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Distribution patterns of microbial communities in ultramafic landscape: a metagenetic approach highlights the strong relationships between diversity and environmental traits

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Abstract

Microbial species richness and assemblages across ultramafic ecosystems were investigated to assess the relationship between their distributional patterns and environmental traits. The structure of microorganism communities in the Koniambo massif, New Caledonia, was investigated using a metagenetic approach correlated with edaphic and floristic factors. Vegetation cover and soil properties significantly shaped the large phylogenetic distribution of operational taxonomic unit within microbial populations, with a mean per habitat of 3.477 (±317) for bacteria and 712 (±43) for fungi. Using variance partitioning, we showed that the effect of aboveground vegetation was the most significant descriptor for both bacterial and fungal communities. The floristic significant predictors explained 43% of the variation for both the bacterial and fungal community structures, while the edaphic significant predictors explained only 32% and 31% of these variations, respectively. These results confirm the previous hypothesis that the distribution of microorganisms was more structured by the vegetation cover rather than the edaphic characteristics and that microbial diversity is not limited in ultramafic ecosystems.

Keywords: metagenetic, microbial communities, topsoil, ultramafic ecosystems

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Introduction

Microorganisms play a key role in ecosystems. They connect plants and soil and provide environmental services such as biogeochemical cycling and soil fertility (Gobat *et al*. 2010). For instance, mycorrhizae are crucial for plant phosphate (P) uptake and may affect other elements as well as the water balance (Smith *et al*. 2011). Rhizospheric bacteria are a major source of input of atmospheric nitrogen (N) into terrestrial ecosystems (Gage 2004) and regulate plant growth (Frankenberg & Arshad 1995). Identifying the causes behind the spatial distribution patterns of microbial communities is crucial to anticipating ecosystem responses to global changes (Singh *et al*. 2010), monitoring their responses to these changes (McHugh *et al*. 2014) and facilitating their restoration, if necessary (Yu *et al*. 2012). To understand microbial contributions to plant phenotypes and community dynamics, the first step is to identify which microbes are present and how abundant they are. Due to limited techniques, answers to

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these questions have remained incomplete over the past few decades. However, recent technical progresses have made these problems more easily tractable, in particular with the diffusion of large-scale metagenetic analyses (Smith & Peay 2014).

Based on these techniques, recent studies were conducted to explore the distributional patterns exhibited by soil microbial communities and the biotic and abiotic factors driving them. Numerous studies have reported soil properties as the main forces shaping the composition of microbial communities. Soil pH has been shown to be the best predictor of bacterial composition (Lauber *et al.* 2008; Rousk *et al.* 2010), whereas the composition of fungal communities was mainly governed by changes in soil nutrient status (Waldrop *et al.* 2006; Lauber *et al.* 2008). Spatial covariation between the plant communities and microbial communities has also been reported (Yergeau *et al.* 2010; Zinger *et al.* 2011). Furthermore, a recent study suggested that fungi are more closely associated with plants than bacteria, themselves being more influenced by soil properties (Nielsen *et al.* 2010). However, most of these studies did not identify the relative contribution of soil properties or plant cover to the spatial patterning of soil microbes. Moreover, the paucity of detailed studies directly comparing the spatial patterns exhibited by soil bacteria and fungi does not allow robust conclusions regarding the similarities or differences in the environmental factors shaping the composition of these communities. Finally, the pattern of structure and diversity of these two microbial domains remains largely unknown.

Ultramafic soils are derived from submarine rock forming the mantle and oceanic crust that became stranded on the continental crust during the process of subduction. These soils are characterized by low concentrations of major plant nutrients (N, P, K), poor water retention, an unbalanced Ca/Mg ratio and high levels of heavy metals, such as nickel (Ni), chromium (Cr), manganese (Mn) and cobalt (Co) (Harrison & Rajakaruna 2011). This unusual and challenging chemistry strongly restricts vegetation growth and select for metal-tolerant populations (O'Dell & Rajakaruna 2011). Ultramafic soils often occur as discontinuous 'island-like' areas, truly 'unearthly' in their appearance, that form striking boundaries with neighbouring soils (Davies *et al.* 2005). Ultramafic soils have given rise to spectacular levels of endemism among both flora (Anacker 2011, 2014) and fauna (Sadlier *et al.* 2009; Strauss & Boyd 2011). These soils constitute a model system for understanding mechanisms of adaptation, ecotypic differentiation and the linkage between natural selection and speciation (Harrison & Rajakaruna 2011). In the unfavourable ultramafic soil conditions, numerous studies have reported the critical role of microbial communities in the enhancement of the survival and vigour of plants and through the improvement of plant tolerance to metals (Cardace & Hoeler 2011; Cardace *et al.* 2014; Schechter & Branco 2014). In addition, the presence of specific microbial symbionts is essential for some plants (Strauss & Boyd 2011). In recent years, the ultramafic metal-resistant bacterial communities associated with the roots of ultramafic-endemic hyperaccumulating plants received a great deal of attention for their potential exploitation in phytoremediation processes (reviewed in Rajkumar *et al.* 2009). Similarly, a large body of works characterized a portion of the fungal population of ultramafic systems, and there are many comparative ecophysiological studies dealing with ultramafic/nonultramafic ecotypes of the same plant species (Doubková & Sudová 2014; Schechter & Branco 2014; Kohout *et al.* 2015). However, Mengoni *et al.* (2009) and von Wettberg & Wright (2011) emphasized the lack of knowledge on the diversity and structure of ultramafic microbial populations, which precludes an understanding of their evolutionary dynamics and the role of interactions with serpentine-tolerant plants. To our knowledge, no comprehensive study of the patterns exhibited by fungal and bacterial ultramafic communities at the scale of the vegetation group has yet been conducted.

New Caledonia is a tropical archipelago in the South Pacific Ocean where ultramafic outcrops cover one-third of the main island (Latham 1985). After million years of evolution, specific ecosystems have evolved with high level of floristic endemism (Jaffré *et al.* 1994), including an important metallophytic flora (Whiting *et al.* 2004), making the island a terrestrial biodiversity hotspot (Myers *et al.* 2000). Such ecosystems provide a unique opportunity to assess the factors affecting the spatial patterning of microbial communities, as steep environmental gradients cause abrupt changes in plant species over short distances (Jaffré & L'Huillier 2010). Recently, a specific and adapted microbial flora isolated from these soils has also been reported (Amir & Ducousso 2010). However, little work has been done on New Caledonian microorganisms until fairly recently (L'Huillier *et al.* 2010). To date, researches focused on the role of symbiotic microorganisms in plant growth, such as N-fixing rhizospheric bacteria (Amir & Ducousso 2010) and arbuscular mycorrhizal (Lagrange *et al.* 2011; Amir *et al.* 2013) or ectomycorrhizal fungi (Jourand *et al.* 2014), whereas little is known about saprophytic fungi. In the present study, we hypothesize (i) that the ultramafic ecosystems do not limit the microbial diversity and (ii) that the distribution of microorganisms is more structured by the vegetal cover rather than the edaphic characteristics. We assessed the influence of vegetation cover and soil properties on the assemblage of microbial communities across

six habitat types in ultramafic ecosystems. Using metagenetic data, we determined the abundance, taxonomic diversity and composition of the microbial communities across the New Caledonian ultramafic system of the Koniambo massif. Understanding the microbial community composition and dynamics in these ultramafic soils may lead to a better understanding of the processes facilitating vegetation succession from shrub land to forest on these high-metal substrates and provide useful information to guide vegetation restoration attempts following nickel mining.

