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Fellfields of the Kerguelen Islands harbour specific soil microbiomes and rhizomicrobiomes of an endemic plant facing necrosis

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Polar regions are characterized by rocky terrains with sparse vegetation and oligotrophic soils, i.e. “fellfields”. In such ecosystems, microbial communities should be essential for soil-plant functioning but their diversity is poorly explored. The sub-Antarctic Kerguelen Islands fellfields are characterized by an endemic long-lived cushion plant, *Lyallia kerguelensis* which rhizosphere may be a shelter for microbes in this harsh environment. Cushions are affected by necrosis and we expect the rhizomicrobiome composition to be related to plant necrosis. We analysed bacterial and fungal communities in bulk- and rhizospheric soils from *L. kerguelensis* in five different fellfields across the Kerguelen Islands using 16S rRNA and ITS1 metabarcoding. We found that soil microbial communities were composed of both restricted and cosmopolitan taxa. While all sites were dominated by the same bacterial taxa (*Chloroflexi*, *Actinobacteria*, α -*Proteobacteria* and *Acidobacteria*), the relative abundance of the main fungal phyla (*Ascomycota*, *Basidiomycota*, *Mortierellomycota* and *Rozellomycota*) highly differed between sites. *L. kerguelensis* rhizomicrobiome was at least as diverse as the bulk soil, making the rhizosphere a possible reservoir of microbial diversity. It was composed of the same main bacterial phyla than detected in the bulk soil while the composition of the rhizosphere fungal communities was specific to each plant. No common microorganisms were identified regarding cushion necrosis extent across plants and sites, but several microbial putative functions were shared, suggesting a possible shift in soil functioning with cushion necrosis increase. Our study brings new information on the diversity and composition of the microbial communities of fellfield soils in a sub-Antarctic Island and the rhizomicrobiome of a characteristic endemic cushion plant.

KEYWORDS

sub-Antarctic, bacteria, fungi, *Lyallia kerguelensis*, cushion plant, dieback

Introduction

Fellfields are habitats characteristic of polar, sub-polar and alpine regions and they consist in a form of tundra ecosystem made of rocks with sparse vegetation and infertile soil (1). Microbial diversity in fellfield soils remains understudied despite the potentially meaningful implication of microbes in soil functioning, nutrient cycling and plant survival in such harsh habitats. For example, due to the rarity of herbivores and invertebrates (in most fellfields), the food webs are mainly driven by decomposition processes that involve microbial activity (2, 3). Within the sub-Antarctic, the Kerguelen Islands are located in the southern Indian Ocean and are isolated by 3 500 km to the nearest vegetated continent and by 2 000 km to the Antarctic. According to these geographical properties, the soil microbial communities they harbour might be shaped by dispersal limitation, leading to narrow microbial diversity as observed in soils from the Antarctic continent (2). However, strong winds that occur in this region may also transport microorganisms over long distances (4, 5), leading to complex assemblages of restricted and cosmopolitan soil microbes shaped by both dispersal limitation and aeolian transport. Moreover, within the archipelago, fellfields show contrasted edaphic properties which may induce strong environmental filtering that drive the soil microbiome assemblages, as already observed in the heterogeneous Arctic landscape (6).

In the fellfields of the Kerguelen Islands, the vegetation is composed of small forbs, short grasses and cushion plants and among them, stands *Lyallia kerguelensis* (Montiaceae) (7), an endemic and long-lived (more than 16 years) cushion plant. For most cushion plants, their dense prostrate shape increases the beneath soil organic matter content, generally making this rhizospheric environment a favorable habitat for soil microorganisms, compared to the surrounding nutrient-poor soil (8, 9). Although the rhizomicrobiome is known to be less diverse with a specific composition compared to the bulk soil microbiome in many ecosystems (e.g. grassland and forest) (10). Indeed, the assembly of the rhizomicrobiome, governed by plants, is directed toward beneficial microorganisms that enhance the plant's capacity to resist to various stresses and to adapt to its environment (11). The composition of the rhizomicrobiome of *L. kerguelensis* might thus result from both plant-driven selection, corresponding to co-evolved species assemblage favoured by i) the longevity of this plant species, and ii) the modifications of the habitat conditions beneath it (12, 13).

The involvement of the rhizomicrobiome in plant nutrition, water uptake and stress resistance (e.g., drought, starvation, frost) was observed in many cases, enhancing plant fitness and homeostasis (e.g., its ability to resist to abiotic stresses) (11, 12, 14–16). These microorganisms might be of great importance for the plants to tolerate climate change that is rapidly occurring in

polar and sub-polar regions, indeed, higher winter temperatures and lower winter precipitations are currently observed (17). These changes induce abiotic stresses to the inhabiting vegetation leading to plant dieback (i.e. the impossibility of the plant to generate new foliage) (18–20). In the Kerguelen Islands, necrotic parts in the cushions of *L. kerguelensis* are described since the early 1990s (7) and are now observed in many sites across the archipelago (7, 21). Thus, considering the harsh environmental conditions that prevail in the fellfields of the Kerguelen Islands and the long lifespan of this plant, we expect *L. kerguelensis* to recruit within its rhizosphere beneficial microorganisms and this rhizomicrobiome would be involved in the plant's ability to resist to the abiotic stresses that this it faces, i.e. limiting necrosis extent.

Using amplicon sequencing that allows access to the total microbial diversity including uncultivable microorganisms (22), we analysed the diversity of bacterial and fungal communities, within the Kerguelen Islands, in both fellfield soils and rhizospheres of *L. kerguelensis* cushions randomly selected for their necrosis extent. We believed that, in fellfield soils, i) an assemblage of both restricted and cosmopolitan soil microorganisms occurred, shaped in particular by dispersal limitation and aeolian transport, and ii) that the soil properties could lead to environmental filtering, inducing site-specificity of the soil microbiome. Then, we thought that the buffered climatic conditions under the plant cushion offer a favorable habitat for soil microorganisms in these particular harsh environmental conditions, leading to a larger diversity within the rhizosphere than in the surrounding bulk soil. Finally, we hypothesised that the rhizomicrobiome might be involved in plant's vigour facing necrosis and studied linear relations between microbial composition and plant necrosis extent.

Materials and methods

Study sites and model plant

The Kerguelen Islands (48°30' - 50°00' S, 68°27' - 70°35' E) are located in the southern Indian Ocean. The landscape is hilly and there are 22 species of native phanerogams (23), with no shrubs or trees and numerous fellfields are observed from the shore to the highest elevations exposed to wind (24). The climate is chronically cold with a mean annual temperature of 4.6°C and a constant wind (around 10 m.s⁻¹), inducing sea-salt spray in a large part of the islands (25, 26). Our study took place during two summer field campaigns (2017–2018 and 2018–2019). In total, five fellfield sites were studied in “Ile Mac Murdo” (MAC1), “Ile Longue” (LON30), “Presqu’île Jeanne d’Arc” (PJDA2 and PJDA6) and “Ile Australia” (AUS25) (Figure 1A and Table 1. Each site (50 m²) comprised at least twenty (up to a hundred) cushions of *L. kerguelensis* scattered on a scale of meters.

