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# Host effects in high ectomycorrhizal diversity tropical rainforests on ultramafic soils in New Caledonia

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## ABSTRACT

Tropical rainforests have been thought to have low prevalence and diversity of ectomycorrhizal symbioses. However, to date, tropical regions have been poorly sampled for ectomycorrhizal fungi. Here, we investigated ectomycorrhizal fungal community diversity and the role of host plants in shaping this diversity in three main ultramafic rainforests in New Caledonia, an archipelago renowned for its exceptional plant diversity and recognized as a biodiversity hotspot. Sampling of ectomycorrhizal root tips and fruit bodies in *Nothofagus aequilateralis*-dominated, *Arillastrum gummiferum*-dominated and mixed rainforests showed high fungal diversity with, in total, 28 lineages and 311 operational taxonomic units (OTUs), of which 95% might be endemic. We also found that host preference and host density influenced ectomycorrhizal community composition and contributed to the high fungal diversity of New Caledonian rainforests. Finally, the *Cortinarius* lineage dominated the below- and above-ground communities, which suggests that this lineage plays a central role in ultramafic ecosystems functioning.

## 1. Introduction

Interactions between plant communities and soil microbial communities are important in ecosystem functioning (Wardle, 2004). Ectomycorrhizal fungi constitute an important component of the soil microbiota. These organisms form a mutualistic association with plant roots and are known to enhance water uptake, mineral nutrition and resistance to biotic and abiotic stresses of the host plant (Smith and Read, 2008). In return, carbohydrates resulting from plant photosynthetic activity are provided to the fungal symbiont. This association involves tree species that cover

large areas at temperate and boreal latitudes (e.g. in Pinaceae, Fagaceae and Betulaceae families) (Molina et al., 1992). In tropical regions, with the exception of diverse Dipterocarpaceae rainforests in Asia (Brearley, 2012), most tree species have been found to be associated with arbuscular mycorrhizas (Malloch et al., 1980; McGuire et al., 2008), suggesting that tropical rainforests are relatively impoverished in ectomycorrhizal associations. Nevertheless, tropical rainforests that are dominated at the canopy layer by a single species, or few species, frequently form ectomycorrhizal associations (McGuire, 2014). Monodominant rainforests are defined as rainforests for which more than 50% of the trees at the canopy layer are dominated by a single species (Connell and Lowman, 1989). Although monodominant rainforests were first seen as an anomaly (Hart, 1990), they are present in all tropical regions (Read and Hope, 1996; McGuire, 2014). Furthermore, these

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monodominant rainforests can cover small patches to large areas (up to several hundred of hectares) (F. Carriconde pers. obs.; Papineau, 1989). Thus, the ecological significance of ectomycorrhizal fungi in the functioning of tropical ecosystems may have been considerably underestimated. However, in comparison to the temperate regions, relatively few studies have been conducted on ectomycorrhizal fungi in the tropics (Alexander and Selosse, 2009). A key step towards understanding the ecological roles of ectomycorrhizal symbioses in natural ecosystems relies on the investigation of ectomycorrhizal fungal communities and their associated host plants, by characterizing fungal diversity and the factors shaping this diversity. Host density and host preference are two factors that can potentially influence ectomycorrhizal fungal diversity (Ishida et al., 2007; Smith et al., 2009; Gao et al., 2013; Tedersoo et al., 2014). Indeed, Gao et al. (2013) showed a positive correlation between the density of host plants and the diversity of ectomycorrhizal fungi in a Chinese subtropical rainforest. More recently, a similar relationship has been detected at a global scale, independent of latitude (Tedersoo et al., 2014). Regarding fungal-host preference and specificity, different and phylogenetically distant host plant species have been shown to harbour distinct ectomycorrhizal fungal communities (Ishida et al., 2007; Smith et al., 2009), although contrasting results have also been obtained. For example, investigations of below-ground ectomycorrhizal fungal communities (i.e. from ectomycorrhizas in the soil) in a lowland rainforest in South America dominated by three co-occurring legumes (Smith et al., 2011), and in a rainforest in West Africa dominated by five co-occurring Fabaceae and Phyllanthaceae tree species (Die' dhiou et al., 2010; Peay et al., 2015), revealed high fungal diversity but no host preference. Similarly, the symbiotic communities associated with a dipterocarp species and a Fabaceae species in South America, displayed high below-ground diversity, with most fungal species detected as host-generalists (Smith et al., 2013). Conversely, in an Ecuadorian rainforest, Tedersoo et al. (2010b) showed a low species diversity of ectomycorrhizal fungi and biased association with Polygonaceae and Nyctaginaceae hosts. These contrasting results, plus the paucity of studies, highlight the need for additional research characterizing the diversity and the underlying mechanisms in different ecosystems in the inter-tropical zone.

New Caledonia is a tropical archipelago located in the South West Pacific (Fig. S1), renowned for its exceptional biodiversity (Chazeau, 1993; Morat et al., 2012). This high biological diversity has been particularly attributed to the long-term isolation of New Caledonia and the variety of soils that have developed over geological time (Morat et al., 2012; Isnard et al., 2016). The main island of New Caledonia (called "Grande Terre") underwent a complete submersion during the Paleogene and re-emerged in the late Eocene, approximately 37 Mya (Pelletier, 2006). The obduction event that led to this re-emergence drove a part of the oceanic lithospheric mantle upwards, giving rise to the ultramafic bedrock that may have covered the entire main island. At present, after erosion and fragmentation, ultramafic soils cover one third of the territory and are represented by numerous massifs (Fig. S1). The "Massif du Grand Sud", with a surface of 3015 km<sup>2</sup>, constitutes the largest continuous ultramafic outcrop known worldwide (Guillot and Hattori, 2013). Ultramafic soils, characterized by low nutrient availability and high heavy metal concentrations, harbour diverse vegetation types and a remarkable plant diversity (Jaffre' and L'huillier, 2010). Rainforests on New Caledonian ultramafic soils display an endemism rate of 83% (Isnard et al., 2016). The ultramafic flora represents a large fraction of the total flora and contributes disproportionately to the high endemism rate of the archipelago (Isnard et al., 2016). *Nothofagus* species (Nothofagaceae) and the monotypic species *Arillastrum gummiferum* (Myrtaceae)

(commonly called "che^ne gomme") are ectomycorrhizal trees endemic to New Caledonia found on ultramafic soils. Both species can dominate the uppermost canopy of ultramafic rainforests and are common components of the ultramafic landscape (Virot, 1956; Papineau, 1989; McCoy, 1992; Read and Hope, 1996; Demenois et al., 2017). They can form small to large (<1 ha to numerous hectares) monodominant stands, which are frequently within or adjacent to mixed rainforests (F. Carriconde pers. obs.; Read and Hope, 1996). Plant community analyses suggest that *Nothofagus* and *A. gummiferum* formations are early forest successional stages that, in the absence of disturbance, develop into mixed rainforests (Read and Jaffre', 2013; Demenois et al., 2017). Cyclones are likely the main source of disturbance, leading to the persistence of *Nothofagus* rainforests (Read and Jaffre', 2013), whereas *A. gummiferum* rainforests are likely to be maintained by wildfire regimes (Demenois et al., 2017). *N. aequilateralis* and *A. gummiferum* also differ in the structure of their monodominance. Indeed, *N. aequilateralis* is characterized by a higher stem density of individuals of smaller diameters, whereas *A. gummiferum* is characterized by fewer but larger stems (Demenois et al., 2017).

