table II.S3.** Soil parameters in the Moroccan cork oak habitats

Sample	Habitat	plot	pH	Total N %	Total C %	C:N ratio	Total P mg / kg	Available P mg / kg	Mg_% meq %	Na_% meq %	K_% meq %	Cation Exchange Capacity meq %
Q10	Maâmora	P4	5.07	0.134	2.273	16.963	127	13	0.4	0.08	0.14	4.73
Q11	Maâmora	P4	5.07	0.134	2.273	16.963	127	13	0.4	0.08	0.14	4.73
Q12	Maâmora	P4	5.07	0.134	2.273	16.963	127	13	0.4	0.08	0.14	4.73
Q13	Maâmora	P5	4.97	0.177	2.764	15.616	109	14	0.54	0.11	0.18	4.63
Q14	Maâmora	P5	4.97	0.177	2.764	15.616	109	14	0.54	0.11	0.18	4.63
Q15	Maâmora	P5	4.97	0.177	2.764	15.616	109	14	0.54	0.11	0.18	4.63
Q16	Maâmora	P6	4.97	0.15	2.354	15.693	108	13	0.59	0.14	0.21	4.78
Q17	Maâmora	P6	4.97	0.15	2.354	15.693	108	13	0.59	0.14	0.21	4.78
Q18	Maâmora	P6	4.97	0.15	2.354	15.693	108	13	0.59	0.14	0.21	4.78
Q19	Benslimane	P7	6.35	0.327	4.876	14.911	566	17	2.03	0.18	0.56	16.68
Q20	Benslimane	P7	6.35	0.327	4.876	14.911	566	17	2.03	0.18	0.56	16.68
Q21	Benslimane	P7	6.35	0.327	4.876	14.911	566	17	2.03	0.18	0.56	16.68
Q22	Benslimane	P8	6.59	0.423	6.251	14.778	442	19	2.76	0.23	0.79	21.44
Q23	Benslimane	P8	6.59	0.423	6.251	14.778	442	19	2.76	0.23	0.79	21.44
Q24	Benslimane	P8	6.59	0.423	6.251	14.778	442	19	2.76	0.23	0.79	21.44
Q25	Benslimane	P9	6.27	0.18	2.681	14.894	461	10	1.42	0.13	0.41	11.49
Q26	Benslimane	P9	6.27	0.18	2.681	14.894	461	10	1.42	0.13	0.41	11.49
Q27	Benslimane	P9	6.27	0.18	2.681	14.894	461	10	1.42	0.13	0.41	11.49
Q46	Chefchaoun	P16	5.47	0.271	4.786	17.661	706	39	3.89	0.21	0.77	24.29
Q47	Chefchaoun	P16	5.47	0.271	4.786	17.661	706	39	3.89	0.21	0.77	24.29
Q48	Chefchaoun	P16	5.47	0.271	4.786	17.661	706	39	3.89	0.21	0.77	24.29
Q49	Chefchaoun	P17	6.04	0.25	4.393	17.572	737	50	4.38	0.34	0.75	21.28

**Table II.S3.** Continued

Sample	Habitat	plot	pH	Total N %	Total C %	C:N ratio	Total P mg / kg	Available P mg / kg	Mg_% meq %	Na_% meq %	K_% meq %	Cation Exchange Capacity meq %
Q50	Chefchaoun	P17	6.04	0.25	4.393	17.572	737	50	4.38	0.34	0.75	21.28
Q52	Chefchaoun	P18	5.72	0.253	4.702	18.585	669	38	3.55	0.16	0.63	23.34
Q53	Chefchaoun	P18	5.72	0.253	4.702	18.585	669	38	3.55	0.16	0.63	23.34
Q54	Chefchaoun	P18	5.72	0.253	4.702	18.585	669	38	3.55	0.16	0.63	23.34

**Table II.S4.** Fungal OTU indicators with respect to soil properties (pH, C:N ratio, available P)

Fungal OTUs (taxonomic assignment)	A	B	IndVal <sup>2</sup>
<b>Soil acidity<sup>1</sup></b>			
Very strongly acid (pH < 5.0)			
97 ( <i>Herpotrichiellaceae</i> sp.)	0.8902	1.0000	0.943*
<b>1188 (<i>Herpotrichiellaceae</i> sp.)</b>	1.0000	0.6667	0.816**
1674 (unidentified fungi)	0.8421	0.6667	0.749*
Strongly acid (pH: 5.0 – 5.5)			
<b>3363 (<i>Cladophialophora</i> sp.)</b>	1.0000	0.6667	0.816**
409 ( <i>Cenococcum</i> sp.)	0.9333	0.6667	0.789*
Moderately acid (pH: 5.6 – 6.0)			
<b>51 (<i>Hygrophorus cossus</i>)</b>	0.9938	1.0000	0.997**
490 ( <i>Oidiodendron chlamydosporicum</i> )	0.9506	0.8333	0.890*
364 ( <i>Hydnotrya cerebriformis</i> )	0.9375	0.8333	0.884*
572 (Sebacinales Group B)	0.8772	0.8333	0.855*
493 ( <i>Archaeorhizomyces</i> sp)	0.9889	0.6667	0.812*
650 (Chaetothyriales sp)	0.8000	0.6667	0.730*
Slightly acid (pH: 6.1 – 6.5)			
<b>928 (<i>Sordariales</i> sp)</b>	1.0000	0.6667	0.816**
Neutral (pH > 6.5)			
<b>315 (<i>Inocybe</i> sp)</b>	0.9944	1.0000	0.997**
<b>85 (<i>Tomentella</i> sp)</b>	0.9799	1.0000	0.990**
<b>45 (<i>Lophiostoma cf cynaroidis</i>)</b>	0.9655	1.0000	0.983***
<b>705 (<i>Sebacina</i> sp)</b>	0.9632	1.0000	0.981**

**Table II.S4.** Continued

Fungal OTUs (taxonomic assignment)	A	B	IndVal <sup>2</sup>
306 ( <i>Tuber</i> sp)	0.9529	1.0000	0.976*
66 ( <i>Tomentella</i> sp)	0.9437	1.0000	0.971*
<b>19070 (<i>Paecilomyces</i> sp)</b>	1.0000	0.6667	0.816**
<b>4242 (<i>Sebacina</i> sp)</b>	0.9714	0.6667	0.805**
<b>376 (<i>Agaricales</i> sp)</b>	0.9474	0.6667	0.795**
250 ( <i>Sebacina</i> sp)	0.9412	0.6667	0.792*
3852 ( <i>Sebacinaceae</i> sp)	0.8889	0.6667	0.770*
1899 ( <i>Cortinarius</i> sp)	0.8571	0.6667	0.756*
205 ( <i>Tomentella</i> sp)	0.8235	0.6667	0.741*
738 ( <i>Preussia flanaganii</i> ); 1114 ( <i>Mortierella amoeboidea</i> )	0.8000	0.6667	0.730*
<b>Soil organic matter decomposition rate</b>			
Strong (C:N ratio < 15)			
<b>17 (<i>Tomentella atramentaria</i>)</b>	0.9968	1.0000	0.998***
<b>69 (<i>Ilyonectria mors-panacis</i>)</b>	0.9201	1.0000	0.959**
<b>38 (<i>Thelephoraceae</i> sp)</b>	0.9936	0.8889	0.940**
<b>358 (<i>Tomentella</i> sp)</b>	0.9949	0.7778	0.880**
<b>66 (<i>Tomentella</i> sp)</b>	0.9779	0.7778	0.872**
<b>28 (<i>Saccharicola</i> sp)</b>	0.9537	0.7778	0.861**
49 ( <i>Cortinarius</i> sp)	0.9982	0.6667	0.816*
315 ( <i>Inocybe</i> sp)	0.9970	0.6667	0.815*
67 ( <i>Tomentella</i> sp)	0.8889	0.6667	0.770*
Moderate (C:N ratio: 15 – 20)			
<b>33 (<i>Cladophialophora</i> sp)</b>	0.9901	1.0000	0.995***

**Table II.S4.** Continued

Fungal OTUs (taxonomic assignment)	A	B	IndVal <sup>2</sup>
<b>16 (Russulaceae sp)</b>	0.9865	1.0000	0.993**
<b>112 (Cladophialophora sp)</b>	0.8970	1.0000	0.947**
75 (Mycosphaerellaceae sp)	0.9844	0.8889	0.935*
<b>95 (Cryptosporiopsis brunnea)</b>	0.9748	0.8889	0.931**
32 ( <i>Cenococcum geophilum</i> )	0.9596	0.8889	0.924*
<b>246 (Cladophialophora sp)</b>	1.0000	0.8333	0.913***
<b>82 (Aspergillus amstelodami)</b>	0.8580	0.9444	0.900**
97 (Herpotrichiellaceae sp)	0.9551	0.8333	0.892*
<b>60 (Lactarius sp)</b>	0.9952	0.7778	0.880**
<b>453 (Oidiodendron griseum)</b>	0.9286	0.8333	0.880**
46 (Herpotrichiellaceae sp)	0.9863	0.7778	0.876*
78 (Dothideomycetes sp)	0.9101	0.8333	0.871*
237 (Chaetothyriales sp)	0.8909	0.8333	0.862*
359 (Cryptosporiopsis sp)	1.0000	0.6667	0.816*
51 ( <i>Hygrophorus cossus</i> )	0.9977	0.6667	0.816*
103 (Atractiellales sp)	0.8351	0.7778	0.806*
407 (Sordariomycetes sp)	0.9322	0.6111	0.755*
72 ( <i>Russula</i> sp)	0.8500	0.6667	0.753*
<b>Soil available P content</b>			
Low (mg P <sub>available</sub> / kg soil < 10)			
903 (Thelephoraceae sp)	1.0000	0.6667	0.816*
<b>780 (Tomentella atramentaria)</b>	0.9971	0.6667	0.815**
148 ( <i>Tomentella</i> sp)	0.9893	0.6667	0.812*

**Table II.S4.** Continued

Fungal OTUs (taxonomic assignment)	A	B	IndVal <sup>2</sup>
1133 ( <i>Ilyonectria mors-panacis</i> )	0.9259	0.6667	0.786*
928 (Sordariales sp)	0.8824	0.6667	0.767*
536 ( <i>Tomentella atramentaria</i> )	0.8712	0.6667	0.762*
759 ( <i>Ilyonectria mors-panacis</i> )	0.8434	0.6667	0.750*
Moderate (mg P <sub>available</sub> / kg soil: 11 – 25)			
93 (Russulaceae sp)	0.9757	0.9333	0.954*
High (mg P <sub>available</sub> / kg soil > 25 – 49)			
<b>362 (<i>Cenococcum geophilum</i>)</b>	0.9398	1.0000	0.969***
<b>471 (<i>Cenococcum geophilum</i>)</b>	0.9911	0.8333	0.909**
171 (Sebacinaceae sp)	0.9598	0.8333	0.894*
<b>1008 (<i>Cenococcum geophilum</i>)</b>	0.9770	0.6667	0.807**
599 (Sordariomycetes sp)	0.9459	0.6667	0.794*
321 (Unidentified fungi)	0.8553	0.6667	0.755*
Very high (mg P <sub>available</sub> / kg soil > 50)			
<b>493 (<i>Archaeorhizomyces</i> sp)</b>	0.9907	1.0000	0.995***
<b>445 (Unidentified fungi)</b>	0.9677	1.0000	0.984***
<b>703 (<i>Degelia plumbea</i>)</b>	0.9653	1.0000	0.982***
<b>230 (<i>Tomentella</i> sp)</b>	0.9135	1.0000	0.956**
<b>551 (Pezizales sp)</b>	0.8889	1.0000	0.943**
<b>1137 (<i>Cladophialophora chaetospira</i>)</b>	0.8451	1.0000	0.919**
572 (Sebacinales Group B)	0.8061	1.0000	0.898*

**Table II.S4.** Continued

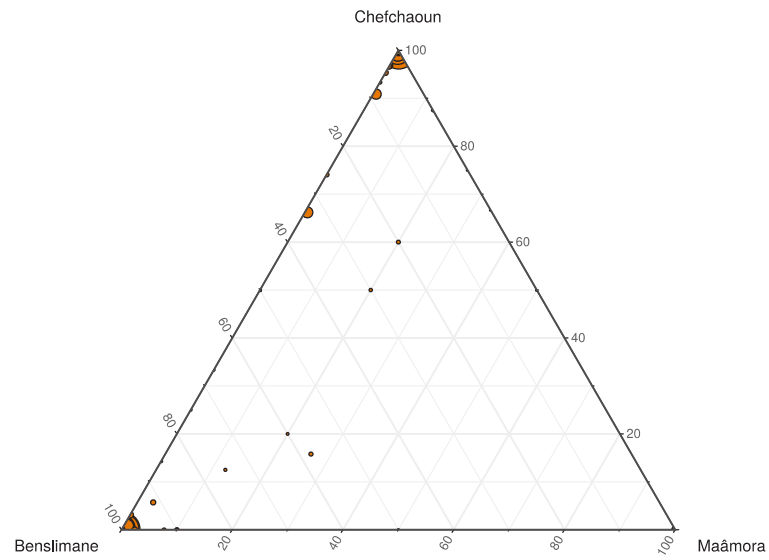
Fungal OTUs (taxonomic assignment)	A	B	IndVal <sup>2</sup>
234 (Dothideomycetes sp); 330 (Mycosphaerellaceae sp); 513 (Helotiales sp); 857 (Agaricales sp); 1149 (Leotiomyces sp); 1239 (Agaricales sp); 1397 (Helotiales sp); 1441 ( <i>Craterellus</i> sp); 1987 ( <i>Craterellus</i> sp); 2398 (Hysteriales sp); 2437 (Unidentified fungi); 2468 ( <i>Craterellus</i> sp); 2606 (Leotiomyces sp); 3213 ( <i>Sarcoleotia globosa</i> ); 4402 (Unidentified fungi); 4770 ( <i>Aspergillus</i> sp); 5738 ( <i>Degelia plumbea</i> ); 5928 (Helotiales sp); 7290 (Unidentified fungi); 7887 (Sebacinales Group B); 10216 ( <i>Terfezia pini</i> )	1.0000	0.6667	0.816*
<b>1664 (<i>Trichoglossum hirsutum</i>)</b>	<b>0.9975</b>	<b>0.6667</b>	<b>0.815**</b>
1143 ( <i>Craterellus</i> sp); 734 (Pleosporales sp)	0.9967	0.6667	0.815*
832 ( <i>Russula foetens</i> )	0.9898	0.6667	0.812*
6933 (Ascomycota sp)	0.9783	0.6667	0.808*
967 (Mycosphaerellaceae sp)	0.9722	0.6667	0.805*
490 ( <i>Oidiodendron chlamydosporicum</i> )	0.9699	0.6667	0.804*
1487 (Leotiomyces sp)	0.9667	0.6667	0.803*
495 (Dothideomycetes sp)	0.9632	0.6667	0.801*
1124 ( <i>Oidiodendron</i> sp)	0.9589	0.6667	0.800*
374 (Dothideomycetes sp)	0.9524	0.6667	0.797*
2236 (Helotiales sp)	0.9474	0.6667	0.795*
1073 (Helotiales sp)	0.9130	0.6667	0.780*
1621 ( <i>Lachnum</i> sp)	0.9091	0.6667	0.778*
482 (Leotiomyces sp); 2720 ( <i>Cryptosporiopsis brunnea</i> )	0.9000	0.6667	0.775*
2684 (Ascomycota sp)	0.8824	0.6667	0.767*
2844 (Saccharomycetales sp)	0.8571	0.6667	0.756*

<sup>1</sup> Only pH, C:N ratio and available P content were considered because of their significance as drivers of EcM-related fungal community structure. Soil categories were defined according the Natural Resources conservation service (<https://www.nrcs.usda.gov/>) and Oregon State University (<http://extension.oregonstate.edu/>).