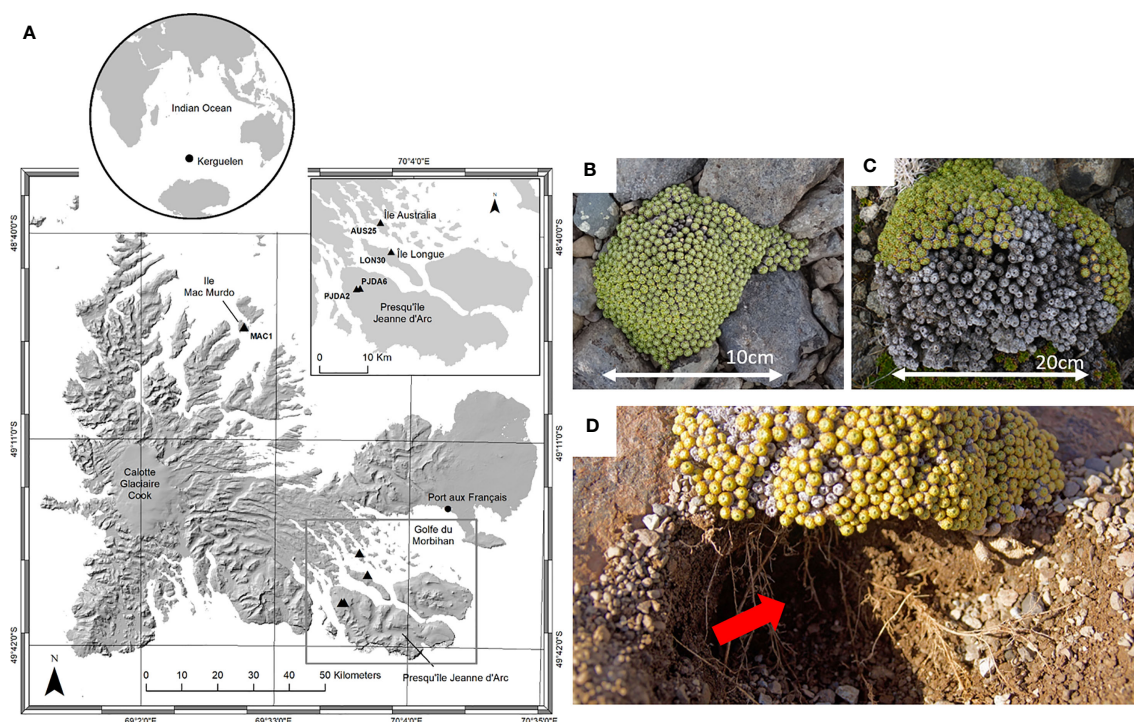


FIGURE 1

(A) Locations of the five sampling sites (black triangles) in Kerguelen Islands on an altitudinal background (NASA-SRTM 30 M, 2005). The black dots indicate the research stations, labels indicate the site names and important features in the island, the grey rectangle shows the zoom into presented on the top right of the figure. Top right map: zoom into the Southeast part with four out of the five studied sites. The map background comes from IGN 1/200 000 georeferenced with spatial adjustment (D. Fourcy, unpublished). The small globe sets the Kerguelen Islands in the South Indian Ocean. ArcGIS 10.8.1. (B) Picture (from above) of *Lyallia kerguelensis* with only a few necrotic apices and (C) with more than half of the cushion necrotic. (D) *Lyallia kerguelensis* and its root system, location of the soil sampling (red arrow), the cushion shows necrosis.

Lyallia kerguelensis Hook. f. belongs to the Montiaceae (27) and has a hemispherical shape of 20 – 40 cm in diameter (7), (Figures 1B, C). This species is endemic to the Kerguelen Islands and has a sparse distribution in fellfields (7). The root system is deep with a main taproot and numerous fine roots branching out (28), (Figure 1D). Within each site, ten plants of *L. kerguelensis* were randomly selected. We know that *L. kerguelensis*' flowering phenology is synchronous within and among populations (28), we carefully selected plants in vegetative stage or with a small part (approximately less than 10% of the cushion surface area) showing fruit or flowers. The necrotic surface area of each plant studied (further called necrosis extent) was measured by photointerpretation (21) (Table 1). Necrosis ranged from 0% to 79% depending on the cushions studied, and the PJDA2 and MAC1 sites showed more variability in necrosis extent than LON30 and AUS25.

Soil sampling

For each plant, a single sample of soil in contact with roots, further referred to as rhizospheric soil, was *in situ* collected

beneath the cushion, at 2 - 8 cm depth (fig. 1D). Around 6 mL of soil was collected under each plant, avoiding roots and rocks. For the bulk soil, we collected surface soil, from 0 to 8 cm depth, at different places assumed to be free of root influence, a single composite bulk soil sample of 200 mL was then made for each site. A subsample of 100 mL was used for soil physico-chemical analyses (Laboratoire d'Analyse des Sols, INRAE, Arras, France), more details on the analyses and parameters are described in Marchand et al., 2021 (23), and in Table 1. All samples were stored at -20°C until DNA extraction. A total of 5 bulk soils (one per site) and 50 rhizospheric soils (10 per site) were collected.

DNA extraction

For every rhizosphere and bulk soil sample, three technical replicates of soil DNA extraction were done and processed separately. For each replicate, DNA extractions were performed from 0.5 g of fresh soil following the protocol of Griffiths et al., 2000 (29) modified by Monard et al., 2013 (30). It consisted of a manual extraction, first with a cell lysis step in lysing matrix E tubes (MP Biomedicals, Santa Ana, California) in

TABLE 1 GPS coordinates of the studied sites and their soil physico-chemical properties and plant necrosis parameters, “*” indicates the variables removed for the NMDS analysis due to correlation with other variables.

