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Lorène Marchand

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Par

Lorène Marchand

Capacité d'adaptation au changement climatique chez l'espèce en coussin à longue durée de vie *Lyallia kerguelensis*, endémique des îles subantarctiques de Kerguelen

Thèse présentée et soutenue à Rennes, le 07/12/2021

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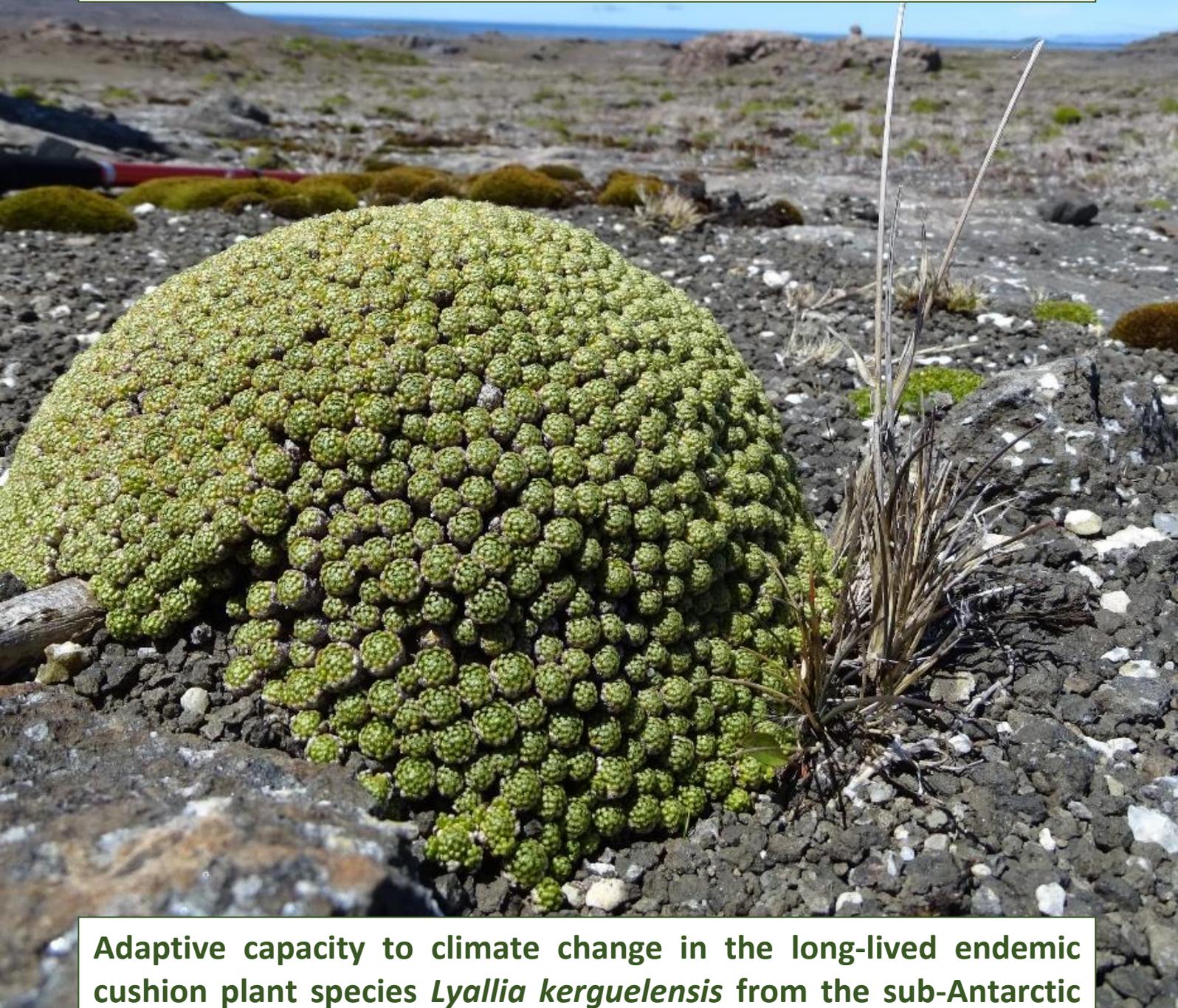
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Résumé

Introduction :

L'adaptation des plantes : contexte théorique

Les changements environnementaux passés ont façonné chaque organisme. En conséquence, le phénotype d'un organisme comprend tous les traits (morphologiques, anatomiques et physiologiques) issus de l'expression d'un génotype en interaction avec son environnement (Kattge et al. 2011; Begon and Townsend 2021). Lorsqu'un changement environnemental se produit, les populations d'une espèce peuvent réagir en modifiant leur aire de répartition géographique, s'adapter via la plasticité phénotypique et/ou la variabilité génotypique (Williams et al. 2008; Hoffmann and Sgrò 2011). Dans de nombreux cas, les populations sont confrontées à des situations "*nowhere-to-go*" (Román-Palacios and Wiens 2020). La combinaison de la plasticité et de la variation génétique qui sous-tend la variabilité phénotypique des populations doivent être toutes deux étudiées, car leur combinaison pourrait changer le résultat des interactions écologiques (Aubert et al. 1999; Snell-Rood et al. 2018). Par conséquent, un des challenges de la recherche interdisciplinaire est d'étudier la vulnérabilité des espèces soumises aux changements environnementaux en prenant en compte leur capacité d'adaptation (Williams et al. 2008; Beever et al. 2016). La capacité d'adaptation est définie comme la capacité d'une espèce ou d'une population à faire face à de nouvelles conditions environnementales et à persister en survivant et en se reproduisant. La force de cette approche se traduit par l'étude de la variabilité spatiale et temporelle des phénotypes et de leur adaptation locale en relation avec l'histoire de vie de l'espèce (Williams et al. 2008; Nicotra et al. 2015). Chez les plantes pérennes, la durée de vie pourrait conditionner le *pool* disponible d'adaptation génétique et de réponses plastiques, et ainsi influencer le résultat de l'adaptation (Bolnick et al. 2011; Cotto et al. 2017; Snell-Rood et al. 2018). Afin d'approcher la variation phénotypique, il est possible d'étudier la variabilité de nombreux traits (morphologiques, anatomiques, physiologiques, biochimiques et phénologiques) dans et entre les populations (Kattge et al. 2011; Catullo et al. 2019). Les approches basées sur les traits permettent d'estimer le *pool* de diversité disponible mais également de déterminer des traits potentiellement adaptés aux nouvelles conditions environnementales (Bolnick et al. 2011; Nicotra et al. 2015). L'étude génomique peut permettre d'identifier des gènes fonctionnels, soumis à une sélection positive, qui sous-tendent des traits physiologiques pouvant jouer un rôle dans l'adaptation des plantes (Hoffmann and Daborn 2007; Hoffmann and Willi 2008). En effet, le transcriptome est très réactif aux changements des conditions environnementales, et les niveaux d'expression des gènes sont corrélés au protéome dans une certaine mesure (Voelckel et al. 2017). De plus, une plante vit dans un écosystème, aussi il est important de prendre en compte les interactions

biotiques éco-évolutives, à savoir le phénotype étendu de la plante qui comprend tous les effets d'un gène sur son environnement, depuis l'intérieur d'un phénotype, plusieurs organismes au sein d'une espèce, jusqu'à un écosystème complexe (Dawkins 1982; Hunter 2018). Cela conduirait à un concept plus inclusif de la capacité d'adaptation des plantes. L'une des catégories de phénotype étendu considère la plante et tous les microorganismes qui lui sont associés (Hunter 2018). Ces microorganismes sont essentiels au fonctionnement de la plante et à son adaptation à l'environnement (Vandegheuchte et al. 2014; Cordovez et al. 2019). Plus précisément, l'assemblage des microorganismes dans le système sol-racine environnant régi par les plantes, c'est-à-dire le « *rhizomicrobiome* », est connu pour être orienté vers des microorganismes bénéfiques qui pourraient améliorer la capacité d'adaptation de la plante (Bulgarelli et al. 2013). Après ce rappel théorique, nous passons au cas de l'adaptation en milieux isolés pour les espèces endémiques.

Isolement et endémisme: conséquences pour l'adaptation

Dans les îles, les habitats disponibles pour suivre les conditions optimales sont limités, ce qui pourraient conditionner l'adaptation des plantes aux changements environnementaux. De plus, les îles océaniques présentent un nombre d'espèces relativement faible et des structures communautaires plus simples que les écosystèmes continentaux. Lorsqu'un changement se produit, les interactions biotiques qui devraient favoriser la résilience de l'écosystème pourraient être amoindries dans le cas des écosystèmes insulaires (Harter et al. 2015). En conséquence, l'intégrité écologique des écosystèmes insulaires est relativement fragile. En outre, les îles abritent une proportion beaucoup plus élevée d'espèces endémiques que les continents. Les espèces endémiques sont soit issues de spéciation *in situ* dans l'île, soit de la réduction d'une aire de distribution plus vaste, avec une évolution plus ou moins longue (cf. cycle des taxons avec des phases d'expansion et d'extinction; (Ricklefs and Bermingham 2002; Ricklefs 2011). Les espèces endémiques insulaires sont particulièrement touchées par le changement climatique en raison de leurs possibilités limitées d'échapper à des conditions défavorables, d'autant plus dans les environnement hostiles (Harter et al. 2015).

La vie dans les environnements froids et hostiles

Les environnements froids et rigoureux caractérisent les régions alpines, subpolaires et polaires qui subissent des vents forts, des climats froids, et de grandes fluctuations de températures et de précipitations. De plus, une hétérogénéité du climat à l'échelle locale et régionale est observable (Körner 2003; Convey 2007; Block et al. 2009; Donhauser and Frey 2018). Par ailleurs, les régions subantarctique et antarctique côtières ont une température du sol fréquemment supérieure à 0°C sans pergélisol contrairement à l'Antarctique continental (Smith 1984; Convey 2007; Donhauser and Frey 2018). Ces différents milieux alpins, polaires et subpolaires présentent des *fellfields* qui sont définis

comme "des substrats minéraux exposés [...] indépendamment de l'influence relative climatique et océanique" (Block et al. 2009). Les sols des *fellfields* sont soumis tout au long de l'année à de nombreux cycles de gel-dégel, à la solifluxion, ainsi qu'à de fortes érosions éoliennes (Smith 1984; Lebouvier and Frenot 2007; Block et al. 2009). Le *microbiome* connu pour quelques sols de *fellfields* est particulier, il abrite des communautés microbiennes spécifiques et tolérantes qui peuvent intervenir par exemple dans la respiration du sol et les cycles des nutriments. Ces fonctions sont d'autant plus importantes en raison de la faible abondance voire de l'absence d'herbivores et de macro-détritivores dans les sols de *fellfields* (Yergeau 2014; Donhauser and Frey 2018; Cox et al. 2019). Une caractéristique essentielle dans ces environnements difficiles est la convergence morphologique de plusieurs espèces de plantes vers une forme en coussin (Aubert et al. 2014) qui optimise le rapport surface/volume, favorisant la vigueur de la plante (Zotz et al. 2000; Butterfield et al. 2013; Bergstrom et al. 2015). Le phénotype étendu de la plante en coussin est particulièrement intéressant, car celle-ci augmente les nutriments et la matière organique et modifie le pH du sol sous-jacent. Ces conditions créent une oasis pour les microorganismes du sol par rapport au sol pauvre environnant. (Roy et al. 2013; Wang et al. 2020; Rodríguez-Echeverría et al. 2021).

Changement climatique et conséquences pour les plantes

Les environnements subantarctiques sont sujets à un changement climatique rapide. En effet, à la fin du siècle, il est prédit une augmentation des températures et des variations de précipitations entraînant une augmentation des périodes de sécheresse, pour l'ensemble des îles subantarctiques. Le dépérissement d'espèces à longue durée de vie a été mis en évidence dans divers environnements hostiles confrontés à des changements climatiques rapides (Molau 1996; Kleier and Rundel 2004; Bergstrom et al. 2015; Bjerke et al. 2017; Dickson et al. 2020, entre autres). Le dépérissement est défini comme le processus d'expansion de la nécrose où une partie du coussin voire le coussin entier ne parvient pas à régénérer un nouveau feuillage (Armesto et al. 1980; Whinam et al. 2014). La nécrose, quant à elle, est le dommage visible à un moment donné et peut être un symptôme du processus de dépérissement (Whinam et al. 2014).

L'environnement des îles Kerguelen

Les îles Kerguelen (48°30' - 50°S, 68°27' - 70°35'E) situées dans le sud de l'océan Indien austral peuvent être considérées comme des sentinelles du changement climatique. En effet, ces îles sont isolées, étant situées à 3500 km des côtes sud-africaines et australiennes (Lebouvier and Frenot 2007). Le paysage des îles Kerguelen est vallonné et est composé de plusieurs types d'habitats, tels que les *fellfields* (Smith 1984). Le climat des îles Kerguelen est froid avec une température annuelle moyenne de 4,6°C et des précipitations annuelles moyennes de 755 mm à "Port aux Français" de 1951 à 2018

(Verfaillie et al. 2021). Depuis plusieurs décennies, des changements climatiques ont été enregistrés dans les îles Kerguelen, tels qu'une diminution des précipitations - même si celle-ci n'est pas linéaire au cours du temps- (Favier et al. 2016; Verfaillie et al. 2019), et une augmentation significative de la température observée jusqu'en 1980, puis quasi constante pendant 30 ans, pour ensuite largement augmenter dans les années 2010. Les prévisions pour la fin du siècle indiquent une augmentation de la variation des précipitations et des températures plus élevées (Verfaillie et al. 2021).

***Lyallia kerguelensis* (Montiaceae)**

Les îles Kerguelen hébergent une espèce endémique de plante en coussin à longue durée de vie, *Lyallia kerguelensis* Hook.f. (Montiaceae, Caryophyllales ; Nyffeler and Eggli 2010). Les observations de plusieurs coussins ont montré que les plantes pouvaient vivre au moins 16 ans (1990-2006, Wagstaff and Hennion 2007) et même jusqu'à 20 ans (individus marqués 2000-2013, ré-identifiés en 2019, Marchand obs. pers.). L'espèce présente une forme de coussin, grossièrement rond de 20-40cm de diamètre (Hennion and Walton 1997; Wagstaff and Hennion 2007), atteignant exceptionnellement plus d'un mètre de diamètre. *L. kerguelensis* est inféodée aux habitats ouverts de *fellfield*, depuis le niveau de la mer jusqu'à près de 300m d'altitude (Wagstaff and Hennion 2007). Ces *fellfields* se trouvent dans des couloirs de vent, sur des plateaux ou entre des falaises avec des gelées fréquentes et un vent permanent.

De plus, depuis les années 1990, Hennion (1992) a observé des coussins de *L. kerguelensis* nécrosés dans de nombreuses populations, principalement situées dans la partie la plus sèche de l'archipel. Il pourrait s'agir d'un phénomène apparemment récent car les premières descriptions botaniques ne mentionnaient pas de nécrose (Hooker 1847; Werth 1911; Chastain 1958). L'origine de la nécrose pourrait être liée au récent changement climatique. Par ailleurs, une étude ciblant les bactéries foliaires n'a pas pu mettre en évidence d'agent pathogène dans les échantillons (Portier 2019), renforçant la possible origine abiotique de la nécrose. Le changement climatique est connu pour stresser les plantes et induire un dépérissement partiel ou total des plantes. Les mécanismes physiologiques amenant au dépérissement, lors de changement climatique, sont largement étudiés chez un certain nombre de plantes à longue durée de vie comme les arbres (McDowell et al. 2008, 2020; Allen et al. 2010; Voltas et al. 2013; Earles et al. 2018). Ces mêmes mécanismes pourraient exister chez *L. kerguelensis* puisqu'il a été observé une réduction des précipitations et l'augmentation de la température (même si elles ne sont pas linéaires) aux îles Kerguelen. Le fonctionnement des coussins de *L. kerguelensis* pourrait être affecté à court et long terme et mettre en péril le maintien de l'espèce.

Objectifs et hypothèses de la thèse

Dans ce contexte, nous posons la question suivante : quelle est la capacité d'adaptation au changement climatique chez *Lyallia kerguelensis*, plante endémique à longue durée de vie des îles Kerguelen ?

La réserve de variabilité (de la morphologie, du transcriptome, et du *rhizomicrobiome*) à travers des environnements contrastés pourrait apporter des connaissances sur la capacité d'adaptation de *L. kerguelensis* aux environnements hostiles, ainsi que des réponses possibles au changement climatique. La morphologie des plantes est l'un des traits qui réagit le plus aux modifications de l'environnement. Par conséquent, la variabilité morphologique de *L. kerguelensis* et sa relation avec des variables environnementales pourraient être essentielles pour identifier les possibles réponses écomorphologiques de l'espèce (chapitre 1). Des investigations à une échelle plus fine seront nécessaires pour trouver les gènes ou les voies métaboliques qui seraient différenciellement exprimés chez *L. kerguelensis* dans des environnements contrastés, ceci afin d'identifier les réponses biologiques et cellulaires susceptibles d'être en lien avec l'adaptation locale (chapitre 2). L'étude du phénotype étendu de *L. kerguelensis*, en particulier l'assemblage du *rhizomicrobiome*, pourrait renseigner sur les microorganismes généralistes et spécifiques sélectionnés dans des environnements contrastés qui seraient aptes à favoriser la vigueur de la plante (chapitre 3). De plus, chez des espèces à longue durée de vie telles que *L. kerguelensis*, l'intégration de la temporalité est importante. Un suivi à long terme de la dynamique des traits morphologiques dans des environnements contrastés permettrait d'estimer leur réponse et la capacité de l'espèce à faire face à un changement climatique rapide (chapitre 4). Enfin, le taux de croissance et l'âge des plantes dans divers environnements pourraient influencer les résultats de l'adaptation en fonction de la persistance et de la résilience des populations au changement climatique (chapitre 5). Ces résultats élargiront nos connaissances sur la capacité d'adaptation des plantes à longue durée de vie qui vivent près de leurs limites physiologiques et sont confrontées à des changements rapides (Hoffmann et al. 2017). Ils permettront de mieux évaluer leurs vulnérabilités au changement climatique et seront précieux pour la recherche sur l'évolution et la conservation.

Chapitre 1 : Variabilité morphologique de la plante en coussin, *Lyallia kerguelensis* (Caryophyllales), en relation avec les conditions environnementales et la géographie dans les îles Kerguelen : implications pour la nécrose du coussin et le changement climatique

Dans la présente étude, nous avons supposé que les traits morphologiques de *L. kerguelensis* co-variaient de manière à maintenir une forme hémisphérique qui est optimale dans les environnements hostiles. Nous avons ensuite prédit qu'une variation morphologique, y compris de la nécrose, serait présente entre les individus et les populations. Nous avons émis l'hypothèse que la

disponibilité en eau du sol, l'exposition au vent et l'exposition géographique étaient probablement les principaux facteurs expliquant l'étendue de la nécrose. Pour tester ces hypothèses, nous avons (i) recherché les relations entre les traits de croissance et l'ensemble de la morphologie des coussins (c'est-à-dire l'allométrie) chez *L. kerguelensis* afin de sélectionner les traits appropriés pour évaluer la variabilité inter-populationnelle, et (ii) cherché à identifier les relations entre cette variabilité inter-populationnelle et les conditions environnementales d'une part et les distances géographiques d'autre part. Enfin, nous avons (iii) étudié l'influence de la nécrose sur la morphologie des coussins et tenté d'identifier les conditions environnementales liées à la nécrose.

Dans cette étude, nous avons analysé 319 plantes provenant de 19 populations dans trois régions différentes des îles Kerguelen. Pour chaque population, les caractéristiques générales de l'environnement ont été enregistrées concernant les types de terrain, la topographie, l'exposition au vent, la pente, la distance à la mer, l'orientation géographique et l'estimation visuelle de la couverture du sol en élément minéraux et en végétation. La conductivité, la saturation en eau, le pH et la température du sol ont été mesurés. La texture et la composition en éléments du sol ont été analysées au laboratoire de l'INRA d'Arras. Au sein de chaque population, nous avons sélectionné le même nombre de coussins " vigoureux " (visuellement moins de 10% de surface nécrosée) et " nécrosé " (visuellement 10% à 80% de surface nécrosée). Par une méthode de photo-interprétation que nous avons développée, nous avons déterminé le périmètre du coussin, sa surface totale, le pourcentage de plantes voisines au contact du coussin et l'étendue de la nécrose. Enfin, nous avons calculé un proxy de compacité du coussin (Cranston et al. 2015). Un total de 11 traits morphologiques et biotiques a été enregistré.

Nous avons déterminé que la hauteur, le diamètre, la forme et la compacité des coussins étaient positivement corrélés. Une allométrie similaire a été rapportée chez d'autres espèces de plantes en coussins (Niklas 1994). Le degré de nécrose n'était pas lié aux traits morphologiques étudiés, ce qui suggère que la nécrose n'est pas favorisée par une morphologie particulière. La surface des coussins était liée à la topographie et à l'exposition au vent, avec des coussins plus grands se développant principalement à mi-pente plutôt que sur des zones plates, et une tendance à des coussins plus grands dans les populations moins exposées au vent. Dans le paysage fragmenté et le climat rigoureux de Kerguelen, la topographie et le vent sont deux composantes environnementales majeures. La topographie vallonnée de l'île génère une hétérogénéité de la répartition des précipitations, de la rétention en eau et de l'exposition au vent (Aubert de la Rüe 1964; Wagstaff and Hennion 2007). De plus, la teneur en sable grossier du sol était positivement corrélée à la surface des coussins, cela peut indiquer une protection contre le vent plus faible que sur un sol rocheux, peut-être en relation avec une plus grande exposition au froid ou au stress de dessiccation. La seule variable environnementale liée de manière significative à la variabilité de la nécrose était la teneur en sodium

du sol, mais uniquement pour les coussins présentant une faible nécrose. Le sodium est transporté par le vent dans les embruns marins, et s'accumule dans le sol. Dans la région subantarctique, le sel marin peut se retrouver loin de la côte en raison des vents forts et permanents (Smith 1987; Jenkin 1997). Des études sur le stress salin de plusieurs espèces végétales de la région subantarctique réparties depuis la côte jusqu'aux *fellfields* ont montré que ces plantes pouvaient être définies comme des glycophytes tolérant le stress salin (Smith 1978; Hennion and Bouchereau 1998; Hummel et al. 2004). Pour *L. kerguelensis*, des concentrations plus élevées de sodium dans le sol pourraient s'ajouter à d'autres stress abiotiques présents dans leurs habitats et augmenter leur sensibilité à d'autres stress osmotiques tels que la sécheresse. L'ensemble de nos résultats suggèrent que la nécrose serait liée à la disponibilité de l'eau dans le sol, bien qu'une origine pathogène ne puisse être exclue. Cela suggère aussi que, dans le cadre des tendances actuelles du changement climatique aux îles Kerguelen, la morphologie des coussins pourrait avoir la capacité de changer pour s'adapter aux variations environnementales, mais la nécrose des coussins pourrait s'accélérer dans les zones les plus salines.

Chapitre 2 : Expression génétique différentielle chez *L. kerguelensis* dans des environnements contrastés : implications pour les stress biotiques et abiotiques

L'étude de la variabilité du transcriptome (défini comme l'ensemble des ARN transcrits) dans les environnements naturels a été suggérée comme un moyen prometteur d'identifier les variants alléliques et épigénétiques liés à des traits ayant une importance fonctionnelle et adaptative (Hoffmann and Daborn 2007; Hoffmann and Willi 2008; Zhang et al. 2020). Cela s'explique par le fait que le transcriptome est très réactif aux changements des conditions environnementales et que les niveaux d'expression des gènes sont corrélés au protéome dans une certaine mesure (Voelckel et al. 2017). *In natura*, les différences d'expression génique au niveau de la population peuvent nous informer sur la façon dont des environnements distincts façonnent la variabilité et l'évolution des traits (Zaidem et al. 2019). Les études du transcriptome offrent également un aperçu des voies de transduction du signal impliquées dans les réponses abiotiques des plantes. Ces voies impliquent des facteurs de transcription qui activent des réseaux de gènes et des protéines-kinases qui activent et désactivent des protéines. Cette régulation peut contribuer à des physiologies végétales spécifiques et à des réponses d'acclimatation aux changements environnementaux et au stress (Gish and Clark 2011; Roy 2016; Kim et al. 2018; Hrmova and Hussain 2021). L'intérêt de cette étude était de déterminer si le profilage du transcriptome des populations de *L. kerguelensis* dans des environnements contrastés des îles Kerguelen révélerait ou non des informations sur les réponses des plantes aux stress abiotiques et biotiques. Nous nous sommes posées les questions suivantes : (i) Existe-t-il des preuves de différences dans les profils d'expression du transcriptome des différentes populations de *L.*

kerguelensis ? (ii) Si oui, y a-t-il un enrichissement en gènes impliqués dans des processus biologiques de réponse au stress ? (iii) Si oui, l'identité des gènes exprimés de manière différentielle concerne-t-elle des facteurs de transcription et des protéines-kinases ? Si le profilage du transcriptome est informatif pour étudier les réponses physiologiques de *L. kerguelensis*, *in natura*, nous prédisons que (i) les profils d'expression du transcriptome différeront entre les différentes régions aux environnements contrastés et que les populations au sein des mêmes régions auront des profils de transcriptome similaires car elles partagent un climat local similaire. (ii) Nous supposons que de nombreux gènes exprimés de manière différentielle entre les populations ou les régions seront liés à des variables environnementales, puisque la variabilité des traits morphologiques de *L. kerguelensis* a été expliquée par des variables environnementales (Marchand et al. 2021, chapitre 1). Enfin (iii), si les différences dans les profils de transcription sont en partie expliquées par les réponses plastiques ou adaptatives au sein des populations de *L. kerguelensis*, nous nous attendons à identifier des gènes impliqués dans la transduction des signaux pour les réponses abiotiques et biotiques.

Cinq régions des îles Kerguelen ont été sélectionnées, et à chaque fois deux populations disjointes dans des environnements contrastés ont été étudiées. Par population, cinq individus sont échantillonnés. Deux types de séquençage d'ADNc ont été entrepris, un « paired-end Illumina RNA Seq » pour chaque échantillon et un séquençage « PacBio SMRT » sur l'échantillon de meilleure qualité. Les profils d'expression du transcriptome étaient spécifiques par population avec une faible variabilité entre les individus. En outre, une spécificité régionale était également observable. Les variables environnementales étudiées étaient aussi population-spécifiques et pourraient être liées aux différences de profils transcriptomiques de ces populations. Les régions les plus différentes pour les profils d'expression génique ont été sélectionnées et leurs ontologies géniques comparées. La plupart des expressions géniques différentielles ont concerné l'échelle des régions. Lors de la comparaison au sein d'une même région, seuls quelques gènes étaient exprimés de manière différentielle, ce qui suggère que les populations d'une même région ont subi des pressions environnementales similaires sur les transcriptomes, même si certaines différences à l'échelle de la population se sont produites dans leurs environnements respectifs. Les analyses d'ontologie génétique (GO) ont permis de retrouver des GO termes pour les trois catégories principales (processus biologique, fonction moléculaire et composant cellulaire). Nous avons constaté que les réponses aux stress abiotique et biotique ainsi que des composants de la membrane mitochondriale et les fonctions de transporteur d'ions étaient régulées à la hausse dans les populations d'une région. Pour l'autre région l'enrichissement des GO termes pour les processus biologiques était observé. Ces GO termes concernaient la défense fongique et des composants cellulaires liés à la paroi cellulaire. Le double de facteurs de transcription et de protéines kinases régulées à la hausse était observé dans la région montrant des GO termes liés aux stress abiotiques. Les facteurs de transcription observés

appartiennent à diverses familles, et il a été montré qu'ils jouent un rôle crucial dans la signalisation du stress (Roy 2016; Kim et al. 2018). Ceci est cohérent avec une réponse aux stress abiotiques de *L. kerguelensis* dans des environnements contrastés. Ces premiers résultats donnent des directions pour de futures analyses qui viseront à comparer les profils de transcription d'autres régions. De plus, ils soulignent la nécessité de mener des expériences complémentaires pour quantifier les gènes différentiellement exprimés dans ces environnements contrastés.

Chapitre 3 : Microbiome du sol des *fellfields* et rhizomicrobiome de *Lyallia kerguelensis*, plante en coussin à longue durée de vie, endémique des îles Kerguelen et confrontée à la nécrose

Les conditions qui prévalent dans les *fellfields* des îles subantarctiques pourraient avoir sélectionnés des microorganismes tolérants et spécifiques comme cela a été observé dans les *fellfields* de l'Arctique et de l'Antarctique (Cox et al. 2016; Malard et al. 2019; Botnen et al. 2020). Cependant, même dans les endroits éloignés et les environnements hostiles, on sait que des microorganismes cosmopolites et généralistes s'installent (Chu et al. 2010; Herbold et al. 2014; Cox et al. 2016), ils devraient donc se trouver également dans les *fellfields* des îles subantarctiques. Comme *L. kerguelensis* est une espèce à longue durée de vie, la composition de son *rhizomicrobiome* pourrait résulter à la fois de l'assemblage d'espèces co-évoluées et d'une sélection active (Berendsen et al. 2012; Chang et al. 2018; Botnen et al. 2020; Nissinen and Kumar 2021). La sélection du *rhizomicrobiome* des plantes devrait être encore plus significative dans des conditions environnementales hostiles. Dans cette étude, nous avons d'abord décrit la composition des communautés (c'est-à-dire, le *microbiome*) bactériennes et fongiques du sol dans différents *fellfields* des îles Kerguelen et étudié sa variabilité spatiale. Nous avons ensuite caractérisé le *rhizomicrobiome* de différentes populations de *L. kerguelensis* et étudié son rôle potentiel dans la nécrose de *L. kerguelensis*. Nous nous attendions à ce que l'étendue de la nécrose soit liée à la plus faible abondance de microorganismes bénéfiques spécifiques dans la rhizosphère de *L. kerguelensis*.

Chacun des cinq sites de *fellfields* sélectionnés consistait en une zone géographique de moins de 50 m², homogène par sa géomorphologie, sa topographie et sa végétation, et présentait une population continue de *L. kerguelensis*. Dans chaque site, dix plantes avec des surfaces nécrosées variables ont été aléatoirement sélectionnées. La surface nécrosée de chaque plante (appelée aussi étendue de la nécrose) a été mesurée par photo-interprétation (protocole détaillé dans le Chapitre 1). Des échantillons de sol de la rhizosphère ont été récoltés entre les racines de chaque plante à une profondeur de 2 à 8 cm. Du sol de surfaces nues (dépourvues de végétation) a été prélevé à trois endroits différents dans chaque site, il est supposé être exempt de l'influence des racines. Un séquençage « Illumina paired-end (2 × 300 bp) MiSeq » d'amplicons du gène de l'ARNr 16S bactérien

et ITS1 fongiques a été réalisé pour l'ensemble des échantillons de sol des rhizosphères de *L. kerguelensis* et de sols nus.

La diversité α des bactéries et des champignons était faible et similaire à ce qui a été observé dans certains *fellfields* alpins et antarctiques, mais plus faible que dans les *fellfields* subarctiques ou arctiques, probablement en raison de la l'isolement géographique des îles Kerguelen. La composition bactérienne des *fellfields* des îles Kerguelen se distingue, notamment, par une proportion plus élevée de *Chloroflexi*. La composition fongique est spécifique des sites dans les *fellfields* des Kerguelen, comme c'est également le cas dans d'autres *fellfields* alpins et antarctiques (Praeg et al. 2019; Wang et al. 2020; da Silva et al. 2021). Un *microbiome* bactérien commun des sols des *fellfields* de Kerguelen a été identifié. En revanche, aucun *microbiome* fongique commun n'a pu être mis en évidence. Cependant, nous avons observé une forte proportion de champignons inconnus (39,6 %) dans tous les échantillons, dans des proportions similaires à celles déjà observées en Antarctique (Cox et al. 2016; Rosa 2020; da Silva et al. 2021). Cette communauté fongique inconnue pourrait avoir un rôle essentiel dans le fonctionnement du sol, comme c'est le cas en Antarctique, où, sur la base des analyses d'ARN, les champignons endémiques sont plus actifs que les champignons généralistes (Cox et al. 2019). Comme attendu, le *microbiome* du sol nu était spatialement structuré dans les cinq sites étudiés de l'archipel aux environnements contrastés. De plus, la structuration de la communauté microbienne semblait être spécifique à la région subantarctique, qui se caractérise par des contraintes d'ammonium et de phosphore plutôt que des contraintes de carbone.

La rhizosphère de *L. kerguelensis* pourrait être un réservoir de diversité microbienne, en effet nous avons observé au moins un *rhizomicrobiome* par site étudié présentant une diversité α bactérienne ou fongique supérieure à celle du sol nu. Dans chaque site, les abondances relatives des *phyla* dominants qui composaient les communautés bactériennes dans la rhizosphère étaient les mêmes que celles observées dans le sol nu correspondant. En revanche, la composition du *rhizomicrobiome* fongique était spécifique de chaque plante et n'était pas nécessairement similaire à celle observée dans le sol nu correspondant. Même si elle n'est pas directement liée à un microorganisme spécifique, la nécrose pourrait résulter de changements dans la composition du *rhizomicrobiome* des coussins de *L. kerguelensis* pour faire face aux stress environnementaux abiotiques tels que ceux induits par le changement climatique dans la région subantarctique. Parmi les microorganismes liés à l'étendue de la nécrose, certains ont été identifiés comme jouant des rôles clés dans le fonctionnement du sol (décomposition, cycle du phosphore ou encore de l'azote). Enfin, des taxa fongiques inconnus étaient corrélés à l'étendue de la nécrose dans chaque site, ce qui suggère leur importance pour le fonctionnement des plantes et une possible coévolution. Les fonctions des microorganismes corrélés à l'étendue de la nécrose de *L. kerguelensis* soutiennent l'hypothèse d'une possible origine abiotique de la nécrose, comme cela a été suggéré par les études antérieures de

l'étendue de la nécrose en relation avec la disponibilité en nutriments et en eau du sol (Chapitre 1, et Chapitre 4).

Chapitre 4 : Croissance lente des coussins de *Lyallia kerguelensis*, endémique des îles Kerguelen et évolution rapide de la nécrose : impact du changement climatique

En raison de la haute valeur patrimoniale de l'espèce, un " Observatoire *Lyallia kerguelensis* " (Réserve naturelle nationale des Terres australes françaises et Institut Polaire Français, programmes 136 et 1116) a été mis en place en 2012 pour suivre le devenir individuel des plantes, la dynamique de leur morphologie et de celle des plantes au contact du coussin sur le long terme. Notre étude s'est fondée sur les données de ce suivi de 2012 à 2019 pour répondre à plusieurs questions de recherche concernant l'évolution de la croissance et de la nécrose des coussins de *L. kerguelensis* : (i) La surface des coussins, l'étendue des nécroses et la quantité de plantes voisines au contact du coussin varient-elles d'un site à l'autre au début du suivi ? (ii) L'étendue de la nécrose est-elle expliquée par le climat régional, les variations climatiques à long terme, la géomorphologie du site et la morphologie du coussin ? (iii) Quelle est la dynamique de la croissance, de l'étendue de la nécrose et des changements dans la quantité de plantes voisines pendant le suivi ? (iv) Une augmentation de la nécrose a été observée, est-elle liée à la variation climatique à court terme, à la géomorphologie du site ou bien à la morphologie du coussin ? (v) Peut-on mettre en évidence une diminution de l'étendue de la nécrose au cours du suivi ? Si oui, est-elle due à la croissance de la plante sur la partie nécrosée du coussin (c'est-à-dire son reverdissement) ? Toutes ces questions sont en relation avec les hypothèses suivantes : (i) nous supposons que la variabilité de la morphologie (surface du coussin, quantité de plantes voisines et étendue de la nécrose) se produit entre les sites puisque leurs environnements géomorphologiques, topographiques et édaphiques diffèrent. (ii) Nous pensons que la variabilité de l'étendue de la nécrose au début du suivi s'explique par la surface des coussins, la quantité de plantes au contact, le climat régional et les variations climatiques à long terme. (iii) Au cours des 5 années du suivi, la surface du coussin et la quantité de plantes voisines peuvent ne pas varier car la croissance est lente dans les *fellfields*. En revanche, l'étendue de la nécrose, qui est un processus plus rapide, pourrait varier de manière significative au cours de l'étude. (iv) Nous supposons que l'augmentation de l'étendue de la nécrose au cours des 5 années de suivi pourrait être influencée par des variations climatiques à court terme. Enfin, (v) un reverdissement possible de la partie nécrosée pourrait avoir eu lieu durant le suivi grâce à l'expansion des parties vertes, vivantes du coussin ou la germination de plantules de *L. kerguelensis* dans ces parties. Il doit être distingué des autres sources de réduction de l'étendue des nécroses, telles que l'érosion des parties nécrosées ou le recouvrement de la partie nécrosée par d'autres espèces végétales.

Nous avons étudié huit populations appartenant à "l'Observatoire *Lyallia kerguelensis*" réparties sur les îles Kerguelen pour couvrir la majeure partie de l'aire de distribution de la plante. Dans le cadre de l'observatoire, chaque population a été visitée à T0 entre décembre 2012 et novembre 2014 et à T1 entre décembre 2016 et janvier 2019, conduisant à des intervalles de temps de 4-5 ans. Cinquante individus marqués choisis au hasard ont été photographiés à T0 et à T1 (sup. Données 1). Les images calibrées ont été traitées avec le logiciel ArcGis 10.6.1 en suivant le protocole de Marchand et al. (2021, Chapitre 1). Chaque trait morphologique a été calculé pour les coussins à la fois à T0 et à T1. Pour caractériser la dynamique de chaque trait, nous avons extrait le coefficient de pente de la régression linéaire de la variation du trait au cours du suivi (coefficient de surface, coefficient de quantité de plantes voisines et coefficient d'étendue de la nécrose). La température moyenne et les précipitations (addition des pluies et des chutes de neige) étaient disponibles de manière saisonnière (Verfaillie 2014). Chaque site a été rattaché à son climat régional. La température et les précipitations moyennes à long terme ont été calculées de manière saisonnière sur 10 ans, de l'été 2004 à l'hiver 2013, couvrant la période avant et pendant le T0. Pour caractériser la variation climatique à long terme, nous avons extrait le coefficient de pente de la régression linéaire des 10 années pour les variables saisonnières de température et de précipitations. Nous avons fait de même pour la variation à court terme du climat avec 3 années de variables saisonnières de température et de précipitation de 2010-2013 qui coïncident avec le T0.

Les trois traits morphologiques (surface de coussin, quantité de plantes voisines et étendue de la nécrose) montrent une variabilité au sein d'un même site et entre les sites, ce qui est attendu puisqu'il a été démontré que ces traits varient avec l'environnement chez cette espèce (Marchand et al. 2021, Chapitre 1). L'étendue de la nécrose était plus importante sur les coussins plus grands et ayant peu de plantes voisines. La nécrose pourrait être considérée comme une signature de sénescence due à l'âge. Le vieillissement est une "réponse au stress temporel" pour les plantes à longue durée de vie ; en effet, les nombreux événements stochastiques qui se produisent tout au long de leur existence déterminent leur durée de vie (Thomas 2013). Par conséquent, les coussins plus vieux/grands ont vraisemblablement subi plus d'événements stressants et accumulent donc plus de nécrose que les coussins plus jeunes/petits. La quantité de plantes voisines est également une variable qui semble importante. La facilitation végétale est définie comme une "interaction dans laquelle la présence d'une espèce modifie l'environnement d'une manière qui améliore la croissance, la survie ou la reproduction d'une seconde espèce voisine" (Bronstein 2009). Ce processus peut se produire en particulier lorsque diverses espèces végétales vivent ensemble dans des environnements stressants (He et al. 2013). *L. kerguelensis* pourrait bénéficier d'une telle facilitation en bénéficiant d'un environnement plus tamponné en présence d'une plus grande quantité de plantes voisines. Cependant la majorité de la variabilité de l'étendue de la nécrose reste non expliquée par ces deux variables.

Au cours des 5 années de l'étude, la dynamique de la croissance a été insignifiante. Chez *L. kerguelensis*, la croissance des coussins semble être un processus complexe comme chez d'autres plantes en coussins (Molau 1997; Morris and Doak 1998). En effet, le nombre équivalent de coussins qui ont rétréci par rapport aux coussins ayant grandi indique que la croissance n'a rien de linéaire. La mort de coussins a été enregistrée pour 37 coussins (sur 303) alors que l'augmentation de la nécrose s'est produite chez la plupart des coussins. D'autres travaux ont souligné l'augmentation rapide de la nécrose (par rapport au taux de croissance des coussins) chez des espèces de plantes en coussin dans le subantarctique (Le Roux et al. 2005; Dickson et al. 2020). Lors de la modélisation de l'augmentation de l'étendue de la nécrose, les principaux facteurs ont été la diminution à court terme des précipitations hivernales et l'augmentation à court terme de la température hivernale, sans implication d'autres variables environnementales. Aux îles Kerguelen, *Pringlea antiscorbutica*, connu sous le nom de chou de Kerguelen, ne présente pas de dormance hivernale et maintient une activité photosynthétique presque toute l'année (Aubert et al. 1999). Cela pourrait être également le cas pour *L. kerguelensis* qui reste verte en hiver (F. Hennion, obs. pers.). L'augmentation de la température hivernale pourrait avoir plusieurs conséquences négatives : elle pourrait augmenter la respiration cellulaire et entraîner la conversion, par la plante, de davantage de réserves carbonées en énergie (Tixier et al. 2019). L'effet négatif direct des températures plus élevées a été mis en évidence chez les plantes de haute montagne avec une perte de glucides (Steinbauer et al. 2020). Ces processus, s'ils sont actifs chez *L. kerguelensis*, peuvent conduire à l'épuisement de la plante et à l'impossibilité de régénérer un nouveau feuillage. L'augmentation des températures devrait également accroître l'évapotranspiration, ce qui est critique si l'absorption racinaire ne peut pas compenser la sécheresse hivernale (Charrier et al. 2021). Ainsi, le stress potentiel induit par des hivers plus secs et plus chauds pourrait entraîner des contraintes sur le bilan hydrique à court terme et sur le métabolisme du carbone à long terme (Charrier et al. 2021). Par conséquent, prochainement en terme de nombre d'années, l'épuisement et la cavitation de portions entières de coussin *L. kerguelensis* pourraient se produire. Le reverdissement du coussin quant à lui semble être un événement rare, mais il est très probable que les méthodes utilisées ne soient pas suffisamment performantes.

Chapitre 5 : Variabilités de la croissance et de l'âge de *Lyallia kerguelensis* Hook.f. (Montiaceae) dans des environnements contrastés et allométrie des traits architecturaux

La durée de vie des plantes est essentielle à considérer pour estimer leur capacité d'adaptation aux changements (Beever et al. 2016). Par exemple, dans le contexte du changement climatique, les plantes pérennes vivant en milieux rudes pourraient persister dans des environnements défavorables grâce à leur plasticité phénotypique, cela a été montré dans les environnements alpins (Dullinger et al.

2012; Cotto et al. 2017). Une conséquence de ce phénomène serait une plus grande production de graines mal adaptées, qui pourrait entraîner un recrutement plus faible et conduire au déclin des populations (Cotto et al. 2017). Par conséquent, il semble important d'étudier à la fois la variabilité des traits phénotypiques et la durée de vie des plantes pour mieux comprendre leur persistance dans les environnements hostiles dans le contexte du changement climatique. La croissance est compliquée à évaluer chez les plantes à longue durée de vie vivant dans des environnements hostiles. Néanmoins, une estimation peut être faite en calculant la variation du diamètre ou de la hauteur de la plante sur plusieurs années. L'âge du coussin quant à lui peut être estimé indirectement en divisant la taille du coussin par son taux de croissance annuel (le Roux and McGeoch 2004; Kleier et al. 2015). Dans l'ensemble, la longévité des plantes en coussin et leur taux de croissance restent peu étudiés. Pour compléter l'étude de la capacité d'adaptation de *L. kerguelensis*, nous nous sommes intéressée à son taux de croissance, son architecture, l'anatomie de sa tige et sa durée de vie. Dans le cadre de "l'Observatoire *Lyallia kerguelensis*" (détaillé au chapitre 4), la variation de la surface des coussins a été suivie, permettant d'étudier le taux de croissance des plantes et d'estimer leur longévité. Nous nous sommes concentrée uniquement sur les 150 coussins qui présentaient une croissance positive. L'étude visait à (i) évaluer le taux de croissance des coussins et la variabilité des taux de croissance entre les populations. Puis (ii) mettre en évidence l'allométrie de l'architecture et enfin (iii) estimer l'âge probable de *L. kerguelensis* en extrapolant la taille des coussins divisée par le taux de croissance dans des environnements contrastés ainsi qu'en comptant les cernes de croissance de la base de la tige. Nous avons supposé (i) que les taux de croissance des coussins variaient entre les populations en raison des conditions environnementales contrastées. (ii) Nous nous attendions à voir des corrélations entre les traits architecturaux puisque l'allométrie morphologique a été observée chez cette espèce. (iii) Nous avons émis l'hypothèse que l'âge pouvait être estimé chez cette plante grâce au taux de croissance et aux cernes de croissance.

L'architecture détaillée et l'anatomie de la tige ont été étudiées sur quelques petits coussins afin de fournir des informations sur l'allométrie de l'architecture et sur l'âge, en observant les cernes de croissance de la tige. Pour cela six plantes de petite taille (diamètre <5-7cm, Marchand obs. pers.), vraisemblablement jeunes, ont été récoltées. Le diamètre du collet de la plante (appelé dans la suite diamètre de la tige) et le diamètre basal du rameau (appelé dans la suite diamètre du rameau) ont été mesurés. La longueur de la tige et des rameaux a également été mesurée. Le nombre de rameaux à différents niveaux de ramification et le nombre d'apex par rameau ont été comptés. L'estimation de l'âge a été faite en considérant un taux de croissance constant pendant toute la durée de vie de la plante (Frenot et al. 1993) et en divisant la surface du coussin par son taux de croissance annuelle. Enfin, pour dénombrer les cernes, des coupes transversales ont été réalisées dans la base de la tige principale de trois coussins sur les six collectés. Ces coupes ont été colorées au colorant vert de

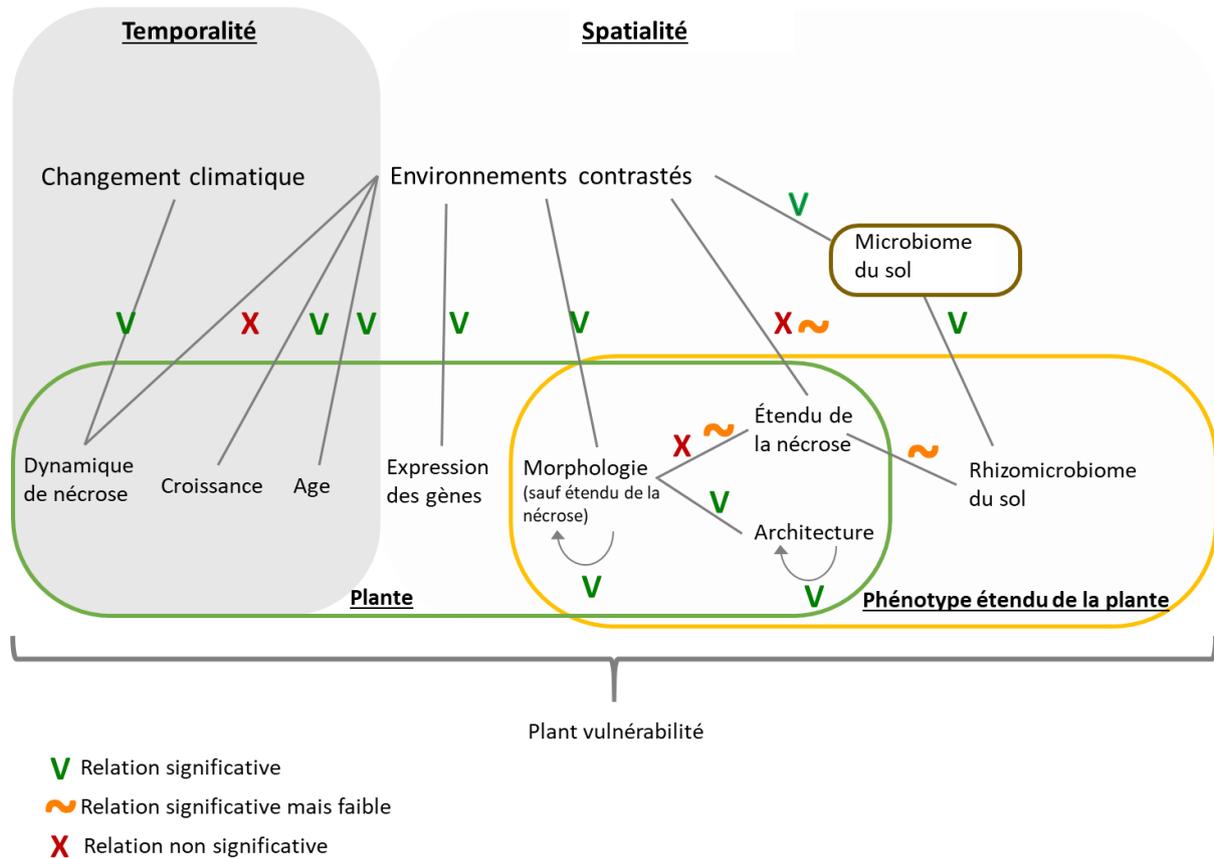
Mirande après un traitement à l'eau de javel pour vider le contenu cellulaire afin de mieux observer les parois végétales, puis elles ont été montées dans l'eau entre lame et lamelle de microscopie pour l'observation au microscope photonique.

Le taux de croissance moyen de *L. kerguelensis* ($7,50 \pm 1,16 \text{ cm}^2 \cdot \text{an}^{-1}$) semble se situer dans la gamme des taux de croissance des autres plantes en coussin (Frenot et al. 1993; Kleier and Rundel 2004). Il varie entre les populations et pourrait s'expliquer par les environnements contrastés entre les sites sélectionnés. L'allométrie des traits architecturaux était visible pour *L. kerguelensis*. Par ailleurs, les cernes annuels successifs de croissance n'étaient pas discernables, ne permettant pas d'estimer l'âge des coussins par cette méthode. Cependant, l'estimation de l'âge possible des coussins de *L. kerguelensis* avec le taux de croissance suggère que cette espèce pourrait vivre quelques décennies voire une centaine d'années pour certains d'entre eux. Cette estimation de l'âge et de la croissance des coussins est essentielle pour évaluer leur capacité d'adaptation et de survie au changement climatique.

Discussion

Vers une estimation de la capacité d'adaptation de *L. kerguelensis*

L'ensemble des résultats obtenus lors de cette thèse est synthétisé sur la figure ci-dessus. Parmi les populations vivant dans des environnements contrastés, une variabilité de la morphologie des plantes dans l'espace (chapitre 1) et dans le temps (chapitre 4) a été observée. Ces résultats suggèrent que le *pool* de variabilité morphologique de *L. kerguelensis* existe et répond aux variables environnementales étudiées. Les profils d'expression génique et l'expression différentielle des gènes ont montré une plus grande variabilité entre les régions qu'entre les populations au sein de chaque région des îles Kerguelen étudiée. L'expression différentielle locale des gènes en relation avec les conditions environnementales locales pourrait indiquer la capacité des populations à s'adapter à leur environnement. Par ailleurs, les variables environnementales semblent faiblement interagir avec la durée de vie et le taux de croissance des plantes (Chapitre 5). Nous concluons que la croissance extrêmement lente peut difficilement être observée avec nos méthodes et nécessiterait d'autres techniques. Une plus grande précision dans les mesures de croissance pourrait mieux révéler l'implication des variables environnementales dans la variabilité de la croissance. Enfin, une variabilité des communautés du *rhizomicrobiome* (chapitre 3) a été observée entre les populations. La relation étroite entre la composition du *rhizomicrobiome* des plantes en coussin et l'environnement a été mise en évidence dans d'autres études (Roy et al. 2013; Wang et al. 2020) et pourrait être considérée comme une composante essentielle pour aider la plante à s'adapter à son environnement, en



particulier dans le cadre d'un changement climatique rapide. En combinant les résultats de la variabilité morphologique, de l'expression génique et de la composition du *rhizomicrobiome*, nous avons observé qu'ils répondaient tous à des variables environnementales qui structurent les populations ou les régions. Ainsi, *L. kerguelensis* et son phénotype étendu semblent répondre à une certaine gamme de variables environnementales. Cela pourrait être un élément essentiel pour que les espèces puissent suivre la vitesse du changement climatique et s'adapter localement (Moritz and Agudo 2013; Cotto et al. 2017). Cependant, la nécrose des plantes (c'est-à-dire une possible réponse à un stress abiotique) visible sur de nombreuses plantes pourrait indiquer que la capacité d'adaptation de *L. kerguelensis* est limitée.

En nous concentrant sur l'étendue de la nécrose des coussins, nous avons observé une variabilité entre les populations (chapitre 1) et ainsi que différentes dynamiques au cours du temps (chapitre 4), qui pourraient être en partie liées aux variables environnementales (sodium du sol, hausse des températures et baisses des précipitations hivernales) (chapitres 1 et 4). Le potentiel osmotique est un élément essentiel pour le fonctionnement des plantes et le stress induit par des conditions plus salines pourrait entraîner une modification de l'absorption de l'eau et des nutriments (Pardo and Quintero 2002; Shabala 2013). Cela pourrait être le cas pour *L. kerguelensis*. Nous avons mis en évidence que la teneur en sodium du sol était positivement corrélée à l'étendue de la nécrose, cependant, l'origine de la nécrose pourrait également dépendre de l'interaction entre plusieurs

facteurs. Une augmentation plus rapide de la nécrose s'est produite dans les endroits où les hivers sont les plus chauds et les plus secs. L'étendue de la nécrose pourrait aussi être liée au *rhizomicrobiome* du sol (chapitre 3). Aucun microorganisme commun n'était lié à l'étendue de la nécrose dans les différentes populations, mais un changement des fonctions microbiennes (décomposition, cycle du phosphore et de l'azote) pourrait se produire. Ces résultats soutiennent l'hypothèse d'une origine abiotique de la nécrose chez *L. kerguelensis* et une relation possible avec le changement climatique récent, qui pourraient impacter le fonctionnement de la plante (et son phénotype étendu), en particulier le stress de la sécheresse. Chaque approche met en évidence la relation entre la nécrose et les stress abiotiques avec un seuil possible au-delà duquel la nécrose pourrait s'étendre. Enfin, la nécrose pourrait ne pas conduire systématiquement à la mort des coussins à court terme. En effet, l'augmentation de la nécrose a été visible sur de nombreux coussins, mais très peu sont morts pendant les 5 ans de l'étude. Cela pourrait être considéré comme un avantage pour la survie de *L. kerguelensis*, dans une certaine mesure, dans des environnements stressants, et soulève la question de la capacité de régénération de la population, sujet abordé dans les pages suivantes.

Au sein des populations, des variabilités morphologiques (surface des coussins, taux de croissance et âge ; chapitres 1, 4 et 5) et du *rhizomicrobiome* (diversité et composition ; chapitre 3) ont été observées tandis que le transcriptome était conservé (chapitre 2). La variabilité morphologique pourrait être liée à des variables micro-environnementales (pas toujours saisies). La convergence évolutive de la plante en coussin dans les environnements hostiles pourrait suggérer que cette forme soit adaptée aux contraintes mécaniques présentes dans ces environnements (c'est-à-dire le vent, la teneur en eau du sol, les cycles de gel-dégel et de grandes fluctuations de température). Enfin, la composition du *rhizomicrobiome* fongique pourrait être sensible à la fois à la composition du sol et au génotype de la plante hôte. Dans l'ensemble, la variabilité au sein d'une population, que nous n'avons pas pu entièrement saisir et expliquer, pourrait être un avantage pour la plante face au changement climatique, car elle augmente la possibilité qu'un caractère soit adapté au nouvel environnement.