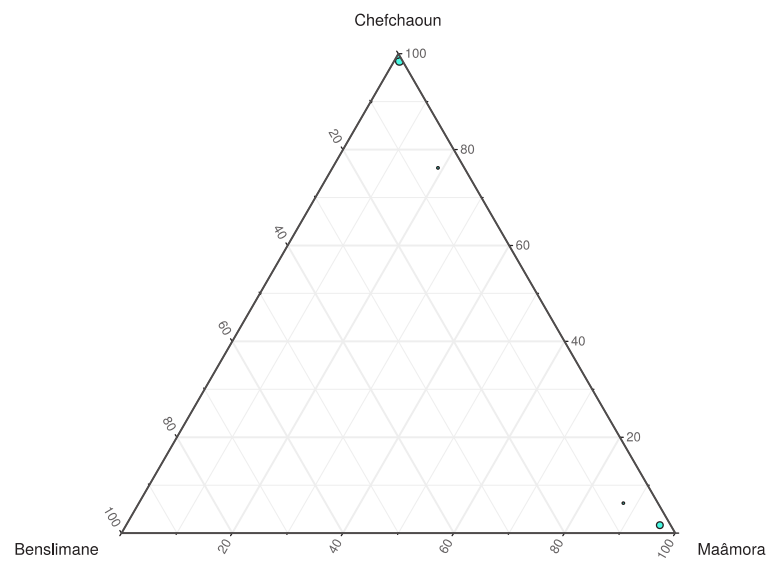
<sup>2</sup> For each soil category, OTU are sorted by decreasing IndVal value index. Only fungal OTUs with A (specificity) and B (sensitivity) superior to 0.8 and 0.6, respectively, are considered. ‘\*\*\*’ corresponds to  $P < 0.001$ ; ‘\*\*’  $P < 0.01$ ; ‘\*’  $P < 0.05$ ; ‘NS’  $P > 0.05$ . OTUs with a  $P < 0.01$  are indicated in bold.



Agaricales (120 OTUs, 14017 reads)

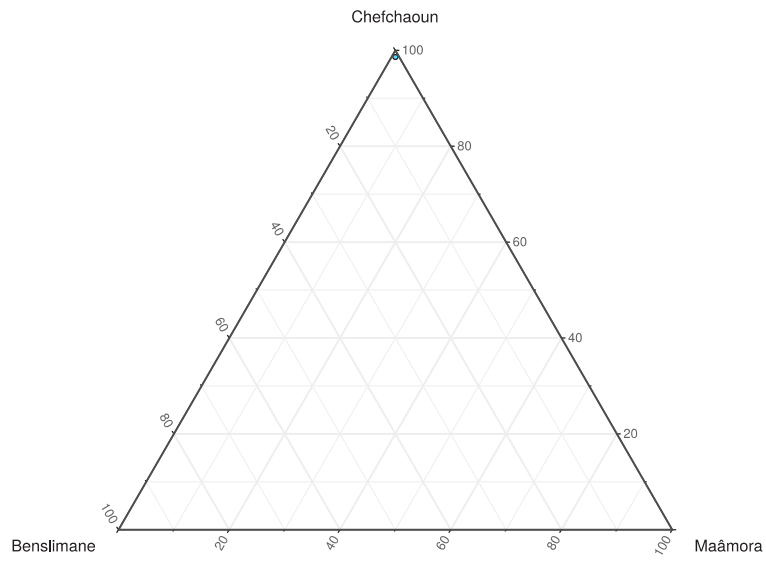


Archaeorhizomycetales (15 OTUs, 1233 reads)



**Figure II.S1.** Distribution of fungal OTUs among habitats (Maâmora, Benslimane, Chefchaoun) in ternary plots. Each ternary plot represents the OTUs for a taxonomic genus. The number of OTUs and sequences are indicated for each taxonomic order (40). The colors used correspond to those in Figure 1. The size of a given dot represents the abundance of one OTU. Only the 20 most dominant genera out of 129 are shown.

Atheliales (1 OTU, 287 reads)



Atractiellales (4 OTUs, 98 reads)

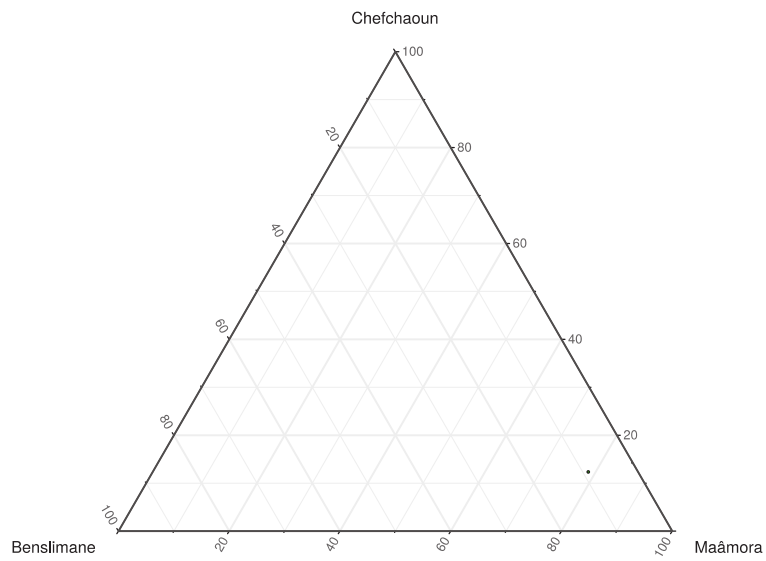
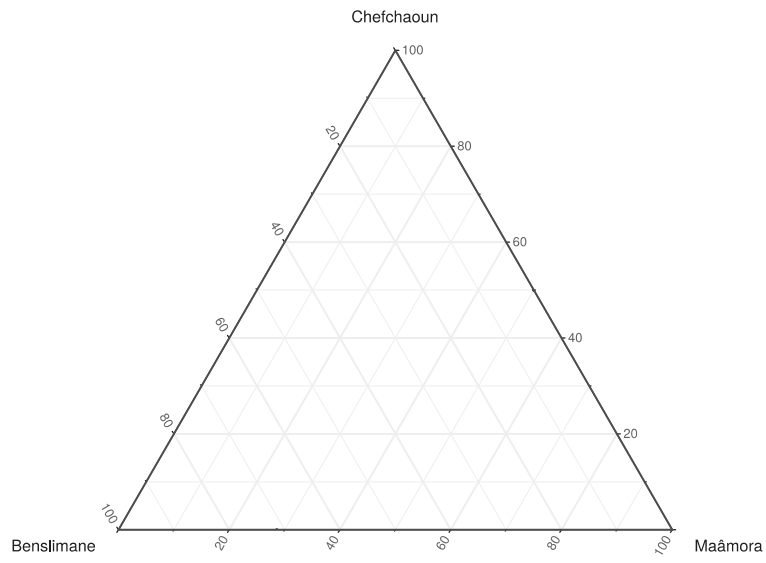


Figure II.S1. Continued.

Auriculariales (6 OTUs, 14 reads)



Boletales (12 OTUs, 943 reads)

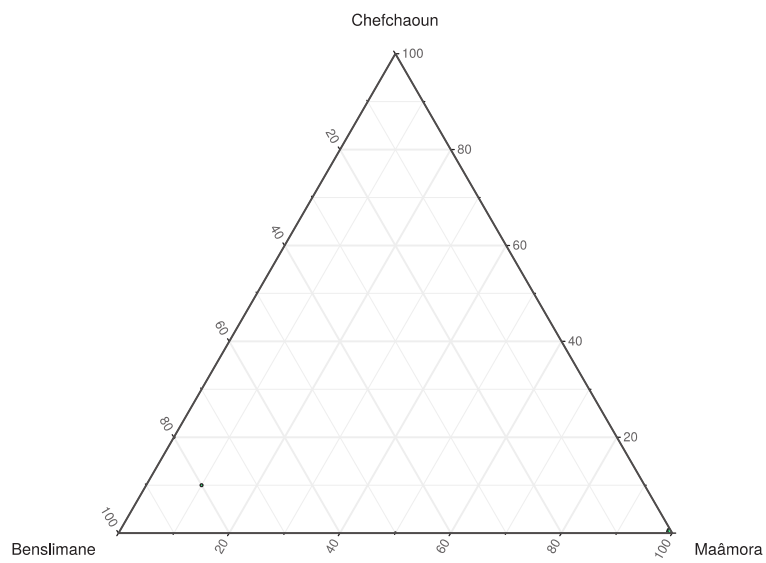
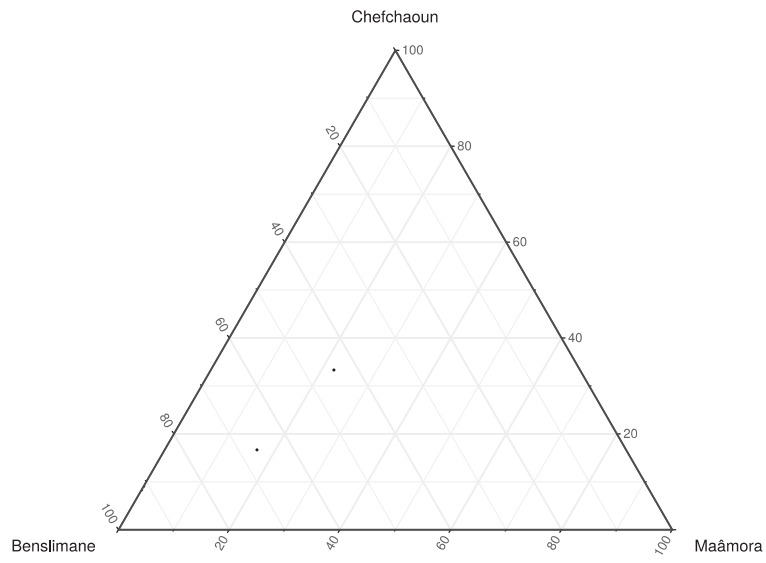


Figure II.S1. Continued.

Cantharellales (10 OTUs, 422 reads)



Capnodiales (56 OTUs, 1895 reads)

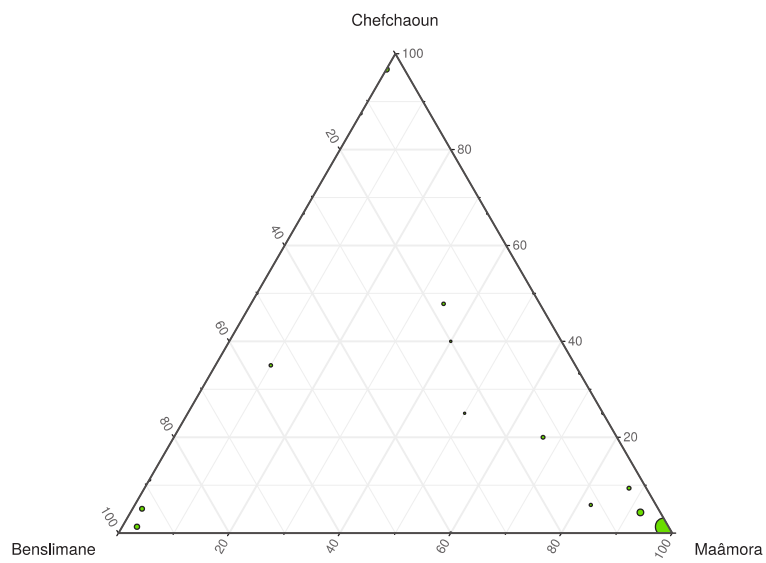
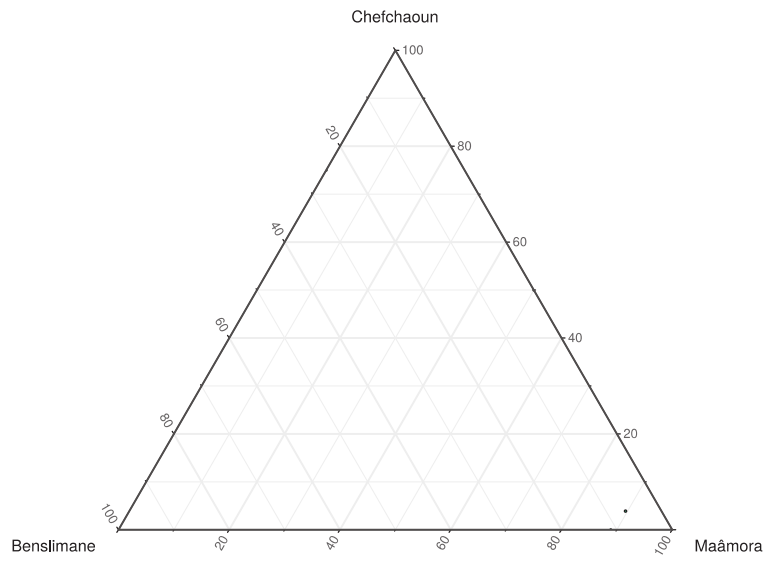


Figure II.S1. Continued.