Characteristics	MAC1	LON30	AUS25	PJDA2	PJDA6
Longitude	69.470	69.908	69.876	68.876	69.820
Latitude	-48.902	-49.520	-49.466	-49.591	-49.591
Altitude (m)	55	154	125	520	482
Clay (%)	1.20	8.50	4.90	4.20	5.00
Silt (%)*	15.20	38.10	14.90	16.70	12.00
Sand (%)*	83.60	53.40	80.20	79.1	83.00
P (mg/kg)	10.00	27.00	11.00	25.00	38.00
N (g/kg)*	1.72	3.66	1.85	1.12	1.27
Organic C (g/kg)	17.80	41.70	23.90	13.40	16.60
Organic matter (g/kg)*	30.80	72.10	41.40	21.10	28.80
C/N	10.35	11.39	12.92	11.96	13.07
K (mg/kg)	59.64	186.38	89.46	96.92	223.65
Na (mg/kg)	181.62	147.14	381.63	68.97	82.76
CEC	4.82	5.85	4.16	2.41	5.29
NH ₄ ⁺ (mg/kg)	6.81	2.57	4.75	1.00	5.90
NO ₃ ⁻ (mg/kg)	0.68	1.33	1.83	1.00	0.49
pH	6.34	5.52	6.12	6.04	6.37
min necrosis (%)	0	0	0	0	1
max necrosis (%)	52	24	37	79	40
mean necrosis (%)	20.57 ± 3.20	6.30 ± 1.74	10.71 ± 2.09	27.81 ± 5.81	16.00 ± 2.79

presence of a lysing buffer containing phenol/chloroform/isoamyl alcohol and followed by nucleic acid precipitation in polyethylene glycol (PEG) 6000 buffer. DNA quality and quantity were checked using NanoDrop device (ThermoFisher Scientific, Waltham, USA) and agarose gel electrophoresis (1%, TAE 1X). DNA extracts were stored at -20°C.

PCR amplification and Illumina sequencing

The bacterial *16S rRNA* gene and the fungal ITS libraries were constructed using the protocol given by the Illumina platform (31), based on a two-step PCR approach. The following primer sets were used for bacteria: 341F (32) and 785R (33) targeting the variable V3 and V4 regions of the *16S rRNA* gene, and for fungi: ITS1F (34) and ITS2 (35) targeting the ITS1 region. Amplifications were performed as described in Hellequin et al. (36). Each primer set contained the overhang adapter: forward overhang (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse overhang (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'). PCRs were carried out in a total volume of 25 µL containing: 12.5 µL of high fidelity Taq polymerase (2X *TransTaq* HiFi TransGen Biotech, China), 1 µL of each primer (10 µM), 0.5 µL of T4gp32 (100 ng/µL) (for fungal amplification), 2 µL (for bacterial amplification) or 4 µL (fungal amplification) of DNA diluted

100 times each and ultrapure water to reach the final volume. For bacteria, the following amplification conditions were used for the first PCR: 95°C for 4 minutes, 25 cycles of 95°C, 52°C, and 72°C during 30 sec each and 10 minutes at 72°C was applied. For Fungi, amplification in the same conditions was performed but with 30 cycles. Amplifications were checked by agarose gel electrophoresis (2%, TAE 1X). Two PCRs per sample were performed and pooled, with each time PCR negative and positive controls to ensure the success of the amplification. The amplicons were handled by the EcogenO platform (Rennes, France) for normalization, barcoding, multiplexing and Illumina paired-end (2 × 300 bp) MiSeq sequencing.

Microbial sequence analysis

Bacterial and fungal sequences were processed using the FROGS pipeline in the Galaxy project Version 3.1 (37). The reads were merged with a minimum overlap of 20 bp (FLASH). The minimal sequence length was 350 bp for bacteria and 120 bp for fungi and the maximum sequence length was 450 bp for both kinds of amplicons. The primer sequences and sequences where the two primers were not present were removed and the sequences were dereplicated. The chimeras were removed using the VSEARCH tool with the UCHIME *de novo* method combined with a cross-sample validation (38, 39). Clustering was performed using the Swarm method with a distance

difference of 3 and OTUs with a relative abundance below 5.10^{-6} were removed (40). The taxonomic affiliations were done using BLAST (NCBI, USA) with the Silva_132_16S database for bacteria and the Unite_Fungi_8.0_18112018 (including a RDP assignation) for fungi. Sequences not associated with bacteria or fungal domain were discarded (e.g. archaea sequences). Only OTUs present in at least two out of the three technical replicated samples were kept for further analysis. At the end of the process, a total of 3 342 733 sequences corresponding to 6 316 OTUs for bacteria and 6 467 991 sequences corresponding to 2 623 OTUs for fungi were obtained. Six and twelve technical replicates from different samples were removed for bacteria and fungi, respectively, due to their low number of sequences (lower than 2 000 and 150 for bacteria and fungi, respectively) compared to the other technical replicates of the same sample. Moreover, for bacteria, one rhizosphere sample (all three replicates) from the AUS25 site was removed due to a too low number of sequences. All data are available in the NCBI sequence read archive (SRA) under accession number PRJNA731349.

Data analyses

Statistical analyses were done using R version 3.4.2. Graphs were created with the ggplot2 package (<https://github.com/tidyverse/ggplot2>). The α -error of 0.05 was used except for (i) correlations between soil physico-chemical properties and microbial community composition and (ii) correlations between necrosis extents and OTUs abundances for which a α -error of 0.01 was chosen to focus on highly significant relations.

To study the α -diversity by calculating species richness and Shannon diversity index [package Phyloseq (41)], the number of sequences was rarefied to 3 758 and 172 sequences per sample for bacteria and fungi respectively (Figure S1). These thresholds were motivated to keep as many technical replicates as possible and limit removing soil and rhizosphere samples. Non-parametric Dunn's Kruskal-Wallis multiple comparisons were applied to study the variability of microbial diversities (species richness and Shannon index, calculated with estimated richness function of package Phyloseq) in bulk soils and rhizosphere soils across sites and between the rhizosphere and its associated bulk soil.

To study the variability of OTUs composition within and across sites, for both bulk and rhizosphere samples the unrarefied dataset was used. A core analysis (microbiome package, <https://github.com/microbiome>) was performed and complemented by Venn diagrams [VennDiagram package (42)]. To study a possible site effect on the composition of microbial communities in the bulk soil, we studied the bacterial community similarities between sites with a permutational multivariate analysis of variance [i.e. PERMANOVA] (function Adonis package vegan (43)) on weighted unifracs

distances when the beta-dispersions were similar. When the beta-dispersions were not similar, we applied an analysis of similarities (i.e. ANOSIM) [function anosim, package vegan (43)] on weighted unifracs distances using 1000 permutations.

The correlations between bulk beta diversity (weighted unifracs distances) and soil physico-chemical properties were performed [envfit function of vegan package (43)], significantly auto-correlated soil properties were previously removed. Bacterial and fungal communities and the significant soil properties were represented with a non-metric multidimensional scaling (i.e. NMDS) [package Phyloseq (41)].

Finally, for each sampling site, Pearson correlations (package stats) were done between the relative abundance of microbial OTUs and the necrosis extent of each plant for OTUs that had a mean relative abundance higher than 0.1% and that were present in at least 85% and 65% of the rhizosphere samples of a site for bacteria and fungi, respectively. These thresholds were defined according to the distribution of OTUs and their occurrence per population.

Results

Microbial communities of fellfield soils of the Kerguelen Islands are composed of both restricted and cosmopolitan bacteria and fungi

Except in LON30, all soils from the five fellfield sites were loamy sand (from 79.1 to 83.6% of sand) and presented low contents in total nitrogen (N), organic carbon (OC) and organic matter (OM) (Table 1). The soil from LON30 contained a lower proportion of sand (53.4%) allowing higher amounts in N, OC and OM, however, all five soils presented low C:N ratios (Table 1).

