Effects of ultramafic topsoil stockpiling during mine activities on its microbial diversity and other microbiological and physicochemical characteristics

Laurent Bordez, Yvon Cavaloc, Philippe Jourand, Marc Ducouso, Farid Juillot, Hamid Amir

► To cite this version:

Laurent Bordez, Yvon Cavaloc, Philippe Jourand, Marc Ducouso, Farid Juillot, et al.. Effects of ultramafic topsoil stockpiling during mine activities on its microbial diversity and other microbiological and physicochemical characteristics. Ecological Engineering, 2022, 177, pp.106563. 10.1016/j.ecoleng.2022.106563 . hal-03581999

HAL Id: hal-03581999

<https://hal.science/hal-03581999>

Submitted on 1 Mar 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ecological Engineering

Effects of ultramafic topsoil stockpiling during mine activities on its microbiota diversity and other characteristics --Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	ultramafic soils; topsoil; microbiota diversity; bacteria; Fungi; AMF
Corresponding Author:	Hamid AMIR, PhD Université de la Nouvelle-Calédonie Noumea, NEW CALEDONIA
First Author:	Hamid AMIR, PhD
Order of Authors:	Hamid AMIR, PhD Laurent Bordez Yvon Cavaloc Philippe Jourand Marc Ducousso Farid Juillot
Abstract:	<p>Nowadays, the ecological restoration is considered as the best solution for the rehabilitation of mining-degraded areas, particularly when it concerns valuable ecosystems. This is the case in New Caledonia considered as a hot spot of biodiversity, with 82% of endemic plant species in ultramafic soils. The use of topsoil to restore mined areas is an important practice. However, topsoil stockpiling can reduce soil fertility. We studied the evolution of different properties of two topsoils stored in 10 m height piles on mined areas, focusing particularly on the microbiota diversity. The effects of storage duration, depth and topsoil origin were analysed. The studied ultramafic soils were characterized by a relatively high microbiota diversity with, for all tested samples, a total of 53 315 bacterial OTUs corresponding to 45 phyla, and 21 486 fungal OTUs corresponding to 7 phyla. Bacterial and fungal general diversity were not affected after 12 months of storage, but community structures of these groups were changed. Arbuscular mycorrhizal fungi (AMF) diversity was significantly reduced by the storage. Microbial activity, AMF spore numbers, soil organic carbon, total nitrogen, cation exchange capacity and C/N decreased significantly starting from 3 to 9 months of topsoil storage, whereas soil bulk density and pH increased, and extractable concentrations of potentially toxic metals varied only slightly. These changes may affect negatively microbial functions of the soils, in relation to ecosystem diversity. It is then recommended to reduce to a minimum possible the topsoil storage before its use in ecosystem restoration.</p>
Suggested Reviewers:	emile benizri emile.benizri@univ-lorraine.fr kataryna Turnau katarzyna.turnau@uj.edu.pl marjana Regvar marjana.regvar@bf.uni-lj.si radkha sudova sudova@ibot.cas.cz pavla doubkova pavla.doubkova@ibot.cas.cz

COVER LETTER

This research article is about changes that occur in ultramafic topsoils during stockpiling operations before ecological restoration of nickel mining-degraded areas.

It concerns biosphere (ecosystem restoration), anthroposphere (mining activity) and lithosphere (ultramafic rocks and nickel ore).

It is a global ecological approach using a multifactorial microbiological and physicochemical characterization of soils evolution during their storage in mines. It is then a contribution to environmental remediation of anthropogenic degradations, including the reduction of erosion and lagoon pollution.

Hamid Amir

Author statement

Hamid Amir : *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, Noumea, 98851, New Caledonia* ; **email** : hamid.amir@unc.nc

Laurent Bordez : *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, Noumea, 98851, New Caledonia* ; **email** : laurent.bordez@gmail.com

Yvon Cavaloc : *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, Noumea, 98851, New Caledonia* ; **mail** : yvon.cavaloc@unc.nc

Philippe Jourand : IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes, (LSTM, UMR040), TA A-82/J, 34398 Montpellier cedex 5, France ; **email** : philippe.jourand@ird.fr

Marc Ducouso : CIRAD, Laboratoire des Symbioses Tropicales et méditerranéennes (LSTM, UMR113), TA A-82/J, 34398 Montpellier cedex 5, France ; **email** : marc.ducouso@cirad.fr

Farid Juillot : Institut de Mineralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS - SU - MNHN - IRD, Sorbonne Université, case 115, 4 place Jussieu, 75252 Paris Cedex 5, France ; and Institut de Recherche pour le Développement (IRD), ERL 206 IMPMC, 101 Promenade Roger Laroque, BPA5, 98848 Noumea, New Caledonia ; **email** : farid.juillot@ird.fr

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Effects of ultramafic topsoil stockpiling during mine activities on its microbiota diversity and other characteristics

Hamid Amir ^{a*}, Laurent Bordez ^a, Yvon Cavaloc ^a Philippe Jourand ^b, Marc Ducousso ^c,
Farid Juillot ^d

^a *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, Noumea, 98851, New Caledonia*

^b IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes, (LSTM, UMR040), TA A-82/J, 34398 Montpellier cedex 5, France

^c CIRAD, Laboratoire des Symbioses Tropicales et méditerranéennes (LSTM, UMR113), TA A-82/J, 34398 Montpellier cedex 5, France

^d Institut de Mineralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS - SU - MNHN - IRD, Sorbonne Université, case 115, 4 place Jussieu, 75252 Paris Cedex 5, France ; and Institut de Recherche pour le Développement (IRD), ERL 206 IMPMC, 101 Promenade Roger Laroque, BPA5, 98848 Noumea, New Caledonia

*** Corresponding Author: Hamid Amir**, *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, BP R4 -98851 Noumea Cedex, New Caledonia*

Email : hamid.amir@unc.nc

Tel : (687) 29 02 72 - Fax : (687) 25 48 29

Effects of ultramafic topsoil stockpiling during mine activities on its microbiota diversity and other characteristics

Hamid Amir ^{a*}, Laurent Bordez ^a, Yvon Cavaloc ^a Philippe Jourand ^b, Marc Ducousso ^c, Farid Juillot ^d

^a *Institut des Sciences Exactes et Appliquées (ISEA), Université de la Nouvelle-Calédonie, Noumea, 98851, New Caledonia*

^b IRD, Laboratoire des Symbioses Tropicales et Méditerranéennes, (LSTM, UMR040), TA A-82/J, 34398 Montpellier cedex 5, France

^c CIRAD, Laboratoire des Symbioses Tropicales et méditerranéennes (LSTM, UMR113), TA A-82/J, 34398 Montpellier cedex 5, France

^d Institut de Mineralogie, de Physique des Matériaux et de Cosmochimie (IMPMC), UMR 7590 CNRS - SU - MNHN - IRD, Sorbonne Université, case 115, 4 place Jussieu, 75252 Paris Cedex 5, France ; and Institut de Recherche pour le Développement (IRD), ERL 206 IMPMC, 101 Promenade Roger Laroque, BPA5, 98848 Noumea, New Caledonia.

ABSTRACT

Nowadays, the ecological restoration is considered as the best solution for the rehabilitation of mining-degraded areas, particularly when it concerns valuable ecosystems. This is the case in New Caledonia considered as a hot spot of biodiversity, with 82% of endemic plant species in ultramafic soils. The use of topsoil to restore mined areas is an important practice. However, topsoil stockpiling can reduce soil fertility. We studied the evolution of different properties of two topsoils stored in 10

m height piles on mined areas, focusing particularly on the microbiota diversity. The effects of storage duration, depth and topsoil origin were analysed. The studied ultramafic soils were characterized by a relatively high microbiota diversity with, for all tested samples, a total of 53 315 bacterial OTUs corresponding to 45 phyla, and 21 486 fungal OTUs corresponding to 7 phyla. Bacterial and fungal general diversity were not affected after 12 months of storage, but community structures of these groups were changed. Arbuscular mycorrhizal fungi (AMF) diversity was significantly reduced by the storage. Microbial activity, AMF spore numbers, soil organic carbon, total nitrogen, cation exchange capacity and C/N decreased significantly starting from 3 to 9 months of topsoil storage, whereas soil bulk density and pH increased, and extractable concentrations of potentially toxic metals varied only slightly. These changes may affect negatively microbial functions of the soils, in relation to ecosystem diversity. It is then recommended to reduce to a minimum possible the topsoil storage before its use in ecosystem restoration.