In contrast to plant diversity, which is relatively well-documented (e.g. Morat et al., 2012; Isnard et al., 2016), information on fungal diversity in New Caledonia is relatively sparse (Horak, 1981; Horak and Mouchacca, 1998; Mouchacca, 1998; Ducouso et al., 2004, 2009; Perrier, 2005; Bordez et al., 2016; Gourmelon et al., 2016). Recently, Bordez et al. (2016) and Gourmelon et al. (2016) investigated soil fungal diversity in New Caledonian ultramafic soils using next generation sequencing approaches. Both studies revealed that soil fungal communities are species rich and structured by vegetation type, suggesting the potential influence of host preference. Gourmelon et al. (2016) also suggested that dispersal limitation and interactions with soil bacteria influence fungi in the soil. Despite these findings, no large molecular study has directly investigated ectomycorrhizal fungal communities in rainforests in this biodiversity hotspot. One preliminary study tried to investigate specifically the ectomycorrhizal fungal diversity in a New Caledonian rainforest, but with a very limited number of samples (8 ectomycorrhizal root tips and 27 fruit bodies successfully genotyped) (Perrier, 2005). Furthermore, in New Caledonia, as more generally in the tropics, molecular data on fungi are very scarce. High quality and reference DNA sequences are thus needed to complement next generation sequencing approaches (Gourmelon et al., 2016).

In the present work, ectomycorrhizal fungal diversity of three main types of rainforests on ultramafic soils were investigated. Two of the rainforests were dominated by a single phylogenetically distant tree species, either *Nothofagus aequilateralis* (Nothofagaceae) or *Arillastrum gummiferum* (Myrtaceae). As previously indicated, these two formations also differ in their stem density (Demenois et al., 2017). These monodominant rainforests were adjacent to the third mixed rainforest formation, which can be characterized by the occurrence of isolated known ectomycorrhizal trees (i.e. *N. aequilateralis*, *N. balansae* or *A. gummiferum*) or their presence in the vicinity. We thus asked the following questions: what is the ectomycorrhizal fungal richness and composition within the three rainforest types studied and are there any differences between them? Does host plant influence ectomycorrhizal fungal richness and community composition? That is, do fungi show a host preference pattern and, secondarily, is ectomycorrhizal fungal diversity related to ectomycorrhizal host plant density? To address these questions, both below-ground (ectomycorrhizas in the soil) and above-ground (fruit bodies) fungal communities were characterized by sequencing the internal transcribed spacer (ITS), a region recognized as a DNA barcode for species identification in fungi (Schoch et al., 2012).

## 2. Materials and Methods

### 2.1. Study sites

This study was conducted at three sites in the south of the main island: at Col de Mourange (22°12'S, 166°40'E), Col de Yate' (22°10'S, 166°53'E) and Pic du Grand Kaori (22°16'S, 166°53'E) (Fig. S1). These sites are located at altitudes of ca. 2600–3500 m on the Massif du Grand Sud. These areas receive approximately 2800–3400 mm rainfall per year (AURELHY model, METEO-FRANCE), with a mean annual temperature of 22 °C (<http://worldclim.org/bioclim>). The three types of rainforests studied, i.e. monodominant *Nothofagus aequilateralis* rainforest, monodominant *Arillastrum gummiferum* rainforest and mixed rainforest, are present at each site. At Col de Mourange and Pic du Grand Kaori, the different formations occur within the same rainforest patch, which is surrounded by a shrubland vegetation, called 'maquis'. At Col de Yate', the *A. gummiferum*-dominated stand is disconnected by 2 km from the two other plant formations.

In mixed rainforests, some of the most abundant plant species were as follows: *Archidendropsis granulosa* (Fabaceae), *Calophyllum caledonicum* (Calophyllaceae), *Cordia jaffrei* (Cunoniaceae), *Gastro-lepis austrocaledonica* (Stemonuraceae), *Montrouzieria gabriellae* (Clusiaceae), *Myodocarpus fraxinifolius* (Myodocarpaceae) and *Syzygium brongniartii* (Myrtaceae). At Col de Mourange and Col de Yate', isolated trees of *A. gummiferum* were present. *Nothofagus balansae* trees were observed in the vicinity of the Col de Yate' mixed-stand.

### 2.2. Ectomycorrhizal roots and fruit bodies sampling

At the Col de Mourange, Col de Yate' and Pic du Grand Kaori sites, and within the three rainforest types, 30 m × 30 m plots were established (Fig. S2), providing 9 plots in total. Within these plots, soil cores were collected, following a sampling protocol modified from Peay et al. (2010). The protocol consisted of nested squares with sides of 2.5, 5, 10, 15 and 20 m (Fig. S2). The largest square (20 m × 20 m) was centred within the plot of 30 m × 30 m (Fig. S2). Soil samples (5 cm diameter × 10 cm depth) were taken at the shared zero point and at the corners of each square after removing the litter (Fig. S2). Sixteen soil cores were consequently harvested per plot, corresponding to a total of 144 samples. Sampling was undertaken during the cooler season (April to July) in 2012 for *Nothofagus* and mixed stands and in 2013 for *A. gummiferum* stands. Each individual core was placed in a separate plastic bag and stored at 4 °C until processing. In the three following days, roots were laid on a sieve and carefully washed under tap water. Cleaned roots from a sampling core were placed in a Petri dish containing deionized water and examined under a dissecting microscope. Roots were identified as ectomycorrhizal based on physical characteristics, in particular the presence of a fungal mantle and extramatricial hyphae. The viability of ectomycorrhizas was based on their colour and turgescence. To represent the below-ground diversity, all morphotypes were collected. Each sampled ectomycorrhiza consisted of a single root tip that was placed independently in an Eppendorf tube containing CTAB 2x buffer (100 mM Tris HCl (pH 8), 1.4 M NaCl, 20 mM Na<sub>2</sub> EDTA, 2% CTAB). Samples were stored at -20 °C until DNA extraction.

The fruit body survey was conducted on the 30 m × 30 m plots from April 2013 to April 2014. Every 3–4 weeks, for 1.5 h, three people collected epigeous fruit bodies belonging to putative ectomycorrhizal genera. Twelve sampling rounds were thus performed per plot. Due to the large gap in knowledge in tropical regions (Alexander and Selosse, 2009) and especially in New Caledonia (Jourand et al., 2014), specimens of uncertain generic identification

were also collected. Whether they could be identified to the species level or not, fruit bodies were assigned to 'morphospecies'. The abundance of each 'morphospecies' was recorded by counting the number of fruit bodies per "morphospecies". Collected fruit bodies were photographed in the laboratory. Small amounts of fresh tissues were stored in CTAB 2x buffer at -20 °C. The remainder of each fruit body was air dried at 45 °C for 72 h and lodged at the IAC herbarium.

### 2.3. Molecular protocols and fungal identification

DNA extraction was performed on ectomycorrhizas and fruit bodies using the Wizard genomic DNA purification kit (Promega) following the protocols of Carriconde et al. (2008a) and Carriconde (2008), respectively. For ectomycorrhizas, extractions were performed on single root tips. Prior to performing Polymerase Chain Reaction (PCR), extracted DNA was diluted at a ratio of 1:20 (v/v) for ectomycorrhizas and at 10 ng/ml for fruit bodies. Regarding fruit bodies, for each site and vegetation type, one sample per morphotype and per sampling date was retained for DNA extraction. The nuclear rDNA internal transcribed spacer (ITS) was amplified using the fungus-specific primer ITS1f (Gardes and Bruns, 1993) and either the basidiomycetes-specific LB-W or the ascomycetes-specific LA-W primer (Tedersoo et al., 2008). PCR reactions were performed as described in Carriconde et al. (2008b). Sequencing was conducted with the primers ITS1 (White et al., 1990) or ITS1f and the reverse primer ITS4 (White et al., 1990) at the 'Plate-Forme du Vivant de la Nouvelle-Calédonie' (PFV-NC) (Nouméa, New Caledonia) using the Big Dye Sequencing Kit v.3.1 (Applied Biosystems, Foster City, CA, USA). Generated sequences were edited with Sequencher v.4.10.1 (Gene Codes Inc., Ann Arbor, MI, USA).