On average per site, the richness of soil bacteria and fungi ranged from 337.6 to 590.3 OTUs and from 6.5 to 57.3 OTUs, respectively, and the Shannon index ranged from 4.4 to 5.8 and from 1.4 to 3.3, respectively; both presenting significant differences between sites (Figures S2A, C, E, G; Richness: $Z = 3.22$ and 1.96 , $p = 0.001$ and 0.049 ; Shannon index: $Z = 3.22$ and 2.11 , $p = 0.001$ and 0.035 , for bacteria and fungi, respectively). Fungal richness was low in LON30 and PJDA2 probably due to the low amount of sequences obtained from the sequencing as observed in Figure S1. Microbial α -diversity was the highest in AUS25 and in PJDA6 for fungi (Figures S2A, E, C, G). While PJDA2 and PJDA6 fellfield sites were the closest geographically (Figure 1), they presented significant differences in the diversity of their soil microbiome (except for bacterial Shannon index), PJDA6 being always significantly richer than PJDA2 (Richness $Z = 2.34$ and 2.70 , $p = 0.019$ and 0.007 ; Shannon index $Z = 1.37$ and 2.59 , $p = 0.17$ and 0.010 for bacteria and fungi, respectively) (Figures S2A, E, C, G). Interestingly, microbial species richness and Shannon index in the soil of LON30, whose texture differed

from the four other soils (Table 1), were similar to those observed in the other sites except for the bacterial species richness in AUS25 (Figures S1A, E, C, G).

Whatever the site, the dominant bacterial taxa in the bulk soils were *Chloroflexi*, *Actinobacteria*, α -*Proteobacteria* and *Acidobacteria*. However, their relative abundance differed between sites (Figure 2A). *Chloroflexi* was always the most represented phylum (from 33% to 49% of the total amount of sequences) excepted in the bulk soil of MAC1 that was dominated by the *Actinobacteria* (41% of the total amount of sequences, Figure 2A). The dominant fungal phyla detected in the bulk soils of the five sites were *Ascomycota*, *Basidiomycota*, *Mortierellomycota* and *Rozellomycota*, and their relative abundance highly differed between sites (Figure 2B). The relative abundance of *Ascomycota* was at least 32% in each bulk soil (Figure 2B). This phylum was dominant in MAC1, LON30 and PJDA6 sites with a relative abundance of 42%, 57% and 51%, respectively, while the *Basidiomycota* dominated in PJDA2 site (56% of the total amount of sequences, Figure 2B). Interestingly, the bulk soils of MAC1 and AUS25 contained a high proportion of unknown fungal sequences (37% and 48% of the total amount of sequences, respectively; Figure 2B).

Ninety-four bacterial OTUs, but no fungal ones, were detected in all five sites and constituting the soil core microbiome of Kerguelen Islands fellfields. They represented 52% of the total amount of bacterial sequences from the bulk soils (Figure 3A, Table S2). On top of the four dominant bacterial phyla described above, members of the *Armatimonadetes*, *Planctomycetes* and *WSP-2* phyla were also identified as part of this soil core microbiome (Figure 3A, Table S2). Each site also harboured a specific soil microbiome, composed of 18 to 319 site-specific bacterial OTUs and up to 145 site-specific fungal OTUs (Figures 3A and C). These specific soil microbiomes induced spatial variability in the composition of microbial communities as observed with the NMDS ordination (Figures 4A and B) and, for both bacterial and fungal communities, a site effect was significantly observed ($R^2=0.83$ and 0.96 and p -value <0.001 and 0.001 respectively). Some soil physico-chemical properties were identified as significantly correlated to microbial community structure. In particular, phosphorus and ammonium contents were significantly correlated to both bacterial and fungal community compositions (Figures 4A and B). Bacterial communities were also correlated to soil pH and contents in nitrate and sodium, while fungal communities were correlated to the CEC (Figures 4A and B).

The composition of the rhizomicrobiome of *Lyallia kerguelensis* differed from the soil microbiome

The diversity (richness and Shannon index) of the *L. kerguelensis* rhizomicrobiome across the five sites showed, for

most of the sites, similar patterns as previously observed for the bulk soil microbiomes (Figures S2B, F, D, H). Among all the rhizospheric soil samples, 22.4% of them presented significant changes in their microbial species richness and Shannon index compared to their corresponding bulk soil microbiome (Table S1). Interestingly, in the present study, among the 50 rhizospheres of *L. kerguelensis* analyzed, significant depletions were only observed for fungi in MAC1 and PJDA6 sites (Table S1). The opposite trend was even observed for some rhizomicrobiomes (13 for bacteria, 6 for fungi) that were highly significantly ($p<0.001$) more diverse than the bulk soil microbiome.

Within each site, whatever the individual rhizomicrobiome considered, the bacterial communities were dominated by the same phyla as observed in the corresponding bulk soils and showed minor changes in their relative abundance between sites (Figure 2C). On the contrary, in each site, the composition of the rhizosphere fungal communities was specific to each plant (Figure 2D). We observed that from 24 to 75% of the bacterial OTUs and from 28 to 81% of the fungal OTUs were shared between the bulk soil and the rhizosphere. Conversely, in each individual rhizosphere, some OTUs were specific and not detected in the corresponding bulk soil (Figures S3 and S4). For bacteria, their relative abundance varied from 26% to 69% of the total amount of sequences in a single rhizosphere. For fungi, these OTUs specific to the individual rhizospheres represented at least 43% of the total amount of sequences of a single plant in PJDA6 and could reach up to 94% and 96% in LON30 and PJDA2 sites, respectively. At each site, we identified a specific rhizomicrobiome composed of 5 to 142 site-specific bacterial OTUs and up to 35 site-specific fungal OTUs (Figures 3C and D). On top of these site-specific rhizomicrobiomes, some OTUs were detected in all the rhizomicrobiomes (50 plants among the five sites) and considered cosmopolitan of *L. kerguelensis* rhizosphere. This core rhizomicrobiome of *L. kerguelensis* was composed of 20 bacterial OTUs (relative abundance of 18% of the total amount of bacterial rhizosphere sequences) but no fungal OTU (Figures 3C and D, Table S2). Among these bacterial OTUs, 18 also belonged to the core microbiome, while two OTUs identified as *Acidimicrobiia* were only detected in the core rhizomicrobiome (Table S2).