Keywords : Ultramafic soils, topsoil, microbiota diversity, bacteria, fungi, AMF

1. Introduction

The soil surfaces stripped by mine activities over the world are constantly rising in relation to the increase of industrial needs, particularly for metals (Deshaies, 2011; Harrison and Rajakaruna, 2011). These practices create important environmental and societal problems (Bradshaw, 1997, Tordoff et al., 2000). Nowadays, ecological restoration is considered as the best option for rehabilitation of these degraded areas (L'Huillier et al., 2010 ; McDonald et al., 2016). Indeed, only this practice can ensure the long-term ecosystem resilience by allowing the reinstallation of at least a part of the original biodiversity. Such a long-term view is vital to face the global crisis affecting the biosphere (Aronson and Alexander, 2013), as stressed by United Nations (UN) general assembly with the proclamation of UN Decade on Ecosystem Restoration for 2021-2030 (Fischer et al., 2020; UN Environment Agency, 2019). However, ecological restoration needs a high level of scientific and technical knowledge, particularly when facing extreme environmental conditions.

In New Caledonia, metal rich ultramafic areas represent one third of the main land containing 20 to 30% of the world nickel resources (L'Huillier and Jaffré, 2010). These ultramafic substrates are covered by very valuable ecosystems, with a high level of endemism (Isnard et al., 2016), up to 82% for plants (Morat et al., 2012). These ecosystems are threatened by mine activities, fire and other anthropogenic pressures, and have been listed as one of the world biodiversity hot spots (Myers et al., 2000; Marchese, 2015). The soils of these areas are generally deficient in macronutrients, particularly P, K and N, and enriched in potentially toxic metals such as Ni, Co, Cr and Mn (Jaffré and L'Huillier, 2010; Proctor, 2003). In addition, Mg is in excess and thus competes with Ca for root absorption, accentuating the selection pressure on plant species (Jaffré and L'Huillier, 2010; Proctor and Nagy, 1992). Finally, most of these

soils that are colonized by ultramafic maquis (shrub heathlands) are subject to large fluctuations of water availability ranging from waterlogging to drought (Jaffré and L’Huillier, 2010). Despite these extreme conditions, these soils contain an important seed bank (Bordez, 2015; L’Huillier et al., 2010) and a high microbial diversity (Bordez et al., 2016; Gourmelon et al., 2016), including original and efficient populations of mycorrhizal fungi (Perrier et al., 2006; Amir et al., 2014a; 2019; Crossay et al., 2019; Waseem et al., 2017).

In relation with these biological properties, the soil stripped during nickel mine activities (generally called topsoil) must be replaced back after mining operations to restore the degraded areas. This practice is considered as fundamental (Ghose, 2001; Sheoran et al., 2010); however the topsoil is often stockpiled before being spread for mined areas restoration (Ghose, 2001). It is frequently the case in New Caledonia, because mines recently operated do not have yet enough surfaces to restore. In other cases, mining activities are not sufficiently coordinated with rehabilitation activities to provide an area for restoration after each stripping operation. Topsoil storage during more than few months can induce a loss of seed bank and a degradation of its fertility (Amir et al. 2014b; Ghose, 2001; Koch, 2007; Stahl et al., 2002). However, only few studies have reported detailed effects of topsoil storage on soil characteristics, including microbiota diversity and structure (Block, 2018; Ezeokoli et al., 2019; Mashigo, 2018). Moreover, to our knowledge, no data on this subject are available for ultramafic topsoils. Yet, the resilience of soil microbiota functions directly depends on microbial community structure (Griffith et al., 2008), in relation to ecological niches of the different taxa, and ecosystem functions also depend on microbiota diversity (Deng, 2012). Different studies reported the covariation of soil microbial and vegetal

communities (Djukic et al. 2010; Yergeau et al. 2010), suggesting that changes in microbiota can induce changes in plant species structure.

We studied the evolution of two topsoils stored in 10 m height piles on mine areas, in New Caledonia. The first topsoil was taken from an open ligno-herbaceous maquis, whereas the second one came from a bushy maquis. We chose a multifactorial approach to analyse the changes that appeared during the storage for microbiota diversity and structure, as well as different other microbial and physico-chemical characteristics. We tested the effects of time storage, depth and topsoil origin.

2. Materials and methods

2.1. Site description, topsoil characteristics and sampling

New Caledonia is located approximately 1,500 km east of Australia and 1,800 km north of New Zealand in the South Pacific. The climate is subtropical to tropical, with a rainy season from December to May and a dry season from September to November.

The two sites where the topsoils were stripped are located on the Koniambo massif (Fig. S1). The monthly temperature in Koniambo fluctuates between 14.5 °C and 21.1 °C, with daily variations of about 5°C. The average monthly rainfall is about 250 mm during the wet season and 100 mm during the dry season.

The first topsoil pile (called topsoil 1) was obtained by stripping a ligno-herbaceous open maquis at an altitude of 450 to 900 m (20° 59.311' S, 164° 49.037' E). The maquis was dominated by the cyperaceous species *Costularia nervosa*, *C. arundinacea*, *Schoenus neocaledonicus* and *Lepidosperma perteres*. The most frequent shrubby species were *Nomandia neocaledonica*, *Hibertia altigena*, *H. pancheri*, *Eucarpha deplanchei*, *Montrouziera gabriellae*, *Myodocarpus floribundus*, *Codia montana* and *Ixora francii*.

The soil was a highly weathered oxisol (ferralsol) with following physico-chemical characteristics (analysis done before the stripping): pF 4.2: 14.85; clay (%): 11.5; silt (%): 27.8; sand (%): 55.2; pH (H₂O): 5.44; organic carbon (%): 2.47 %; total N (%): 0.09; total P (mg kg⁻¹): 120.48; available P (Olsen mg kg⁻¹): 0.00; Ca (g Kg⁻¹): 0.65; CEC (meq 100g⁻¹): 7.50; K CE (meq 100g⁻¹): 0.08; Mg (%): 0.78; Fe (%): 41.60; Mn (%): 0.41; Ni (%): 0.55; Cr (%): 1.94; Co (%): 0.08; Fe DTPA (extractable by dimethylenetriaminopentaacetic acid, mg kg⁻¹): 77.05; Mn DTPA (mg kg⁻¹): 667.61; Ni DTPA (mg kg⁻¹): 76.69; Cr DTPA (mg kg⁻¹): 0.54; Co DTPA (mg kg⁻¹): 121.86.

The second topsoil pile (called topsoil 2) was obtained by stripping a dense bushy maquis at the same altitude (20° 58.331' S, 164° 47.618' E). The main plant species of the ecosystem were: *Tristaniopsis Callobuxus*, *Alstonia spp.*, *Codia montana*, *Hibbertia lucens*, *Metrosideros nitida*, *Costularia arundinacea*, *Lepidosmerma perteres*. The soil was also a weathered oxisol (ferralsol) with following physico-chemical characteristics (analysis done before the stripping): pF 4.2: 16.07; clay (%): 11.5; silt (%): 26.0; sand (%): 55.0; pH (H₂O): 5.36; organic carbon (%): 4.07 %; total N (%): 0.13; total P (mg kg⁻¹): 126.91; available P (Olsen mg kg⁻¹): 0.33; Ca (g Kg⁻¹): 0.60; CEC (meq 100g⁻¹): 9.81; K CE (meq 100g⁻¹): 0.11; Mg (%): 0.82; Fe (%): 44.6; Mn (%): 0.41; Ni (%): 0.48; Cr (%): 1.93; Co (%): 0.09; Fe DTPA (extractable by dimethylenetriaminopentaacetic acid, mg kg⁻¹): 129.62; Mn DTPA (mg kg⁻¹): 629.21; Ni DTPA (mg kg⁻¹): 108.43; Cr DTPA (mg kg⁻¹): 0.27; Co DTPA (mg kg⁻¹): 112.88.

The two soils and associated vegetation were stripped with a 25-ton articulated hydraulic excavator. The stripped vegetation was integrated to the topsoils. The topsoil piles were placed on relatively flat places and had a height of 10 m (Fig. S2).

Topsoil samples were collected at the constitution of the topsoil piles (t0); and every three months until 18 months (t3; t6; t9; t12; t18). For sampling, an opening was

done on the pile with an excavator and samples were collected at three different depths (0.1 m; 2 m; 10 m) with a disinfected planter, after scraping of the first cm. Except for the determination of bulk density, soil samples were sieved at 2 mm mesh.