Materials and methods

Sites description and sampling

The study area is located in the northern province of New Caledonia on the Koniambo massif (20°56′08″S, 164°43′55″E; altitude 460–930 m) (Fig. 1). This site has been used several times to study models of New Caledonian ultramafic substrates (Perrier 2005). The mean annual precipitation is 957 mm (range per month: 45–270 mm), with a dry season from May to September. The mean minimum and maximum temperatures are 14 °C and 22 °C, respectively. The dominant soil units are ferralsols (Perrier 2005).

In this area, we investigated the microbial communities of six types of topsoil, each one associated with specific vegetation communities and thus considered as distinct habitats. These habitats represent different stages of plant succession. A detailed description of each habitat can be found in Table 1. A detailed diagram of the complete material and method is given in Fig. S1 (Supporting information). A total of 36 soil samples were collected, corresponding to six replicates per habitat, as following.

In each habitat, six plot replicates (10 × 10 m) were randomly chosen (Dagnelie 2012). Within each plot, a total of 12 systematically distributed cores were collected using an auger (D = 5 cm; h = 15 cm). This sampling depth allowed access to the major portion of

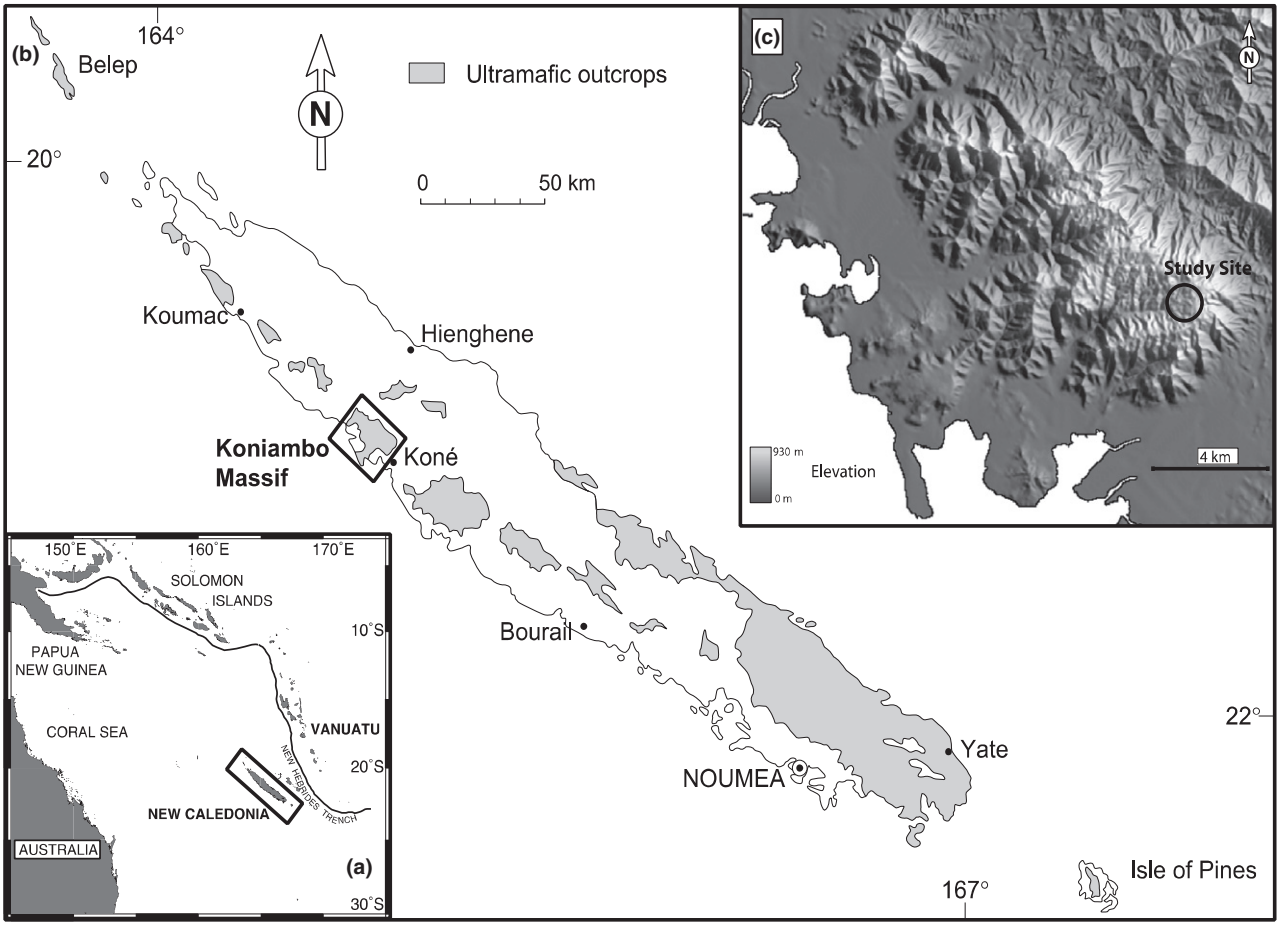


Fig. 1 (a) Location map of New Caledonia in the southwest Pacific. (b) Map of the ultramafic bodies of New Caledonia (in grey). (c) Shaded relief map of the Koniambo massif with the study site location.

Table 1 Descriptions of the habitat types investigated

Successional stage	Description
(Ab) Bushy shrub maquis dominated by *Tristaniopsis guillainii*	On plateaus. Widely open group (bare soil >35%) from 50 cm to 1.5 m in height. Characterized by an extreme reduction herbaceous whose recovery rate does not exceed 5% and a shrub statum >50%.
(L) Ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.	On slopes. Significant development of the herbaceous layer (>50%). The low shrub layer (1.20 m average) is discontinuous and has a recovery rate lower than the herbaceous layer. With light demanding, mesophilic, pioneers species and larger species. Bare soil >10%
(A) Maquis dominated by *Araucaria montana* (Araucariaceae)	On the ridges and summital plateaus. Characterized by a Araucaria tree stratum very loose amount dominating ligno-herbaceous or ligno statum very heterogeneous. Consisting mainly of orophilous small species or creeping. Bare soil >10%. Group close to the previous.
(P) Preforest dominated by *Tristaniopsis callobuxus*	Located on the edge of forest formations. Maquis higher and denser than the previous two. Extremely diverse. The shrub layer is larger (2/3 of the surface). Dominated by *T. callobuxus*. Third is covered by a herbaceous layer. No tree layer.
(N) Forest dominated by *Nothofagus codonandra & Nothofagus balansae*	In zone troughs, Maquis species (*T. guillainii, T. callobuxus*) represented by trees from 8 to 10 m, associated with forest species. Facies dominated by Nothofagus (*N. codonandra* and *N. balansae*)
(C) Forest dominated by *Cocconerion minus & Dacrydium balansae*	Same features as above. Facies dominated by *Cocconerion cf. minor* and *D. balansae*

These groups range from 200 to 900 m above sea level, occupying the slopes and hills on lateritic soils (source RASM, 2007).

plant fine roots and microorganisms within the soil samples as reported by Gobat *et al.* (2010). Soil cores from each plot were pooled to produce a homogenized soil sample per plot (Ter Heerdt *et al.* 1996). Then, the 36 soil samples were sieved (2 mm) and stored frozen at −80 °C until molecular analyses were performed.