Taxonomic assignment was carried out by comparing all ITS sequences to UNITE and INSD databases, using the MassBLASTER module implemented in PlutoF 2.5 (Abarenkov et al., 2010). Some root tips were identified as endophytic (e.g. *Oidiodendron*) or saprotrophic species (e.g. *Mycena*) and consequently excluded from the dataset. Thereafter, sequences were partitioned into operational taxonomic units (OTUs), using a cut off of 97% sequence identity (e.g. Nilsson et al., 2008). For *Cortinarius* a 98% identity was applied due to the fact that the ITS region is more conserved in this genus (Stefani et al., 2014). After removing the flanking 18S and 28S rDNA partial sequences with the ITSx module from PlutoF v.2.5, OTUs were delineated using the software cd-hit-est ([http://weizhonglab.ucsd.edu/cdhit\\_suite/](http://weizhonglab.ucsd.edu/cdhit_suite/)) (Huang et al., 2010). OTUs were checked by aligning the sequences belonging to a same lineage (see below) using the software MEGA v.6 (Tamura et al., 2013) and manually adjusted. Final taxonomic assignment was performed by combining BLAST and OTU clustering.

Taxa were assigned to ectomycorrhizal phylogenetic lineages defined by Tedersoo et al. (2010a) and Tedersoo and Smith (2013). These lineages correspond to monophyletic groups that have independently evolved the ability to form ectomycorrhizal symbiosis. Several of our sequences belonged to "groups" constituted by both ectomycorrhizal and non-ectomycorrhizal lineages: *Catathelasma*, *Sistotrema*, *Xenasmatella*, *Atheliales*, *Helotiales*, *Agaricomycetes* and *Sordariomycetes*. In order to determine whether these sequences fit into known lineages within these "groups", phylogenetic trees were constructed for all these fungal groups independently. Reference sequences (e.g. according to Moncalvo et al., 2006; Tedersoo et al., 2009; Tedersoo and Smith, 2013) were downloaded from GenBank or UNITE databases and aligned with sequences from this study. To improve the quality of the analyses, due to the occurrence of multiple insertions and/or deletions in the ITS alignment, a gap cut off of 0.5 was used. Briefly, best-fit models for each fungal group were identified using

jModelTest v.2.1.4 (Darriba et al., 2012) and Bayesian Markov Chain Monte Carlo (MCMC) analyses were performed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003). Sequences that do not phylogenetically fit to known ectomycorrhizal lineages were not considered for further analyses.

#### 2.4. Molecular protocols and plant host identification

To confirm that the dominant plant species, i.e. *N. aequilateralis* and *A. gummiferum*, were indeed the ectomycorrhizal hosts sampled in the related monodominant stands, 10 samples were selected per formation and per site for molecular identification. These samples originated from different root fragments (i.e. only one root tip retained per root fragment) and corresponded to root tips previously successfully typed in regard to fungal partners. In mixed stands, due to the low occurrence of putative ectomycorrhizas in soil cores, one sample per root fragment and for each fungal OTU was selected. Therefore, 19 samples from Col de Mouirange, 19 from Col de Yate' and 9 from Pic du Grand Kaori were retained, totalling 47 samples for mixed rainforest. Out of the 587 root tips identified as ectomycorrhizal fungi (see Results section), 107 samples (60 from monodominant and 47 from mixed stands) were submitted to PCR reactions for plant identification.

For comparison, leaves from the 3 following ectomycorrhizal plant species were collected in the field: *N. aequilateralis* (Pic du Grand Kaori site), *N. balansae* (Foret de la Superbe site, 22°09.210'S, 166°35.630'E), and Pic du Pin site, 22°14.742'S, 166°49.574'E), and *A. gummiferum* (Col de Yate' site). Plant DNA was extracted using DNeasy Plant Mini Kit (QIAGEN) according to Doyle and Doyle (1990).

We tested the trnL chloroplast region and the ITS plant region on ectomycorrhizal root tips for plant host identification. No clear PCR products were obtained from the trnL region and thus identification of the host plant was thus performed amplifying the ITS plant region (primer pair P17 and 26S-82R) (Popp and Oxelman, 2001). PCR reactions were performed as indicated in Barrabe' (2013). Sequencing was carried out at the 'Plate-Forme du Vivant de la Nouvelle-Calédonie' (PFV-NC). ITS host plant sequences were compared to GenBank and the three ectomycorrhizal plants collected in the field.

#### 2.5. Community statistical analyses

Levels of diversity of below-ground (i.e. ectomycorrhizas) and above-ground (i.e. fruit bodies) communities were assessed by calculating species richness (S), Chao index that corresponds to the expected species richness, Shannon-Wiener diversity index (H), Simpson diversity index (1-D) and Pielou's evenness (J). Species accumulation curves were also constructed. The extrapolated species richness Hill number and its confidence interval at the 95% level were calculated (Chao et al., 2014). Calculations were performed (i) independently for each plot, (ii) by plant formation type (i.e. grouping of plots belonging to the same plant formation), (iii) by site (i.e. grouping the 3 distinct plant formations by site), and (iv) on the whole below-ground community or above-ground community (i.e. by grouping the ectomycorrhizas or the fruit bodies from the 3 rainforest types and the 3 sites).

To determine similarity between communities, the Bray-Curtis dissimilarity index was computed on below- and above-ground data sets. Based on this index, non-metric multidimensional scaling (NMDS) was used to visually represent ectomycorrhizal fungal similarities between plots. To test the effect of plant formation and site, PERMANOVA were performed using 999 permutations on the Bray-Curtis measurement. Correlations between ectomycorrhizal host density and fungal diversity were performed

using Spearman correlation tests, independently for ectomycorrhizas and fruit bodies.

All statistical analyses were carried out with R v.3.2.2 (R Core Team, 2015) with RStudio v.0.99.485 (RStudio Team, 2015). The following R packages were used: Vegan (Oksanen et al., 2015), iNEXT (Chao et al., 2014; Hsieh et al., 2016), ggplot2 (Wickham, 2009), VennDiagram (Chen, 2015) and psych (Revelle, 2018).

### 3. Results

#### 3.1. Ectomycorrhizal fungal diversity

The ITS region was successfully sequenced from 587 ectomycorrhizal root tips that were clustered into 126 OTUs (Table 1), including 47 singletons (OTUs represented by only one sample). For fruit bodies, the ITS was sequenced from 480 representative samples (see Materials and Methods), and according to the abundance recorded in the field, OTUs were consequently attributed to 2369 samples (Table 1). In total, 235 OTUs were delineated from fruit bodies (Table 1), and 83 corresponded to singletons. Comparison between ectomycorrhizas and fruit bodies revealed a total number of 311 distinct OTUs (Table S1), with 50 OTUs (16%) shared between both communities (Fig. 1). Thus, 185 and 76 OTUs were observed only from ectomycorrhizas and fruit bodies, respectively. Combining both communities, within *N. aequilateralis*, *A. gummiferum* and mixed rainforests, 167, 129 and 100 ectomycorrhizal fungal OTUs were detected, respectively.