2.2. General physico-chemical analyses

Sieved and dried soil samples were sent to the Laboratoire des Moyens Analytiques (LAMA US IMAGO) of the IRD Noumea Center for physico-chemical characterization (see physico-chemical properties listed above) using standard methods (<https://imago.ird.fr/moyens-analytiques/noumea/les-prestations>).

2.3. Soil DNA extraction and bar-coded pyrosequencing of bacteria and fungi

DNA pyrosequencing analyses were realized at the beginning of the storage and after 12 months for the three depth levels. 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). We performed the alternative lysis method proposed by the manufacturer. Soil subsamples were vortexed for 3–4 s and heated to 70 °C for 5 min three times. The DNA samples were then 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 by Woese (1987) as the broadly conserved DNA barcode. The primers 27F and 553R were used to amplify this region. The fungal community was analyzed by targeting the DNA region of the ITS2 rRNA gene, which is widely used as a DNA metabarcoding marker (Bates et al., 2013). The primers ITS3 and ITS4 were used to amplify this region. As shown by White et al. (1990), the primer set provides 95% of fungal and 99% of Glomeromycota DNA. The full set of primers used is reported in

Table S1. The PCRs were conducted in 25 μ L reactions with 0.625 μ L (0.25 μ M) of each primer, 2.5 ng of the template DNA for each soil subsample, 1 X PCR buffer and 0.08 U of GoTaq^R Flexi DNA polymerase (Promega Corporation, Madison, WI, USA). The cycling conditions were: 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. The amplified DNA was purified by filtration (Sephadex G50; Sigma-Aldrich, St. Louis, MO, USA) and the product purity was visualized on agarose 1.5% gel in a TAE buffer. Gels were stained with ethidium bromide (0.5 μ g/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 for each microbial domain. The DNA concentration of each amplicons was determined using a NanoDrop spectrophotometer (Nano- Drop 2000; Thermo Scientific, Wilmington, DE, USA). Finally, for both bacteria and 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).

2.4. Processing of pyrosequencing data

The quality of the data was analyzed as described by Nilsson et al. (2012) and processed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit (Caporaso et al., 2010). 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. We used ITSX SOFTWARE (version 1.0.10) (Bengtsson-Palme et al., 2013) to remove non-fungal 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-INSO 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 and Holmes (2014), who showed that the rarefaction of microbiome count data is inefficient in the statistical sense, the EDGER package within the R statistical environment ('EDGERPACKAGE', Robinson et al., 2010), was used to normalize the differential sequencing depth analysis and the OTU composition for the two microbial domains. The 'calcNormFactors' function was used to find a set of scaling factors for the library sizes that minimize the log-fold changes between the samples for most genes. We then generated an OTU normalized matrix for both fungi and bacteria. These new matrixes were used to the downstream statistical analysis.

2.5. Global microbial activity (carbon mineralisation)

Soil microbial activity is a good index of fertility as it releases mineral elements for plant nutrition. We chose to measure the CO₂ produced by microbial respiration,

corresponding to carbon mineralisation. The CO₂ can be easily captured and dosed. Each soil sample (40g of equivalent dry soil adjusted to 80% of retention capacity) was incubated 28 days in an hermetic bottle (500 mL) containing a small flask with 10 mL of NaOH 0.25 N. To avoid the saturation of the NaOH solution, the flask was replaced after 14 days and the sample was then incubated 14 days more. The CO₂ produced was quantified with 0.1 M and 0.5 M HCl solutions (Joergensen, 1996) for each of the two periods of 14 days and the values were then summed to obtain the final value. Three soil samples, each representing a mix of two, were reacted for each depth and each time of storage.

2.6. *Arbuscular mycorrhizal fungi spore extraction and quantification*

Arbuscular mycorrhizal symbiosis is very important in New Caledonian ultramafic maquis (Amir and Ducousso, 2010; Perrier et al., 2006). It was then of interest to follow up the abundance of arbuscular mycorrhizal fungi (AMF) during topsoil storage. Regarding the high number of samples, we chose to count the viable AMF spores after humid sieving followed by sucrose gradient centrifugation (method modified from Daniels and Skipper, 1982). Four soil samples were used for each time of storage and depth. For each sample, 50 g of sieved and dried soil were placed in a 255 µm mesh sieve adjusted above a 36 µm mesh sieve. The soil was then wet sieved through the two sieves and the harvested material in the 36 µm mesh sieve was suspended in 20 mL of water in a 50-mL Falcon tube. A 25-mL sucrose solution (70% v/w) was injected at the bottom of the tube, forming a stepped density gradient that was centrifuged at 800 rpm for 5 min. Spores of AMF were collected from the interface with the sucrose solution, washed with tap water on a 36 µm sieve, and transferred to a 50-mL Falcon tube with 10 mL of water. Finally, the Falcon tube was vortexed and 5 successive

aliquots of 100 μL were immediately transferred to Petri dishes for spore counting under a stereomicroscope. Only viable spores containing protoplasm and not broken were counted.

2.7. Bulk density

As the topsoil situated in depth inside the piles can be compressed, it was Regarding the possible compression of soil within the piles, it was important to measure the changes of the bulk density during topsoil storage. This analysis was done by weighting 300 cm^3 of non-sieved soil, before and after drying at 105°C during 72h. Then we calculated two parameters: i) the bulk density value ρ ($\text{g}\cdot\text{cm}^{-3}$) as the mass/volume ratio of the dry soil; ii) the Water-Filled Pore Space (Robertson and Groffman, 2015) as $\text{WFPS} = (\text{TE} \times 100 \times \rho) / (1 - (\rho / \rho_s))$, where TE is the soil water content in % (soil dried 72h at 105°C). Four soil samples were tested for each level and topsoil pile.

2.8. Statistical analyses

The statistical analyses were performed using R statistical environment ('VEGAN PACKAGE', R.3.1.1, R foundation). The differences between the variable means were tested with a permutational multivariate analysis of variance (PERMANOVA). In case of significant differences, PERMANOVA pairwise comparisons were used to identify the differences. The results were illustrated by nonmetric multidimensional scaling (NMDS) analysis. The relationship between fungal or bacterial communities and edaphic variables was tested using spearman correlation (PRIMER V7). When a significant relationship was found, a distance-based linear model (DISTLM) was built to identify the significant predictors 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 and Anderson, 2001).

3. Results

3.1. Changes in the composition of bacterial communities

From the 454-pyrosequencing data, we obtained global numbers of 138 932 and 269 046 exploitable sequences of bacteria respectively for topsoil 1 and topsoil 2. These sequences could be classified in 24 382 OTUs for topsoil 1 and 33 449 OTUs for topsoil 2. At the beginning of the storage (t0) the mean number of OTUs was 2917 for topsoil 1 and 4730 for topsoil 2 which has a significantly higher bacterial diversity (Fig. 1). This diversity did not change significantly after 12 months of storage whatever the depth considered.

A total of 45 bacterial phyla were identified. Overall, for the two topsoils and the three depths, the dominant phyla were: Proteobacteria ($43.0 \pm 2.9\%$), Acidobacteria ($29.1 \pm 1.6\%$), Actinobacteria ($13.0 \pm 0.2\%$), Planctomyces ($8.4 \pm 1.1\%$), Chloroflexi ($5.8 \pm 0.7\%$), Verucomicrobia ($5.1 \pm 0.8\%$), Bacteroidetes ($4.1 \pm 1.4\%$), Firmicutes ($2.7 \pm 0.4\%$). The other phyla accounted for less than 2% each (Fig. 2A). The same bacterial orders were present in the two topsoils (Fig. 2B). After 12 months of storage, these taxa differed on their relative abundance. Respectively 45% and 50% of the observed differences between t0 and t12 were due to the variations in 12 taxa. It corresponded mainly to a reduction of the abundance of Planctomycetales, Rhodospirillales, Acidobacteriaceae and Chthoniobacterales, and an increase of the abundance of Burkholderiales, Sphingomonadales, Sphingobacteriales and Nocardiodaceae. Details

of the abundance values and corresponding standard errors for the different taxa are given in table S2.

A non-metric multidimensional scaling (NMDS) ordination (Fig. 3) showed significant differences ($p < 0.001$) of the bacterial OTU assemblages, between the two storage periods tested (t0 and t12), and this for the two topsoils. These latter were also clearly different for their bacterial community structure ($p < 0.001$). No differences were detected between the three topsoil depths. Furthermore, the distance based linear model (DISTLM) approach (Fig. S3) showed that 16% of the variability between t0 and t12 storage periods was explained significantly by variations, during the storage, of pH (for 10% of the variability) and organic carbon (6% of the variability).