La variabilité de l'étendue de la nécrose entre les individus au sein des populations, a été observée (chapitres 1 et 4) et pourrait être liée à la variabilité inter-individuelle de la surface du coussin ainsi qu'à sa communauté végétale au contact de celui-ci (chapitre 4). Dans une autre étude, l'étendue de la nécrose semble être indépendante de la morphologie des coussins (Chapitre 1). Par conséquent, l'étendue de la nécrose pourrait ne pas résulter exclusivement du vieillissement de la plante (Thomas 2013), où les coussins les plus âgés présentent une nécrose plus importante (Molau 1996; Kleier and Rundel 2004). Cependant, la majorité de la variabilité reste inexpiquée et pourrait être liée à l'échelle micro-environnementale ou bien à des événements stochastiques survenus au sein de la population. Donc, des recherches supplémentaires sont nécessaires pour mieux comprendre la dynamique de la nécrose intra-populationnelle en relation avec le microclimat, la microtopographie (comme les

mouvements du vent) et les relations biotiques (comme la distance avec la première plante en coussin voisine).

Les espèces végétales endémiques à longue durée de vie pourraient persister plus longtemps que prévu en cas de changement climatique dans des habitats inadéquats, grâce à leur plasticité phénotypique (Duputié et al. 2012; Cotto et al. 2017). Ce cadre pourrait se produire pour les espèces endémiques à longue durée de vie dans les îles subantarctiques, confrontées à des environnements hostiles et au changement climatique rapide. Pour *L. kerguelensis*, sa durée de vie semble être au moins de plusieurs décennies et pour certains coussins peut-être d'une centaine d'années (chapitre 5). Les coussins de *L. kerguelensis* pourraient persister, dans une certaine mesure, en réponse au changement climatique qui a commencé à être enregistré dans les années 1960 aux îles Kerguelen. La régénération est un autre paramètre important pour le renouvellement des populations et leur maintien, et semble plus compliquée si les plantes persistent dans des environnements inadéquats. Ainsi, la distribution actuelle de *L. kerguelensis* pourrait ne pas refléter sa niche potentielle. L'étude de sa démographie, c'est-à-dire de l'abondance et de la classe de tailles des coussins dans diverses populations, pourrait donner un aperçu de la nouvelle niche optimale de l'espèce dans le cadre du changement climatique actuel.

Un scénario en cas de fort changement climatique

Avec les nouvelles prévisions du rapport du GIEC, la prédiction du changement climatique est pire que celle du rapport précédent (Ranasinghe et al. 2021). Un changement de végétation se produit avec le changement climatique dans de nombreux environnements. Les plantes natives à longue durée de vie, y compris certaines plantes en coussins, semblent ne pas suivre cette voie pour de multiples raisons et pourraient s'éteindre localement (Fosaa et al. 2004; Le Roux and McGeoch 2008; Ferreira et al. 2016; Cotto et al. 2017). Le changement climatique attendu dans les îles Kerguelen est une augmentation des températures minimales d'environ + 1 ou + 2,3 °C avant la fin du siècle, accompagnée d'un changement des précipitations pouvant atteindre 11 ou 23 % (Verfaillie et al. 2021). Ce changement peut engendrer un stress considérable pour les plantes limitées aux habitats froids. Nous pouvons nous interroger si la variabilité des traits phénotypiques et de l'expression des gènes de *L. kerguelensis* avec l'aide de micro-organismes sera suffisante en présence de changements rapides. Avec un climat plus chaud et plus humide, *L. kerguelensis* pourrait allonger sa saison de croissance, ce qui serait avantageux pour la productivité des plantes, comme observé dans la toundra arctique (Ernakovich et al. 2014). Cependant, cet environnement doux et humide pourrait induire la prolifération d'agents pathogènes, ce qui pourrait aggraver la nécrose des plantes comme cela a été observé pour *Azorella macquariensis* sur l'île Macquarie (Bergstrom et al. 2015). En revanche, avec un climat plus chaud et plus sec, *L. kerguelensis* pourrait connaître une diminution de sa saison de croissance en raison de sécheresses plus fréquentes et/ou plus intenses, comme cela a été observé dans la toundra alpine

(Ernakovich et al. 2014). La régénération pourrait être compromise car la levée de dormance des graines pourrait être plus difficile avec des conditions plus chaudes et plus sèches. De plus, la sécheresse pourrait facilement endommager les semis, leur flétrissement ayant déjà été observé en été chez cette espèce (Hennion and Walton 1997). Enfin, la nécrose du coussin de *L. kerguelensis* peut être accélérée dans les environnements les plus secs, les plus chauds et les plus salins.

Conclusion générale

La combinaison des approches utilisées pour estimer la capacité d'adaptation de *L. kerguelensis* au changement climatique révèle l'ampleur des défis scientifiques auxquels nous sommes confrontés. La morphologie de *L. kerguelensis* présente une allométrie et répond à la teneur en eau du sol et à l'intensité du vent. Le transcriptome des plantes vigoureuses est spécifique à chaque région avec une expression différentielle des gènes liés aux réponses aux stress abiotique ou biotique. La croissance de *L. kerguelensis* est très lente et population spécifique. Sa durée de vie comprend plusieurs décennies au moins. Le *microbiome* des sols des *fellfields* des îles Kerguelen est spécifique et varie en fonction de la teneur en nutriments du sol. Le *rhizomicrobiome* de la plante a montré une variation similaire au *microbiome* mais sa composition est sous l'influence de *L. kerguelensis*. Enfin, la variabilité de l'étendue de la nécrose pourrait être liée à la morphologie des coussins et aux variables environnementales. Plus précisément, la nécrose pourrait être un dommage dû au stress de la sécheresse lié aux tendances hivernales de réchauffement et d'assèchement du climat. L'étendue de la nécrose pourrait être aggravée par le stress salin et un changement de composition du *rhizomicrobiome*. Dans le cadre des tendances actuelles du changement climatique aux îles Kerguelen, *L. kerguelensis* pourrait avoir la capacité de faire face et de s'adapter aux variations environnementales dans une certaine mesure.

Notes : les références utilisées pour ce résumé sont à retrouver dans les parties de la thèse correspondante.

Introduction

Plant adaptation: What does theory say?

Past environmental conditions shaped every organism. As a result, an organism's phenotype comprises all the traits (morphological, anatomical and physiological) from the expression of a genotype in interaction with its environment (Kattge *et al.* 2011; Begon & Townsend 2021). When an environmental change occurs, species populations can respond by shifting geographical range, exhibiting plasticity, or genetically adapting (Williams *et al.* 2008; Hoffmann & Sgrò 2011). These responses are modulated by the intrinsic sensitivity of the population and the intensity of the change (Moritz & Agudo 2013). In many cases, populations are facing "nowhere-to-go" situations (Román-Palacios & Wiens 2020). Therefore, an essential aspect of population responses to environmental changes is their capacity to persist in the same location by modifying their traits with plastic and/or genetic responses (Moritz & Agudo 2013; Hoffmann *et al.* 2017). Plasticity is defined as "all aspects of the phenotype in which expression varies due to environmental differences" by Sultan (2021). Plastic responses reflect the direct influence of the environment on organism traits that takes place throughout their life cycle (Chevin *et al.* 2010; Catullo *et al.* 2019; Diamon & Martin 2021). Plastic responses appropriate to the new condition can be considered adaptive and increase the organism's fitness (Fox *et al.* 2019; Diamon & Martin 2021). However, high costs are potentially associated with them (Moritz & Agudo 2013; Snell-Rood *et al.* 2018). The lack of ability to produce an optimal trait could also limit the possibility of coping with changes (Chevin *et al.* 2010; Murren *et al.* 2015). Furthermore, plasticity can evolve genetically in response to selection (Chevin & Hoffmann 2017; Catullo *et al.* 2019; Pfennig 2021). Genetic adaptation mechanisms occur in a multivariate space where genetic constraints (for example, interactions between traits) and the direction of selection across multiple traits determine species' adaptation (Hoffmann & Sgrò 2011; Duputié *et al.* 2012). This mechanism usually requires several generations before a genetic adaptation suitable to the new condition occurs, and therefore will act differently between short or long generation time species (Hoffmann *et al.* 2017). Hence, the genetic diversity that the species acquired throughout its evolution might be essential to respond to changes because appropriate genetic variation might already have arisen (Hoffmann & Sgrò 2011).

These responses are not mutually exclusive, and it has been shown that a complex interplay exists (Moritz & Agudo 2013; Catullo *et al.* 2019). The combination of plasticity and genetic variation that underlies phenotypic variability needs to be studied together, as their combination might change the outcome of ecological interactions (Bolnick *et al.* 2011; Snell-Rood *et al.* 2018). A more inclusive way to assess species vulnerability to environmental changes such as climate change is needed for

evolutionary biology, ecology, and conservation research (Williams *et al.* 2008; Nicotra *et al.* 2015). Therefore, this challenging interdisciplinary research of species vulnerability to changes could be assessed with the adaptive capacity concept (Williams *et al.* 2008; Beever *et al.* 2016).

The adaptive capacity concept

Adaptive capacity is defined as the ability of a species or population to cope with new environmental conditions and persist in surviving and reproducing. The strength of this approach is reflected through studying spatial and temporal phenotypic variability and local adaptation in relation to the species life history (Williams *et al.* 2008; Nicotra *et al.* 2015; Beever *et al.* 2016). Adaptive capacity is close to the evolutionary biological concept of adaptive potential. It can be seen as a possible component of adaptive capacity (reviewed in Seaborn *et al.* 2021). Life-history characteristics should be considered when estimating adaptive capacity (Nicotra *et al.* 2015; Thurman *et al.* 2020). The length of plant generation might condition the available pool of genetic adaptation and plastic responses and influence the outcome of adaptation (Bolnick *et al.* 2011; Cotto *et al.* 2017; Snell-Rood *et al.* 2018). *In-natura*, populations consist of phenotypically diverse individuals. In order to approach phenotypic variation, it is possible to study the variability of traits (morphological, anatomical, physiological, biochemical and phenological) across populations (Kattge *et al.* 2011; Catullo *et al.* 2019). Trait-based approaches allow identifying the available diversity and the probability of a trait to be appropriate to the new environmental conditions (Bolnick *et al.* 2011; Nicotra *et al.* 2015). The genomic study provides a means to study how organisms upregulate and downregulate the expression of specific genes and alleles in response to the environment to improve their fitness, growth and development (Artemov *et al.* 2017; Kim *et al.* 2018; Akhter *et al.* 2021). These sources of variation across populations could be a pivotal element to substitute "space-for-time" for species persistence (Blois *et al.* 2013). Identifying genes differentially expressed in relation to environmental variables might be valuable to better estimate which population might harbour adaptation to the environmental change (DeBiasse & Kelly 2016; Voelckel *et al.* 2017).

Overall, the adaptive capacity might give more precise insights into the vulnerability of plants to environmental changes. However, a plant does not stand alone in the environment. Therefore, considering eco-evolutionary biotic interactions, namely the plant extended phenotype (Dawkins 1982; Hunter 2018), would lead to a more inclusive concept of plant adaptive capacity.

Extended phenotype and adaptive capacity

The extended phenotype includes all effects of a gene on its environment, from inside a phenotype, several organisms within a species until a complex ecosystem (Dawkins 1982). One of the extended phenotype categories, recently added consists, in the plant and all the microorganisms associated with it (Hunter 2018) and which are essential for plant functioning and adjustment to the environment (Vandenkoornhuysen *et al.* 2015; Cordovez *et al.* 2019). More precisely, the assembly of microorganisms governed by plants in the surrounding root-soil system, *i.e.*, the rhizomicrobiome, is known to be directed towards beneficial microorganisms that might enhance the plant's adaptive capacity (Bulgarelli *et al.* 2013). Plant rhizomicrobiomes might result from co-evolved species assemblages and active selection (Berendsen *et al.* 2012; Cordovez *et al.* 2019). Therefore, a particular diversity and composition occur in this area under the influence of the plant (Nautiyal & Dion 2008; Berendsen *et al.* 2012), resulting in a close relationship between plant traits, rhizomicrobiome, and environmental changes (Cordovez *et al.* 2019; Weil *et al.* 2021) (Fig. 1). Hence, the plant adaptive capacity combines complex processes that need to be studied together to better understand plant vulnerability to environmental changes. After this theoretical background on plant adaptation, we will come to the main topic: the adaptation of long-lived cushion plant species endemic to an island. This take place in the context of climate change occurring in an island subject to a harsh environment.

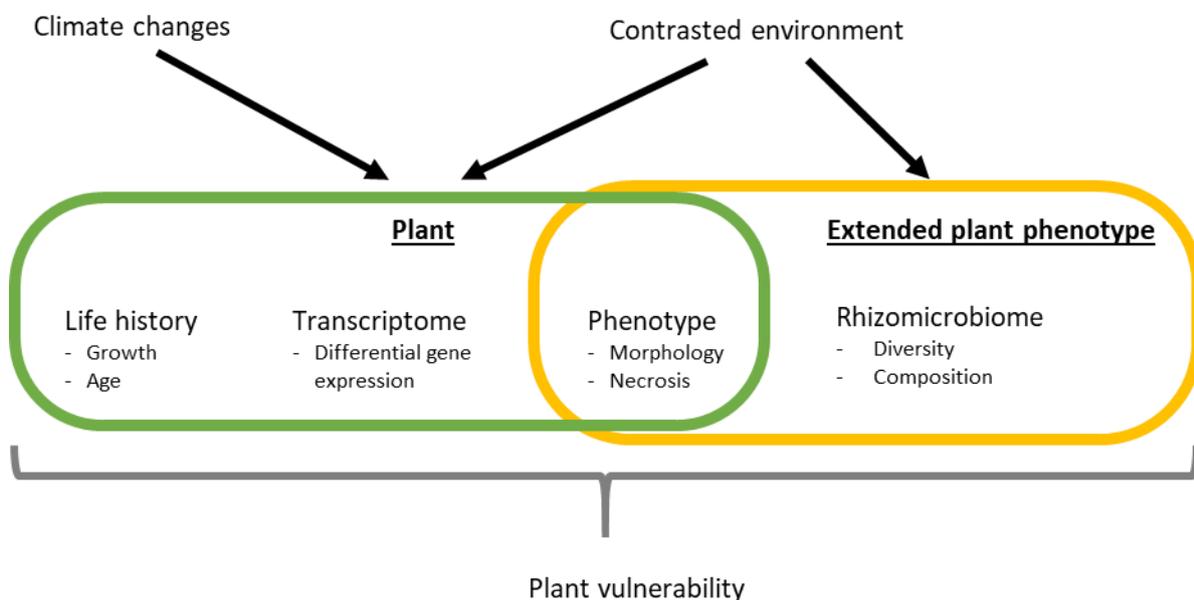


Figure 1: schematic view of the conceptual approach used in this thesis based on the inclusive adaptive capacity.

Species isolation and endemism: consequences for adaptation

Detailed characteristics and species evolution in islands have been previously discussed in Weigelt *et al.* 2013; Warren *et al.* 2015; Patiño *et al.* 2017 (among others). Essential features that might condition plant adaptation to environmental changes are the limited habitats available to track optimal conditions within the island. If the landscape is heterogeneous enough, it might allow micro-scale tracking (Harter *et al.* 2015; Irl *et al.* 2015). However, this process might not be sufficient to cope with large or intense change (Chen *et al.* 2011; Moritz & Agudo 2013). For example, opportunities for an altitudinal shift by species might be limited as islands tend to have low elevations, except for the largest ones (Weigelt *et al.* 2013). Furthermore, compared to continental ecosystems, oceanic islands have relatively low species numbers and simpler community structures. When a change occurs, biotic interaction and resources are expected to favour ecosystem resilience which might be less the case in island ecosystems (Harter *et al.* 2015). When a change such as rapid climate change occurs, it might strongly affect native island flora. For example, in the Azores islands, more than 90% of native plant species are expected to lose habitat, with for some of them the loss of all adequate habitats (Ferreira *et al.* 2016). Species already found at the upper part of islands are expected to be even more threatened in Faeroe Islands, among other locations (Fosaa *et al.* 2004; Harter *et al.* 2015 and references therein). According to this, the ecological integrity of island ecosystems is relatively sensitive. Islands harbour a much higher proportion of endemic species than continents, and island endemic species are especially affected due to their limited opportunities to escape unfavourable conditions (Harter *et al.* 2015). Endemic species result from local speciation or reduction of a formerly larger geographical area and might result from shorter or longer evolution related to taxon cycle with phases of expansion and extinction (Ricklefs & Bermingham 2002; Ricklefs 2011). Endemic species are strongly associated with particular habitats, usually extremely stable (Jansson 2003). In this regard, it is interesting to consider "phenotypic integration", *i.e.* the number and strength of trait correlations within an organism (Pigliucci 2003). A strong positive correlation was found between phenotypic integration and the degree of endemism of 14 species across an endemism gradient to Kerguelen Islands (Hermant *et al.* 2013). The question was whether high phenotypic integration found in the most endemic species might limit their phenotypic plasticity when facing changes (Hermant *et al.* 2013). It was suggested that strong correlations among traits might lower the ability of organisms to reach an optimal trait value in response to the change. Further studies targeting the intraspecific phenotypic variability of Kerguelen *Ranunculus sp.* and the Kerguelen Cabbage, *Pringlea antiscorbutica*, showed positive correlations between phenotypic integration and phenotypic variability (Labarrere 2017). Therefore, more ecological and evolutionary studies are needed to understand the fate of endemic

species under climate change (Cartwright 2019), and this might be even more challenging for these plants in harsh environments.

Life in harsh cold environments

Harsh cold environments characterize alpine, sub-polar, and polar regions and involve strong winds, cold climates and large fluctuations of both temperatures and precipitations with considerable heterogeneity of climate at both local and regional scales (Körner 2003; Convey 2007; Block *et al.* 2009; Donhauser & Frey 2018). However, alpine, sub-polar and polar environments harbour fundamental differences. Latitudes impact temperatures, solar irradiances and seasonal and diurnal patterns that induce differences between alpine (temperate and equatorial), sub-polar and polar regions (Convey 2007; Donhauser & Frey 2018). In sub-polar and coastal regions, the soil is not composed of permafrost and soil temperature is buffered and above 0°C (Smith 1984; Convey 2007; Donhauser & Frey 2018). One similarity of habitats of these regions is concerned with the occurrence of fellfields. They are tundra-like habitats of rock and glacial till (Block *et al.* 2009; Donhauser & Frey 2018). For the southern hemisphere, fellfields are more precisely defined as "all exposed mineral substrata [...] regardless of their relative climatic and oceanic influence" (Block *et al.* 2009). Examples of Kerguelen Islands fellfields are shown in Figure 2 A, B and C. Fellfield soils are subject to strong physico-climatic stresses: many freeze-thawing cycles per year, solifluction and strong wind erosion (Smith 1984; Lebouvier & Frenot 2007; Block *et al.* 2009). Furthermore, their soil microbiomes are expected to harbour specific and tolerant microbial communities which may sustain critical roles such as soil respiration and nutrient cycles due to the low abundance or even absence of herbivores and macro-detritivores in fellfields (Yergeau 2014; Donhauser & Frey 2018; Cox *et al.* 2019).

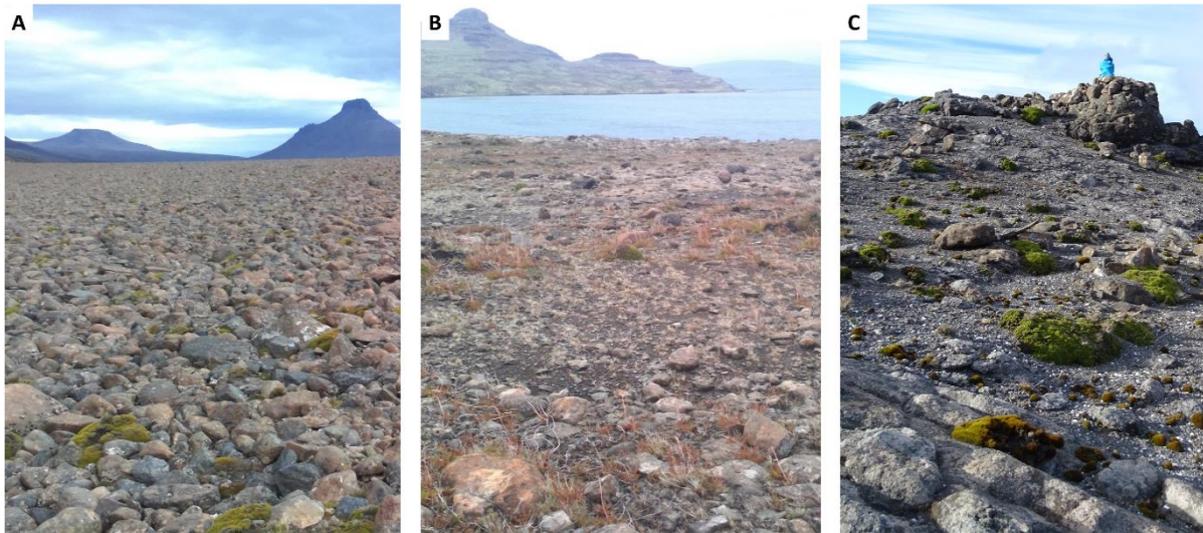


Figure 2: examples of fellfields in Kerguelen Islands: (A) in "Pic du Chat" in "Presqu'île Ronarch" with mainly blocks above 20cm diameter, extremely scarce vegetation composed of cushion plants and small graminoids; (B) in "Ile Australia" with pebbles (2-20 cm of diameter) in larger proportion than blocks and occurrence of cushion plants and various graminoid species; (C) in "Ile Mac-Murdo" with blocks and gravel (<2cm) and scarce vegetation mainly composed of cushion plants.

In harsh cold environments, presumably, native plants have undergone long-term adaptation and therefore exhibit sufficient hardiness to survive harsh conditions (Hennion *et al.* 2006b, a; Körner 2016; Wang *et al.* 2019). Several adaptations are common to Antarctic or sub-Antarctic organisms in relation to stress tolerance. Low temperatures and numerous freeze-thaw events may have led, for example, to the accumulation of secondary metabolites, possibly "anti-freeze compounds" and ice-nucleation outside of cells (Convey *et al.* 2006; Hennion *et al.* 2006b; Sierra-Almeida *et al.* 2009). Several plant species maintain photosynthesis at low temperatures, with some active below 0°C (Hennion *et al.* 2006b). Their metabolisms might shift to avoid damages (Cho *et al.* 2018), with dehydration leading to lower metabolic activities and rapid repair and resumption of metabolic activities. Furthermore, when studying RNA expression, *Colobanthus quitensis* in Antarctica showed genes involved in pathways of stress response to low temperature (Cho *et al.* 2018). Hence many native plants in these harsh environments live close to their physiological limits and show numerous stress responses to abiotic factors (Convey *et al.* 2006; Hennion *et al.* 2006b). An essential feature in harsh environments is the convergence of various plants towards prostrated forms, including cushion forms. Currently, 1328 cushion plants species have been recorded from various parts of the world (Aubert *et al.* 2014; Boucher *et al.* 2016). Their shape can slightly vary from hemispherical, tufted, or flat with various compactness levels (Aubert *et al.* 2014). The best known are probably the genera *Azorella* (Apiaceae from the Andes and the sub-Antarctic) which is known for its giant mat sizes with up to 12m of perimeter length (Fig. 3A and B) (Kleier *et al.* 2015; Dickson *et al.* 2020), *Androsace* with numerous species of cushion plants of 10-20 cm of diameter living on cliffs in the high Alpine (Fig. 3 C) (He *et al.*

2014; Boucher *et al.* 2021) or *Silene acaulis* which can grow up to 70cm of diameter with its widespread distribution in the sub-Arctic and Arctic (Fig. 3 D) (Benedict 1989; Molenda *et al.* 2012). Cushion shape optimizes the ratio of surface-to-volume, which is an important feature to sustain vigour (Zotz *et al.* 2000; Butterfield *et al.* 2013; Bergstrom *et al.* 2015). Indeed, this shape can increase heat and soil nutrients within and below the cushion, however, these elements are not generalizable to every population and species (summarised in Momberg & le Roux 2020). The variability of leaf compactness in cushion forms might be related to differential expression of thermomorphogenesis-related genes, as observed for *Colobanthus quitensis* (Cho *et al.* 2018). Cushion plant extended phenotype is particularly interesting, as cushion plant increases nutrient and organic matter and modifies the pH of the soil underneath and these conditions create an oasis for soil microorganisms compared to the poor surrounding soil (Roy *et al.* 2013; Wang *et al.* 2020; Rodríguez-Echeverría *et al.* 2021). For example, two subspecies of *Silene acaulis* which grow in similar soils in the Alps show differential rhizosphere fungal communities. This might be explained by environmental filtering and direct plant–fungi interactions depending on the sub-species (Roy *et al.* 2018).



Figure 3: A: *Azorella compacta* cushion with its irregular cushion growth form, from Kleier *et al.* (2015); B: *Azorella macquariensis* cushions with *Pleurophyllum hookeri* and graminoid epiphytes (central region of Macquarie Island, MI, south of Lake Prion), from Dickson *et al.* (2020). C: *Androsace* pictures depicting the overall morphology of different species delimited within the /*Helvetica* clade, from Boucher *et al.* (2021) and D: Female *Silene acaulis* in bloom on Whistler Mountain, from Molenda *et al.* (2012). Pictures used either were under creative commons license, or the authorization to use the figures was given by the editors.

Rapid climate change and consequences for plant species

Alpine, sub-polar and polar regions are subject to rapid climate changes, probably the fastest-warming on Earth (IPCC: Ranasinghe *et al.* 2021). These regions face a temperature increase, precipitation variation (quantity and regime), and modification of wind regime, among other factors (IPCC: Ranasinghe *et al.* 2021). For the end of the century, prediction for the whole sub-Antarctic is an increase in temperature and precipitation variations. However, the intensity of temperature increase is island-specific, from 1°C in Heard and Macquarie Islands up to 1.5°C in Marion Islands for example, when considering the greenhouse gases representative concentration pathway (RCP) 6.0, mainly due to the position of these islands in relation to the southern annular mode (Harter *et al.* 2015). This temperature increase may look small, but it is very large for such buffered climates with both low temperature ranges and low local averages. Moreover, the decrease in precipitation and the increase in evaporation should increase the salinity in the sub-Antarctic Islands (le Roux & McGeoch 2008). The intensity of the temperature, precipitation and wind changes will impact the possible response of the

species to that change (Moritz & Agudo 2013). This is of special concern as alpine, sub-polar, and polar ecosystems often have low heat resilience (IPCC: Ranasinghe *et al.* 2021). Impact on long-lived species was highlighted with the occurrence of dieback in various harsh environments facing rapid climatic changes (Molau 1996; Frenot *et al.* 1997, Kleier & Rundel 2004; Bergstrom *et al.* 2015; Bjerke *et al.* 2017; Dickson *et al.* 2020, among others). Dieback is defined as the process of expansion of mortality where a particular portion of the cushion or the entire cushion fails to regenerate new foliage (Armesto *et al.* 1980; Whinam *et al.* 2014). Necrosis is the visible damage at a particular time and can be a symptom of the dieback process (Whinam *et al.* 2014). In the sub-Antarctic, the most documented case concerns *Azorella macquariensis* (Apiaceae) (Fig 4 A and B), a long-lived endemic cushion plant of Macquarie Island that shows rapid dieback all over the island due to a combination of abiotic factors and pathogens enhanced by climate change (Bergstrom *et al.* 2015; Dickson *et al.* 2020), (Fig 4 B). In this species, the mean dieback increased significantly by 30% between 2009 and 2011 (Fig 4 C). A rainfall exclusion experiment on a congeneric long-lived cushion plant, *Azorella selago* from Marion Island, resulted in necrosis after a year of this treatment (Le Roux *et al.* 2005). Therefore, these keystone species will very likely be vulnerable to climate change. However, studies addressing the adaptive capacity of these plants remain few.

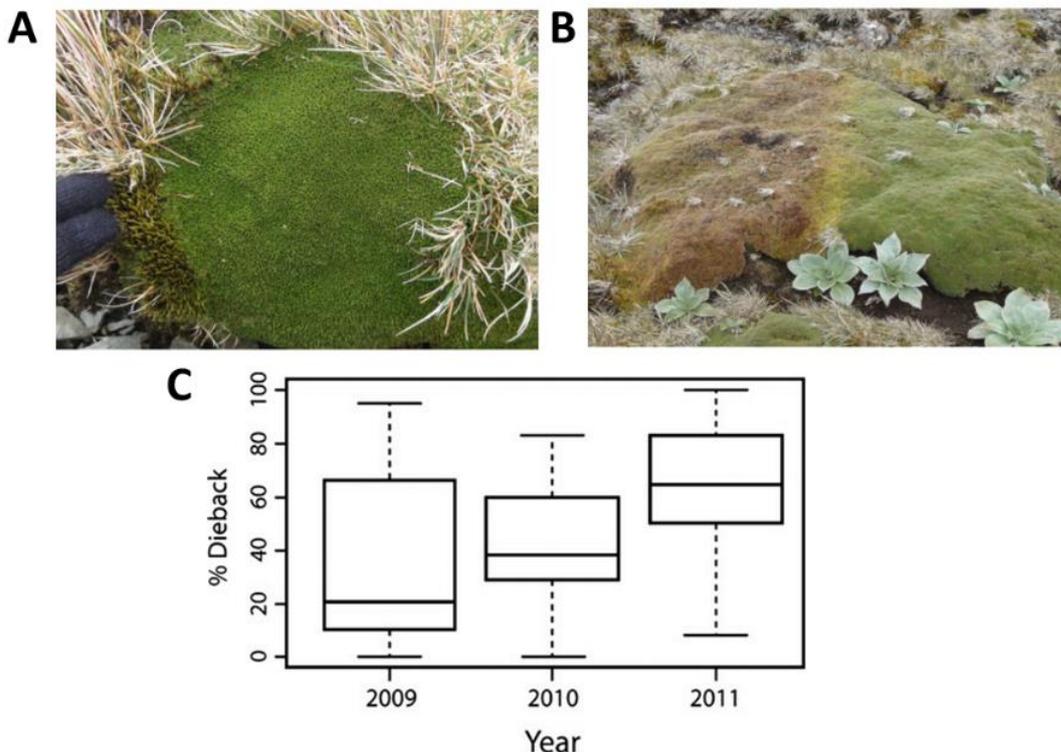


Figure 4: Illustrations of the *Azorella macquariensis* dieback in Macquarie Island fellfield; adapted from Bergstrom *et al.* (2015). A: healthy *A. macquariensis*; B: *A. macquariensis* cushion with a chlorosis line; C: significant increase in *Azorella macquariensis* dieback between 2009 and 2011 (median with the box delimitating upper and lower quartile, bars denote range). The authorization to use the figures was given by the editors.

When the optimal shape of cushion plants cannot be sustained, negative impacts can be observed, like cushions sagging and crescent forms (Zotz *et al.* 2000; Combrinck *et al.* 2020). For cushions without lignified stems such as *A. macquariensis* or *Grimmia pulvinata*, their shapes are sustained by cell turgidity in the stems. When water is lacking, the cells are no longer as turgid, the cushion may collapse (Zotz *et al.* 2000; Bergstrom *et al.* 2015). Another example concerns sediments windblown accumulated on the leeward side of *A. selago* cushion in Marion Islands, leading to stem mortality on this side and occurrence of crescent shape (Combrinck *et al.* 2020). Modifying wind patterns and intensity in these islands might damage other sides of the cushion, lowering their vigour. Furthermore, the increase of temperature and decrease in precipitations under climate change may lead to changes in bacterial or fungal diversity and composition with possible negative effects on soil functioning as experimentally observed in alpine and polar fellfields (Hawkes *et al.* 2011; Yergeau *et al.* 2012; Donhauser & Frey 2018; Misiak *et al.* 2021). This might impact the plant's functioning. In addition, consequential changes in the rhizomicrobiome composition in interaction with plant responses to changes are most probable but remain little understood. For example, in the high Arctic Svalbard archipelago, a decrease in pioneer ectomycorrhiza of long-lived plants was highlighted with a warming climate (Botnen *et al.* 2020).

In this context, long-lived endemic plant species can be seen as good models for studying climate change impact in the sub-Antarctic and other harsh environments. Investigating their adaptive capacity through spatial and temporal phenotypic plasticity, local adaptation, and rhizomicrobiome composition might be essential to assess their vulnerability to ongoing and future climate change. Investigating adaptive capacity in these models might be valuable for evolution and conservation knowledge.

The Kerguelen Islands environment

Geographical location

Kerguelen Islands (48°30' - 50°00' S, 68°27' - 70°35' E) are located in the southern Indian Ocean. These Islands belong to the South Indian Ocean Province (SIOP) together with Archipel Crozet, Iles Marion and Prince Edwards, and Heard and Mc Donald (Smith 1984; Chown *et al.* 1998). The Kerguelen Islands are part of the French territory of the “Terres Australes et Antarctiques Françaises” and have the status of “Réserve naturelle nationale”. Since 2019, Kerguelen Islands have got the status of “World Heritage” by UNESCO. These islands are isolated, being located at about 3500 km from either the South African or the Australian coastlines (Lebouvier & Frenot 2007) (Fig. 5). Their origins is dating at ~ 29Mya, making them the oldest islands in the SIOP and they are mainly composed of basalt (Nicolaysen

et al. 2000). This archipelago of 7500 km² of land comprises around 300 islands, all-around a main island called "Grande Terre"; the main island is dominated by "Mont Ross" and harbour the Cook ice cap on the western part (Fig. 5).

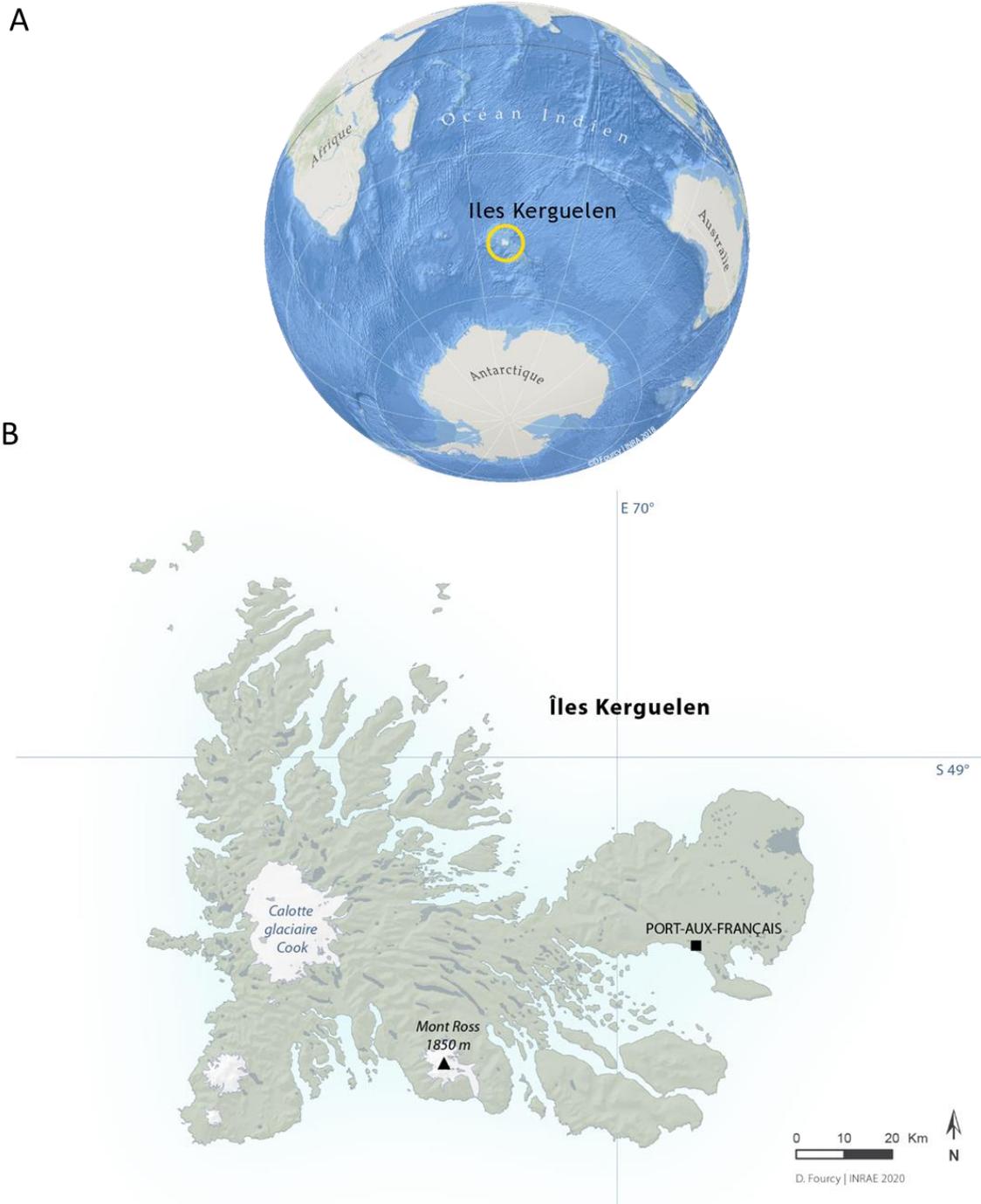


Figure 5: (A) position of the Kerguelen Islands in the Indian Ocean and (B) Map of the topography of Kerguelen Islands, lakes are in grey, the ice cap is in white. The summit of the Islands is indicated with the black triangle "Mont Ross 1850 m", and the black square indicates the research station "Port-aux-Français" (B).

Abiotic and biotic characteristics

The Kerguelen Islands landscape is hilly and harbours several types of habitats (Aubert de la Rüe 1964). There are 22 species of native phanerogams (Frenot *et al.* 2001), with no shrubs or trees. Grassland and wetland occurs at low altitudes (< about 150 meters) protected from the winds and numerous fellfields occurs from the shore to the highest elevations when facing winds (Aubert de la Rüe 1964; Hennion *et al.* 2006a). Kerguelen fellfields are ecosystems of rock and glacial till and were presumed deficient in nutrients (Aubert de la Rüe 1964). In fellfields, the native flora is impoverished due to geographical isolation that acts as biodiversity filters and constant low temperature and strong wind, a component of water stress, disturbance, and abrasion (Aubert de la Rüe 1964). Vegetation is tundra-like, composed mainly of perennial plants, such as megaherbs, grass, and cushion plants, as well as bryophytes (Aubert de la Rüe 1964). Soil microbiome is little studied in the whole sub-Antarctic compared to other harsh environments such as alpine and Antarctic regions. In the Kerguelen Islands, two studies have been done, on the fungal communities in herbfield soils (Pansu *et al.* 2015) and the mycorrhiza associated with various native plants (Strullu *et al.* 1999) respectively. The fungal α -diversity was low and similar to what was observed in some alpine and Antarctic fellfields but lower than in sub-Arctic or Arctic fellfields, possibly due to dispersal limitation (Yergeau 2014; Pansu *et al.* 2015; Praeg *et al.* 2019; Wang *et al.* 2020). Modification in fungal composition was observed due to rabbit introductions, which induced less indigenous fungal species which might impact soil functioning (Pansu *et al.* 2015). Based on RNA analyses, endemic fungi are more active than generalist ones in the Antarctic (Cox *et al.* 2019) and this might be the case also in the Kerguelen Islands. Ectomycorrhiza were absent from the roots studied. However, endomycorrhiza were observed in *Azorella selago* and were not observed in *Lyallia kerguelensis* and *Pringlea antiscorbutica* (Strullu *et al.* 1999) as well as associated with other native plants in the sub-Antarctic Islands (Smith & Newton 1986, Frenot *et al.* 2005).

Climate and climate change

Kerguelen Islands climate is cold with mean recorded annual temperature of 4.6°C and annual rainfall of 755 mm at "Port-aux-Français" from 1951 to 2018 (Verfaillie *et al.* 2021, Fig. 6). The wind is strong, around 10m.s⁻¹ and constant in these Islands (Frenot *et al.* 1998). Kerguelen Islands climate is under the strong influence of the southern annular mode, which influences temperatures, winds, and precipitation distribution from the subtropics to Antarctica (Abram *et al.* 2014). The southern annular mode induces mainly westerly winds; therefore, the northern and western parts of the archipelago are wetter and colder. The warmest and driest part is found in the Golfe du Morbihan in the South-East part (fig. 6).

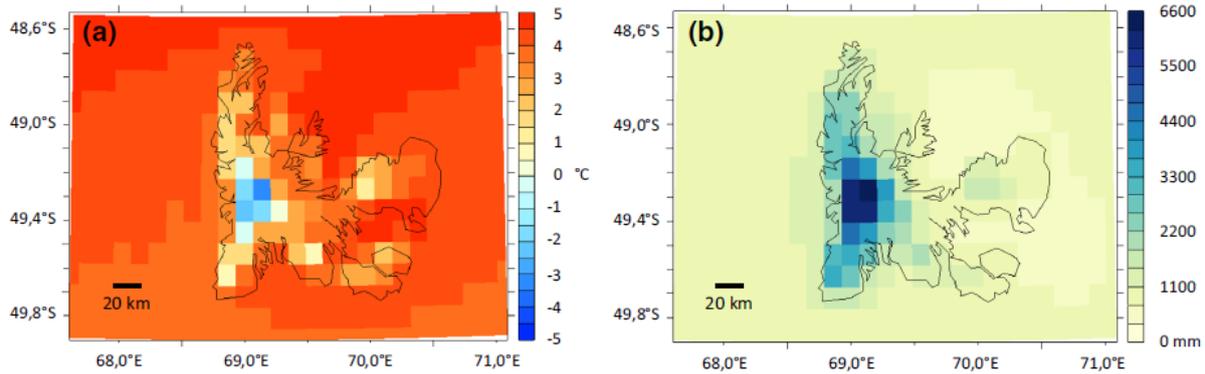


Fig. 6: from Verfaillie *et al.* (2019), Maps of 10 km-resolution of mean near-surface temperature (a) and accumulated precipitation (b) over 1980-2005 on the Kerguelen Islands simulated by the MAR model forced by ERA-Interim after corrections (humidity increased by 10% and temperature increased by 0.8 °C) at the MAR lateral boundaries. The authorization to use the figures was given by the editors.

For several decades, climate changes have been recorded in the whole southern hemisphere, where the anomalously high phase of the southern annular mode was observed (Abram *et al.* 2014; Jones *et al.* 2016) with large-scale significant impacts in reducing precipitation. In the Kerguelen Islands, it resulted in a marked shift in the storm path, leading to lower precipitation (Favier *et al.* 2016; Verfaillie *et al.* 2019). However, this trend is non-linear. Meteorological data were first recorded in the 1950s at "Port-aux-Français", after very humid conditions in the 1950s reaching $1100\text{mm}^{\text{a}^{-1}}$, a 50% decrease in precipitation occurred in the 1960s (Fig. 7A). Afterwards, it increased linearly, reaching almost $800\text{mm}^{\text{a}^{-1}}$ in 1975 (Fig. 7A), followed by a significant drying trend, leading to minimum precipitation of $565\text{mm}^{\text{a}^{-1}}$ in the 2000s (Fig. 7A). However, the 2010s were relatively wet with $820\text{mm}^{\text{a}^{-1}}$ of precipitation (Fig. 7A). For temperature, after a cool period during the 1960s, a significant increase was observed until 1980, and the temperature remained almost constant during 30 years, to finally largely increase in the 2010s (Fig. 7C). For Kerguelen Islands, prediction for the end of the century indicates an increase of precipitation variation and higher temperatures (Verfaillie *et al.* 2021). Of course, this will depend on the global climate change scenario. For instance, according to the Representative Concentration Pathway scenario (RCP) 4.5 and 8.5, an increase in minimum temperatures of about + 1 or + 2.3 °C is expected before the end of the current century at the global level, along with up to 11% or 23% change in precipitation. Such projected changes may cause considerable stress for plants restricted to cold habitats (Verfaillie *et al.* 2021). Hence, the ongoing climate change in the Kerguelen Islands impacts native species, which usually grow in particularly cold and wet conditions and are already showing clear signs of water stress during summer droughts (Hennion 1992; Chapuis *et al.* 2004; Hennion *et al.* 2006a; Wagstaff & Hennion 2007; Lebouvier *et al.* 2011). This raises concerns about how long-lived species of these islands can stand rapid changes.

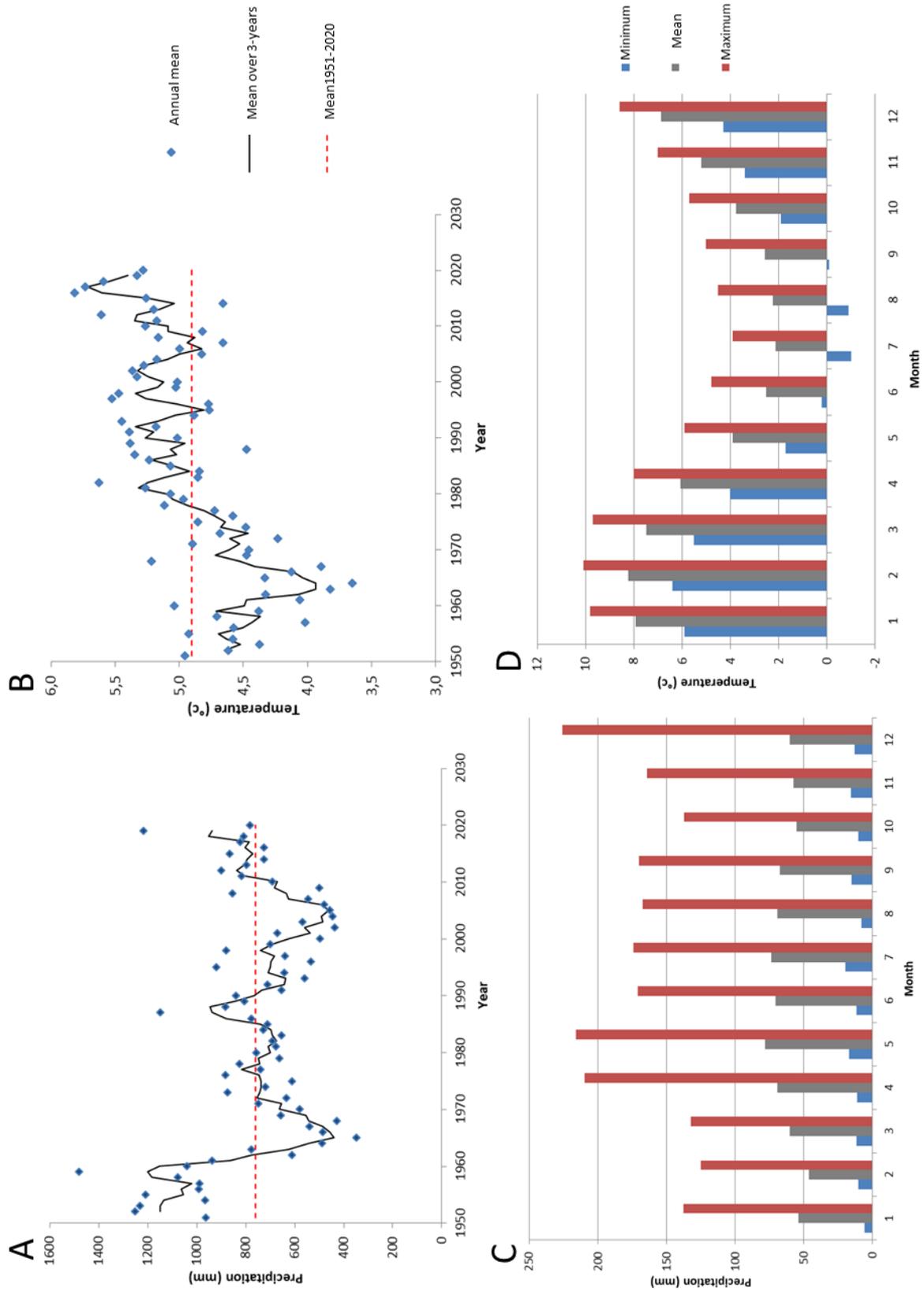


Figure 7. Climate data from Port aux Français in Kerguelen Islands. Météo-France 1951-2020, compiled by UMR 6553 ECOBIO for IPEV 136 and 1116. Annual precipitation (mm) (A), monthly precipitation (B), annual temperature (C), monthly temperature (D).

***Lyallia kerguelensis* (Montiaceae), a long-lived cushion plant species endemic from the Kerguelen Islands and affected by necrosis**

Lyallia kerguelensis Hook.f. (Hooker 1847) (Montiaceae, Caryophyllales; Nyffeler & Eggli 2010) is endemic to the Kerguelen Islands with no representatives in other islands of the SIOP, even in the closest Heard Island (Wagstaff & Hennion 2007). The species shows a restricted distribution (Fig. 8) and its sister species (closest related species) *Hectorella caespitosa* is endemic from New Zealand. With a maximum divergence age of these disjunct sister species that pre-dates full Antarctic glaciation (Wagstaff & Hennion 2007; Winkworth *et al.* 2015), a possible scenario is the widespread distribution of common ancestors in the Antarctic then migration of *L. kerguelensis* and *H. caespitosa* respectively to the Kerguelen Islands and New Zealand during the long cooling period from the Miocene to the Neogene in Antarctica. Numerous records of pollen and macrofossils of *L. kerguelensis* in Kerguelen peat were dating at 13.600 until 12.920 cal yr BP (Van der Putten *et al.* 2015). This period coincides with a shift of climate conditions characterized by increasing wind and colder and wetter conditions. *L. kerguelensis* might have been more widely distributed and abundant then, its current scattered distribution suggesting a relict stage. Before and after this period, pollen and macrofossils records were in much lower quantity, suggesting a more restricted distribution (Van der Putten *et al.* 2015).

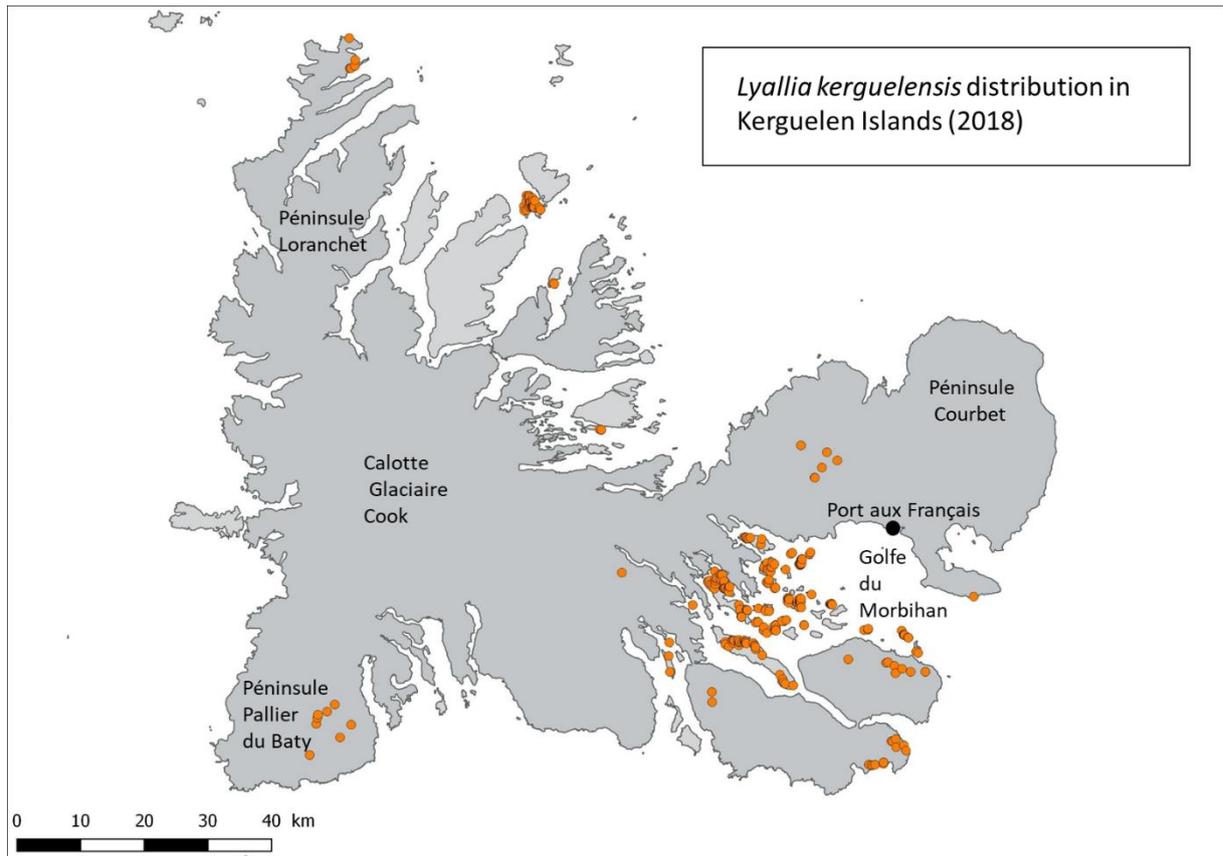


Figure 8: distribution of *L. kerguelensis* in Kerguelen Islands (orange dots) in April 2018; latest data from the Terres australes et antarctiques françaises – Réserve naturelle nationale des Terres australes françaises and Institut Polaire Français, programs 136 and 1116. A dot can show one individual as well as a population. The black dot indicates the Research station; labels indicate broad regions and important features in the islands.

Biology

L. kerguelensis is a long-lived perennial cushion plant species. Observations of several specimens showed that plants could persist at least 16 years (1990-2006, Wagstaff & Hennion 2007) and even up to 20 years (marked individuals 2000-2013, re-identified in 2019, Marchand pers. obs.). The species has a cushion shape, roughly-round 20-40cm diameter (Fig. 9 A-E-F) (Hennion & Walton 1997a, b; Wagstaff & Hennion 2007), exceptionally larger than a meter (Fig. 9 D). It is considered as a herbaceous species with radial compact hemispherical cushion and a single pivotal root and classified in this same category (C1) as *Androsace helvetica* (L.) (Fig. 9 B and Fig. 9 C) (Aubert *et al.* 2014). The main stem is short (a few centimeters) with numerous ramifications (Wagstaff & Hennion 2007). The stems are dichotomously ramified and terminated by an apex formed of a rosette of small, densely imbricated leaves around an apical bud (Hooker 1847; Werth 1911; Hennion 1992). These leaves are scale-like, averaging 5mm in length and usually four times as long as wide (Hooker 1847). Apex necrosis occurred in numerous cushions (see the following section). The root system is strong and deep,

composed of a main thick deep root branching out with numerous fine roots (Werth 1911; Chastain 1958). Furthermore, we often observed aerial and slightly flexible roots that radiate from cushions and penetrate the soil at a distance, likely functioning as guys to anchor the plant in the face of strong winds, similarly to those described in *Azorella macquariensis* (Bergstrom *et al.* 2015) (Fig 9 C). Flowers appear in cushions that have reached a diameter of about 10 cm (Hennion 1992). Roughly the flowering phenology starts in December and the fruits are mature at the end of the summer (Hennion 1992). The fecundation, probably autogamous, leads to the formation of fruits (capsules) with one to two seeds (about one third of the capsules for the latter case in (Hennion & Walton 1997a). No vegetative reproduction was noted (F. Hennion and L.J. Marchand pers. obs.). Germination in the lab was difficult, revealing deep dormancy of the seed (Hennion & Walton 1997b). This low seed production, low viability and the scarcity of germination on the ground outside the cushions suggest a weak sexual reproductive capacity in this species (Hennion 1992; Hennion & Walton 1997a).