Chaetosphaeriales (4 OTUs, 92 reads)



Chaetothyriales (209 OTUs, 5622 reads)

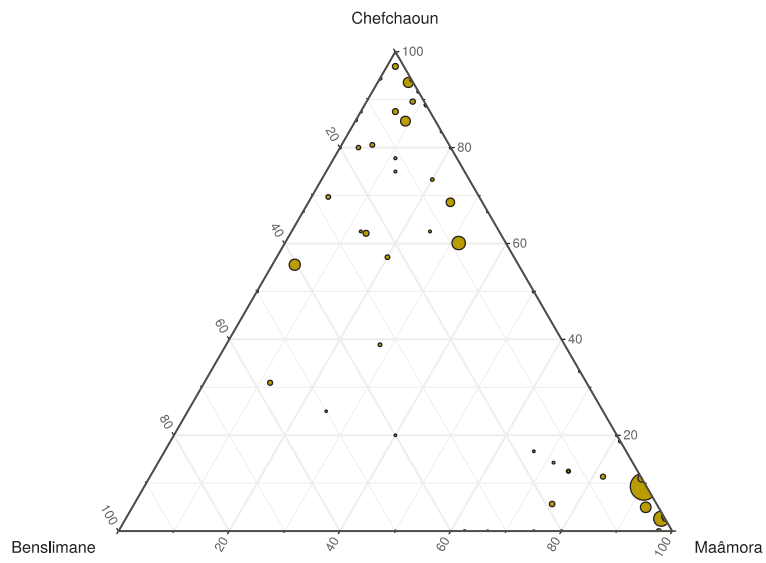
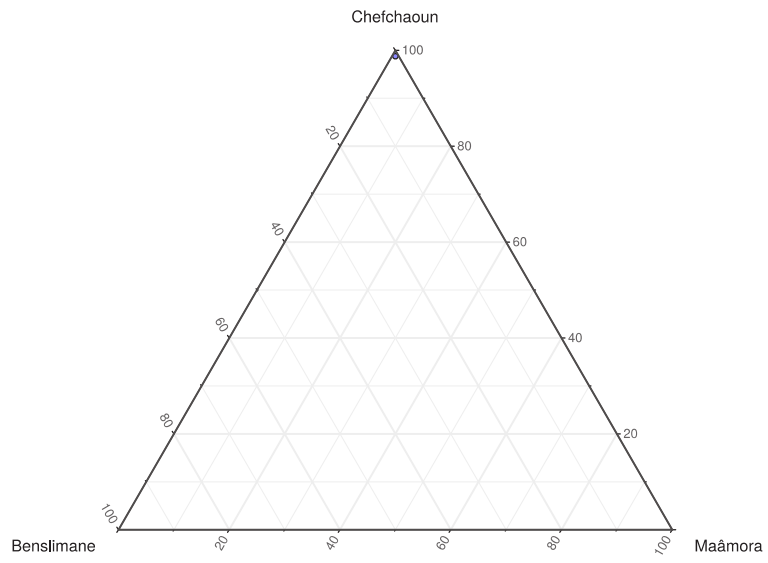


Figure II.S1. Continued.

Coniochaetales (20 OTUs, 392 reads)



Cystobasidiales (4 OTUs, 4 reads)

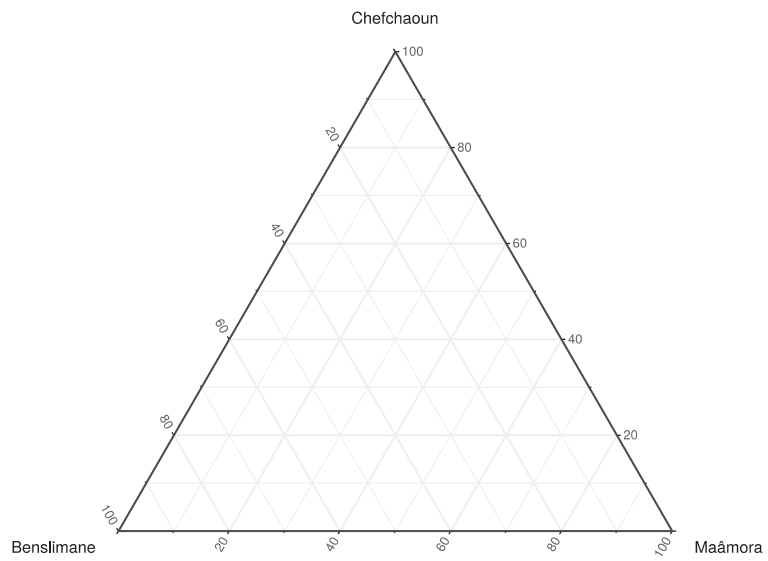
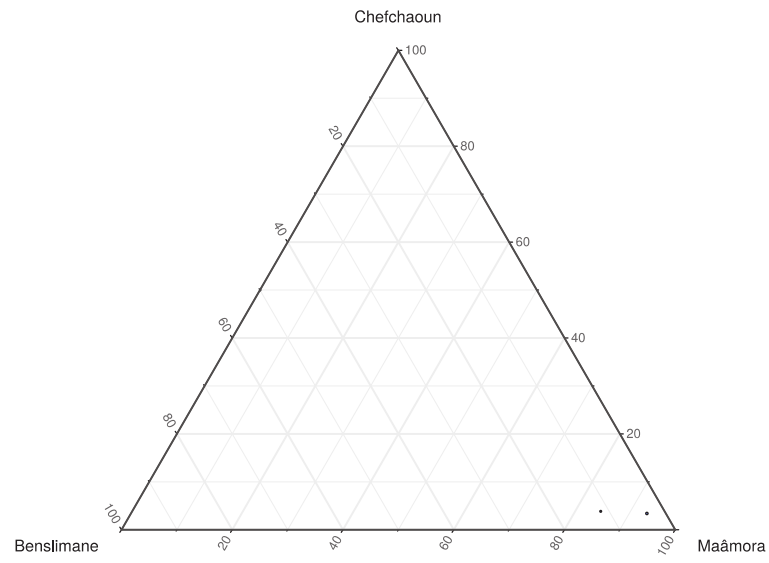


Figure II.S1. Continued.

Diaporthales (3 OTUs, 116 reads)



Dothideales (1 OTU, 10 reads)

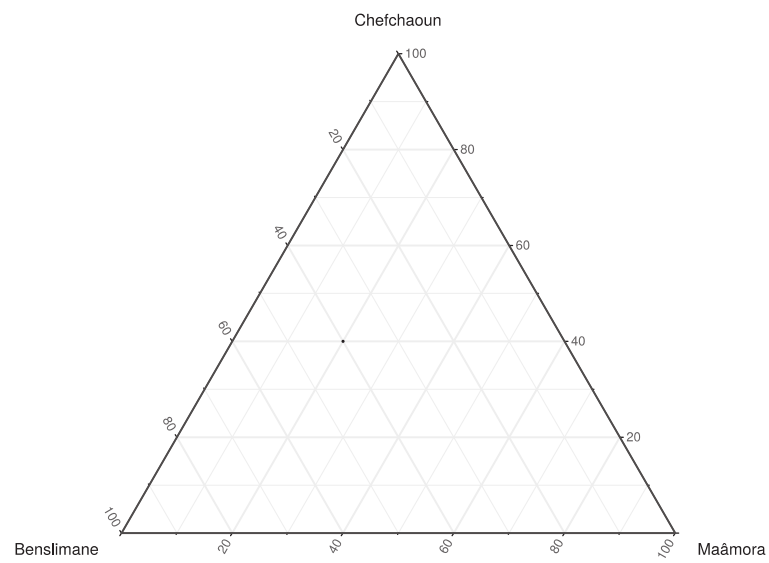
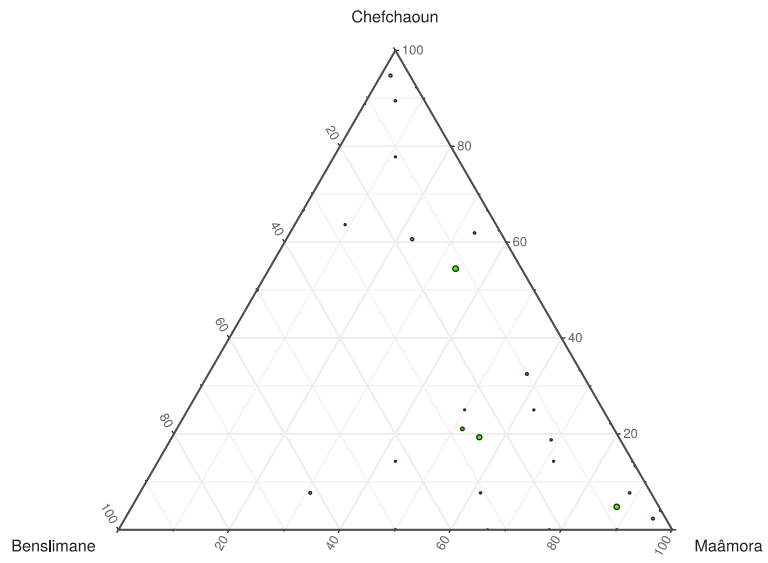


Figure II.S1. Continued.



Eurotiales (119 OTUs, 1690 reads)



Geoglossales (6 OTUs, 482 reads)

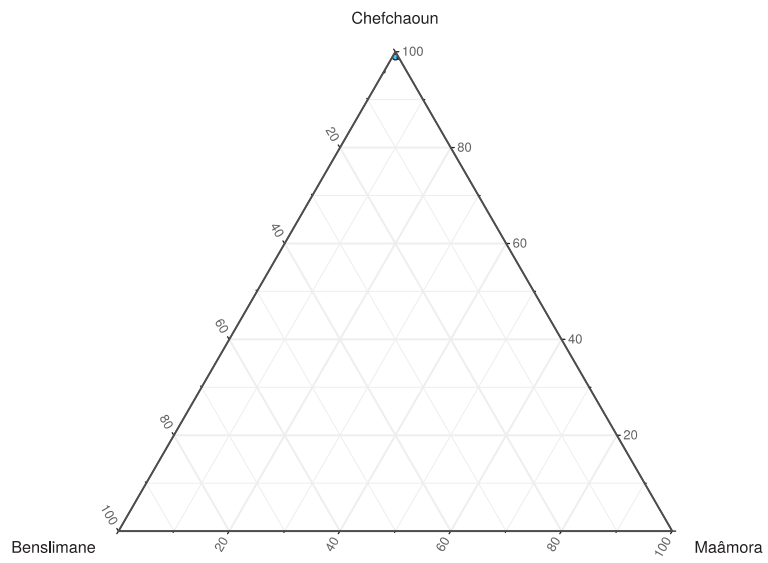
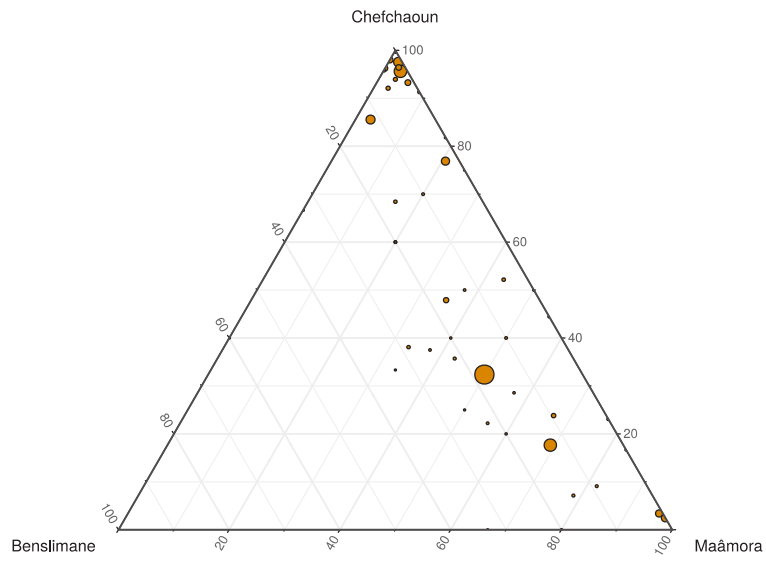


Figure II.S1. Continued.

Helotiales (164 OTUs, 4796 reads)



Hymenochaetales (1 OTU, 14 reads)

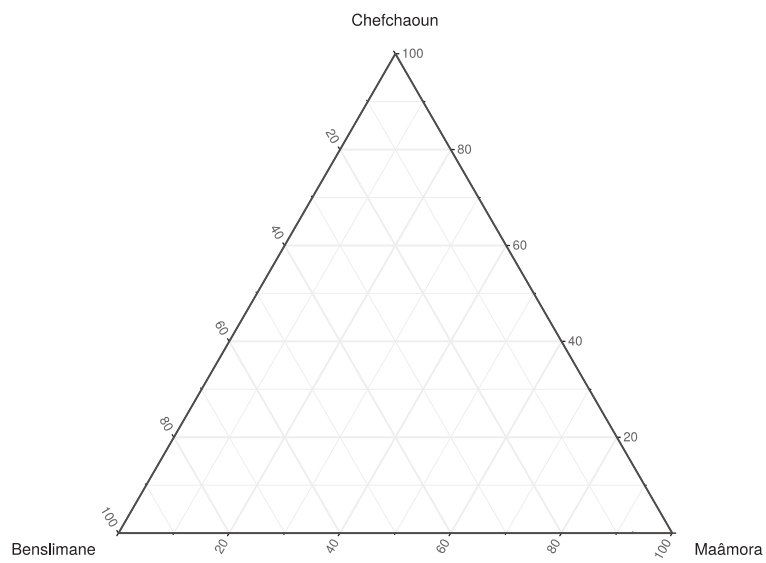
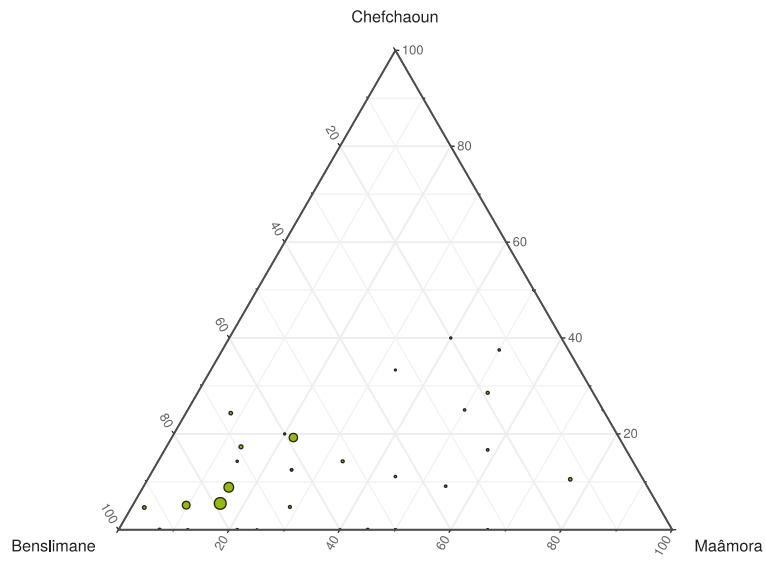


Figure II.S1. Continued.