To evaluate the influence of the established vegetation on the soil microbial community, the composition of the vegetation of each plot was described using the Braun-Blanquet abundance index (Meddour 2011). In addition, to evaluate the influence of the physicochemical properties of the soil, a part of each soil samples was sent to the LAMA laboratory (IRD, NOUMEA). The following characteristics were evaluated according to the method reported by Perrier (2005): pF4.2, pH H2O, organic C, total N, available P (Olsen), total P, DTPA (diethylene triamine pentaacetic acid) extractable metals (Mn, Ni, Fe, Cr, Co, Al), K, Ca, Mg, Si, CEC and granulometry (clays, silts and sands). The complete physical and chemical characteristics of the soil samples are reported in Table S1 (Supporting information).

Soil DNA extraction and bar-coded pyrosequencing of bacterial and fungal communities

For each soil sample, eight DNA extractions were performed as replicates from 0.25 g subsamples using the Mobio PowerSoil extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The alternative lysis method proposed by the manufacturer was performed. Thus, soil subsamples were vortexed for 3–4 s, then heated to 70 °C for 5 min, three times. The remaining steps were performed as directed by the manufacturer's original instructions. DNA samples were stored at −20 °C until needed. The bacterial community diversity was assessed using the DNA ~545-bp region covering V1-V3 within the 16S gene region and considered as the broadly conserved DNA barcode according to Woese (1987). The primers 27F and 553R were used to amplify this region. The fungal community was analysed by targeting the DNA region of the ITS2 rRNA gene, which is widely used as a DNA metabarcoding marker (Bates *et al.* 2013). Moreover, White *et al.* (1990) have shown that the primer set provides 95% of fungal and 99% of Glomeromycota DNA. The primers ITS3 and ITS4 were used to amplify this region. The full set of primers used in the study is reported in Table S2 (Supporting information). The PCRs were conducted in 25 μL reactions with 0.625 μL (0.25 μM) of each primer, 2.5 ng of the template DNA for each soil subsample, 1 X PCR buffer and 0.08 U of GoTaq® Flexi DNA polymerase (Promega Corporation, Madison, WI, USA). The cycling conditions were as follows: initial denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 57 °C for 1 min and 72 °C for 1.5 min, and finally a 10 min extension at 72 °C. Amplified DNA was purified by filtration (Sephadex G50; Sigma-Aldrich, St. Louis, MO, USA). The product purity was visualized on agarose

1.5% gel in a TAE buffer. Gels were stained with ethidium bromide (0.5 μg/mL) and visualized under UV light. After DNA quality control, the subsample replicates of PCR products from a same soil sample were pooled to obtain one final amplicon per soil sample, according to the microbial domain. Thus, we obtained 36 bacterial amplicons and 36 fungi amplicons. Then, the DNA concentration of each amplicons was determined using a NanoDrop spectrophotometer (NanoDrop 2000; Thermo Scientific, Wilmington, DE, USA). Finally, both the bacteria and the fungi, 4 libraries of 9 amplicons pooled in equimolar ratios, were generated, as recommended by 454 Life Sciences (Margulies *et al.* 2005). The pyrosequencing was performed by Beckman Coulter Genomics (Beckman Coulter Genomics, Danvers, MA, USA).

Processing of pyrosequencing data

The quality of the data was analysed as described by Nilsson *et al.* (2012) and processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso *et al.* 2010). In brief, bacterial and fungal sequences were quality-trimmed to a score >25 and assigned to soil samples based on their barcodes. Ambiguous bases and mismatches in primer sequences were not admitted. Chimeras were checked and removed with the Chimera Slayer algorithm in QIIME. Both bacterial and fungal sequences were binned into operational taxonomic units (OTUs) using a 97% identity threshold with uclust (Kunin *et al.* 2010), and the most abundant sequence from each OTU was selected as a representative sequence for that OTU. Singletons were removed. Specifically, we used ITSX SOFTWARE (version 1.0.10) (Bengtsson-Palme *et al.* 2013) to remove nonfungal ITS2 sequences. Taxonomy was assigned to bacterial OTUs by using the Basic Local Alignment Search Tool (BLAST) for each representative sequence against a subset of the SILVA database (the full database filtered at 97% sequence identity using BLAST) (SILVA SSU 111) (Quast *et al.* 2013). The fungal pyrosequencing reads were BLASTed against a database of the ITS2 rRNA sequences from UNITE-INSD V6 (Abarenkov *et al.* 2010) using the species hypothesis (Kõljalg *et al.* 2013). The percentage similarity to the assigned sequence ranged from 97% to 98.5%, with an average value of 98.3%. After this step, the unidentified fungi sequences were extracted and BLASTed against the MAARJAM database using a 97% identity threshold (Opik *et al.* 2010) to identify the Glomeromycota.

According to McMurdie & Holmes (2014), who showed that the rarefaction of microbiome count data is inefficient in the statistical sense, we used the EDGER package within the R statistical environment ('EDGER PACKAGE', Robinson *et al.* 2010), to normalize the differential sequencing depth analysis and the OTU composition for the two microbial domain. We used the 'calcNormFactors' function to find a set of scaling factors for the library sizes that minimize the log-fold changes between the samples for most genes. Thus, we generated an OTU normalized matrix, for both fungi and bacteria. These new matrixes were used to the downstream statistical analysis.

Statistical analyses

We used several multivariate statistical analyses to compare habitats using four groups of parameters (physicochemical and floral parameters, fungal and bacterial communities). Hellinger-transformed data (square root of relative abundance) were used to generate Bray–Curtis dissimilarity matrices for floristic, fungal and bacterial variables. An Euclidian dissimilarity matrix was generated after standardization of the data for the physicochemical variables. The statistical significance for all tests was set at P-value <0.05. The difference between habitats was tested using a permutational analysis of variance fixed model (PERMANOVA) (Anderson 2001) (PRIMER V7, Primer-E Ltd, 239 Plymouth, UK). In case of significant differences, PERMANOVA pairwise comparisons were used to identify the origin of the differences. The results were illustrated by nonmetric multidimensional scaling (NMDS) analysis using the vegan package within the R statistical environment ('VEGAN PACKAGE', R.3.1.1, R foundation).