L. kerguelensis is octoploid (Siljak-Yakovlev *et al.* 2020) and polyploidy (i.e. the possession of more than two sets of chromosomes) is an important phenomenon in the evolution of plant species and responses to biotic and abiotic factors, with various hypotheses on its role in adaptation having been suggested (reviewed in Ramsey & Ramsey 2014). For example, a greater propensity for plasticity in polyploid species was experimentally shown in some cases. Whole-genome duplication (leading to polyploidy) was observed for many Caryophyllales living in harsh environments and Wang *et al.* (2019) hypothesized that it might be related to harsh environment adaptation. Indeed, specific genes involved in drought and cold tolerance duplicated more than expected within the Montiaceae (Wang *et al.* 2019). *L. kerguelensis'* genome is associated with a considerable size variation (31.4% of genome length variability, Siljak-Yakovlev *et al.* 2020). The population located in the North of Kerguelen differed significantly from the other two populations investigated (Siljak-Yakovlev *et al.* 2020). Finally, the genetic knowledge available for *L. kerguelensis* is limited; genetic diversity on single sequence repeats was extremely low (Robert *et al.* 2021) and lower than *A. macquariensis* endemic from Macquarie Island (sub-Antarctic) (Chau *et al.* 2019). Nevertheless, five genetic groups were observed and were geographically structured (Robert *et al.* 2021).

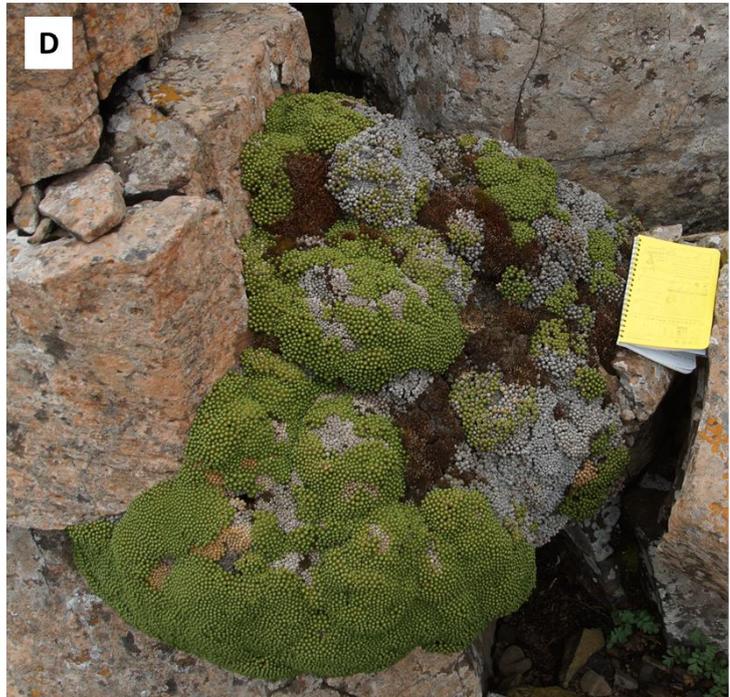
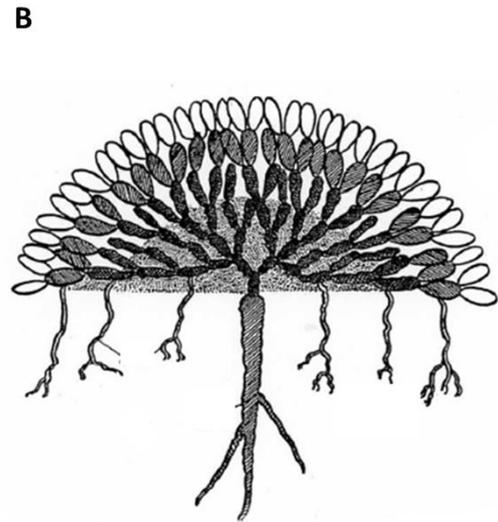


Figure 9: A: *Lyallia kerguelensis* cushion. An epiphytic *Festuca contracta* Kirk plant is present in the cushion; the neighbouring plants on the left of the picture are *Agrostis magellanica* Lam. Necrotic and vigorous apices are shown (respectively red and white arrows). B: schematic morphology of cushion plant type C1: herbaceous species with radial compact hemispherical cushion (Aubert et al. 2014), drawing (Rauh 1939). C: aerial and slightly flexible roots that radiate from cushions and penetrate the soil at a distance, likely functioning as guys to anchor the plant in the face of strong winds. D: extremely large bumpy *L. kerguelensis* cushion on the bottom of a cliff, diameter estimated roughly around 1m, the occurrence of mosses (brown colour) within the cushion entity and necrotic parts are visible. E: necrotic *L. kerguelensis* where necrotic parts are visually less dense than green parts inside the red circle.

Ecology

Lyallia kerguelensis populations are generally small, i.e. composed of twelve or more individual plants in our surveys. In some rare cases there may be up to a hundred individuals. This species is only found in open fellfield habitat from close to the shore until almost 300m (Wagstaff & Hennion 2007). These fellfields are usually wind corridors on a plateau or between cliffs with frequent frosts and constant wind. The plant grows on gravel and between blocks in open vegetation within fellfield communities of small cushions of *A. selago* and short-length plants such as *Festuca contracta* or *Agrostis magellanica* (Wagstaff & Hennion 2007) (Fig. 8 A-D). Only in few cases, poor condition cushions of *L. kerguelensis* were found in closed vegetation (i.e. without bare soil, Wagstaff & Hennion 2007). This species is mainly recorded in and around the "Golfe du Morbihan" (gulf with numerous islands in the south-eastern part of the archipelago). Several populations were recorded in fellfields in the North of the archipelago. Finally, in the southwestern part, "Péninsule Rallier du Baty" several populations of *L. kerguelensis* were recorded in large glacial valleys. The distribution of the species is still under investigation, as populations were observed in most of the investigated fellfields, we could hypothesize that *L. kerguelensis* might be present also in the less investigated parts of the archipelago (North and West). The species' ecological niche was not fully defined before the start of this PhD.

Ecophysiology

The ecophysiology of *L. kerguelensis* has been little studied. However, it has been shown abundant polyamine and aromatic amine levels in its leaves and less in the stem and roots (Hennion & Martin-Tanguy 1999). Polyamines are secondary metabolites and may play essential roles in many developmental processes in plants such as growth and senescence, but also responses to several environmental challenges such as cold or heat (Hennion & Martin-Tanguy 1999). It is possible that *L. kerguelensis*, as a Montiaceae, would have a metabolism that improves photosynthetic efficiency and reduces water loss i.e. crassulean acid metabolism (CAM) as this was previously observed in numerous species in Cactaceae, Didiereaceae and Montiaceae genera in harsh environments (Wang et al. 2019).

Within Montiaceae, C₃, C₄, CAM and facultative CAM, photosynthetic pathways are observed (Herskovitz 2019; Wang et al. 2019). This different photosynthetic pathway has evolved independently in these diverse lineages (Goolsby et al. 2018; Wang et al. 2019). Briefly, CO₂ enters through stomata and reaches the chloroplast, where it reacts with the Rubisco enzyme for C₃ plants while CO₂ is fixed by PEP carboxylase enzyme in the mesophyll for C₄ and CAM. Afterward, CO₂ reaches the chloroplast to be catalyzed by the Rubisco (Simpson 2006; Goolsby et al. 2018). In CAM species, stomata are open at night, which allows lowering of water losses, CO₂ is then stored in the mesophyll until the next day. Both C₄ and CAM are adapted to xeric conditions as the PEP carboxylase has a higher affinity with CO₂ than the Rubisco (Simpson 2006). Many Montiaceae are defined as CAM facultative (also called C₃-CAM), which are C₃ species with varying levels of low-level or inducible CAM activity. The photosynthetic pathways remain to be investigated in *L. kerguelensis*.

Necrosis (i.e., visible damage, possible symptoms of stress and dieback process) has been observed in many *L. kerguelensis* populations since the 1990s (Hennion 1992). It might be a seemingly recent phenomenon as the early botanical descriptions did not mention any necrosis (Hooker 1847; Werth 1911; Chastain 1958). The origin might be related to the recent climate change, as necrotic cushions were mainly observed in the drier part of the archipelago (Hennion 1992). Furthermore, a recent study targeting leaf bacteria could not evidence any pathogens in the samples (Portier 2019), strengthening the possible abiotic origin. Several physiological mechanisms may be at work to end up with necrosis. Firstly, *L. kerguelensis* necrosis might not occur only on large cushions that are expected to be older and possibly at the end of their developmental stages. Second, embolism of plant vessels may occur, indeed enhanced evapotranspiration and reduced soil water content can lead to a higher risk of this damage which can result in the death of the shoot if the water flow cannot be restored (Rolland et al. 2015). The ongoing climate change is known to stress plants, and the mechanisms leading to plant dieback are extensively studied for other long-lived plants such as trees (McDowell et al. 2008, 2020; Allen et al. 2010; Voltas et al. 2013; Earles et al. 2018). Cavitation was an hypothesis to explain cushion plant dieback (Bergstrom et al. 2015; Rolland et al. 2015). By extension, these mechanisms may concern *L. kerguelensis*. Reduction of precipitation and increase of temperature, even if non-linear in the Kerguelen Islands, might stress *L. kerguelensis* leading to short-term and long-term impacts on plant functioning. Thirdly, plant exhaustion might also occur more rapidly with reduced metabolism in summer due to drought and increased nutrient need in winter due to increased temperatures, resulting in the impossibility of regenerating new foliage (Charrier et al. 2021).

Aims and hypotheses of the PhD

The PhD work takes place in the broad framework of assessing *in-natura* plant vulnerability and their adaptive capacity to climate change. Little research has concerned long-lived species, and even fewer studies have been done in sub-Antarctic Islands despite this region facing some of the most rapid climate changes on Earth. The Kerguelen Islands' fellfields harbour a long-lived endemic cushion plant, *Lyallia kerguelensis*, which might have a low adaptive capacity as necrosis damage, possibly due to climatic stress, was recorded for a few decades. In this context, we raise the question: **what is the adaptive capacity to climate change of the Kerguelen long-lived endemic cushion plant species, *Lyallia kerguelensis*?**

L. kerguelensis, by its long lifespan, cushion form, and endemism to isolated islands characterized by harsh environments subject to rapid climate change, appears as a good model. Its pool of variability (morphology, transcriptome, necrosis extent and soil rhizomicrobiome) across contrasted environments might provide insights into *L. kerguelensis* adaptation to harsh environments and its possible responses to changes. Plant morphology is one of the traits that respond the most to environments. Therefore, the pool of morphological variability of *L. kerguelensis* and its relation to specific environmental variables might be essential to identify the species phenotypic diversity and within it the possibility of a phenotype to be appropriate to the new condition (Chapter 1). At a thinner scale, it will be essential to find which genes or pathways are differentially expressed in *L. kerguelensis* in contrasted environments to decipher or reveal population biological and cellular responses possibly linked to local adaptation (chapter 2). When considering the extended phenotype, *L. kerguelensis* rhizomicrobiome assembly filtered from the surrounding microbiome might inform us on the selected generalist and specific microorganisms, apt to sustain plant vigour in a contrasted environment (chapter 3). Integrating temporality is important, for long-lived species such as *L. kerguelensis*, monitoring morphological trait dynamics within a number of years in contrasted environments might be essential to estimate their responses and the probability to cope with fast climate change (chapter 4). Finally, plant growth rate and age in various environments might influence adaptation outcomes as they underlie plant persistence and resilience to climate change (chapter 5). These findings will broaden our knowledge of the adaptive capacity of long-lived plants in isolated harsh environments facing rapid changes. It will allow to better assess their vulnerability to climate change, and it will be valuable for evolutionary and conservation research.

Organisation of the PhD thesis

Part-I addresses morphological variability and gene expression in *Lyallia kerguelensis* in contrasting environments.

- **Chapter 1** establishes a new methodology to measure morphological traits in this cushion plant and relating them to environmental variables. We expected to see allometry of traits for the cushions, and that environmental variables (such as wind intensity, topography or soil water content) can explain that trait variability across space (especially necrosis).
- **Chapter 2** addresses the transcriptome of *L. kerguelensis* in contrasted environments to determine whether or not transcriptome profiling across populations would reveal information on plant abiotic and biotic stress responses. We hypothesized that different transcriptome profiles might occur as populations are in contrasted environments and differences might be explained by the plastic or adaptive responses of *L. kerguelensis* populations.

Within **part II** we considered the soil microbial diversity and composition and its relation with rhizomicrobiome diversity and composition and plant vigour in contrasted environments.

- **Chapter 3** examined the bacterial and fungal variability and diversity of fellfield soil microbiomes and *L. kerguelensis* rhizomicrobiome; the former in relation to environments and the latter in relation to plant vigour. *L. kerguelensis* rhizosphere may act as a reservoir of microbiota for fellfield soil, and its necrosis might be correlated to specific taxa or functions its rhizomicrobiota sustain.

In **part III** we assess the temporality of *L. kerguelensis* cushions' morphological change and their possible longevity in relation with abiotic and climatic factors in contrasted environments.

- **Chapter 4** focuses on the dynamics of growth and necrosis extent variability across time in relation to cushion morphology and edaphic and climatic variables. We hypothesized that growth was related to environmental variables. In contrast, the necrosis extent increase might be related to environmental variables and climate changes.
- In **Chapter 5**, we studied the growth rate and tried to find an estimator of the age of *L. kerguelensis* cushions. We hypothesized that growth rate, plant architecture, and anatomy could help better understand the life span of this long-lived plant and estimate its age.

The **general discussion** synthesizes the results and the complementarity of the approaches to assess the adaptive capacity of *L. kerguelensis*. Results are discussed under the scenario of rapid climate change and opens on research perspectives.

Annex 1 summarizes the results of a germination experiment with cold and freeze treatments on *L. kerguelensis* seeds. **Annex 2** indicates the hydric potential measurements *in-natura* on several cushions of *L. kerguelensis* during summer days. In **Annex 3** can be found the scientific production and complementary research activity.

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PART I: Morphological variability and gene expression in *Lyallia kerguelensis* in contrasting environments

Chapter 1: Morphological variability of *Lyallia kerguelensis* in relation to environmental conditions and geography in the Kerguelen Islands: implications for cushion necrosis and climate change

Chapter 2: Differential gene expression of *L. kerguelensis* in contrasted environments: implications for biotic and abiotic stresses



Chapter 1: Morphological variability of *Lyallia kerguelensis* in relation to environmental conditions and geography in the Kerguelen Islands: implications for cushion necrosis and climate change

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Abstract

In recent decades climate change has been faster in various parts of the world. Within species, to counter rapid climate changes shift of geographical area, individuals' plastic responses or populations' genetic adaptation might occur. The sub-Antarctic islands are subject to one of the most rapid climate changes on earth, with already visible impacts on native vegetation. Such might be the case of *Lyallia kerguelensis* a cushion plant strictly endemic to the Kerguelen Islands. In *L. kerguelensis*, necrotic parts were observed in cushions these last decades and possibly related to water stress. We analysed morphological variability of *L. kerguelensis*, including necrosis extent, across 19 populations spanning a wide range of environments across the Kerguelen Islands. Inter-population variations in the cushion surface area, shape and compactness were well explained by topography, degree of wind exposure, slope aspect, proportions of coarse sand and bare soil, and geographical distance between populations. All these variables are related to wind intensity and water availability. Moreover, in cushions with less than 10% necrosis in surface area, necrosis extent was positively correlated to soil sodium. Sodium availability might reduce the plant's capacity for osmotic adjustment in face of other abiotic stresses, such as water stress. We conclude that cushion morphology may have the capacity to adjust to environmental variation, including aspects of climate change, but that cushion necrosis may be accelerated in the driest and most saline environments.

Keywords: cushion plant, Kerguelen Islands, morphological variability, necrosis, photointerpretation, sub-Antarctic

Introduction

In recent decades climate change has been faster in various parts of the world (IPCC 2013). Islands have been particularly concerned which threatened a large part of the insular floras (Harter et al. 2015; Veron et al. 2019). Indeed, within species, to counter rapid climate changes, shift of geographical area, individuals' plastic responses or populations' genetic adaptation might occur (Hoffmann and Sgrò 2011; Noble et al. 2019). However geographical shift of area will be limited for native insular plant species (Chen et al. 2011). Insular plants will thus have to respond and keep up with the speed of climate change, which will be particularly challenging if they are living close to their physiological limits (Moritz and Agudo 2013). This is eminently interesting in the sub-Antarctic islands where climate change is extremely fast (Smith 2002; Convey 2007; Lebouvier et al. 2011) and plants are living close to such physiological limits (Hennion et al. 2006b). In the near future, global climate change is predicted to have particularly strong effects at higher latitudes in the (sub-)polar regions (IPCC 2013, 2014). For instance, according to the Representative Concentration Pathway scenario (RCP) 6.0, an increase in minimum temperatures of about +1 to +1.5°C is expected before the end of the current century, along with up to 10.0% change in precipitation, which may cause considerable stress for plants restricted to cold habitats (Harter et al. 2015). Temperature seasonality will also be affected, inducing an earlier and longer growth period, lower frequency of freeze-thaw cycles, and higher frequency of droughts (Boelhouwers et al. 2003; Lebouvier et al. 2011; IPCC 2013; Harter et al. 2015). Such is the case of the Kerguelen Islands, where the climate is chronically cool and windy year-round, as is typical for the sub-Antarctic islands generally (Convey 1996), with a mean annual temperature of 4.84°C for the period 1976-2008 (Lebouvier et al. 2011). Overall since 1950 mean annual temperature increased (+0.016 °C.year⁻¹). Rainfall tends to decrease across time with two long periods of rainfall below the average (1962-1976 and 1991-2011) and two short periods of rainfall around the average (1977-1991 and 2011 until now) (Météo-France, compilation IPEV 136 - UMR 6553). These climatic changes have led to the retreat of the Cook ice cap which is among one of the most drastic glaciers retreats on Earth (Verfaillie et al. 2015; Favier et al. 2016).

The sub-Antarctic Islands form a biogeographical unit, even if there are variations in their origins and locations (Shaw et al. 2010). Kerguelen Islands with three other archipelagos (Prince Edward Islands, Crozet archipelago and Heard and Mc Donald Islands) form the South Indian Ocean Province with unique biodiversity and a high degree of endemism (Hennion and Walton 1997; Van der Putten et al. 2010). Impacts of climate change on vegetation have rapidly become apparent in the sub-Antarctic Islands with for instance occasional wilting of native plants, or dieback in several cushion plants (Frenot et al. 2001; Hennion et al. 2006a; Le Roux and McGeoch 2008; Bergstrom et al. 2015). Here we focus on the cushion plant *Lyallia kerguelensis* Hook.f. (Montiaceae), strictly endemic to Kerguelen, a

monotypic genus and phylogenetically isolated in its family (Wagstaff and Hennion 2007b; Nyffeler and Egli 2010). Partial necrosis of *L. kerguelensis* cushions was first described by Hennion (1992), being absent from all previous descriptions of the species (Hooker 1847; Werth 1911; Chastain 1958), and thereby suggesting it might be a recent phenomenon in this species (Fig. 1). Furthermore, as partly necrotic cushions were mostly observed in the drier parts of the island it was suggested that this necrosis may be induced by dry conditions (Hennion 1992; Wagstaff and Hennion 2007b). Therefore, necrosis in *L. kerguelensis* cushions appeared variable and possibly related to local climatic conditions.

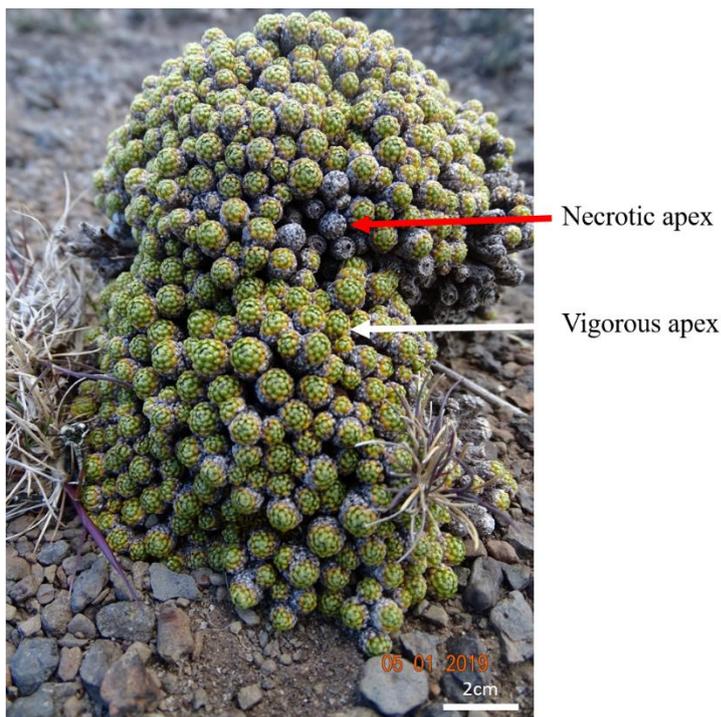


Figure 1: *Lyallia kerguelensis* cushion. An epiphytic *Festuca contracta* T. Kirk plant is present in the cushion, the neighbouring plants on the left of the picture are *Agrostis magellanica* Lam.. Necrotic and vigorous apices are shown (respectively red and white arrows).

Cushion plants are often typical of areas that experience cold or cool climates, low precipitation and/or strong winds in various regions of the Earth (Körner 2003, 2016; Boucher et al. 2016). Such growth forms have a low surface area to volume ratio, which aids in reduction of heat and water losses (Körner 2003; Aubert et al. 2014). Typically, in a plant's morphology is among the functional traits that vary in response to environmental, including climatic conditions. A few examples show that morphology of cushion plants may respond to environment. Indeed cushion size varied in relation to altitudinal gradient, a proxy of temperature and precipitation in alpine environments (Kleier et al. 2015; Zhao et al. 2018). *Azorella compacta* (Apiaceae) plants present a larger surface area on the cushion part with northern exposure side which maximizes solar radiation (Kleier et al. 2015). Moreover, air circulation and windblown sediment around cushions may explain the initial development of the crescent shape

in *Azorella selago* (Combrinck 2008; Combrinck et al. 2020). Hence morphological variation in size and shape can be considered as a proxy of the response capacity of cushion plants to environmental, including climate variation (Körner 2016). In contrast, when changes are fast low response capacity is expected. Direct drought sensitivity was demonstrated experimentally in Marion Island for the cushion plant *A. selago* (Le Roux et al. 2005), with earlier senescence (i.e. stem death) apparent in cushions with lower water availability. Dieback, or large scale necrosis, is defined as a process during which portions of the cushion, or even the entire cushion, can no longer regenerate new foliage (Armesto et al. 1980). Studies of the spatial dynamics of dieback in the congeneric plant *A. macquariensis* from Macquarie Island highlight a relation of the dieback to a potential decrease in soil water availability under recent climate change rather than a pathogenic origin. Nevertheless, stressed plants might be more sensitive to infections (Whinam et al. 2014; Bergstrom et al. 2015; Dickson et al. 2019). Finally, necrosis can be related to wind intensity, with more necrosis on the more exposed part of the cushion (Kleier and Rundel 2004). These observations suggest that necrosis may be due to or enhanced by climate change.

Measuring the variability of necrosis extent and morphology in *L. kerguelensis* more generally could provide a proxy for the response capacity of the plant to climate change. *L. kerguelensis* could be considered as a model case to study response capacity of an endemic plant species to climatic changes. In the current study, we assumed that the morphological traits of *L. kerguelensis* co-vary so as to maintain a hemispherical shape that is optimal in harsh environments. We then predicted that morphological variation, including necrosis extent would be present across individuals and populations. We hypothesized that water availability, wind exposure and slope aspect were likely primary drivers of necrosis. To test these hypotheses, we (i) searched relations between growth traits and the entire morphology (further called allometry) in *L. kerguelensis* to select appropriate traits for assessing inter-population variability, and (ii) sought to identify relationships between this inter-population variability and environmental conditions on one hand and geographical distances on the other hand. Finally, we (iii) studied the influence of necrosis on the cushion morphology and attempted to identify which environmental conditions are related to necrosis.

Materials and methods

Lyallia kerguelensis Hook.f. (Nyffeler and Eggli 2010), Montiaceae, is a long-lived perennial herb, persisting at least 16 years (Wagstaff and Hennion 2007b). It forms roughly round-shaped cushions (Fig. 1) 20 – 40 cm across, exceptionally up to 1 m, in small and sparse populations (Hennion and Walton 1997; Wagstaff and Hennion 2007b). The root system is strong and deep, composed of a main thick deep root branching out with numerous fine roots (Werth 1911; Chastain 1958). Furthermore, we often observed aerial and slightly flexible roots that radiate from cushions and penetrate the soil at a distance, likely functioning as guys to anchor the plant in the face of strong winds. *L. kerguelensis* distribution in Kerguelen is sparse, with an altitude range from sea level to around 300 m a.s.l. (Hennion and Walton 1997; Wagstaff and Hennion 2007b); Réserve Naturelle TAF, IPEV Subanteco and PlantEvol, unpublished) (Fig. 1). The species is restricted to windswept stony habitats in fellfields or moraines (Wagstaff and Hennion 2007b). Fellfields (or “wind deserts”) are extensive, sterile, rocky areas present from the shore to the highest elevations, and more generally characteristic of polar regions (Aubert de la Rüe 1964). Vegetation is patchy, mainly composed of lichens and bryophytes (Smith 1984; Block et al. 2009).

Our study was based on four field campaigns in the Kerguelen Islands (48°30' - 50°S, 68°27' - 70°35'E ; Lebouvier and Frenot 2007). The first three campaigns were from December to January (2015-2016, 2016-2017 and 2017-2018), the last campaign was from December 2018 to March 2019. In the current study, we analysed 319 plants from 19 populations (Fig. 2, Online Resource Table 1) in three different regions of Kerguelen: the northern part with Port Christmas (two populations) and Ile MacMurdo (two populations), the south-western part with one population at Péninsule Rallier du Baty, and the south-eastern part including populations in Vallée des Souilles in Presqu'île Ronarch (three), Ile Australia north (three) and south (four), Ile Longue (two) and Plateau du Vent in Presqu'île Jeanne d'Arc (two).

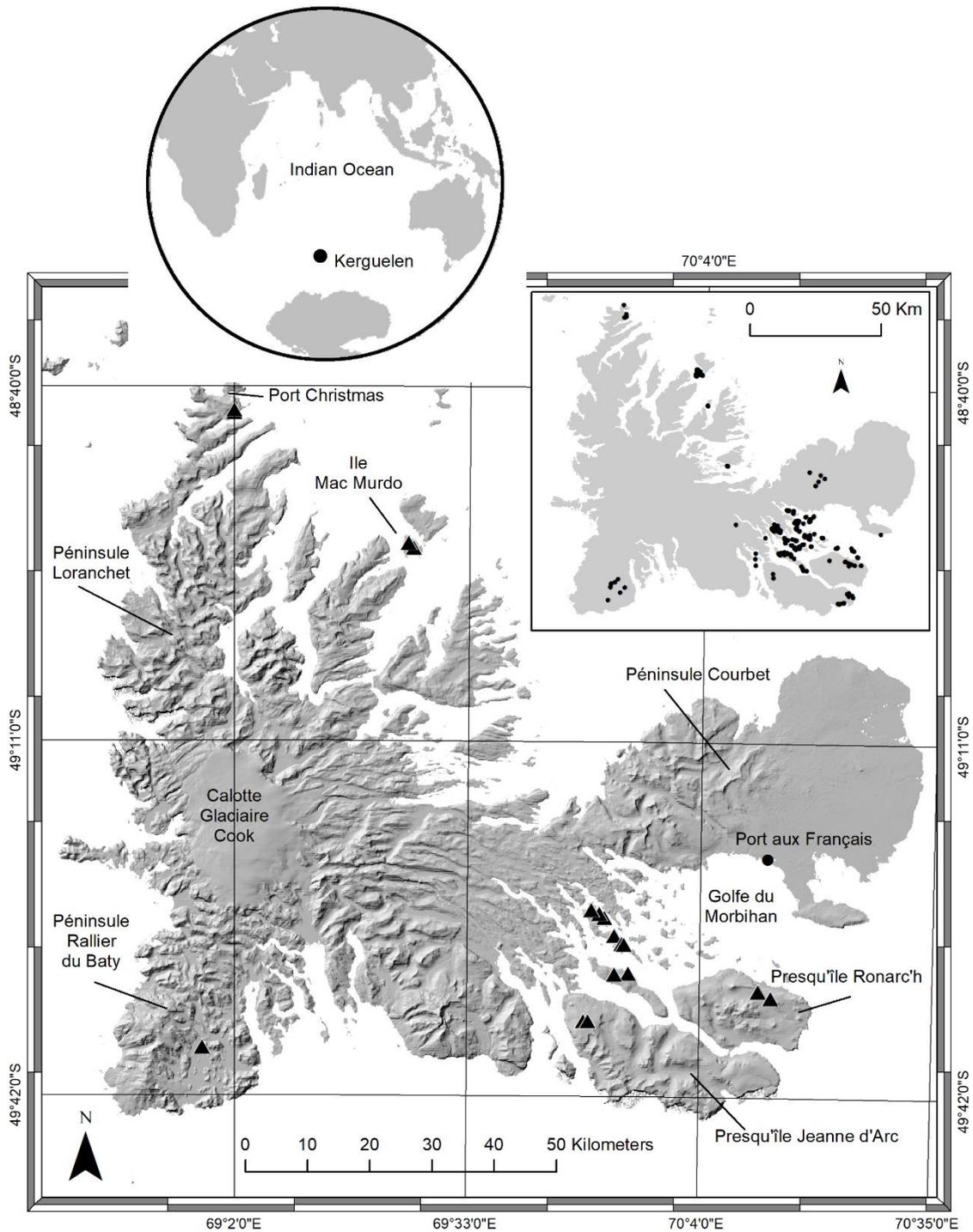


Figure 2: Locations of the 19th studied populations of *Lyallia kerguelensis* (black triangles) in Kerguelen Islands on an altitudinal background (NASA – SRTM 30M, 2005); the black dot indicates the research station, labels indicate important features in the island. Top right map: distribution of *L. kerguelensis* on Kerguelen Islands (black dot) in April 2018; data from the Réserve Naturelle Terres Australes Françaises and Institut Polaire Français, programs 136 and 1116) map background 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

For each population, general environmental characteristics were recorded regarding types of fellfield (slope, scree slope, plateau, summit, outwash plain), topography (flat, terrace, base or mid or top of slope, summit, other); wind exposure (very sheltered, sheltered, exposed, very exposed); slope; distance to sea; slope aspect; visual estimation of cover in percentage of blocks (>20cm), pebbles (2cm to 20 cm), gravel (2mm to 2 cm), bare soil (<2mm), bryophytes and herbs. Soil conductivity, water saturation and temperature were measured with a TRIME (Time Domain Reflectometry with intelligent Micro Elements) probe. Altitude was determined using GPS (eTrex 10, Garmin). For each sampled population, three samples of bare soil, 200 mL in total, were collected at different locations around cushions to provide a composite soil sample. Half of the soil sample was immediately stored at -20°C for subsequent determination of nitrate concentration (Laboratoire d'Analyse des Sols, INRA, Arras, France). A small portion of the remaining fresh soil was mixed with 30 mL of distilled water and then left for 18-24 h to permit the sedimentation of soil particles, and pH was then determined using a pH meter (BASIC 20 PLUS CRISON, resolution 0.01 pH) (Hermant et al. 2013). The remaining soil was dried at 105°C for 48 h, being weighed before and after drying. The dried soil was used to determine soil texture and composition (Laboratoire d'Analyse des Sols, INRA, Arras, France) (Online Resource Table 2). The studied cushions were selected following categories identified in an earlier study (Dorey et al. 2017, unpublished). Dorey et al. (2017) applied a random sampling of 83 *L. kerguelensis* cushions in 11 populations selected for their contrasted environments. With exploratory analyses they observed that the percentage of explanation of environmental variables was reduced with the increase of necrosis extent. They found that cushions above a threshold of 10% necrotic surface area have lower relationship with environmental variables than below this threshold (Online Resource Fig.1). In our study, we sampled cushions below and above this threshold to better characterize the relationship between the necrosis extent and specific environmental variables for each group. Within each population, we selected the same number of cushions from each category, defining these as 'vigorous cushion' (visually less than 10% of necrotic extent), and 'necrotic cushion' (visually 10% to 80% of necrotic extent). The maximum proportion of necrosis retained in the study was arbitrarily set at 80%, above which we considered the cushion as too affected to be reactive.

For each cushion, pictures were taken vertically from above the cushion and with a scale, without shade on the cushion, allowing to see the top of each apex. An extraction protocol to measure morphological traits by photointerpretation (Dorey et al. 2017, unpublished) was then applied. The cushion height was measured in the field for 229 cushions (data not available for AUS23, AUSN1, AUSN2 and AUSN4 populations). Image analyses were performed with Arcgis 10.6.1. We determined the perimeter of the cushion (Online Resource fig. 2a), the total surface area of the cushion (Online Resources fig. 2b), the percentage of neighbouring plants (Online Resource fig. 2c), the necrotic extent

(Online Resources fig. 2e); the shorter, longer radius and the cushion shape (Online Resources fig. 2f). Finally, we calculated a cushion compactness proxy (Cranston et al. 2015) using the following formula:

$$Compactness = \frac{apex\ number}{surface\ area}$$

We calculated also two ratios, height and short radius and height and long radius of the cushion to provide a proxy of the 3D shape of the cushion. A total of 11 morphological and biotic traits were recorded and are listed in Table 1.

Table 1: Morphological traits of *L. kerguelensis* and biotic traits per population (mean \pm standard deviation): number of individuals, cushion surface area, cushion perimeter, cushion compactness, cushion shape, cushion height, ratio height/short radius, ratio height/long radius, ratio height/long radius, proportion of neighbouring plants in contact with the perimeter, proportion of epiphytes across the cushion surface area, necrosis extent for vigorous cushions (<10% necrosis extent) and necrosis extent for necrotic cushions (>10% necrosis extent). NA= data not available. For necrosis extent of vigorous and necrotic cushions mean and standard deviations were calculated per category per population.

Population	Cushion											
	Number of individuals	surface area (cm ²)	Cushion perimeter (cm)	Cushion compactness	Cushion shape	Height (cm)	Height / short radius	Height/ long radius	Proportion of neighbouring plants	Proportion of epiphytes	Necrotic extent (%) for vigorous cushion	Necrotic extent (%) for necrotic cushion
AUS23	12	215.80 \pm 46.83	88.94 \pm 14.64	4.58 \pm 0.48	0.65 \pm 0.03	NA	NA	NA	31.57 \pm 7.19	0.0008 \pm 0.0007	0.92 \pm 0.11	32.40 \pm 6.05
AUS25	20	122.75 \pm 21.63	56.39 \pm 5.20	7.53 \pm 0.77	0.71 \pm 0.05	5.95 \pm 0.55	0.89 \pm 0.09	1.24 \pm 0.10	63.99 \pm 6.63	0.0002 \pm <0.0001	2.91 \pm 0.99	32.30 \pm 8.22
AUS30	20	162.33 \pm 44.01	50.38 \pm 7.00	5.85 \pm 0.28	0.76 \pm 0.04	7.52 \pm 1.04	0.65 \pm 0.52	0.86 \pm 0.05	32.13 \pm 6.53	0.0002 \pm 0.0001	0.70 \pm 0.33	21.24 \pm 3.13
AUS32	19	97.26 \pm 13.21	42.98 \pm 3.41	5.49 \pm 0.38	0.70 \pm 0.05	7.00 \pm 0.74	0.65 \pm 0.41	0.92 \pm 0.07	34.72 \pm 5.88	<0.0001 \pm <0.0001	3.96 \pm 0.86	22.16 \pm 3.91
AUSN1	10	250.69 \pm 62.95	84.82 \pm 11.25	6.69 \pm 0.82	0.77 \pm 0.04	NA	NA	NA	41.30 \pm 5.60	0.0009 \pm 0.0003	2.37 \pm 1.69	25.50 \pm 7.46
AUSN2	10	301.46 \pm 50.83	95.83 \pm 13.31	7.74 \pm 0.27	0.84 \pm 0.02	NA	NA	NA	44.67 \pm 8.89	0.0020 \pm 0.0008	4.89 \pm 1.24	15.48 \pm 3.34
AUSN4	8	168.21 \pm 69.98	57.58 \pm 14.82	6.37 \pm 0.84	0.69 \pm 0.08	NA	NA	NA	14.90 \pm 5.05	0.0005 \pm 0.0004	3.76 \pm 1.44	20.00 \pm 2.83
LONH1	12	29.78 \pm 6.65	24.49 \pm 2.62	6.89 \pm 0.46	0.70 \pm 0.05	4.21 \pm 0.66	0.63 \pm 0.08	0.93 \pm 0.11	19.46 \pm 7.12	<0.0001 \pm <0.0001	1.90 \pm 1.45	26.29 \pm 5.81
LON30	20	62.26 \pm 9.01	36.36 \pm 3.54	5.48 \pm 0.32	0.80 \pm 0.04	6.35 \pm 0.44	0.54 \pm 0.03	0.71 \pm 0.04	24.09 \pm 6.10	0.0001 \pm <0.0001	2.78 \pm 0.94	25.52 \pm 2.74
MAC1	19	126.66 \pm 24.90	51.91 \pm 5.82	3.76 \pm 0.18	0.68 \pm 0.06	6.20 \pm 0.52	0.71 \pm 0.07	1.08 \pm 0.09	20.09 \pm 5.09	<0.0001 \pm <0.0001	3.43 \pm 0.89	21.70 \pm 3.56
MAC3	20	99.15 \pm 14.91	44.06 \pm 3.51	4.25 \pm 0.20	0.81 \pm 0.04	5.93 \pm 0.56	0.81 \pm 0.06	1.02 \pm 0.07	11.23 \pm 3.37	<0.0001 \pm <0.0001	2.67 \pm 0.26	25.19 \pm 1.14
PI21	20	39.55 \pm 5.48	30.50 \pm 2.43	6.48 \pm 0.27	0.75 \pm 0.05	2.41 \pm 0.25	1.24 \pm 0.14	1.75 \pm 0.19	0.46 \pm 0.45	<0.0001 \pm <0.0001	3.32 \pm 0.27	23.13 \pm 0.72
PI22	10	122.30 \pm 31.35	69.51 \pm 7.43	5.50 \pm 0.23	0.54 \pm 0.06	4.40 \pm 0.56	1.14 \pm 0.21	2.16 \pm 0.31	40.13 \pm 8.38	0.0010 \pm 0.0004	4.06 \pm 1.17	21.29 \pm 6.11
PI23	20	100.61 \pm 20.06	47.30 \pm 5.47	6.55 \pm 0.42	0.73 \pm 0.03	7.90 \pm 0.92	0.59 \pm 0.07	0.81 \pm 0.09	18.60 \pm 6.31	<0.0001 \pm <0.0001	4.49 \pm 0.36	37.10 \pm 4.77
PCR1	20	68.99 \pm 11.91	172.84 \pm 136.32	4.60 \pm 0.26	0.81 \pm 0.03	4.35 \pm 0.81	0.85 \pm 0.09	1.11 \pm 0.11	28.00 \pm 5.43	<0.0001 \pm <0.0001	1.60 \pm 0.23	29.25 \pm 1.69
PCR2	18	146.18 \pm 55.67	51.17 \pm 9.00	4.28 \pm 0.35	0.74 \pm 0.05	7.23 \pm 0.58	0.83 \pm 0.06	1.11 \pm 0.08	15.90 \pm 3.62	0.0001 \pm <0.0001	2.16 \pm 0.91	37.36 \pm 6.58
PJDA2	20	162.06 \pm 68.53	40.88 \pm 11.57	5.45 \pm 0.33	0.75 \pm 0.04	4.05 \pm 0.22	1.49 \pm 0.13	1.96 \pm 0.17	12.98 \pm 4.97	0.0001 \pm <0.0001	0.70 \pm 0.54	51.39 \pm 6.75
PJDA6	20	261.61 \pm 65.94	80.82 \pm 13.68	5.40 \pm 0.17	0.77 \pm 0.03	5.68 \pm 0.64	0.76 \pm 0.07	0.95 \pm 0.07	24.00 \pm 4.88	<0.0001 \pm <0.0001	2.01 \pm 0.79	28.59 \pm 3.09
RBA5	20	162.15 \pm 26.47	74.74 \pm 7.93	6.51 \pm 0.23	0.76 \pm 0.02	4.89 \pm 0.13	0.88 \pm 0.08	1.14 \pm 0.10	27.26 \pm 4.99	0.0000 \pm 0.0000	5.18 \pm 1.33	32.70 \pm 5.22

All statistical analyses were carried out in the open-source programming language R, version 3.4.2. Graphs were produced with the *ggplot2* package (Wilkinson 2005). Random Distribution of morphological traits were verified within both necrotic categories and were then pooled except for necrotic extent traits. Spearman correlogram (*PerformanceAnalytics* package) was used to determine the allometry of the traits of the 319 individuals, $n=279$ for variables using height. Based on these allometric relationships, only the surface area, compactness and shape traits were used in subsequent analyses. To determine the significance of inter-population variation in morphological traits, a Kruskal-Wallis test was applied. To study the relationships between cushion morphology and environment, the environmental conditions were divided into six categories (topography and habitat, slope aspect, surface texture, soil texture, soil chemical composition and soil physical characteristics) as shown in Table 2. In each category, spearman correlogram was used to determine the covarying variables. A multivariate model was established between each morphological trait and the environmental categories. Normality and heteroscedasticity were checked respectively with the Shapiro test (Royston 1982) and the *ncvTest* (package *car*, Fox and Weisberg 2019) and, if necessary, a *box-cox* transformation was applied (package *MASS*, Venables and Ripley 2002). Then an AIC backward (package *MASS*) analysis was applied to obtain a minimum adequate model. For the multivariate cushion surface area and shape model with topography and habitat, a *post hoc* Emmeans test was applied with the Tukey method (*emmeans* package). Finally, to study geographical distance between populations a Mantel test (*Ade4* package) was performed; Euclidian distance for the trait, Manhattan distance for the geographical coordinates, and 999 permutations for robustness of the p-value were chosen.

Table 2: Environmental characteristics used in the multivariate models of the 19 populations of *L. kerguelensis* studied. Topography and type of habitat: altitude, type of fellfield (slope, scree slope, plateau, summit, outwash plain), topography (flat, terrace, base or mid or top of slope, summit, other), wind exposure (very sheltered, sheltered, exposed, very exposed), range of slopes (0°,]0-3°],]3-10°],]10-30°],]30-80°]); slope aspect (N, NW, W, SW, S, SE, E, without dominate slope aspect); visual estimation of surface texture: pebbles (2cm to 20 cm), bare soil (<2mm) and herbaceous cover in percentage; soil texture: clay and coarse sand percentage; soil chemical composition: Na, Mn, Al, P and N cmol.kg⁻¹, ratio C.N⁻¹; soil physical characteristics: pH, organic matter and soil humidity.

category	Topography and type of habitat				Slope aspect	Surface texture		Soil texture					Soil chemical composition					Soil physical characteristic		
	Altitude (m)	Type of fellfield	Topography	Wind exposure		Slope	Pebble (2 to 20cm) cover (%)	Soil (< 2mm) cover (%)	Herbaceous cover (%)	% clay	% fine sand	Na cmol kg ⁻¹	Mn cmol kg ⁻¹	Al cmol kg ⁻¹	P cmol kg ⁻¹	N cmol kg ⁻¹	C N ⁻¹	pH	Organic matter g kg ⁻¹	Water saturation (%)
AUS23	137	slope	mid slope	exposed	N	10	0	30	4.9	22.6	1.66	0.039	0.546	0.011	1.85	12.9	6.12	41.4	24.27	
AUS25	125	scree slope	mid slope	very exposed	NW	20	20	30	5.3	13.9	0.59	0.015	0.542	0.009	1.85	12.3	6.12	39.2	17.67	
AUS30	15	plateau	Slope base	very exposed	NW	30	10	20	6.5	20.1	1.06	0.015	0.071	0.017	3.25	11.0	6.70	62.1	5.77	
AUS32	60	plateau	flat	very exposed	E	30	10	30	7.9	19.8	0.70	0.015	0.882	0.019	2.92	11.5	5.25	57.9	52.98	
AUSN1	14	slope	mid slope	intermediate	SW	30	10	20	8.5	17.7	0.64	0.021	1.710	0.027	3.66	11.4	5.52	72.1	34.99	
AUSN2	32	slope	mid slope	intermediate	NE	30	10	20	7.3	27.3	0.63	0.020	0.809	0.014	2.66	12.9	6.34	59.3	23.67	
AUSN4	22	plateau	summit	exposed	S	30	10	10	3.6	26.0	0.99	0.014	0.903	0.005	0.67	12.5	6.80	14.4	32.00	
LON11	84	plateau	flat	very exposed	Without	15	5	5	5.2	13.6	1.29	0.010	0.160	0.036	0.43	11.7	6.36	8.7	60.79	
LON30	154	plateau	flat	very exposed	Without	15	5	5	5.0	15.3	0.35	0.013	0.202	0.024	1.42	11.0	5.62	27.0	21.71	
MAC1	55	plateau	summit	very exposed	Without	10	5	5	6.0	15.9	0.33	0.010	1.290	0.025	2.04	11.6	5.77	40.8	33.00	
MAC3	153	summit	top of slope	very exposed	S	25	2	15	5.4	17.8	0.23	0.030	1.520	0.021	1.97	11.7	5.75	39.9	19.67	
P121	320	slope	top of slope	very exposed	S	70	2	10	4.2	8.1	0.30	0.019	0.764	0.025	1.12	11.9	6.04	23.1	14.53	
P122	250	slope	Slope base	very exposed	SE	70	0	30	4.9	10.8	0.35	0.019	0.755	0.038	0.80	10.7	6.21	14.8	14.00	
P123	340	scree slope	top of slope	very exposed	SW	10	5	10	5.5	19.7	0.50	0.054	0.481	0.015	2.27	12.9	6.54	50.4	55.32	
PCR1	238	plateau	flat	exposed	Without	40	5	20	5.4	15.4	0.91	0.017	0.080	0.009	2.37	13.0	6.80	53.5	25.86	
PCR2	216	plateau	flat	exposed	without	30	5	20	4.7	9.7	0.80	0.013	0.064	0.010	1.81	13.2	6.90	41.2	26.65	
PJDA10	520	plateau	terrace	very exposed	NW	40	5	40	4.2	25.5	0.88	0.005	0.159	0.013	2.02	12.2	6.40	42.6	32.02	
PJDA6	482	plateau	mid slope	very exposed	W	30	10	15	0.4	17.2	0.21	0.016	0.145	0.003	0.25	10.7	6.55	7.34	17.53	
RBA5	71	outwash plain	flat	exposed	SW	35	0	20	4.1	8.2	0.55	0.031	0.503	0.025	1.35	11.4	6.58	26.7	18.89	

Results

Allometry of traits and morphological variability

The surface area, perimeter, and height of the cushions were strongly positively correlated one to each other (Table 3). However, the cushion compactness was correlated only with the cushion height and the cushion shape was correlated to none of the other morphological traits. Mean cushion shape ratio (relation between short and long diameter) was 0.75 ($n = 319$, $sd = 0.01$) with low variation indicating that cushions approximate an ovoid cushion shape. Moreover, the ratios between height and short or long radius were also close to 1 ($n = 279$, mean \pm sd; 0.85 ± 0.03 and 1.17 ± 0.04 respectively). For vigorous and non-vigorous cushions the relationships between the necrosis extent and the three morphological traits (cushion surface area, shape, and compactness) were not significant.

Table 3: Spearman correlations and p -values for correlations of each morphological and biotic trait. Cushion surface area, cushion perimeter, cushion height, cushion shape, cushion compactness, proportion of epiphytes across the cushion surface area and proportion of neighbouring plants in contact with the cushion perimeter. For volume and height $n = 229$, the other traits $n = 319$. Asterisks indicate the significance of the correlation * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

	Cushion surface area	Cushion perimeter	Cushion height	Cushion shape	Cushion compactness	Proportion of epiphytes	Proportion of neighbouring plants
Cushion surface area	1	0.94***	0.68***	0.05	-0.04	0.37 ***	0.40***
Cushion perimeter		1	0.60***	-0.10	-0.01	0.44***	0.42***
Cushion height			1	0.08	-0.12*	0.05	0.33***
Cushion shape				1	0.10	-0.04	0.01
Cushion compactness					1	0.20***	0.19***
Proportion of epiphytes						1	0.34***
Proportion of neighbouring plants							1

Biotic traits relating to the neighbouring plant community (epiphyte proportion and neighbouring plant proportion) were positively correlated one to each other but also with most of the morphological traits, except for cushion height (not correlated with the proportion of epiphytes) and shape (correlated with none of these biotic traits) (Table 2).

To determine inter-population variability in plant morphological traits, Kruskal-Wallis tests were performed. Significant variation between populations was detected for the cushion surface area (Chi-squared, $X_{318} = 94.011$, $p < 0.0001$), compactness (Chi-squared, $X_{318} = 134.47$, $p < 0.0001$) and shape (Chi-squared, $X_{318} = 29.292$, $p = 0.0377$). Between populations, for vigorous cushions the necrosis extent varied significantly (Chi-square test, $X_{18} = 35.943$, $p = 0.0072$). In contrast, for necrotic cushions inter-population variations of necrosis extent were not significant (Chi-square test, $X_{18} = 16.348$, $p = 0.5683$).

Relationships between cushion morphology and environment or geography

The variability of the cushion surface area across populations was well explained by the topography and habitat, slope aspect and soil texture models, explaining 75%, 54% and 19% of overall variation respectively (Table 4). In the first model, two conditions were significant, topography and wind exposure, without significant interaction. A *post-hoc* test showed that larger cushions were present mid-slope rather than on flat areas (Fig. 3a). A non-significant trend of larger surface area with a decrease of wind exposure was found. For the slope aspect model, the *post hoc* test did not identify any significant slope aspect influencing the cushion surface area. Finally, soil texture was positively related to the cushion surface area, with a larger percentage of coarse sand being correlated to a larger cushion surface area. Inter-population variability in cushion shape was explained (52% of variation) by the univariate model with slope aspect variable (Table 4), with a lower circular shape preferentially observed with south-east slope aspect compared to locations with south-west or north-east slope aspects or flat locations (Fig. 3b). Besides, variability in cushion compactness was significantly related to the surface texture model (18% of variation; Table 4), and was positively correlated to the proportion of bare soil (Pearson correlation, $r = 0.478$, $n = 19$, $p = 0.039$). To determine relationships between population morphology and geography, we applied Mantel tests separately for each morphological trait. For cushion surface area and shape the results of the Mantel tests were insignificant, respectively, $r = -0.178$, $p = 0.933$ and $r = -0.022$, $p = 0.441$. The results were significant only for the cushion compactness ($r = 0.320$, $p = 0.011$), where longer distances across populations were related to higher inter-population variability of cushion compactness but not of cushion shape or surface area.

Table 4: Minimum adequate multivariate linear models of morphological traits and environmental conditions; $n = 19$, adjusted R^2 (ad. R^2), degree of freedom (df), F-value and p-value are presented. Cushion surface area model with variables of topography and habitat, of slope aspect and of soil texture; cushion shape model with variable of slope aspect; cushion compactness model with variable of texture surface; bold numbers indicate significant variables.

Cushion surface area				
	Ad. R^2	df	F-value	p-value
Topography & habitat	75%	8		
Topography			9.153	0.0037
Wind exposure			9.403	0.0154
Type of fellfield			1.913	0.2017
Slope aspect	64%	10		
Slope aspect			4.978	0.001
Soil texture	21%	17		
Fine sand			5.811	0.0275
Cushion shape				
	Ad. R^2	df	F-value	p-value
Slope aspect	52%	10		
Slope aspect			4.455	0.0352
Cushion compactness				
	Ad. R^2	df	F-value	p-value
Surface texture	18%	17		
Bare soil			4.974	0.0395

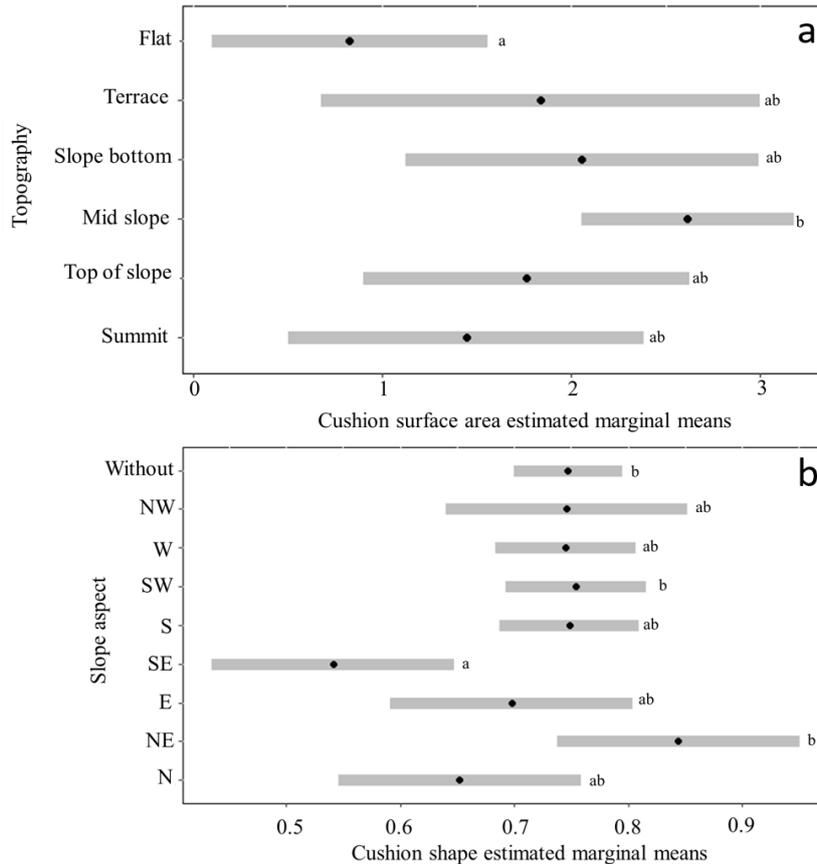


Figure 3: a: Prediction of cushion surface area (m²) with various topographies. The black dot represents the value of the linear model and the grey shape the 95% confidence interval. Different letters indicate significant differences ($p < 0.05$) with topography. b: Prediction of shape with various slope aspects. The black dot represents the value of the linear model and the grey shape the 95% confidence interval. Different letters indicate significant differences ($p < 0.05$) between slope aspects.

Relationship between cushion necrosis and environment or geography

We investigated whether environmental variables explained necrosis extent across populations. For necrotic cushions models were insignificant (data not shown). For vigorous cushions, only the sodium concentration (ANOVA, $F_{14} = 0.081$, $p = 0.0219$) was significant in the soil composition model (*adjusted* $R^2 = 27\%$, Fig. 4) Mantel tests of the necrosis / geographic distance relationships were significant for neither vigorous nor necrotic cushions ($r = -0.05$, $p = 0.645$, and $r = -0.05$, $p = 0.530$, respectively).

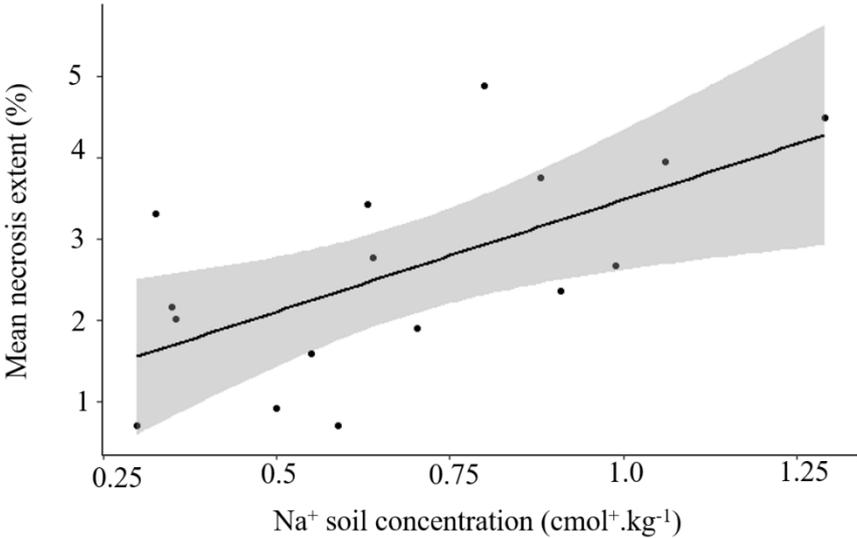


Figure 4: Relationship between soil sodium concentration and mean necrosis extent (%); linear regression $t_{14} = 2.922$, $p = 0.012$; The grey strip represents the 95% confidence interval

Discussion

Cushion allometry was observed for most of the morphological traits analysed. Three traits were selected as representative of the allometry: cushion surface area, shape and compactness. For each trait, the variation among the populations could be explained by environmental and geographical variables. Finally, necrosis extent did not vary with morphological traits but in vigorous cushions inter-population variation was related to soil composition.