3.2. Changes in the composition of fungal communities

The global numbers of exploitable fungal sequences obtained were 181 096 and 307 254 respectively for topsoil 1 and topsoil 2. These sequences were classified in 9 357 fungal OTUs for topsoil 1 and 11 779 for topsoil 2. The number of fungal OTUs at t0 was 533 for topsoil 1 and 965 for topsoil 2, which has a significantly higher fungal diversity (Fig. 4). Except for a significant increase of the fungal diversity after 12 months at the surface layer of topsoil 1, the other samples did not show any significant differences in relation with the time of storage or the depth.

Overall, for the two topsoils and the three depths, the dominant phyla, were: Ascomycota ($46.2 \pm 5.7\%$) and Basidiomycota ($25.6 \pm 7.2\%$) (Fig. 5A). The non-identified phyla represented $28.4 \pm 0.2\%$. The other phyla were less abundant Zygomycota ($3.3 \pm 0.5\%$), Chytridiomycota ($> 1\%$), Glomeromycota ($> 1\%$), Blastocladiomycota ($> 1\%$) were. The composition of the phyla changed significantly

after 12 months of storage for the two topsoils, with a reduction of the Basidiomycota abundance and an increase of the Ascomycota and the unidentified phyla abundance. The two topsoils significantly differed for fungal community structure ($p < 0.001$). A total of 41 fungal classes were identified. Among them 10 were dominant (Fig. 5B). Moreover, 68.3% (topsoil 1) and 69.8% (topsoil 2) of the observed changes after 12 months of storage were related to a reduction of the Agaricomycetes, and an increase of the Sordariomycetes and an unidentified group. Details of the abundance values and corresponding standard errors for the different taxa are given in table S3.

NMDS ordination (Fig. 6) showed that significant changes in the fungal OTU assemblages appeared after 12 months of storage for the two topsoils ($p < 0.001$), but without significant effect of the depth. The DISTLM approach (Fig. S4) indicated that 29.3% of these changes in fungal communities during topsoil storage were explained by variation in pH H₂O (13.4%), carbon mineralization (7.3%), extractable Mn content (4.4%) and exchangeable K (4.3%).

Arbuscular mycorrhizal fungi (AMF) sequences (Glomeromycota) were also analysed; respectively 77 and 177 sequences were obtained for Topsoil 1 and Topsoil 2. At t₀, these sequences corresponded respectively to 22 and 47 OTUs (Fig. 7), Topsoil 2 being significantly richer. After 12 months of storage, the number of OTUs decreased significantly (*i.e.* 3 to 4 times), for the two topsoils. Overall, the depth did not affect the OTUs number, except for 10 m depth in Topsoil 1 which showed significantly less OTUs than those of the two other depths. The structure of AMF communities was affected by the storage for the two topsoils. However, the OTU numbers for each taxa were too small to obtain reliable statistical analysis (data not shown).

3.3. Evolution of the global microbial activity

Global microbial activity expressed by microbial respiration in the incubated soil samples was negatively affected by the topsoil storage (Fig. 8). In topsoil 1, the activity dropped significantly (*i.e.* 5 to 7 times) after 6 months and then tended to stabilize. The same dynamic was observed for topsoil 2, but with a lower reduction of the activity. Some values (between 3 and 9 months) were significantly lower at the deepest layer in comparison with the surface of the piles. Negative correlations between microbial activity on the one hand and depth and bulk density on the other hand were detected (respectively $r = -0.338$, $p < 0.01$ and $r = -0.330$, $p = 0.01$).

3.4. Evolution of AMF viable spore numbers

The number of viable AMF spores in topsoil 1 dropped significantly 3 times, after 3 months of storage in the surface of the topsoil pile and more than 4 times after 6 months, before stabilizing until 18 months (Fig. 9). In topsoil 2, the decrease was less rapid until 6 months, but the decline was finally stronger (*i.e.* 10 times) after 12 months. A significant difference between the two topsoils for this parameter was detected ($p < 0.001$). The reduction of viable spore numbers was significantly stronger in the deepest layer for the two topsoil piles during the first period (*i.e.* 6 to 9 months), before dropping equally for all depths. A negative correlation between the number of viable spores and the depth was noted ($r = -0.527$, $p < 0.0001$). AMF viable spore richness was also negatively correlated to bulk density ($r = -0.322$, $p < 0.01$), and positively correlated to organic carbon ($r = 0.412$, $p < 0.0001$), and Cation Exchange Capacity ($r = 0.42$, $p < 0.0001$).

3.5. Evolution of the physico-chemical characteristics

Topsoil bulk density varied similarly (*i.e.* from 1.2-1.3 g.cm⁻³ to 1.8-1.9 g.cm⁻³) for the two topsoil piles during storage (Fig 10). Soil density increased mostly during the first three months. However, differences between the three tested depths were significant only after 9 months, with higher densities in the deepest layer (10 m). The Water-Filled Pore Space (Fig. 10) did not change significantly (*i.e.* from 50 to 53 %) in the surface layer during storage. However, this parameter showed a significant decrease in the deepest layer after 9 and 18 months of storage for topsoil 1 and after 6 months of storage for topsoil 2.

The organic carbon stock in topsoil 1 decreased from 31 g.kg⁻¹ to less than 10 g.kg⁻¹ after 9 months storage, with significant differences compared to the initial soil (t0) that after 6 months storage (Fig. 11). In topsoil 2, the loss in organic carbon after 9 months storage was lower (*i.e.* from 42 g.kg⁻¹ to 20 g.kg⁻¹). For the two topsoils, no significant differences were detected between the three depths. The dynamic of total nitrogen was very close to that of organic carbon (Fig. 11). Organic carbon and total nitrogen were negatively correlated with the duration of storage (respectively $r = -0.417$, $p = 0.0001$; and $r = -0.324$, $p = 0.01$).

The pH values (Fig. S5) increased from 5.4 to 6.2 during storage in topsoil 1 and from 5.2 to 6.6 in topsoil 2. Significant differences appearing after 3 to 6 months of storage. Overall, pH values were higher in the deep layers of the piles. The C/N values (Fig. S6) dropped significantly from 23-25 to 3-5 after three months of storage for the two topsoils. The values did not vary significantly for longer duration of storage.

Among the other physico-chemical parameters that have been followed (Table S4), it can be noticed that topsoil storage induced a reduction of CEC, exchangeable K and Ca as well as the Ca/Mg ratio after 6 to 9 months for the two topsoils. In topsoil 1, extractable Fe, Ni, Mn and Co decreased significantly after 3 to 9 months of storage,

whereas extractable Cr increased 6 to 10 times after three months of storage and then stabilized. In topsoil 2, only extractable Fe was reduced and extractable Co increased after three months of storage, before stabilizing.

4. Discussion

The storage of 10 m height piles of ultramafic topsoils during 18 months induced modifications of their microbiota structure and affected different other characteristics.

4.1. *Effects of topsoil stockpiling on microbiota diversity*

Despite their extreme edaphic characteristics related to the ultramafic nature of their parent rocks, the studied soils from ultramafic ecosystems, showed unexpectedly high microbial diversity richness (4730 bacterial OTUs and 965 fungal OTUs for topsoil 2), comparable to those reported for temperate forests or Amazonian forests (Bordez et al., 2016). In a previous study, Gourmelon et al. (2016) found similar results for different other ultramafic soils from New Caledonia. This high richness is consistent with Mouillot et al. (2013) assertion that ultramafic ecosystems host highly complex microbial communities. Topsoil 2, from a bushy maquis with a denser vegetation, was characterized by a higher bacterial and fungal diversity. This relation between ecosystem productivity and microbial diversity richness agrees with different reported studies for other types of ecosystems (Cong et al., 2015; Kennedy et al., 2005; Li et al. 2015). Bordez et al. (2016) showed that the gradient in vegetation density and productivity of 6 ultramafic ecosystems from New Caledonia is the main factor driving microbial diversity richness, even if the edaphic properties (especially total N, pH and CEC) also influence the richness of microbial communities.