Hypocreales (90 OTUs, 2575 reads)



Hysteriales (62 OTUs, 12815 reads)

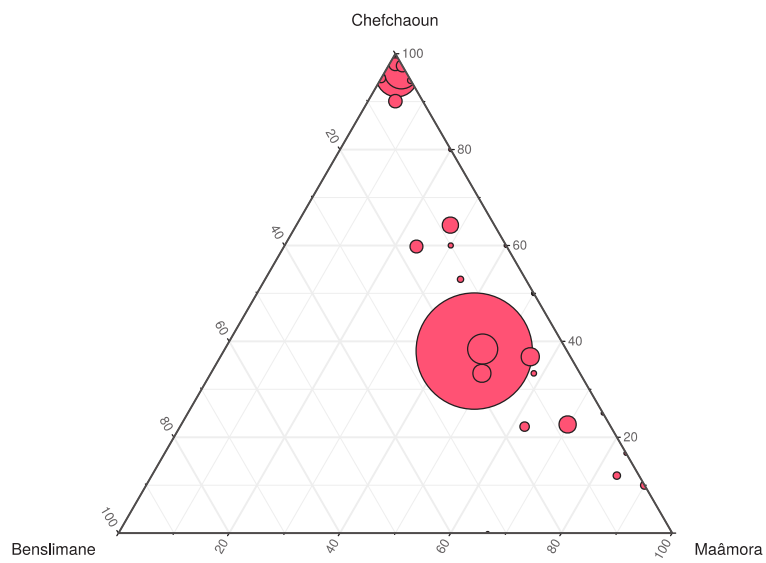
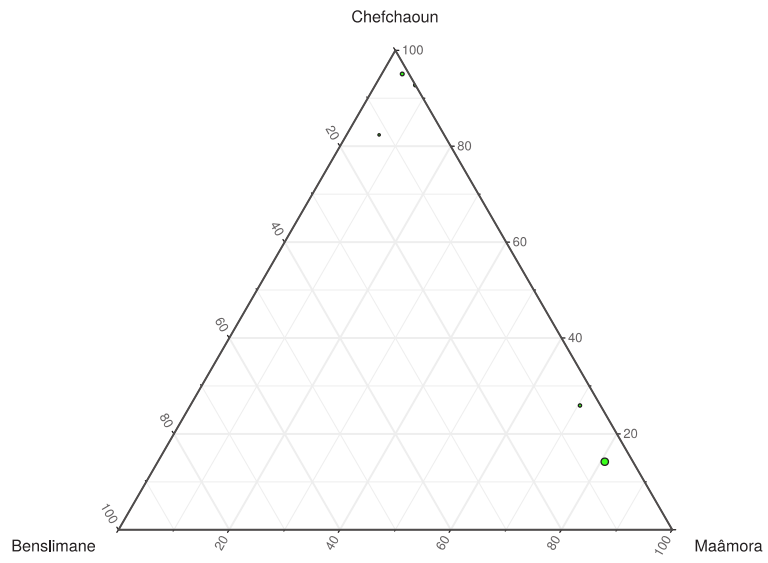


Figure II.S1. Continued.

Incertae\_sedis (34 OTUs, 766 reads)



Magnaporthales (2 OTUs, 2 reads)

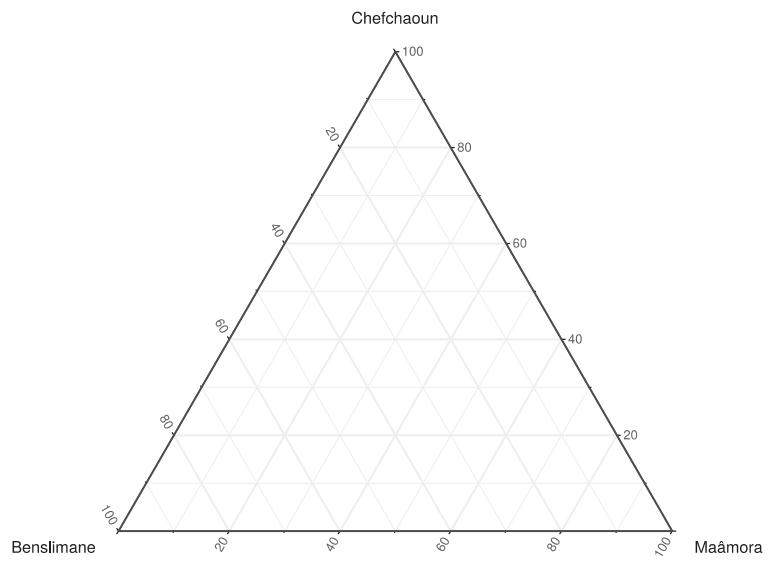
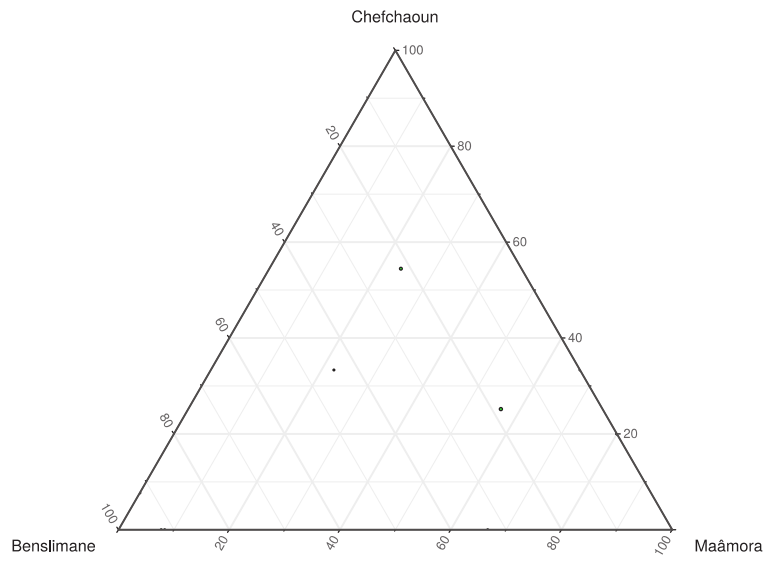


Figure II.S1. Continued.

Mortierellales (31 OTUs, 355 reads)



Mucorales (10 OTUs, 400 reads)

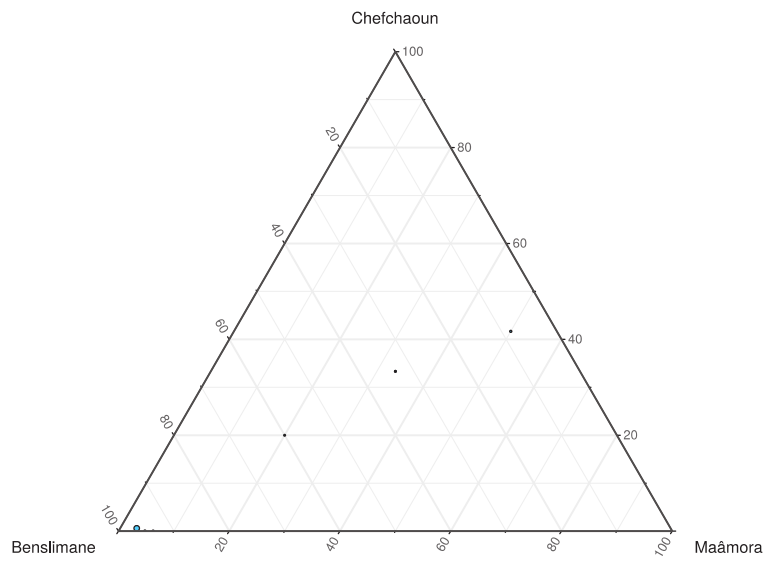
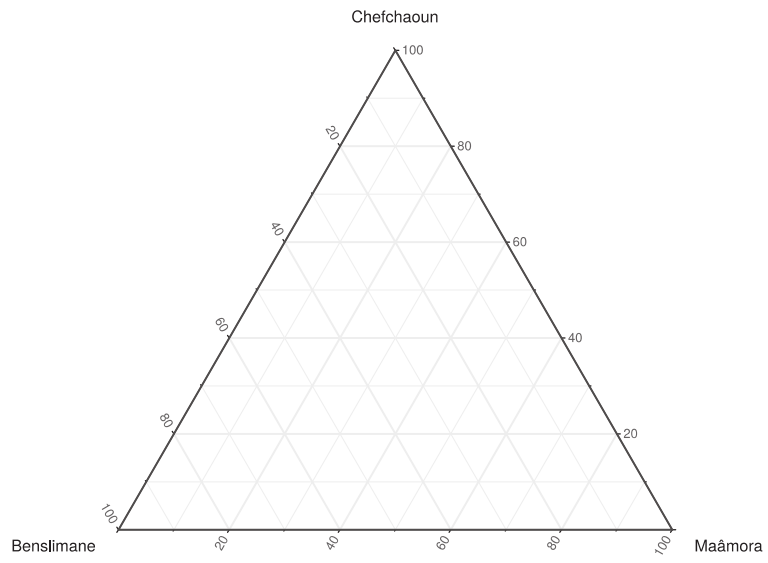


Figure II.S1. Continued.

Ophiostomatales (1 OTU, 9 reads)



Orbiliales (2 OTUs, 2 reads)

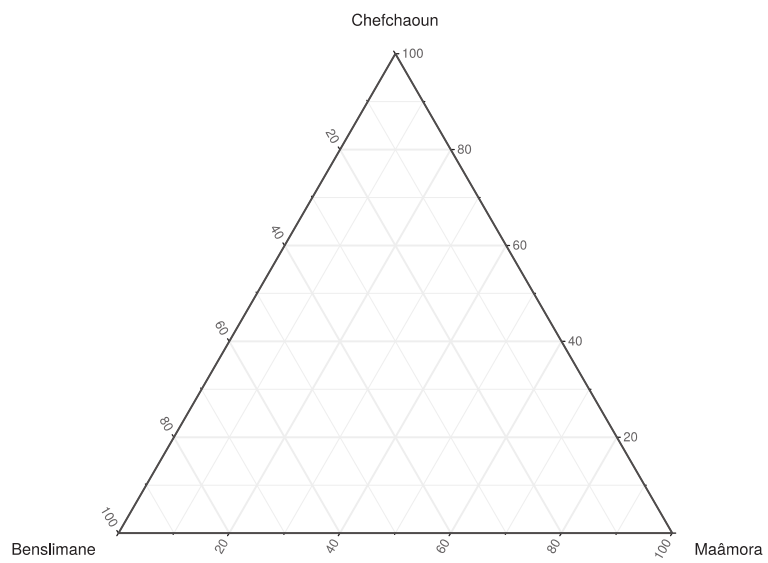
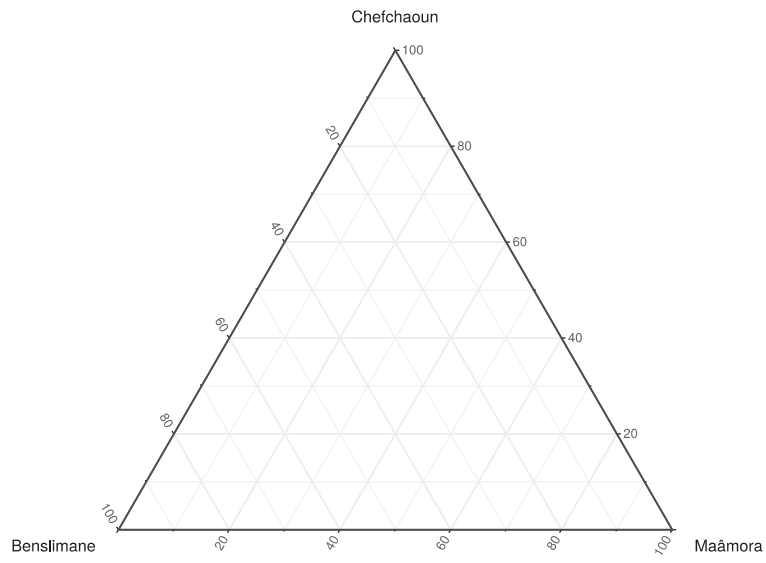


Figure II.S1. Continued.