Allometry of traits and morphological variability

A previous study indicated a strong relationship between height and diameter in *L. kerguelensis* (Hermant et al. 2013). Here we determined that along with height and diameter, shape and compactness were positively correlated. Similar allometry has been reported elsewhere in other species of cushion plants (Niklas 1994). Besides, shape ratio showed low variation suggesting a conserved circular shape, and height-radius ratio close to 1 strengthened the hemispherical shape of *L. kerguelensis* cushions. Our study confirms the position of *L. kerguelensis* in the class C1 (hemispherical, compact and hard cushion) of Aubert et al. (2014). Positive correlations were also observed between cushion morphology and biotic variables, suggesting that larger cushions of *L. kerguelensis* (surface area, perimeter and compactness) provide greater opportunity to trap dispersing seeds and are favorable environments for other plant species. Furthermore larger and older cushions might have provided a longer period of colonization opportunities for epiphytes. Similar results were observed for various cushion plants in the high Andes (Arredondo-Núñez et al. 2009).

The degree of necrosis was not related to the morphological traits studied, suggesting that necrosis is not encouraged by a particular morphology, contrary to our initial expectation that necrosis would cause sagging and change in cushion shape leading to lower cushion compactness as observed in *Azorella macquariensis* (Bergstrom et al. 2015). Vigorous cushions (<10% necrosis) showed inter-population variability in mean necrosis, varying from 0.70% to 5.18%. This suggests that some populations are less subject to necrosis or that recovery can occur, as has been noted in a previous survey (Dorey, 2017). However, for necrotic cushions (>10% necrosis) inter-population variation was not significant, confirming the presence of necrosis in all the populations studied without influence of specific environmental conditions.

Relationships between environment, geography and morphology

The surface area of cushions was related to topography and wind exposure, with larger cushions predominantly developing mid-slope rather than on flat areas, and a trend of larger cushions in less

wind-exposed populations. In the fragmented landscape and harsh climate of Kerguelen, topography and wind are two major environmental components. The hilly topography of the island (Fig. 2) generates heterogeneity in rainfall, water retention and wind exposure (Aubert de la Rüe 1964; Wagstaff and Hennion 2007b). Mid-slope areas receive runoff water from higher altitude and at the time drain to lower altitude, unlike flatter areas, while wetlands usually develop at the base of slopes. The Kerguelen Islands, like other sub-Antarctic islands, are considered to have moist climates (Hennion et al. 2006b) and sub-Antarctic plants are known for their requirement of water for growth (Dorne and Bligny 1993; Hennion and Walton 1997). Thus, mid-slope may provide optimal water supply for the growth of *L. kerguelensis*. Furthermore, wind exposure induces mechanical stress and can accelerate transpiration of plants (Körner 2003; Haussmann et al. 2009; Gardiner et al. 2016). Plants from *L. kerguelensis* developing in less windy environments such as mid-slope may be less subject to extreme meteorological events like storms or severe frost. Finally, contents of coarse sand in the underlying soil was positively correlated to cushion surface area. Sandy soils are generally well draining, with low water storage capacity and a high usable fraction (Körner, 2003). This finding suggests that ease of water uptake favours larger cushions.

Variation in cushion shape between populations was mainly explained by slope aspect. In Kerguelen, north and north-west slope aspects provide the greatest solar exposure but are also the most exposed to wind, and vegetation is more luxurious away from these slope aspects (south or east aspect) (Werth 1911; Aubert de la Rüe 1964). Our data indicated lower occurrence of circular cushions on the south-east aspect, less exposed to wind, suggesting that the circular shape might be associated with wind exposure in *L. kerguelensis*. This finding is in agreement with the recent air flow dynamics model on *A. selago* where cushions with more pronounced crescent shapes were in habitats with less airflow turbulence (Combrinck et al. 2020).

Variability in cushion compactness was partly explained by the proportion of bare soil and geographic distance between populations. Greater cushion compactness was correlated with a higher proportion of bare soil. This may indicate lower protection against wind than on rocky ground. Thus, higher compactness in cushion plants generally may indicate greater exposure to cold or desiccation stress. It is interesting to note that compactness in cushion plants generally co-varies with altitude, that may be correlated to temperature and humidity variation (He et al. 2014; Cranston et al. 2015; Zhao et al. 2018). However, *L. kerguelensis* did not show compactness variation across the altitudinal gradient examined here. In summary, cushion surface area, shape and compactness were all driven primarily by two major environmental variables: wind intensity and water availability.

Relationships between environment, geography and necrosis

The only environmental variable significantly linked with variation in necrosis was soil sodium content for cushions with low necrosis extent only. The nearest distance to the sea was negatively related to soil sodium concentration ($t_{17} = 2.27$, $p = 0.036$), and a decrease with altitude was observed. Salt is carried in wind-blown sea spray from which it enters the soil, and in the sub-Antarctic marine salt may be found far from the coast due to permanent strong winds (Smith 1987; Jenkin 1997). Furthermore, in Marion Island salinity gradient with altitude and distance from the sea is observed (Conradie and Smith 2012). The mean soil sodium concentration was similar in our sites in Kerguelen to that in Marion Island (Conradie and Smith 2012), supporting the hypothesis of a similar marine influence in both islands. Nevertheless necrosis extent in *L. kerguelensis* was not directly related to the nearest distance to the sea ($t_{17} = -1.43$, $p = 0.170$). From a physiological point of view, sodium induces adjustment of osmotic potential in plants through various mechanisms (Navarro et al. 2007; Shabala 2013). Ion homeostasis (partly with sodium) is achieved during abiotic stress and allows to keep an osmotic potential sufficient for water absorption and then sustain turgor of the cells (Pardo and Quintero 2002). Furthermore, sodium at high concentration can reduce stomata density and then alter photosynthesis (Orsini et al. 2011). In the sub-Antarctic region, studies of salt stress have been performed on several plant species some of which are spread from the coast up to fellfields, concluding that such plants could be defined as glycophytes tolerant of salt stress (Smith 1978; Hennion and Bouchereau 1998; Hummel et al. 2004). For *L. kerguelensis*, higher sodium concentrations in soil may add to other abiotic stresses in their habitats and increase their sensitivity to other osmotic stresses such as drought (Jamshidi Goharrizi et al. 2020). Taken together, our data suggest that necrosis may be related to water availability, although a pathogenic origin cannot be excluded. Our results are consistent with the findings on *A. macquariensis* where dieback was related to summer water availability (Whinam et al. 2014; Bergstrom et al. 2015; Dickson et al. 2019) and do not support a pathogenic origin.

Possible impact of climate change on L. kerguelensis

In summary, cushion surface area, shape and compactness were primarily driven by two environmental variables: wind intensity and water availability. Cushion necrosis also seems to be linked with multiple stresses and mainly osmotic stresses such as drought. However environmental changes expected with the RCP 6.0 model include an overall +1.26 to + 1.50°C temperature increase by 2081-2100, along with 0.1% to 10.0% lower water availability (Harter et al. 2015), which will increase stress for plant populations already facing low water availability or unfavorable slope aspect, exposing them to higher wind desiccation. Indeed, change already observed in precipitation in the Kerguelen Islands over the past 60 years (Lebouvier et al. 2011) may have triggered necrosis or enhanced the process, as observed on Macquarie Island with increased wind speed, hours of sunshine

and evapotranspiration over 17 years (Bergstrom et al. 2015; Dickson et al. 2019). Under the scenario outlined above cushion necrosis in *L. kerguelensis* may therefore become increasingly frequent.

Conclusions

Our study confirms that *L. kerguelensis* cushions tend to form a hemispherical shape. The size and compactness of the plant vary with environmental conditions (topography, slope aspect, wind exposure and soil texture) and geography, all of which are related to wind intensity and/or water availability. The occurrence of necrosis in cushions was related partly to soil salinity. Higher sodium concentrations in soil may add to other osmotic stresses such as drought to increase. Our results suggest that, under current climate change trends in the Kerguelen Islands, cushion morphology may have the capacity to change to adjust to environmental variation. However, in the future, cushion necrosis may be accelerated in the driest and most saline environments. *In situ* experiments are required to better understand the drivers of necrosis, coupled with long-term monitoring to document the dynamics of necrosis and any potential recovery.

Availability of data and material:

Images and R-Script are available at first author's convenience. The datasets generated during the current study are available online on Osuris geonetwork. <https://www.osuris.fr/geonetwork/srv/fre/catalog.search#/metadata/7528fbbb-e1ac-4db2-a179-f20d3f03ff83>.

Authors' contributions:

L.J.M, F.H, M.T. and T.D. conceived the ideas. F.H. and L.J.M. collected data. T.D. and Y.R. set up the photointerpretation method. L.J.M. and T.D. analysed the data. L.J.M, F.H and M.T. led the writing with a contribution from T.D. All authors contributed to discussions. Following comments from the Editor and three reviewers, L.J.M., F.H. and M.T. revised the manuscript with contributions from T.D. and Y.R. designed the map.

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Online Resource

Online Resource Table 1: environmental characteristics of the 19 populations of *L. kerguelensis* studied. At population scale: longitude; latitude; altitude; type of fellfield (slope, scree slope, plateau, summit, wall, fault, outwash plain); topography (flat, terrace, base or mid or top of slope, summit, other); wind exposure (very sheltered, sheltered, exposed, very exposed); range of slope (0°,]0-3],]3-10°],]10-30°],]30-80°]); distance to the closest sea shore; slope aspect (N, NW, W, SW, S, SE, E, without dominant slope aspect); visual estimation of blocks (>20cm), pebbles (2cm to 20 cm), gravel (2mm to 2 cm), bare soil (<2mm), bryophyte and herbaceous cover in percentage.

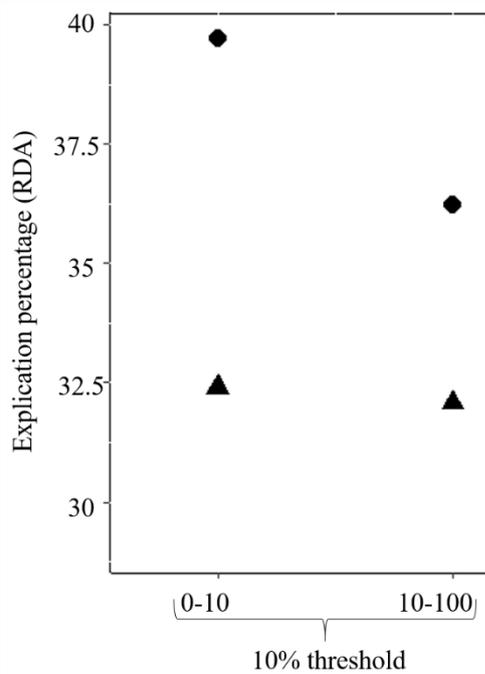
Population	Latitude	Longitude	Altitude (m)	Type of fellfield	Topography	Wind exposure	Slope (°)	Distance to the sea (km)	Slope aspect	Block cover (%)	Pebble cover (%)	Gravel cover (%)	Soil cover (%)	Bryophyte cover (%)	Herbaceous cover (%)
AUS23	69.877	-49.465	137	slope	mid slope	exposed]3-10]	3	N	80	10	0	0	0	30
AUS25	69.876	-49.466	125	scree slope	mid slope	very exposed]10-30]	4	NW	20	20	20	20	5	30
AUS30	69.893	-49.477	15	plateau	bottom of slope	very exposed]3-10]	3	NW	40	30	20	10	0	20
AUS32	69.898	-49.478	60	plateau	flat	very exposed]0-3]	1	E	50	30	10	10	10	30
AUSN1	69.854	-49.440	14	slope	mid slope	intermediate]10-30]	2	SW	50	30	0	10	5	20
AUSN2	69.843	-49.434	32	slope	mid slope	intermediate]10-30]	2	NE	50	30	0	10	20	20
AUSN4	69.825	-49.428	22	plateau	summit	exposed	0	2	S	60	30	0	10	20	10
LON11	69.908	-49.520	84	plateau	flat	very exposed	0	4	without	70	15	5	5	70	5
LON30	69.877	-49.521	154	plateau	flat	very exposed	0	10	without	70	15	0	5	75	5
MAC1	69.427	-48.902	55	plateau	summit	very exposed	0	5	without	80	10	10	5	0	5
MAC3	69.416	-48.895	153	summit	top of slope	very exposed]10-30]	8	S	50	25	20	2	10	15
P121	70.200	-49.545	320	slope	top of slope	very exposed]0-3]	16	S	20	70	10	2	0	10
P122	69.197	-49.545	250	slope	bottom of slope	very exposed]0-3]	20	SE	10	70	0	0	10	30
P123	70.224	-49.554	340	scree slope	top of slope	very exposed]3-10]	20	SW	80	10	5	5	5	10
PCR1	69.033	-48.705	238	plateau	flat	exposed	0	6	without	60	40	0	5	0	20
PCR2	69.033	-48.701	216	plateau	flat	exposed	0	9	without	60	30	10	5	0	20
PIDA10	69.809	-49.591	520	plateau	terrace	very exposed]3-10]	27	NW	20	40	20	5	0	40
PIDA6	69.820	-49.590	482	plateau	mid slope	very exposed]10-30]	35	W	30	30	30	10	5	15
RBA5	68.962	-49.630	71	outwash plain	flat	exposed	0	8	SW	10	35	35	0	10	20

Online Resource Table 2: soil characteristics of the 19 populations of *L. kerguelensis* studied; soil texture (clay, sand, silt), soil chemical composition (Na, Mn, Al, CEC, P, N, C.N⁻¹, NO³⁻, NH⁴⁺) and soil hydric characteristics (pH, conductivity and soil humidity).

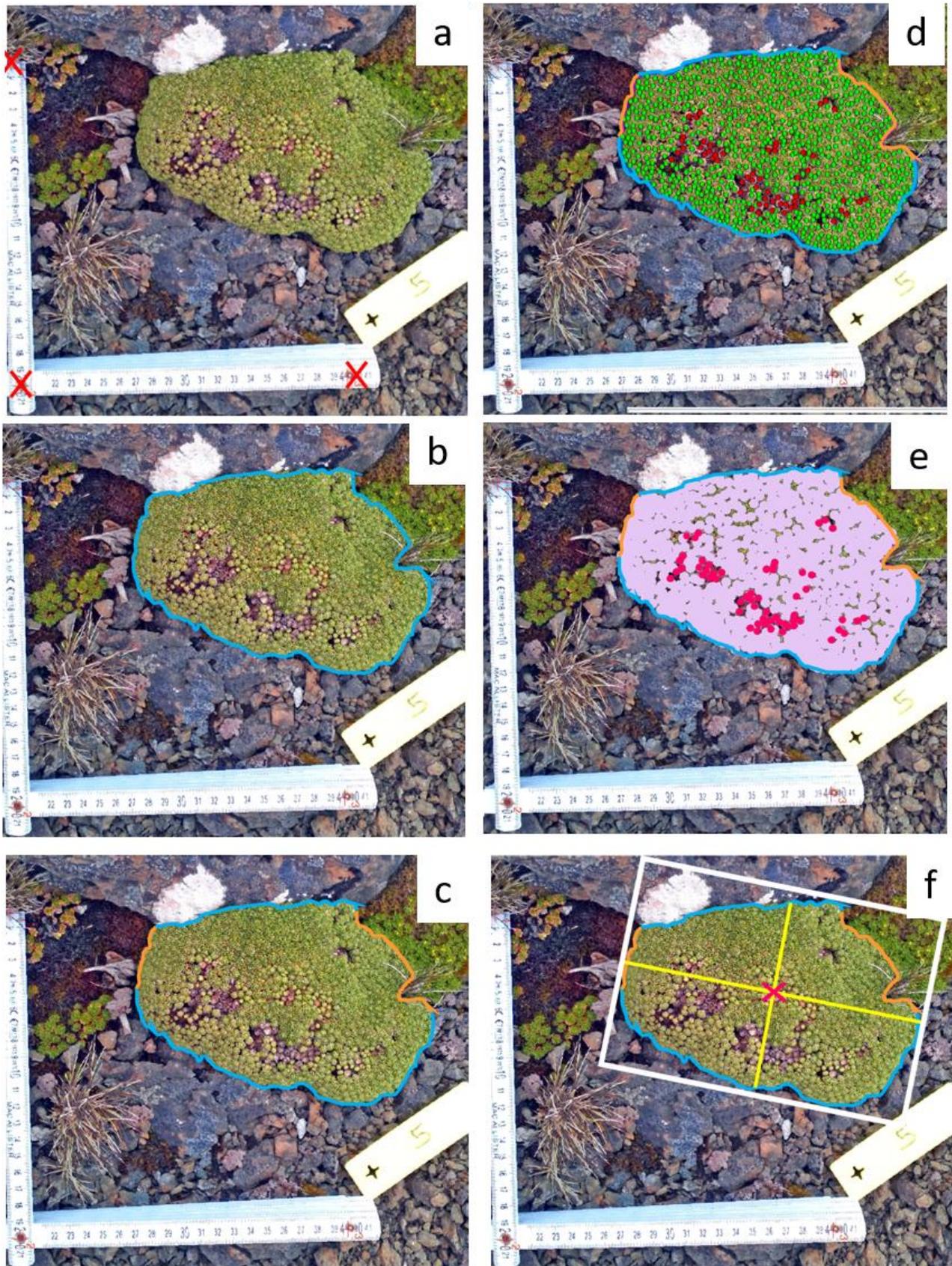
Population	% clay	% sand	% silt	Na kg ⁻¹	Mn kg ⁻¹	Al kg ⁻¹	CEC	P cmol kg ⁻¹	N kg ⁻¹	C N ⁻¹	NO ³⁻ kg ⁻¹	NH ⁴⁺ mg kg ⁻¹	pH	Conductivity μs cm ⁻¹	Water saturation (%)
AUS23	4.9	40.1	7.5	1.66	0.039	0.546	4.16	0.011	1.85	12.9	1.83	4.75	6.12	292	24.27
AUS25	5.3	39.7	7.7	0.59	0.015	0.542	7.23	0.009	1.85	12.3	1.13	3.53	6.12	171	17.67
AUS30	6.5	35.4	11.4	1.06	0.015	0.071	22.41	0.017	3.25	11.0	1.31	2.81	6.70	492	5.77
AUS32	7.9	32.2	13.9	0.70	0.015	0.882	3.47	0.019	2.92	11.5	1.52	4.17	5.25	240	52.98
AUSN1	8.5	26.7	19.1	0.64	0.021	1.710	5.85	0.027	3.66	11.4	1.33	2.57	5.52	189	34.99
AUSN2	7.3	36.2	10.2	0.63	0.020	0.809	5.79	0.014	2.66	12.9	1.32	6.81	6.34	270	23.67
AUSN4	3.6	43.8	4.5	0.99	0.014	0.903	11.84	0.005	0.67	12.5	0.98	1.75	6.80	86	32.00
LON11	5.2	36.0	11.4	1.29	0.010	0.160	5.82	0.036	0.43	11.7	0.93	0.93	6.36	422	60.79
LON30	5.0	33.4	14.1	0.35	0.013	0.202	1.32	0.024	1.42	11.0	1.02	1.02	5.62	237	21.71
MAC1	6.0	25.9	21.1	0.33	0.010	1.290	3.21	0.025	2.04	11.6	1.24	2.69	5.77	124	33.00
MAC3	5.4	29.2	18.1	0.23	0.030	1.520	3.44	0.021	1.97	11.7	1.19	1.48	5.75	85	19.67
P121	4.2	39.6	8.4	0.30	0.019	0.764	2.41	0.025	1.12	11.9	1.00	1.00	6.04	130	14.53
P122	4.9	38.8	8.8	0.35	0.019	0.755	3.46	0.038	0.80	10.7	0.99	0.99	6.21	123	14.00
P123	5.5	56.2	9.5	0.50	0.054	0.481	4.61	0.015	2.27	12.9	0.82	14.6	6.54	560	55.32
PCR1	5.4	37.0	10.4	0.91	0.017	0.080	8.71	0.009	2.37	13.0	0.46	4.58	6.80	996	25.86
PCR2	4.7	41.7	6.0	0.80	0.013	0.064	11.37	0.010	1.81	13.2	0.61	5.93	6.90	360	26.65
PJDA10	4.2	24.9	23.1	0.88	0.005	0.159	3.91	0.013	2.02	12.2	0.59	2.93	6.40	588	32.02
PJDA6	0.4	47.8	2.0	0.21	0.016	0.145	0.76	0.003	0.25	10.7	0.54	2.33	6.55	50	17.53
RBA5	4.1	41.4	6.6	0.55	0.031	0.503	6.68	0.025	1.35	11.4	0.46	1.48	6.58	420	18.89

Online resource Table 3: quantitative description of physical attributes and environmental parameters of the 19 populations studied. Topography and type of habitat: altitude, type of fellfields (slope, scree slope, plateau, summit, wall, fault, outwash plain), topography (flat, terrace, base or mid or top of slope, summit, other), range of slope (0° ,]0-3°],]3-10°],]10-30°],]30-80°],), slope aspect (N, NW, W, SW, S, SE, E, without dominate slope aspect); distance to the sea and wind exposure (very sheltered, sheltered, exposed, very exposed); visual estimation of surface texture: mineral block (>20cm), pebbles (2cm to 20 cm), gravel (2cm to 2mm), bare soil (<2mm), bryophyte and herbaceous cover in percentage; soil texture: clay, silt and sand percentage; soil chemical composition: Ca, P, N, ratio C.N⁻¹, Mn, Al, Na, CEC, NO³⁻, NH⁴⁺, Fe; soil physics characteristics: conductivity, pH, soil water saturation and organic matter.

Category	Variable	Description
Topography & habitat	Altitude	14-520 m a.s.l.
	Types of fellfields	slope, summit, plateau, glacial outwash plain
	Topographical position	Bottom of slope, mid slope, top of slope, summit, flat area and terrace
	Slope aspect	Present at all slope aspects
	Slope	always less than 30%, with populations also occurring on flat areas
	Wind exposure	Intermediate, windy and very windy
	Distance to the sea	1 km to 35 km
Surface texture	Mineral cover	Block cover (10 to 80%) and pebbles (10 to 70%) were predominant and alternative one to each other. Proportions of gravel and bare soil were always below 20%.
	Vegetation cover	herbaceous species: 5 to 40%, bryophytes: 0 to 20% ; except Ile Longue where bryophytes were in the majority (70-75%)
Soil texture		organo-mineral, 24 to 57 % sand, less than 9% clay and up to 23 % silt
Soil chemical composition	Calcium	0.3 to 2.7 cmol kg ⁻¹
	Phosphorous	0.005 to 0.038 cmol kg ⁻¹
	Total nitrogen	0.25 to 3.66 cmol kg ⁻¹
	C.N ⁻¹ ratio	10.7 to 13.2
	Manganese	0.005 to 0.054 cmol kg ⁻¹
	Aluminium	0.064 to 1.71 cmol kg ⁻¹
	Sodium	0.206 to 1.66 cmol kg ⁻¹
	Effective cation exchange capacity	0.76 to 22.41
	Nitrates	0.46 to 1.83 cmol.kg ⁻¹
	Ammonium	0.93 to 14.6 cmol.kg ⁻¹
	Iron	0.003 to 0.06 cmol.kg ⁻¹
	Manganese	0.005 to 0.054 cmol.kg ⁻¹
Soil physical characteristics	Conductivity	50 $\mu\text{s cm}^{-1}$ to 996 $\mu\text{s cm}^{-1}$
	pH	5.2 to 6.9.
	Water saturation	5.77% to 60.79%.
	Organic matter	7.34 to 72.1

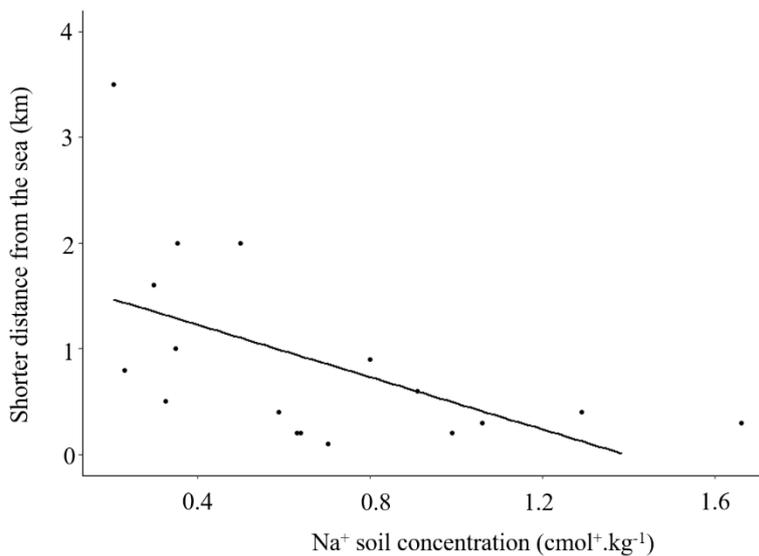


Online Resource Figure 1: Redundancy analysis (RDA) explanation percentage; cushions were selected randomly and determination of necrosis extent was done with the same methods as in our study detailed in Online Resource Figure 2; two groups are defined: 0-10% necrosis extent ($n=42$) and 10-100% necrosis extent ($n=41$). Black dots represent RDA explanation percentage for environmental variables (altitude, slope, % block cover, % pebbles cover, % gravel cover, % bare soil cover, % bryophyte cover, % herbaceous cover, mean height of neighbouring plants, neighbouring plant proportion) and black triangles represent RDA explanation percentage for soil variables (soil water content, pH, conductivity, soil depth, clay, fine silt, coarse silt, fine sand, coarse sand, $C.N^{-1}$, organic matter, CEC, NH^{4+} , NO^3). Other thresholds were tried but were less significant.



Online Resource Figure 2: Analyses are done with Arcgis 10.6.1, using a WDS_1994_World_Mercator spatial reference. (a) The rule with a 90° angle, next to the cushion is used to calibrate the system as

the length between the red crosses is indicated on the rule. (b) Polygonal entity was created to determine the perimeter of the cushion (blue line) and the surface area. (c) The proportion of the perimeter merging with other plants, referred to as the percentage of neighbouring plants (orange line). (d) Each apex is composed of an apical meristem, terminal leaves visible on the pictures and older leaves hidden inside the cushion (thus not visible on the picture). Point features were used to determine the number of vigorous (green) and necrotic (red) apices (e) a circular area surrounding each point entity was created per apex category with a fixed size, then merged to create a unique area entity, avoiding potential superposition of the circular areas; vigorous surface area (light pink) and necrotic surface area (magenta) were determined respectively, then a ratio between the total surface area of the cushion and the necrosis surface area is done and further called percentage of necrosis extent. (f) A cushion shape proxy was generated, the smallest rectangle (white) where the cushion fit was automatically defined around the perimeter polygon, the middle of the rectangle is defined as the centroid of the cushion (red cross). Cushion diameters (short and long) were assessed by measurement of the two orthogonal diameters at the centroid up to the perimeter polygon (yellow lines) (extension ET GeoWizards), short and long radius are respectively half of the short and long diameter. If necessary another polygonal feature was applied to delimit the surface area of the epiphytes (plants growing on the cushion) which are present on some cushions.



Online Resource Figure 3: Linear relationship between the shorter distance from the sea to the population and the soil sodium concentration. Two outliers removed.

Chapter 2: Differential gene expression of *L. kerguelensis* in contrasted environments: implications for biotic and abiotic stresses

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Note: For this chapter, bioinformatic analyses were conducted at Massey University by J. Henry and Prof. P. Lockhart

Introduction

One challenge is understanding the possible impact of climate change on plant species. It is essential as many species might not be able to shift geographically to track optimal environments, leading to local extinction (Román-Palacios and Wiens 2020). Therefore, an essential aspect of population responses to environmental changes is their capacity to persist in the same location by modifying their traits with plastic and/or genetic responses (Moritz and Agudo 2013; Hoffmann et al. 2017). However, we currently have limited understanding of the adaptive capacity of species under climate change (Hoffmann and Sgrò 2011; Román-Palacios and Wiens 2020; Sandoval-Castillo et al. 2020). Genome science can help inform this understanding. It has provided sequencing technologies for investigating the genetic variation of organisms and their populations (e.g. Wang et al. 2009; Elshire et al. 2011). It provides a means to identify genes and alleles that are associated with environmental change (Hoffmann and Daborn 2007; Hoffmann and Willi 2008; Borevitz 2021) and a means to study how organisms upregulate and downregulate the expression of specific genes and alleles in response to the environment to improve their fitness, growth and development (Artemov et al. 2017; Kim et al. 2018; Akhter et al. 2021). One suggested way of studying the evolutionary response of species to environmental change is to study the expression of their genes across environmental gradients (Hoffmann and Daborn 2007; Hoffmann and Willi 2008). This approach has the potential to help identify functional genes under positive selection and the physiologies of species that may play a role in plant adaptation (Hoffmann and Daborn 2007; Hoffmann and Willi 2008; Voelckel et al. 2017; Sork 2017; Zhang et al. 2020). Reasons for this are that the transcriptome is very reactive to changing environmental conditions and the expression levels of genes are correlated with the proteome to some extent (Voelckel et al. 2017). While many studies have examined the response of the transcriptome in common-garden experiments, it is increasingly recognized that *in natura* studies can be helpful to investigate complex environmental changes (Hoffmann and Daborn 2007; Voelckel et al. 2008; Zaidem et al. 2019). *In natura*, population-level gene expression differences can inform us how distinct environmental gradients shape trait variability and evolution (Zaidem et al. 2019). Some examples have been discussed in Voelckel et al. (2017) where it is noted that in closely related plant species, genes for the ontology class "abiotic stress response" are often differentially expressed. The expression patterns of these genes might be important for plant physiologies and their adaptation and plastic responses (Voelckel et al. 2017). Transcriptome studies also offer insight into environmental signal transduction pathways involved in the abiotic responses of plants. These pathways involve transcription factors that activate gene networks, protein kinases and phosphatases that activate and deactivate proteins (e.g. Wang et al. 2020; Guo et al. 2021). This regulation can contribute to specific plant physiologies and acclimation responses to environmental changes and stress (Gish and Clark

2011; Roy 2016; Kim et al. 2018; Hrmova and Hussain 2021). Studying differential gene expression of stress responses in the context of specific environmental variables has the potential to be very informative for understanding the vulnerability of plant species to climate change. For example, a study of the transcriptome of *Colobanthus quitensis*, an Antarctic long-lived cushion plant, revealed modulation of energy metabolism and change in patterns of stress-responsive gene expression mainly in relation to low temperature (Cho et al. 2018). Furthermore, in the same study, the cushion compactness of *Colobanthus quitensis* was suggested to be related to differentially expressed thermomorphogenesis-related genes (Cho et al. 2018).

Lyallia kerguelensis is a long-lived cushion plant endemic to the Kerguelen Islands, its distribution is scarce and restricted to fellfields (Wagstaff and Hennion 2007). Populations are mostly composed of few, twelve or more, individual plants. In some rare cases there may be up to a hundred individuals. In our previous study (Marchand et al. 2021) morphological variability was related to environmental variables including topography and soil composition. In this chapter we report initial findings from studying differential gene expression of *L. kerguelensis* in contrasted environments. Furthermore, genetic diversity of eighteen populations across the archipelago was low when targeting SSP markers known to be very sensitive (Robert et al. 2021). The interest was to determine whether or not transcriptome profiling of *L. kerguelensis* population across contrasted environments in the Kerguelen Islands would reveal information on plant abiotic and biotic stress responses. We asked the following questions:

(i) Is there evidence for differences in the transcriptome expression profiles of different *L. kerguelensis* populations? (ii) If so, is there gene enrichment of biological processes that involve stress responses? (iii) If so, does the identity of differentially expressed genes concern transcription factors or other regulatory proteins previously associated with signal transduction pathways for abiotic and biotic stress response?

If transcriptome profiling is informative for investigating the physiological responses of *Lyallia kerguelensis in natura*, we predict that (i) transcriptome expression profiles will differ between different regions that are contrasted environments and that populations within the same regions will have similar transcriptome profiles as they share similar local climate. (ii) we hypothesize that many of the genes differentially expressed across populations or regions will be related to environmental variables as the variability of *L. kerguelensis* morphological traits were explained by population environmental variables (Marchand et al. 2021, Chapter 1 and 4). Finally (iii), if differences in transcription profiles are in part explained by plastic or adaptive responses of *Lyallia kerguelensis* populations we expect to identify genes involved in environmental signal transduction for abiotic and biotic responses e.g. transcription factors and protein kinases.

Material and methods

Regions and populations selection and characteristics

Five different regions were selected: two in the North: “Port Christmas” (PCR) and “île Mac-Murdo” (MAC) and three in the South East: “île Australia” (AUS), “Plateau du vent” in “Presqu’île Jeanne d’Arc” (PJDA) and “Plateau Dent du Chat” (PDC) in “Presqu’île Ronarch” (Figure 1).

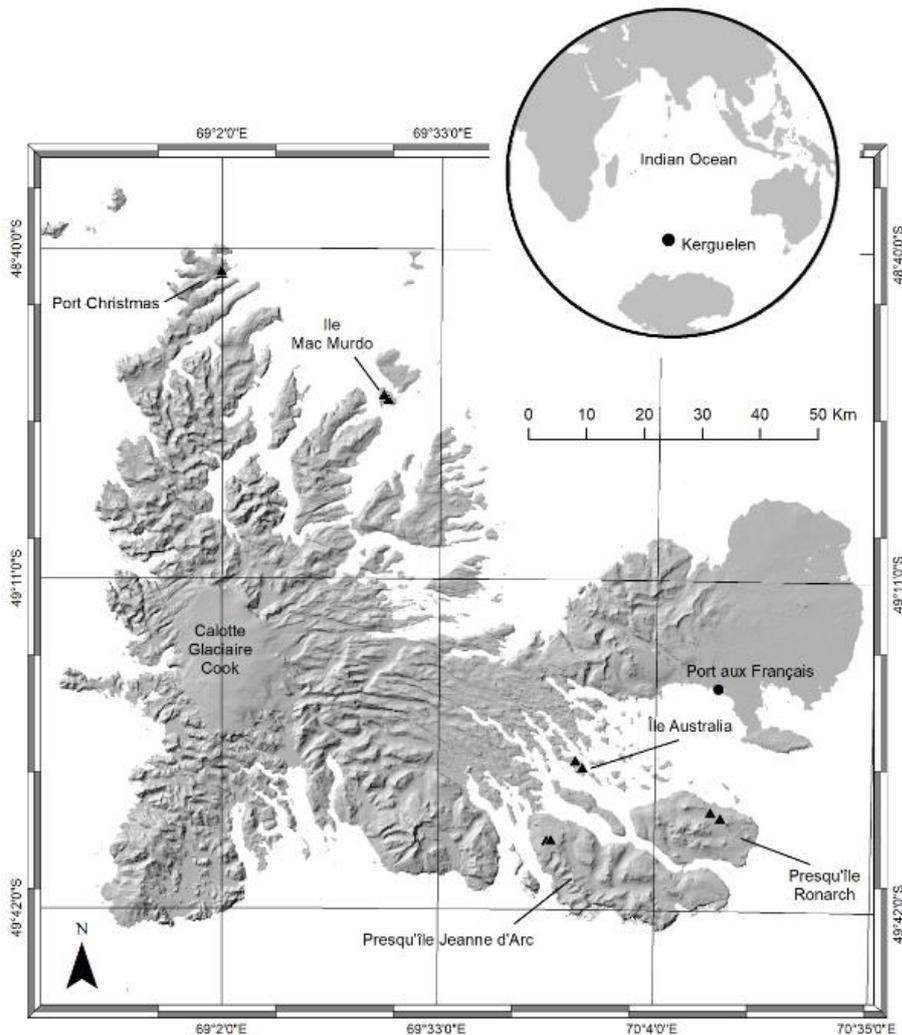


Figure 1: Locations of the 10 populations of *Lyallia kerguelensis* (black triangles) on an altitudinal background in the Kerguelen Islands (NASA – SRTM 30M, 2005), region names are in black. the black square indicates the research station of "Port-aux-Français". The small globe illustrates the Kerguelen Islands position in the South Indian Ocean. Maps were designed with ArcGIS 10.8.1 software.

Cushion selection

We selected two visually disjunctive populations within each region: PCR1 and PCR2, MAC1 and MAC3, AUS25 and AUS30, PJDA6 and PJDA10, PDC1 and PDC2. Each population consisted of a geographical area of less than 50 m² where the geomorphology, topography and soil properties were homogeneous and harboured a continuous *L. kerguelensis* population. Measurements of population characteristics were described in Marchand et al. 2021, Chapter 1 (values are indicated in Table 2 on Chapter 1).

Lyallia kerguelensis Hook. f. (Hooker 1847; Montiaceae) is a long-lived perennial herb. It forms roughly round-shaped cushions 20 – 40 cm across and exceptionally up to 1 m (Wagstaff & Hennion 2007). Its cushion growth form is characteristic of plants living in harsh environments with soil subject to low water holding capacity and strong fluctuation of temperatures and precipitation (Körner 2003). We selected randomly 5 or 6 visually vigorous cushions, less than 10% of necrotic part, randomly distributed among the population with a size above 10cm of diameter approximately to avoid damaging too small cushions. On each cushion, 4 to 5 apices were collected at a different cushion location to cover its possible gene expression variability. Apices collected were immediately put into RNA later and kept at a low temperature until returned to the lab. They were stored at -20°C, then shipped to the laboratory in Rennes (UMR 6552 ECOBIO) for RNA extraction. Additionally, two samples from the growing chamber of the laboratory in Rennes (UMR 6552 ECOBIO, ECOLEX) were also collected. Two apices were collected due to their relatively small size, the same procedure of storage was used. Both cushions originally come from the south-west part of the archipelago, the "Péninsule Rallier du Baty" from the population RBA5.

RNA extraction

Two or three apices per sample were put into liquid nitrogen and manually ground with pestle and mortar until obtaining thin powder. The RNA extractions were done following Quiagen protocol (RNeasy® Mini kit, Quiagen). Briefly, a mix of 455µL of β-Mercaptoethanol and buffer RLT was added (10µL:1mL) and samples were placed for three minutes at 56° to disrupt the tissue. The lysate was cleared with 500µL of ethanol and placed in a RNA spin column. 700µL of buffer RW1 with DNase treatment was applied (RNase-Free DNase Set, Qiagen) to eliminate possible genomic DNA. Afterwards, 500µL of buffer PRE was added to clean the membrane with the RNAs in it. Finally, RNAs were detached from the membrane with 30µL of RNA-free water and immediately stored at -80°C. RNA quality and quantity were checked using NanoDrop device (ThermoFisher Scientific, Waltham, USA) and Bioanalyzer 2100 device (Agilent, USA). RNAs samples were air-freighted on dry ice to Novogene (Hong Kong, China). Two types of cDNA sequencing were undertaken, paired-end Illumina RNA Seq for every sample and outsourced PacBio single-molecule real-time (SMRT) sequencing on the highest quality sample (PJDA10_19).

Sequence analysis

Details are available in supplementary material 1 for the steps and software used in the processing of these data. Briefly, processing of the Illumina data involved read error correction, trimming, quality and length filtering. Processing of PacBio data involved generating non-redundant full-length non-concatemer (FLNC) reads for each of the cDNAs sequenced. At the end of the process, 48 Illumina

data samples were retained for further analyses (6 for AUS25, 4 for AUS30, 2 for MAC1, 5 for MAC3, 5 for PDC1, 4 for PDC3, 5 for PCR1, 5 for PCR2, 6 for PJDA6, 4 for PJDA10 and 2 for RBA5) and FLNC PacBio reads for one sample PJDA10_19. Samples from AUS30 were used to construct a *de novo* transcriptome (unigene library) with Trinity v2.11.0 (Grabherr et al. 2011). Basic assembly statistics were generated, and quality of the transcriptomes inferred through using BUSCO v5.2.2 (Simão et al. 2015), TransDecoder v5.5.0 (<https://github.com/TransDecoder>), and basic local alignment search tool (BLAST) v2.10.0 (Altschul et al. 1990) matches against known *Arabidopsis thaliana* proteins. For studies of differential expression, transcript counts were generated by mapping Illumina reads against the PacBio FLNC reads with Salmon v1.5.1 (Patro et al. 2017).

PCA of gene expression

To compare counts for gene expression, transcripts per million-normalised (TPM) values for each transcript were extracted from the Salmon output. These values were then grouped and summed, using functions of Tidyverse 1.3.0 (Wickham et al. 2019) and R v4.0.2 (R Core Team, 2020) in RStudio v1.3.959 (RStudio Team, 2020), according to *A. thaliana* gene function as inferred from the BLASTx results. Subsequently, a Kruskal-Wallis test was carried out to retain only genes that showed significantly different (at $p < 0.05$) expression in at least one population. A PCA was then performed on these retained genes using the R function 'prcomp' and the results were visualised using the ggbiplot v0.55 (<https://github.com/vqv/ggbiplot>) R package. Based on the results of PCA, four outlying samples two for RBA5 and two for PDC3 were removed (Fig. SI). In order to apply a 95% confidence interval for each population, populations with less than three samples were withdraw (PDC3 and MAC1), (Fig SII).

MFA of environmental variables

Thirty-five environmental variables were used to study the environmental characteristics of the eight regions. Every quantitative variable was normalized, Spearman correlations between quantitative variables were checked and removed if significant (function cor.test). A multiple factor analysis (MFA) was applied (package FactoMineR v2.4 and factoextra v1.0.7, Husson et al. 2017). MFA allows studying qualitative and quantitative variables within the same analyses. The two or three main explanatory variables of the dimension one and two of the MFA were retained as well as the two main qualitative variables. A second MFA was done with these selected variables. The same procedure was applied to study the variability of AUS and PCR populations (four populations).

Pairwise comparisons of gene expression between AUS and PCR populations

To investigate in more detail differential expression between two propulations with distinctly different transcription profiles, DESeq2 v1.30.1 (Love et al. 2014) was used to carry out six differential gene

expression (DGE) analyses between AUS and PCR populations. Raw transcript counts were imported into DESeq2 with tximport v1.18.0 (Soneson et al. 2015) and analysed at the *A. thaliana* gene level using the 'tx2gene' option. Genes were considered differentially expressed if the fold change was greater than 1.5 and the p value, corrected for multiple testing using the Benjamini and Hochberg method (Benjamini and Hochberg 1995), was below a threshold of 0.05. Overlap of differentially expressed genes was visualised using the UpSetR v1.4.0 package (Conway et al. 2017).

Gene ontology term enrichment analyses in AUS and PCR populations

Gene ontology (GO) term enrichment analyses were conducted for differentially expressed genes (DEGs) in AUS and PCR populations. Non-redundant Araport11 gene identifiers (IDs) were extracted from the BLASTx results and were uploaded to the UniProt website (UniProt Consortium 2018) on 22 September 2021. Gene ontology terms associated with the *A. thaliana* genes were then retrieved via the 'Retrieve/ID Mapping' function. The Araport11 IDs, annotated with GO terms, were downloaded in a tab-separated text document. Lists of upregulated and downregulated genes (Araport IDs) output from the DESeq2 analyses were subsequently annotated with GO terms. These lists were then uploaded to agriGO v2 (Tian et al. 2017). Singular Enrichment Analyses (SEA) were carried out using a custom background of non-redundant *A. thaliana* genes with the minimum number of mapping entries set to five. Due to the relatively few DEGs, the Fisher statistical method (Fisher 1992) was used without correction for multiple testing at a significance level of $p < 0.05$. Overlapping GO terms were discovered with the R package venn v1.10 (<https://github.com/dusadrian/venn>) and Venn diagrams were constructed with ggvenn v0.1.9 (<https://github.com/yanlinlin82/ggvenn>).

Transcription factor discovery

The software iTAK v1.7 (Zheng et al. 2016) was used to identify genes putatively important in gene expression regulation. The transcript with the highest BLASTx bit score, amongst all transcripts clustered according to the same *A. thaliana* gene function, was selected as a representative sequence for that gene. For any upregulated or downregulated genes, the representative sequences were searched against the iTAK database and lists of differentially expressed transcription factors, transcriptional regulators, and protein kinases output.

Results

Illumina read quality data

The Illumina short reads obtained for each sample were found to be of high quality, with little data lost after read error correction, trimming, and quality filtering (Table SI). Examination of FastQC reports indicated very few sequencing issues, and quality processing retained at least 95% of reads in all samples (Table SI).

Comparison of PacBio reads and Trinity de novo assembly

The Trinity assembly produced 134,533 unigenes, which was over three times the number of transcripts (38,004) recovered from the PacBio dataset (Table SII). From these Trinity unigenes, 80.5% were expected single-copy orthologues (with 3.7% duplication), whereas only 63.3% of single-copy orthologues were recovered from the PacBio reads (Table SII). However, commonly reported quality metrics such as contig N50 and median contig length were much greater for the PacBio reads (Table SII). More importantly, 93.6% of PacBio reads had successful matches against known *A. thaliana* proteins, and 91.9% contained predicted proteins. In contrast, the Trinity unigenes had 38.0% successful matches and 42.6% predicted proteins (Table SII). Due to their apparent quality, for the rest of the analysis, the PacBio reads were used instead of the Trinity unigenes *de novo* assembly. Removal of redundant PacBio transcripts was then performed (supplementary material 1) and results are in table SII.

Gene Expression and environmental comparisons between populations

Variability in the transcriptome of *L. kerguelensis* in the five populations was observed (Fig.2). The first two principal components explained more than 40% of the variability (Fig.2). Transcriptome profiles were more similar within regions than between regions with low variability among individuals of a population (Fig. 2). Each population was characterized by specific environmental variables (Fig. 3). The MFA analyses captured almost 80% of the variability. The explanatory variables are related to soil nutrient (N, C/N and P) and pH as well as slope, population topography and wind exposure (Fig. 3). Within regions, populations tended to share similar values for environmental variables. For example, AUS25 and AUS30 are geographically close to each other and differed slightly in their soil nitrogen concentration. Exceptions to this general trend also occurred. For example, PJDA6 and PDC2, located at two different regions were found more similar than the other populations from the same respective regions as indicated by the first two axes of the FMA (which capture a majority of the variability).

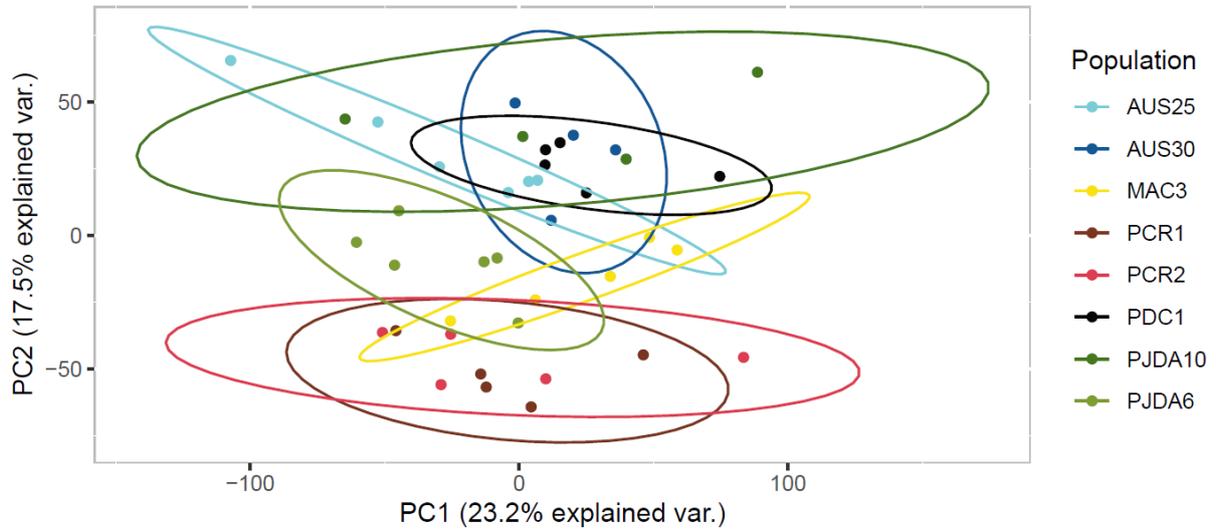


Figure 2: Principal components analysis (PCA) of PacBio transcript counts (transcripts per million-normalised) aggregated to gene-level by summing TPM values for all transcripts annotated against the same *Arabidopsis thaliana* proteins. Each ellipse represents the 95% confidence interval for each population.

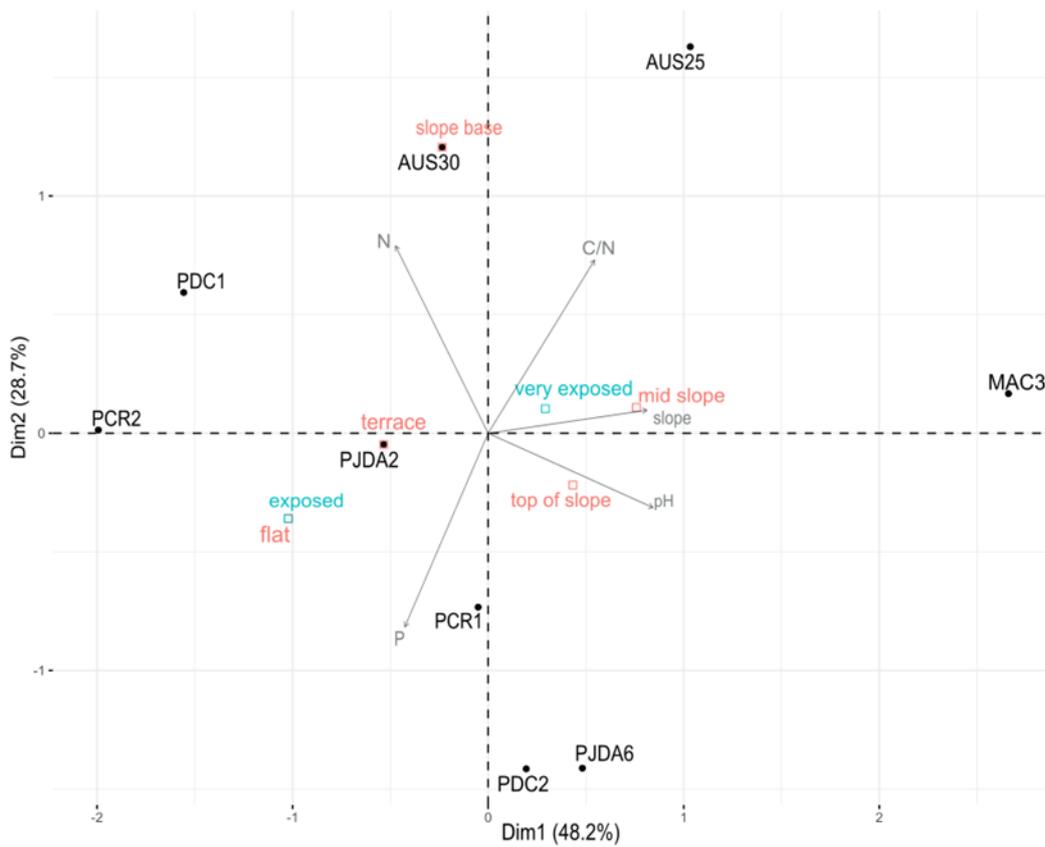


Figure 3: Multiple Factor Analysis (MFA) represents the qualitative and quantitative environmental variables for the eight populations studied. Quantitative variables are in grey. Blue color indicates the wind exposure qualitative variable and the red color indicates the topography qualitative variable.

Pairwise gene expression comparisons between AUS and PCR populations

We selected two regions exhibiting distinct gene expression profiles, namely AUS and PCR (Fig. 2) for further analyses. The PCA results in Fig. 2 indicated similar gene expression patterns amongst the AUS populations (AUS30 and AUS25) and the PCR populations (PCR1 and PCR2). In contrast, there were clear differences in the expression profiles of AUS and PCR populations. Furthermore, these populations were also distinct for their environmental characteristics (Fig. SIII). The MFA analyses' second axe separates PCR to AUS, while the first axis explains the intra-regions variability (Fig SIII).

A substantial number of genes showed similar patterns of differential expression between AUS and PCR regions when pairwise comparisons were made (Fig. 4). Indeed, 51 genes were found differentially expressed in populations of the AUS region compared to populations of the PCR region. At the same time, 41 genes were differentially expressed between AUS30 and PCR populations and 20 genes between AUS25 and PCR populations. Also, 37 genes were specific to the AUS30-PCR1 comparison (which presented the largest gene set). Finally, within AUS and PCR regions, only a few genes were differentially expressed (Fig 4). The number of genes exhibiting upregulation in pairwise comparisons of AUS and PCR populations are shown in Table 1. The results in Fig. 4 and Table 1 indicate that slightly more genes were upregulated in the AUS populations relative to the number of genes upregulated in PCR populations. Within regions, PCR2 had more upregulated genes than PCR1, while for AUS it was almost equivalent (Table I).

Table 1: Number of genes upregulated in a AUS population (downregulated in a PCR population) and upregulated in a PCR population (downregulated in a AUS population) for inter-region comparison. Within region upregulated genes are reported in each population. Values indicate the % genes exhibiting upregulation out of 9,263 genes surveyed, which showed differential expression.