Out of the 311 OTUs detected, only 20 showed a species level match to sequences available in the UNITE database (98% for the Cortinariaceae and 97% for the others genera). The matched specimens originated from the Australasian region (New Caledonia, Australia, New Zealand and Papua New Guinea) and other regions of the world (China, Madagascar, Estonia and Finland). Five samples matched described species, namely *Cantharellus decolorans*, *Cortinarius picoides*, *Lactarius clarkeae*, *Sebacina dimitica* and *Sistotrema muscicola* (Table S1). Of these 20 OTUs, 15 showed a correspondence with samples collected outside New Caledonia. Thus, 296 OTUs (ca. 95%) have so far only been recorded in New Caledonia.

Regarding species richness across the different sites, a higher number of OTUs was detected at Col de Mouirange and Col de Yate', in a decreasing order, within *N. aequilateralis*, *A. gummiferum* and mixed stands (Table 1). Expected species richness (Chao index), Shannon index (H) and Simpson index (1-D) revealed the same pattern at both sites, i.e. an apparent greater below-ground ectomycorrhizal species diversity within *N. aequilateralis* stands, followed by *A. gummiferum* and mixed rainforests (Table 1). At Pic du Grand Kaori, the highest richness was observed under both *N. aequilateralis* and *A. gummiferum* (Table 1). The Shannon index was higher in the *A. gummiferum* formation and the Simpson index was equal to that in the mixed formation. However, there is no clear trend in the expected species richness (Chao index). As seen for Col de Mouirange and Col de Yate' sites, when analyses were performed by plant formation types (by grouping plots belonging to the same plant formation), greater values of species richness, Shannon index and expected species richness (Chao index) were obtained for *N. aequilateralis*, followed by *A. gummiferum* and then mixed rainforests (Table 2). This tendency was corroborated by the extrapolated species richness (Hill number) (Table 2) and the related species accumulation curves (Fig. 2A).

As observed in the below-ground community, comparing fruit bodies from the different plots, ectomycorrhizal fungal diversity tended to be highest within monodominant *N. aequilateralis* rainforests (Table 1). Indeed, a higher number of OTUs was detected at all sites under *N. aequilateralis* (Table 1). However, this higher diversity was confirmed by the Shannon and Simpson indices only at

Table 1

Summary of the diversity indexes calculated at each plot on the below-ground (mycorrhizas) and above-ground (fruit bodies) ectomycorrhizal fungal communities from three New Caledonian rainforest types (*Nothofagus aequilateralis*-dominated, *Arillastrum gummiferum*-dominated and mixed rainforests).

	n	nL	S	Chao ± s.e.	qD [LCL; UCL]	H	D	J
<b>Mycorrhizas</b>								
CM <i>Nothofagus aequilateralis</i>	68	8	34	83.27 ± 33.79	70.88 [37.20; 104.56]	3.29	0.95	0.93
CM <i>Arillastrum gummiferum</i>	145	8	23	27.14 ± 4.85	26.00 [19.94; 32.05]	2.74	0.92	0.87
CM Mixed	31	7	11	15.03 ± 4.74	15.03 [1.53; 28.53]	2.04	0.83	0.85
CY <i>Nothofagus aequilateralis</i>	54	7	25	57.06 ± 25.85	52.64 [23.77; 81.51]	2.98	0.94	0.93
CY <i>Arillastrum gummiferum</i>	77	6	19	22.55 ± 3.81	22.53 [12.78; 32.28]	2.43	0.87	0.82
CY Mixed	35	6	12	13.94 ± 2.58	13.94 [5.89; 22.00]	2.13	0.83	0.86
PGK <i>Nothofagus aequilateralis</i>	65	6	15	23.04 ± 8.16	22.69 [7.14; 38.23]	1.81	0.70	0.67
PGK <i>Arillastrum gummiferum</i>	103	9	15	21.19 ± 7.48	19.86 [12.90; 26.82]	2.25	0.87	0.83
PGK Mixed	9	7	8	29.78 ± 27.70	29.78 [7.66; 51.89]	2.04	0.86	0.98
TOTAL	587	24	126	187.26 ± 24.27	159.75 [140.78; 178.72]	4.22	0.98	0.87
<b>Fruit bodies</b>								
CM <i>Nothofagus aequilateralis</i>	497	14	63	111.90 ± 26.26	84.50 [68.49; 100.51]	3.01	0.91	0.73
CM <i>Arillastrum gummiferum</i>	133	9	38	73.83 ± 23.72	72.69 [37.24; 108.15]	2.88	0.88	0.79
CM Mixed	81	9	29	45.13 ± 11.57	45.13 [13.58; 76.68]	2.95	0.92	0.88
CY <i>Nothofagus aequilateralis</i>	684	11	40	51.98 ± 9.15	44.44 [37.42; 51.45]	2.04	0.75	0.55
CY <i>Arillastrum gummiferum</i>	202	8	33	77.90 ± 31.20	69.45 [37.54; 101.36]	2.33	0.82	0.67
CY Mixed	142	10	33	96.55 ± 55.61	82.65 [35.65; 129.65]	2.89	0.92	0.83
PGK <i>Nothofagus aequilateralis</i>	229	11	48	60.45 ± 8.42	60.23 [44.65; 75.80]	3.29	0.94	0.85
PGK <i>Arillastrum gummiferum</i>	126	6	32	41.31 ± 6.76	41.31 [20.99; 61.64]	2.64	0.84	0.76
PGK Mixed	275	8	25	46.05 ± 16.37	41.91 [28.89; 57.93]	1.35	0.50	0.42
TOTAL	239	17	235	333.96 ± 28.66	296.16 [270.83; 321.49]	4.04	0.95	0.74

CM: Col de Mouirange; CY: Col de Yate'; PGK: Pic du Grand Kaori.

n: number of samples; nL: number of lineages; S: species richness (number of OTUs); Chao ± s.e.: expected species richness and its standard error (s.e.); qD: Hill number, extrapolated species richness; LCL and UCL: lower and upper limits of the confidence interval at 95%; H: Shannon index; D: Simpson index; J: Pielou's evenness.

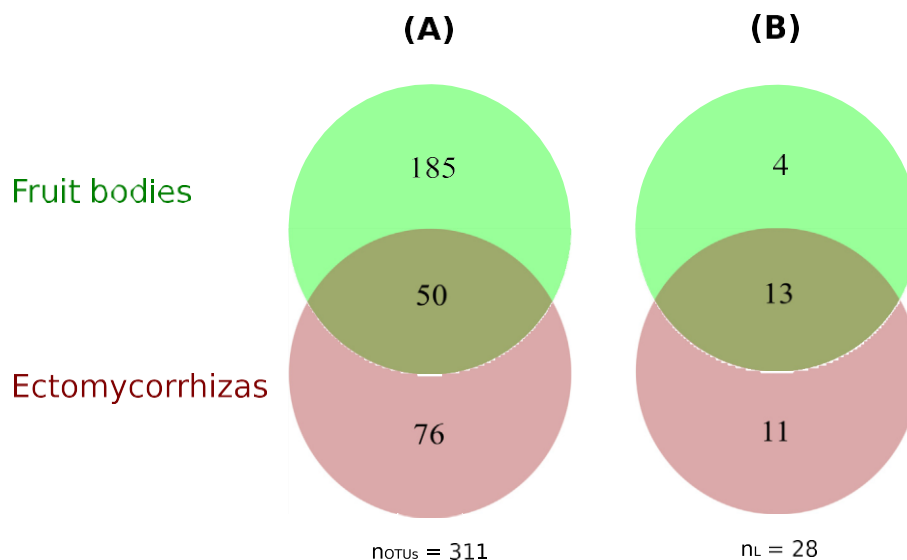


Fig. 1. Venn diagrams showing (A) the number OTUs and (B) the number of ectomycorrhizal lineages, from New Caledonian rainforests, shared and unique to the above-ground (fruit bodies) and below-ground (ectomycorrhizas) communities. The total number of OTUs (nOTUs) and lineages (nL) are given at the bottom.