The relationship between fungal or bacterial communities and floristic or edaphic variables was tested using Relate tests (PRIMER V7). When a significant relationship was found, a distance-based linear model (DISTLM) was built to identify the significant predictors for floristic or edaphic variables. This model does a partitioning of variation using a multiple regression model. Permutation methods are used to assess statistical significance of each predictor variable. In addition, preliminary diagnostics are made to assess and to avoid multicollinearity among predictor variables that could bias the results (McArdle & Anderson 2001). The fitted DISTLM models were visualized in multidimensional space, using the distance-based redundancy analysis (dbRDA) routine (PRIMER V7). The details of each DISTLM model are given in Fig. S2 and Tables S4–S7 (Supporting information).

To assess the amount of the variation explained by each sets of significant predictors (floristic and edaphic), we partitioned the overall variation of the bacterial or fungal communities using a DISTLM model which included only the significant predictors of both sets of predictors (physicochemical and floral).

Results

Vegetation and soil characteristics of the studied area

The location of the studied area is described in Fig. 1 and detailed descriptions of the vegetation and edaphic characteristics of each habitat type are given in Tables 1 and S1 (Supporting information), respectively. The permutational analysis of variance of the floristic characteristics revealed that a unique vegetation community characterized each habitat ($P < 0.001$). This marked contrast is shown by the nonmetric multidimensional scaling (NMDS) plot ordination of the vegetation dissimilarity matrix (Fig. 2a). This figure illustrates the gradient of vegetation succession and the contrast between more open habitats (habitats A, Ab and L), where bare soil or rock is exposed between plants, and completely vegetated habitats (habitats P, N and C), where vegetation cover is continuous. This second group was the richest in species and plant abundance. The PERMANOVA analysis of the soil characteristics revealed four significantly distinct groups ($P < 0.01$). This is illustrated by the NMDS (Fig. 2b). Two groups corresponded to the Ab habitat and the A habitat, which had the lowest value in withering point (pF4.2), nitrogen and pH H_2O, respectively. The third (habitats P, N and L) and the fourth group (habitats P and C) corresponded to the habitats with most mature soils. The Relate test revealed a significant relationship between aboveground vegetation and edaphic characteristics ($P < 0.01$, rho = 0.27). The distance-based linear model (DISTLM) approach showed that nitrogen, pH, pF4.2, silts and DTPA extractable manganese significantly explained 50% of the variability in the vegetation community (26%, 11%, 6%, 4% and 3%, respectively, $P < 0.001$) (Fig. S2, Supporting information).

Assessing the compositions of bacterial communities

From the 454 pyrosequencing data, we obtained a total of 391 716 exploitable sequences (77% of the total 511 626) that could be classified in 40 559 OTUs with a mean of 34.767 ± 317 per sample (ranging from 2.564 ± 273 in the L habitat to 4.731 ± 589 in the P habitat) (Table S3, Supporting information). A distinct assemblage of bacterial OTUs characterized each studied habitat. This is revealed by the permutational analysis ($P = 0.01$) and it illustrated by the NMDS (Fig. 3a). At the phylum level, there were 40 major bacterial taxa present within most habitats. Once averaged across all habitats, the most abundant bacterial groups were Proteobacteria (41.38 ± 1.26%) and Acidobacteria (18.49 ± 1.01%), followed by Actinobacteria (10.29 ± 0.77%),

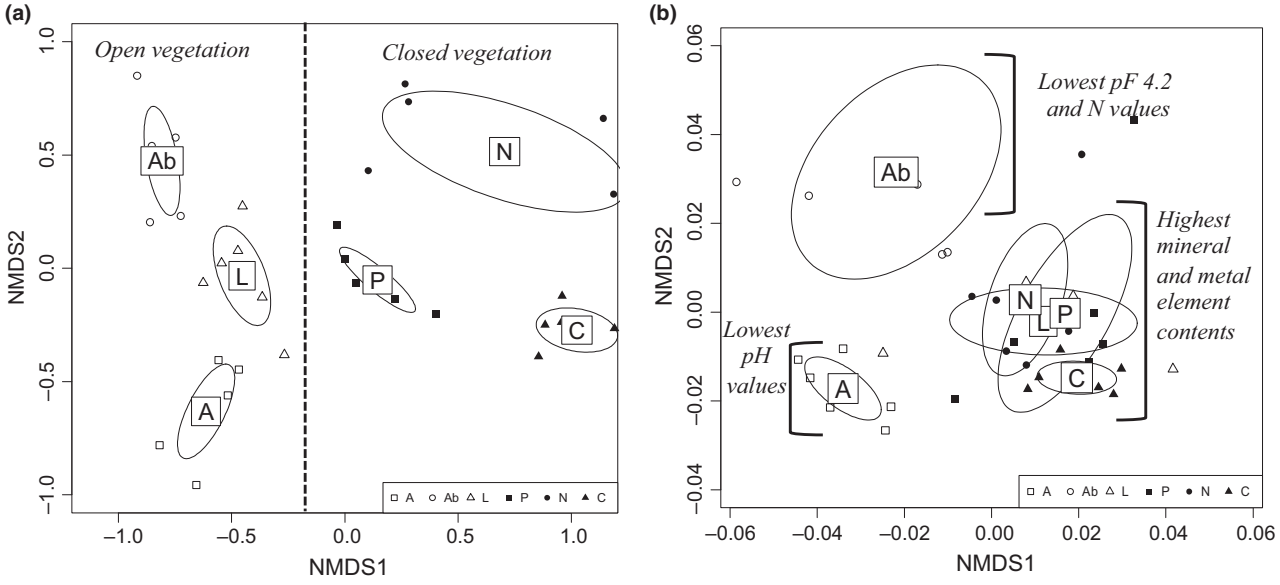


Fig. 2 Two-dimensional NMDS ordination of (a) floristic structure and (b) edaphic characteristics per habitat at Koniambo massif, in New Caledonia. Stress level = 0.13 and 0.12, respectively. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per habitat. The location of ellipsoids within each diagram indicates their degree of similarity. Habitat type: A (maquis dominated by *Araucaria montana*), Ab (bushy shrub maquis dominated by *Tristaniopsis guillainii*), L (ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.), P (preforest dominated by *Tristaniopsis callobuxus*), N (forest dominated by *Nothofagus codonandra* & *Nothofagus balansae*), C (forest dominated by *Cocconerion minus* & *Dacrydium balansae*).

Planctomycetes (10.06 ± 0.86%), Verrucomicrobia (6.24 ± 1.38%) and Chloroflexi (<6%) (Table S3, Supporting information). The 34 remaining bacterial phyla accounted for <8% of the sequence data and <1% each. The taxa exhibited different relative abundances among habitats (Fig. 3b). The abundance of Proteobacteria was greater in the N habitat (47.27 ± 1.31%) and lower in the Ab habitat (35.97 ± 1.31%) compared to other habitats. Acidobacteria and Actinobacteria were most abundant, albeit not significantly so, in habitat A and habitat N (22.51 ± 1.24% and 12.50 ± 0.77%, respectively). Habitat Ab exhibited a significantly greater proportion of Chloroflexi (11.35 ± 0.65%).