Inter-region	All	%	UpAUS	%	UpPCR	%
AUS25-PCR1	148	1.60	81	0.87	67	0.72
AUS25-PCR2	129	1.39	63	0.68	66	0.71
AUS30-PCR1	193	2.08	99	1.07	94	1.01
AUS30-PCR2	138	1.49	69	0.74	69	0.74
Intra AUS	All	%	UpAUS30	%	UpAUS25	%
AUS30-AUS25	13	0.14	7	0.08	6	0.06
Intra PCR	All	%	UpPCR1	%	UpPCR2	%
PCR1-PCR2	11	0.12	2	0.02	9	0.10

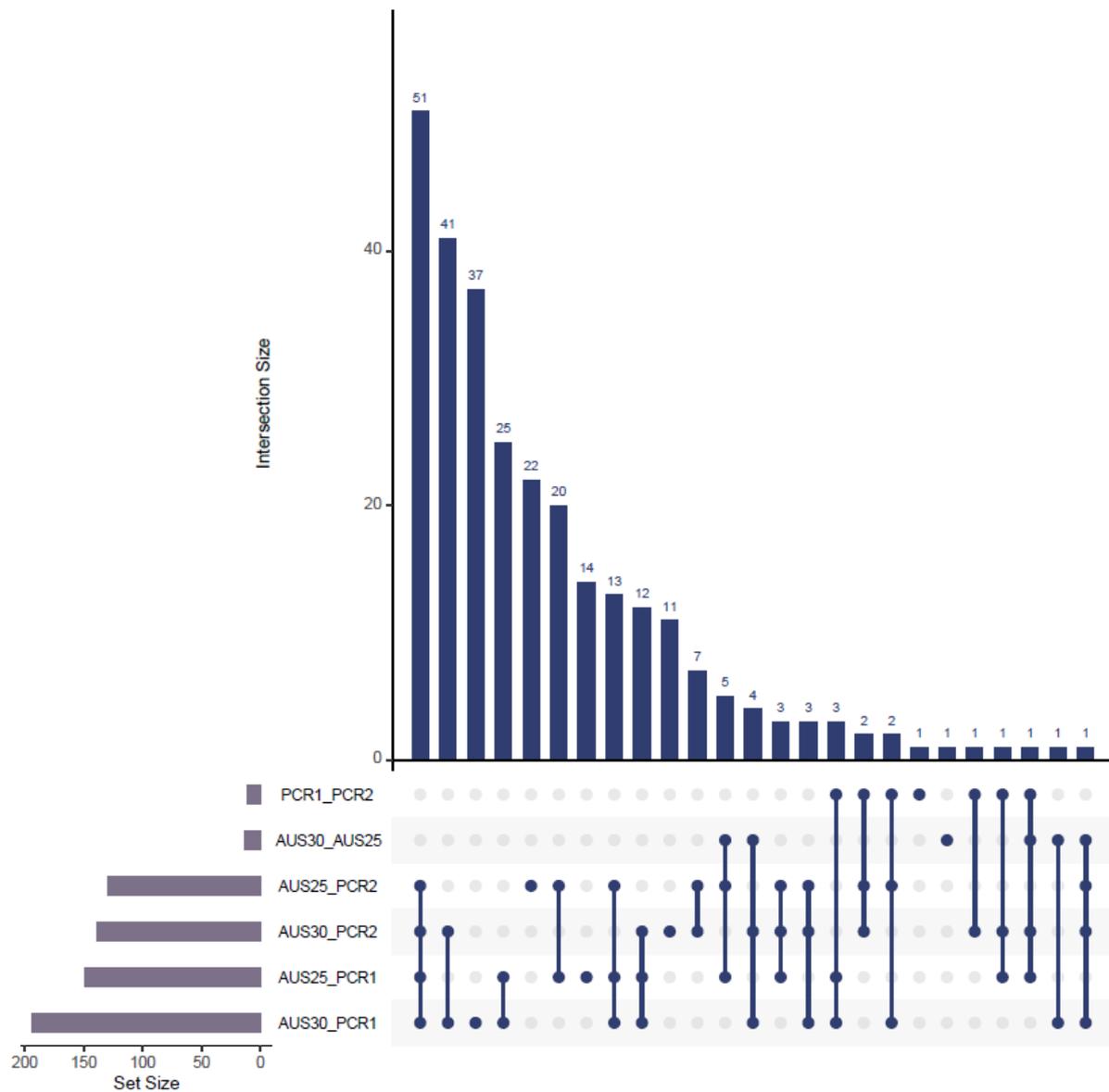


Figure 4: UpSet plot showing numbers of differentially expressed genes. The x axis indicates genes differentially expressed in only one comparison (single dots) and genes differentially expressed in two or more comparisons (joined dots). The y axis (with values on top of the bars) shows the numbers of genes in each intersection.

Gene ontology term enrichment analyses between AUS and PCR populations

To investigate whether the patterns of differential expression had functional significance, a GO term enrichment analyses were conducted. These analyses identified whether any gene ontology terms were represented by an overabundance of expressed genes linked to a particular GO term. These analyses were not carried out between populations of the same region due to the low number of differentially expressed genes between populations at the same regions. The GO term enrichment analyses for the AUS-PCR population comparisons are shown in Figure 5 and reflect the gene expression patterns (Table I). More enriched GO terms were discovered for upregulated genes in the

AUS populations compared to PCR. The AUS25_PCR1 comparison identified 48 enriched GO terms in the AUS25 population, while other AUS_PCR2 comparisons identified 34 to 47 GO terms in upregulated genes of the AUS populations. Interestingly, a set of 19 GO terms (26.7% of the total GO terms) were shared between all the comparisons, and less than 11 GO terms were comparison-specific. In contrast, numbers of enriched GO terms discovered for upregulated genes in the PCR populations were greatest in the AUS30_PCR1 comparison (60 GO terms), while the other comparisons identified 23 to 36 GO terms upregulated in PCR populations. Among upregulated GO terms in PCR populations, only five were shared by all the comparisons (Fig. 3) and up to 24 GO terms were comparison specific.

Of the GO terms upregulated in both AUS25 and AUS30 populations compared to PCR populations, 11 GO terms were related to biological processes, four GO terms to molecular function and four GO terms for cell components (Table II). Of the GO terms upregulated in PCR populations two of them were related to the category biological function and three of them to category cellular component (Table II).

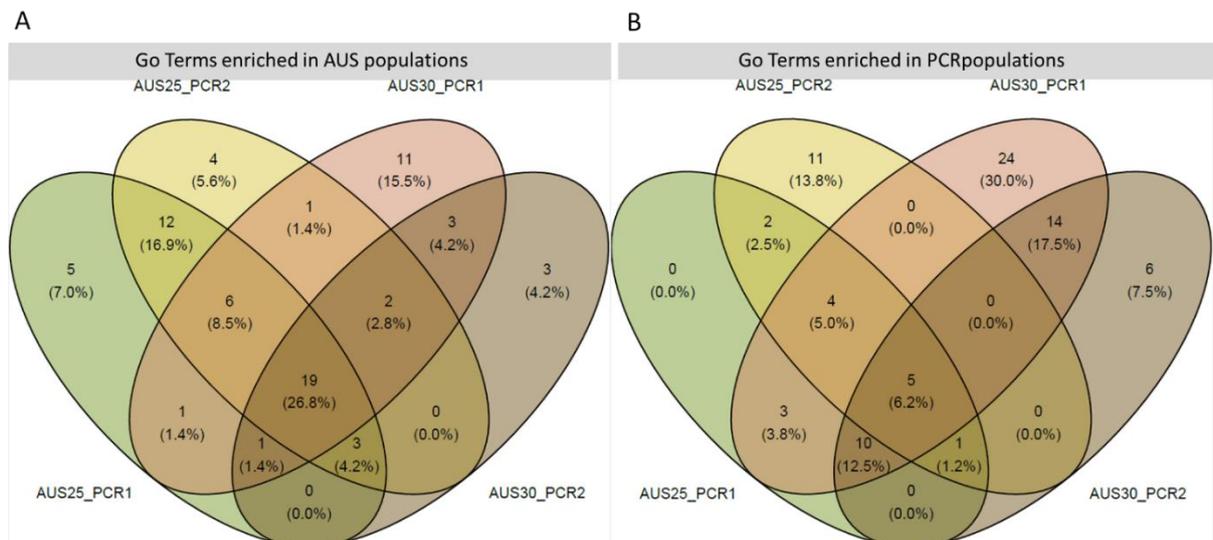


Figure 3: (A) Venn diagrams showing numbers (with percentages) of GO terms enriched in upregulated genes in AUS populations, and (B) upregulated genes in PCR populations. Overlapping regions indicate GO terms that were enriched in two or more comparisons.

Table II: GO terms enriched in all comparisons for both AUS and PCR, go-term name, type, and affiliation are reported.

Region	Go term	category	affiliation
Upregulated AUS	GO:0043207	Biological process	response to external biotic stimulus
	GO:0051707	Biological process	response to other organism
	GO:0009607	Biological process	response to biotic stimulus
	GO:0006950	Biological process	response to stress
	GO:0050896	Biological process	response to stimulus
	GO:0009605	Biological process	response to external stimulus
	GO:0006811	Biological process	ion transport
	GO:0010035	Biological process	response to inorganic substance
	GO:0009651	Biological process	response to salt stress
	GO:0014070	Biological process	response to organic cyclic compound
	GO:1901700	Biological process	response to oxygen-containing compound
	GO:0015075	Molecular function	ion transmembrane transporter activity
	GO:0022891	Molecular function	substrate-specific transmembrane transporter activity
	GO:0022804	Molecular function	active transmembrane transporter activity
	GO:0022892	Molecular function	substrate-specific transporter activity
	GO:0031966	Cellular component	mitochondrial membrane
GO:0005740	Cellular component	mitochondrial envelope	
GO:0005743	Cellular component	mitochondrial inner membrane	
GO:0044429	Cellular component	mitochondrial part	
Upregulated PCR	GO:0050832	Biological process	defence response to fungus
	GO:0009620	Biological process	response to fungus
	GO:0005576	Cellular component	extracellular region
	GO:0030312	Cellular component	external encapsulating structure
	GO:0005618	Cellular component	cell wall

Transcription factor enrichment between AUS and PCR populations

The identity of genes differentially expressed in pairwise population comparisons was investigated to see if proteins commonly associated with environment signal transduction pathways were among the differentially expressed genes. Across all pairwise population comparisons made, 26 transcription factors were upregulated in AUS populations (Table III), mainly bHLH (11) and C2H2 (7). Eleven transcription factor were upregulated in PCR populations (Table III), mainly MADS-MIKC (4) and C3H (4). A few transcriptional regulators were found; seven were upregulated in AUS population and four in PCR. Finally, 17 protein kinases were upregulated in AUS populations and nine were upregulated in PCR populations (Table IV). The main protein kinase family was RLK-Pelle_DLSV for AUS populations and RLK-Pelle_Os for PCR populations. Of the differentially expressed transcription factors and protein kinases identified, only one kinase was detected in an intra-population comparison (PCR1-PCR2, CAMK_CAMKL-CHK1) (Table IV).

Table III : Transcription factors, transcriptional regulators and protein kinases upregulated in AUS and PCR populations

comparison	Up AUS		Up PCR		Up AUS		Up PCR		Up AUS		Up PCR	
	nb	Transcription factors	nb	Transcription factors	nb	Transcriptional regulators	nb	Transcriptional regulators	nb	Protein Kinases	nb	Protein Kinases
AUS25_PCR1	3	bHLH	1	C2C2	1	SET	1	Pseudo ARR-B	1	CAMK_CDPK	1	RLK-Pelle_DLSV
	1	C2H2	1	MADS-MIKC	1	TAZ			2	RLK-Pelle_DLSV		
	1	MYB	1	C3H			1	CMGC_CLK	1	CMGC_CLK		
	1	C2H2-Dof							1	CAMK_CDPK	NA	
	2	C2C2-Dof	1	MADS-MIKC	1	TAZ			1	RLK-Pelle_DLSV		
AUS25_PCR2	3	bHLH	1	C3H					1	CMGC_CLK		
	1	HB-other							1	STE_STE11		
	1	MYB							1	CAMK_CDPK		
	1	HB-HD-ZIP							1	STE_STE11		
	3	bHLH	1	C2C2-LSD	1	MBF1	1	Pseudo ARR-B	1	CAMK_CDPK	1	RLK-Pelle_DLSV
AUS30_PCR1	1	C2H2	1	NAC	1	TAZ	1	SET	1	RLK-Pelle_DLSV	1	CAMK_CDPK
	1	HSF	1	MADS-MIKC					1	CMGC_CLK	3	RLK-Pelle_RLCK-Os
	1	MYB	1	C3H					1	CMGC_GSK		
	1	C2C2-Dof							1	CMGC_GSK		
	3	bHLH	1	MADS-MIKC	1	MBF1	1	SET	1	STE_STE11	1	CAMK_CDPK
AUS30_PCR2	1	MYB	1	C3H	1	TAZ			1	CMGC_GSK	2	RLK-Pelle_RLCK-Os
	1	C2C2-Dof							1	CAMK_CAMKL-CHK1		
AUS30_AUS25	Up AUS30		Up AUS25		Up AUS30		Up AUS25		Up AUS30		Up AUS25	
	NA		NA		NA		NA		NA		NA	
PCR1_PCR2	Up PCR1		Up PCR2		Up PCR1		Up PCR2		Up PCR1		Up PCR2	
	NA		NA		NA		NA		1	CAMK_CAMKL-CHK1	NA	

Discussion

The study described in this chapter reports the first analyses of transcriptome profiles of the sub-Antarctic cushion plant, *L. kerguelensis*, sampled across contrasted environments in the Kerguelen Islands. A number of points are noteworthy:

- (i) Transcriptome expression profiles were found to differ between different regions with contrasted environments. Populations within the same region tended to have similar transcriptome profiles indicating reproducibility of biological replicates.
- (ii) Pairwise comparisons of populations at two regions identified differentially expressed genes concerning abiotic and biotic stress responses. In comparing PCR and AUS regions, the PCR region with greatest environmental variability also exhibited greatest variation in transcription profiles.
- (iii) Gene ontology terms were enriched for genes associated with environmental signal transduction pathways for abiotic stress responses (i.e. transcription factors and protein kinases).

While the *Lyallia kerguelensis* transcriptome data are still at an early stage of exploration and analysis the above findings are encouraging. *In natura*, population-level gene expression differences can inform how distinct environmental variables shape trait variability and evolution in *L. kerguelensis*. At the very least, with further inter region and population analyses of the *L. kerguelensis* data this hypothesis can be rigorously tested. There are additional points to discuss including methodology insights that have importance for ongoing analyses and future studies.

Gene expression and environmental comparisons between populations

Transcriptome expression profiles were population-specific with low variability among individuals. Furthermore, region specificity was also observable. While phylogeographic structuring of genetic variation within and between regions could explain such patterns of variability, the very low levels of genetic variability observed between *L. kerguelensis* cushions (Robert et al. 2021) would suggest that such an explanation is unlikely to explain the transcriptome variability obtained. Populations' specificities were highlighted for environmental variables and might be linked to population transcriptome patterns.

Pairwise gene expression comparisons between AUS and PCR populations

Two of the most contrasted regions for gene expression profiles (AUS and PCR) were selected and their gene ontologies compared. These regions showed clear differences in environmental variables, AUS populations seem to have more similar soil nutrient contents than PCR populations. However, the

topography is similar between PCR populations and different within AUS populations. Most differential gene expression was inferred between regions. Interestingly a higher number of genes were differentially expressed in both AUS populations compared to both PCR populations. This suggests that transcriptome profiles were characteristic of both regions and populations. Finally, when comparing within a region, only a few genes were differentially expressed, suggesting that populations from the same region experienced similar environmental pressures on transcriptomes even if some population scale differences occurred in their respective environments.

Gene ontology term enrichment analyses between AUS and PCR populations

Based on the findings and GO term specificities obtained from our RNASeq analyses of AUS and PCR populations some general conclusions can be drawn about the main region differences for the PCR and AUS populations. Gene ontology analyses recovered terms for all three main categories (biological process, molecular function and cellular component). However, terms were not equally distributed amongst these three categories. Examination of the GO terms enriched in all comparisons for AUS populations were primarily related to external stimuli and stresses. This finding was expected, *in-natura*, a combination of stress is likely to occur in complex environments. We found that responses to abiotic and biotic stress as well as mitochondrial component and ion transporter function were upregulated in AUS populations. Such environmental responses might be expected if *L. kerguelensis* is adapted to its local conditions or micro-environment through plastic expression of its adaptive genetic variation. Several responses to abiotic or biotic stress were found in AUS populations that might be related to local environmental variables including climate. One GO term for response to salt stress was upregulated. Even if soil sodium content was not a primary variable to explain population variabilities in this analysis, it was positively correlated with the extent of *L. kerguelensis* necrosis (Marchand et al. 2021, Chapter 1). Ion transport processes are important for osmotic regulation, and are a key element for plant functioning, especially under conditions of drought or salt stress (Pardo and Quintero 2002; Shabala 2013). Furthermore, mitochondria are essential for maintaining energy and activating acclimation responses during stress responses (reviewed in Suzuki et al. 2012; Choudhury et al. 2017). Recent studies have shown that mitochondria can extend their membrane structure under abiotic stress conditions (reviewed in Choudhury et al. 2017). Given the relative number of abiotic stress terms in AUS compared to PCR populations, an interesting hypothesis to test would be whether abiotic stresses are more intense in AUS populations compared to PCR populations. A contrasting observation between AUS and PCR populations, concerned the enrichment of GO terms for biological processes in the PCR populations. These terms concerned fungal defence and cellular components related to the cell wall and encapsulating structure. The rhizospheres of PCR populations were not sampled in Kerguelen Islands. However, they were for other populations. Numerous unknown fungi were

recorded in the rhizosphere soil of *L. kerguelensis*, especially in the northern population at the region MAC (Chapter 3, Figure S1). Furthermore, 40% of the rhizosphere fungal OTUs were specific to the rhizospheres of MAC population compared to other populations, such as AUS25 (Chapter 3, Figure 4). Studying the rhizomicrobiome, rhizoplane and endosphere of *L. kerguelensis* roots of PCR populations might give insight on a possible specific fungal community composition in this region compared to AUS region. Similarly, differential expression and ontology analyses of MAC populations using the available expression data already collected might also be informative, since the above interpretation from analyses of PCR populations also predicts a similar gene expression response in the MAC3 population. Transcription factors and transcriptional regulators are known to underlie signal transduction pathways in the plastic response of plants (Kim et al. 2018; Hrmova and Hussain 2021; Akhter et al. 2021). More than 2 times the number of transcription factors and protein kinases were upregulated in the AUS populations compared to the PCR populations. Transcription factors belong to diverse families and have been found to play a crucial role in stress signaling (Roy 2016; Kim et al. 2018). For example, Zinc finger C2H2 transcription factors are known to be regulators of various abiotic (salt, osmotic, cold and drought, among others) stress responses in plants that bind DNA, RNA and proteins (Han et al. 2020). Overall expression of transcription factors among individuals of AUS populations was much higher than in the PCR populations (Figure 3). This is consistent with an expectation of abiotic stress responses of *L. kerguelensis* in contrasted environments.

Methodological Issues

An important methodological issue highlighted by the present study concerns the use of short and long read sequences. Both PacBio (long reads) and Illumina (short reads) data were obtained for the current study. This allowed comparison of two different references (PacBio FLNC reads and a Trinity unigenes library) for mapping the Illumina reads. The Trinity assembly had greater transcriptome coverage. The BUSCO analysis indicated that more expected eudicot genes were represented in the Trinity library. However, the PacBio reads were of higher quality, and produced better mapping results than did the Trinity assembly. These findings strengthen the importance of comparing methods to establish a solid library reference for downstream analyses.

Conclusion and perspectives

Region-specific and population-specific transcription profiles were identified in the present study. Similar expression profiles were found for most individuals within populations and for different populations from the same region. The profiles obtained appear to be related to environmental variabilities within and across regions. Ontology analyses from comparisons of two of the most

different regions show that many of the genes differentially expressed in *L. kerguelensis* concern abiotic or biotic stress responses, transcription factors and kinases associated with environmental signal transduction and acclimation responses. The results obtained are encouraging and suggest that transcriptome profiling can provide insight into the relations of *L. kerguelensis* to its environment. These first results indicate directions for future analyses that will aim at comparing transcription profiles from other regions, test results with regional characteristics (such as climatic data used in Chapter 4) or other environmental data, as well as the need to conduct complementary experiments to quantify differentially expressed genes. For example, qPCR validation has not yet been carried out for the differential expression results obtained and it will be interesting to carry out such analyses in the future to confirm our results.

Availability of data:

A further description of the computational pipeline, with detailed software parameters and example R scripts, is available at <https://github.com/jc-henry/Lyallia>.

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Supplementary material

Supplementary material 1:

Illumina read processing

Sequencing read quality was assessed prior to, and after, quality filtering by using FastQC v0.11.9 (Andrews 2010). Rcorrector v1.0.4 (Song and Florea 2015) was used in accordance with best practices recommended by (Freedman 2016) to correct or flag any RNA sequencing reads containing erroneous kmers. Rcorrector appends information to each header in the FASTQ files. To remove this information, which can cause issues with later processes (Freedman 2016) and remove any read pairs for which one read was deemed unfixable by Rcorrector, the script FilterUncorrectablePEfastq.py from the Harvard Informatics GitHub repository TranscriptomeAssemblyTools (<https://github.com/harvardinformatics/TranscriptomeAssemblyTools>) was used. The final step in sequencing read filtering utilised TrimGalore! v0.6.4 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). TrimGalore! was set to remove Illumina adapter contamination from reads, and trim bases from read ends that fell below a threshold of Phred quality score 20. Any read shorter than 50 bp after trimming was discarded. All further analyses used these filtered reads.

PacBio read processing

Raw data (subreads) from Pacific Biosciences (PacBio) single-molecule real-time (SMRT) sequencing were processed to produce polished high quality full-length transcripts. Initially, circular consensus (CCS) reads, with predicted accuracy above 99% were generated using SMRT Link v8.0.0 (PacBio). Subsequently, the IsoSeq v3.2.2 (PacBio) pipeline was used to generate Full-Length, Non-Concatemer (FLNC) reads. The IsoSeq pipeline consisted of three steps. First, CCS reads were correctly orientated, and cDNA primers removed, with the lima command. Second, polyA tails were trimmed, and concatemers removed, using refine. Third, cluster was used to generate the final high-quality FLNC reads used in analyses.

Trinity de novo assembly

Reads from three samples (AUS30_0, AUS30_2, AUS30_3) were used to construct a *de novo* transcriptome with Trinity v2.11.0 (Grabherr et al. 2011). Trinity was run with default parameters except for minimum contig length set at 300. Transcriptome redundancy was reduced by mapping reads (AUS30_0, AUS30_2, AUS30_3) against the Trinity assembly using Salmon v1.5.1 (Patro et al. 2017) and then retaining only single transcripts (unigenes) with the greatest read support per Trinity cluster.

Evaluation of assemblies

Basic assembly statistics were generated using the TrinityStats.pl command from the Trinity suite of tools. Transcriptome completeness was inferred through recovery of expected single-copy orthologues from the OrthoDB v10 (Kriventseva et al. 2018) eudicots database using BUSCO v5.2.2 (Simão et al. 2015). TransDecoder v5.5.0 (<https://github.com/TransDecoder>) was used to identify predicted proteins. For TransDecoder, a minimum size of 100 amino acids was set, and only the single best predicted protein per transcript was output. To match transcripts against *A. thaliana* proteins, the basic local alignment search tool (BLAST) v2.10.0 (Altschul et al. 1990) was used. Protein sequences from the Araport11 genome release (Cheng et al. 2017) were downloaded and a local BLAST database constructed. Against this database, BLASTx was used to search transcript sequences, with a threshold of E-value 1e-05 to exclude poor matches and '-max_hsps' and '-max_target_seqs' set to one to output only the best BLAST match per transcript.

Removal of redundant PacBio transcripts

CD-HIT-EST from the CD-HIT v4.8.1 package (Li and Godzik 2006; Fu et al. 2012) was used to remove identical transcripts (483 transcript (1.27%)) amongst the FLNC reads. To achieve this, strict criteria were applied, and transcripts were only clustered if meeting thresholds of 99% identity and 99% length coverage. Subsequently, quality of the remaining transcripts was assessed once again with TrinityStats.pl, BUSCO, TransDecoder, and BLASTx. These filtered FLNC transcripts were used in the remainder of the analyses. More than 93% reads were successfully annotated against Araport11 protein sequences. These annotations were assigned based on BLASTx matches to 9,883 *A. thaliana* isoforms which corresponded to 9,263 genes. Furthermore, approximately 92% of the PacBio reads produced predicted proteins.

Transcript quantification

Transcript quantification was carried out for all samples by mapping Illumina reads against the FLNC transcripts with Salmon v1.5.1. As recommended in the Salmon documentation (<https://salmon.readthedocs.io/en/latest/salmon.html#using-salmon>), the 'gcBias' and 'validateMappings' flags were enabled.

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Supplementary tables and figures

Table S1: for each Illumina samples sequence, numbers of reads, before and after trimming and filtering, the percentage of sequence retained is indicated.

Sample	Raw Reads	After correction	
		trimming and filtering	% Retained
AUS25_1	21704051	20748457	95,59716294
AUS25_3	21594519	20768929	96,17685395
AUS25_4	19129137	18391305	96,14288925
AUS25_5	20779528	20026572	96,37645282
AUS25_7	21653950	20569626	94,99248867
AUS25_9	19362838	18670698	96,4254207
AUS30_0	19306656	18632133	96,50626706
AUS30_2	19300564	18427826	95,47817359
AUS30_3	20100982	19466557	96,84381091
AUS30_5	18844612	18193083	96,54262449
MAC1_6	21224215	20307801	95,68222429
MAC1_7	21133974	20162314	95,40237913
MAC3_2	19975390	19300854	96,6231648
MAC3_5	20806234	20140358	96,79963226
MAC3_7	21725395	20985268	96,59326332
MAC3_9	21707142	20989611	96,69449345
MAC3_10	22326134	21619672	96,83571728
PCR1_4	19201755	18466897	96,1729644
PCR1_5	21982826	21042334	95,72169656
PCR1_7	20097397	19592530	97,48789856
PCR1_9	20396331	19809123	97,12101162
PCR1_10	20965228	20264953	96,65982645
PCR2_3	21289656	20634500	96,92265577
PCR2_4	23041664	22315490	96,84843074
PCR2_8	21059280	20103402	95,46101291
PCR2_10	20643139	20128438	97,5066728
PCR2_18	21144854	20485611	96,88225324
PDC1_2	20223384	19642872	97,12950118
PDC1_4	18972204	18337329	96,65365711
PDC1_5	20268959	19541670	96,41180882
PDC1_7	20562727	19819563	96,38586847
PDC1_8	19623819	18931659	96,47285781
PDC3_1	21469103	21096648	98,26515807
PDC3_4	22555687	21747332	96,41618098
PDC3_8	22291848	21296732	95,53596454
PDC3_10	19641895	19261327	98,06246801
PJDA6_1	18395722	17793691	96,7273315
PJDA6_2	23425876	22708653	96,93833008
PJDA6_3	19704292	18986514	96,35725049
PJDA6_6	19244559	18657760	96,95083166
PJDA6_7	18623452	17988948	96,59298394
PJDA6_9	21581562	20961866	97,12858597
RBA5_2	21925673	20879832	95,23006204
RBA5_3	19992030	19226079	96,16871823

Table SII: Transcriptome statistics comparison for Trinity non redundant transcript, PacBio reads and non-redundant PacBio sequences

	Trinity non-redundant transcripts	PacBio reads	PacBio non-redundant sequences
Transcriptome Statistics			
Transcript number	134533	38004	37521
%GC	43,28	44,97	44.95
Contig N50	810	2077	2065
Median contig length	495	1711	1706
Average contig length	710,42	1803,71	1797.36
Total assembled bases	95574654	6854835 7	67438580
BUSCO			
Complete BUSCOs	1872	1474	1474
Complete and single-copy BUSCOs	1787	603	606
Complete and duplicated BUSCOs	85	871	868
Fragmented BUSCOs	136	31	31
Missing BUSCOs	318	821	821
BLASTx Araport Hits			
Transcripts with hits	51133	35558	35096
Against unique Arabidopsis isoforms	18999	9889	9883
Against unique Arabidopsis genes	15685	9264	9263
TransDecoder			
Predicted Proteins	57369	34935	34614

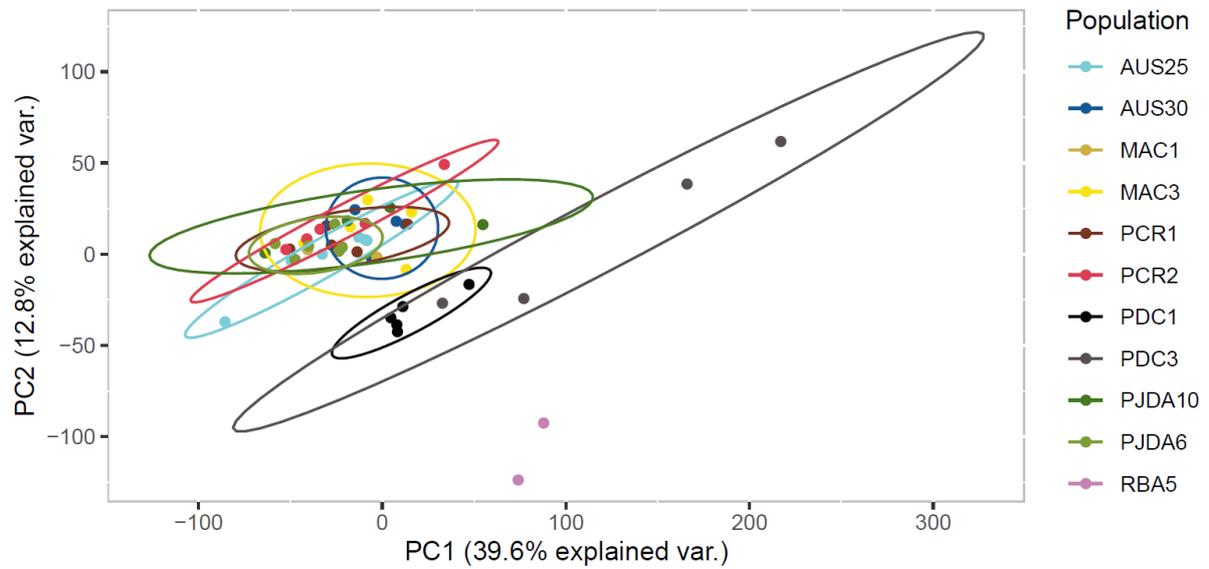


Figure S1: Principal components analysis (PCA) of PacBio transcript counts (transcripts per million-normalised) aggregated to gene level by summing TPM values for all transcripts annotated against the same *Arabidopsis thaliana* proteins for the eleven populations. Each ellipse represents the 95% confidence interval for each population.

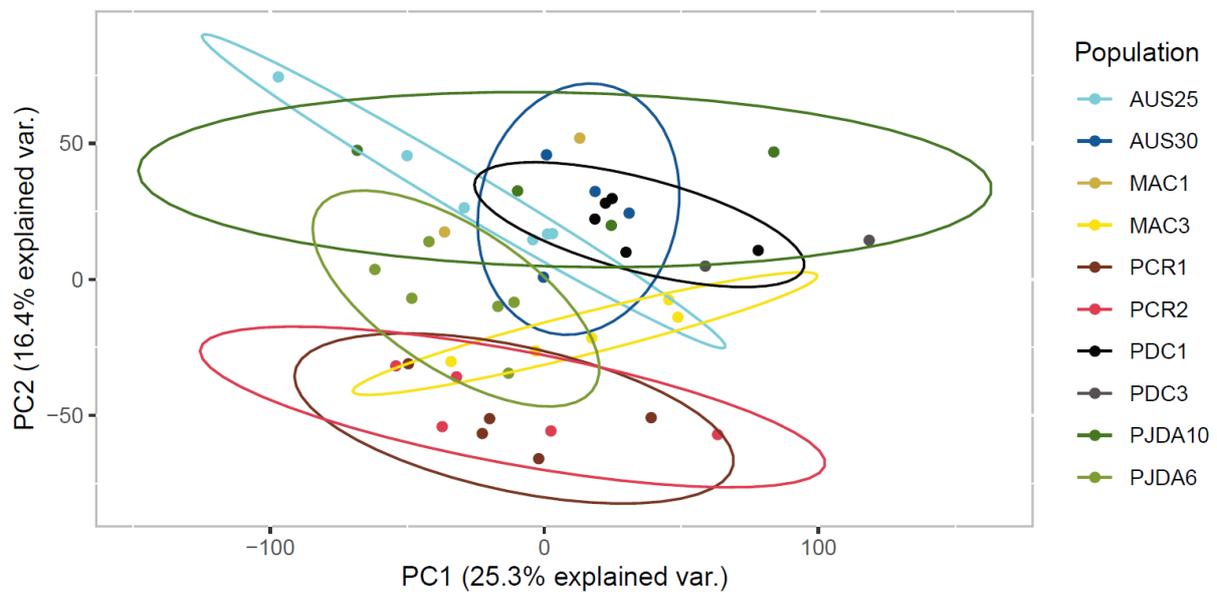


Figure S2: Principal components analysis (PCA) of PacBio transcript counts (transcripts per million-normalised) aggregated to gene level by summing TPM values for all transcripts annotated against the same *Arabidopsis thaliana* proteins for ten populations (outliers removed). Each ellipse represents the 95% confidence interval for each population.

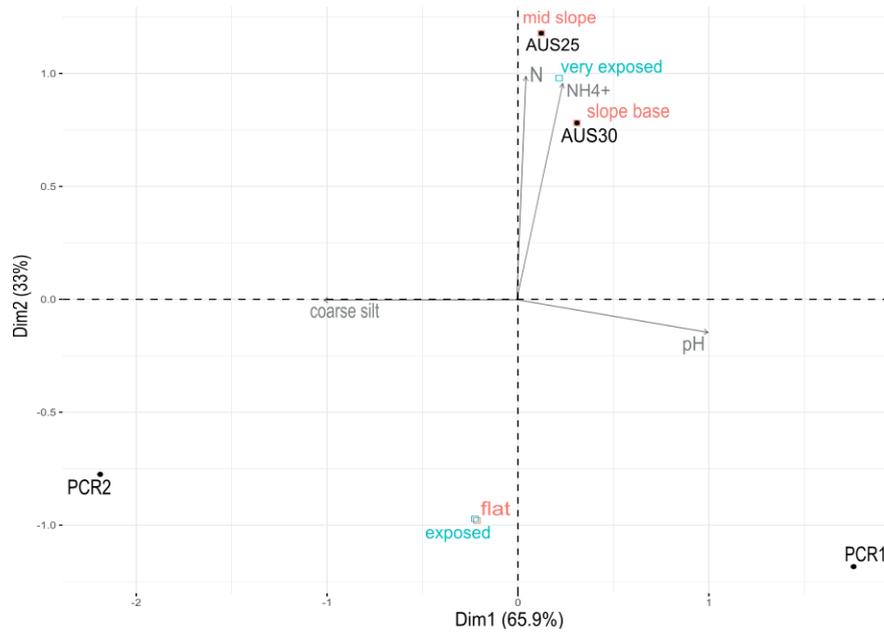


Figure S3: Multiple Factor Analysis (MFA) represents the qualitative and quantitative environmental variables for the four populations studied. Quantitative variables are in grey. Blue color indicates the wind exposure qualitative variable and the red color indicates the topography qualitative variable.

PART II: Soil microbial diversity and composition and its relation with rhizomicrobiome diversity and composition and plant vigour in contrasted environments

Chapter 3: Fellfields of the Kerguelen Islands harbour specific soil microbiome and rhizomicrobiomes of a long-lived endemic cushion plant facing necrosis



Chapter 3: Fellfields of the Kerguelen Islands harbour specific soil microbiome and rhizomicrobiomes of a long-lived endemic cushion plant facing necrosis

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Abstract

The Kerguelen Islands, in the sub-Antarctic, harbour numerous fellfields regarded as a form of tundra ecosystem of rock with cool climate and strong winds. Their soils are poor in nutrients and subject to frequent freeze-thaw cycles. These harsh conditions should select a specific soil microbiome, possibly including endemic taxa due to geographical isolation. Kerguelen island fellfields harbour an endemic and long-lived plant, *L. kerguelensis*, which shows necrosis on parts of its cushions. Firstly, *L. kerguelensis* rhizosphere may act as a reservoir of microbiota for fellfield soils. Secondly, its necrosis might be correlated to specific taxa. Bacteria and fungi were characterised by metabarcoding in the microbiome and rhizomicrobiome in five sites. The diversity is poor all over the island soils, probably due to dispersal limitations and harsh conditions. We observed generalist and endemic taxa in the microbiome and rhizomicrobiome with site specificities. Ammonium and phosphorus were the main drivers of the site microbial variability. The rhizomicrobiome harbours as many microorganisms as the bulk soil but is composed of different taxa that might sustain important roles for soil functioning. The necrosis extent was not related to specific microbiota but might be enhanced by modification of their functions.

Keywords: rhizomicrobiome, fellfield, sub-Antarctic, bacteria, fungi, *Lyallia kerguelensis*

Introduction

Fellfields are habitats characteristic of polar, sub-polar and alpine regions consisting of a form of tundra ecosystem made of rock with sparse vegetation and infertile soil [1, 2]. Fellfields experience harsh climatic conditions, including cool or cold climate, heavy precipitation and strong wind and present rough living conditions for many organisms, including soil microorganisms [3–6]. Their soils are subject to numerous freeze-thaw cycles and to rapid fluctuations of both temperature and water content. In Antarctic and sub-Antarctic regions, fellfield soils are also characterised by low contents in nitrogen (both nitrate and ammonium forms), phosphorus (as it is mainly under inorganic form unavailable for organisms) and both carbon and organic matter when vegetation cover is scarce [1, 5, 7–10]. In sub-Antarctic islands, fellfields are subjected to an oceanic climate and cool temperatures [11]. Their soils are influenced by marine sea spray and bird colonies [12, 13]. Conditions that prevail in the fellfields of the sub-Antarctic islands might have select for tolerant and specific microorganisms as observed in the Arctic and Antarctic fellfields [14–16]. However, even in remote locations and harsh environments, widespread and generalist microbes are known to settle [14, 17, 18] and should be found in the sub-Antarctic island fellfields.

The Kerguelen Islands, located in the Southern Indian Ocean, are the largest archipelago of the sub-Antarctic region [19]. The Kerguelen Islands are isolated from at least 3000km to the nearest continents. Therefore, dispersal limitation of microorganisms may occur, leading to narrow microbial diversity in the Antarctic continent [9]. Furthermore, strong winds may transport microorganisms over long distances for other close islands, as observed in the Arctic [18, 20]. Kerguelen Island fellfield soil microbial communities might be shaped by both dispersal limitation and aeolian transport leading to complex assemblages of endemic and cosmopolitan microbes. Within the archipelago, environmental filtering might also drive the assembly of soil microbiome, as fellfields show contrasted edaphic and climatic conditions [21], it was already observed in the heterogeneous Arctic landscape [17]. Due to the low herbivore pressure in some parts of the Kerguelen Islands, soil microbes should sustain key roles in ecosystem functioning as the food webs in these fellfields are mainly driven by decomposition processes that involve microbial activity [9, 14]. Fellfields are particularly threatened by fast climate change affecting the Kerguelen Islands. Temperatures shifted from a 10-y mean temperature of 4.43°C in the 1950s to 5.1°C in the 2010s [22] and dry trend associated with a marked shift in the storm track location is observed [23, 24]. These changes affect the vegetation [25] and may also lead to modifications in soil bacterial and fungal diversities with negative effects on soil functioning as experimentally observed in alpine and polar fellfields [2, 26–28]. For example, a shift toward generalist microorganisms was found with experimental temperature increase in the sub-Antarctic Falkland

Island fellfields associated with a possible increase of soil respiration [26]. There is thus a need to explore the soil microbiome and its spatial structure in these environments.

Kerguelen Island fellfields host scarce vegetation tolerant of windy and freezing environments and composed of plants such as small forbs, short grasses and cushion plants [29]. Among them, *Lyallia kerguelensis*, Montiaceae [19] is an endemic cushion plant. Cushion shapes are known to reduce temperature and internal humidity fluctuations in plants and increase soil organic matter content making the underneath soil a favorable habitat for soil microbiota compared to the surrounding nutrient-poor soil [30–33]. Necrotic parts in *L. kerguelensis* cushions were described for the first time in the early 1990s [34] and are apparently concerned with populations of the whole archipelago [19, 21]. Necrosis might be a symptom of plant dieback (i.e. the impossibility of the plant to generate new foliage) [35, 36]. Previous studies identified relations between necrosis extent of *L. kerguelensis* and both the soil salinity and the increase of winter temperature and decrease of winter precipitation [21, 37]. These two features being affected by climate change, *L. kerguelensis* may thus be seen as an indicator species for climate change effect on fellfield biota.

As *L. kerguelensis* is a long-lived species, the composition of its rhizomicrobiome might result from both co-evolved species assemblage and active selection [15, 38–40]. The assembly of the rhizomicrobiome governed by plants is known to be directed towards beneficial microorganisms that enhance the plant's capacity to adapt to its environment [41]. The role of the rhizomicrobiome in plant stress resistance was highlighted in various contexts (e.g., drought, starvation, frost) [38, 42, 43]. Plant rhizomicrobiome selection should be even more meaningful under harsh environmental conditions facing climate change. For example, in the high Arctic Svalbard archipelago, a decrease in pioneer ectomycorrhiza of long-lived plants was highlighted with warming climate [15]. Similarly, in sub-Antarctic Macquarie Island, cushions of *Azorella macquariensis* were primarily stressed by reducing water availability that made them more susceptible to pathogens [15, 44]. Hence, microorganisms should be valuable for the plant to cope with stresses and sustain vigour and thus help to preserve endemic keystone plant species such as *L. kerguelensis*.

In this study, we conducted a field survey first to describe the composition of the soil bacterial and fungal communities in different fellfields of the Kerguelen Islands and analyse its spatial variability using amplicon sequencing. Microbial diversity in fellfield soils remains understudied despite the potentially meaningful participation of microbes in soil functioning and nutrient cycling in such particular habitats. We thus aimed to identify an endemic soil microbiome and the abiotic properties that lead to environmental filtering between different fellfields of the Kerguelen Islands. Then we characterised the rhizomicrobiome of different populations of *L. kerguelensis* and investigated its potential role for *L. kerguelensis* to face necrosis. We expected necrosis extent to be related to the lower abundance of specific beneficial microorganisms in the rhizosphere of *L. kerguelensis*.

Material and methods

Study sites and model plant

The Kerguelen Islands (48°30'– 50°00' S, 68°27' – 70°35' E) comprises a large island surrounded by about 60 small islands [19]. They are characterized by basaltic rock, hilly topography, and harbour numerous fellfields. The climate is chronically cold with a mean annual temperature of 4.6°C and a constant wind-inducing salt spray all over the islands [46]. Our study took place during two summer field campaigns from December 2017 to January 2018 and from December 2018 to March 2019. Five fellfield sites were studied; one site in “Ile Mac Murdo” in the northern part of the archipelago (MAC1), and four sites in the south-eastern part, in “Ile Longue” (LON30), in “Presqu’île Jeanne d’Arc” (PJDA2 and PJDA6) and in “Ile Australia” (AUS25) (Fig. 1). Each site consisted of a geographical area of less than 50 m², homogeneous in geomorphology, topography and vegetation and that harboured a continuous population of *L. kerguelensis* (Table I). MAC1 in the north is wetter than the other sites located in the southeast (on average +33% of precipitation on the period 2004-2013 [37]). MAC1, AUS25 and LON30 range from 55 to 154m while PJDA2 and PJDA6 were at higher altitudes (482 and 520 meters, respectively) that should induce colder temperatures and more frequent freeze-thaw cycles than the other sites (Table I) [25]. The soils were slightly acid and had a loamy-sand texture, except in LON30 in which it was a sandy-loam soil, more acid and richer in organic carbon and nitrogen contents than the other soils. In all sites (except PJDA2) nitrogen was preferentially under ammonium form.

Lyallia kerguelensis Hook. f. belongs to Montiaceae [47]. This species is strictly endemic to the Kerguelen Islands and has a sparse distribution in fellfields [19]. Species populations comprise between a dozen to rarely a hundred individuals, in most cases scattered on a scale of meters (L. J. Marchand and F. Hennion personal observations). *L. kerguelensis* is a long-lived (more than 16 years) perennial cushion with a hemispherical shape of 20 – 40 cm diameter, rarely more [19]. The root system is deep with a main taproot and numerous fine roots branching out [48, 49]. Within each site, ten plants showing variable necrotic surface areas were randomly selected. The necrotic surface area of each plant (further called necrosis extent) was measured by photointerpretation [21] (Table SII).

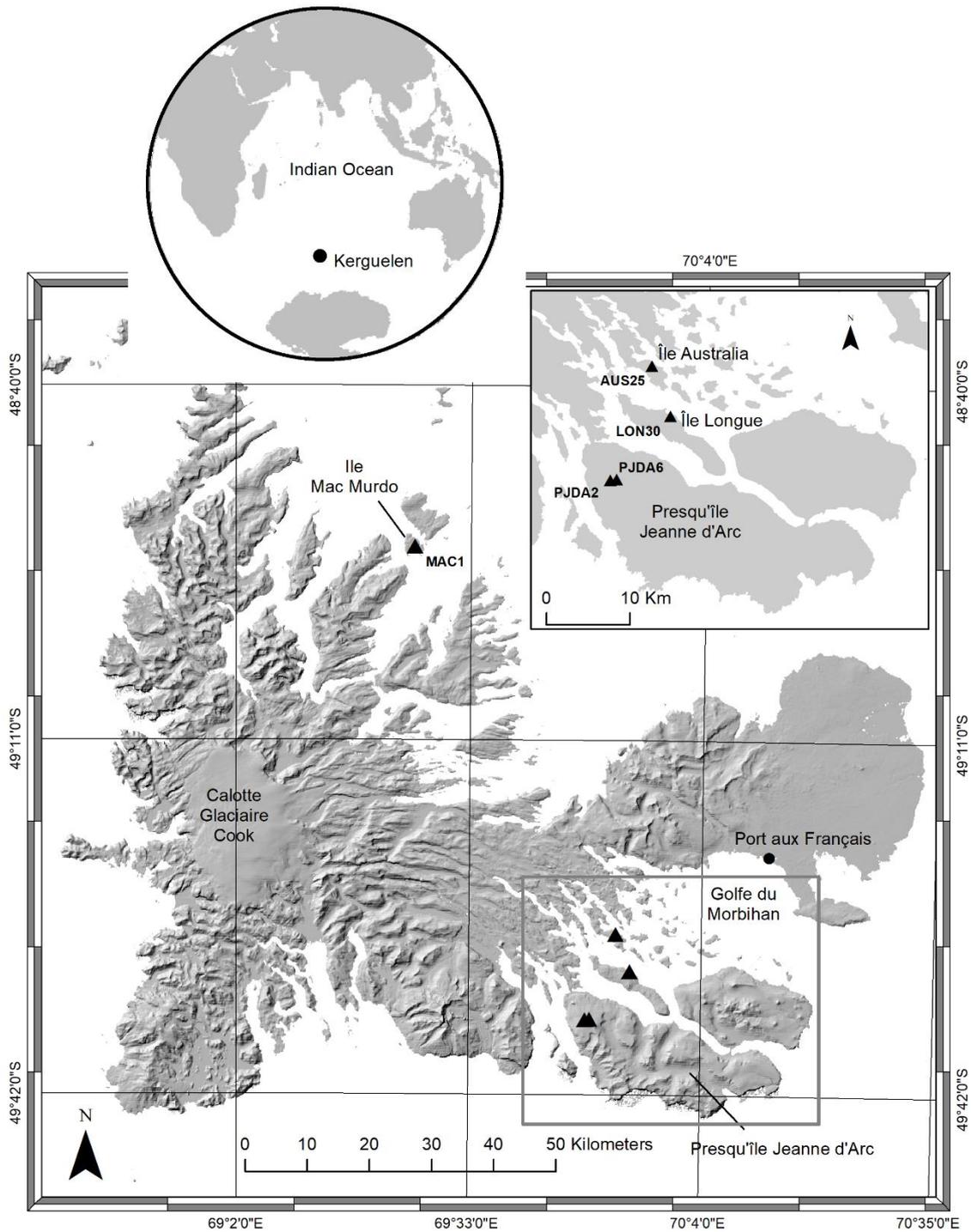


Figure 1: Locations of the five sampling sites (black triangles) in Kerguelen Islands on an altitudinal background (NASA–SRTM 30 M, 2005); the black dot indicates the research station, labels indicate site names and important features in the island, the grey rectangle shows the localisation represent on the top right map. Top right map: zoom on the South-East part with the four studied sites. Map background 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.

Table 1: Site GPS coordinates and soil physico-chemical properties, “*” indicates the variables removed due to correlation above 90% 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

Soil sampling

Rhizosphere soil was collected under the cushion of each selected plant within their root systems at 2 - 8 cm depth. A composite bulk soil of 200 mL was done by pooling three samples of bare surface soil collected at different places within each site and assumed to be free of root influence. For both kinds, rhizosphere and bulk soil samples, three subsamples were constituted and stored at -20°C for replicated DNA extraction. Furthermore, for bulk soil, 50 mL were used for molecular analyses and nitrate determination (Laboratoire d'Analyse des Sols, INRAE, France). Thirty milliliters of fresh soil in deionised water were used for the pH measurement (using a ratio of 1:1 soil: water (v/v) BASIC 20 PLUS CRISON, Spain). The remaining fresh soil was used for soil physico-chemical analyses (Laboratoire d'Analyse des Sols, INRAE, France).

DNA extraction

Three replicates of 0.5 g each of soil sample were made and DNA extraction was processed following the protocol of Griffiths et al. 2000 [50] with the modification applied in Monard et al. 2013 [51]. DNA quality and quantity were checked using NanoDrop device (ThermoFisher Scientific, Waltham, USA) and with agarose gel electrophoresis (1%, TAE 1X). DNA extracts were stored at -20 °C.

PCR amplification and Illumina sequencing

The bacterial 16S rRNA gene library and the fungal ITS libraries were conducted using the protocol given by the Illumina platform [52], which is a two-step PCR approach. The following primer sets were used for bacteria: 341F [53] and 785R [54] and for fungi: ITS1F [55] and ITS2 [56]. Each primer set contained the overhang adapter: forward overhang (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse overhang (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3') and targeted the variable V3 and V4 regions of the 16S rRNA gene for the bacteria, and the ITS1 region for fungi, respectively. PCR was 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 (for fungal amplification only) (100 ng/µL, SIGMA, France), 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 were performed per sample and pooled. 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 [57]. 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 chimeras were removed, clustering was performed using the Swarm method with a distance difference of 3 and OTUs with relative abundance below $5 \cdot 10^{-6}$ were removed [58]. The taxonomic affiliation was done using BLAST (NCBI, USA) with the Silva_132_16S database for bacteria and the Unite_Fungi_8.0_18112018 (including a RDP assignment) for fungi. Only OTUs present in at least two out of the three replicated samples were kept. 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 and used for further analyses. Six replicates were removed for bacteria and twelve for fungi from different samples due to their too low number of sequences. In addition, for bacteria, one individual (all three replicates) from the AUS25 site

was removed for the same reason. All data are available with the accession number SRA data: PRJNA731349.

Data analyses

Statistical analyses were done using R version 3.4.2 and the Phyloseq package [59]. Graphs were created with the ggplot2 package. 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 an α -error of 0.01 was chosen to focus on highly significant relations.

To assess the microbial α -diversity, species richness and Shannon diversity index were calculated and the bacterial and fungal datasets were rarefied at 3 758 and 172 sequences per sample, respectively. The choice of the rarefaction level was motivated by a trade-off between keeping a sufficient sampling depth to detect the most prevalent taxa and keeping a maximum of replicates per sample. Dunn's Kruskal-Wallis multiple comparisons (DunnTest function, package FSA) were applied to study the spatial variability of microbial diversities (species richness and Shannon index) in bulk soils and rhizosphere soils across sites and between the rhizosphere and the associated bulk soil.

To study the OTU composition variability, a core analysis (*core()*, microbiome package) was performed, for each site and across sites, for both bulk and rhizosphere samples, and complemented by Venn diagrams (VennDiagram package) representation of shared OTU between sites, and between individuals and their associated bulk soil.

Bacterial and fungal community dissimilarity between sites were studied with non-metric multidimensional scaling (NMDS) ordination with weighted unfrac distances (*ordinates()*, package Phyloseq [44]). Correlations between microbial community composition and soil physico-chemical properties were done (*enfit()*, Vegan package), significantly auto-correlated soil properties were previously removed. To evidence OTUs that explained the observed differences between the bulk soils of the five different sites, after that called indicator OTUs, we applied a multi-level pattern analysis (*multipatt()*, indicpecies package, [60]) with the "r.g" function and 999 permutations.

Finally, for each sampling site, Pearson correlations 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 were present in at least 85% and 65% of the rhizosphere samples of a site for bacteria and fungi, respectively. These thresholds were motivated by a trade-off between focusing on OTUs generalist in the rhizosphere samples and keeping enough OTUs for the analyses.

Results

Soil bacterial and fungal communities and their variability across fellfields of the Kerguelen Islands

On average per site, the richness soil bacterial and fungal ranged from 337.6 to 590.3 OTUs and from 6.5 to 57.3 OTUs, respectively and Shannon index ranged from 4.4 to 5.8 and from 1.4 to 3.3, respectively; both presenting differences between sites (Fig. 2A, C, E and G; Richness: $Z = 3.22$ and 1.96 , and p -value = 0.001 and 0.049 , Shannon index: $Z = 3.22$ and 2.11 , and p -value = 0.001 and 0.035 , for bacteria and fungi respectively). Microbial diversity was the highest in AUS25 and the lowest in PJDA2 and fungal diversity was also high in PJDA6 (Fig. 2A, E, C and G). While PJDA2 and PJDA6 fellfield sites were the closest geographically (Fig. 1), they presented significant differences in the diversity of their soil microbiome (except bacterial Shannon index). PJDA6 being always significantly richer (Richness $Z = 2.34$ and 2.70 , and p -value = 0.019 and 0.007 , Shannon index $Z = 1.37$ and 2.59 and p -value = 0.17 and 0.010 for bacteria and fungi respectively) (Fig. 2A, E, C and G). Interestingly, microbial species richness and Shannon index in the soil of MAC1; the only fellfield site in the northernmost location (Fig. 1), did not significantly differ from those observed in the other sites (Fig. 2A, E, C and 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 (Fig. 3A). *Chloroflexi* were always the most represented taxa (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, Fig. 3A). Interestingly, the *Proteobacteria* phylum was mainly composed of α -*Proteobacteria*. The dominant fungal taxa detected in the bulk soils of the five sites were *Ascomycota*, *Basidiomycota*, *Mortierellomycota* and *Rozellomycota*, and their relative abundance highly differed between sites (Fig. 3B). The relative abundance of *Ascomycota* was at least 32% in each bulk soil (Fig. 3B). This phylum was dominant in MAC1, LON30 and PJDA6 sites with a relative abundance of 42 %, 57 % and 51 %, respectively, while the *Basidiomycota* dominated PJDA2 site (56 % of the total amount of sequences, Fig. 3B). 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; Fig 3B).

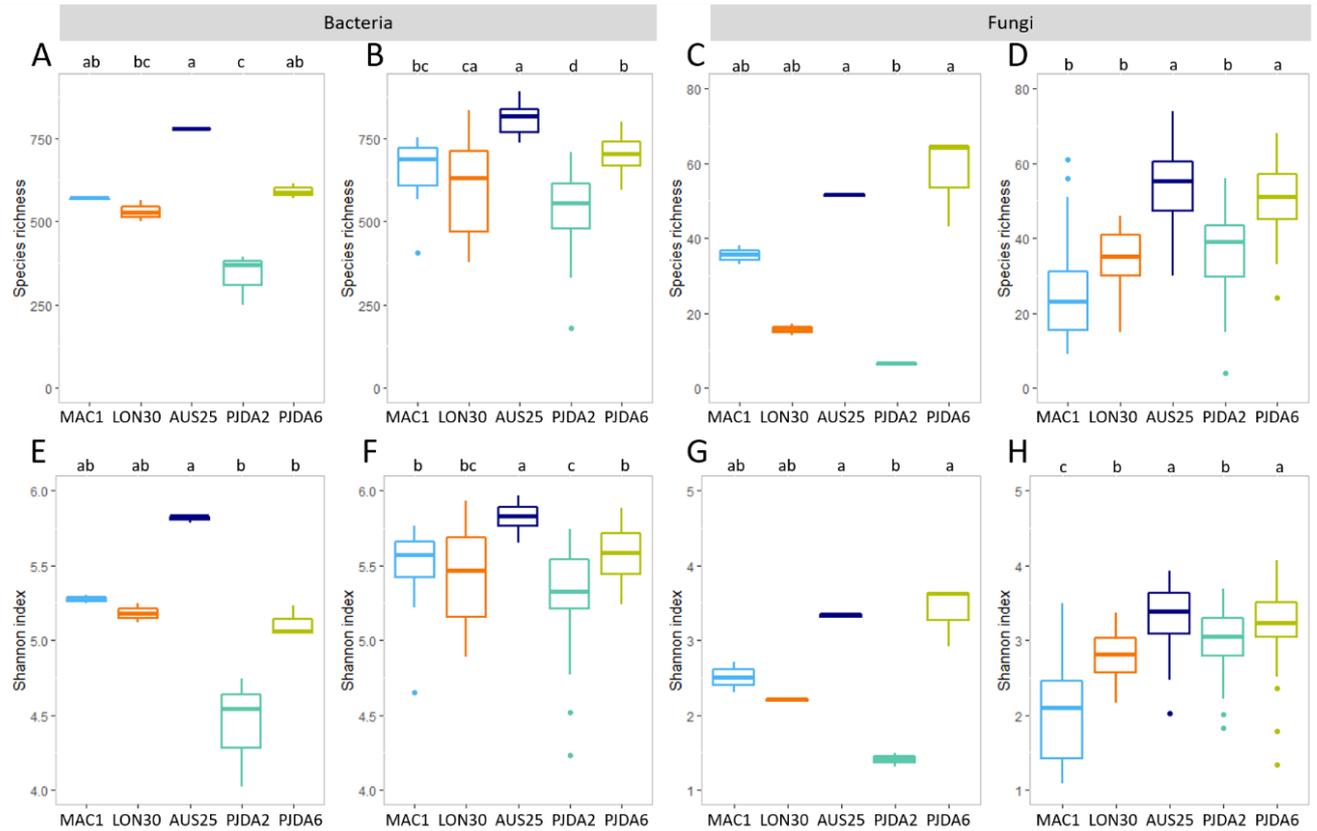


Figure 2: Bacterial species richness in bulk soils (A) and rhizosphere soils (B) of each site. Fungal species richness in bulk soils (C) and rhizosphere soils (D) of each site. Bacterial Shannon index in bulk soils (E) and rhizosphere soils (F) of each site. Fungal Shannon index in bulk soils (G) and rhizosphere soils (H) of each site. Different letters indicate significant differences (Dunn's Kruskal-Wallis).