Relationship between plant necrosis extent and composition of the rhizomicrobiome

When considering all five sites, the necrosis extent value of the studied plants (i.e. 50 plants in total) varied between 0% and 79% (Table 1) and correlative Spearman analyses were performed to identify relationships between bacterial and fungal OTUs abundance in the rhizomicrobiome of *L. kerguelensis* and

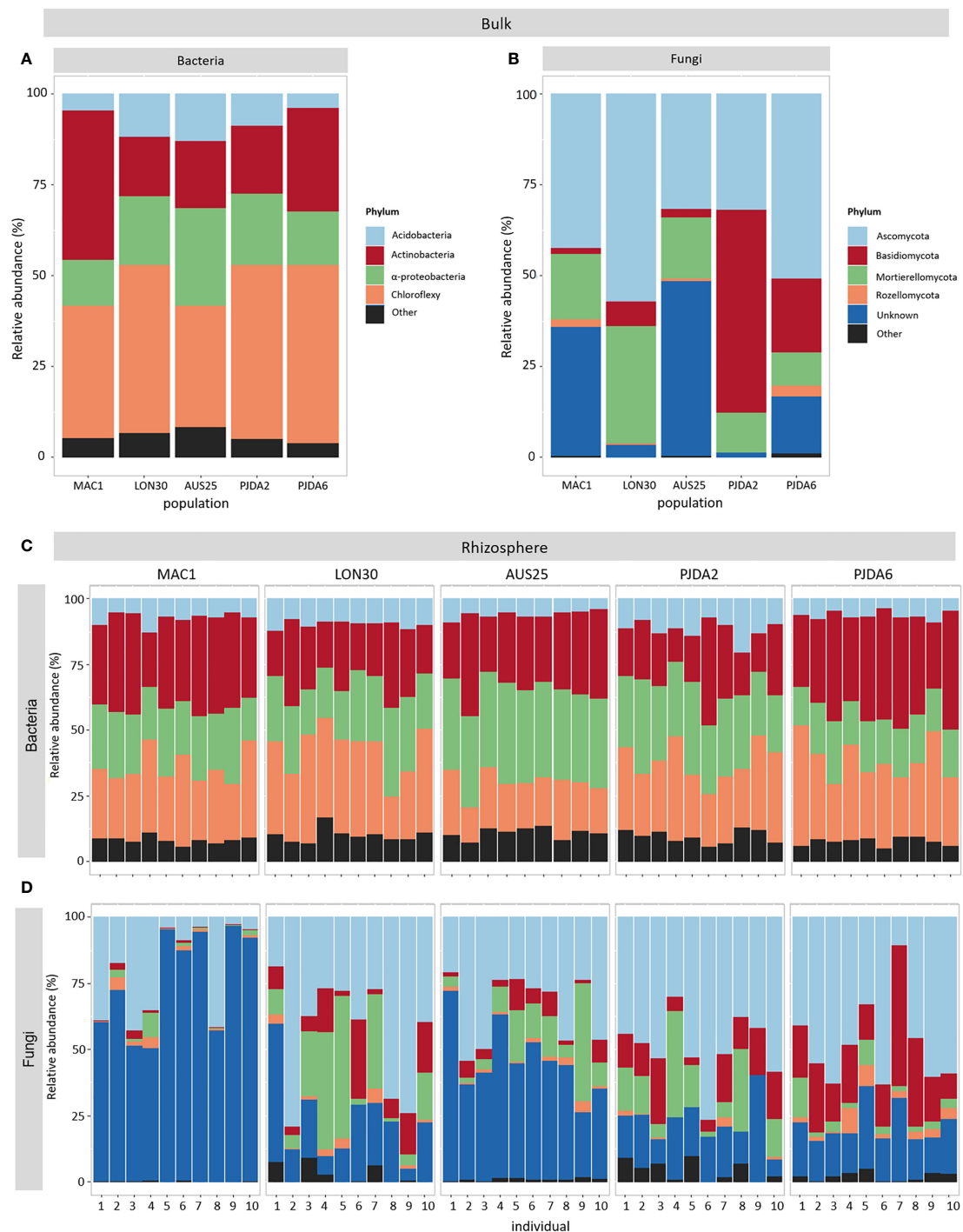


FIGURE 2

Relative abundance of the four dominant bacterial (A) and fungal (B) taxa present in the bulk soil of each site. (C, D) Relative abundance of the four dominant bacterial (C) and fungal (D) taxa present in the rhizosphere of each individual plant in each site.

necrosis extent. We identified 14 and 15 bacterial OTUs and 10 and 4 fungal OTUs positively and negatively correlated to the necrosis extent, respectively (Table 2). All of them were site-specific, avoiding to observe any general pattern across sites

(Table 2). However, regardless of the direction of the correlation, several OTUs related to putative microbial functions such as N_2 fixation, organic matter degradation and phosphate solubilisation were observed (Table 2).

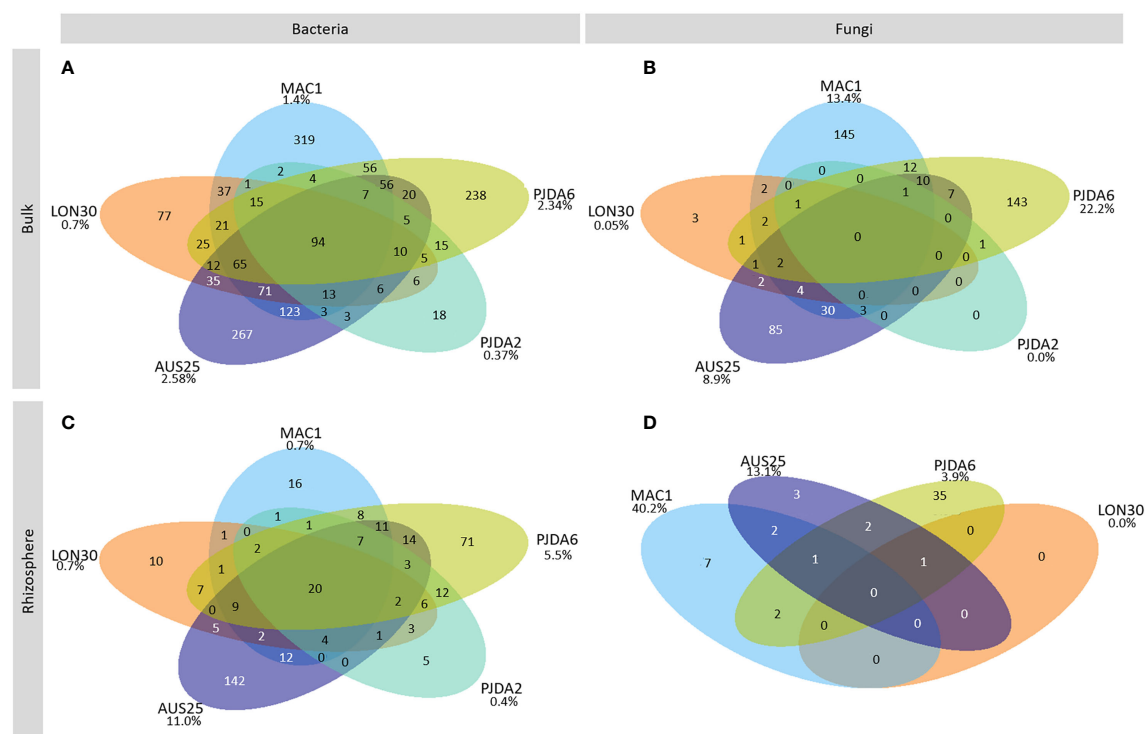


FIGURE 3

Venn diagrams of the soil core microbiome for (A) bacteria and (B) fungi and the core rhizomicrobiome for (C) bacteria and (D) fungi. The numbers indicate the number of OTUs in each condition. The percentages represent the amount of OTUs specific to a site compared to the overall core soil OTUs of the same site. The soil from PJDA2 did not have any fungal core microbiome.