Ostropales (2 OTUs, 3 reads)



Peltigerales (12 OTUs, 100 reads)

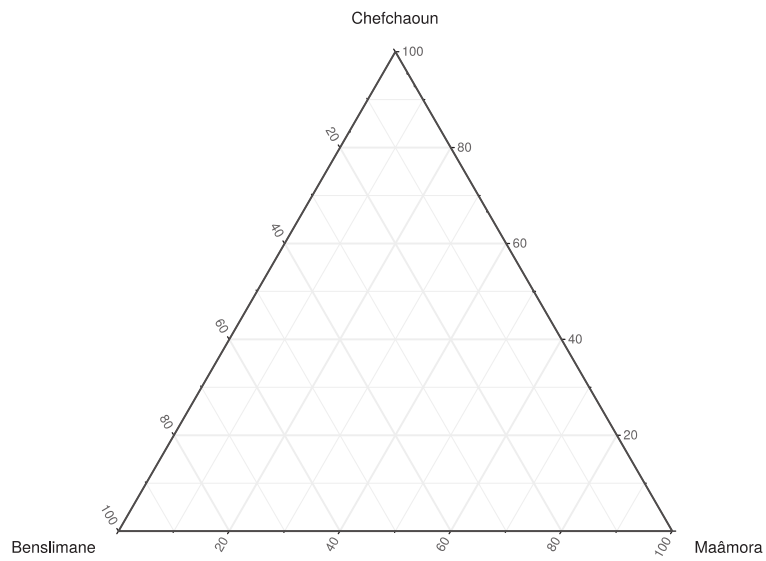
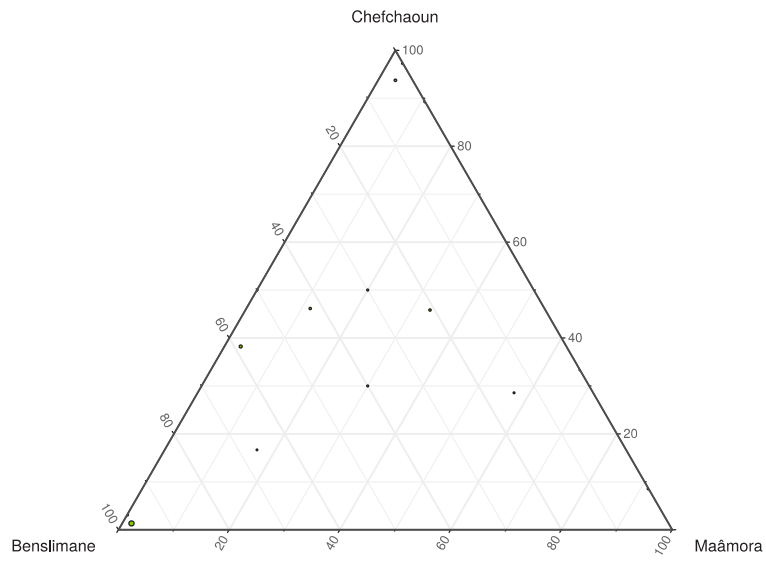


Figure II.S1. Continued.



Pezizales (32 OTUs, 678 reads)



Pleosporales (44 OTUs, 1276 reads)

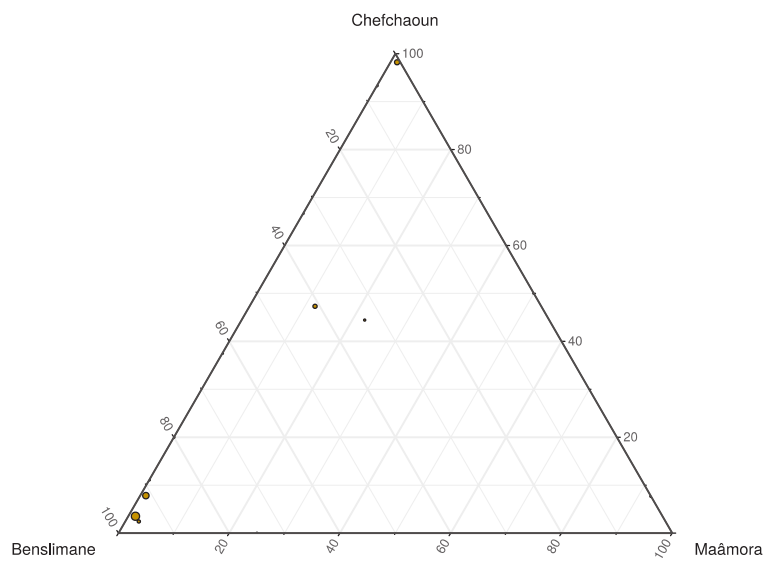
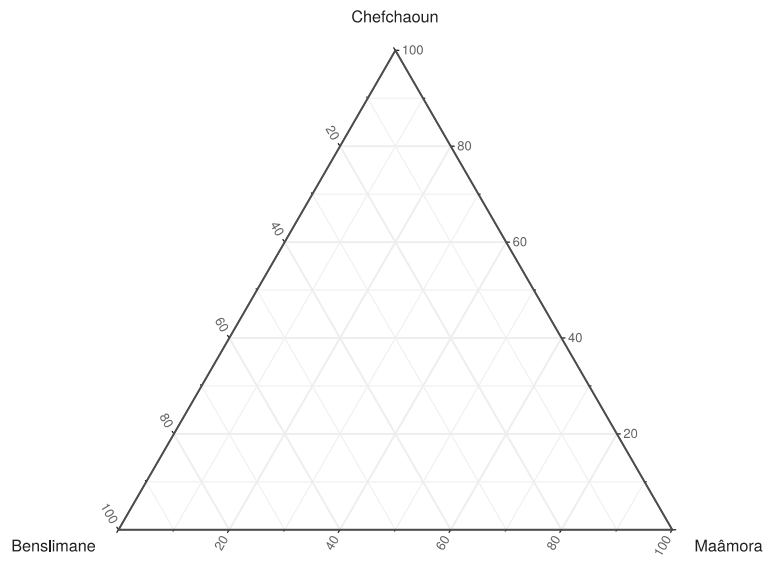


Figure II.S1. Continued.

Rhytismatales (1 OTU, 24 reads)



Russulales (140 OTUs, 16275 reads)

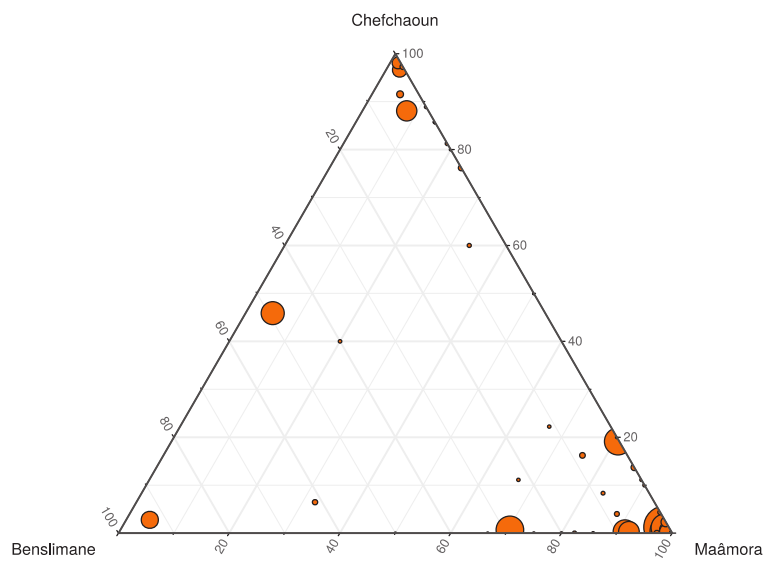
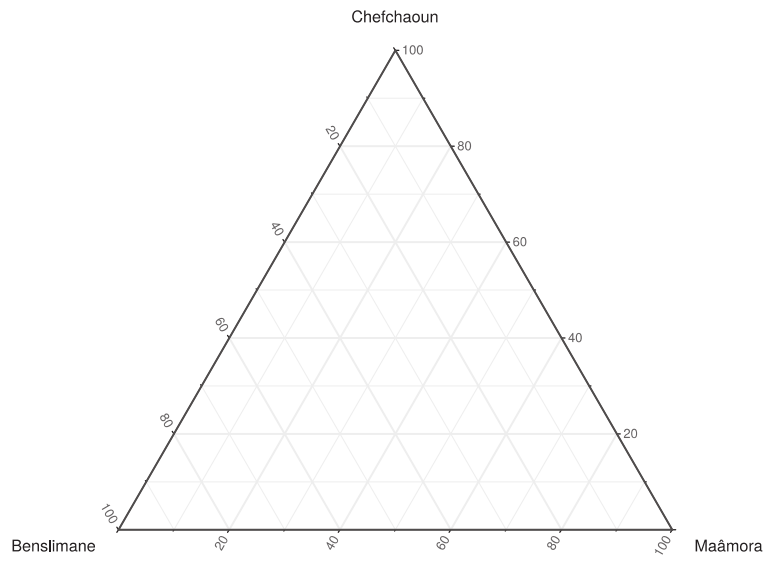


Figure II.S1. Continued.

Saccharomycetales (3 OTUs, 10 reads)



Sebacinales (112 OTUs, 5902 reads)

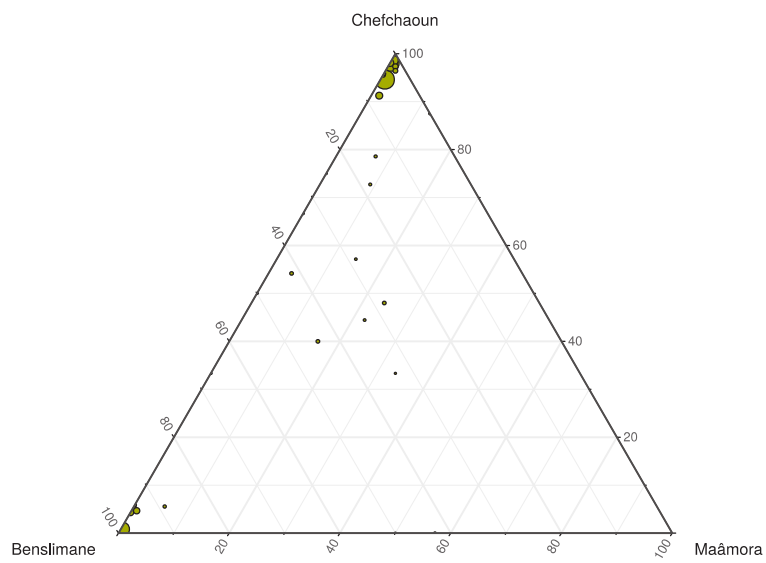
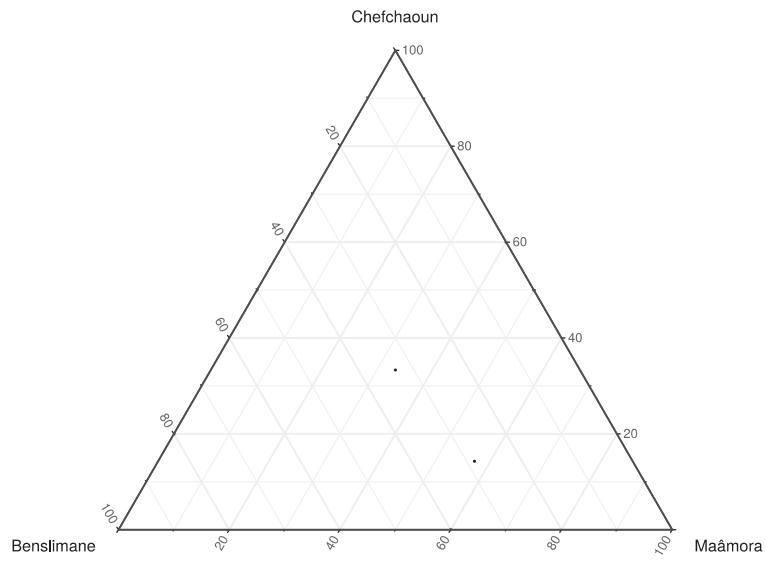


Figure II.S1. Continued.

Sordariales (26 OTUs, 77 reads)



Thelephorales (234 OTUs, 17084 reads)

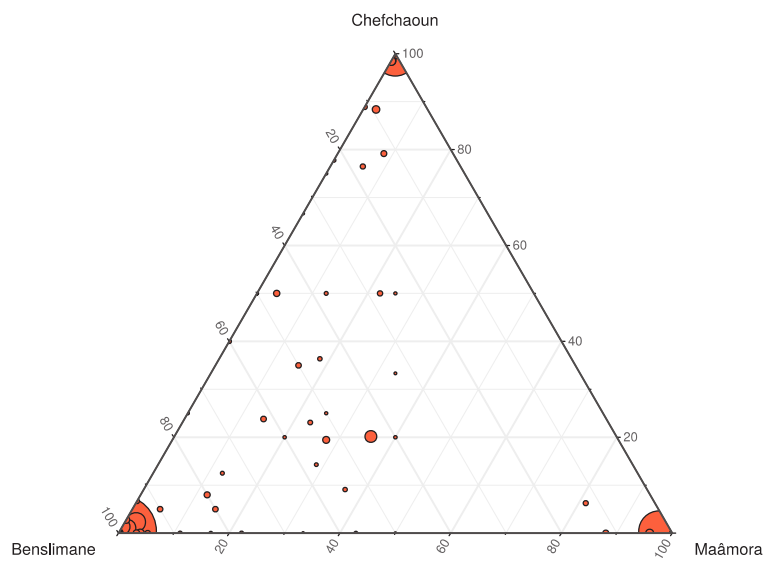
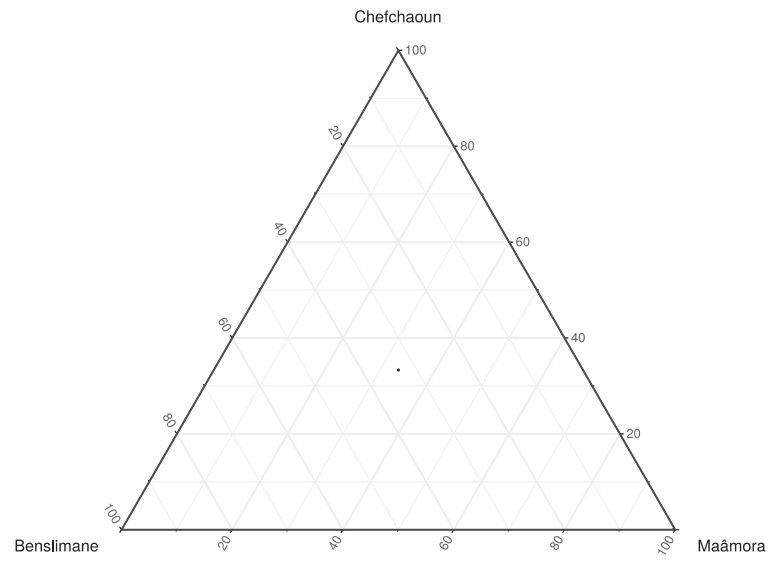


Figure II.S1. Continued.