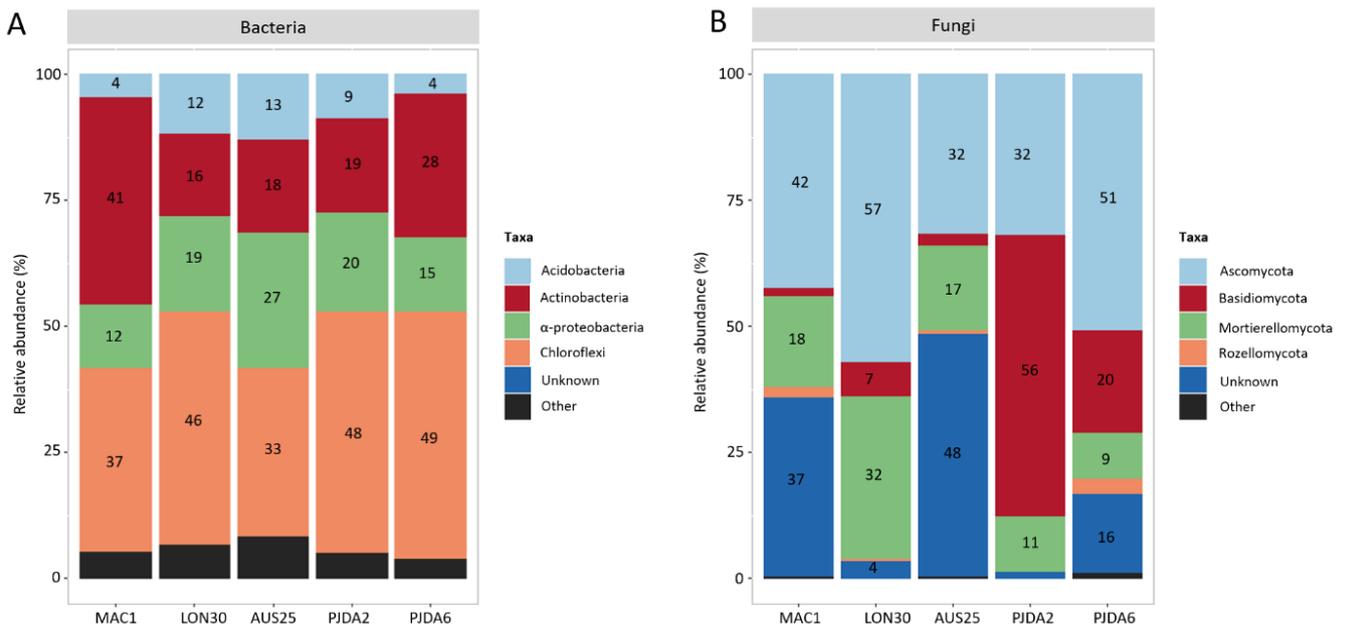


Figure 3: Relative abundance of the four dominant bacterial (A) and fungal (B) taxa present in the bulk soil of each site. Numbers indicate the numbers of OTUs in the corresponding taxa.

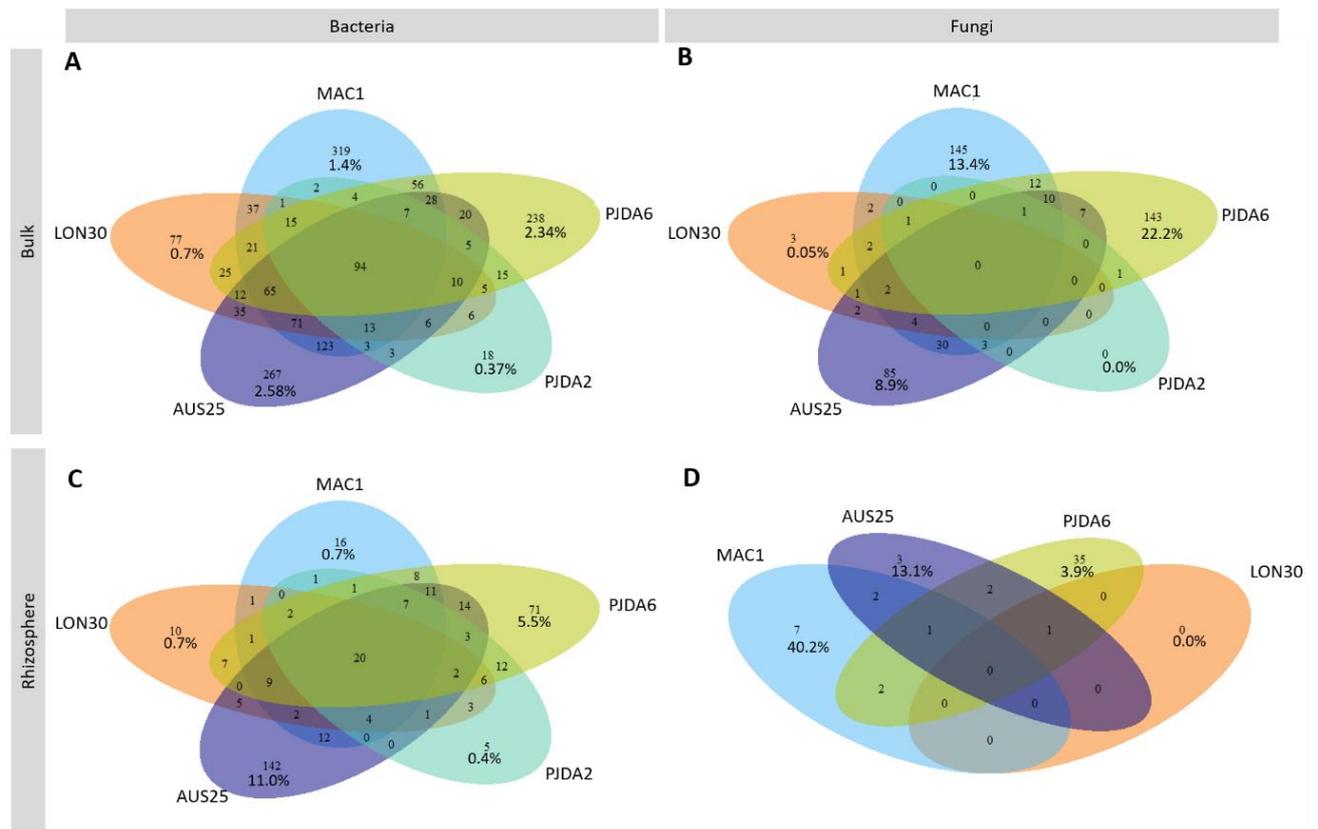


Figure 4: the bulk core-microbiome for bacteria (A) and fungi (B) and the core-rhizomicrobiome for bacteria (C) and fungi (D). The numbers indicate the number of OTUs. PJDA2 has no fungal core-microbiome.

The OTUs detected in all the five sites were considered cosmopolitan within the Kerguelen Islands, constituting the soil core-microbiome of this particular habitat. This microbiome was composed of 94 bacterial OTUs, but no fungal one, corresponding to a relative abundance of 52 % of the total amount of bacterial bulk soil sequences (Fig. 4A and Table S1). On top of the four dominant bacterial phyla, members of the *Armatimonadetes*, *Planctomycetes* and *WSP-2* phyla were also identified among the core microbiome (Fig. 4B and Table S1). On top of this soil core-microbiome, each site harboured a specialized soil microbiome, composed of 18 to 319 site-specific bacterial OTUs and up to 145 site-specific fungal OTUs (Fig. 4A and B).

Spatial variability of the soil microbiome of the Kerguelen Islands was observed with the NMDS ordination and some soil physico-chemical properties were identified as significantly involved in microbial community structure (Fig. 5A and B). In particular, phosphorus and ammonium contents were significantly related to both bacterial and fungal community compositions (Fig. 5A and B). Bacterial communities were also influenced by soil pH and contents in nitrate and sodium, while fungal communities responded to CEC (Fig. 5A and B).

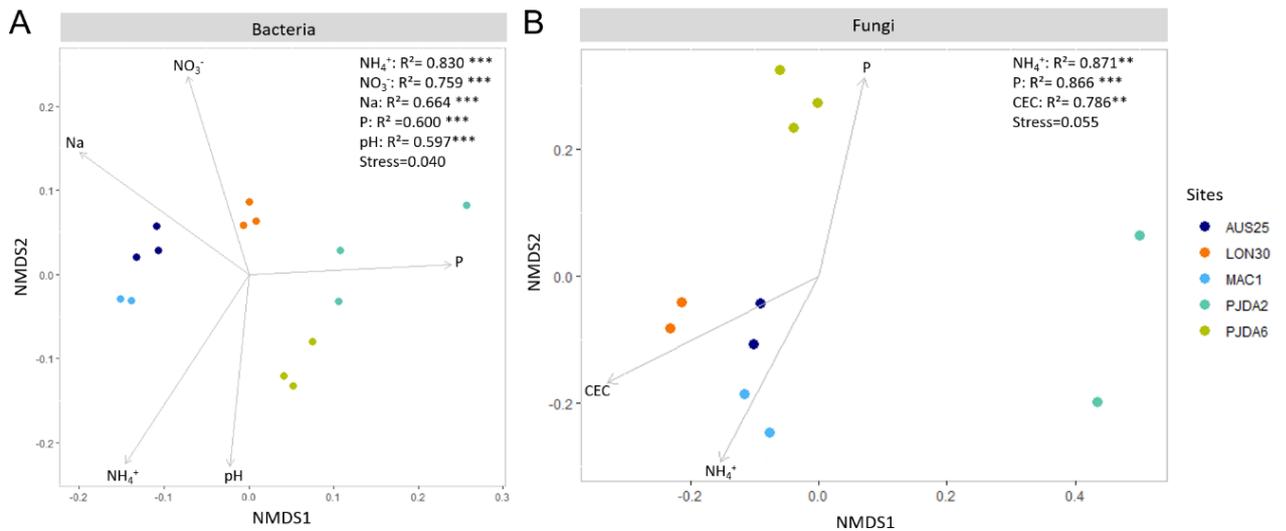


Figure 5: Non-metric multidimensional scaling (NMDS) ordination showing significant ($p < 0.01$) relationship between soil physico-chemical properties and the bacterial (A) and fungal (B) communities composition in the bulk soils of each site.

To characterise the soil microbiome of each fellfield site, 1 011 indicator OTUs were identified, representing 18% and 22% of the total amount of sequences for bacteria and fungi, respectively (Table SIII). Most of these indicator OTUs were bacterial (937 out of 1011) and among them, a high proportion (758 out of 937) was affiliated to the four dominant phyla. The thirty-three indicator OTUs with a relative abundance higher than 1% were further considered key indicator OTUs (Table II). Most of them (twenty) were observed in the soil from PJDA6, while none were detected in the soil from AUS25 and members of the fungal domain were only observed in the two PJDA fellfield sites (Table II). The soil from MAC1 site was characterised by a *Pseudoarthrobacter* sp. (OTU 2) and soil from LON30 by an *AD3* class OTU (OTU 66). *Rhodococcus* sp. (OTU 11) and *Mycosphaerella tassiana* (OTU 94) were indicators of the PJDA2 soil and *Jatrophihabitans* sp. (OTU 10) and *Cryptococcus* sp. (OTU 5) indicators of PJDA6 soil among other OTUs (Table II).

*The assemblage of the microbiome in the rhizosphere of *L. kerguelensis**

The diversity (richness and Shannon index) of the *L. kerguelensis* rhizomicrobiome across the five sites showed similar patterns than previously observed for the bulk soil microbiome (Fig. 2B, F, D and H). However, significant changes in the species richness and Shannon index were observed between the individual rhizomicrobiome and the corresponding bulk soil microbiome (Table SII). Interestingly most of these significant differences corresponded to an enrichment of the microbial diversity in the rhizosphere compared to the bulk soil (Table SII). Significant depletions were only observed for fungal communities in MAC1 and PJDA6 sites (Table SII).

Within each fellfield site, whatever the plant rhizomicrobiome considered, the bacterial communities were dominated by the same phyla as observed in the corresponding bulk soils and showed a minor variation of abundance between sites. (Fig. S1A). On the contrary, in each site, the composition of the rhizosphere fungal communities was plant-specific (Fig. S1B).

A core-rhizomicrobiome of *L. kerguelensis* was identified across the fifty plants among the five sites. It was composed of twenty bacterial OTUs (relative abundance of 18 % of the total amount of bacterial rhizosphere sequences) but none fungal OTU (Fig. 4C and D, Table SI). Among these bacterial OTUs, eighteen also belonged to the bulk soil core-microbiome, while two OTUs identified as *Acidimicrobiia* were only detected in the rhizosphere (Table SI). Each site showed its core-rhizomicrobiome, composed of five to 142 site-specific bacterial OTUs and up to thirty-five site-specific fungal OTUs (Fig. 4C and D). Some OTUs were specific to the rhizosphere and not detected in the corresponding bulk soil (Fig. S2 and S3). For bacteria, their relative abundance varied from 26% to 69% of a single plant's total amount of sequences and significantly differed between some sites (table SIV). 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. Moreover, the relative abundance of these OTUs specific to individual plants significantly differed between each site (Fig. S2, S3 and Table SIV).

Relationship between plant necrosis extent and composition of the rhizomicrobiome

The correlative analyses identified 14 and 15 bacterial OTUs positively and negatively correlated to necrosis extent, respectively, and 10 and 4 fungal OTUs positively and negatively correlated to necrosis extent, respectively (Table III). No general pattern could be observed across sites, OTUs correlated with necrosis extent of *L. kerguelensis* being site-specific (Table III). However, regardless of the direction of the correlation, several OTUs related to specific microbial functions were observed. OTUs corresponding to bacteria and/or fungi potentially involved in N₂ fixation (orders *Acetobacterales*, *Ktedonobacterales*, *Rhizobiales*, *Frankiales* and class *KD4-96*) and organic matter degradation (orders

Thermomicrobiales, *Tremellales*, *Mortierellales* and class *TK10* and *Agaricomycetes*) were detected in all the sites except PJDA6 (Table III). Also, correlated bacterial OTUs characteristics of low phosphorus soil content (orders *IMCC26256*, *Gemmatales*, *Microtrichales*, *Solibacterales*) were present in all the sites except PJDA2 (Table III).

Table III: Significant ($\alpha=0.01$) Pearson correlation between the relative abundance of OTUs and the necrotic extent. OTUs present in 60% or 85% of the samples (for fungi and bacteria respectively) with a mean site abundance higher than 0.1% are present in the table. Taxonomy of the Blast sequence associated are indicated.

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	low phosphorus soil
	OTU_223	0.357	93.103	-3.478	0.002	Bacteria	Actinobacteria	Acidimicrobia	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.	low phosphorus soil
	OTU_287	0.134	100.000	-2.810	0.009	Bacteria	Actinobacteria	Acidimicrobia	Microtrichiales	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
LON30	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	degradation
	OTU_7	3.880	100.000	4.471	<0.001	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi_sp	degradation
	OTU_1	12.704	100.000	6.201	<0.001	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi_sp	degradation
	OTU_46	0.205	100.000	3.004	0.006	Bacteria	Actinobacteria	Acidimicrobia	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	Rhodospseudomonas	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	degradation
	OTU_11	10.388	92.593	3.940	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	unidentified	unidentified	Helotiales_sp	degradation
	OTU_1	5.221	100.000	-4.977	<0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Bradyrhizobium	metagenome	N ₂ fixation
AUS25	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	Acidobacteria	Soilbacteriales	Soilbacteraceae	Candidatus	unknown species	low phosphorus soil
	OTU_93	0.444	100.000	-2.813	0.009	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	(Subgroup 3)	Soilbacter	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	N ₂ fixation
	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	Acidimicrobia	Microtrichiales	Ilumatobacteraceae	Ilumatobacteraceae 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	N ₂ fixation
	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	Acidimicrobia	Microtrichiales	lamiaeae	lamia	unknown species	low phosphorus soil
OTU_343	0.141	100.000	4.828	<0.001	Bacteria	Actinobacteria	Acidimicrobia	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	N ₂ fixation	
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	degradation	
OTU_163	0.802	92.857	2.934	0.007	Fungi	Rozellomycota	unidentified	unidentified	unidentified	unidentified	Rozellomycota_sp	degradation	
OTU_12	1.696	92.857	3.119	0.004	Fungi	Basidiomycota	Agaricomycetes	unidentified	unidentified	unidentified	unidentified	Agaricomycetes_sp	degradation
OTU_40	0.554	92.857	3.839	0.001	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	Mortierella	Mortierella	organic matter degradation	
OTU_47	0.144	60.714	7.613	<0.001	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Ophiocordycipitaceae	Tolyposcladium	Tolyposcladium sp	degradation	
OTU_86	0.385	100.000	-4.235	<0.001	Bacteria	Chloroflexi	Chloroflexia	Thermomicrobiales	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	Xanthobacteraceae	unknown genus	unknown species	N ₂ fixation
OTU_131	0.258	100.000	3.240	0.003	Bacteria	Actinobacteria	Acidimicrobia	Microtrichiales	Ilumatobacteraceae	Ilumatobacteraceae group	unknown species	low phosphorus soil	
OTU_288	0.190	65.517	-3.239	0.003	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi_sp	degradation
OTU_251	0.273	79.310	-3.134	0.004	Fungi	unidentified	unidentified	unidentified	unidentified	unidentified	unidentified	Fungi_sp	degradation

Discussion

Extensive knowledge of fellfield soil microbiomes in alpine, polar and sub-polar regions exist. However, even if this type of habitat is similar between regions, they harbour intrinsic differences that lead to the variability of the microbial composition. Therefore, in-depth comparisons of the microbial community between studies seems complicated.

Soil microbial communities in fellfields and specificity of the Kerguelen Islands

Geographical isolation, harsh climatic and edaphic conditions of fellfields should select for an adapted soil microbiome characterised by low diversity, as observed for the endemic microbiota in the Antarctic continent [9, 14, 61]. Within fellfields, regional climate strongly influences the composition of bacterial and especially fungal microbiotas [9, 26, 39, 62–66]. Fellfields from the sub-Antarctic region are under a marine influence with salt-spray compared to continental fellfields. They should highly select for specific soil microbiome adapted to these particular conditions. On top of these specialised soil microbiome, we expected microbial communities to include a non-negligible proportion of generalist fungi and bacteria as previously observed in the Antarctic soil [9, 14]. These generalist microbiomes might disperse through aeolian transport that could appear at a large scale as observed in Antarctica or Greenland and should occur in the Kerguelen Islands subjected to the westerly wind circumpolar circulation [18, 20]

Both bacteria and fungi α -diversity was low and similar to what was observed in some alpine and Antarctic fellfields, but lower than in sub-Arctic or Arctic fellfields possibly due to dispersal limitation induced by the islands [9, 26, 39, 62–66]. As previously observed in alpine and the Antarctic fellfields, the soils' microbiomes of the Kerguelen Island fellfields was composed of a high proportion of *Actinobacteria* and *Acidobacteria* [26, 39, 63, 64, 67, 68] as well as *Ascomycota*, *Basidiomycota* and *Mortierellomycota* [33, 63, 69, 70]. The bacterial composition of Kerguelen Island fellfields differed by a higher proportion of Chloroflexi and a quasi absence of *Planctomycetes*, β -*Proteobacteria*, *firmicute* and *Cyanobacteria* compared to other polar, sub-polar and alpine fellfields [8, 39, 62–65, 67, 68]. However, in some Antarctic soils, *Chloroflexi* stands among the most abundant phyla [71, 72]. The α -*proteobacteria* were observed to increase with increased temperature in the Antarctic, which might explain the high proportion in Kerguelen Island soils less subject to extremely cold climates [26]. Active decomposition by *Basidiomycota* has been highlighted in various Arctic and Antarctic regions [73], where herbivores could be rare. This implies foodwebs that rely mainly on microbial decomposition [9] and suggests that *Basidiomycota* might carry this important function in the Kerguelen Island fellfields. Fungal composition is site-specific in the Kerguelen Island fellfields and this observation was done in various alpine and Antarctic fellfields too [63, 64, 70].

A bacterial core-microbiome of Kerguelen Island fellfield soils was identified and composed of OTUs in the bulk soils of the five sites. This core-microbiome comprised six orders widely spread in the world [61]. The other orders were more specific to the explored sites in the Kerguelen Islands. In contrast, no fungal core-microbiota could be highlighted. However, we observed a high proportion of unknown fungi (39.6%) in a similar range as observed in Antarctica when targeting the ITS2 region [14, 69, 70]. This proportion of unknown fungi was higher than in various alpine fellfields when targeting ITS1 [33] or ITS2 region [63, 64]. This unknown fungal community might have a valuable role in soil functioning as observed in the Antarctic, where, based on RNA analyses, specific fungi are more active than generalist fungi [74].

Spatial variability of the microbial communities relates to soil properties in the Kerguelen Island fellfields

Spatial short-scale variability of the microbiome community was visible through indicator OTUs. PJDA6 site comprised most indicator OTU while it is located close to PJDA2 site. Interestingly AUS25 site does not present any indicator OTUs while it is the only site for AUS Island.

We expected environmental filtering to occur at the archipelago scale and observed that the soil microbiome was spatially structured depending on the five sites. In a previous study, Pansu et al. 2015 [66] observed remarkable differences in the soil fungal communities between herbfields within the Kerguelen Islands [66]. The spatial variability between fellfield soils observed in the present study resulted from contrasted edaphic properties such as nitrogen, phosphorus and sodium contents and pH. Nitrogen limitation shapes the soil microbiome in many regions, and phosphorus is a major driver in poor nutrient fellfield soils [2, 62]. Differences in the soil microbiome composition between the five studied fellfields were explained by ammonium and phosphorus content for both bacteria and fungi. Ammonium is known to especially drive the bacterial specialist community in alpine fellfields, as it is a limiting element [75]. Furthermore, ammonium was strongly related to MAC1 microbial composition and this site was characterized by indicator OTUs possibly related to nitrogen soil content [61, 76]. Nitrogen (nitrate and ammonium) strongly structure the bacterial composition. The nitrogen limitation was observed in two sub-Antarctic Islands [12, 14] and probably occurred in Kerguelen Island fellfields. Moreover, phosphorus values were low and shaped the microbial community composition of PJDA sites. For phosphorus, the limitation of this element for plant growth was highlighted in polar and subpolar regions due to its non-extractable form for organisms [5, 62, 77]. Both PJDA sites had indicator OTUs present in the Antarctic continent, which is limited in phosphorus available for organisms [7, 78, 79]. Finally, even though the five soils only showed slight differences in pH, this element shaped the Kerguelen Island fellfield bacterial communities, which is also the case in various soil types [16, 17, 62,

64]. Surprisingly, contrary to alpine, sub-arctic, Arctic and Antarctic fellfields, carbon contents were not significantly associated with bacterial or fungal community compositions [9, 16, 33, 64, 65]. Overall the structuration of the microbial community seemed to be specific to the sub-Antarctic region with ammonium and phosphorus constraints rather than carbon constraints.

*The assemblage of the microbiome in the rhizosphere of *L. kerguelensis**

Cushion plants are known to buffer climatic variation to a certain extent and to increase temperature and humidity inside and below themselves [32, 80–82]. Therefore the soil underneath the cushion should constitute a favorable habitat for soil microorganisms in the harsh fellfield condition. *L. kerguelensis*' rhizosphere might thus be a reservoir of microbial diversity and we observed that at least one rhizomicrobiome per site showed a higher bacterial or fungal α -diversity than the bulk soil, contrarily to what is commonly observed in temperate environments [38, 39]. This higher α -diversity might be eased by the plant's long life, which allows it to modify even more its environment over time, giving rise to co-evolution processes to occur between the plant and its rhizomicrobiome. This process is especially valuable for the plant as a high microbial diversity allows for more metabolic activities and facilitates nutrient decomposition and mineralisation [83]. Moreover, the number of unknown fungi was higher in the rhizosphere than in the corresponding bulk soil. As endemic fungi participate largely in Antarctica's microbial activity [74], this unknown fungal enrichment in the rhizosphere of *L. kerguelensis* suggests that a higher microbial activity occurs in the soil beneath the cushion than in the bulk soil. Within each site, a core-rhizomicrobiome shared between all plants was observed. It was composed of a lower amount of OTUs than the associated bulk soil core-microbiome suggesting again a filter effect done by the plant rhizosphere. Moreover, at the individual plant rhizosphere level, the rhizomicrobiome was composed of several specific OTUs not detected in the bulk soil for bacteria or fungi. This suggests that the rhizosphere could constitute a reservoir of diversity for many microorganisms.

Bacteria and fungi presented contrasted patterns regarding their assemblage in the rhizosphere of *L. kerguelensis*. First, in each site, the relative abundances of the dominant phyla that composed bacterial communities in the rhizosphere were the same as observed in the bulk soil whatever the plant, which is consistent with alpine fellfield studies [63, 64]. 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 structuring for fungi was already observed in several alpine fellfields [33, 64, 84]. Secondly, across sites, *L. kerguelensis* core-rhizomicrobiome was only identified for bacteria. It comprised OTUs absent in the bulk soil core-microbiome, highlighting a specific bacterial assemblage within the rhizosphere of this species independently of its close habitat. This was not observed for fungi, for which the rhizosphere community strongly depended on the site as no OTU was

shared between all of them. These results point out the close link that exists between fungi and plants within their rhizosphere.

Relation between plant necrosis extent and the composition of its rhizomicrobiome

Mutual benefits between the plant and microorganisms may occur and might be more valuable for both partners in harsh environments. We did not identify any pathogenic bacteria or fungi related to the necrosis extent. We could thus hypothesize that abiotic parameters might be involved, as previously observed by Bergstrom et al. 2015 [44] for *Azorella macquariensis*. The functions of microorganisms correlated to *L. kerguelensis* necrosis extent supported a possible abiotic origin of necrosis as was suggested by the earlier studies of necrosis extent in relation to soil nutrient and water availability [21, 37]. Even if not directly linked to a specific microorganism, necrosis could result from changes in the composition of plant rhizomicrobiome that affect the plant ability to face abiotic environmental stresses such as those induced by climate change in the sub-Antarctic region. Among the OTUs related to necrosis extent, some were identified as supporting key roles in soil functioning. Microorganisms related to the decomposition process seemed to vary with necrosis extent; turn-over of decomposer might occur depending on the decomposition stage of the necrotic part of the cushion [14, 85–88]. Bacterial orders related to soil phosphorus content and nitrogen assimilation vary with necrosis extent [76, 89–94]. Interestingly, we could not observe phosphate solubilizing microbes mainly because functional information is not always known, and taxonomical identification was partial for most OTUs. Finally, unknown fungal taxa correlated to necrosis extent were present in every site suggesting their importance for plant functioning and possible co-evolution [14, 15, 74].

Conclusion

As expected, microbial communities in the soil of the Kerguelen Island fellfields showed similarities with other fellfield habitats of the world but also presented many specificities. Microbial diversity was low, probably due to dispersal limitation. *Chloroflexi* and unknown fungi characterized the specificity of the microbiome and rhizomicrobiome in these islands. Soil microbial communities were spatially structured according to ammonium and phosphorus contents of the soil that induced site-specificity. Bacteria were found more generalist within the archipelago, with a core-microbiome and rhizomicrobiome shared between all sites, contrarily to the site-specificity of fungi. Within harsh environments, *L. kerguelensis* and its rhizosphere seemed to constitute a reservoir of diversity for microorganisms with an active selection of its rhizomicrobiome. This selection might lead to an assemblage of beneficial microorganisms that sustain key functions in the rhizosphere of *L.*

kerguelensis. It could help the plant resist environmental stresses that triggered necrosis within the cushion of this long-lived endemic plant.

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Supplementary data

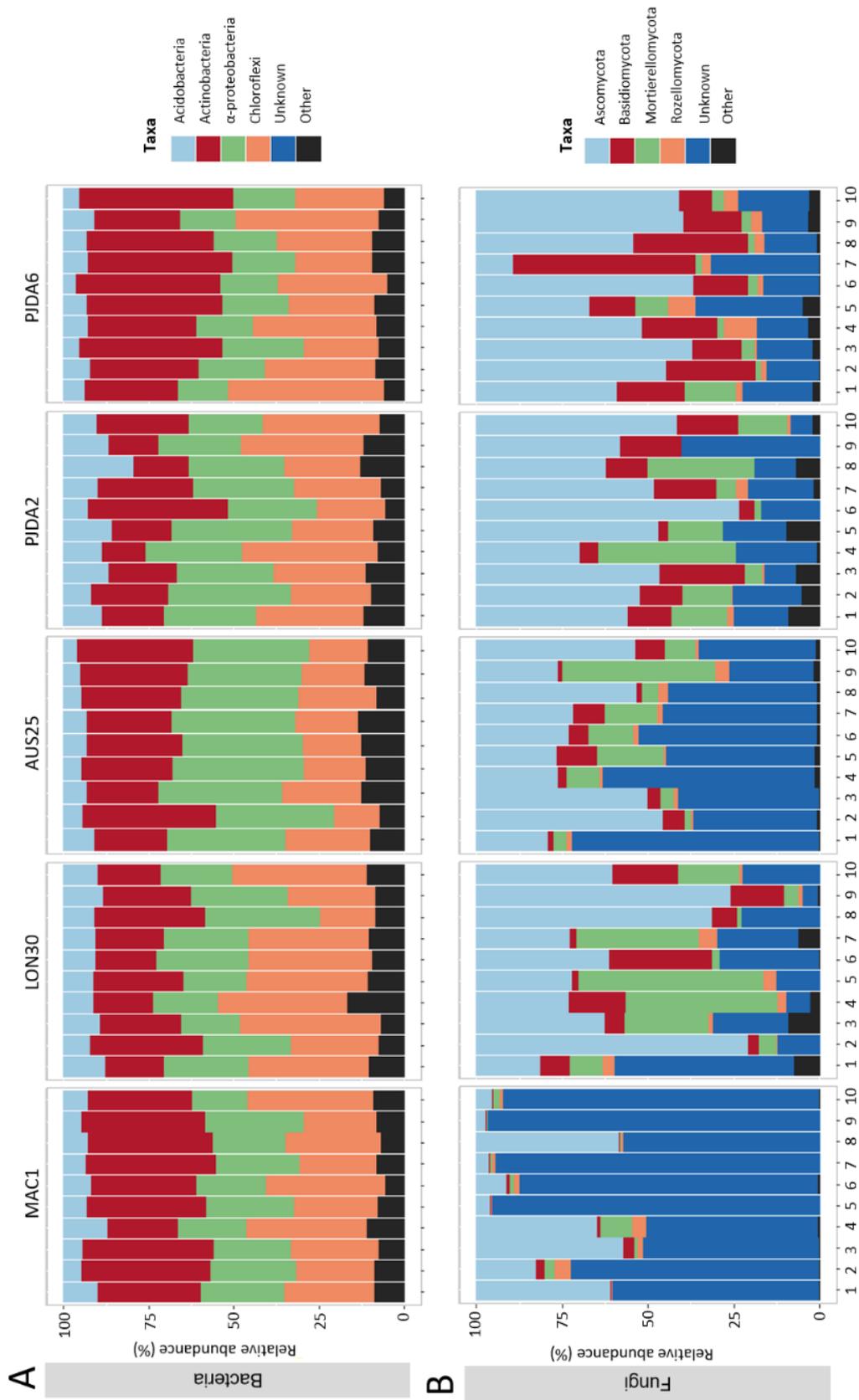


Figure S1: relative abundance of the 4 most dominant bacterial (A) and fungal (B) taxa in rhizosphere soil per site of each *L. kerguelensis*.

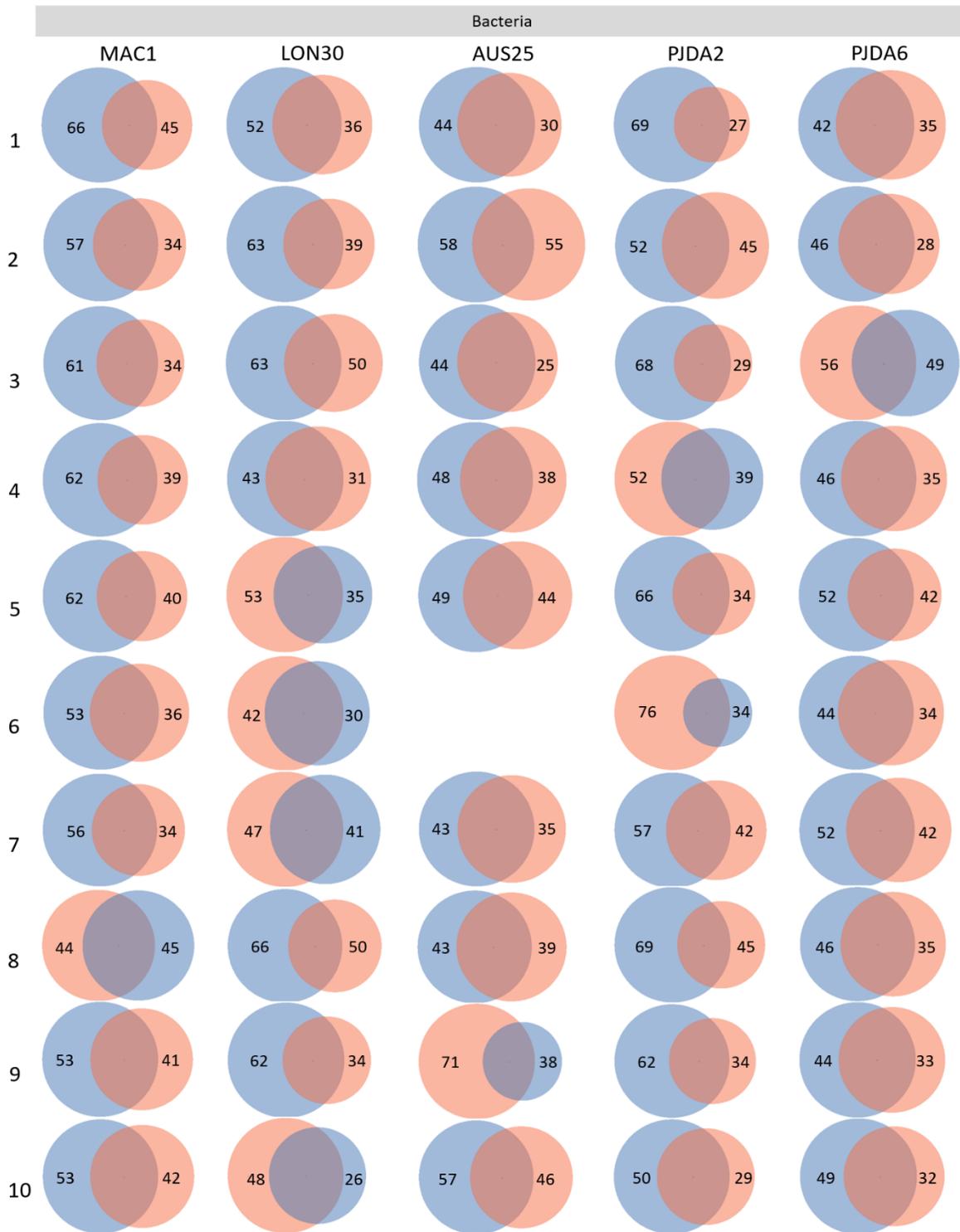


Figure S2: Proportion of bacterial OTUs specific or shared between the rhizosphere (blue) and bulk soil (red) of each individual per site. The size of the circle is proportional to the number of OTUs of the compartment, the left circle represents the largest compartment. Numbers indicate the percentage of OTUs specific to the bulk or to the rhizomicrobiome.

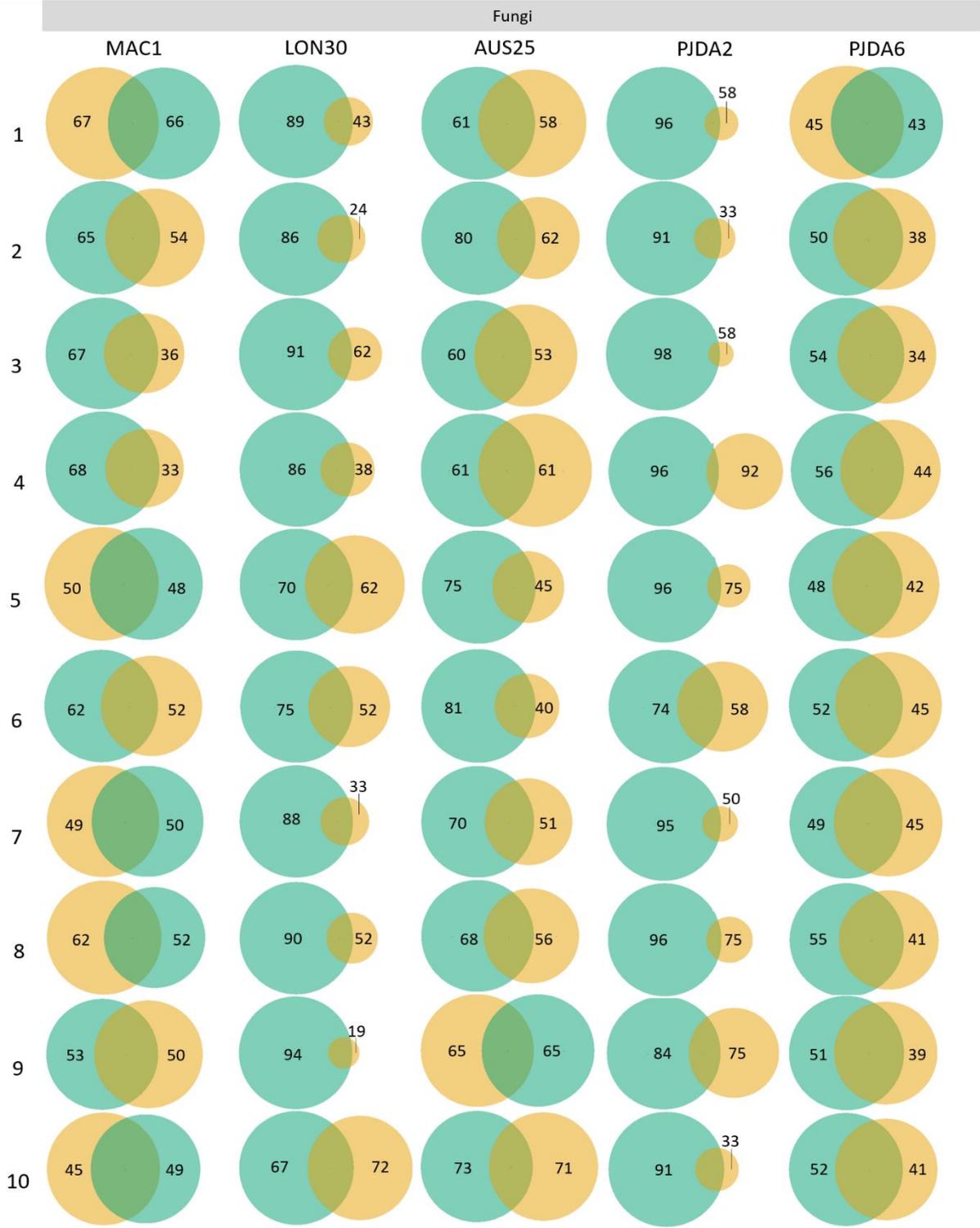


Figure S3: Proportion of fungal OTUs specific or shared between the rhizosphere (green) and bulk soil (yellow) of each individual per site. The size of the circle is proportional to the number of OTUs of the compartment, the left circle represents the largest compartment. Numbers indicate the percentage of OTU specific to the bulk or to the rhizomicrobiome.

OTU	OTU	Domain	Phylum	Class	Order	Family	Genus	Species	total relative abundance	relative abundance	MAC1 bulk	relative abundance						
bulk	rhizo	OTU							bulk(%)	OTU	OTU	OTU	OTU	OTU	OTU	OTU	OTU	OTU
X	X	Bacteria	WPS-2	unknown class	unknown order	unknown family	unknown genus	unknown species	0.613	0.421	1.652	0.367	0.474	0.203	0.203	0.203	0.203	0.203
X	X	OTU_25	Bacteria	WPS-2	unknown order	unknown family	unknown genus	unknown species	0.613	0.421	1.652	0.367	0.474	0.203	0.203	0.203	0.203	0.203
X	X	OTU_268	Bacteria	Acidobacteria	Solibacteriales	Solibacteraceae	Candidatus Solibacter	unknown species	0.047	0.009	0.037	0.049	0.070	0.054	0.054	0.054	0.054	0.054
X	X	OTU_290	Bacteria	Actinobacteria	IMCC26256	(Subgroup 3)	unknown genus	unknown species	0.467	1.312	0.560	0.560	0.178	0.273	0.273	0.273	0.273	0.273
X	X	OTU_278	Bacteria	Actinobacteria	Streptomycetales	Streptomycetaceae	Kitasatospora	unknown species	0.089	0.253	0.028	0.095	0.062	0.062	0.062	0.062	0.062	0.062
X	X	OTU_280	Bacteria	Chloroflexi	Ktedonobacteriales	Ktedonobacteraceae	HSB OF53-F07	unknown species	0.095	0.208	0.155	0.068	0.052	0.045	0.045	0.045	0.045	0.045
X	X	OTU_289	Bacteria	Chthonomonadetes	Chthonomonadiales	Chthonomonadaceae	Chthonomonas	unknown species	0.023	0.013	0.022	0.016	0.015	0.048	0.048	0.048	0.048	0.048
X	X	OTU_290	Bacteria	Alphaproteobacteria	Rhizobiales	KF-JG30-83	unknown genus	unknown species	0.120	0.093	0.155	0.239	0.077	0.031	0.031	0.031	0.031	0.031
X	X	OTU_33	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	1.252	2.048	1.071	1.790	0.865	0.778	0.778	0.778	0.778	0.778
X	X	OTU_34	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	unknown species	0.480	0.616	0.390	0.060	0.806	0.555	0.555	0.555	0.555	0.555
X	X	OTU_345	Bacteria	Acidobacteria	Solibacteriales	Solibacteraceae	Candidatus Solibacter	unknown species	0.068	0.040	0.108	0.087	0.044	0.056	0.056	0.056	0.056	0.056
X	X	OTU_35	Bacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiales	unknown genus	unknown species	0.202	0.262	0.152	0.250	0.237	0.124	0.124	0.124	0.124	0.124
X	X	OTU_36	Bacteria	Chloroflexi	Thermomicrobiales	Thermomicrobiaceae	unknown genus	unknown species	0.099	0.098	0.164	0.060	0.067	0.118	0.118	0.118	0.118	0.118
X	X	OTU_37	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	unknown species	0.390	0.931	0.260	0.511	0.093	0.366	0.366	0.366	0.366	0.366
X	X	OTU_41	Bacteria	Actinobacteria	Microtrichiales	lamiaceae	lamia	unknown species	0.145	0.089	0.198	0.062	0.149	0.214	0.214	0.214	0.214	0.214
X	X	OTU_43	Bacteria	Chloroflexi	Ktedonobacteriales	Ktedonobacteraceae	HSB OF53-F07	unknown species	0.274	0.208	0.585	0.092	0.216	0.284	0.284	0.284	0.284	0.284
X	X	OTU_44	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.325	0.452	0.365	0.557	0.144	0.166	0.166	0.166	0.166	0.166
X	X	OTU_449	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.051	0.118	0.043	0.041	0.017	0.017	0.017	0.017	0.017	0.017
X	X	OTU_494	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.186	0.160	0.316	0.187	0.173	0.099	0.099	0.099	0.099	0.099
X	X	OTU_50	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiales	unknown species	0.452	0.160	0.254	0.766	0.469	0.476	0.476	0.476	0.476	0.476
X	X	OTU_53	Bacteria	Acidobacteria	Solibacteriales	Solibacteraceae	Bryobacter	unknown species	0.534	0.102	0.678	0.171	1.228	0.296	0.296	0.296	0.296	0.296
X	X	OTU_57	Bacteria	Actinobacteria	unknown order	unknown family	unknown genus	unknown species	0.404	0.368	0.455	0.196	0.448	0.549	0.549	0.549	0.549	0.549
X	X	OTU_58	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	unknown species	0.275	0.315	0.198	0.062	0.312	0.501	0.501	0.501	0.501	0.501
X	X	OTU_59	Bacteria	Actinobacteria	Ktedonobacteriales	Ktedonobacteraceae	HSB OF53-F07	unknown species	0.276	0.332	0.102	0.117	0.453	0.372	0.372	0.372	0.372	0.372
X	X	OTU_60	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.466	0.284	0.774	0.638	0.476	0.113	0.113	0.113	0.113	0.113
X	X	OTU_61	Bacteria	Acidobacteria	Solibacteriales	Solibacteraceae	Bryobacter	metagenome	0.256	0.213	0.161	0.397	0.198	0.287	0.287	0.287	0.287	0.287
X	X	OTU_64	Bacteria	Alphaproteobacteria	Rhizobiales	(Subgroup 3)	unknown genus	unknown species	0.185	0.120	0.186	0.103	0.337	0.144	0.144	0.144	0.144	0.144
X	X	OTU_65	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.873	2.447	0.860	0.206	0.878	0.572	0.572	0.572	0.572	0.572
X	X	OTU_67	Bacteria	Actinobacteria	MB-A2-108	unknown family	unknown genus	unknown species	0.400	0.581	0.709	0.644	0.062	0.121	0.121	0.121	0.121	0.121
X	X	OTU_69	Bacteria	Proteobacteria	Alphaproteobacteria	Xanthobacteriales	unknown genus	unknown species	0.790	0.266	1.640	0.937	0.876	0.104	0.104	0.104	0.104	0.104
X	X	OTU_7	Bacteria	Alphaproteobacteria	Rhizobiales	Beljermicaceae	unknown genus	unknown species	1.335	0.013	0.254	0.364	2.799	2.965	2.965	2.965	2.965	2.965
X	X	OTU_70	Bacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteriales	unknown genus	unknown species	0.206	0.115	0.139	0.421	0.212	0.096	0.096	0.096	0.096	0.096
X	X	OTU_71	Bacteria	Acidobacteria	Acidobacteriales	unknown family	unknown genus	unknown species	0.159	0.133	0.210	0.185	0.232	0.023	0.023	0.023	0.023	0.023
X	X	OTU_72	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	0.576	0.027	0.520	0.035	0.773	1.323	1.323	1.323	1.323	1.323
X	X	OTU_758	Bacteria	Anaerolineae	unknown order	unknown family	unknown genus	unknown species	0.073	0.027	0.059	0.019	0.118	0.121	0.121	0.121	0.121	0.121
X	X	OTU_77	Bacteria	Acidobacteria	Solibacteriales	Solibacteraceae	Bryobacter	unknown species	0.104	0.053	0.173	0.168	0.059	0.054	0.054	0.054	0.054	0.054
X	X	OTU_79	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	unknown species	0.375	0.807	0.337	0.380	0.093	0.436	0.436	0.436	0.436	0.436
X	X	OTU_8	Bacteria	Actinobacteria	Microtrichiales	lummatobacteraceae	unknown genus	unknown species	1.171	2.668	1.003	0.934	0.582	1.262	1.262	1.262	1.262	1.262
X	X	OTU_80	Bacteria	Chloroflexi	unknown order	unknown family	unknown genus	unknown species	1.041	0.390	0.340	1.019	1.501	2.554	2.554	2.554	2.554	2.554
X	X	OTU_81	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	unknown species	0.385	0.541	0.452	0.250	0.443	0.301	0.301	0.301	0.301	0.301
X	X	OTU_86	Bacteria	Chloroflexi	Thermomicrobiales	IG30-KF-CM45	unknown genus	unknown species	0.176	0.044	0.102	0.057	0.113	0.518	0.518	0.518	0.518	0.518
X	X	OTU_88	Bacteria	Actinobacteria	Frankiales	Acidothermaceae	Acidothermus	unknown species	0.225	0.137	0.155	0.043	0.327	0.420	0.420	0.420	0.420	0.420
X	X	OTU_93	Bacteria	Proteobacteria	Alphaproteobacteria	A0839	unknown genus	unknown species	0.173	0.230	0.084	0.475	0.033	0.056	0.056	0.056	0.056	0.056
X	X	OTU_99	Bacteria	Actinobacteria	Microtrichiales	unknown family	unknown genus	unknown species	0.107	0.417	0.034	0.109	0.044	0.045	0.045	0.045	0.045	0.045
X	X	OTU_120	Bacteria	Actinobacteria	Microtrichiales	lummatobacteraceae	unknown genus	unknown species	0.107	0.417	0.034	0.109	0.044	0.045	0.045	0.045	0.045	0.045
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria	IMCC26256	unknown family	unknown genus	metagenome	0.178	0.306	0.178	0.327	0.344	0.207	0.207	0.207	0.207	0.207
X	X	OTU_55	Bacteria	Actinobacteria														

Table SII: Species richness, Shannon index, OTUs number, and Z-value and p-value adjusted of the comparison between the rhizosphere and the bulk soil associated per individual ($\alpha = 0.01$).

Site	Individual	Necrosis extent(%)	Bacteria						Fungi					
			Species richness			Shanon index			Species richness			Shanon index		
			value	Z value	Padjusted	value	Zvalue	Padjusted	value	Zvalue	Padjusted	value	Zvalue	Padjusted
MAC1	1	0	731.67	2.61	0.01	5.73	2.97	0.01	24.00	-0.71	0.48	2.06	-0.47	0.64
	2	3	700.67	1.83	0.07	5.65	2.55	0.01	29.67	0.10	0.92	2.21	-0.02	0.99
	3	5	712.33	2.32	0.02	5.66	2.43	0.02	46.33	0.77	0.44	3.10	0.85	0.40
	4	9	726.67	2.76	0.01	5.60	1.76	0.08	52.25	0.90	0.37	3.24	1.05	0.30
	5	16	720.50	2.67	0.01	5.63	2.27	0.02	12.67	-1.50	0.13	0.71	-2.21	0.03
	6	26	605.33	0.81	0.42	5.34	0.48	0.63	25.00	0.03	0.98	1.94	-0.09	0.93
	7	28	678.00	1.58	0.12	5.55	1.68	0.09	14.00	-2.05	0.04	1.20	-1.72	0.09
	8	31	586.00	0.51	0.61	5.46	0.85	0.39	12.33	-1.94	0.05	1.36	-1.38	0.17
	9	45	548.67	0.48	0.63	5.15	0.35	0.72	21.00	-0.82	0.41	2.02	-0.40	0.69
	10	52	588.00	0.23	0.82	5.35	0.44	0.66	18.00	-1.56	0.12	1.43	-1.49	0.14
	BULK	na	568.50			5.27			35.5			2.50		
LON30	1	0	637.33	1.18	0.24	5.56	1.52	0.13	40.67	2.58	0.01	3.07	2.62	0.01
	2	0	733.67	1.92	0.06	5.74	2.09	0.04	34.00	1.93	0.05	2.63	1.20	0.23
	3	0	663.33	1.09	0.28	5.39	0.83	0.41	38.00	2.11	0.04	3.10	1.93	0.05
	4	0	559.33	0.26	0.79	5.27	0.22	0.83	34.33	1.27	0.21	2.88	1.50	0.13
	5	0	411.67	-1.09	0.28	5.00	-0.74	0.46	15.00	0.24	0.81	2.28	-0.12	0.91
	6	1	461.67	-0.65	0.51	5.09	-0.57	0.57	23.33	0.82	0.41	2.67	1.33	0.18
	7	1	572.00	0.35	0.73	5.43	0.95	0.34	42.67	3.05	0.01	3.17	2.57	0.01
	8	16	741.00	1.83	0.07	5.79	2.13	0.03	33.67	1.76	0.08	2.44	0.34	0.73
	9	21	821.00	2.48	0.01	5.90	2.70	0.01	42.00	2.99	0.01	2.92	2.14	0.03
	10	24	427.33	-1.00	0.32	5.12	-0.31	0.76	19.00	0.34	0.74	2.53	0.29	0.77
	BULK	na	530			5.18			35.5			2.20		
AUS25	1	0	832.33	1.71	0.09	5.86	0.94	0.35	38.00	-0.96	0.34	2.55	-1.27	0.21
	2	1	764.00	-0.29	0.08	5.81	-0.05	0.96	66.33	1.58	0.11	3.72	1.39	0.17
	3	1	787.67	0.31	0.76	5.72	-0.89	0.38	53.33	-0.29	0.77	3.43	0.27	0.79
	4	3	829.00	1.57	0.12	5.85	0.40	0.69	46.00	-0.31	0.75	3.05	-0.34	0.73
	5	5	863.50	2.13	0.03	5.95	1.61	0.11	51.33	0.46	0.65	3.24	-0.44	0.66
	6	8	na	na	na	na	na	na	55.67	1.15	0.25	3.38	0.31	0.76
	7	10	827.00	1.57	0.12	5.91	1.30	0.19	70.00	1.48	0.14	3.84	1.51	0.13
	8	20	757.33	-0.34	0.74	5.72	-1.35	0.18	53.00	-0.10	0.92	3.51	0.44	0.66
	9	21	768.00	-0.48	0.63	5.78	-1.00	0.32	60.50	0.37	0.71	3.43	0.23	0.82
	10	37	852.33	2.29	0.02	5.93	1.75	0.08	42.67	-0.29	0.77	2.86	-0.39	0.69
	BULK	na	776.67			5.81			51.5			3.33		
PJDA2	1	0	624.00	2.57	0.01	5.53	2.68	0.01	42.00	2.00	0.05	3.27	2.32	0.02
	2	0	475.00	0.83	0.41	5.27	1.29	0.20	40.67	2.10	0.04	3.09	1.96	0.05
	3	0	660.33	3.14	0.01	5.59	2.98	0.01	49.50	3.11	0.01	3.24	2.42	0.02
	4	0	350.00	0.10	1.00	4.83	0.32	0.75	16.50	0.38	0.71	2.08	0.38	0.71
	5	2	569.67	1.96	0.05	5.10	1.39	0.17	35.00	1.20	0.23	2.70	1.13	0.26
	6	38	181.00	-0.33	0.74	4.23	-0.18	0.86	15.00	0.26	0.80	2.22	0.36	0.72
	7	45	512.50	1.31	0.19	5.26	1.08	0.28	52.00	2.97	0.01	3.61	3.24	0.01
	8	46	596.00	2.37	0.02	5.51	2.62	0.01	36.00	1.17	0.24	3.01	1.31	0.19
	9	74	575.50	1.73	0.09	5.36	1.84	0.07	9.50	0.13	0.90	1.25	0.25	0.80
	10	79	476.67	1.13	0.26	5.24	1.39	0.17	35.00	1.73	0.08	2.92	1.76	0.08
	BULK	na	337.67			4.44			6.5			1.40		
PJDA6	1	1	630.33	0.82	0.41	5.35	0.72	0.47	47.67	-1.67	0.10	3.11	-1.69	0.09
	2	2	757.67	3.29	0.01	5.74	3.29	0.01	56.33	-1.02	0.31	3.15	-1.31	0.19
	3	9	630.50	0.40	0.69	5.32	0.28	0.78	57.00	-1.12	0.27	3.50	-0.36	0.72
	4	12	682.00	1.18	0.24	5.51	1.69	0.09	46.67	-1.02	0.31	3.20	-0.21	0.83
	5	15	746.33	3.05	0.01	5.70	3.05	0.01	53.33	-0.47	0.64	3.37	0.11	0.91
	6	18	676.67	1.10	0.27	5.49	1.52	0.13	52.33	-1.33	0.18	3.37	-0.25	0.80
	7	23	737.67	2.72	0.01	5.71	2.79	0.01	39.00	-2.71	0.01	2.29	-2.36	0.02
	8	26	739.33	2.83	0.01	5.76	3.08	0.01	57.67	-0.57	0.57	3.43	-0.17	0.87
	9	26	683.33	1.75	0.08	5.38	0.93	0.35	53.00	-0.72	0.47	2.92	-0.42	0.67
	10	31	726.67	3.10	0.01	5.69	0.28	0.78	63.67	0.21	0.83	3.33	0.04	0.97
	BULK	na	590.33			5.11			57.33			3.39		

Table SIII: Significant indicator OTUs per site, relative abundance, p-value and taxonomy of the Blast sequence associated are indicated.