In the present study, the microbiota global diversity of the two topsoils was not affected by 12 months storage. Bacterial and fungal OTU numbers were not significantly different compared to the non-stored control topsoil, whatever the depth considered. At the order level, all taxa survived during topsoil storage, at least at a low abundance. In a study comparing a 5-years stockpiled topsoil with the non-mined corresponding topsoil, Ezeokoli et al. (2019) found a higher microbiota diversity in the latter. The duration of storage can probably explain the differences with our study. Indeed, in our experiment organic carbon and mineralization activity started to decline only after 6 to 9 months of storage. The number of cultivable aerobic bacteria also dropped significantly starting from 6 to 9 months and was only 7 times lower after 12 months (data given in Bordez, 2015). Some species becoming rare after 12 months could thus disappear from the stored topsoil after few years. In fact, contrary to the diversity, the structure of microbial communities changed after 12 months of storage. For bacteria, these changes were mainly driven by variations on the relative abundance of 12 taxa (at the order level), some of them showing a decreasing abundance and others showing an increasing abundance. Fungal taxa showed a similar type of changes, with variations in the relative abundance of three classes.

Studies discussing variations in microbial taxa composition in relation with soil properties are relatively recent and quite limited in number. Among these studies, Gourmelon et al. (2016) found the same 4 dominant bacterial phyla than those found in the present study (*i.e.* Proteobacteria, Planctomyces, Acidobacteria and Actinobacteria) in different ultramafic soils from New Caledonia. The domination of Proteobacteria, Acidobacteria and Actinobacteria was also reported by other studies in different soil types and conditions (Herrera et al., 2007; Ko et al., 2017; Liang et al., 2019). Ko et al. (2017) showed changes of bacterial phyla structure in deep layers of

the soil, with an increase of Acidobacteria in relation with carbon reduction caused by microbial activity. In our study, Acidobacteria did not vary with depth and reduction of organic carbon after 12 months of storage. However, the DISTLM analysis indicated that the reduction of organic carbon caused by microbial activity affected the bacterial community structure of the two stockpiled topsoils. As observed by Waldrop et al. (2006) and Lauber et al. (2008), organic carbon reduction can have different consequences, particularly in terms of competition and starvation of a part of the microbiota. In our study, the phyla of Planctomycetes was less represented after 12 months of topsoil storage in comparison with the non-stored ones. This phylum has indeed been reported as sensitive to soil history (Buckley et al., 2006). Our DISTLM analysis also detected an effect of soil pH on the bacterial community structure. Soil pH is commonly reported as regulating the structure of microbiota communities (Lauber et al., 2008; Rousk et al., 2010; Sheng et al., 2019). In our case, it is interesting to note that Acidobacteria, the second most important bacterial phylum in the studied topsoils, declined after storage in topsoil 2 where the pH value raised from 5.2 to 6.5. This result is in agreement with Sait et al. (2006) who indicated that Acidobacteria grow optimally from pH 4 to 5.5 and poorly or not at all for pH above 6.5.

Concerning fungi, Ascomycota and Basidiomycota were generally dominant in our study. This result is in agreement with previous studies on ultramafic soils from New Caledonia (Bordez et al., 2016; Gourmelon et al., 2016) and on other soil types (Karst et al., 2013; Ko et al., 2017; Weber et al., 2013; Wang et al., 2020). The main detected variables that were found to structure these communities were pH, carbon mineralization, exchangeable K and DTPA extractable Mn. The statistical analysis showed that the relative importance of the Basidiomycota was positively correlated with organic carbon ($p = 0.02$) and carbon mineralisation ($p < 0.01$) (data not shown).

Indeed, we observed a clear reduction of the Basidiomycota group after 12 months of topsoil storage, as carbon concentration and C/N dropped drastically. This phylum is known for its ability to decompose organic matter, particularly litter and wood (Purahong et al., 2016; 2018; Wang et al., 2020). In addition, many Basidiomycota species are ectomycorrhizal and may then be disturbed by the topsoil storage. Among the Basidiomycota, the class of Agaricomycetes was dominant, which is frequently reported in similar studies (Karst et al., 2013; Ko et al., 2017; Sheng et al., 2019). Inversely, Ascomycota were more represented after 12 months of storage, particularly in deep layers. This result can probably be linked to the high resistance of this group to many soil stresses (Egidi et al., 2019), such as pH variations and organic carbon rarefaction. Indeed, Ascomycota are mostly saprotroph with a large variety of enzymes that enable them to use recalcitrant substrates (Ko et al., 2017).

AMF communities were also relatively diverse, with 22 and 47 OTUs found for the two non-stored topsoils. On average, these values correspond to those reported for different other soil types (Schechter and Burns 2008; Yu et al. 2012; Hassan et al. 2014; Kruger et al. 2017). The 12 months storage clearly reduced this diversity for the two topsoils. As obligatory symbionts, AMF can survive mainly as spores in absence of plants, but the spores were also affected as indicated by the reduction of their numbers during the storage. Regarding the ecological importance of AMF in ultramafic soils from New Caledonia (Amir et al., 2010; Amir et al., 2019; Crossay et al. 2019), this reduction of AMF diversity and density in stored topsoils may slow down the relative growth of plant species in restored areas. According to Birnbaum et al. (2017), AMF communities of stockpiled sandy topsoils naturally recolonized by plants take 5 to 10 years, before returning to their initial level.

Other changes can also influence the structure of the microbiota in relation with the specific needs of each taxa. A compaction of the soil after 12 months of storage has been noted for the three topsoil layers, particularly in the 10 m depth layer. Such an increase of the bulk density with the depth has also been observed by Ezeokoli et al. (2019) in stockpiled soils. This increase can create an anoxia and inhibit taxa that require high concentrations of oxygen.

4.2. Effects of topsoil stockpiling on some physico-chemical and microbiological properties

The evolution of bulk density and water-filled pore space values showed that the two studied topsoils have been compacted during the first months of storage, especially in deep layers. The sandy nature of the soils (*i.e.* 55% sand fraction) and their relatively low organic C contents suggest that compaction could have been driven by aggregates destruction upon driving rain and high pressure in deep layers. Such a compaction effect with reduction of soil aggregates has been reported by other studies for stockpiled topsoils (Abdul-kareem and McRae, 1984; Strohmayer, 1999; Wick et al., 2007). Organic C and total N contents decreased 2 to 3 times after 9 months of storage, without significant differences between the three tested depth levels. Several studies showed a reduction of organic matter in stored soils (Visser et al., 1984; Harris and Birch, 1989; Gobat et al., 2010; Birnbaum et al., 2017) as a consequence of microbial mineralization in absence of any carbon flux. This assumption is supported by our results, which showed a positive correlation between organic C and microbial activity. We also observed a strong decrease, before stabilization, of the microbial activity after 6 months of storage in the two topsoils, in

agreement with those from other studies (Visser et al., 1984; Harris et al., 1989; Strohmayer, 1999).

Other chemical properties showed some variations in relation to organic matter reduction, and/or leaching after driving rains during topsoils storage. Among those, CEC, as well as exchangeable K and Ca decreased after 3 to 9 months of storage. However, no significant differences could be found between the three tested depths. Organic carbon reduction and leaching can also explain the reduction of extractable Fe, Mn and Co, since the fraction of these metals that is associated with organic matter through complexation is usually the most extractable (McGrath, 1996; Menzies et al., 2007). This explanation is supported by the lack of variation of extractable Fe, Mn and Co in topsoil 2 where organic carbon reduction is much lower. The increase of extractable Cr during storage can also be attributed to organic carbon reduction since organic matter is known to counteract Cr oxidation by Mn-oxides and thus to reduce Cr extractability in soils (Fendorf et al., 1992; 1995).

5. Conclusions

The two topsoils stockpiled in mine conditions during 18 months showed different modifications of their microbiota diversity and other microbiological and physico-chemical properties. Overall, these variations differed significantly for the two topsoils and some were influenced by the depth of storage. Our results thus suggest that New Caledonian ultramafic soils are sensitive to management during mine activities. If the changes in physico-chemical characteristics in these conditions are relatively well known, the perturbations induced on microbiota community structure of the stored topsoils must be regarded with attention, considering the possible degradations of soil microbial functions (Griffith et al., 2008; Ezeokoli et al., 2019). Indeed, some studies

reported variations of the structure of plant communities in relation to differences of microbial communities (Djukic et al., 2010; Yergeau et al., 2010). Yu et al. (2012) found a clear relationship between root-associated fungal communities and plant health status. Changes on microbial community structure upon topsoil stockpiling could thus induce degradations of soil functions and then perturbations of the dynamic of the reconstructed ecosystems. Considering all the above-mentioned results, the duration of the stockpiling of ultramafic topsoils should be reduced as far as possible and in all cases should not exceed 6 months. It is also recommended to reduce the height of the stockpiles when possible to 1 m or less to minimize the compaction and the anaerobiosis (Amir et al., 2014b).