Discussion

Microbial diversity and dominant phyla in the fellfield soils of the Kerguelen Islands

In our study, the soil bacterial diversity (Shannon index) was similar to the one observed in other temperate ecosystems (cropland, grassland and forest) (10). It was higher than previously detected in some studies located in the Arctic and Alpine sites (44, 45) but lower than studies performed in Antarctic, Arctic and Himalayan sites (46–48). Furthermore, the composition of soil bacterial communities in the fellfields of the Kerguelen Islands differed from those reported in other sub-polar, polar and alpine fellfields by a higher proportion of *Chloroflexi* and an almost total absence of *Planctomycetes*, *β-Proteobacteria*, *Firmicutes* and *Cyanobacteria* (44–47, 49–52). Interestingly, a large proportion of *Chloroflexi* is known to fix CO₂ through photosynthesis (53) and among them are the *Chloroflexaceae* and *Roseiflexaceae* families that have been detected in our samples. In these habitats where the vegetation is scarce and thus contributes poorly to soil carbon inputs, the dominance of the *Chloroflexi* (in four out of the five fellfield soils studied) indicates that soil microorganisms may stand in for

fixing C-CO₂. This might lead the fellfield soils from the Kerguelen Islands to act as an active carbon sink. As previously observed in alpine and Antarctic fellfields, the soil microbiome of the Kerguelen Island fellfields was also composed of a high proportion of *Actinobacteria* (47, 51, 54). These bacteria are ubiquitous and are known to have beneficial roles in soil functioning and plant fitness. They are actively involved in soil organic matter decomposition, producing a broad range of metabolites that could promote plant growth and mitigate plant abiotic stress (55, 56). Even if being among the dominant bacterial phyla, the relative abundance of *Acidobacteria* in the fellfield soils of the Kerguelen Islands varied from only 4 to 13% (6, 49, 57). Such a reduced proportion of *Acidobacteria* might have been to the advantage of *α-Proteobacteria*, which constituted one of the most abundant soil bacterial taxa. As observed experimentally by Yergeau et al., 2012 (54), in the Antarctic, this shift in the composition of bacterial communities might result from the increase in mean temperature, which is the case in the Kerguelen Islands over the past 70 years (26).

The main fungal phyla detected in the fellfield soils of the Kerguelen Islands were *Ascomycota*, *Basidiomycota* and *Mortierellomycota*, which corresponded to previous observations performed in soils from alpine and polar fellfields

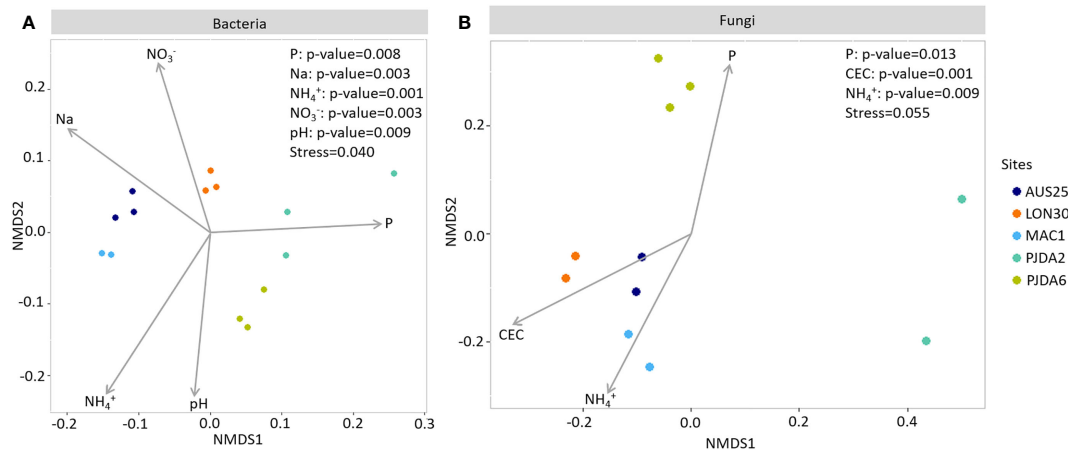


FIGURE 4

Non-metric multidimensional scaling (NMDS) ordination showing significant ($p < 0.01$) relationships between soil physico-chemical properties and the composition of (A) bacterial and (B) fungal communities in the bulk soils of each site.

(8, 45, 58, 59). *Ascomycota* are known to be more abundant in soils of moderate or low carbon contents (60) such as the fellfield soils of the present study (mean organic carbon content of 2.27%). Interestingly, the active decomposition of organic matter by *Basidiomycota* has been highlighted in the soils of various Arctic and Antarctic regions (61). In fellfields of the Kerguelen Islands, native herbivores and invertebrates are inexistent which implies food webs to rely mainly on microbial decomposition where this key function might partly been carried by the numerous *Basidiomycota* detected. The *Mortierellomycota* phylum was mainly composed of *Mortierella* sp. which are known to be a phosphorus solubilizing fungi (62, 63) and to produce high amounts of trehalose and fatty acids. These compounds are known to participate in cold stress tolerance for the soil microbiome in harsh environments (64).

Restricted and cosmopolitan soil microbiomes of the Kerguelen Islands

A large proportion of the fungal sequences (up to 48%) could not be assigned to any phylum and corresponded to unknown fungi in the database. This proportion of unknown fungi stood across a similar range as observed in soils from Antarctica (~40% detected from ITS2 region analysis) (3, 58, 59). These unknown fungal communities might be related to fungi with restricted distribution due to the remoteness of the Kerguelen Islands. These fungi might have a valuable role in soil functioning as observed in the Antarctic, where, based on RNA analyses, endemic fungi were identified as more active than cosmopolitan ones (65).

A soil core microbiome shared between the five fellfield sites was identified. It was only composed of bacterial OTUs that belonged to both widespread and more narrowly distributed taxa (66). These taxa were related to N^2 fixation (*Rhizobiales*, *Frankiales*, *Ktedonobacterales* and *Acetobacterales*), adapted to soil with low content in phosphorus (*Gemmatales*, *Microtrichales* and *Solibacterales*) and involved in soil organic matter degradation (*Micrococcales* and *Thermomicrobiales*) (67–73). Together with the unknown fungal communities, this soil core microbiome represented the specificity of the microbial communities of the fellfield soils of the Kerguelen Islands that were composed of both restricted and cosmopolitan microbes. Our results support the view of a mixed microbial assemblage in the Kerguelen Islands' soils that would reflect both dispersal limitation due to islands' remoteness and aeolian deposition allowing long-distance transport.