Due to its large size this table is available online: <https://github.com/lormarchand/thesis>

Table SIV: results of Wald test (package RVAideMemoire) on the site effect of the relative abundance of specific OTU in the rhizospheres.

Site	Domain	Proportion	Standard deviation	group
MAC1	Bacteria	0.221	0.010	a
LON30	Bacteria	0.195	0.013	ab
AUS25	Bacteria	0.182	0.008	b
PJDA2	Bacteria	0.221	0.11	a
PJDA6	Bacteria	0.181	0.007	b
MAC1	Fungi	0.164	0.006	a
LON30	Fungi	0.236	0.007	b
AUS25	Fungi	0.196	0.008	c
PJDA2	Fungi	0.259	0.006	d
PJDA6	Fungi	0.144	0.003	e

PART III: Temporality of *L. kerguelensis* cushions' morphological change and their possible longevity in relation with abiotic and climatic factors in contrasted environments

Chapter 4: Plant-climate relations and resilience to change: insights from a long-lived sub-Antarctic cushion plant (*Lyallia kerguelensis*)

Chapter 5: Growth and age variability in contrasted environments and architectural traits allometry in *Lyallia kerguelensis* Hook. f. (Montiaceae)



Chapter 4: Plant-climate relations and resilience to change: insights from a long-lived sub-Antarctic cushion plant (*Lyallia kerguelensis*)

Running title: plant growth and necrosis linked to climate change

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Data availability: Images are available at first and senior authors' convenience. The datasets and R-Script generated during the current study will be available online on the Osuris geonetwork (<https://www.osuris.fr/geonetwork/srv/fre/catalog.search#/home>).

Abstract

1. Among species threatened by rapid climate change, long-lived plants, expected to respond slowly to changes, may be of particular concern. Necrosis has been observed in several slow-growing plants and may be related to accumulated effects of climatic stress. However, few examples of impacts of climate change on long-lived plants and their possible resilience have been truly documented. Such studies may be particularly important as these species often act as keystone species in their ecosystems.
2. We worked on the long-lived cushion plant species *Lyallia kerguelensis* (Montiaceae), endemic from the Kerguelen Islands and in which necrosis affects most plants and all populations. A long-term “*Lyallia* Observatory” monitored the dynamics of growth and necrosis extent of plants in populations spread across the islands. We analysed calibrated images of these plants at 4–5-year intervals to estimate the spatial variability and temporal changes of their surface area and necrosis extent. We correlated these morphological data to climatic data issued from regional climate modelling and other abiotic and biotic (plant neighbourhood) environmental variables.
3. Concerning spatial variability, necrosis extent was largest both in larger cushions and in cushions surrounded by a few neighbouring plants. Concerning temporal variability, growth was not detectable across this period confirming the slow process. In contrast, necrosis extent increased in many cushions. The increase of necrosis extent was particularly high in sites where short-term winter temperature increase and winter precipitation decrease were highest. Few plant deaths were observed across the survey. Re-greening events were observed in a few cushions.
4. **Synthesis:** Our research provides insights into the possible responses and resilience of long-lived plants to rapid climate change. We showed that plant vigour could be threatened by specific, short-term climatic changes (warmer and drier winters). The long-lived plant accumulated necrosis across a short time. The capacity to re-green was observed in a few individuals. This study case suggests that long-lived plants may be highly sensitive to short-term climatic changes and may have a weak capacity of resilience. This finding is of special importance for ecosystems such as polar or alpine, subject to rapid climate change.

Keywords: plant-climate interaction, climate change, long-lived plant, necrosis, long-term growth monitoring, sub-Antarctic, cushion plant, *Lyallia kerguelensis*

Introduction

There is now compelling evidence that climate is rapidly changing in certain parts of the world (IPCC 2014; Ranasinghe *et al.* 2021), challenging natural plant populations and vegetation dynamics (Williams *et al.* 2007; Harter *et al.* 2015; Román-Palacios & Wiens 2020). Namely, stress symptoms eventually leading to the decline of several long-lived plant species have been documented in various ecosystems (Molau 1996; Kleier & Rundel 2004; Allen *et al.* 2010; Bergstrom *et al.* 2015; Bjerke *et al.* 2017; Dickson *et al.* 2020, among others). In harsh environments, these plants often live close to their physiological limits (Convey *et al.* 2006; Hennion *et al.* 2006b). In long-lived plants, responses to changes are supposed to be slow (Moritz & Agudo 2013; Cotto *et al.* 2017) and symptoms of stress may be observed for a long time before death eventually occurs. Therefore, it is fundamental to better study long-lived plants over time to sharpen our understanding of their growth, decline and possible resilience. Indeed, the life history of such long-lived plants, for instance their morphology, may reflect and have recorded responses to successive climatic fluctuations. Empirical studies of organisms in their natural populations are still required to improve the assessment of adaptive capacity of species and finally predict the response capacity of ecosystems to rapid climate change (Becker *et al.* 2013). Long-lived plants might be accurate witnesses of the impacts of climate change on biotas.

Alpine, sub-polar and polar regions, with harsh environments are characterized by long-lived plants and are affected by rapid climate changes (Ranasinghe *et al.* 2021). There stands a particularly interesting growth shape, *i.e.*, cushion plants. Cushion plants are characterized by their hemispherical shape that lowers the ratio surface-to-volume, limiting heat and water losses (Zotz *et al.* 2000; Butterfield *et al.* 2013) allowing warmer, wetter and nutrient-rich conditions within and below themselves (Aubert *et al.* 2014; Momberg & le Roux 2020). In a number of long-lived cushion plant species from various environments, stress symptoms were highlighted with the occurrence of dieback (Armesto *et al.* 1980; Hennion 1992; Le Roux *et al.* 2005; Bokhorst *et al.* 2009; Bergstrom *et al.* 2015; Bjerke *et al.* 2017, among others). Dieback is defined as the impossibility to regenerate new foliage on a portion of the cushion or the entire cushion (Armesto *et al.* 1980; Whinam *et al.* 2014). At a particular time, necrosis damage might be observed and can be defined as a symptom of a dieback process (Whinam *et al.* 2014). Multiple factors might co-occur for these stress symptoms, for example, a combination of abiotic and biotic variables was raised to explain the progression of dieback in *Azorella macquariensis* (Bergstrom *et al.* 2015). Furthermore, the involvement of climate change on the rapid increase of necrosis has been highlighted for several long-lived plant species (Molau 1996; Bergstrom *et al.* 2015; Upson *et al.* 2016; Bjerke *et al.* 2017 among others). Cushion plants are expected to live several decades up to hundreds of years, with a slow growth rate from few millimetres to few centimetres per year (Frenot *et al.* 1993; Kleier & Rundel 2004; le Roux & McGeoch 2004; Wagstaff &

Hennion 2007). Finally, the persistence of long-lived plants in unsuitable environments was observed in alpine environments and this might modify their evolutionary responses (Cotto *et al.* 2017). Integrating population dynamics will be essential to estimate the species adaptive capacity (Phiri *et al.* 2009; Cotto *et al.* 2017). However, for many long-lived cushion plant species, their individual growth dynamics in the long term are generally unknown, limiting our estimate of their life span. Similarly, their population dynamics, which necessarily requires long-term monitoring, is rarely studied.

The occurrence of dieback and loss of cushion integrity was particularly studied in the sub-Antarctic region, affected by a drastic and rapid climate change (Favier *et al.* 2016; Verfaillie *et al.* 2021). Several studies highlight that necrosis possible driver in the sub-Antarctic may be drought induced by climate change due to decreasing precipitation, increasing temperature, or both (Wagstaff & Hennion 2007; Bergstrom *et al.* 2015). In cushion plant *Azorella selago* (Apiaceae) from Marion Island, a rainfall exclusion experiment resulted in necrosis occurrence after a year of this treatment (Le Roux *et al.* 2005). Moreover, a long-term survey of the congeneric cushion plant species *Azorella macquariensis* from Macquarie Island showed that large-scale necrosis progressed over time and space following a longitudinal gradient (Whinam *et al.* 2014; Bergstrom *et al.* 2015; Dickson *et al.* 2019). The origin of this necrosis might be linked with a combination of lower summer water availability and the possible establishment of pathogens on already water-stressed plants (Bergstrom *et al.* 2015; Dickson *et al.* 2019, 2020). In the context of necrosis, it should be noted that cushions might have the ability to re-green. Indeed, the terminal part of the shoot, defined as the plant apex, is formed by small, densely imbricated leaves around an apical bud (Werth 1911; Hennion 1992). The development of the axillary buds (located at each leaf) results in local cushion surface widening, then replacing adjunct necrotic parts. Re-greening was observed in *Azorella compacta* in the high Andes; after removing a portion of green apices, mimicking dieback, the plant had novel green apices on the removed part (Kleier & Rundel 2004). Aside from re-greening by itself, other processes may lead to a decrease of cushion necrosis. Seeds of the same species may germinate and seedlings establish within the cushion. In the Kerguelen endemic cushion plant *Lyallia kerguelensis*, the germination of seeds in the dead parts of cushions was observed (Hennion & Walton 1997). Several genotypes were observed within large cushions of *A. selago* in Marion Island, suggesting the possibility of a similar establishment of seedlings of the species within the cushions (Cerfonteyn *et al.* 2011). Finally, necrotic parts might be easily detached from the cushion due to strong winds (windblown), leading to cushion shape modification. Overall, in cushion plants, precise temporal dynamics of cushion growth and morphological changes needs to be better studied to understand the impact of necrosis on plant resilience. Indeed, due to long life span, dieback in cushion plants might be a more sensitive measure of climate change impacts than their survival. This knowledge will be essential to improve the adequacy of conservation management for these and other native species and finally assess the sustainability of ecosystems.

The present study took place in the Kerguelen Islands with a typical sub-Antarctic cool and windy climate all year round: mean annual temperature of 4.6°C and mean wind speed of 9.7 m.s⁻¹ at “Port-aux-Français” (PAF) from 1951 to 2018 (Verfaillie *et al.* 2021). The western side of the Islands is also very humid as reflected by the presence of an almost permanent cloud cover and of the Cook ice cap, but permanent westerlies induce a strong foehn effect at the lee of the ice cap. This is reflected by the mean annual rainfall at PAF (755 mm from 1951 to 2018), in the eastern part of the Islands, whereas the Western side receives 3 to 4 more precipitation. Kerguelen Islands’ climate is strongly influenced by the southern annular mode (SAM), which shapes temperatures and precipitation distribution from the subtropics to Antarctica (Abram *et al.* 2014). Climate changes have been recorded since several decades in the Kerguelen Islands. It resulted in a significant drying trend associated with a marked shift in the storm track location (Favier *et al.* 2016; Verfaillie *et al.* 2019), whereas a large increase in the mean temperature was also observed at Kerguelen (e.g., Favier *et al.* 2016; Verfaillie *et al.* 2021). Direct impacts of climate change are already visible in the Kerguelen Islands, with more frequent and more prolonged summer droughts, the rise of the snow line and the drastic reduction of the Cook ice cap (Hennion *et al.* 2006a; Lebouvier *et al.* 2011; Verfaillie *et al.* 2015). These islands harbour numerous fellfields and host the long-lived cushion plant *Lyallia kerguelensis* (Montiaceae). The species is strictly endemic to the Kerguelen Islands and has a scarce distribution on the land (Wagstaff & Hennion 2007) (Fig. 1). *L. kerguelensis* cushions are expected to live several decades (Wagstaff & Hennion 2007), however, their growth is unknown and might be challenging to assess for large cushions as they might be composed of several genotypes as observed for *Azorella selago* (Cerfonteyn *et al.* 2011). *L. kerguelensis* shows necrotic parts (Hennion 1992), a possibly recent phenomenon as the early botanical descriptions did not mention any necrosis (Hooker 1847; Werth 1911; Chastain 1958). Previous work showed that nineteen populations (all the populations studied) had partly necrotic cushions and that necrosis extent varied among populations (Marchand *et al.* 2021b). For cushions with low necrosis extent the amount was positively correlated to soil sodium content which was interpreted as sodium possibly worsening osmotic stress by soil drought (Marchand *et al.* 2021b). Furthermore, highly necrotic cushions did not respond to environmental variables, probably because of their loss of vigour (Marchand *et al.* 2021b). Moreover, a pathogenic origin of necrosis appears unlikely up to now, as no disease-causing agents were present among bacteria on leaves (Portier 2019) and no specific rhizomicrobiomes were associated with necrotic cushions (Marchand *et al.* 2021a). These results reinforce a possible climatic or another abiotic origin of the necrosis in *L. kerguelensis*, which needs to be sought. Due to the patrimonial status of the species, a “*Lyallia kerguelensis* Observatory” (Réserve naturelle nationale des Terres australes françaises and Institut Polaire Français, programs 136 and 1116) was set up in 2012 to monitor the fate of individual plants and their

morphological dynamics in the long term. Our study was based on the data from this survey. We raised five research questions:

- (i) Does cushion surface area, necrosis extent and its neighbouring plant amount vary across sites at the beginning of the survey?
- (ii) When focusing on necrosis extent, is it explained by regional climate, long-term climatic trends, site geomorphology and cushion morphology?
- (iii) What is the dynamics of growth, necrosis extent, and neighbouring plant amount during the survey?
- (iv) When focusing on necrosis increase, is it related to short-term climatic trends; long-term climatic trends, site geomorphology, or cushion morphology?
- (v) Can we evidence a decrease of necrosis extent during the survey, and if so, which are due to growth of the green parts over the necrotic parts of the cushion?

(i) We assume that variability of morphology (cushion surface, neighbouring plant amount and necrosis extent) occurred between sites since their geomorphological, topographical and edaphic environments differed. (ii) We expect the variability of necrosis extent at the beginning of the survey to be explained by cushion surface area, neighbouring plant amount and regional climate. (iii) During the 5 years of the survey, the cushion surface area and neighbouring plant amount might not vary as the growth rate is slow. In contrast, the necrosis extent, as a faster process, might vary significantly during the survey. (iv) We hypothesize that the necrosis extent increase during the survey might be influenced by short-term climatic trends as necrosis extent seems to be rapid. Finally, (v) a possible re-greening of the necrotic parts by growth of living parts might have occurred during the survey. It should be distinguished from other sources of reduction of necrosis extent such as erosion of necrotic parts finally windblown, development of *L. kerguelensis* seedlings in the necrotic parts, or colonization of the necrotic part by other plant species.

Materials and methods

Study sites

The Kerguelen Islands (48°30' - 50°S, 68°27' - 70°35'E), located in the Southern Indian Ocean (Fig. 1) are the largest archipelago of the sub-Antarctic region (Wagstaff & Hennion 2007). The Kerguelen Islands are isolated by at least 3000 km from the nearest continents. They harbour numerous fellfields which are regarded as a form of tundra ecosystem composed of rock or glacial till, and deficient in nutrients (especially nitrogen) with a low water retention capacity (Aubert de la Rüe 1964; Smith & Mucina 2006; Block *et al.* 2009). They experience high precipitation, snow cover, strong wind, numerous freeze-thaw cycles and rapid fluctuations of both temperature and soil water content.

The Kerguelen Islands are subject to a chronically cool climate (yearly average of 4.6°C between 1951 to 2018, Verfaillie *et al.* 2021) with a strong marine influence. Variability of the climate is seen across the islands, the west part is colder and rainier, and the warmest and driest part is located in the “Golfe du Morbihan” on the south-east side (Verfaillie *et al.* 2019). Variations were recorded since the 1950s for temperature, with a cool period during the 1960s followed by a significant increase until 1980, then an almost constant period during 30 years, and a final great increase in the 2010s. Thus, the 10-y mean annual temperature shifted from 4.43°C in the 1950s to 5.1°C in the 2010s (Verfaillie *et al.* 2021). For precipitation, after very humid conditions in the 1950s, reaching 1100 mm a⁻¹ at “Port-aux-Français”, a 50% decrease in precipitation occurred in the 1960s. Then it increased again linearly to reach almost 800 mm.a⁻¹ in 1975. Afterwards, a significant drying trend occurred, which led to minimum precipitation of 565 mm.a⁻¹ at “Port-aux-Français” in the 2000s. The 2010s were, however relatively wet at “Port-aux-Français” (precipitations 820 mm.a⁻¹). The significant drying trend associated with a marked shift in the storm track location (Favier *et al.* 2016; Verfaillie *et al.* 2019) resulted from the anomalously high phase of the SAM (Abram *et al.* 2014; Jones *et al.* 2016), with large-scale significant impacts in the sub-Antarctic and Antarctic regions. Differences in the Kerguelen region were more precisely marked by anticyclonic circulation anomalies in the 1975-1990 period compared to the 1960-1975 period (Favier *et al.* 2016).

We studied eight populations belonging to the “*Lyallia kerguelensis* Observatory”, spread across the Kerguelen Islands to cover most of the plant distribution range. Each site consisted of a geographical area of less than 50 m² where the geomorphology, topography and soil properties were homogeneous and harboured a continuous *L. kerguelensis* population. Locations were: two in the north: “Port Christmas” (PCR) and “île Mac-Murdo” (MAC), one in the east: “îles Normandes” (NOR), four in the south-east: “île Blakeney” (BLA), “île Haute” (HAU), “île Mayes” (MAY) and “Plateau Dent du Chat” (PDC) and one in the south-west: “Rallier du Baty” (RBA) (Figure 1).

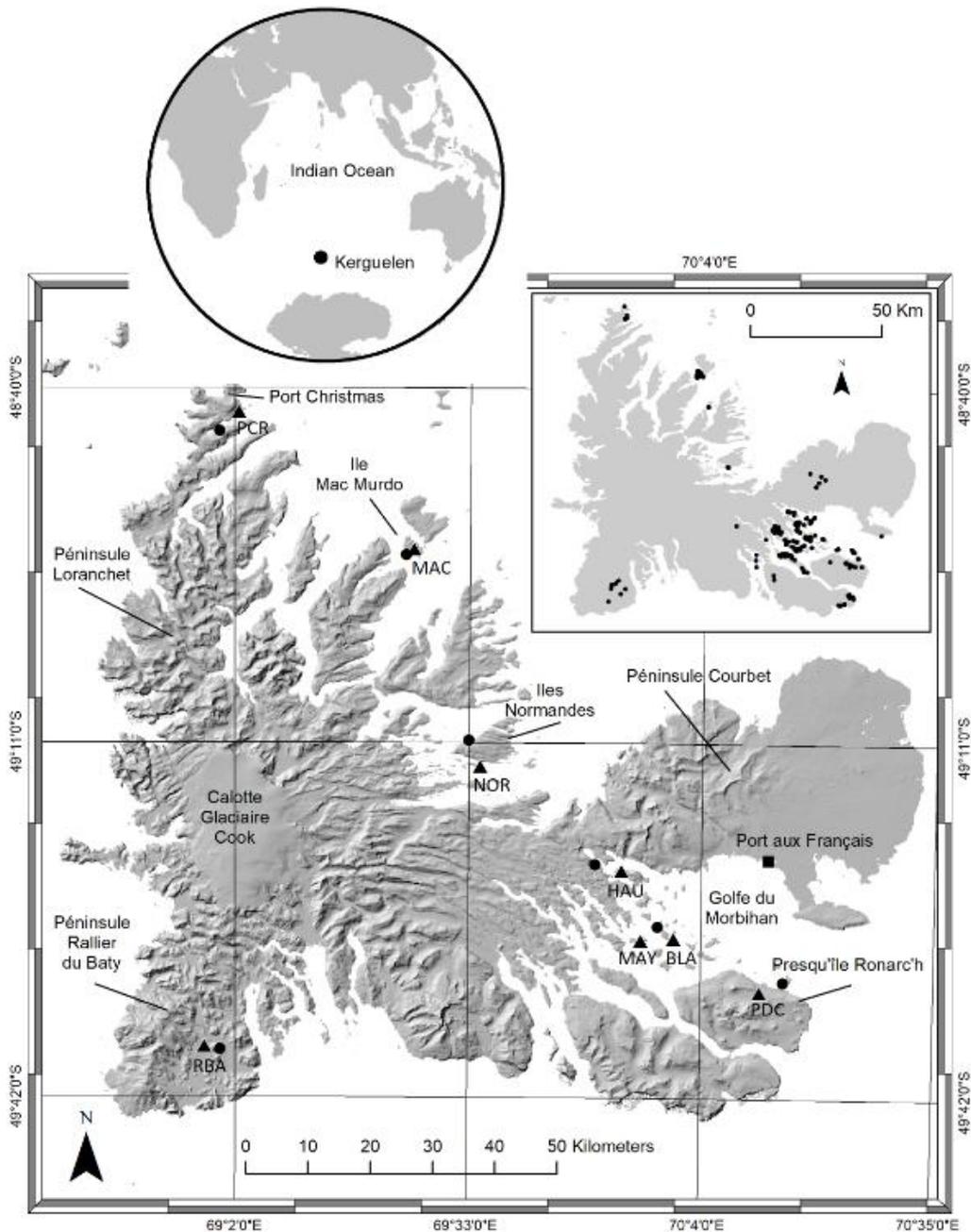


Figure 1: Locations of the 8 sites hosting monitored populations of *Lyallia kerguelensis* (black triangles) on an altitudinal background in the Kerguelen Islands (NASA – SRTM 30M, 2005), names below the black triangles; the black dots indicate the location of time series extracted from the regional climate model; the black square indicates the research station of “Port-aux-Français”; the labels indicate essential features in the islands. Top right map: distribution of *L. kerguelensis* on Kerguelen Islands (black dots) in April 2018; data from the Réserve naturelle nationale des Terres australes françaises and from Institut Polaire Français programs 136 and 1116; map background from IGN 1/200 000 georeferenced with spatial adjustment (D. Fourcy, unpublished). The small globe illustrates the Kerguelen Islands position in the South Indian Ocean. Maps were designed with ArcGIS 10.8.1 software.

Species of study

Lyallia kerguelensis Hook. f. (Hooker 1847; Montiaceae) is a long-lived perennial herb, that forms roughly round-shaped cushions 20 – 40 cm across, exceptionally up to 1 m (Wagstaff & Hennion 2007).

Its cushion growth form is characteristic of plants living in harsh environments with soil subject to low water holding capacity and many freeze-thaw cycles (Körner 2003). While the plant's age is unknown, observations of several specimens showed that plants could persist at least 16 years (1990-2006, Wagstaff & Hennion 2007) and even up to 20 years (marked individuals 2000-2013, re-identified in 2019, Marchand pers. Obs.). The plant distribution is scarce (Fig. 1), fragmented and located in fellfields at various altitudes (from 14 m to 520 m), and with contrasting soil physico-chemical properties (Wagstaff & Hennion 2007; Marchand *et al.* 2021b). The cushions often host plants of other species around their periphery. This neighbouring plant community is composed of perennial plants native or introduced to the islands. It is interesting to note that mammal herbivore populations (rabbits and reindeers) have had a strong impact on Kerguelen native plant communities since their introduction in the XIX and XXth century respectively (Chapuis *et al.* 2004). In this study, populations of “ile Australia” (AUD), ile Mac-Murdo” (MAC), “iles Normandes” (NOR), “ile Blakeney” (BLA), “ile Haute” (HAU) and “ile Mayes” (MAY) are situated on islands devoid of introduced herbivores. The populations of “Port-Christmas” (PCR), “Plateau Dent du Chat” (PDC) and on “Rallier du Baty” (RBA), are located outside of the current reindeer population distribution, but in zones where rabbits are known to occur (Yoccoz *et al.* unpublished). However, no individual rabbit or sign of their presence were observed on these study sites and no visible damage (trampling, apex herbivory) was observed on the plants.

Sampling and measurements

Under the observatory, each population was visited at T0 between December 2012 and November 2014 and at T1 between December 2016 and January 2019, leading to time intervals of 4-5-years where 50 marked individuals (randomly selected in the population at the setup of Observatory) were photographed both times (sup. Data 1).

Calibrated images were processed with ArcGis 10.6.1 software following the protocol from Marchand *et al.* (2021). However, due to a few low-quality images or windblown identity sticks during the survey, less than 50 individuals per population were available for the study (sup data 1). Furthermore, when cushions died during the survey (37 out of 303 individuals), we considered them as 100% necrotic at T1. To characterise the cushion morphology, we calculated the cushion surface area (cm²), as it was well correlated to other morphological traits for this plant species (Marchand *et al.* 2021b). To estimate the neighbouring plant amount (%) we defined the ratio: cushion perimeter in contact with other plants divided by the total perimeter of the cushion. The necrotic extent of the cushion (%) was calculated as the necrotic surface area divided by the whole cushion surface area (Marchand *et al.* 2021b). Each trait was calculated for cushions at both T0 and T1. To characterise the dynamics of change of each trait during the survey, we extracted the regression coefficient of the change in trait

values across the two dates, resulting in the variables: surface area dynamics, neighbouring plant amount dynamics and necrosis extent dynamics.

Several causes of decrease of necrosis were searched for in images. First, the occurrence of a green part at T1 where a necrotic part was observed at T0, which could indicate a re-greening by the development of axillary buds of green apices next to the necrotic part, or seedling germination of *L. kerguelensis* within it. With our imaging methods, we cannot distinguish one event from the other, but both are considered favourable for the maintenance of integral cushion entity. We sought other possibilities of necrosis decrease. We looked for the growth of the green part of the cushion, which may be sufficient to lead to a decrease of the proportion of necrosis extent without a reduction of the necrotic surface area. Then we looked for erosion causes when an empty space was visible on T1 image at the position of a necrotic part at T0. Necrotic parts are less dense and easily detached from the cushion by frost damage and strong winds. The final cause sought was the colonization of necrotic parts visible at T0 by other plant species at T1.

Edaphic and climatic variables

Environmental characteristics were recorded *i.e.*, altitude, topography (flat, terrace, base or mid or top of slope, summit); wind exposure (very sheltered, sheltered, exposed, very exposed); slope; slope aspect; visual estimation of soil cover in the percentage of blocks (> 20 cm). These variables were supposed to represent local environmental heterogeneity in the sub-Antarctic region (Hennion *et al.* 2006a; Marchand *et al.* 2021b).

Mean temperature and precipitation (addition of rainfall and snowfall) were available seasonally for the austral summer from November to April and austral winter from May to October from 2004 to 2013 (Verfaillie 2014). Each site was related to its regional climate except for two sites (BLA and MAY) which were related to a common climate as they stand at less than 5,3 km one from each other (sup Data 2). The climate at each site first relies on measured precipitation and temperature measured at the meteorological station of “Port-aux-Français” (PAF). These data were distributed (or spatially corrected) using outputs from the regional climate model MAR (Modèle Atmosphérique Régional; Gallée & Schayes 1994). The simulation covers the whole islands at 10km resolution (Verfaillie *et al.* 2019). Details of the MAR simulation can be found in Verfaillie *et al.* (2019). Here, the MAR was forced by the ERA-Interim Reanalyses (Dee *et al.* 2011) over the period 1980-2013. Since simulated and measured temperatures at PAF were very similar, the distributed temperature data from the MAR were directly used at each site. However, regional climate models generally fail in reproducing the exact inter-annual variability of observed precipitation, we preferred using field observations of summer and winter precipitation at PAF and to correct them with the distribution ratio. This spatial correction of PAF data was computed as the ratio between modelled values at the cell including PAF

site and at closest cells to each other sites. Long-term mean temperature and precipitation were calculated seasonally over 10 years from summer 2004 to winter 2013, covering the period before and during the T0 survey (Table SII). To characterise the long-term climate trends, we extracted the regression coefficient of 10-year variation for both the seasonal temperature and precipitation data (Table SII). We did the same for the short-term trends of the climate with 3-year variation of seasonal temperature and precipitation data from 2010-2013 which coincided with the T0 survey (sup. Data 2). A total of 16 variables was used (Tables SII) and comprised plant morphology, edaphic properties, climate mean and trends over a long and a short term.

Statistical analyses

Statistical analyses were performed with R version 4.0.2. (i) To study the variability of cushion morphology between sites at T0 we applied a Dunn's Kruskal-Wallis multiple comparison (package FSA). (ii) To study the variability of necrosis extent at T0 in relation to cushion morphology, edaphic properties and climate (mean and long-term trends) (Table SII) we used a two-step method, with first a Random Forest analyses "RF1" (package RandomForest, Liaw & Wiener 2001) to seek for the main explanatory variables. A Random Forest is a partitioning method that produces a large ensemble of regression trees considering both qualitative and quantitative variables and considers a randomly subset of the dataset to evaluate its accuracy. We set 9999 trees to reach stable results and three variables to each split (sup data 1 and 2). Variables were ranked by incremental node purity weighted average value (INS), which measures the homogeneity of the node at each split for each variable. The most informative variables are the ones ranked the highest until a gap on the INS values. Second, the two highest-ranked variables were incorporated into a generalised linear mixed model (GLMM) "GLMM1". GLMM are a mix of a linear mixed model (LMM) (which allows both fixed and random effects) and generalised linear models (GLM) (which handle non-normal data) (Bolker 2008; Bolker *et al.* 2009). The inclusion of random effects enables control of the hierarchical structure (pseudo-replication), *i.e.*, individuals within populations. Fixed effects are equivalent to explanatory variables in generalised linear models. We applied penalised quasi-likelihood methods (PQL) as our data followed a quasi-Poisson distribution and we were cautious to have enough replicates for the accuracy of the method (glmmPQL, package MASS) (Bolker *et al.* 2009). Interactions between explanatory variables were checked and conserved if they were significant and all quantitative explanatory variables were scaled. Estimation of conditional R^2 was done with a standardised generalised variance approach (package r2glmm). Prediction plots (packages sjPlot and ggplot2) allow visualising the outcome of the model (coefficients and confidence interval) on a given fixed effect variable. (iii) For the dynamics of morphological traits across space; we applied Dunn's Kruskal-Wallis multiple

comparisons (package FSA) and a T-test to determine if the dynamics (all populations) was significantly different from zero. (iv) To study the increase of necrosis extent in relation to cushion morphology, edaphic properties and climate short-term trends (Table SII), we subsampled cushions with a positive necrosis trend as well as cushions with trends between 0 and -1 (equivalent to a necrosis decrease below 5% of the surface area) as possibly due to the inaccuracy of the method, the absolute trends value was used. This choice was motivated by the shape of the distribution of the necrosis dynamics (Fig 4F). A total of 278 individuals were used. To analyse the increase of necrosis extent in relation to short-term climatic trends, we used the same methodology as for (ii), ten variables were used in the Random Forest RF2 (Table SI and SII). The six highest-ranked variables were implemented in mixed models GLMM2. Temporal changes of precipitation and temperature values are spatially correlated since data are obtained from the MAR simulations (Fig. S1). As a consequence, we directly separated the contribution of precipitation and temperature in our models. Three mixed models were done with one climatic variable each time: 3-year winter precipitation trend in mixed model “GLMM2_a”, 3-year summer precipitation trend in mixed model “GLMM2_b” and 3-year winter temperature trend in mixed model “GLMM2_c”. The same analysis was done with long-term climatic trends rather than short-term climatic trends (Table SIV). (v) To study necrosis decrease, we focused on the 25 cushions with the most negative dynamics (more than 5% decrease of necrosis). They were checked individually on the image to identify the possible causes of the decrease.

Results

Variability of morphology at the beginning of the survey

At the beginning of the survey, variability of necrosis extent, cushion surface and neighbouring plant amount were observed between populations (Fig. 2 A, C, E and Table SIII). For total surface area, HAU and PCR populations showed significantly lower values than most of the other populations (Fig. 2 A and Table SIII). The neighbouring plant amount was significantly more abundant in BLA, MAY, and PDC than most populations (Fig. 2 C and Table SIII). For necrosis extent, cushions in NOR site showed lower values than other populations (HAU, PDC and RBA) (Fig. 2 E and Table SIII).

Relation between variability of necrosis extent and environmental variables at the beginning of the survey

With the random forest analysis, necrosis extent's variability across populations was related to cushion surface area and neighbouring plant amount (INP = 10982 and 7251 respectively) (Table I). A mixed model was built with these two main explanatory variables and was significant for both cushion surface area and the neighbouring plant amount (coefficient of regression = 0.172 and -2.030, and p-value =

0.015 and 0.033 respectively) (Table II). More precisely, the necrosis extent is predicted to be higher for larger cushion surface area (Fig. 3 A, Table II) and higher when the neighbouring plant amount is scarce (Fig. 3 B, Table II). The model prediction was more accurate for smaller cushions than for larger cushions and the explanatory capacity of the model was relatively low (up to 7.4%).

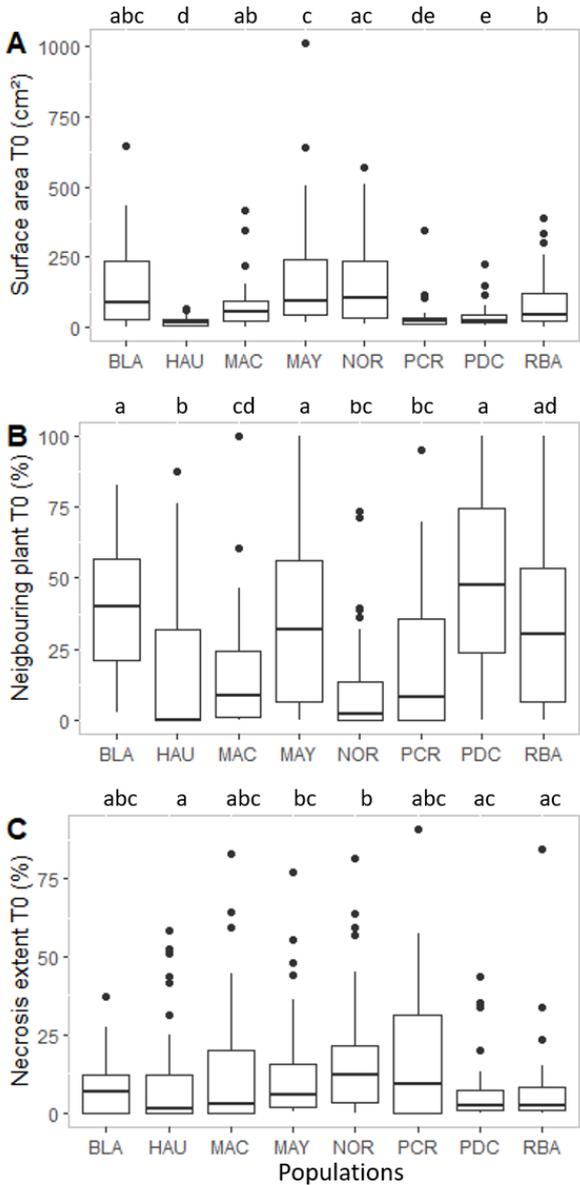


Figure 2: Boxplots of the total surface area per site at T0 (A), neighbouring plant amount at T0 (B) and necrosis extent at T0 (C). Different letters indicate significant differences between sites (Dunn’s Kruskal-Wallis comparison).

Table 1: Results of the random forest “RF1” and “RF2”, explanatory variables were ranked by importance. Bold text indicates the variables selected for the mixed models.

RF1		RF2	
Variables	INS	Variables	INS
Cushion surface area	10982	Cushion surface area	10.35
Neighbouring plant amount	7251	3-year winter precipitation trend	6.35
10-year winter temperature trend	1179	Necrotic surface area	6.10
Topography	1082	3-year summer precipitation trend	5.44
10-year summer temperature trend	964	Neighbouring plant amount	5.31
Slope aspect	879	3-year winter temperature trend	5.28
10-year mean summer temperature	878	3-year summer temperature trend	3.66
Altitude	872	Block cover	3.60
Block cover	865	Altitude	2.14
10-year mean summer precipitation	706	Topography	1.89
10-year winter precipitation trend	628	Slope aspect	1.05
10-year mean winter precipitation	572	Degree of slope	0.73
Wind intensity	569	Wind intensity	0.50
10-year summer precipitation trend	562		
10-year mean winter temperature	519		
Degree of slope	361		

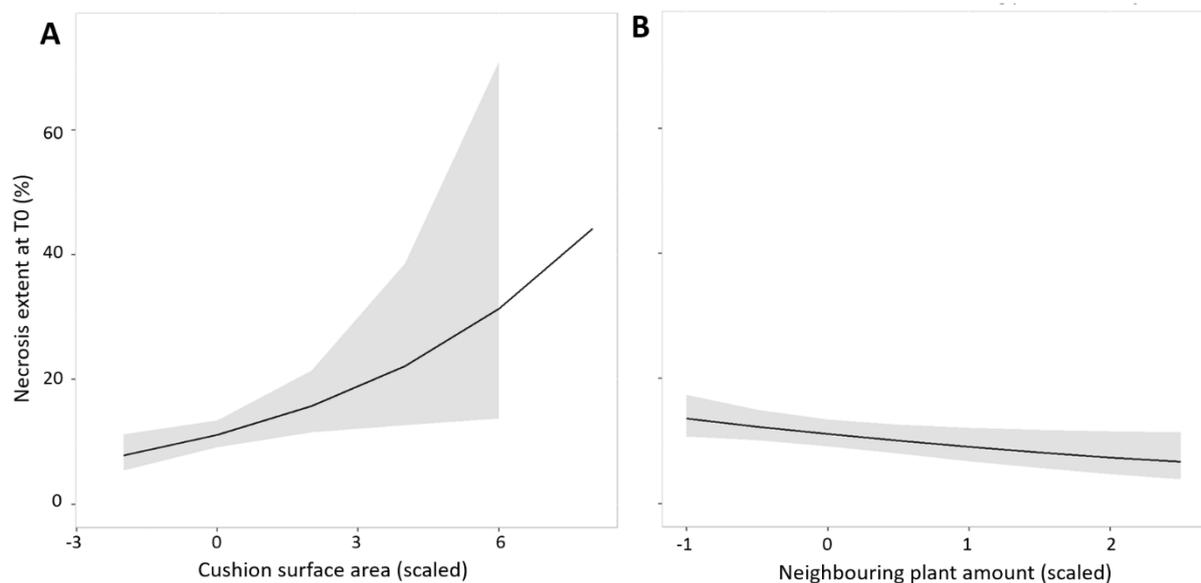


Figure 3: Predictive plot of the necrosis extent vs. cushion surface area (A) and vs. neighbouring plant amount (B) with our model. The grey shading indicates the 95% confidence interval around the estimated value (black line).

Table II: Results of quasi Poisson generalised linear mixed model "GLMM1". Coefficient of regression, p-value and R² estimated are shown for each explanatory variable. Bold text indicates significant variables. The number of observations and groups is indicated.

	N	regression coefficient	p-value	R ² estimate	upper CL	lower CL
model "GLMM1"				0.025	0.074	0.004
Cushion surface area		0.172	0.015	0.051	0.060	0.001
Neighbouring plant amount		-2.03	0.033	0.018	0.051	0.000
observations	30					
groups	3					
	8					

Dynamics of morphology across time

During the 4-5 years of the survey, the cushion growth was not statistically different from zero (T = 1.513, p-value = 0.131) (Fig. 4 B). The number of cushions which grew was compensated by the number of cushions which shrank or were stable (Fig 4 B), and only little variability was observed between sites (Fig 4. A). The dynamics of neighbouring plants' amount was positively and significantly higher than zero (T = 3.353 and p-value < 0.001) (Fig. 4 D and F). These dynamics varied among sites, with BLA showing a higher increase than most other sites (Fig 4C). The dynamics of necrosis extent was positively and significantly higher than zero (T = 10.479, and p-value<0.001 respectively) and it showed site variability with a higher increase of necrosis in BLA, PCR and RBA sites.

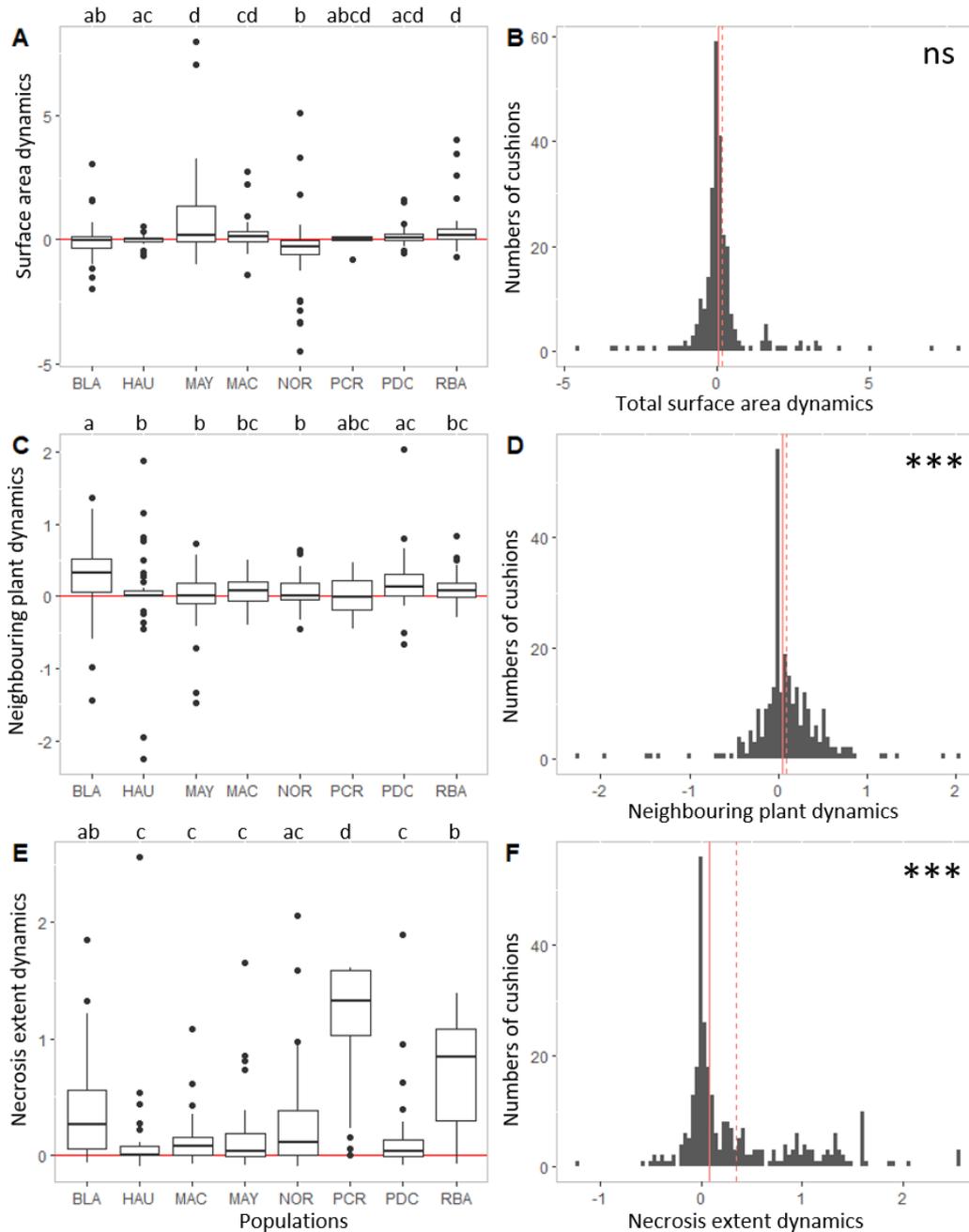


Figure 4: Boxplots of the surface area coefficient (slope coefficient between T_0 and T_1 value) per site (A), neighbouring plant amount coefficient (C) and necrosis extent coefficient (E). Different letters indicate significant differences (Dunn's Kruskal-Wallis comparison). Bar plots of the distribution of the coefficient of variation between T_0 and T_1 of the total surface area (B), neighbouring plant amount (D) and necrosis extent (F). The dashed line represents the mean and the continuous line the median of the dataset. To enlarge the graph on subfigure (B), one plant (NOR44) was removed from the total surface area coefficient graph. Results of a T-test are shown on the top right corner. 'ns' indicates not significant and '***' indicates a p -value < 0.001.

Table III: Results of quasi Poisson generalised linear mixed models "GLMM2". Coefficient of regression, p-value and R² estimated are shown for each explanatory variable. Bold text indicates significant variables. The number of observations and groups is indicated.

	N	Regression coefficient	p-value	R ² estimate	upper CL	lower CL
model "GLMM2_a"				0.138	0.225	0.081
Necrosis extent T0		-0.035	0.655	0.001	0.021	0.000
Cushion surface area		0.028	0.755	0.000	0.000	0.000
Neighbouring plant amount		-0.033	0.695	0.001	0.021	0.000
3-year winter precipitation trend		-0.536	0.014	0.137	0.216	0.072
	27					
observations	8					
groups	8					
model "GLMM2_b"				0.075	0.152	0.036
Necrosis extent T0		-0.040	0.613	0.001	0.022	0.000
Cushion surface area		0.023	0.804	0.000	0.019	0.000
Neighbouring plant amount		-0.026	0.759	0.000	0.020	0.000
3-year summer precipitation trend		0.384	0.123	0.074	0.142	0.027
	27					
observations	8					
groups	8					
model "GLMM2_c"				0.137	0.224	0.080
Necrosis extent T0		-0.036	0.650	0.001	0.021	0.000
Cushion surface area		0.010	0.915	0.000	0.018	0.000
Neighbouring plant amount		-0.035	0.689	0.001	0.021	0.000
3-year winter temperature trend		0.546	0.009	0.137	0.215	0.072
	27					
observations	8					
groups	8					

Relation between variability of necrosis extent increase and environmental variables

With the random forest analysis, the necrosis extent increase between T0 and T1 was mainly explained by six variables (Table 1): cushion surface area (INP = 10.35), 3-year winter precipitation trend (INP = 6.35), T0 necrosis extent (INP = 6.10), 3-year summer precipitation trend (INP = 5.44), neighbouring plant amount (INP = 5.31) and 3-year winter temperature trend (INP = 5.28). Therefore, three mixed models with each time one climatic variable were built (Table III). Necrosis extent increase was higher in locations with respectively a low 3-year winter precipitation trend (model "GLMM2_a", coefficient of regression = -0.536 and p-value = 0.014, Fig. 5B and Table III), and a high 3-year winter temperature trend (model "GLMM2_b", coefficient of regression = 0.546 and p-value = 0.009, Fig. 5B and Table III). With the mixed model "GLMM2_b" the relation was not significant between increase of necrosis and

3-year summer precipitation trend (Table III). Overall, both significant models could explain up to 22.4% - 22.5% of the variability of the necrosis increase during the survey (Table III). The cushion ecomorphology was not significant and neither other environmental variable (Table III). The same analysis was conducted with the long-term climatic trends but was insignificant (Table SIV).

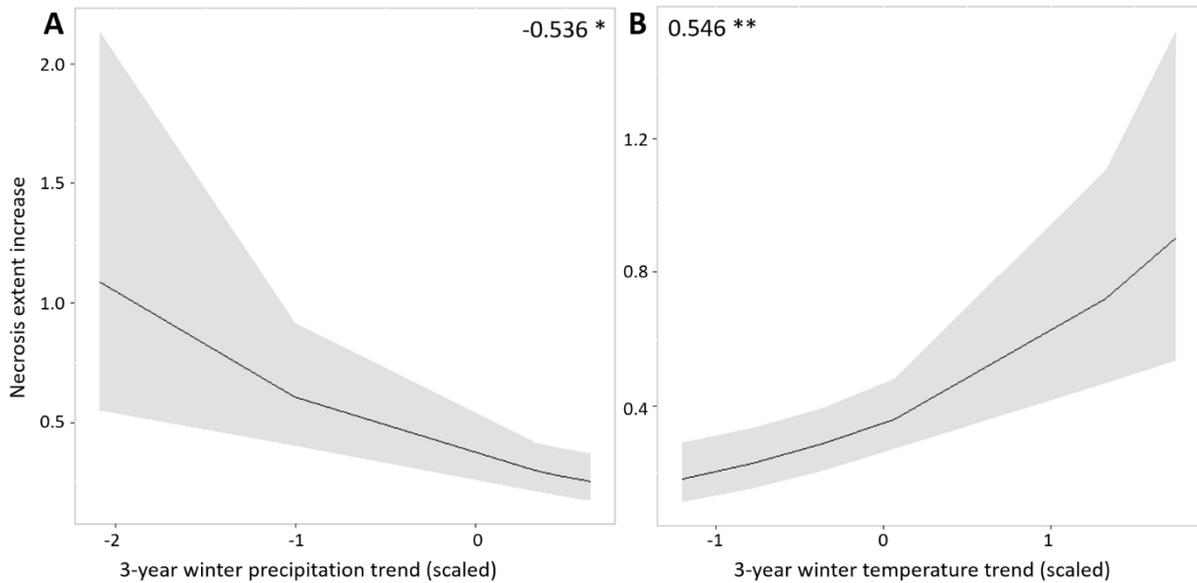


Figure 5: Predictive plot of necrosis extent increase vs. 3-year precipitation coefficient (A) and vs. 3-year winter temperature coefficient (B). The grey shading indicates the 95% confidence interval around the estimated value (black line).

Decrease of necrosis extent across time

The decrease of necrosis extent occurred in 25 individuals across 303 individuals and may be due to several different causes that may variously combine in cushions (Table IV). The highest occurrence was due to re-greening of the necrotic parts: for thirteen cushions and for most (eleven of them) it was associated with an increase of the surface area of the cushion. On eleven pictures, the presence at T1 of *Azorella selago*, *Acaena magellanica*, or unidentified moss species was observed at positions where necrotic apices were present at T0 (Table IV). Then, on seven pictures, it could be due to erosion of the necrotic part. Finally, for one plant, the growth of the cushion around the necrotic part was sufficient to lower the necrotic extent.

Table IV: Necrosis decrease coefficient of 25 individuals, morphological characteristics and possible causes of the reduction of the necrosis.

Population	Individual	Necrosis extent coefficient	Cushion surface area T0 (cm ²)	Cushion surface area T1 (cm ²)	Necrotic surface area T0 (cm ²)	Necrotic surface area T1 (cm ²)	Re-greening	Cushion growth	Windblow n	Growth of other plants
NOR	33	-0.1725	127.28	149.00	13.67	6.75	X	X	X	
PDC	37	-0.1443	116.00	146.31	14.52	8.33	X	X		Acaena magellanica
MAY	7	-0.1758	28.8	40.41	3.56	0.79	X	X		
HAU	1	-0.3976	4.50	7.06	0.70	0.00	X	X		
MAY	13	-0.1463	235.16	331.73	22.79	2.87	X	X		
MAY	19	-0.1596	304.00	433.87	48.17	28.23	X	X		
MAY	34	-0.1577	17.94	29.02	2.04	0.60	X	X		
MAY	48	-0.2735	19.80	25.82	5.57	4.80	X	X		
PDC	30	-0.131	17.50	19.58	1.99	0.92	X	X		
PDC	38	-0.5847	53.73	63.03	19.01	4.25	X	X		
RBA	26	-0.1625	9.90	16.30	1.45	0.48	X	X		
HAU	42	-0.4986	9.69	23.21	3.02	2.72	X			
PDC	3	-0.4582	10.73	10.82	4.66	2.27	X			
HAU	23	-0.2909	47.87	51.44	20.87	16.59		X	X	
BLA	20	-0.1308	41.89	181.19	3.74	5.36		X		Acaena magellanica
MAC	13	-0.4566	19.66	38.90	8.28	5.55		X		Acaena magellanica
HAU	38	-0.3986	41.76	39.60	21.91	14.62		X	X	Azorella selago
HAU	44	-0.4017	51.90	33.59	26.58	11.94		X	X	Azorella selago
NOR	20	-0.1475	399.88	299.96	65.49	33.15		X	X	Azorella selago
PDC	31	-0.2552	54.13	41.56	10.75	3.13				Acaena magellanica
HAU	18	-0.3257	67.06	42.42	39.14	4.55				Azorella selago
HAU	27	-0.1872	35.52	28.97	3.82	1.00				Azorella selago
HAU	35	-0.34	8.970	6.63	2.23	0.87				Azorella selago
HAU	31	-0.1213	61.09	61.82	6.22	3.37				Mosses
PCR	5	-0.2045	19.20	12.77	11.05	5.73				Mosses

Discussion

We have established that (i) cushion surface area, necrosis extent and neighbouring plant amount vary among sites at the beginning of the survey. (ii) We found that correlations existed between these three traits, larger necrosis extent was partly explained by larger cushion surface area and lower neighbouring plant amount. (iii) Cushion growth was insignificant; in contrast, necrosis extent and neighbouring plant amount showed significant positive dynamics during the 5-year survey for all populations. The necrosis extent increase was rapid compared to cushion growth and widespread across the populations. (iv) Higher necrosis increase was associated with short-term higher winter precipitation decrease and short-term higher winter temperature increase. Also, (v) we made progress toward estimating the possible event of re-greening of *L. kerguelensis* while other cases of decrease of necrosis were related to erosion of dead parts or colonisation by neighbouring plants.

The three morphological traits (cushion surface area, neighbouring plant amount and necrosis extent) showed variability within and between sites which is expected as these traits were shown to vary with the environment for this species (Marchand *et al.* 2021b). Indeed, in a previous work addressing 19 populations, we showed that cushion surface area was explained by site topography and wind exposure (Marchand *et al.* 2021b). It should be noted that in the present study cushions were selected randomly, which implies both size and age variability within and between sites (plant size *a priori* representing a proxy for age in cushions, like other plants; Frenot *et al.* 1993; Molau 1997; Morris & Doak 1998; le Roux & McGeoch 2004). Finally, for the variability of necrosis extent, in a previous study necrosis extent was positively correlated to the soil sodium content in cushions showing less than 10% of necrosis (Marchand *et al.* 2021b).