Trechisporales (5 OTUs, 280 reads)



Tremellales (11 OTUs, 323 reads)

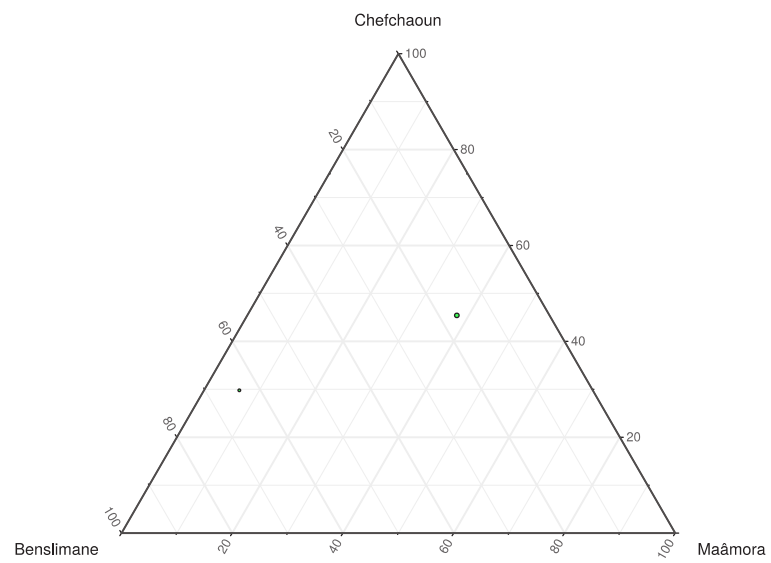
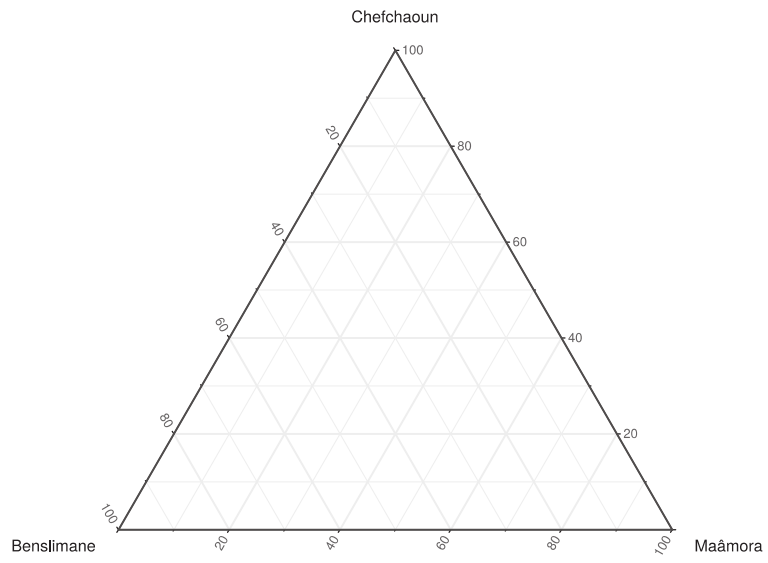


Figure II.S1. Continued.

Wallemiales (1 OTU, 7 reads)



Xylariales (7 OTUs, 101 reads)

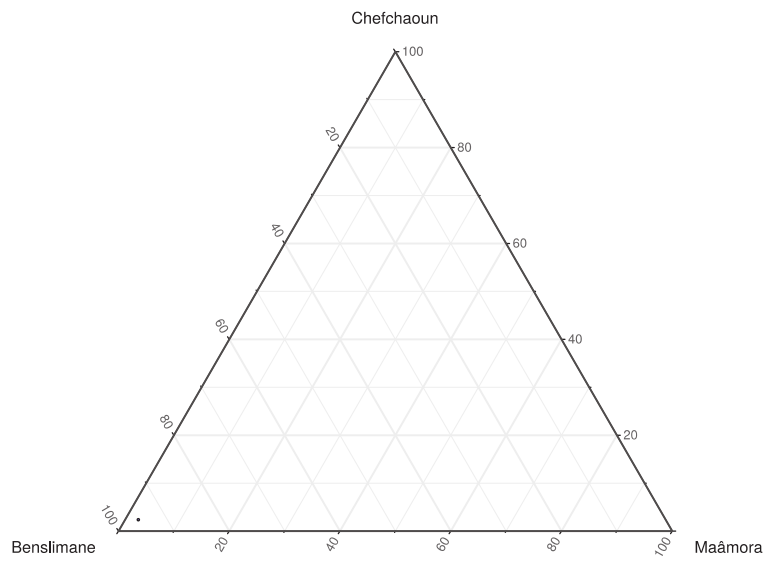
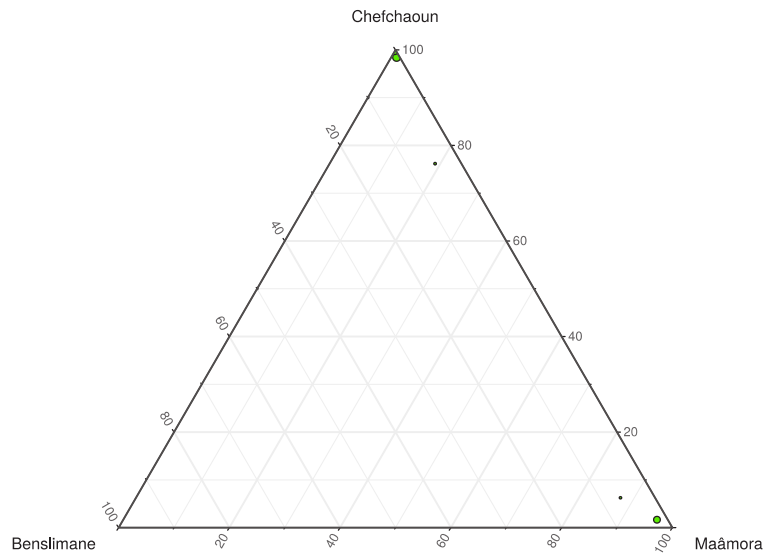
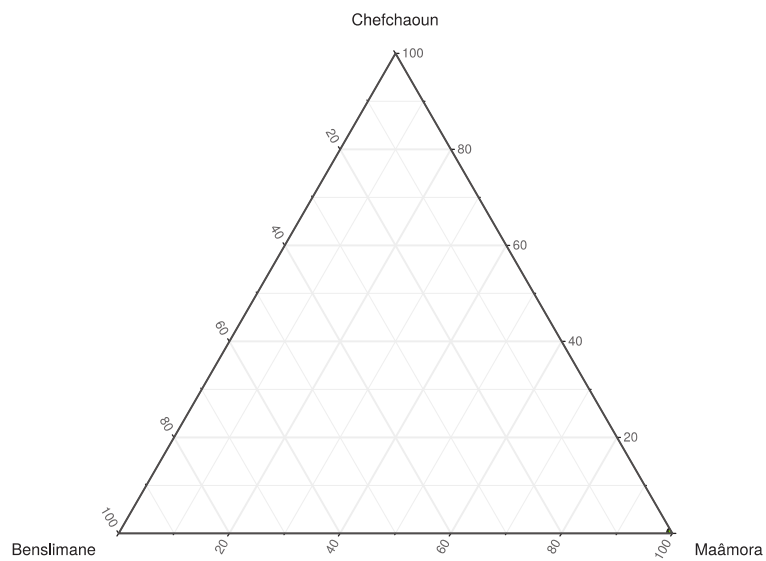


Figure II.S1. Continued.

Archaeorhizomyces (15 OTUs, 1233 reads)



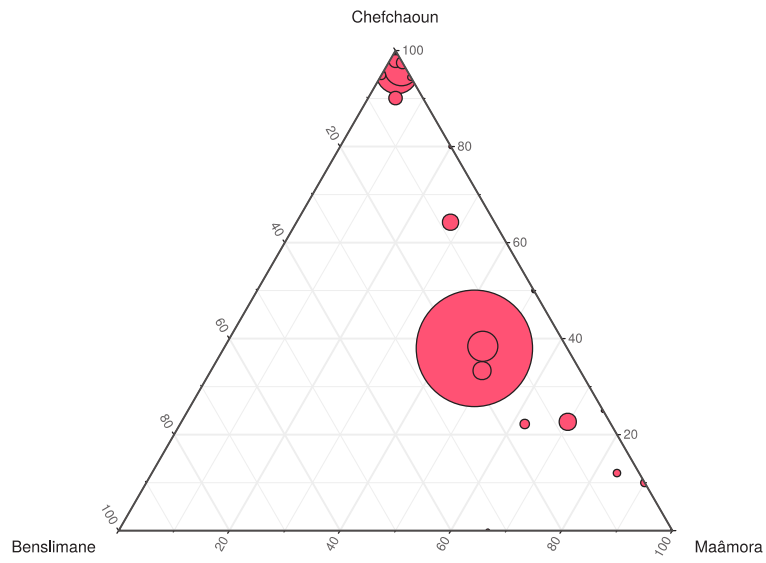
Boletus (3 OTUs, 903 reads)



**Figure II.S2.** Distribution of fungal OTUs among habitats (Maâmora, Benslimane, Chefchaoun) in ternary plots. Each ternary plot represents the OTUs for a taxonomic genus. The number of OTUs and sequences are indicated for each taxonomic order. The colors used correspond to those in Figure 1. The size of a given dot represents the abundance of one OTU. Only the 20 most dominant genera out of 129 are shown.



Cenococcum (44 OTUs, 12459 reads)



Cladophialophora (86 OTUs, 3046 reads)

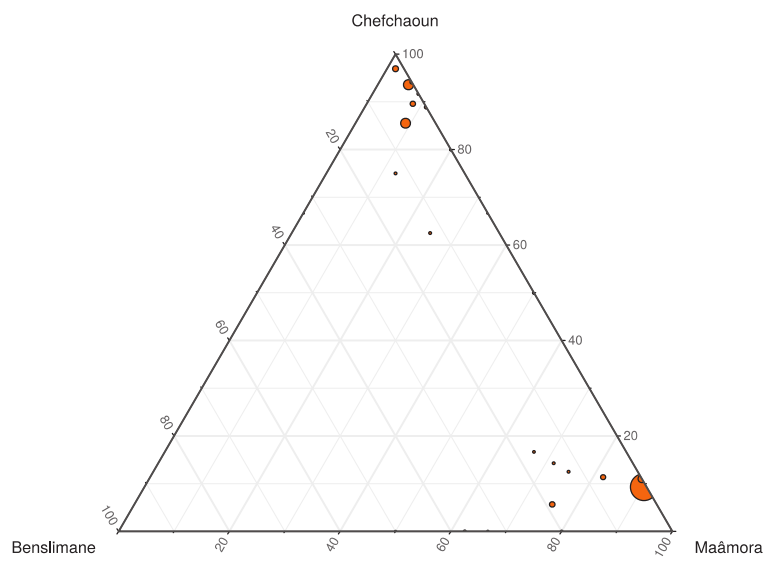
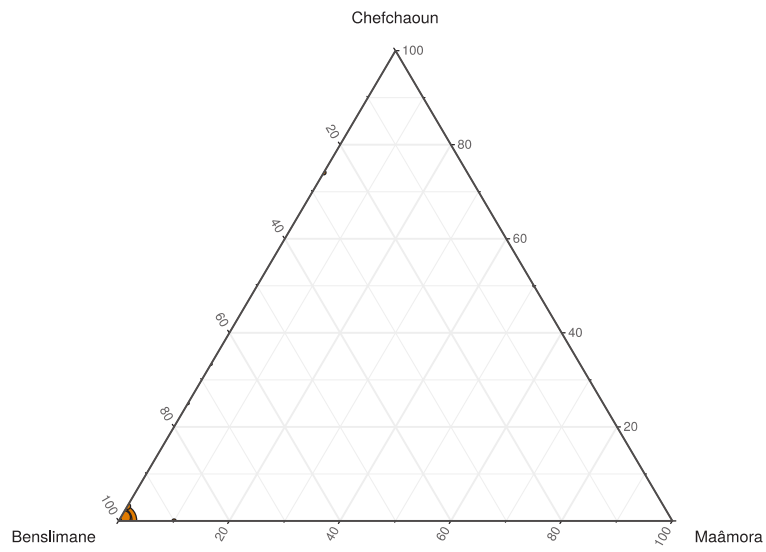


Figure II.S2. Continued.

Cortinarius (39 OTUs, 4568 reads)



Cryptosporiopsis (21 OTUs, 1317 reads)

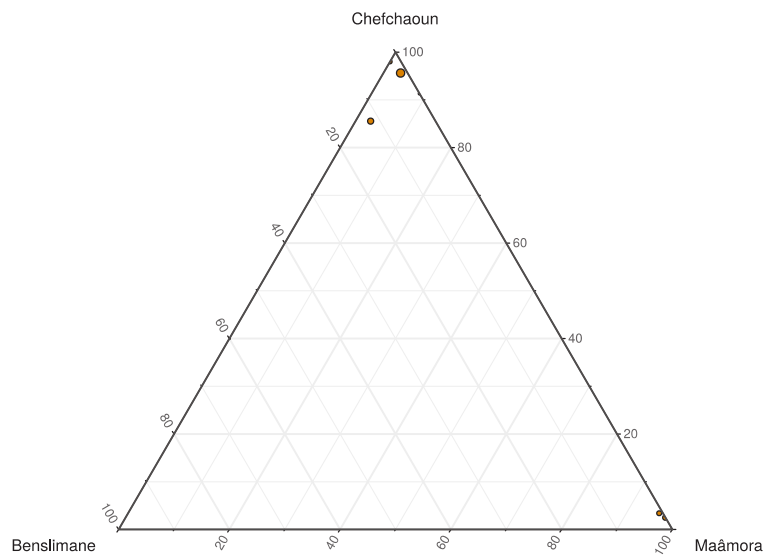
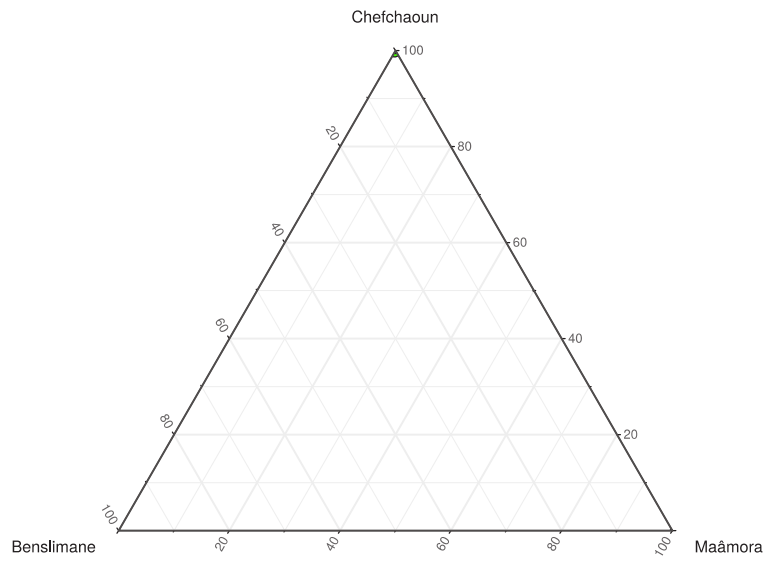


Figure II.S2. Continued.