Pic du Grand Kaori. Extrapolated species richness (Chao index) corroborates the existence of a higher diversity of ectomycorrhizal fungi in *N. aequilateralis* formations at Col de Mouirange and Pic du Grand Kaori, but not at Col de Yate' (Table 1). Finally, combining plots from the same plant formation also showed greater ectomycorrhizal fungal diversity within monodominant *N. aequilateralis* rainforests (Table 2). However, Hill numbers (Table 2) and species accumulation curves showed substantial overlap in richness estimates (Fig. 2B).

### 3.2 Ectomycorrhizal fungal lineages

The 311 distinct OTUs represented 28 well-identified

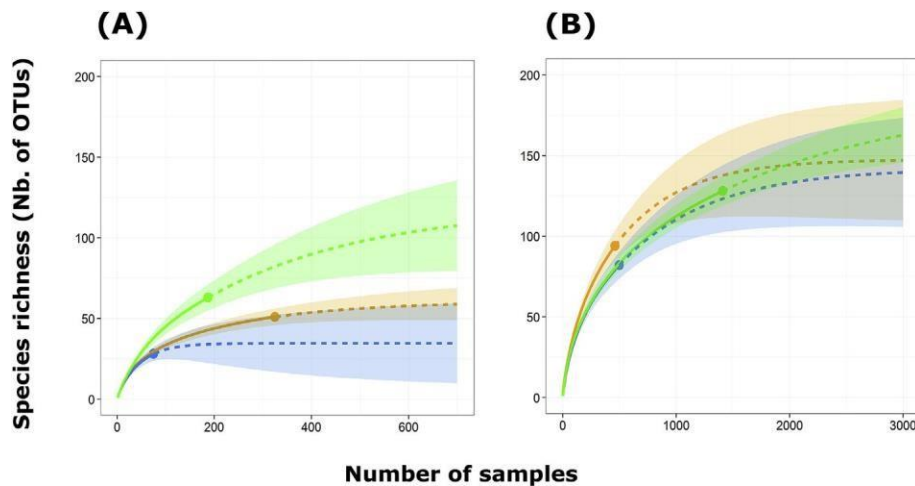
phylogenetic lineages. In addition to these 28 lineages, 1 OTU belonging to the Boletales and 2 OTUs in the Cantharellales could not be assigned at the lineage level. Out of these 28 lineages, 13 were shared between the root and fruit body communities, with 11 and 4 were unique to roots and fruit bodies, respectively (Fig. 1). The most striking finding was the overall large representation of the */cortinari* lineage in terms of abundance and OTU richness both below-ground and above-ground. This was true irrespective of the site location and the forest type (Fig. 3, Fig. S3, S4 & S5). The */cortinari* lineage represented 57.2% (336/587) and 55.8% (1322/2369) of root tips and fruit bodies, respectively. Regarding species richness, 53.2% (67/126) of below-ground and 47.2% (111/235) of above-ground OTUs, and 45.7% (142/311) of all OTUs were assigned

**Table 2**  
Summary of the diversity indexes calculated by plant formation type and by site, on the below-ground (mycorrhizas) and above-ground (fruit bodies) ectomycorrhizal fungal communities from three different rainforest types in New Caledonia (*Nothofagus aequilateralis*-dominated, *Arillastrum gummiferum*-dominated and mixed rainforests).

		n	nL	S	Chao ± s.e.	qD [LCL; UCL]	H	D	J
<b>Mycorrhizas</b>									
Plant formation	<i>Nothofagus aequilateralis</i>	187	14	63	119.56 ± 28.51	107.56 [79.53; 135.60]	3.54	0.94	0.85
	<i>Arillastrum gummiferum</i>	325	14	51	60.77 ± 6.83	58.89 [48.83; 68.96]	3.37	0.95	0.86
	Mixed	75	12	28	34.63 ± 5.25	34.63 [9.98; 59.29]	3.00	0.93	0.90
Site	CM	244	16	64	109.38 ± 24.65	80.55 [70.77; 99.34]	3.67	0.96	0.88
	CY	166	14	51	69.07 ± 10.63	67.11 [51.37; 82.85]	3.49	0.95	0.89
	PGK	177	14	37	69.22 ± 21.69	57.58 [42.11; 73.05]	2.90	0.91	0.80
<b>Fruit bodies</b>									
Plant formation	<i>Nothofagus aequilateralis</i>	1410	16	128	186.09 ± 22.87	162.76 [145.50; 180.01]	3.34	0.91	0.69
	<i>Arillastrum gummiferum</i>	461	11	94	147.66 ± 21.80	147.07 [109.83; 184.31]	3.5	0.93	0.77
	Mixed	498	14	82	142.05 ± 27.20	139.54 [105.73; 173.39]	2.99	0.84	0.68
Site	CM	711	15	112	162.88 ± 20.55	159.73 [131.16; 188.30]	3.67	0.95	0.78
	CY	1028	14	92	157.57 ± 30.15	135.99 [112.20; 159.78]	2.95	0.87	0.65
	PGK	630	12	95	125.37 ± 13.69	124.92 [96.28; 153.57]	3.30	0.89	0.73

CM: Col de Mouirange; CY: Col de Yaté; PGK: Pic du Grand Kaori.

n: number of samples; nL: number of lineages; S: species richness (number of OTUs); Chao ± s.e.: expected species richness and its standard error (s.e.); qD: Hill number, extrapolated species richness; LCL and UCL: lower and upper limits of the confidence interval at 95%; H: Shannon index; D: Simpson index; J: Pielou's evenness.



**Fig. 2.** Species accumulation curves of ectomycorrhizal fungal species richness (number of OTUs) for three rainforest types in New Caledonia for (A) ectomycorrhizas and (B) fruit bodies. *N. aequilateralis* rainforests, *A. gummiferum* rainforests and mixed rainforests are shown in green, orange and blue, respectively. Plain lines indicate interpolations, dashed lines indicate extrapolations and plain circles represent the observed species richness. 95% confidence intervals are also shown.

to *cortinarius*.

After *cortinarius*, the most OTU-rich lineages found below-ground were *russula-lactarius* (7.9%; 10 OTUs), *cantharellus* (4.8%; 6 OTUs), *clavulina* (4.8%; 6 OTUs), *tomentella-thelephora* (4.0%; 5 OTUs) and *boletus* (3.2%; 4 OTUs). For fruit bodies, they corresponded to *entoloma* (12.3%; 29 OTUs), *boletus* (8.1%; 19 OTUs), *russula-lactarius* (5.1%; 12 OTUs), *inocybe* (4.7%; 11 OTUs), *tricholoma* (4.7%; 11 OTUs), *amanita* (3.8%; 9 OTUs) and *coltricia* (3.8%; 9 OTUs).