Acknowledgments

The authors gratefully acknowledge CNRT Koniambo Nickel Society for providing the financial support.

Supplementary data

Supplementary data associated with this article can be found online at: doi...

Author Contribution

H. A. supervised and designed the research, provided the funding and wrote the manuscript; L. B. realized the research studies, contributed to their design and checked the manuscript text; Y. C. contributed to the design and supervision of molecular studies ; P. J. contributed to the design and supervision of molecular studies and checked the manuscript text; M. D. contributed to the supervision and checked

the manuscript text; F. J. contributed to the supervision of physico-chemical characterizations and their interpretation and checked the manuscript text.

References

Abdul-Kareem A.W., McRae S.G., 1984. The effects on topsoil of long-term storage in stockpiles. *Plant Soil* 76, 357–363.

Amir H., Ducouso M., 2010. Les bactéries et les champignons du sol sur roches ultramafiques, In : L’Huillier L, Jaffré T, Wulff A (Eds), *Mines et environnement en Nouvelle-Calédonie : les milieux sur substrats ultramafiques et leur restauration*. IAC Ed., Noumea, pp. 129-145.

Amir H., Jourand P., Cavaloc Y., Ducouso M., 2014a. Role of mycorrhizal fungi on the alleviation of heavy metal toxicity on plant, In: Solaiman Z, Abbott L, A. Varma A, (Eds), *Mycorrhizal fungi: Use in sustainable agriculture and forestry*. Soil Biology Series, Springer, Heidelberg, pp. 241-258.

Amir H., L’Huillier L., Fogliani B., Cavaloc Y., Jourand P., Ducouso M., Majorel C., Hannibal L., Saintpierre D., Gunkel P., Pagand P., Echevarria G., Mouchon L.C., Bonis M.L., Montargues-Pelletier E., Maggia L., Wulff A., 2014b. Caractérisation et fonctionnement du système sol/ plante/ microorganismes dans les maquis miniers. Perspectives d’application à la restauration écologique. Rapport Final- Projet ECOMINE BIOTOP – CNRT « Nickel et son environnement. Edition CNRT, 315 p.

Amir H., Cavaloc Y., Laurent A., Pagand P., Gunkel P., Lemestre M., Médevielle V., Pain A., McCoy S., 2019. Arbuscular mycorrhizal fungi and sewage sludge enhance growth and adaptation of *Metrosideros laurifolia* on ultramafic soil in New Caledonia: a field experiment. *Sci. Total Environ.* 651, 334-343.

- 610 Aronson J., Alexander S., 2013. Ecosystem Restoration is Now a Global Priority: Time
611 to Roll up our Sleeves. *Restor. Ecol.* 21, 293-296.
- 612 Bates S.T., Ahrendt S., Bik H.M., Bruns T.D., Caporaso J.G., Cole J., Dwan M., Fierer N.,
613 Gu D., Houston S., Knight R., Leff J., Lewis C., Maestre J.P., McDonald D., Nilsson
614 R.H., Porras-Alfaro A., Robert V., Schoch C., Scott J., Taylor D.L., Parfrey L.W., Stajich
615 J.E., 2013. Meeting Report: Fungal ITS Workshop (October 2012). *Standards in*
616 *Genomic Sciences.* 8.
- 617 Bengtsson-Palme J., Ryberg M., Hartmann M., Branco S., Wang Z., Godhe A., De Wit
618 P., Sánchez-García M., Ebersberger I., de Sousa F., Amend A.S., Jumpponen A.,
619 Unterseher M., Kristiansson E., Abarenkov K., Yann B., Y., Sanli K., Eriksson K.M.,
620 Vik U., Veldre V., Nilsson R.H., 2013. Improved software detection and extraction of
621 ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for
622 analysis of environmental sequencing data (M Bunce, Ed.). *Methods Ecol. Evol.* 4,
623 914-919.
- 624 Birnbaum C., Bradshaw L.E., Ruthrof K.X., Fontaine J.B., 2017. Topsoil stockpiling in
625 restoration: Impact of storage time on plant growth and symbiotic soil biota. *Ecol.*
626 *Restor.* 35, 237-245.
- 627 Block P.R., 2018. Monitoring the effects of surface coal-mine-reclamation on soil
628 biological properties. Master thesis. Nord Dakota State University. Nord Dakota.
- 629 Bordez L., 2015. Stratégies de revégétalisation des maquis miniers nickelifères de
630 Nouvelle-Calédonie: étude sur les potentiels biologiques des topsoils et leur gestion
631 en vue de leur utilisation pour la restauration écologique des milieux dégradés. PhD
632 thesis, Université de la Nouvelle-Calédonie, Noumea.
- 633 Bordez L., Jourand P., Ducousso M., Carriconde F., Cavaloc Y., Santini S., Claverie J.M.,
634 Wantiez L., Leveau A., Amir H., 2016. Distribution patterns of microbial

communities in ultramafic landscape: a metagenetic approach highlights the strong relationships between diversity and environmental traits. *Mol. Ecol.* 25, 2258-2272.

Bradshaw A., 1997. Restoration of mined lands - using natural process. *Ecol. Eng.* 8, 255-269.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello K.E., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. methods* 7, 335–336.

Cong J, Yang Y, Liu X et al. (2015) Analyses of soil microbial community compositions and functional genes reveal potential consequences of natural forest succession. *Sci. Rep-UK* 5, 10007.doi: 10.1038/srep10007.

Crossay T., Majorel C., Redecker D., Gensous S., Medevielle V., Durrieu G., Cavaloc Y., Amir H., 2019. Is a mixture of arbuscular mycorrhizal fungi better for plant growth than single-species inoculants? *Mycorrhiza* 29, 325-339.

Daniels, B. A., Skipper, H. D., 1982. Methods for the recovery and quantitative estimation of propagules from soil, In: Schenck. N. C. (Ed.), *Methods and Principles of Mycorrhizal Research*. Amer. Phytopath. Soc, St. Paul, Minnesota. pp. 29–35.

Deng H., 2012. A review of diversity-stability relationship of soil microbial community: What do we know? *J. Soil Environ.* 24, 1027-1035.

Deshaies M., 2011. Grands projets d'exploitation minière et stratégie des firmes pour se rendre environnementalement acceptables. *L'Espace Politique* 15-3 Firmes et géopolitiques, DOI : 10.4000/espacepolitique.2113.

- 659 Djukic I., Zehetner F., Mentler A., Gerzabek M.H., 2010. Microbial community
660 composition and activity in different Alpine vegetation zones. *Soil Biol. Biochem.*
661 42, 155–161.
- 662 Egidi E., Delgado-Baquerizo M., Plett J.M., Wang J., Eldridge D.J., Bardgett R.D.,
663 Maestre F.T., Singh B.K., 2019. A few Ascomycota taxa dominate soil fungal
664 communities worldwide. *Nat. Commun.* 10, 2369. doi.org/10.1038/s41467-019-
665 10373-z.
- 666 Ezeokoli O.T., Mashigo S.K., Paterson D.G., Bezuidenhout C.C., Adeleke R.A., 2019.
667 Microbial community structure and relationship with physicochemical properties of
668 soil stockpiles in selected South African opencast coal mines. *Soil Sci. Plant Nutr.* 65,
669 332-341.
- 670 Fendorf S.E. and Zasoski R.J., 1992. Chromium (III) oxidation by α -MnO₂. I:
671 Characterization. *Environ. Sci. Technol.* 26, 79-85. doi.org/10.1021/es00025a006
- 672 Fendorf S.E., 1995. Surface reaction of chromium in soils and waters. *Geoderma* 67,
673 55-71. doi.org/10.1016/0016-7061(94)00062-F
- 674 Fischer J., Riechers M., Loos J., Martin-Lopez B., Temperton V.M., 2021. Making the UN
675 Decade on Ecosystem Restoration a Social-Ecological Endeavour. *Trends Ecol. Evol.*
676 36, 20-28.
- 677 Ghose M., 2001. Management of Topsoil for Geo-environmental Reclamation of Coal
678 Mining Areas. *Environ. Geol.* 40, 1405-1410.
- 679 Gobat J-M., Aragno M., Matthey W., 2010. *Le sol vivant: bases de pédologie, biologie*
680 *des sols*. Presses polytechniques Universitaires Romandes, Lausanne.
- 681 Gourmelon V., Maggia L., Powell J.R. , Gigante S., Hortal S., Gueunier C., Letellier K. ,
682 Carriconde F., 2016. Environmental and Geographical Factors Structure Soil