The bacterial community was significantly related to the aboveground vegetation (Relate test, $P < 0.01$, rho = 0.53) and soil characteristics (Relate test, $P < 0.01$, rho = 0.52). A DISTLM model indicated that 9 vegetation species were significant predictors of the bacterial community structure, explaining 43% of the overall variation (Fig. 4a). Similarly, DISTLM model indicated that 6 edaphic characteristics were significant predictors of bacterial community structure and explained a proportion (32%) of the overall variability. These 6 predictors were nitrogen (9%), pH (9%), pF 4.2 (5%), Ca, CEC and silts (3% each) (Fig. 4b). Consequently, the aboveground vegetation had a stronger influence on the composition of bacterial communities across the ultramafic ecosystem than the edaphic characteristics. The detail of significant species and edaphic characteristics included in distLM is given in Tables S4 and S5 (Supporting information), respectively.

The significant predictors of the aboveground vegetation species and edaphic characteristics predicted 56.82% total of the variation of bacterial community structure. The floristic significant predictors explained 24.42%, the edaphic predictors explained only 14.24%, and 18.16% were explained by both sets of predictors (Fig. 5).

Assessing the fungal communities

From the fungal community analyses in the 36 soil samples from across the six ultramafic habitats, we obtained a total of 490 898 exploitable sequences (83% of the total

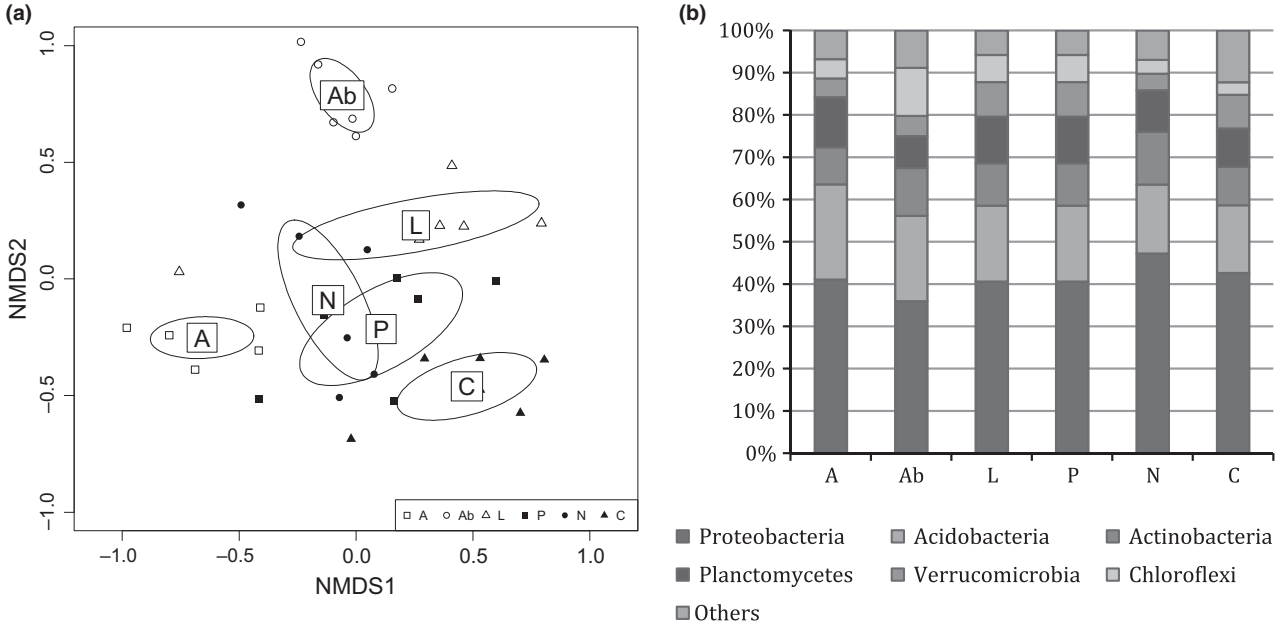


Fig. 3 Bacterial community characteristics for the different habitats. (a) Two-dimensional NMDS ordination of bacterial community structure. Stress = 0.18. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per habitat. The locations of ellipsoids within each diagram indicate their degree of similarity. (b) Relative abundance of bacterial phyla identified in all samples. Others = Cyanobacteria, Gemmatimonadetes, Bacteroidetes, TM6, Armatimonadetes, Candidate_division_OD1, Nitrospirae, Candidate_division_TM7, Elusimicrobia, Thermotogae, Candidate_division_OP11, Candidate_division_OP3, WCHB1-60, Candidate_division_WS3, Spirochaetes, Candidate_division_BRC1, SM2F11, Fibrobacteres, Tenericutes, Deinococcus-Thermus, Lentisphaerae, Fusobacteria, Synergistetes, Deferribacteres, Caldiserica, Chlamydiae, Aquificae, Candidate_division_OP9, Chlorobi. Habitat type: A (maquis dominated by *Araucaria Montana*), Ab (bushy shrub maquis dominated by *Tristaniopsis guillainii*), L (ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.), P (preforest dominated by *Tristaniopsis callobuxus*), N (forest dominated by *Nothofagus codonandra* & *Nothofagus balansae*), C (forest dominated by *Cocconerion minus* & *Dacrydium balansae*).