### 3.3. Ectomycorrhizal fungal community structure

Few OTUs from root tips were shared between rainforest types (Fig. S5). The NMDS ordination, based on the Bray-Curtis dissimilarity index clearly showed that ectomycorrhizal fungal communities in the soil were distinct across the different rainforest formations (Fig. 4a). PERMANOVA analyses confirmed these visual differences. Indeed, the below-ground fungal community was significantly partitioned by the vegetation formation (31.4%; F-value ¼ 1.373; p-value ¼ 0.007), with no site effect (Table 3).

Regarding fruit bodies, in comparison to ectomycorrhizas, a

higher number of OTUs were shared between the three vegetation types (Fig. S5). The NMDS ordination showed a clear separation of the *N. aequilateralis* formations from the two other formations (Fig. 4B). The *A. gummiferum*-dominated and mixed stands overlapped. The PERMANOVA test indicated that the variance was not significantly partitioned by plant formation or by site (Table 3), although plant formation was marginally significant (27.8%; p-value ¼ 0.076). Significant correlations were obtained between host density and fungal below-ground richness ( $r = 0.72$ ; p-value ¼ 0.03) and above-ground fungal richness ( $r = 0.9$ ; p-value < 0.01). The targeted plant ITS was successfully sequenced from 26, 20 and 25 root tips collected under *N. aequilateralis*, *A. gummiferum* and mixed rainforests. Thus, in total 66.4% (71/107) of the samples were successfully sequenced. When comparing to GenBank database and sequences obtained from leaves, all ectomycorrhizas from *N. aequilateralis* and *A. gummiferum* mono-dominant vegetation types correspond to the dominant plant species, respectively, as *Nothofagus* and *A. gummiferum*. These results are not surprising because, except at Col de Mouirange where only one tree of *Melaleuca* was observed at the boundary of the *Nothofagus* plot, all ectomycorrhizal trees were identified as



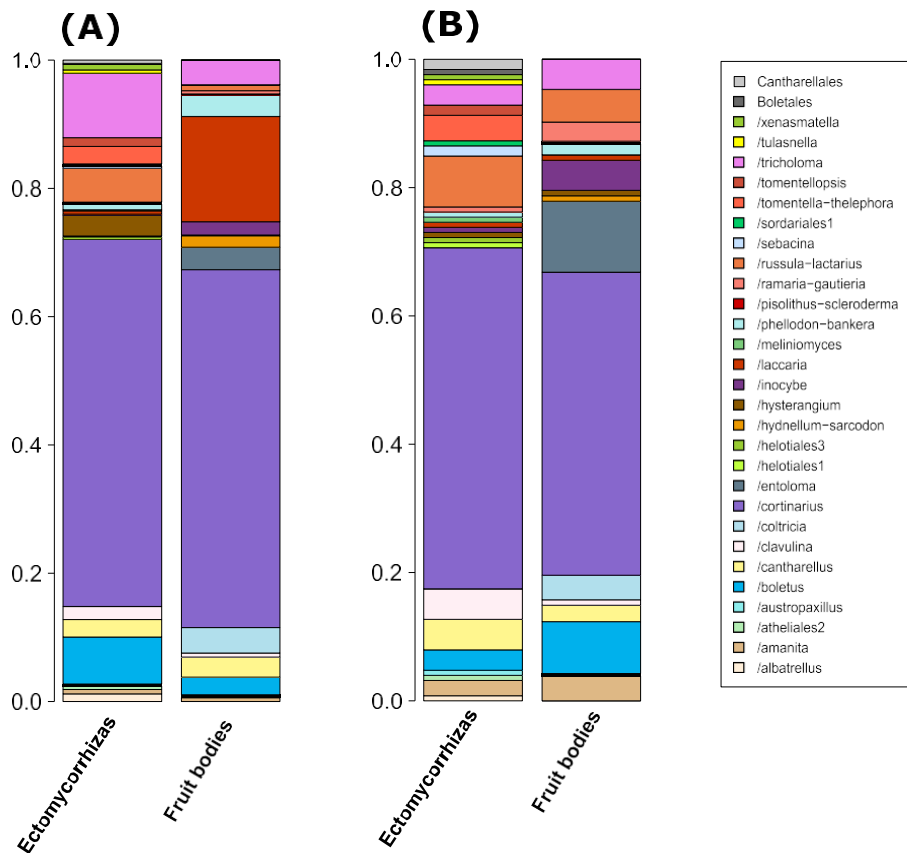


Fig. 3. Barplots of the (A) relative abundance and (B) relative number of OTUs of ectomycorrhizas and fruit bodies from three distinct rainforest types from New Caledonia.

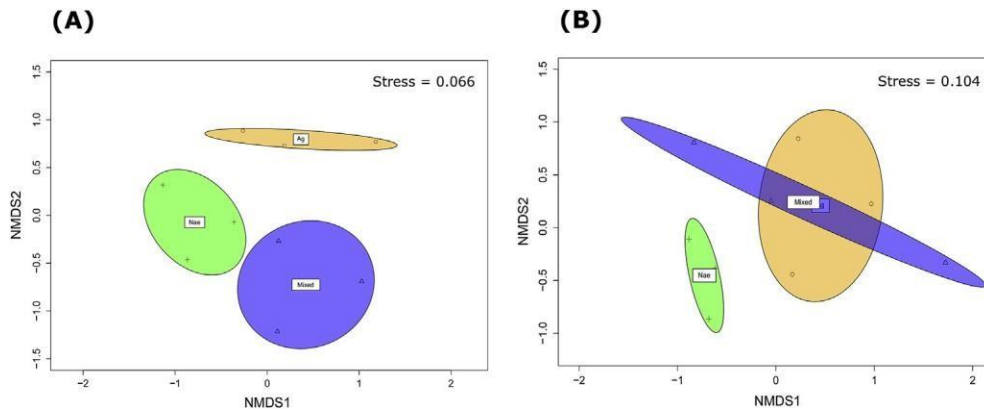


Fig. 4. NMDS ordination based on Bray-Curtis dissimilarity index on ectomycorrhizal fungal communities from three different New Caledonian rainforest types, for the (A) above-ground and the (B) below-ground communities. The 95% confidence intervals are represented in green, orange and blue for monodominant *Nothofagus aequilateralis* rainforests, monodominant *Arillastrum gummiferum* rainforests and mixed rainforests, respectively.

*N. aequilateralis* in *N. aequilateralis* monodominant forests and *A. gummiferum* in *A. gummiferum* monodominant forests.

In the mixed stand at Col de Mouirange, all ectomycorrhizas typed (15/15) were assigned to *A. gummiferum*. This is certainly due to roots coming from sparse trees of this species present in the plot and its vicinity. At Col de Yaté mixed stand, all ectomycorrhizas (9/9) belonged to *Nothofagus*. This might be due to the presence of *N. balansae* seedlings in the upper part of the plot, and also to the occurrence of some *N. balansae* trees in proximity. From the last site, namely Pic du Grand Kaori, only one root tip was successfully typed from the mixed stand. This sample showed a closest match

(90%) with *Weinmannia racemosa* (*Cunoniaceae*) from New Zealand, a genus represented by 5 endemic species in New Caledonia (Morat et al., 2012).