- 683 Microbial Diversity in New Caledonian Ultramafic Substrates: A Metagenomic
684 Approach. PLoS ONE 11 (12), e0167405. doi:10.1371/journal.pone.0167405.
- 685 Griffiths B.S., Hallett P.D., Kuan H.L., Gregory A.S., Watts C.W., Whitmore A.P., 2008.
686 Functional resilience of soil microbial communities depends on both soil structure
687 and microbial community composition. *Biol. Fertil. Soils* 44, 745–754.
- 688 Harris J, Birch P, Short K 1989: Changes in the microbial community and physico-
689 chemical characteristics of topsoils stockpiled during opencast mining. *Soil Use*
690 *Manage.*, 5, 161–168.
- 691 Harrison S., Rajakaruna N., 2011. *Serpentine: The Evolution and Ecology of a Model*
692 *System*. University of California Press, Berkeley, CA.
- 693 Herrera A., Héry M., Stach J.E.M., Jaffré T., Norman P., Navarro E., 2007. Species
694 richness and phylogenetic diversity comparisons of soil microbial communities
695 affected by nickel-mining and revegetation efforts in New Caledonia. *Eur. J. Soil*
696 *Biol.* 43, 130–139.
- 697 Isnard S., L'huillier L., Rigault F., Jaffré T., 2016. How did the ultramafic soils shape the
698 flora of the New Caledonian hotspot? *Plant Soil* 403, 53-76.
- 699 Jaffré T., L'Huillier L., 2010. La végétation des roches ultramafiques ou terrains
700 miniers, In : L'Huillier L, Jaffré T, Wulff A (Eds), *Mines et environnement en*
701 *Nouvelle-Calédonie : les milieux sur substrats ultramafiques et leur restauration*.
702 IAC Ed., Noumea, pp. 45-103.
- 703 Joergensen R.G., 1996. The fumigation extraction method to estimate microbial
704 biomass : calibration of the kEC value. *Soil Biol. Biochem.* 28, 25-31.
- 705 Karst J., Piculell B., Brigham C., Booth M., Hoeksema J.D., 2013. Fungal communities in
706 soils along a vegetative ecotone. *Mycologia* 105, 61-70.
- 707 Kennedy N., Edwards S., Clipson N., 2005. Soil bacterial and fungal community

- 708 structure across a range of unimproved and semi-improved upland grasslands.
709 Microb. Ecol. 50, 463–73.
- 710 Ko D., Yoo G., Yun S.T., Jun S.C., Chung H. 2017. Bacterial and fungal community
711 composition across the soil depth profiles in a fallow field. J. Ecol. Environ. 41, 34.
712 DOI: 10.1186/s41610-017-0053-0
- 713 Koch J.M., 2007. Alcoa's mining and restoration process in South Western Australia.
714 Restor. Ecol. 15, 11–16.
- 715 Kunin V., Engelbrektson A., Ochman H., Hugenholtz P., 2010. Wrinkles in the rare
716 biosphere: pyrosequencing errors can lead to artificial inflation of diversity
717 estimates. Environ. Microbiol. 12, 118–23.
- 718 Lauber C.L., Strickland M.S., Bradford M.A., Fierer N., 2008. The influence of soil
719 properties on the structure of bacterial and fungal communities across land-use
720 types. Soil Biol. Biochem. 40, 2407–2415.
- 721 L'Huillier L., Jaffré T., 2010. L'exploitation des minerais de nickel en Nouvelle-
722 Calédonie, In : L'Huillier L, Jaffré T, Wulff A (Eds), Mines et environnement en
723 Nouvelle-Calédonie : les milieux sur substrats ultramafiques et leur restauration.
724 IAC Ed., Noumea, pp. 21-31.
- 725 L'Huillier L., Wulff A., Gateblé G., Fogliani B., Zongo C., Jaffré T., 2010. La restauration
726 des sites miniers, In : L'Huillier L, Jaffré T, Wulff A (Eds), Mines et environnement en
727 Nouvelle-Calédonie : les milieux sur substrats ultramafiques et leur restauration.
728 IAC Ed., Noumea, pp. 147-230.
- 729 Li H., Wang X., Liang C., 2015. Aboveground-belowground biodiversity linkages differ
730 in early and late successional temperate forests. Sci. Rep-UK 5, 12234. Doi:
731 10.1038/srep12234.

- 732 Liang H., Wang X., Yan J., Luo L., 2019. Characterizing the Intra-Vineyard Variation of
733 Soil Bacterial and Fungal Communities. *Front Microbiol* 10, 1239. doi:
734 10.3389/fmicb.2019.01239. eCollection.
- 735 Marchese C., 2015. Biodiversity hotspots: A shortcut for a more complicated concept.
736 *Global Ecol. Conserv.* 3, 297–309.
- 737 Margulies M., Egholm M., Altman W.E., et al., 2005. Genome sequencing in
738 microfabricated high-density picolitre reactors. *Nature*, 437, 376–380.
- 739 Mashigo S.K., 2018. Diversity and functional attributes of microorganisms from
740 stockpiled soils of coal mines in Mpumalanga Province, South Africa. Master
741 Dissertation), North-West University, Potchefstroom.
- 742 McArdle B.H., Anderson M.J., 2001. Fitting multivariate models to community data: a
743 comment on distance-based redundancy analysis. *Ecology*, 82, 290-297.
- 744 McDonald T., Jonson J., Dixon K.W., 2016. National standards for the practices of
745 ecological restoration in Australia. *Restor. Ecol.* 24, S4-S32.
- 746 McGrath D., 1996. Application of single and sequential extraction procedures to
747 polluted and unpolluted soils. *Sci. Tot. Environ.* 178, 37-44. doi.org/10.1016/0048-
748 9697(95)04795-6
- 749 McMurdie P.J., Holmes S., 2014. Waste not, want not: why rarefying microbiome data
750 is inadmissible. *PLoS comput. Boil.* 10, e1003531. doi.org/10.1371/journal.pcbi.
751 1003531.
- 752 Menzies N.W., Donn M.J. and Kopittke P.M., 2007. Evaluation of extractants for
753 estimation of the phytoavailable trace metals in soils. *Environ. Pollut.* 145, 121–
754 130. doi:10.1016/j.envpol.2006.03.021
- 755 Morat P, Jaffré T., Muzinger J. Pillon Y., Veillon J-M., Chapolin M., Birnbaum P.,
756 Rigault F., Dagostini G., Tinel J., Lowry P., 2012. The taxonomic reference base

- 757 «FLORICAL» and characteristics of the native vascular flora of New-Caledonia.
758 *Adansonia* 3, 179–221.
- 759 Mouillot D., Bellwood D.R., Baraloto C., Chave J., Galzin R., Harmelin-Vivien, Michel
760 Kulbicki H., Lavergne L., Lavorel S., Mouquet N., Paine C.E.T., Renaud J. , Thuiller
761 W., 2013. Rare species support vulnerable function in high-diversity ecosystems.
762 *PLoS Boil.* 11, e1001569. doi.org/10.1371/journal.pbio.1001569.
- 763 Myers N., Mittermeier R.A., Mittermeier C.G., da Fonseca G.A., Kent J., 2000.
764 Biodiversity hotspots for conservation priorities. *Nature* 403, 853–858.
- 765 Nilsson R.H., Tedersoo L., Abarenkov K., Ryberg M., Kristiansson E., Hartmann M.,
766 Schoch C.L., Nylander J.A.A, Bergsten J., Porter T.M., Jumpponen A.,
767 Vaishampayan P., Ovaskainen O., Hallenberg N., Bengtsson-Palme J., Eriksson
768 K.M., Larsson K.H., Larsson E., Kõljalg U., 2012. Five simple guidelines for
769 establishing basic authenticity and reliability of newly generated fungal ITS
770 sequences. *MycoKeys* 4, 37–63.
- 771 Perrier N., Amir H., Colin F., 2006. Occurrence of mycorrhizal symbioses in the metal-
772 rich lateritic soils of the Koniambo Massif, New Caledonia. *Mycorrhiza* 16, 449-
773 458.
- 774 Proctor J., 2003. Vegetation and soil and plant chemistry on ultramafic rocks in the
775 tropical Far East. *Perspect. Plant Ecol.* 6, 105-124
- 776 Proctor J., Nagy L., 1992. Ultramafic rocks and their vegetation: an overview, In: Baker
777 AJM, Proctor J, Reeves RD (Eds.) *The vegetation of ultramafic (serpentine) soils.*
778 Intercept Ltd, Andover, pp. 470-495.
- 779 Purahong, W. Wubet T, Lentendu G, Schlöter M, Pecyna MJ, Kapturska D, Hofrichter
780 M, Krüger D, Buscot F. 2016. Life in leaf litter: novel insights into community