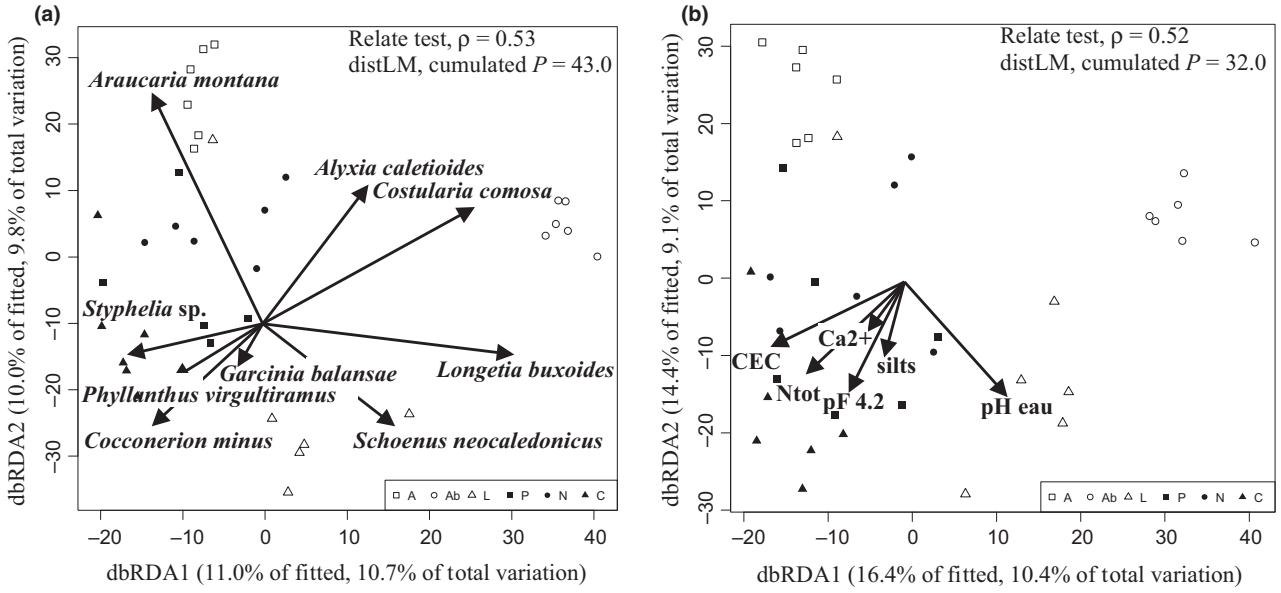
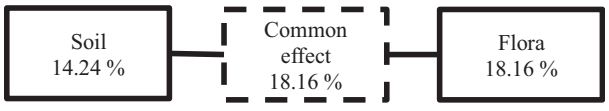


Fig. 4 Distance-based redundancy analysis from distance-based linear models between bacterial communities and (a) the floristic structure and (b) the edaphic characteristics. Only explanatory significant variables are shown. Habitat type: A (maquis dominated by *Araucaria Montana*), Ab (bushy shrub maquis dominated by *Tristaniopsis guillainii*), L (ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.), P (preforest dominated by *Tristaniopsis callobuxus*), N (forest dominated by *Nothofagus codonandra* & *Nothofagus balansae*), C (forest dominated by *Cocconerion minus* & *Dacrydium balansae*).



Total 56.82 % ; unexplained 43.18 %

Fig. 5 Variance partitioning distance-based linear model (DISTLM) shows the relative effects of the two significant variable sets on the composition of bacterial taxa. The full squares represent the effect of individual significant variable sets by partitioning out the effects of the other significant variable sets. The discontinuous square between the full squares represents the combined effects from the significant variables on either side of the discontinuous square.

591 019). Classification of the sequences revealed 9555 OTUs. The number of OTUs varied from 534 ± 34 in the L habitat to 965 ± 101 in the P habitat (Table S3, Supporting information). Permutational analyses (Fig. 6a) indicated that each habitat type was associated with a specific fungal community ($P < 0.001$). Basidiomycota and Ascomycota represented a majority of the fungal sequences in this study (81.46 ± 1.86% and 7.44 ± 1.16%, respectively) (Table S3, Supporting information). Unidentified fungi accounted for 4.98 ± 0.81% sequences. The remaining taxa included Zygomycota (5.51 ± 0.77%), Glomeromycota (<1%), Chytridiomycota (<0.1%) and Blastocladiomycota (<0.1%). The relative abundance of fungal phyla in Fig. 6b shows little variation among habitats. The presence of Ascomycota was significantly greater in the Ab habitat (14.86 ± 1.11%) and lower in the C habitat (3.61 ± 1.20%) compared to other habitats. Habitat N included the highest percentage of Basidiomycota (85.40 ± 1.83%), and habitat Ab, the lowest value (74.14 ± 2.04%).

As with the bacterial communities, the fungal community was significantly related to the aboveground vegetation (Relate test, $P < 0.01$, rho = 0.63) and soil characteristics (Relate test, $P < 0.01$, rho = 0.43). The DISTLM model indicated that 7 vegetation species were significant predictors of the fungal community structure, explaining 43% of the overall variation. Moreover, DISTLM model indicated that 5 edaphic characteristics were significant predictors of fungal community structure and explained a proportion (31%) of the overall variability (Fig. 7a). These 5 predictors were pH (9%), nitrogen (8%), calcium (5%), aluminium (5%) and iron (4%) (Fig. 7b). Thus, the aboveground vegetation had a stronger influence on the composition of fungal communities across the ultramafic ecosystem than the edaphic characteristics. The detail of significant species and edaphic characteristics included in distLM is given in Tables S6 and S7 (Supporting information), respectively.

The significant predictors of the aboveground vegetation species and edaphic characteristics predicted 55.79% total of the variation of fungal community structure. The floristic significant predictors explained 24.75%, the edaphic predictors explained only 11.87%,

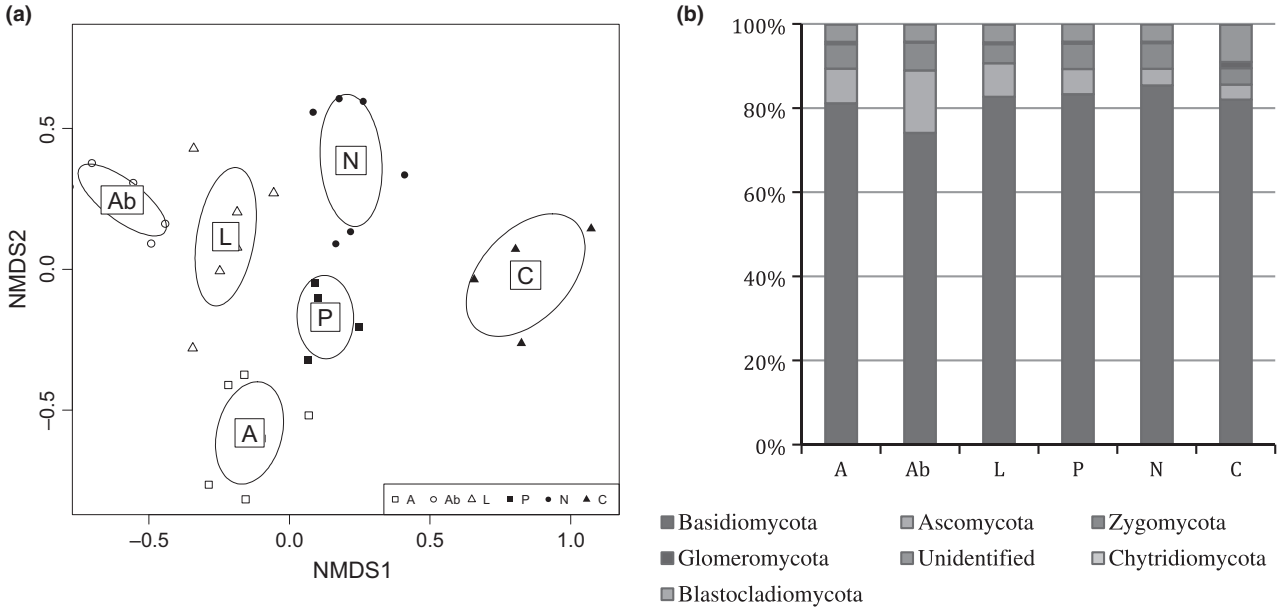


Fig. 6 Fungal community characteristics for the different habitat. (a) Two-dimensional NMDS ordination of fungal community structure. Stress = 0.10. Ordination was based on the distance dissimilarity matrix. Ellipsoids represent the standard error confidence limit (95%) per habitat. The locations of ellipsoids within each diagram indicate their degree of similarity. (b) Relative abundance of fungal phyla identified in all samples. Habitat type: A (maquis dominated by *Araucaria Montana*), Ab (bushy shrub maquis dominated by *Tristaniopsis guillainii*), L (ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.), P (preforest dominated by *Tristaniopsis callobuxus*), N (forest dominated by *Nothofagus codonandra* & *Nothofagus balansae*), C (forest dominated by *Cocconerion minus* & *Dacrydium balansae*).