#### 4. Discussion

##### 4.1. New Caledonian ultramafic rainforests harbour a high and endemic fungal diversity

Pooling below- and above-ground communities, we detected 167, 129 and 100 ectomycorrhizal fungal OTUs in *N. aequilateralis*



Table 3

Results of PERMANOVA analyses testing the effect of plant formations and geographical origin (sites) on the structure of the below-ground (ectomycorrhizas) and above-ground (fruit bodies) fungal communities from the three different types of New Caledonian rainforests studied.

	Df	SS	Mean S	F-value	R <sup>2</sup>	P-value
<b>Mycorrhizas</b>						
Plant formation	2	1.157	0.579	1.373	0.314	0.007
Site	2	0.841	0.420	0.998	0.228	0.461
Residuals	4	1.685	0.421		0.458	
Total	8	3.683			1.000	
<b>Fruit bodies</b>						
Plant formation	2	0.988	0.494	1.143	0.278	0.076
Site	2	0.834	0.417	0.964	0.235	0.630
Residuals	4	1.730	0.432		0.487	
Total	8	3.552			1.000	

rainforests, *A. gummiferum* rainforests and mixed rainforests, respectively, which represent in total 311 OTUs. These OTUs belong to 28 distinct phylogenetic lineages. Such results strongly suggest the existence of a high ectomycorrhizal fungal species diversity and lineage diversity in these New Caledonian rainforests on ultramafic substrates. In comparison to other studies of ectomycorrhizal fungal diversity, these ecosystems seem to be as rich, or even richer, than other tropical and temperate forests (Richard et al., 2005; Walker et al., 2005; Nara, 2006; Ishida et al., 2007; Tedersoo et al., 2008; Dié'dhiou et al., 2010; Peay et al., 2010; Smith et al., 2011, 2013; Corrales et al., 2016; Horton et al., 2017). Interestingly, high fungal diversity in the tropics has often been encountered in monodominant and co-dominant rainforests (Peay et al., 2010; Smith et al., 2011, 2013; Corrales et al., 2016), a situation which is similar to that in temperate forests in terms of abundance of ectomycorrhizal trees.

Another striking finding in this study is the large proportion of OTUs, namely 95%, that lack species level matches to existing databases. If these molecular species are indeed only present in New Caledonia, this would indicate that New Caledonian ectomycorrhizal fungi display an endemism rate of 95%. Such high endemism is consistent with the high endemism rates encountered in other taxonomic groups, particularly in vascular plants with about 74% of species being endemic to the New Caledonian archipelago (Morat et al., 2012), with endemism rates about 90% in rainforests (Ibanez et al., 2018). However, it cannot be excluded that the same molecular species are present in the surrounding South West Pacific region (e.g. in Australia and New Zealand) but have not been sequenced or deposited yet in international sequence databases. Further large investigations in this biogeographic region are thus needed.

The long term geographic isolation and the complex geological history of New Caledonia are seen as factors that led to the exceptional New Caledonian biodiversity (Isnard et al., 2016). Since the re-emergence of the main island at the end of the Eocene, approximately 37 Mya, living organisms must have colonized the archipelago by long distance dispersal and multiple introductions. Adaptation or pre-adaptation (exaptation) to ultramafic substrates, founding effects, limitation of gene flow between populations, large ultramafic surfaces, habitat patchiness and interactions with host plants (e.g. Pillon et al., 2010; Barrabé et al., 2014; Isnard et al., 2016) may have contributed to the speciation and diversification of ectomycorrhizal fungi. Such high diversity strongly supports the idea that ultramafic substrates do not limit ectomycorrhizal fungal diversity but may even promote it (Branco, 2010). Due to its insular nature and its evolutionary history, New Caledonia may represent an outlier to the growing point of view that ectomycorrhizal fungal biodiversity displays a reversed latitudinal gradient (Tedersoo and

Nara, 2010; Tedersoo et al., 2012, 2014), i.e. that tropical regions harbour a lower ectomycorrhizal fungal diversity than temperate regions.

#### 4.2 The over-representation of the /cortinariu lineage

Another significant finding of our study is the over-representation of the /cortinariu lineage in terms of the number of root tips colonized and the number of OTUs, whether at the above-ground or below-ground community level and irrespective of the rainforest type. In a temperate rainforest in mainland Australia, *Cortinariu* was the second most abundant genus, colonizing 15% of *Nothofagus cunninghamii* seedling roots (Tedersoo, 2007 in Tedersoo et al., 2008), and the most species-rich genus, representing 33.3% of the ectomycorrhizal fungal species (Tedersoo et al., 2009). It was also the most diverse genus encountered on *Nothofagus solandri* roots in New Zealand (Dickie et al., 2010). The checklist of fungi collected under five *Nothofagus* species in New Zealand (McKenzie et al., 2000), as well as the survey of hypogeous fruit bodies in *N. dombeyi* and *N. pumilio* in south Argentina (Nouhra et al., 2012), revealed again the prominence of this lineage. These studies, along with our work, strongly suggest that /cortinariu is a dominant in *Nothofagus* rainforests, regardless of the geographical origin of the host plant. *A. gummiferum* is a member of the Eucalypteae tribe and the sister taxon to the eucalypts group (Ladiges and Cantrill, 2007). In Tasmanian *Eucalyptus delegatensis* forests, Horton et al. (2017) found that /cortinariu was again the most abundant and the most species-rich lineage in the below- and above-ground ectomycorrhizal communities. Such dominance, in terms of number of species, was also observed from fruit bodies in a *Eucalyptus obligata* forest (Gates et al., 2011). Similarly to *Nothofagus*, *Eucalyptus* species and the sister species *A. gummiferum* seem to share a common pattern.

The large dominance of /cortinariu in relative abundance or in number of species, suggests that members of this lineage may play central roles in ecosystem functioning in these forests. Some ectomycorrhizal fungi have retained, from their saprotrophic ancestors, the capacity to decompose soil organic matter via notably the production of extracellular oxidative enzymes, such as laccases and peroxidases (Lindahl and Tunlid, 2015; Shah et al., 2016). From humus samples collected in boreal pine forest and sub-arctic birch forest, Boëdeker et al. (2014) showed a significant correlation between the relative abundance of *Cortinariu* DNA and Mn-peroxidase activity. They also detected 11 genes encoding for peroxidases in the *Cortinariu glaucopus* genome (Boëdeker et al., 2014). It has been proposed that the benefit of decomposing organic matter arises primarily from the mobilization of nitrogen and not the acquisition of metabolic carbon (Lindahl et al., 2007; Shah et al., 2016). Such a strategy would allow ectomycorrhizal fungi and their host plants greater access to nitrogen particularly in nutrient poor soils, such as soils originated from ultramafic rocks. Interestingly, *Cortinariu* species were identified from root tips and extramatrical hyphae that were directly in contact with plant residues being degraded (Fig. S6). Members of this lineage may thus act as decomposers and participate actively in below-ground carbon dynamics in the New Caledonian rainforests.

In addition, the /cortinariu lineage may facilitate the recruitment and establishment of other ectomycorrhizal plants, influencing the plant community dynamics. Indeed, *N. aequilateralis* and *A. gummiferum* rainforests are early forest successional stages that develop into mixed rainforests (Read and Jaffré, 2013; Demenois et al., 2017). The potential role of /cortinariu in plant successional dynamics is also supported by the fact that within shrubland formations dominated by ectomycorrhizal plants, which represent early colonizing stages on ultramafic substrates, the /cortinariu

lineage also dominates the ectomycorrhizal fungal below-ground community (Gourmelon, 2016).