- 781 dynamics of bacteria and fungi during litter decomposition. *Molecular Ecology*
 782 25(16), 4059–4074.
- 783 Quast C., Pruesse E., Yilmaz P. Gerken J., Schweer T., Yarza P., Peplies J., Glöckner F.O.,
 784 2013. The SILVA ribosomal RNA gene database project: improved data processing
 785 and web-based tools. *Nucleic acids res.* 41, D590–6. DOI:10.1093/nar/gks1219.
- 786 Robertson G. P., Groffman P. M., 2015. Nitrogen transformations, In: Paul, E. A. (ed.),
 787 Soil microbiology, ecology and biochemistry. Fourth edition. Academic Press,
 788 Burlington, pp. 421-446.
- 789 Robinson M.D., McCarthy D.J., Smyth G.K., 2010. edgeR: a Bioconductor package for
 790 differential expression analysis of digital gene expression data. *Bioinformatics* 26,
 791 139-140.
- 792 Rousk J., Bååth E., Brookes P.C., Lauber C.L., Lozupone C., Caporaso J.G., Knight R.,
 793 Fierer N., 2010. Soil bacterial and fungal communities across a pH gradient in an
 794 arable soil. *ISME j.* 4, 1340–1351.
- 795 Sait M., Davis K.E.R., Janssen P.H., 2006. Effect of pH on Isolation and Distribution of
 796 Members of Subdivision 1 of the Phylum Acidobacteria Occurring in Soil. *Appl.*
 797 *Environ. Microbiol.* 72, 1852–1857.
- 798 Sheng Y., Cong W., Yang L., Liu Q., Zhang Y., 2019. Forest Soil Fungal Community
 799 Elevational Distribution Pattern and Their Ecological Assembly Processes. *Front.*
 800 *Microbiol.* 10:2226. doi: 10.3389/fmicb.2019.02226.
- 801 Sheoran V., Sheoran A.S., Poonia P., 2010. Soil Reclamation of Abandoned Mine Land
 802 by Revegetation!: A Review. *International Journal of Soil Sediment and Water*, 3, 1–
 803 21.

- 804 Stahl P.D., Perryman B.L., Sharmasarkar S., Munn L.C., 2002. Topsoil Stockpiling Versus
805 Exposure to Traffic: A Case Study on In situ Uranium Wellfields. *Restor. Ecol.* 10,
806 129–137.
- 807 Strohmayer P., 1999. Soil Stockpiling for Reclamation and Restoration activities after
808 Mining and Construction. *Restoration and Reclamation Review* 4, 1-6.
- 809 Tordoff G.M., Baker A.J.M., Willis A. J., 2000. Current approaches to the revegetation
810 and reclamation of metalliferous mine wastes. *Chemosphere*, 41, 219–228.
- 811 United Nations Environment Agency Resolution 73/284. 2019. United Nations Decade
812 on Ecosystem Restoration (2021–2030). <https://undocs.org/A/RES/73/284>.
- 813 Visser S., Fujikawa J., Griffiths C.L., Parkinson D., 1984. Effect of topsoil storage on
814 microbial activity, primary production and decomposition potential. *Plant Soil* 82,
815 41-50.
- 816 Waldrop M.P., Zak D.R., Blackwood C.B., Curtis C.D., Tilman D., 2006. Resource
817 availability controls fungal diversity across a plant diversity gradient. *Ecol. Lett.* 9,
818 1127–1135.
- 819 Waseem M., Ducousso M., Prin Y., Domergue O., Hannibal L., Majorel C., Jourand P.,
820 Galiana A., 2017. Ectomycorrhizal fungal diversity associated with endemic
821 *Tristaniopsis* spp. (Myrtaceae) in ultramafic and volcano-sedimentary soils in New
822 Caledonia. *Mycorrhiza* 27, 407–413.
- 823 White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of
824 fungal ribosomal RNA genes for phylogenetics, In: Innis, M.A., Gelfand, D.H.,
825 Sninsky, J.J., and White, T.J. (eds), *PCR Protocols: A Guide to Methods and*
826 *Applications*. Academic Press, New York, pp. 315–322.
- 827 Woese C.R., 1987. Bacterial evolution. *Microbiol. Rev.* 51, 221-271.

- 828 Yergeau E., Bezemer T.M., Hedlund K., Mortimer S.R., Kowalchuk G.A., van der Putten
829 W.H., 2010. Influences of space, soil, nematodes and plants on microbial
830 community composition of chalk grassland soils. *Environ. Microbiol.* 12, 2096–
831 2106.
- 832 Wang Y., Xu X., Liu T., Wang H., Yang Y., Chen X., Zhu S., 2020. Analysis of bacterial
833 and fungal communities in continuous-cropping ramie (*Boehmeria nivea* L. Gaud)
834 fields in different areas in China *Sci. Rep.* 10, 3264. doi: 10.1038/s41598-020-
835 58608-0.
- 836 Weber C.F., Vilgalys R., Kuske C.R., 2013. Changes in fungal community composition in
837 response to elevated atmospheric CO₂ and nitrogen fertilization varies with soil
838 horizon. *Front. Microbiol.* 4, 78. doi: 10.3389/fmicb.2013.00078.
- 839 Wick, A.F., Stahl P.D., Rana S., Ingram L.J., 2007. Recovery of reclaimed soil
840 structure and function in relation to plant community composition. In: *Thirty*
841 *years of SMCRA and beyond: Proceedings of the 24th National Meetings of the*
842 *American Society of Mining and Reclamation*, Gillette, WY. 2–7 June 2007.
843 Curran Assoc., Red Hook, NY. pp. 941–957.
- 844 Yu L., Nicolaisen M., Larsen J., Ravnskov S., 2012. Molecular characterization of root-
845 associated fungal communities in relation to health status of *Pisum sativum* using
846 barcoded pyrosequencing. *Plant Soil* 357, 395–405.

Color should be used for all figures in print

Fig. 1

Fig. 1. Bacterial OTU numbers (mean $\pm$ se) in 12 months stockpiled topsoils (t12) compared to the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test (n = 10).

Fig. 2. Relative abundance of bacterial phyla (A) and orders (B) in 12 months stockpiled topsoils (t12) compared to the non-stored topsoils (t0).

Fig. 3

Fig. 3. Non-metric multidimensional scaling analysis (NMDS) of bacterial community structure of 12 months stockpiled topsoils (t12) compared with the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). The distance between the ellipses indicates the degree of similarity between the communities. T0 bacterial communities differed significantly between the two topsoils and with the corresponding stored topsoils. LH = ligno-herbaceous maquis topsoil; P = bushy maquis topsoil.

Fig. 4

Fig. 4. Fungal OTU numbers (mean $\pm$ se) in 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test ($n = 10$). The topsoil 2 was found significantly richer than topsoil 1 at t0.

Fig. 5. Relative abundance of fungal phyla (A) and classes (B) in 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0).

Fig. 6

Fig. 6. Non-metric multidimensional scaling analysis (NMDS) of fungal community structure of 12 months stockpiled topsoils (t12) in comparison with the non-stored corresponding topsoil (t0) at different depth (0.1m, 2 m and 10 m). The distance between the ellipses indicates the degree of similarity between the communities. T0 fungal communities differed significantly between the two topsoils and with the corresponding stored topsoils. LH = ligno-herbaceous maquis topsoil; P = bushy maquis topsoil.

Fig. 7. AMF OTU numbers (mean $\pm$ se) in 12 months stockpiled topsoils (t12) compared with the non-stored topsoils (t0) at different depth (0.1m, 2 m and 10 m). For each graph, bars with the same letter are not significantly different according to a one-way PERMANOVA followed by Pair-wise permutation test ($n = 10$).

Fig. 8

Fig. 8. Evolution of the microbial activity (carbon mineralisation) of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ($n = 6$ mixed 2 by 2).

Fig. 9

Fig. 9. Evolution of viable AMF spore numbers in stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ($n = 6$ mixed 2 by 2).

Fig. 10

Fig. 10. Evolution of bulk density and water-filled pore space of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ($n = 4$).

Fig. 11

Fig. 11. Evolution of organic carbon and total nitrogen of stockpiled topsoils in relation to time storage and depth. For each graph, means with the same letter are not significantly different according to a two-way PERMANOVA followed by Pair-wise permutation test ($n = 4$).