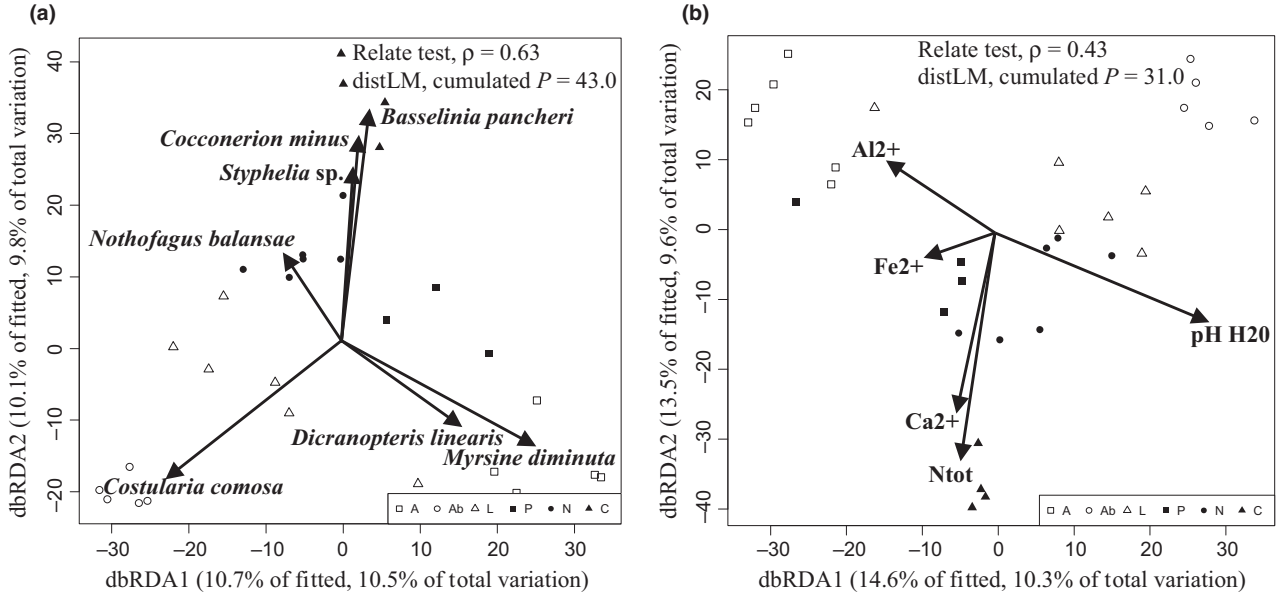


Fig. 7 Distance-based redundancy analysis from distance-based linear models between fungal community and (a) the floristic structure and (b) the edaphic characteristics. Only explanatory significant variables are shown. Habitat type: A (maquis dominated by *Araucaria Montana*), Ab (bushy shrub maquis dominated by *Tristaniopsis guillainii*), L (ligno-herbaceous maquis dominated by *Hibbertia altigena, Eucarpha deplanchei* and *Costularia* sp.), P (preforest dominated by *Tristaniopsis callobuxus*), N (forest dominated by *Nothofagus codonandra* & *Nothofagus balansae*), C (forest dominated by *Cocconerion minus* & *Dacrydium balansae*).

Fig 8 Variance partitioning distance-based linear model (DISTLM) shows the relative effects of the two significant variable sets on the composition of fungal taxa. The full squares represent the effect of individual significant variable sets by partitioning out the effects of the other significant variable sets. The discontinuous square between the full squares represents the combined effects from the significant variables on either side of the discontinuous square.

and 19.17% were explained by both sets of predictors (Fig. 8).

Discussion

An increasing number of studies have attempted to characterize how microbial distribution patterns respond to environmental factors (Nielsen *et al.* 2010; Rousk *et al.* 2010). The previous conclusions of studies regarding ultramafic ecosystems have been limited by the techniques used (Lenczewski *et al.* 2009) or by the focus on a single microbial domain, such as bacteria (Herrera *et al.* 2007). The application of the metagenetic analysis to ultramafic ecosystems was expected to greatly expand our knowledge of the microorganisms populating these systems (von Wettberg & Wright 2011). The present study is the first to use this approach to assess the richness and taxonomic diversity of bacterial and fungal communities across an ultramafic system and their links to edaphic properties and vegetation cover.

Structure of bacterial and fungal communities

Rothschild & Mancinelli (2001) emphasized the tendency to consider extreme environments as hosting low biodiversity. For instance, Mengoni *et al.* (2009) stated that the low fertility of ultramafic soils was thought to be due to 'little diversity, as well as a general paucity, in the bacterial flora of the ultramafic soils'. Similarly, Branco & Ree (2010) highlighted the fact that ultramafic characteristics were considered most likely detrimental to the establishment of fungi because high levels of heavy metals are almost universally toxic directly via the enzymatic inhibition and disruption of cellular integrity (Gadd 1993) and indirectly through the production of free radicals (Dowling & Simmons 2009). However, recent studies have tended to demonstrate that ultramafic soils do not limit bacteria (Schechter & Branco 2014) or mycorrhizal fungal diversity (Branco 2010).

The metagenetic data gathered in this study confirm these recent findings by showing an unexpectedly high richness of bacterial and fungal species in ultramafic soils. Moreover, using similar experiments and methods, our results are comparable to those found in nonultramafic natural environments such as forest, which are considered the most complex and richest terrestrial ecosystems (Myers 1992). Indeed, at the 97% similarity threshold, we found a mean of 3.477 ± 317 OTUs per habitat for bacteria, while Kim *et al.* (2014) found a mean of 3500 OTUs in a South Korean temperate forest and 2500 in a Malaysian tropical rainforest, and Nacke *et al.* (2011) found 3212 OTUs in a German temperate forest. For fungi, we found a mean of 712 ± 43 OTUs, similar to the numbers reported for temperate forests in France (Buée *et al.* 2009) and Japan (Kadowaki *et al.* 2013), and for an Amazonian forest (Fierer *et al.* 2007).