#### 4.3. A host effect on ectomycorrhizal fungal communities

Our results clearly showed that ectomycorrhizal fungal communities in New Caledonian rainforests are structured according to vegetation type. Indeed, NMDS and PERMANOVA analyses clearly distinguished *N. aequilateralis* fungal communities from those of *A. gummiferum*. This finding is in accordance with the hypothesis that phylogenetically distant host plants will harbour distinct ectomycorrhizal fungal communities (Ishida et al., 2007; Smith et al., 2009; Tedersoo et al., 2010b). Indeed, *N. aequilateralis* and *A. gummiferum* belong, respectively, to the Nothofagaceae (Fagales) and the Myrtaceae (Myrtales) (The Angiosperm Phylogeny Group, 2016). Host preference of fungi in New Caledonia was also hypothesized by Gourmelon et al. (2016). The authors showed that soil fungal communities differed by plant formation, including Nothofagus and mixed rainforests. They also suggested that microbial communities were influenced by the dominant plant species, such as *N. aequilateralis*. The present work combined with that of Gourmelon et al. (2016) thus strongly supports the view that host preference influences New Caledonian ectomycorrhizal fungal diversity. Interestingly, in contrast to our study, no evidence of host preference of ectomycorrhizal fungi was observed in a Western African tropical rainforest, nor in South American rainforests (Die' dhiou et al., 2010; Smith et al., 2011, 2013). As in temperate regions, both patterns of host preference and no host preference are thus observed in the tropics (Horton and Bruns, 1998; Kennedy et al., 2003; Richard et al., 2005; Ishida et al., 2007; Tedersoo et al., 2008; Roy-Bolduc et al., 2016). Understanding the reasons behind these different findings is an important topic for future research.

Despite the potential central role of host preference in shaping ectomycorrhizal fungal diversity, little is known about the mechanisms leading to host preference. Evolutionary history of the fungal-host association is one probable explanation (Bruns et al., 2002; Ishida et al., 2007; Tedersoo et al., 2008; Roy et al., 2013). Co-evolution phenomena and multiple host shifts are seen as potential underlying mechanisms leading to ectomycorrhizal fungal specialization (Kretzer et al., 1996; Rochet et al., 2011). Further phylogenetic studies are needed to determine whether diversification signals in relation to host plants occur within ectomycorrhizal fungal lineages in New Caledonia, such as in the species rich /cortinariu lineage.

Environmental conditions are also seen as potential factors that can lead to ecological specificity (Dickie, 2007; Huggins et al., 2014). Soil chemistry, for instance, was suggested as a potential environmental filter driving the host specialization in the *Alnus*-ectomycorrhizal fungal association (Huggins et al., 2014). Environmental factors can also be a driving force shaping ectomycorrhizal fungal communities, independently of host-specific interactions. Recently, using a reciprocal transplant experiment crossing seedlings of distinct dipterocarp species, soil types and canopy openness, Peay et al. (2015) showed the major role of soil environment, and not host specificity, in determining ectomycorrhizal fungal diversity and structure. In our study system, *N. aequilateralis*, *A. gummiferum* and mixed rainforests had relatively few differences in their soil properties in the first 10 cm depth (Read et al., 2006; Gourmelon et al., 2016; Demenois, 2017). Gourmelon et al. (2016) revealed differences among non-forest (sedge and shrubland vegetations) and forest (*N. aequilateralis* and mixed rainforests) formations, but not among forests types. At the same sites, no evidence of differences in soil characteristics was observed between *N. aequilateralis* forests and adjacent mixed rainforests (Read et al., 2006). Such

observations, combined with the higher ectomycorrhizal fungal community similarities between the vegetation of the same type, which are 12 km<sup>2</sup> 25 km apart, suggest that the host associated community structure we observed does not arise from edaphic differences. However, we cannot exclude that soil properties, in some extent, directly or indirectly, influence fungal communities in these forests.

In addition to host preference, host density is also seen as a factor potentially influencing ectomycorrhizal fungal diversity (Peay et al., 2007; Tedersoo et al., 2010b, 2014). Indeed, Tedersoo et al. (2010b) found an extremely low below-ground ectomycorrhizal fungal richness in an Ecuadorian rainforest where host trees were scattered within a non-ectomycorrhizal rainforest matrix. In our study, the below-ground and above-ground ectomycorrhizal fungal diversity was highest under Nothofagus, which presents a higher stem density than *A. gummiferum* in its related stands (Demenois et al., 2017). In mixed rainforests, only isolated *A. gummiferum* trees were recorded at Col de Mouirange and Col de Yate', and no known ectomycorrhizal trees were observed at Pic du Grand Kaori. It should also be noted that some *Nothofagus balansae* trees are present in the vicinity of the mixed-stand at Col de Yate'. Ectomycorrhizal fungal species richness of New Caledonian rainforests thus seems directly related to the host density. It can be hypothesized that greater ectomycorrhizal root density may drive the observed differences between the different types of forests (Tedersoo et al., 2014).

However, we cannot exclude that, in addition to host density and host identity, ecological factors such as litter quality (Aponte et al., 2010), interactions with other organisms (Frey-Klett and Garbaye, 2005; Gourmelon et al., 2016) and site history, such as the disturbances experienced by the different plant formations (cyclones for *N. aequilateralis* versus wildfires for *A. gummiferum*) (Demenois et al., 2017) could influence the ectomycorrhizal fungal communities in our three rainforest types. Co-variation between these factors may also impact other soil microorganisms.

#### 5. Conclusion

The present work is the first extensive molecular study undertaken on ectomycorrhizal fungi in New Caledonia, an archipelago renowned for its exceptional plant diversity and recognized as a biodiversity hotspot. The investigation of the below- and above-ground ectomycorrhizal fungal communities in mixed and monodominant rainforests from ultramafic substrates clearly revealed, with 311 OTUs and 28 phylogenetic lineages, the existence of a remarkable fungal diversity. Comparison to international sequence databases suggests that up to 95% of the molecular species we identified are endemic to New Caledonia. The archipelago may thus represent an outlier to the hypothesized general pattern of a reversed latitudinal gradient of biodiversity in ectomycorrhizal fungi. Our data even support that monodominant plant systems can act as centres of ectomycorrhizal fungal biodiversity in the tropics. The investigation of fungal composition showed striking dominance of the /cortinariu lineage, in both ectomycorrhizal root tips and fruit bodies, in terms of relative abundance and species diversity. Overall, this pattern seems to be common to the entire geographical distribution of *Nothofagus* and even between *Eucalyptus* species and their New Caledonian sister monotypic species, *A. gummiferum*. Such dominance of /cortinariu could indicate that members of this lineage play central roles in ecosystem functioning. Finally, the ectomycorrhizal fungal communities in these forests seem to be influenced by tree host identity and density. Host preference was identified as the most probable factor underlying the observed fungal community structure. Further investigations are needed to determine whether diversification signals in fungal

lineages can be detected in relation to host plants or other ecological and environmental features in New Caledonia.

#### Data accessibility

DNA sequences: Genbank accessions KY773993 e KY774303.

#### Author contributions

F.C., L.M., J.R., S.M.C. and M.G. defined the conceptual framework of the study. F.C., L.M., S.M.C. and J.G. designed the sampling procedure. F.C., K.L., S.G. and V.G. performed the sampling. F.C., K.L., S.G. and J.-M.B. produced the sequence data. F.C., J.-M.B. and T.I. carried out the statistical analyses. F.C., J.R., J.-M.B., L.M., T.I. and M.G. wrote the manuscript.

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