Spatial variability of microbial communities of fellfield soils at the scale of the Kerguelen Islands

In the present study, differences in the soil microbiome composition between the five studied fellfields were explained by several soil properties. Among them, nitrogen was involved in structuring both bacterial and fungal communities through soil contents in ammonium and nitrate. Nitrogen is known to be the main limiting factor in high latitude ecosystems (74, 75) and, according to the low C:N ratio observed in the fellfield soils of the Kerguelen Islands (from 10.35 to 13.07), rapid mineralisation should occur, enhancing N loss (76). Thus, soil functioning and the involved microbial communities in these ecosystems might

TABLE 2 Rhizospheric bacterial and fungal OTUs presenting significant ($\alpha = 0.01$) Pearson correlation between their relative abundance and the necrotic extent of the corresponding plant. OTUs detected in 60 % and 85 % of the samples for fungi and bacteria, respectively, with a mean relative abundance per site higher than 0.1 % are presented. Their taxonomy from the closest Blast sequence is indicated. For bacteria, N= 16, 10, 142, 5 and 71 for MAC1, LON30, AUS25, PJDA2 and PJDA6, respectively. For fungi N= 7, 3, 35 for MAC1, AUS25 and PJDA6, respectively.

Site	OTU	Relative abundance (%)	Presence OTU(%)	T value	p value	Domain	Phylum	Class	Order	Family	Genus	Species	Putative function
MAC1	OTU 194	0.282	100.000	-3.718	0.001	Bacteria	Actinobacteria	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	unknown species	
	OTU 223	0.357	93.103	-3.478	0.002	Bacteria	Actinobacteria	Acidimicrobiia	IMCC26256	unknown family	unknown genus	unknown species	low phosphorus soil
	OTU 437	0.107	96.552	-3.191	0.004	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	unknown genus	unknown species	low phosphorus soil
	OTU 20	0.632	100.000	-3.025	0.005	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	unknown genus	metagenome	N ² fixation
	OTU 649	0.117	86.207	-2.944	0.007	Bacteria	Chloroflexi	Ktedonobacteria	Ktedonobacterales	Ktedonobacteraceae	1921-2	unknown species	N ² fixation
	OTU 157	0.191	100.000	-2.897	0.007	Bacteria	Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	unknown genus	unknown species	low phosphorus soil
	OTU 86	0.263	93.103	-2.875	0.008	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	unknown genus	unknown species	organic matter degradation
	OTU 278	0.348	100.000	-2.824	0.009	Bacteria	Actinobacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Kitasatospora	Kitasatospora sp.	
	OTU 287	0.134	100.000	-2.810	0.009	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	unknown family	unknown genus	metagenome	low phosphorus soil
	OTU 28	0.693	96.552	2.980	0.006	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	unknown genus	unknown species	N ₂ fixation
	OTU 24	1.603	62.069	-3.634	0.001	Fungi	Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetaceae	Archaeorhizomyces	Archaeorhizomyces sp	organic matter degradation
	OTU 27	3.453	61.290	-2.767	0.010	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	
	OTU 7	3.880	100.000	4.471	<0.001	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	
	OTU 1	12.704	100.000	6.201	<0.001	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	

(Continued)

TABLE 2 Continued

Site	OTU	Relative abundance (%)	Presence OTU(%)	T value	p value	Domain	Phylum	Class	Order	Family	Genus	Species	Putative function
LON30	OTU 46	0.205	100.000	3.004	0.006	Bacteria	Actinobacteria	Acidimicrobiia	IMCC26256	unknown family	unknown genus	unknown species	low phosphorus soil
	OTU 166	0.134	89.655	3.317	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Rhodopseudomonas	unknown species	N ₂ fixation
	OTU 5	1.874	77.778	3.256	0.003	Fungi	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae	Cryptococcus	Cryptococcus sp	organic matter degradation
	OTU 20	4.371	81.481	3.289	0.003	Fungi	Ascomycota	unidentified	unidentified	unidentified	unidentified	Ascomycota sp	
	OTU 11	10.388	92.593	3.940	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales sp	
AUS25	OTU 1	5.221	100.000	-4.977	<0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	metagenome	N ₂ fixation
	OTU 48	0.274	100.000	-3.344	0.003	Bacteria	Chloroflexi	TK10	unknown order	unknown family	unknown genus	unknown species	organic matter degradation
	OTU 418	0.189	100.000	-3.253	0.003	Bacteria	Acidobacteria	Acidobacteriia	Solibacterales	Solibacteraceae (Subgroup 3)	Candidatus Solibacter	unknown species	low phosphorus soil
	OTU 93	0.444	100.000	-2.813	0.009	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	A0839	unknown genus	unknown species	N ₂ fixation
	OTU 942	0.205	100.000	2.804	0.009	Bacteria	Chloroflexi	JG30-KF-CM66	unknown order	unknown family	unknown genus	unknown species	
	OTU 236	0.200	100.000	3.739	0.001	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	unknown genus	unknown species	organic matter degradation
	OTU 5	1.949	100.000	3.804	0.001	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	unknown species	low phosphorus soil
	OTU 267	0.173	100.000	3.988	<0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	unknown genus	unknown species	N ₂ fixation
	OTU 438	0.128	96.552	4.048	<0.001	Bacteria	Actinobacteria	MB-A2-135	unknown order	unknown family	unknown genus	unknown species	
	OTU 560	0.224	96.552	4.220	<0.001	Bacteria	Actinobacteria	Actinobacteria	Frankiales	Cryptosporangiaceae	Fodinicola	unknown species	N ₂ fixation
	OTU 763	0.101	86.207	4.804	<0.001	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Iamiaceae	Iamia	unknown species	

(Continued)

TABLE 2 Continued

Site	OTU	Relative abundance (%)	Presence OTU(%)	T value	p value	Domain	Phylum	Class	Order	Family	Genus	Species	Putative function
	OTU 343	0.141	100.000	4.828	<0.001	Bacteria	Actinobacteria	Acidimicrobiia	IMCC26256	unknown family	unknown genus	unknown species	low phosphorus soil
	OTU 12	1.367	100.000	4.915	<0.001	Bacteria	Chloroflexi	KD4-96	unknown order	unknown family	unknown genus	unknown species	low phosphorus soil
	OTU 226	0.132	86.207	6.155	<0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodomicrobiaceae	Rhodomicrobium	unknown species	N ₂ fixation
	OTU 349	0.212	85.714	2.884	0.008	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	
	OTU 163	0.802	92.857	2.934	0.007	Fungi	Rozellomycota	unidentified	unidentified	unidentified	unidentified	Rozellomycota sp	
	OTU 12	1.696	92.857	3.119	0.004	Fungi	Basidiomycota	Agaricomycetes	unidentified	unidentified	unidentified	Agaricomycetes sp	organic matter degradation
	OTU 40	0.554	92.857	3.839	0.001	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella pseudozygospora	organic matter degradation
	OTU 47	0.144	60.714	7.613	<0.001	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	Tolypocladium	Tolypocladium sp	
PJDA2	OTU 86	0.385	100.000	-4.235	<0.001	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	JG30-KF-CM45	unknown genus	unknown species	organic matter degradation
	OTU 64	0.417	96.552	-3.037	0.006	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	unknown genus	unknown species	N ₂ fixation
PJDA6	OTU 131	0.258	100.000	3.240	0.003	Bacteria	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	unknown species	low phosphorus soil
	OTU 288	0.190	65.517	-3.239	0.003	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	
	OTU 251	0.273	79.310	-3.134	0.004	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi sp	