In addition to revealing a number of fungal phyla equal to those reported in ultramafic soil studies (Daghino *et al.* 2012) and nonultramafic studies (Lim *et al.* 2010), the results showed that the abundance of bacterial species is distributed among a wide array of 40 phyla. In comparison, Lauber *et al.* (2008) reported 25 bacterial phyla based on their studies in 88 soils across North and South America. Nacke *et al.* (2011) reported 17 bacterial phyla in German forests, and Uroz *et al.* (2010) reported 15 phyla in French forests. In addition, 34 bacterial phyla counted for <1% each, corresponding to rare taxa. Given that many rare species play unique ecological roles, Mouillot *et al.* (2013) suggest that ultramafic ecosystems are homes to highly complex microbial communities.

Finally, the results confirmed that ultramafic soils do not limit bacterial and fungal diversity, as recently hypothesized (Branco 2010; Schechter & Branco 2014). On the contrary, these ecosystems might be among the most complex terrestrial systems.

Distribution patterns of microbial domains

The results showed that each habitat is characterized by a unique microbial community, for both bacteria and fungi. In addition, an apparent successional gradient from maquis to forest was found in terms of changing microbial communities, with higher diversity in the more developed forest soils. Those results are in agreement with those recently reported in Cong *et al.* (2015) and in Li *et al.* (2015). To understand this pattern, it was attempted to characterize the relationship between microbial communities and the edaphic properties and vegetation cover of each habitat, using variance partitioning. The effect of vegetation and edaphic characteristics was the same between the two microbial domains

investigated. In both cases, the effect of aboveground vegetation was the most significant descriptor. Although weaker, the relationships with the edaphic characteristics were also significant. These results are at odds with previous studies, suggesting that fungi are more closely associated with plants than are prokaryotes, the latter being more influenced by soil properties (Fierer & Jackson 2006; Millard & Singh 2009), in particular the pH (Rousk *et al.* 2010). This apparent discrepancy might be due to the relatively narrow range of pH (5.27–5.65, with an extreme pH value in site Ab of 4.64) characterizing the environments we sampled. In a similar case, Bonito *et al.* (2014) showed that host associations played a role in moderating community structures. However, the results support the existence of spatial covariations between plant communities and microbial communities reported in studies investigating different environments (Kennedy *et al.* 2005; Zinger *et al.* 2011).

Among soil properties, the distribution patterns of the two communities were significantly influenced by nitrogen, pH and calcium. The results are broadly consistent with previous studies showing that shifts in the structure of bacterial and fungal communities can be associated with changes in a number of soil properties, including soil texture (Girvan *et al.* 2003), soil pH (Fierer & Jackson 2006) and soil nitrogen availability (Frey *et al.* 2004). As reported by Zinger *et al.* (2011), the significant covariation between pH and bacterial diversity may be related to Acidobacteria, a dominant group of soil bacteria known to be highly responsive to soil pH. In addition, the variation of soil pH may reflect the differences in the availability of simple organic substrates in soils for which bacteria are more competitive (de Boer *et al.* 2005). By contrast, if fungal communities are considered to be less sensitive to soil pH, fungal biomass has been reported to covary with soil pH and so explaining significant covariations between fungi and soil pH (Zinger *et al.* 2011). The observed nitrogen effect on fungal communities may be direct, but it may also reflect the recruitment of different types of mycorrhizal associations in ecosystems that display contrasted nitrogen and nutrient cycles. Patterns of dissimilarity among fungal communities were related to nitrogen, which is consistent with the typical saprophytic status of most fungi and their higher capacity than bacteria to grow on complex substrates (Romaní *et al.* 2006). Ectomycorrhizal associations are usually more prevalent in ecosystems with lower rates of organic matter recycling (Chapman *et al.* 2006). Soil texture was also found to be correlated with bacterial community composition, a pattern consistent with previous studies (Girvan *et al.* 2003; Lauber *et al.* 2008).

Most of the above edaphic factors are known to be influenced by vegetation (Eviner & Chapin 2003). Moreover, our results showed a strong relationship between the vegetation cover and these edaphic factors. Many authors have reported that plants can influence soil properties through litter and rhizodeposition, with changes in the rate and quality of substrate input due to alterations in plant species composition and diversity. Binkley (1995) and Thomsen *et al.* (2003) reported that plant species can control some physicochemical properties such as soil pH, carbon to nitrogen ratio, the cation capacity exchange and soil structure. Such mechanisms due to plants argue in favour of the prevalence of vegetal cover in structuring the microbial populations (Gobat *et al.* 2010). Similar results have been reported by Zinger *et al.* (2011) in alpine systems and by Kadowaki *et al.* (2013) in mixed Japanese forests. Thus, all these elements could explain the combined effect of edaphic variables and plant community composition on the bacterial and fungal community variation.

In this study, a metagenetic approach was used to assess the influence of vegetation cover and soil properties on the assemblages of bacterial and fungal communities across six habitat types in New Caledonian ultramafic ecosystems. First, the ultramafic microbial communities analysed in this study show one of the richest and most complex natural terrestrial systems. This result confirms the hypothesis that the properties of ultramafic soils do not limit microbial diversity (Schechter & Branco 2014). Second, there is a complex interaction between the aboveground vegetation, the edaphic characteristics and the partitioning of microbial diversity. At the ultramafic landscape scale of a massif, the bacterial and fungal beta diversities are likely more driven by the vegetation cover than the edaphic properties. The structure of soil microbial communities in ultramafic systems might find an application in the design of new strategies for the restoration of these sites, especially for the management of topsoil. As suggested by van Etten *et al.* (2014), a central issue about separating topsoil of the different communities to help re-creating spatial patterns of vegetation types following mining remains. The present study shows that the separation of topsoils with clearly distinct microbial communities could help to re-create spatial patterns of vegetation types following mining.

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B.L., PhD student, wrote the study and participated in experiment achievement, data process and analyses; A.H., J.P., PhD directors, coordinated the study; A.H., D.M. and J.P. designed the experiment, participated in study writing management and reviewed the study; D.M. participated in field experiment management; C.Y. participated in design experiment, technical and scientific advices; C.F. advised and reviewed the study; C.J.M. and S.S. contributed to bioinformatic analysis; W.L. performed in statistical analyses; and L.A. is representative of industrial partner.

Data accessibility

DNA sequences: NCBI SRA: SRP052988, OTU table, floristic and physicochemical analyses: doi:10.5061/dryad.4fb1 m

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Detailed diagram of the complete material & method.

Table S1 Environmental characteristics of sampling habitats.

Table S2 454 primer sequences and barcode used in the present study.

Fig. S2 Distance based linear models between the floristic structure and the edaphic characteristics.

Table S3 Summarize of sequencing data and relative abundance of major phyla.

Table S4 Results of the distance based linear models between the bacterial structure and the floristic characteristics.

Table S5 Results of the distance based linear models between the bacterial structure and the edaphic characteristics.

Table S6 Results of the distance based linear models between the fungi structure and the floristic characteristics.

Table S7 Results of the distance based linear models between the fungi structure and the edaphic characteristics.