Entoloma (12 OTUs, 1103 reads)



Hygrophorus (3 OTUs, 2643 reads)

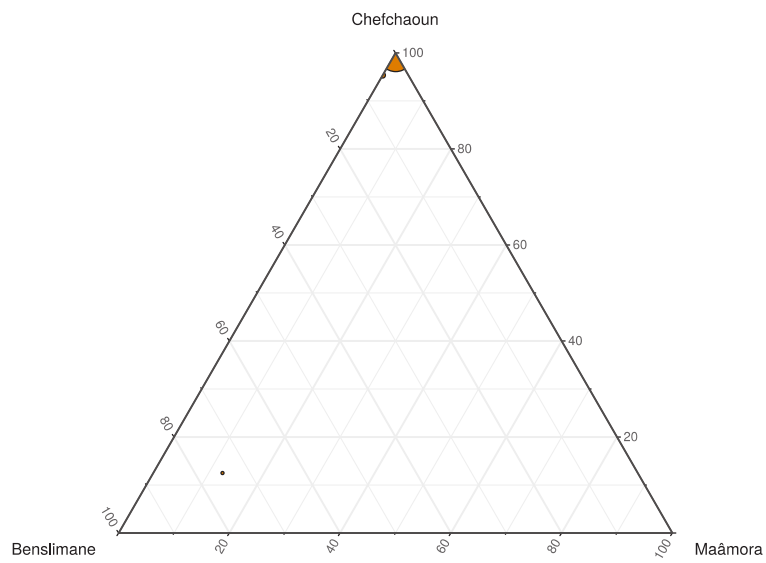
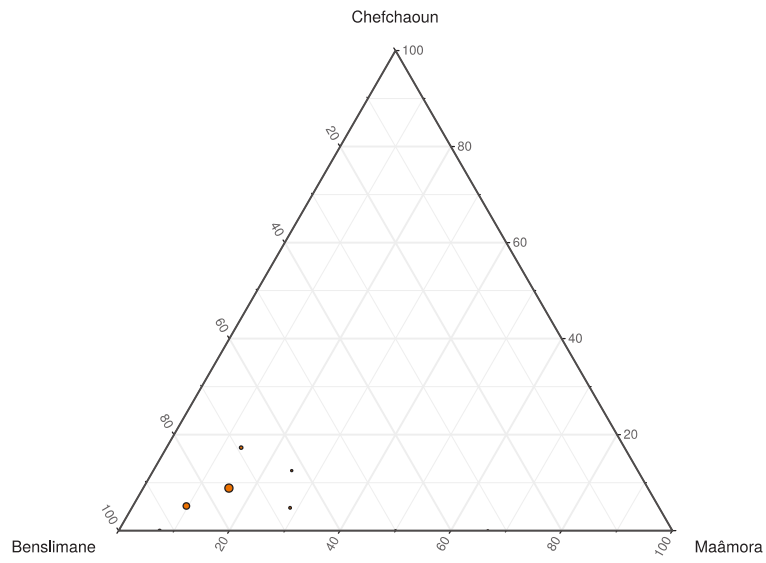


Figure II.S2. Continued.

Ilyonectria (24 OTUs, 1007 reads)



Inocybe (19 OTUs, 4744 reads)

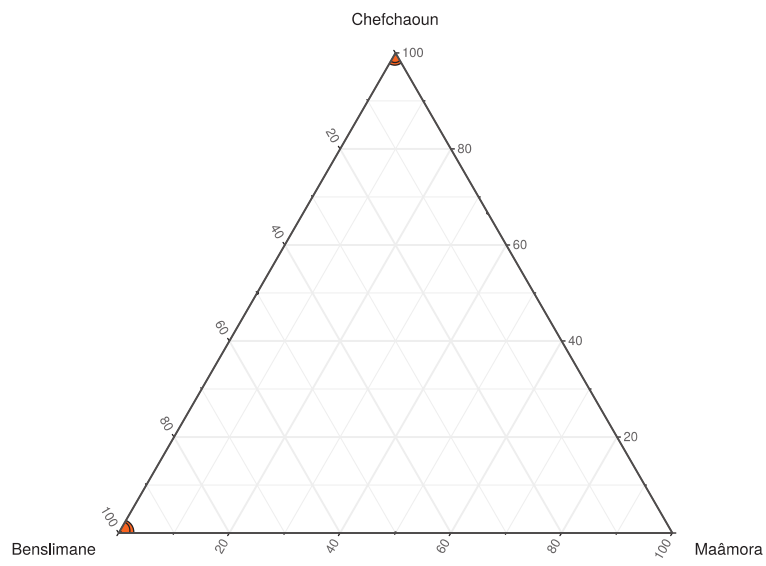
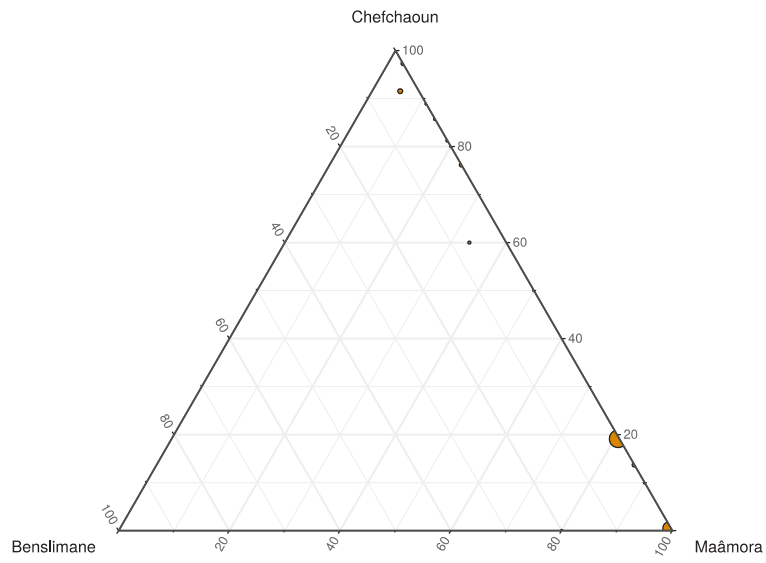


Figure II.S2. Continued.

Lactarius (47 OTUs, 2196 reads)



Lophiostoma (8 OTUs, 561 reads)

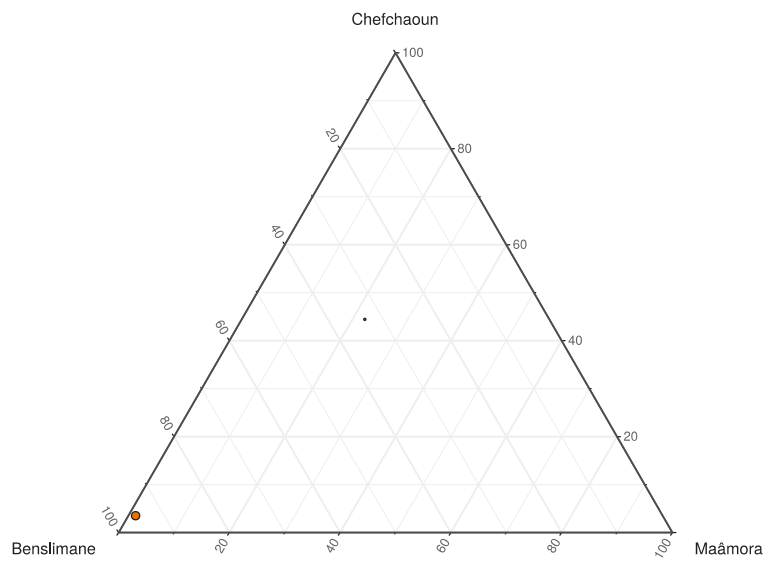
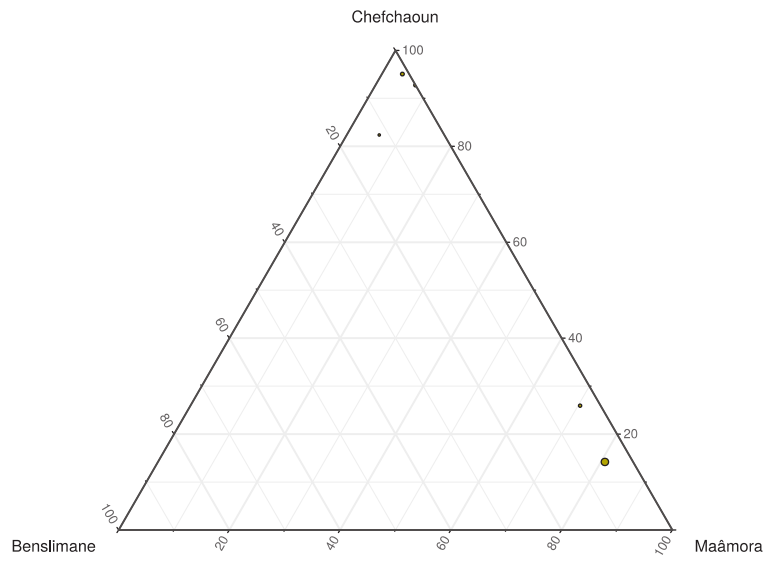


Figure II.S2. Continued.

Oidiodendron (25 OTUs, 751 reads)



Penicillium (76 OTUs, 1115 reads)

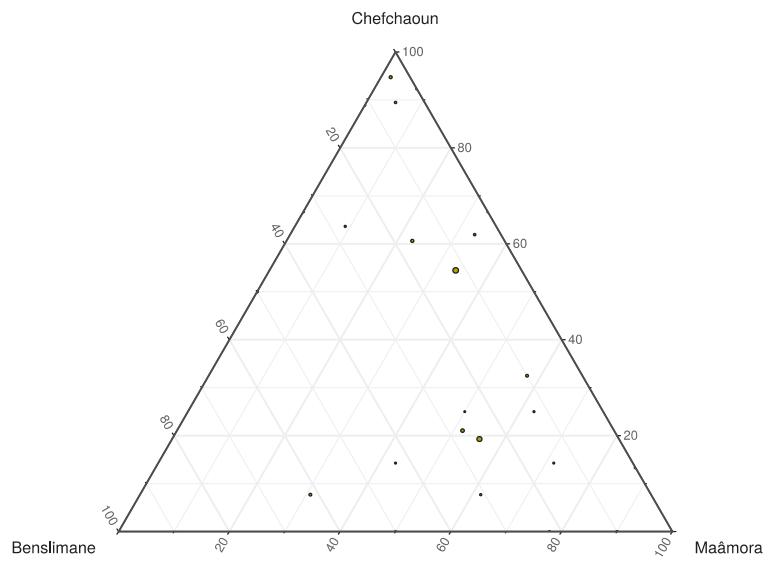
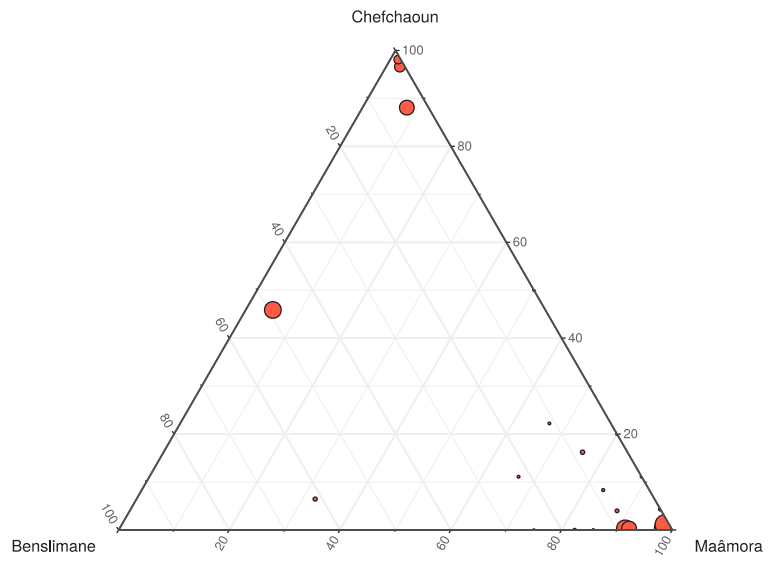


Figure II.S2. Continued.