OTUs detected in 60% and 85% of the samples for fungi and bacteria, respectively, with a mean relative abundance per site higher than 0.1% are presented. Their taxonomy from the closest Blast sequence is indicated. For bacteria, N= 16, 10, 142, 5 and 71 for MAC1, LON30, AUS25, PJDA2 and PJDA6, respectively. For fungi N= 7, 3, 35 for MAC1, AUS25 and PJDA6, respectively.

heavily rely on the nitrogen cycle. Soil phosphorus content also appeared to structure both bacterial and fungal communities across the fellfield soils of the Kerguelen Islands. Phosphate has previously been observed to be correlated to the distribution of soil bacterial communities in Antarctica (77). Finally, even though the five soils showed slight differences in pH, it has been emphasized to shape the soil bacterial communities. This result corroborated what is widely assumed for temperate soil microbiomes, *i.e.* soil pH strongly structures bacterial taxa, fungal communities being less sensitive (78, 79).

The rhizosphere of *Lyallia kerguelensis* is a reservoir of diversity for soil microorganisms in fellfields

In each site, the relative abundances of the dominant bacterial phyla in the rhizosphere of *L. kerguelensis* were the same as observed in the bulk soils. Meanwhile, the composition of the fungal rhizomicrobiome at the phylum scale was plant-specific and not necessarily similar to the one observed in the corresponding bulk soil. Such plant-dependency in structuring fungal communities was already observed in several alpine fellfields (8, 49, 57) and, in the present study, translated into the absence of shared fungal OTU between all plants from the five sites. The core rhizomicrobiome of *L. kerguelensis* was thus only composed of bacteria, highlighting a specific bacterial assemblage within the rhizosphere of this plant species apparently little influenced by its close habitat.

It is assumed that in their rhizosphere, plants select a specific rhizomicrobiome allowing them to better deal with biotic and abiotic stresses. This selection pressure usually leads to an assemblage of less diverse microbial communities than observed in the surrounding soil (10, 12, 80). In our study, the rhizomicrobiomes were at least as diverse as the microbiome in the associated bulk soil, some of them being even more diverse (13 and 6 rhizomicrobiomes out of 50 for bacteria and fungi, respectively). The cushion shape of plants such as that of *L. kerguelensis* is known to buffer climatic variation to a certain extent and to increase temperature and humidity inside and beneath it. Therefore the soil underneath should be a favorable habitat for soil microorganisms in the harsh environmental conditions that prevail in fellfields, the rhizosphere of *L. kerguelensis* achieving to constitute a reservoir of microbial diversity (12, 81).

Relation between plant necrosis extent and the composition of its rhizomicrobiome

L. kerguelensis is affected by necrosis throughout the archipelago. Other cases of browning and dieback of long-

lived plants have been documented in the Antarctic and sub-Antarctic, and several hypotheses on their origin have been raised. In the sub-Antarctic Macquarie Islands, Bergstrom et al., 2015 (18) suspected the dieback of *Azorella macquariensis* to result from a decrease in water availability under climate change, making the plants weaker and more susceptible to fungal pathogens. For *L. kerguelensis* in the Kerguelen Islands, a possible link between necrosis expansion and climate drying was previously suggested (7, 21). In the present study, by analyzing the rhizomicrobiome of *L. kerguelensis*, we identified some OTUs related to the necrosis extent but they did not correspond to any known plant pathogenic microorganisms. Moreover, these OTUs were not conserved across populations. More than the presence of specific microorganisms, their involvement in nutrient cycling might be important for the plant to better cope with abiotic stresses. Among the OTUs related to necrosis extent, we identified microorganisms at the order level that are known to support some key roles in soil functioning and plant mediation to stress resistance (82) such as N₂ fixation (orders *Acetobacterales*, *Ktedonobacterales*, *Rhizobiales*, *Frankiales* and class *KD4-96*), decomposition processes (orders *Thermomicrobiales*, *Tremellales*, *Mortierellales* and class *TK10* and *Agaricomycetes*) and adaptation to soils with low content in phosphorus (orders *IMCC26256*, *Gemmatales*, *Microtrichales*, *Solibacterales*) (67–73, 83–86). Overall, our study tends towards the hypothesis of a combination of abiotic stresses (20, 21) and changes in the composition of the soil rhizomicrobiome, which may alter some key microbial functions.

Conclusions

Our results highlight specificities and possible critical roles of both soil microorganisms and adapted plants, such as *L. kerguelensis*, in the harsh environmental conditions which characterize the Kerguelen Islands' fellfields. Soil microbial communities were composed of possibly restricted microorganisms, putatively sustaining important functions for both this endemic plant and these remote soil systems. *L. kerguelensis* rhizosphere might be a reservoir of microbial diversity due to its long lifespan and its capacity to buffer climatic conditions. The necrosis that is affecting this plant for decades was not related to shared microorganisms between plants but might rather be related to shift in microbial functions that sustain plant vigour. This detailed description of soil and rhizosphere microbiomes in fellfield soils from the Kerguelen Islands brings new insights on the possible role of cushion plants to sustain microbial diversity and functions in harsh isolated environments. This study constitutes a new evidence for the preservation of endemic plant species especially in ecosystems that are strongly affected by climate change.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA731349>.

Author contribution

LM, FH, MT and CM conceived the ideas. LM and FH performed the fieldwork. LM, M-CM and BM performed the laboratory work. LM and CM analysed the data. LM, FH and CM led the writing with contributions from MT. All authors contributed to the article and approved the submitted version.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsoil.2022.995716/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Relative abundance of the OTUs from the soil core microbiome and core rhizomicrobiome (OTUs present in all the samples of the bulk or rhizosphere soil or both). Their taxonomy from the closest Blast sequence is indicated.

SUPPLEMENTARY TABLE 2

Species richness and Shannon index of the soil microbiomes and of each individual plant rhizomicrobiome per site. The results of a Dunn's Kruskal-Wallis multiple comparison test between the species richness or the Shannon index from each rhizosphere and the associated bulk soil are presented, the Z-value and adjusted p-value ($\alpha = 0.01$) are reported.

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