Russula (64 OTUs, 7019 reads)



Sarcoleotia (4 OTUs, 398 reads)

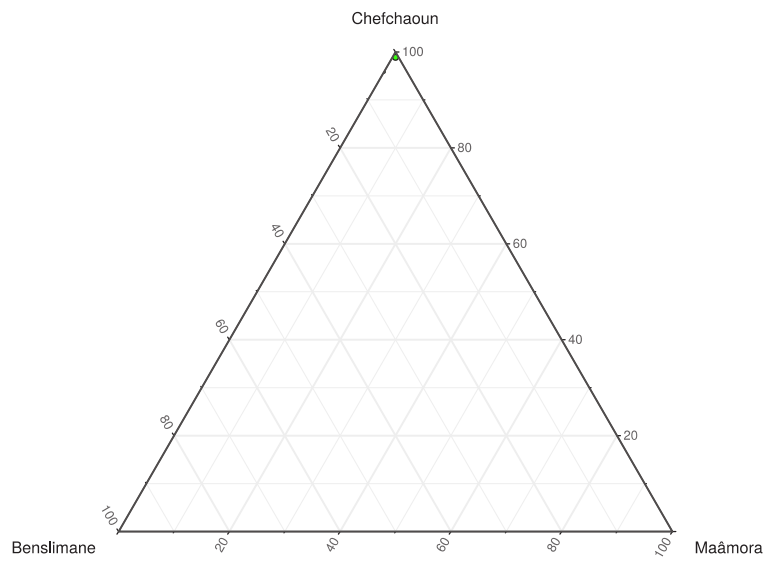
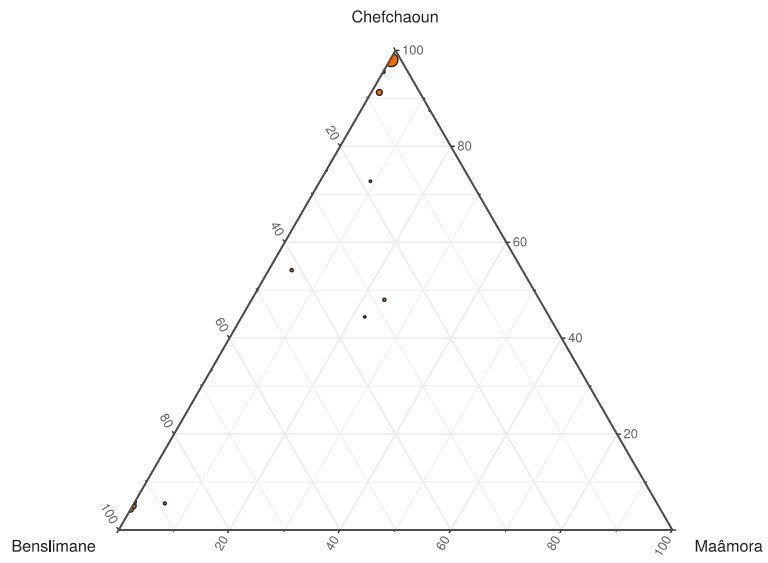


Figure II.S2. Continued.



Sebacina (41 OTUs, 1667 reads)



Tomentella (142 OTUs, 11609 reads)

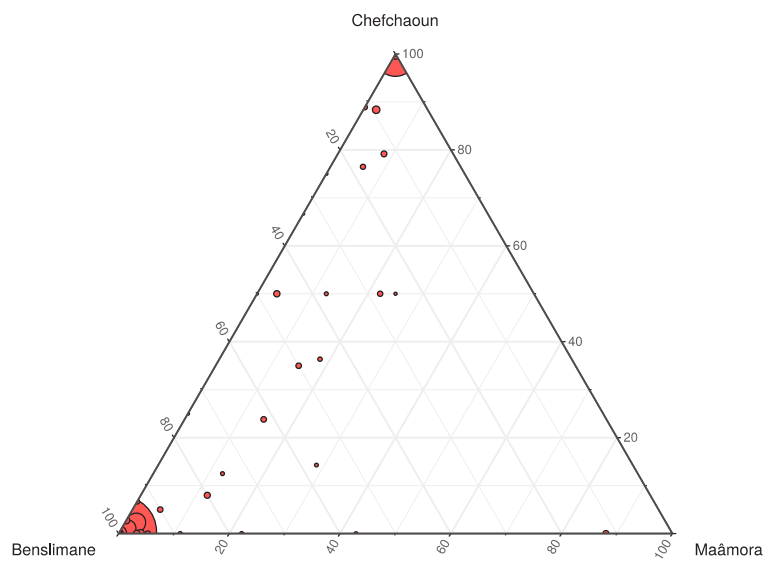
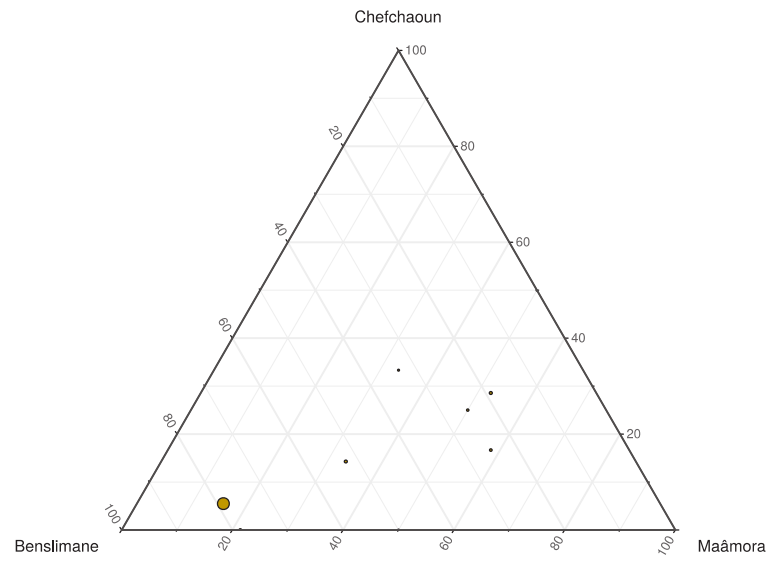


Figure II.S2. Continued.

Trichoderma (18 OTUs, 912 reads)



Umbelopsis (10 OTUs, 400 reads)

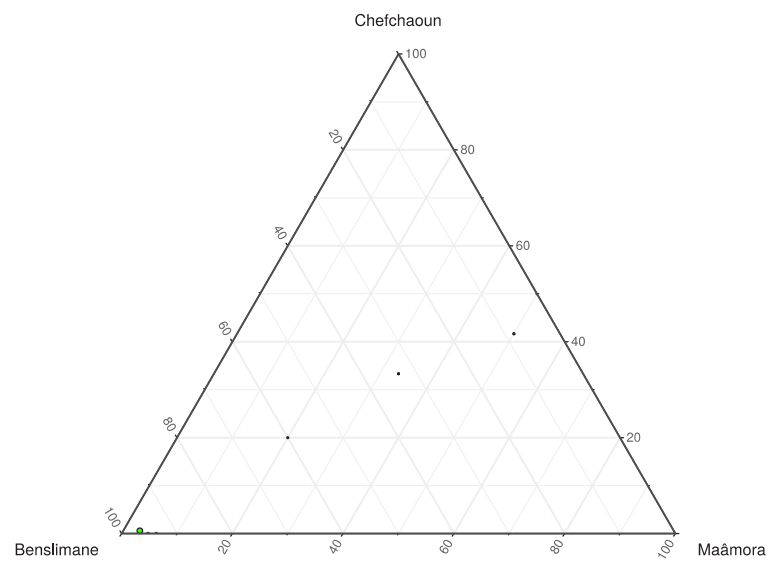


Figure II.S2. Continued.

**Table IV.S1.** Taxonomic affiliation of fungal OTUs associated with cork oak and the understorey vegetation in the Moroccan habitats<sup>1</sup>

<b>OTU</b>	<b>reads</b>	<b>phylum</b>	<b>class</b>	<b>order</b>	<b>family</b>	<b>genus</b>	<b>species</b>
11	27241	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum geophilum
17	16178	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella atramentaria
14	13817	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
16	13298	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp
26	7903	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp
28	7325	Ascomycota	Dothideomycetes	Pleosporales	Massarinaceae	Saccharicola	Saccharicola sp CBMAI 1030
37	7259	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Sebacinaceae sp
29	6389	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula sp
45	6322	Ascomycota	Dothideomycetes	Pleosporales	Lophiostomataceae	Lophiostoma	Lophiostoma cf cynaroidis A33
20	6268	Basidiomycota	Agaricomycetes	Agaricales	Hydnangiaceae	unidentified	Hydnangiaceae sp
32	6137	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum geophilum
30	5925	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	Inocybe	Inocybe subporospora
34	5403	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	Sebacina	Sebacina sp
36	5209	Ascomycota	Sordariomycetes	Hypocreales	Incertae_sedis	Ilyonectria	Ilyonectria estremocensis
33	5045	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	Cladophialophora sp
38	4933	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
25	4475	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula violeipes
62	4197	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum geophilum
44	4068	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	unidentified	Nectriaceae sp
49	3906	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	Cortinarius sp
51	3886	Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrophorus	Hygrophorus cossus
53	3713	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp
59	3653	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Cryptosporiopsis	Cryptosporiopsis brunnea
47	3578	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella sp
52	3480	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula sp
58	3360	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula sp
46	3236	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	unidentified	Herpotrichiellaceae sp
71	3043	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	unidentified

**Table IV.S1.** Continued<sup>1</sup>

<b>OTU</b>	<b>reads</b>	<b>phylum</b>	<b>class</b>	<b>order</b>	<b>family</b>	<b>genus</b>	<b>species</b>
74	3042	Ascomycota	Dothideomycetes	Pleosporales	unidentified	unidentified	Pleosporales sp
70	3034	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	Sebacina	Sebacina sp
56	3019	Ascomycota	Dothideomycetes	Hysteriales	Gloniaceae	Cenococcum	Cenococcum geophilum
69	2966	Ascomycota	Sordariomycetes	Hypocreales	Incertae_sedis	Ilyonectria	Ilyonectria mors-panacis
19	2834	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Tricholoma	Tricholoma columbetta
101	2816	Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	Clavulina	Clavulina sp
64	2778	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
100	2748	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula odorata
78	2746	Ascomycota	Dothideomycetes	unidentified	unidentified	unidentified	Dothideomycetes sp
91	2685	Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	unidentified	Sebacinaceae sp
60	2521	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Lactarius	Lactarius sp
95	2491	Ascomycota	Leotiomycetes	Helotiales	Dermateaceae	Cryptosporiopsis	Cryptosporiopsis brunnea
87	2487	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma erinaceum
66	2442	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella sp
61	2245	Ascomycota	Pezizomycetes	Pezizales	Pezizaceae	Terfezia	Terfezia pini
55	2205	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	unidentified	Thelephoraceae sp
72	2172	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	Russula	Russula sp
93	2169	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	unidentified	Russulaceae sp
82	2156	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Aspergillus	Aspergillus amstelodami
85	2133	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella sp
67	2104	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	Tomentella	Tomentella sp
88	2063	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp

<sup>1</sup> only the 50 most dominant OTUs out of 4837 are shown

**Table IV.S2.** Taxonomic affiliation of AM fungal OTUs associated with cork oak and the understorey vegetation in the Moroccan habitats<sup>1</sup>

OTU	reads	phylum	order	family	genus	species
4	22638	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus clarus
1	15134	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus intraradices
3	12374	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus irregularis
5	10218	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
26	8269	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
10	5454	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus irregularis
134	3527	Glomeromycota	Glomerales	Claroideoglomeraceae	Claroideoglomus	Claroideoglomus lamellosum
83	3094	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
77	2668	Glomeromycota	Diversisporales	Diversisporaceae	Redeckera	Redeckera fulva
9	2440	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
34	2360	Glomeromycota	Glomerales	Glomeraceae	Sclerocystis	Sclerocystis sinuosa
92	2357	Glomeromycota	Glomerales	Claroideoglomeraceae	Claroideoglomus	Claroideoglomus luteum
100	2240	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora schenckii
22	2227	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus intraradices
117	1979	Glomeromycota	Diversisporales	Gigasporaceae	Gigaspora	Gigaspora decipiens
47	1730	Glomeromycota	Diversisporales	Acaulosporaceae	Acaulospora	Acaulospora sieverdingii
129	1715	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora schenckii
45	1642	Glomeromycota	Diversisporales	Gigasporaceae	Gigaspora	Gigaspora gigantea
194	1518	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora schenckii
155	1386	Glomeromycota	Diversisporales	Diversisporaceae	Diversispora	Diversispora incertae sedis
57	1303	Glomeromycota	Diversisporales	Acaulosporaceae	Acaulospora	Acaulospora sieverdingii
165	1278	Glomeromycota	Diversisporales	Diversisporaceae	Redeckera	Redeckera fulva
131	1142	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora schenckii
99	1125	Glomeromycota	Glomerales	Glomeraceae	Glomus	Glomus macrocarpum
145	1107	Glomeromycota	Glomerales	Glomeraceae	Glomus	Glomus macrocarpum
97	978	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
238	952	Glomeromycota	Archaeosporales	Geosiphonaceae	Geosiphon	Geosiphon pyriformis
136	945	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus proliferus
228	920	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora trappei
82	793	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
251	766	Glomeromycota	Diversisporales	Diversisporaceae	Diversispora	Diversispora incertae sedis
88	726	Glomeromycota	Diversisporales	Gigasporaceae	Racocetra	Racocetra castanea
247	707	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
13	701	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus fascilatus
74	694	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
192	686	Glomeromycota	Glomerales	Glomeraceae	Glomus	Glomus incertae sedis

**Table IV.S2.** Continued<sup>1</sup>

<b>OTU</b>	<b>reads</b>	<b>phylum</b>	<b>order</b>	<b>family</b>	<b>genus</b>	<b>species</b>
140	598	Glomeromycota	Diversisporales	Gigasporaceae	Gigaspora	Gigaspora candida
148	581	Glomeromycota	Glomerales	Claroideoglomeraceae	Claroideoglomus	Claroideoglomus claroideum
90	575	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus intraradices
313	571	Glomeromycota	Diversisporales	Gigasporaceae	Scutellospora	Scutellospora calospora
176	560	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
29	536	Glomeromycota	Glomerales	Glomeraceae	Incertae sedis	Glomus iranicum
104	525	Glomeromycota	Glomerales	Glomeraceae	Rhizophagus	Rhizophagus fascilatus
403	496	Glomeromycota	Archaeosporales	Ambisporaceae	Ambispora	Ambispora callosa
153	490	Glomeromycota	Glomerales	Claroideoglomeraceae	Claroideoglomus	Claroideoglomus luteum
95	467	Glomeromycota	Glomerales	Glomeraceae	Sclerocystis	Sclerocystis sinuosa
260	455	Glomeromycota	Archaeosporales	Archaeosporaceae	Archaeospora	Archaeospora schenckii
689	440	Glomeromycota	Diversisporales	Diversisporaceae	Diversispora	Diversispora trimurales
25	437	Glomeromycota	Glomerales	Glomeraceae	Sclerocystis	Sclerocystis sinuosa
375	435	Glomeromycota	Diversisporales	Diversisporaceae	Diversispora	Diversispora incertae sedis

<sup>1</sup> only the 50 most dominant OTUs out of 1750 are shown