When modelling the necrosis variability across sites, morphological variables (cushion surface area and neighbouring plant amount) were significant. Necrosis extent was greater in larger cushions and might be seen as senescence signature due to age. Ageing is a “time-stress response” for long-lived plants; indeed, the numerous stochastic events that occurred throughout their life mediate their life span (Thomas 2013). Therefore, older/ larger cushions have necessarily experienced more stressful events and accumulated more necrosis than the younger/ smaller ones. This was noted for larger individuals of *Azorella compacta* in Chile and for *Diapensia lapponica* in northern Sweden (Molau 1996; Kleier & Rundel 2004). Also, the neighbouring plant amount was an important variable as we observed a lower necrosis extent when more plants were in contact. *L. kerguelensis* might benefit from the effects of neighbouring plants through increase of organic matter content, nutrients, and a more buffered environment, a process described as facilitation (*i.e.*, interaction in which the presence of one species alters the environment in a way that enhances growth, survival or reproduction of a second, neighbouring species, Bronstein 2009). Facilitation has been more studied in the reverse direction *i.e.*,

positive effects provided by the cushion plant to hosted plants from other species within the cushion (mild microenvironment, le Roux & McGeoch 2010; Molenda *et al.* 2012; Raath-Krüger *et al.* 2021). In Marion Island, the cushion plant *Azorella selago* was shown to act as a nurse plant for the grass *Agrostis magellanica* in the harsher environments (le Roux & McGeoch 2010). It should be noted that *L. kerguelensis* plants are very rarely found in closed vegetation and when so, were in poor condition, suggesting the species inability to sustain competition (Hennion 1992; Wagstaff & Hennion 2007). So, we may hypothesise that under more moderate conditions, there will be greater cover of other plant species, which might tip the balance of the interaction from facilitative to competitive. Nevertheless, the variables driving most of the variability of necrosis extent across individuals remains unknown, as our model explains less than 10% of the variability. It might be related to the occurrence of several genotypes within a cushion that react differently to environmental variables, as the development of seedlings was observed within cushions of *L. kerguelensis* (Hennion & Walton 1997). Also, it may be related to past stressful events and/or to impacts of micro-topography within site, both effects which were not recorded in our study and would require different monitoring techniques.

During the 5-years of the survey, the dynamics of growth was insignificant. *L. kerguelensis*' cushion growth seems to be a complex process like in other cushion plants (Molau 1997; Morris & Doak 1998). Indeed, the equivalent number of shrunk cushions compared to cushions having grown indicates that growth, measured as cushion surface area, is nothing like linear. It is known from a previous study that cushion surface area is positively correlated to apex density (Marchand *et al.* 2021b). Longer-term monitoring will probably highlight the dynamics of *L. kerguelensis* across its life span, especially if the frequency of photographs of labelled cushions can be increased, possibly through automatic, non-impacting, recording by cameras. The significant increase of the neighbouring plant amount during the survey was probably due to faster growth of these other species than *L. kerguelensis*. Necrosis extent increase was significant within a short time compared to the growth rate of the species. The death of *L. kerguelensis* was recorded only for a small proportion of the plants (37 out of 303), while necrosis increase occurred in most cushions. Other works pointed out the rapid increase of necrosis (compared to growth rates) in cushion plant species in the sub-Antarctic (Le Roux *et al.* 2005; Dickson *et al.* 2020). Therefore, necrosis extent measure might be a more sensitive metric of climate change impacts than survival in such harsh environments.

When modelling the necrosis extent increase, the main drivers were the short-term climatic trends without the implication of other environmental variables or long-term climate trends. The non-implication of long-term trends strengthens the importance of recent climate change on the necrosis extent increase. Extreme drought events became more frequent after the great increase of the temperature during the 2010's. Indeed, short-term decrease in winter precipitation and short-term increase in winter temperature were both significant in our models. The precipitation probably drives

the temperature; indeed, the driest winter is associated with less cloud, inducing more sun and more heat. Only a few studies focus on the winter physiology of long-lived plants in harsh environments (Aubert *et al.* 1999; Fosaa *et al.* 2004). In the Kerguelen Islands, *Pringlea antiscorbutica*, known as the Kerguelen cabbage, does not show winter dormancy and maintains photosynthetic activity almost all year round (Aubert *et al.* 1999). This might be the case also for *L. kerguelensis*, which apical buds remain green in winter (F. Hennion, pers. obs.). To discuss further, we will refer to works concerning winter physiology of other long-lived plant species such as trees. Winter is a challenging season and affects the carbon budget, transport and allocation, which controls frost tolerance and timing and success of growth resumption (Zwieniecki *et al.* 2015; Tixier *et al.* 2019). A reduction of precipitation in *L. kerguelensis*' biotopes might induce soil water deficit during the winter season leading to a possibly higher risk of embolism for plant vessels as observed for trees (Mayr *et al.* 2002; Voltas *et al.* 2013; Earles *et al.* 2018). Indeed, altitudinal study of trees living at their latitudinal margin (supposedly close to their physiological limits) showed more dieback at higher altitude associated to a decrease of conductivity (Mayr *et al.* 2002) or a rise of stomatal control of water losses (Voltas *et al.* 2013), strengthening the hypothesis of a possible increase of vessel embolism due to winter drought (Mayr *et al.* 2002; Voltas *et al.* 2013). Furthermore, trees with narrower vessels, more resistant to embolism, showed less dieback (Mayr *et al.* 2002; Voltas *et al.* 2013). The ability to rapidly refill embolised vessels was highlighted in the two sub-Antarctic cushion plants *Azorella macquariensis* and *Colobanthus muscoides* (Rolland *et al.* 2015). It would be interesting to search for such anatomical features in *L. kerguelensis*. Furthermore, the increase in winter temperature might have several negative consequences. Indeed, this temperature change should increase cell respiration and result in the plant converting more non-structural carbohydrates to energy (Tixier *et al.* 2019). The direct negative effect of warmer temperatures was highlighted in high alpine plants with loss of carbohydrates (Steinbauer *et al.* 2020) and possible dampening of spring starch accumulation in coniferous species (Earles *et al.* 2018). If active in *L. kerguelensis*, such processes may lead to plant exhaustion, finally resulting in the impossibility to regenerate new foliage. Increased temperatures should also increase the evaporative demand, which is critical if root absorption cannot compensate for winter drought (Charrier *et al.* 2021). Hence the possible stress induced by drier and warmer winters might lead to short-term water balance constraints and long-term carbon metabolism constraints (Charrier *et al.* 2021). Therefore, across a rather short period of years, exhaustion and embolism of whole portions of *L. kerguelensis* might occur, leading to the senescence of shoots, ultimately challenging the survival of the cushion entity. The negative impact of warmer and drier winter on long-lived plants has most relevance in areas with minimal snow accumulation, such as sub-Antarctic, sub-Arctic, or other mid-altitude environments. There, many long-lived plant species adapted to harsh environments might be

vulnerable to climate change and might live too close to their physiological limits to sustain vigour in the future. In locations with snow cover such as high Arctic, Antarctic and high alpine region, snow accumulation buffers winter climate fluctuation. However, warmer and drier winters tend to reduce this snow cover which might expose plants to winter fluctuation more and might lead to vegetation damage, as observed in the high Arctic (Bokhorst *et al.* 2009; Bjerke *et al.* 2017). Finally, re-greening of the cushion seems to be a rare event. However, we recognise that our methods might not fully capture this process. The whole catchment would require monthly or bi-monthly monitoring (for instance with fixed cameras) to demonstrate it. Cushion re-greening was observed in a few cases, which suggested the ability of the plant to restrain necrosis and sustain vigour. Furthermore, the integrity of the cushion might be conserved with new germinations growing within it. These results are important to estimate the vulnerability and assess the adaptive capacity to climate change of *L. kerguelensis* and other long-lived plant species in similar environments. Conservation policies should consider that *L. kerguelensis* can lose vigour rapidly but needs a longer time to possibly recover.

Conclusion

Our research provides insights into the possible responses and resilience of long-lived plants to rapid climate change. We showed that climate change might directly impact long-lived species with an increase of stress symptoms, such as necrosis, but do not seem to lead to death within a few years. Necrosis might be an accurate measure to study the impacts of rapid climate change on long-lived plants. Overall, this study case suggests that long-lived plants may be highly sensitive to short-term climatic changes and may have a weak capacity of resilience. Finally, long-term monitoring of organism response in natural environments is required to predict impacts of rapid climate change on floras and ecosystems.

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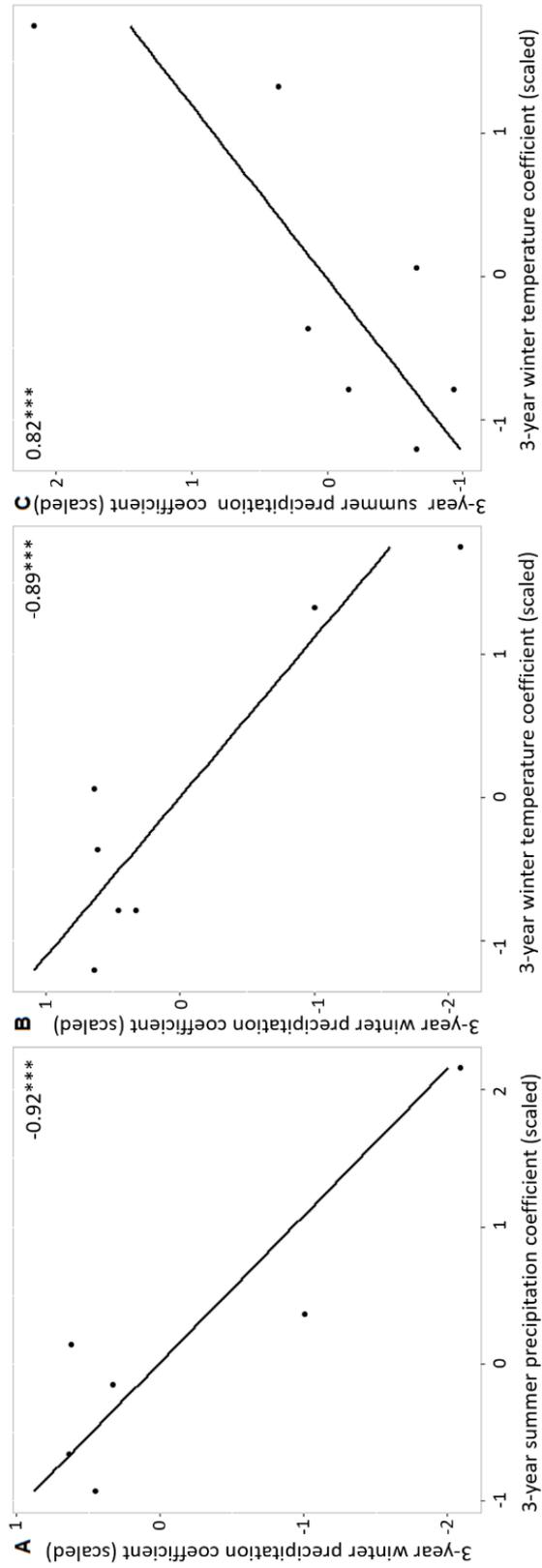
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Supplementary data

Sup data Fig 1: Spearman correlations between climatic variables. (A) 3-year winter temperature coefficient v.s. 3-year precipitation coefficient. (B) 3-year winter precipitation coefficient v.s. 3-year winter temperature coefficient. (C) 3-year summer precipitation v.s. 3-year winter temperature coefficient



Sup data Table 1: number of cushions, cushion ecomorphology per sites at T0 and T1 (mean ± SE)

Population	survey	year	month	number of cushion	number of dead cushion	cushion surface area (cm ²)	h necrotic extent (%)	"i _i " and "i _v "	"i _i " and "i _v "	neighboring plant community (%)
BLA	T0	2013	2	33	na	138,09 ± 26,24	8,56 ± 1,63			38,90 ± 3,72
	T1	2016	12	0	0	137,95 ± 25,04	26,01 ± 4,37			50,70 ± 4,86
HAU	T0	2013	11	41	na	19,81 ± 2,81	10,54 ± 2,60			14,99 ± 3,93
	T1	2017	2	3	3	20,65 ± 2,73	15,49 ± 4,21			14,52 ± 3,51
MAC	T0	2012	12	40	na	78,60 ± 13,56	13,83 ± 3,22			15,52 ± 3,17
	T1	2018	1	0	0	90,20 ± 13,27	19,72 ± 3,80			19,76 ± 3,04
MAY	T0	2014	1	37	na	178,55 ± 34,06	13,13 ± 2,94			35,61 ± 5,02
	T1	2018	12	0	0	230,87 ± 48,46	20,66 ± 4,96			33,91 ± 4,76
NOR	T0	2014	12	38	na	150,45 ± 23,64	17,58 ± 3,22			11,78 ± 3,07
	T1	2017	12	1	1	127,62 ± 23,43	27,97 ± 4,52			13,61 ± 3,33
PCR	T0	2014	11	29	na	38,70 ± 11,94	16,79 ± 3,97			20,47 ± 4,72
	T1	2018	12	20	20	36,18 ± 10,06	86,89 ± 9,33			29,50 ± 6,07
PDC	T0	2014	11	39	na	40,17 ± 7,05	6,53 ± 1,63			48,25 ± 4,89
	T1	2018	12	0	0	47,15 ± 8,15	11,24 ± 3,21			57,63 ± 4,61
RBA	T0	2013	1	46	na	86,98 ± 14,29	6,81 ± 1,99			33,91 ± 4,28
	T1	2019	1	10	10	132,73 ± 24,43	56,20 ± 5,95			44,78 ± 4,85

Sup data Table II: site geomorphology, 10-year mean seasonal climate and coefficient of slope variation at 10-years and 3-years are presented.

Category	Geomorphology				10-year mean seasonal climate				10-year seasonal climate slope coefficient				3-years seasonal climate slope coefficient					
	altitude (m)	slope (°)	block cover (%)	slope aspect	Wind intensity	Topography	summer temperature	summer precipitation	winter temperature	winter precipitation	summer temperature	summer precipitation	winter temperature	winter precipitation	summer temperature	summer precipitation	winter temperature	winter precipitation
random forest																		
"ii" and "iv"																		
BLA	19]0-3]	30	NE	exposed	terrace	6.009	322	2.34	320	24.25	0.08	26.88	0.04	-36.00	0.09	-8.50	0.02
HAU	125	0	80	Without	exposed	summit	5.986	419	1.33	410	30.72	0.08	33.62	0.04	-54.50	0.09	-13.50	0.00
MAC	72	0	64	Without	exposed	terrace	6.163	578	2.32	518	32.63	0.06	31.30	0.03	18	0.00	-0.00	0.01
MAY	59]3-10]	50	NE	exposed	slope base	6.009	322	2.34	320	24.25	0.08	26.88	0.04	-36.00	0.09	-8.50	0.02
NOR	32	0	20	Without	exposed	summit	5.486	505	0.99	487	35.82	0.07	35.10	0.03	-2.00	0.02	-17.00	0.00
PCR	151]0-3]	26	Without	exposed	terrace	4.522	991	1.52	748	45.68	0.06	46.76	0.04	33	-0.06	-54.00	0.05
PDC	246]0-3]	35	SE	exposed	mid slope	5.200	530	0.86	523	31.84	0.08	37.60	0.04	-36.00	0.13	-8.50	-0.01
RBA	79	0	1	SW	exposed	flat	5.208	1842	1.31	1404	106.07	0.07	84.09	0.05	154.5	0.03	-84.00	0.06

eco-morphological trait	Comparison	Z	P.adj
	BLA - HAU	5,228068	1,71E-07
	BLA - MAC	0,938369	0,348055
	HAU - MAC	-4,50863	6,52E-06
	BLA - MAY	-1,38308	0,16664
	HAU - MAY	-6,85249	7,26E-12
	MAC - MAY	-2,41932	0,01555
	BLA - NOR	-0,81154	0,417058
	HAU - NOR	-6,28728	3,23E-10
	MAC - NOR	-1,82658	0,067763
	MAY - NOR	0,597757	0,550002
	BLA - PCR	3,565027	0,000364
	HAU - PCR	-1,2993	0,193841
	MAC - PCR	2,815751	0,004866
	MAY - PCR	4,993996	5,91E-07
T0 cushion surface area	NOR - PCR	4,463215	8,07E-06
	BLA - PDC	3,085604	0,002031
	HAU - PDC	-2,20338	0,027568
	MAC - PDC	2,26253	0,023665
	MAY - PDC	4,623117	3,78E-06
	NOR - PDC	4,048976	5,14E-05
	PCR - PDC	-0,72425	0,468915
	BLA - RBA	1,285165	0,198735
	HAU - RBA	-4,32767	1,51E-05
	MAC - RBA	0,335391	0,73733
	MAY - RBA	2,827246	0,004695
	NOR - RBA	2,218305	0,026534
	PCR - RBA	-2,59046	0,009585
	PDC - RBA	-2,00598	0,044859
	BLA - HAU	5,542584	2,98E-08
	BLA - MAC	3,18154	0,001465
	HAU - MAC	-2,46595	0,013665
	BLA - MAY	0,477825	0,632775
	HAU - MAY	-5,21188	1,87E-07
	MAC - MAY	-2,77859	0,00546
	BLA - NOR	3,984234	6,77E-05
	HAU - NOR	-1,54625	0,122044
	MAC - NOR	0,882211	0,377663
	MAY - NOR	3,609394	0,000307
	BLA - PCR	3,616984	0,000298
	HAU - PCR	-1,54794	0,121637
	MAC - PCR	0,70705	0,479535
	MAY - PCR	3,25075	0,001151
T0 neighbouring plant community	NOR - PCR	-0,11114	0,911506
	BLA - PDC	0,242948	0,808045
	HAU - PDC	-5,53816	3,06E-08
	MAC - PDC	-3,06941	0,002145
	MAY - PDC	-0,24813	0,80403
	NOR - PDC	-3,90705	9,34E-05
	PCR - PDC	-3,52025	0,000431
	BLA - RBA	1,519149	0,128725
	HAU - RBA	-4,42161	9,80E-06
	MAC - RBA	-1,85774	0,063205
	MAY - RBA	1,051261	0,293139
	NOR - RBA	-2,74379	0,006074
	PCR - RBA	-2,42111	0,015473
	PDC - RBA	1,328141	0,184131
	BLA - HAU	0,870175	0,384205
	BLA - MAC	-0,15141	0,87965
	HAU - MAC	-1,07592	0,281963
	BLA - MAY	-1,14881	0,250634
	HAU - MAY	-2,11054	0,034812
	MAC - MAY	-1,04983	0,293796
	BLA - NOR	-1,956	0,050466
	HAU - NOR	-2,97064	0,002972
	MAC - NOR	-1,89739	0,057776
	MAY - NOR	-0,82419	0,409831
	BLA - PCR	-0,62694	0,530698
	HAU - PCR	-1,49639	0,134553
	MAC - PCR	-0,50829	0,611247
	MAY - PCR	0,465673	0,64145
T0 necrosis extent	NOR - PCR	1,240393	0,21483
	BLA - PDC	0,605502	0,544845
	HAU - PDC	-0,26953	0,787521
	MAC - PDC	0,794646	0,426819
	MAY - PDC	1,822628	0,06836
	NOR - PDC	2,670173	0,007581
	PCR - PDC	1,23487	0,216879
	BLA - RBA	0,665368	0,505815
	HAU - RBA	-0,24079	0,809721
	MAC - RBA	0,866802	0,38605
	MAY - RBA	1,932959	0,053241
	NOR - RBA	2,815566	0,004869
	PCR - RBA	1,313155	0,189131
	PDC - RBA	0,039382	0,968586

Sup data Table III: Dunn test results of the variability of cushion ecomorphology between sites, represent in the figure 2.

Table SIV: Results of quasi-Poisson generalised linear mixed model of the necrosis increase in relation with long-term climate trends. Coefficient of regression, p-value and R^2 estimated are shown for each explanatory variable. Bold text indicates significant variables. The number of observations and groups is indicated.

	N	Regression coefficient	p-value
model "GLMM3_a"			
Necrosis extent T0		-0.037	0.635
Cushion surface area		0.026	0.777
Neighbouring plant amount		-0.029	0.732
10-year summer precipitation trend		0.387	0.125
observations	278		
groups	8		
model "GLMM3_b"			
Necrosis extent T0		-0.047	0.557
Cushion surface area		0.028	0.757
Neighbouring plant amount		-0.019	0.823
10-year summer temperature trend		-0.309	0.184
observations	278		
groups	8		
model "GLMM3_c"			
Necrosis extent T0		-0.037	0.642
Cushion surface area		0.029	0.749
Neighbouring plant amount		-0.032	0.707
10-year winter precipitation trend		0.417	0.092
observations	278		
groups	8		
model "GLMM3_d"			
Necrosis extent T0		-0.040	0.618
Cushion surface area		0.024	0.794
Neighbouring plant amount		-0.028	0.745
10-year winter temperature trend		0.113	0.669
observations	278		
groups	8		

Chapter 5: Growth and age variability in contrasted environments and architectural traits allometry in *Lyallia kerguelensis* Hook. f. (Montiaceae)

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Abstract

Life span, anatomy and allometry are important features to estimate plant adaptive capacity to climate change. Indeed, long-lived species are associated with slow response to changes that might be challenging under rapid climate change. Furthermore, cushion form is shown by many plant species in harsh environments and might result in strong traits allometry due to physical constraints of the hemispherical shape. We focused on *Lyallia kerguelensis*, a long-lived cushion plant species endemic to the Kerguelen Islands. Its age, anatomy and architectural allometry were unknown, while we know that some cushions sustain visible growth and that morphological traits show strong correlations. When growth occurred, its rate was population-specific, probably due to contrasted environments across populations. *L. kerguelensis* stem anatomy did not show any rings and numerous crystals presumably of calcium oxalate and starch grains were observed. Allometry between basal stem diameter, number of apices and basal shoot diameter was found. Finally, age estimations by growth rate indicated that the species might live a few decades to rarely a few hundred years, with a small inter-population variation. This work will contribute to a better understanding of long-lived species growth and life span in contrasted environments in the sub-Antarctic.

Introduction

Plant life span is essential to consider when estimating their adaptive capacity to environmental changes (Beever et al. 2016). Adaptive capacity is defined as the ability of a species, or population, to cope with and persist under new environmental conditions (Beever et al. 2016). The duration of plant generation can condition genetic adaptation rate and plastic responses which might influence the outcome of adaptation (Bolnick et al. 2011; Snell-Rood et al. 2018). In theory, long-generation time species will need to genetically adapt faster per generation than short-generation time species to cope with changes (Hoffmann et al. 2017). In harsh cold environments native plants have presumably experienced long-term adaptation and therefore exhibit sufficient hardiness to survive harsh conditions (Hennion et al. 2006a, b; Körner 2016; Wang et al. 2019). Furthermore, these regions are subject to rapid climate change (Ranasinghe et al. 2021) at a time pace that might challenge species adaptive capacity. For example, in alpine regions in the context of climate change, long-lived plants might persist in unfavourable environments thanks to their phenotypic plasticity (Duputié et al. 2012; Cotto et al. 2017). They are predicted to eventually produce more maladapted seeds. A consequence of this might then be lower recruitment and decline of populations if genetic adaptation favourable to the new condition has not arisen in the population (Cotto et al. 2017). Therefore, both phenotypic trait variability and generation time need to be investigated to better understand the adaptive capacity of long-lived plants in harsh environments under rapid climate change. We focused on *Lyallia kerguelensis* (Montiaceae), a long-lived endemic cushion plant of the Kerguelen Islands. To complement the study of its adaptive capacity, we looked at its cushion growth rate, plant architecture and life span.

Harsh environments are defined by strong winds, low water storage in the soil and high fluctuations of both temperatures and precipitations (Körner 2003). Cushion plants have arisen from evolutionary convergence to hemispherical, tufted, or flat shapes with various compactness levels (Aubert et al. 2014). Their vegetative apparatus is composed of a main taproot and a short basal stem from which several branched stems start (fig. 1) (Aubert et al. 2014).

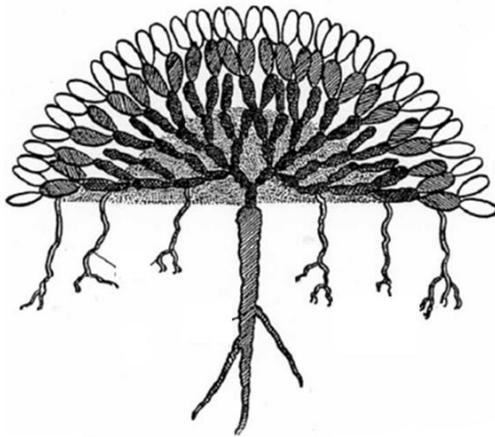


Figure 1: schematic view of a cushion plant architecture that sustains the cushion morphology (Aubert et al. 2014). The authorization to use the figures was given by the editors.

Cushion shape optimizes the surface-to-volume ratio, which keeps heat and water losses from the plant surface at a minimum and also helps it resist the strong mechanical constraints (i.e. wind, soil, freeze-thaw cycles, among others) of that environment (Zotz et al. 2000; Butterfield et al. 2013; Bergstrom et al. 2015; Combrinck et al. 2020). Cushion plants show strong correlations between morphological traits (Niklas 1994; Hermant et al. 2013; Aubert et al. 2014). Therefore, the architectural traits that sustain plant morphology might show strong allometry too. However, only a few studies addressed the architectural traits related to morphology or functionality in detail. Furthermore, knowledge on cushion growth is limited.

Cushion growth is slow from several millimetres to a few centimetres per year. For *Azorella selago* vertical growth rates ranged between 0.46 and 0.56 $\text{cm}\cdot\text{y}^{-1}$ in Marion Island and the Kerguelen Islands glacial margin, respectively (Frenot et al. 1993; le Roux and McGeoch 2004). Finally, the faster growth rate was that of *Azorella compacta* in central Andean altiplano with 1.46 $\text{cm}\cdot\text{y}^{-1}$ (Kleier and Rundel 2004). Cushion plants are too slow-growing for growth studies under controlled conditions to be informative enough. Growth rate can be used to estimate cushion age indirectly when dividing the cushion size by its annual growth rate (Frenot et al. 1993; le Roux and McGeoch 2004; Kleier et al. 2015). Relations between age and morphology were raised in cushion plants. Growth rate and cushion size could present linear (Morris and Doak 1998; Powolny et al. 2016) or non-linear relationships (Molau 1997; Kleier et al. 2015). However, these methods were applied only to a small number of individuals from only one or two populations, limiting the age estimation accuracy at the species level. To our knowledge, estimation of plant age in several populations contrasted by their micro-environments and their slope aspects was done only in *Azorella selago* in Marion Island (le Roux and McGeoch 2004) and highlights variations of growth rates in relation to biotic and abiotic micro-environments. As a whole, the longevity of cushion plants and their growth rates remain understudied, mainly because they are in hardly accessible environments where only a few methods can be applied.

Their slow growth often involves the rarity and long life of specimens, thus hampering destructive studies. Direct age estimation consists in counting stem rings in woody plants such as shrubs or trees and was applied a few times on cushion plants for example, for *Silene acaulis* (Powolny et al. 2016). Indeed, radial growth is possible through the cambium (secondary meristem), which produces xylem cells in particular (Rathgeber et al. 2016). Early in the growing season, xylem vessels are larger with thinner cell walls than xylem vessels produced later. This differential growth is mainly due to the soil water content, temperature, and nutrient variability within the growing season (Rathgeber et al. 2016). This differential pattern along the season allows distinguishing annual increment, called stem ring, in the wood.

For *L. kerguelensis*, its growth rate and age are unknown, and cushions might persist for more than a decade. Observations of several specimens showed that plants could persist at least 16 years (1990-2006, Wagstaff & Hennion 2007) and even up to 20 years (marked individuals 2000-2013, reidentified in 2019, Marchand pers. Obs.). Plant architecture and anatomy of this species were described in the last centuries (Hooker 1847; Werth 1911) without considering trait allometry or functionality. In our previous study (Marchand *et al.* 2021, Chapter 1), across 19 populations in contrasted environments, allometry of morphological traits was highlighted, cushion surface area, compactness and height strongly positively correlated. Furthermore, variation of the morphology of 303 cushions was observed across 5-year intervals (Chapter 4). Growth (an increase of cushion surface area) was noticeable only in half of the cushions, while the overall growth rate of the dataset was not different from zero (Chapter 4). Thus, a detailed understanding of plant growth is needed to better understand its life span. Studying its adaptive capacity is therefore relevant to better understand its vulnerability to climate change. Under the "*Lyallia kerguelensis* Observatory" (details in chapter 4), variation of cushion surface area was monitored, allowing the study of plant growth rate and estimation of its longevity. On top of it, detailed architecture and stem anatomy was studied on a few cushions to provide insights into architecture allometry and into possible age by observing stem rings.

The study aimed at (i) assessing the growth rate of individual cushions and study the variability of growth rates across populations. Then (ii) document architecture allometry and finally (iii) estimate the probable age of *L. kerguelensis* by extrapolating cushion size based on growth rate in contrasted environments, and by counting stem diameters. We assumed (i) that growth rates of cushions varied across populations due to contrasted environmental conditions. (ii) We expected to see correlations between architectural traits as morphological allometry was observed in this species. (iii) We hypothesized that the age could be estimated in this plant through growth rate and stem rings.

Material and Methods

Kerguelen Islands (48°30' - 50°S, 68°27' - 70°35'E) are located in the southern Indian Ocean and are subject to a chronically cold and windy climate (year average of 4.6°C between 1950-2020, Météo France 2021). These Islands harbour an endemic and long-lived cushion plant *Lyallia kerguelensis* (Montiaceae). Its distribution is scarce and restricted to fellfield habitats (Wagstaff and Hennion 2007). Fellfields are regarded as a form of tundra ecosystem composed of rocks or glacial till, oligotrophic and nutrient deficient (especially nitrogen) with a low water retention capacity (Aubert de la Rüe 1964; Smith and Mucina 2006; Block et al. 2009). Eight populations belonging to the "Lyallia Observatory" were studied to cover the species distribution range in contrasted environments (detail in chapter 4). The yearly growth rate of each cushion was then calculated with the following formula:

$$\text{Growth rate per year} = \frac{\text{cushion surface area } T1 - \text{cushion surface area } T0}{\text{number of months of the survey}} \times 12 \text{ months}$$

For the rest of the analyses, we focused only on the 150 cushions that showed positive growth. The mean population growth rate was calculated as well as the mean dataset growth rate.

To study cushion plant architecture and anatomy, six plants of small size (diameter <5-7cm, Marchand pers. obs.), presumably young, were collected in two geographically close populations (three plants in each) on "Ile Australia", AUS30 and AUS32. The sampling of larger individuals was avoided for obvious conservation reasons of slow-growing, long-lived plants. Diameter of the collar of the plant (later called stem diameter) and branches basal diameter (later called shoot diameter) were measured (Fig 2A and C), as well as the lengths of both. The number of shoots at different levels of branching and the number of apices per shoot were noted (Fig 2A). An apex is defined as the terminal part of the shoot comprising numerous small, densely imbricated leaves around an apical bud (Hooker 1847; Werth 1911).

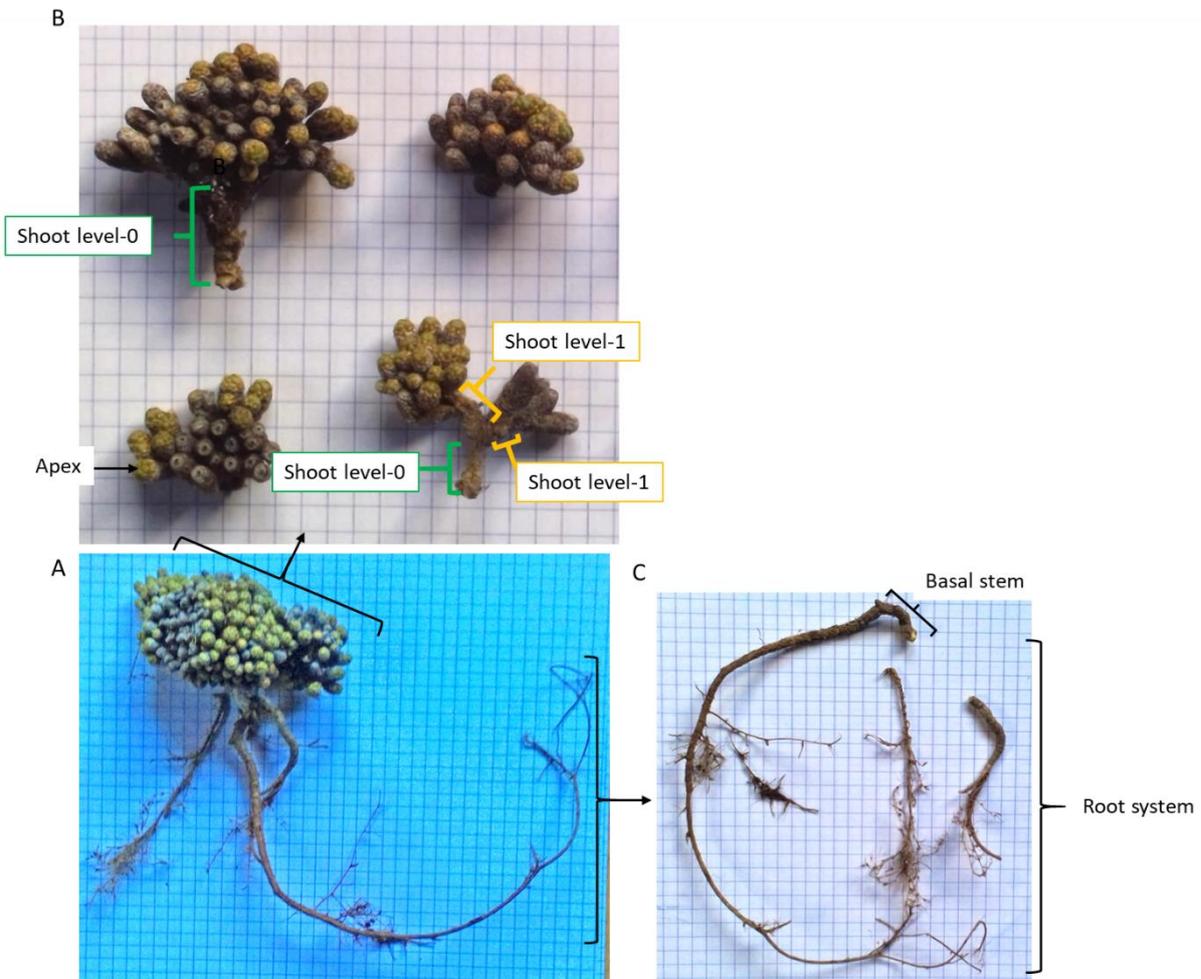


Figure 2: (A) Pictures of a *Lyallia kerguelensis* cushion (individual AUS30-32), (B) the stem that can be detached for the main stem are called level-0, and stem detached from level-0 are called stem level-1, apices are visible. (C) The main stem, roots system are shown. The background grid pattern is 5 mm.

Age estimation was done by considering a constant growth rate over the plant's life span (Frenot et al. 1993) and dividing cushion surface area by its growth rate. Three growth rate assessment methods were applied: (i) with the individual growth rate, (ii) with the mean population growth rate where the individual belongs, and (iii) with the mean dataset ($n=150$) growth rate.

Finally, to observe stem rings, the main basal stems of three cushions out of the six collected were cut and conserved in 70% (v/v) alcohol at 4°C before the observations. They were collected in January 2019, which is mid-summer in Kerguelen. Transverse sections were made in the stem manually using a razor blade, then put for 45 minutes in a diluted sodium hypochloride solution (9.6% m/m active chlorine) to empty the cell contents. The sections were then rinsed four times with tap water. In a second step, the sections were immersed in 30% acetic acid ($c \geq 99,8\%$, Sigma-Aldrich Merk, France) for 10 minutes and then stained with Mirande's reagent (Green carmine of Mirande, Sordalab, France). This reagent consisted of 1 mL iodine green, 20 mL 60% ethanol, and 1000 mL of 1% aluminium carmine (Deysson 1954). Finally, coloured sections were rinsed with tap water. To highlight starch content, a

drop of lugol (lugol:water, 1:100) was added on some sections. The coloured sections were made in a drop of water between slide and lamella, and observed using an optic microscope (CH40 Olympus, France) connected to a HD-Ultra camera (Euromex, The Netherlands).

Statistical analyses were performed with R version 4.0.2. (i) To study the growth rate variability between populations, we applied a Dunn's Kruskal-Wallis multiple comparison (package FSA, <https://github.com/droglenc/FSA>). (ii) Correlations between architectural traits were searched for by the Spearman method (package stats). (iii) To study population age variability with the three methods, we applied a Dunn's Kruskal-Wallis multiple comparison (package FSA).

Results

Growth determination and variability across the eight populations studied

Over the 303 cushions analysed, growth occurred in 19% (NOR) to 83% (RBA) of cushions depending on the population. Overall, across populations studied, 56% of cushions showed a positive growth rate (Table I). For the rest of the study, cushions with a positive growth rate ($n=150$) are kept. The mean growth was $7.50 \pm 1.16 \text{ cm}^2 \cdot \text{y}^{-1}$ across the dataset. At population scale, MAY ($18.46 \pm 5.52 \text{ cm}^2 \cdot \text{y}^{-1}$) cushions grew more than BLA ($6.59 \pm 2.52 \text{ cm}^2 \cdot \text{y}^{-1}$), RBA ($7.07 \pm 2.20 \text{ cm}^2 \cdot \text{y}^{-1}$), PCD ($3.70 \pm 0.96 \text{ cm}^2 \cdot \text{y}^{-1}$), HAU ($1.32 \pm 0.36 \text{ cm}^2 \cdot \text{y}^{-1}$) and PCR ($1.21 \pm 0.26 \text{ cm}^2 \cdot \text{y}^{-1}$) cushions (Table I and Fig 2). In contrast, MAY cushion growth was not significantly different to NOR ($17.87 \pm 7.69 \text{ cm}^2 \cdot \text{y}^{-1}$) and MAC ($5.41 \pm 1.56 \text{ cm}^2 \cdot \text{y}^{-1}$). Across other populations, the difference was less clear (Fig. 3).

Table I: number of cushions that grew, percentage of grown cushions, mean and standard error of cushion surface area at the beginning of the survey, growth rate per year, age estimations with the individual growth rate, population growth rate and whole dataset growth rate.

Population	Number of cushions	Grown cushions (%)	Surface area at T0 (cm^2)	Growth rate ($\text{cm}^2 \cdot \text{y}^{-1}$)	Age with individual growth rate	Age with mean site growth rate	Age with mean dataset growth rate
BLA	16	45.45	74.57 ± 27.63	6.59 ± 2.52	24.99 ± 3.48	15.15 ± 5.08	13.31 ± 4.47
HAU	20	52.63	20.04 ± 3.78	1.32 ± 0.36	40.68 ± 13.31	18.45 ± 3.03	3.24 ± 0.53
MAC	25	62.50	65.23 ± 13.86	5.41 ± 1.56	33.90 ± 7.71	17.14 ± 3.05	12.36 ± 2.20
MAY	22	62.86	218.46 ± 53.29	18.46 ± 5.52	31.51 ± 8.12	16.86 ± 3.86	41.22 ± 9.50
NOR	8	18.92	176.78 ± 63.67	17.87 ± 7.69	29.25 ± 11.71	12.89 ± 4.57	30.72 ± 10.88
PCR	4	50.00	24.61 ± 6.96	1.21 ± 0.26	37.92 ± 19.82	25.53 ± 4.74	4.11 ± 0.76
PDC	25	64.10	43.66 ± 10.22	3.70 ± 0.96	38.55 ± 18.98	15.88 ± 3.35	7.83 ± 1.65
RBA	30	83.33	100.10 ± 17.19	7.07 ± 2.20	36.64 ± 4.77	20.16 ± 3.92	19.00 ± 3.69

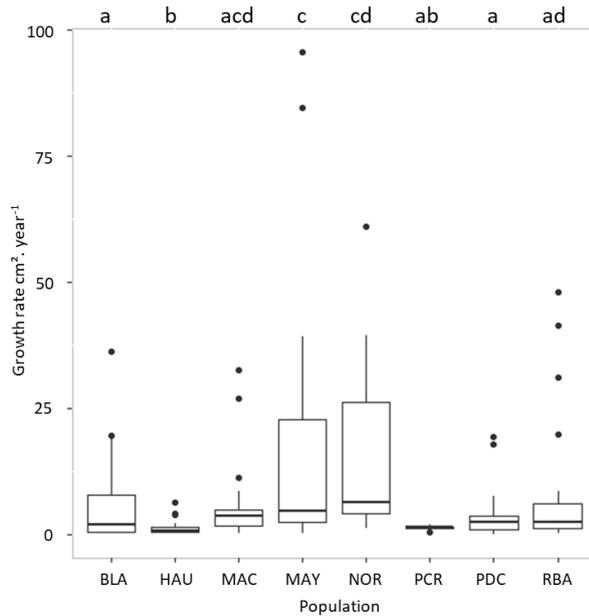


Figure 3: boxplots of cushion growth rate per population. Different letters indicate significant differences between populations (Dunn's Kruskal-Wallis comparison).

Stem anatomy

The anatomical features of the three stems studied were similar. The cambium is continuous, while the xylem and phloem are in regular poles around the stem (Fig. S1 A, B and C). In length, the xylem seemed to be continuous, without cell size differences (Fig. S1 A, B, C and D). Interestingly, ramification and interruption of the xylem were visible (Fig. S1 A and B). A large bark of around 382 μ m was observed around the stem, composed of several layers of densely lignified cells (Fig. S1 A and E). Numerous crystals, with a star shape, were visible in different tissues of the stems (Fig S1 A, C and focus on one in F), their diameter range from 37 to 74 μ m. Finally, within parenchyma cells, numerous amyloplasts were observed (Fig S1. D and focus on a cell content in G)

Architecture of small individuals

L. kerguelensis was composed of a short thick stem (0.53 ± 0.05 cm of diameter and 1.87 ± 0.49 cm of length) with underlying up to 13 level-0 shoots (mean \pm SE, 6.33 ± 3.30). Every cushion (except AUS32-B) showed level-1 shoots (6.00 ± 3.30) and one level-2 shoot was observed for AUS32-A cushion (Tables II). Almost half of the apices were on the level-0 shoots (79.83 ± 23.01 apices), the rest of the apices were on the level-1 shoots (76.67 ± 23.81 apices) except for one individual with 26 apices on level-2 shoots (Table II). Stem diameter was positively correlated to the number of level-1 shoots (correlation = 0.896, p-value = 0.016, Fig. 4). The same result was reached with the number of apices after removing one individual (correlation = 0.0618 and 0.949, p-value= 0.191 and 0.014, Fig. 4 C and D). Similarly,

the diameter of level-1 shoots was positively correlated to the total number of apices (correlation = 0.943, p-value = 0.0167, Fig. 4 B).

Table II: Architectural traits of the six small individuals, stem diameter and length, number of ramifications at level-0, 1 and 2, the diameter of ramifications level-0, 1 and 2.

Population	individual	stem diameter (cm)	stem length (cm)	Level of ramification	Number of level-0 shoots	Number of level-1 shoots	Number of level-2 shoots	Total number of apices	mean diameter level-0 shoots (cm)	mean diameter level-1 shoots (cm)	mean diameter level-2 shoots (cm)
AUS30	A	0.6	0.7	2	13	4	na	264	0.43	0.40	na
	B	0.5	1.5	2	5	1	na	131	0.23	0.33	na
	C	0.6	4.2	2	11	4	na	282	0.40	0.38	na
AUS32	A	0.7	1.3	3	2	19	1	126	0.30	0.31	0.3
	B	0.4	2.0	1	2	na	na	93	0.40	0.30	na
	C	0.4	1.5	2	5	2	na	75	0.13	0.27	na

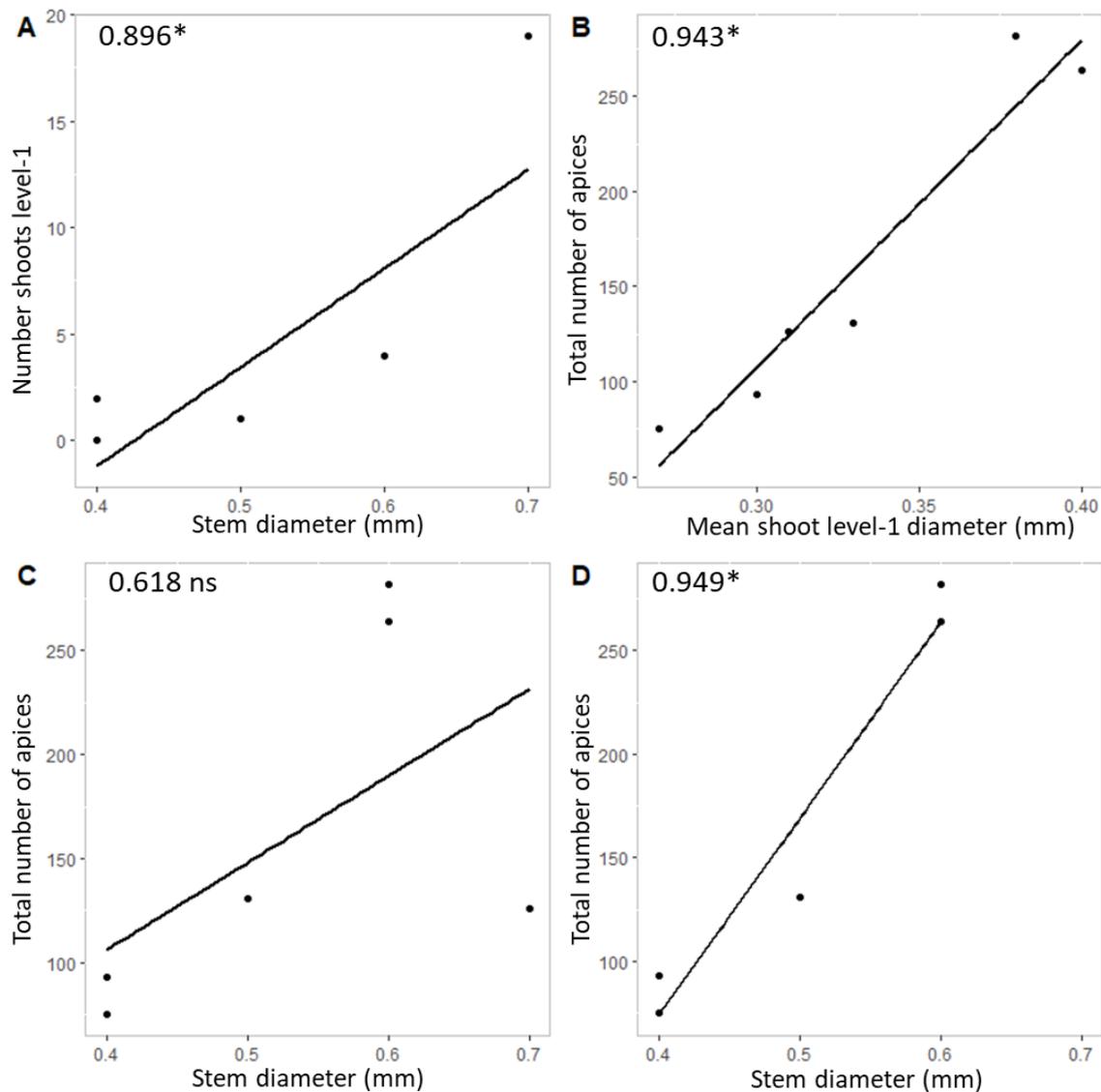


Figure 4: (A) correlations between the number of shoots level-1 and stem diameter, $n=6$ (two overlapped points), (B) Total number of apices and mean ramet-1 diameter, $n=6$, (C) Total number of

*apices and stem diameter with the 6 individuals, n=6 or (D) when removing one individual AUS32-A. The Spearman correlation coefficient is written with the p-value associated: 'ns'= not significant, '**' < 0.01 and '***' < 0.001.*

Cushion age estimation

When estimating the age of 150 cushions based on the individual growth rate, the mean age was 35 ± 4.2 years and cushions ranged from 5 to 490 years old. When the mean population growth rate was used to estimate cushion age, the mean age was 17 ± 1.7 years ranging from less than a year to 82 years, while the mean dataset growth rate was used to estimate cushion age, the mean age was also 17 ± 2.0 years ranging from less than a year to 160 years. Across the eight populations, the age did not vary significantly either with the individual growth rate method or with the population growth rate method, except for one population in each of these cases (Fig. 4A and B). Using the individual growth rate method PDC was younger than RBA, while BLA was younger than PCR when using the population mean growth rate method (Fig. 4A and B). Age assessment with the mean dataset growth rate method showed more variability within and between populations than with the two other methods, reflecting the cushion surface area variability across populations (Fig 4C). With this method, plants in MAY and NOR were supposed to be older than plants in most other populations (Fig. 4C).

The age estimation with the individual growth rate method provided an older age estimate compared to the two other methods (Fig. S2A). Age estimation based on the dataset mean growth rate gave a smaller age range (Fig. S2A). The three age estimation methods showed significant age differences across most populations, except NOR where only eight cushions grew out of thirty-seven (Fig. S2B).

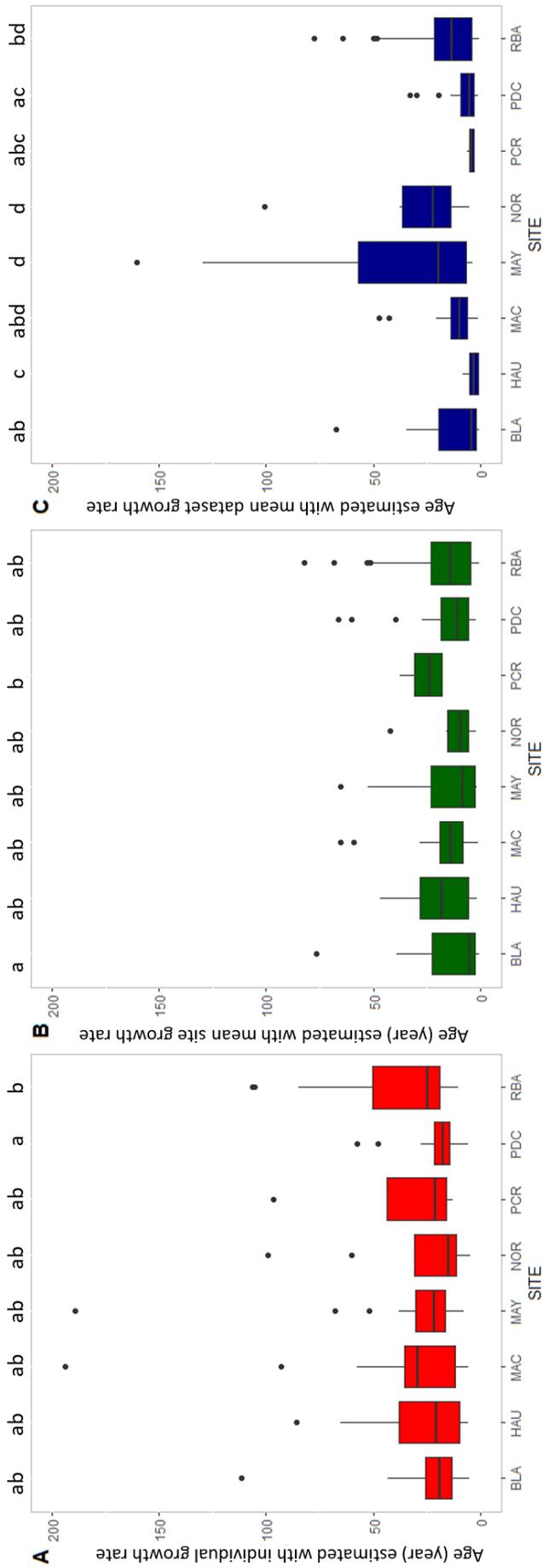


Figure 4: boxplots of age per population with three methods, (A) calculated with the individual growth rate, two points were removed from the graph to increase its size (respectively one individual of 490 years in PDC population and 275 years in HAU population), (B) calculated with the mean population growth rate and (C) calculated with the mean dataset growth rate. Different letters indicate significant differences between populations (Dunn's Kruskal-Wallis comparison).

Discussion

Cushion growth estimation

L. kerguelensis mean growth rate ($7.50 \pm 1.16 \text{ cm}^2 \cdot \text{y}^{-1}$) appears to be in the range of other cushion plant growth rates. However, the growth measurement method was not similar to other studies; precise comparison with other species' growth rates cannot be discussed. For *L. kerguelensis*, the growth rate varied between individuals and populations and might be explained by plant sizes and contrasted environments among the selected populations. Indeed, a previous study of 19 populations of *L. kerguelensis* showed that cushion surface area was related to population topography and slope (Marchand *et al.* 2021, chapter 1). Furthermore intrinsic positive relationship between cushion plant size and growth rate might be expected as larger plant might have more resource to invest in growth than smaller ones. It should also be noted that cushions may be formed of several genetic individuals due to frequent dispersal of seeds and germination of *L. kerguelensis* offspring *in situ* in the cushion (Hennion and Walton 1997). This was also observed for the large and irregular cushions of the other cushion species *Azorella selago* (Cerfonteyn *et al.* 2011). Also, a better understanding of within-population microenvironment would be essential to determine which variables influence growth rate as observed for *A. selago* in Marion Island (le Roux and McGeoch 2004).

Cushion stem anatomy

The stem of *L. kerguelensis* is characteristic of angiosperm species. (Behnke 1993) observed calcium oxalate crystals with a star shape for this species, therefore the crystals we observed might be calcium oxalate crystals. Their possible functional role, however, is still under debate. The xylem vessel's size variation was not conspicuous compared to numerous woody species (Rathgeber *et al.* 2016), it was impossible to distinguish successive annual growth rings. This contrasts with the results in the few other shrubs or cushions plants in harsh environments for which the stem anatomy is known (Powolny *et al.* 2016; Anadon-Rosell *et al.* 2018; Francon *et al.* 2021). Thus, in *L. kerguelensis*, the indiscernable growth rings in wood cannot tell us the age of the cushion.

Architecture

Allometry (relation between growth traits and the entire morphology) of architectural traits was visible for (small individual of) *L. kerguelensis*. Stem and shoot diameters were correlated to the total number of apices. Our previous study showed that the total number of apices was correlated to the cushion surface area (Marchand *et al.* 2021, chapter 1). The conservation of architecture allometry that underlies plant morphology is critical in a harsh environment. Indeed, conservation of the optimal cushion shape is often correlated to plant vigour (Zotz *et al.* 2000; Bergstrom *et al.* 2015; Combrinck

et al. 2020). For *L. kerguelensis*, high phenotypic integration (strong correlation among cushion height and diameter) was found (Hermant et al. 2013). The lack of overall correlation between stem diameter and number of apices when considering six individuals might be due to one individual that lost cushion parts, resulting in a lower number of apices compared to its stem diameter. In Chapter 4, we observed that for some cushions, the necrotic part might be removed from the plant within few years, most probably detached by the strong mechanical stresses linked to wind and freeze-thawing conditions in its habitat. Complementary measurements and analyses of correlations among architectural and morphological traits should be carried out to confirm our observation. Obtaining an easy and straightforward measurement of the cushion size by its stem diameter directly in the field might be useful for further research targeting specific cushion size or comparison between cushion size classes.

Cushion age estimation

The three simple methods (estimation with individual growth rate, mean population growth rate or mean dataset growth rate) used to estimate the ages of 150 *L. kerguelensis* cushions suggest that this species could live for a few decades to even a hundred years for some. When considering individual growth rates, age estimation was higher than when considering the mean growth rate of the population or of the datasets. This might be explained by the variation of growth rate during plant life span or due to contrasted microenvironments within population. Across populations, the mean age of *L. kerguelensis* is similar when estimating it with individual or population growth rate, except for RBA and PCR respectively, which might be older. RBA and PCR are located in the coldest, wetter and probably windier environments in the extreme south-west and the extreme north of the archipelago, respectively. Therefore, they might have an extremely slow growth rate with a longer life span. When using the dataset mean growth rate to estimate cushion age, huge variability was observed within and across populations. This age variability reflects the variability of cushion surface area observed within and across populations for this species, as previously reported (Chapter 4, Marchand *et al.* 2021). Similar observation was done for *A. selago* in Marion Island where the accuracy of age estimation varies with plant growth rate and size (le Roux and McGeoch 2004). Therefore, estimating age with mean dataset growth rate is inaccurate for these species and probably for other species in contrasted harsh environments.

Conclusion

Overall, *L. kerguelensis* growth rates vary between populations. Growth estimation in a common garden experiment might disentangle the implication of genotype and environment in growth rate. Allometry was observed for architectural traits strengthening the conserved morphology already

reported for this species. *L. kerguelensis* might live a few decades and rarely more than a few hundred years in every population. This age assessment is essential to estimate the species' adaptive capacity as life span is one of the key features contributing to plant capacity to tolerate climate change.

Acknowledgements

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Supplementary data

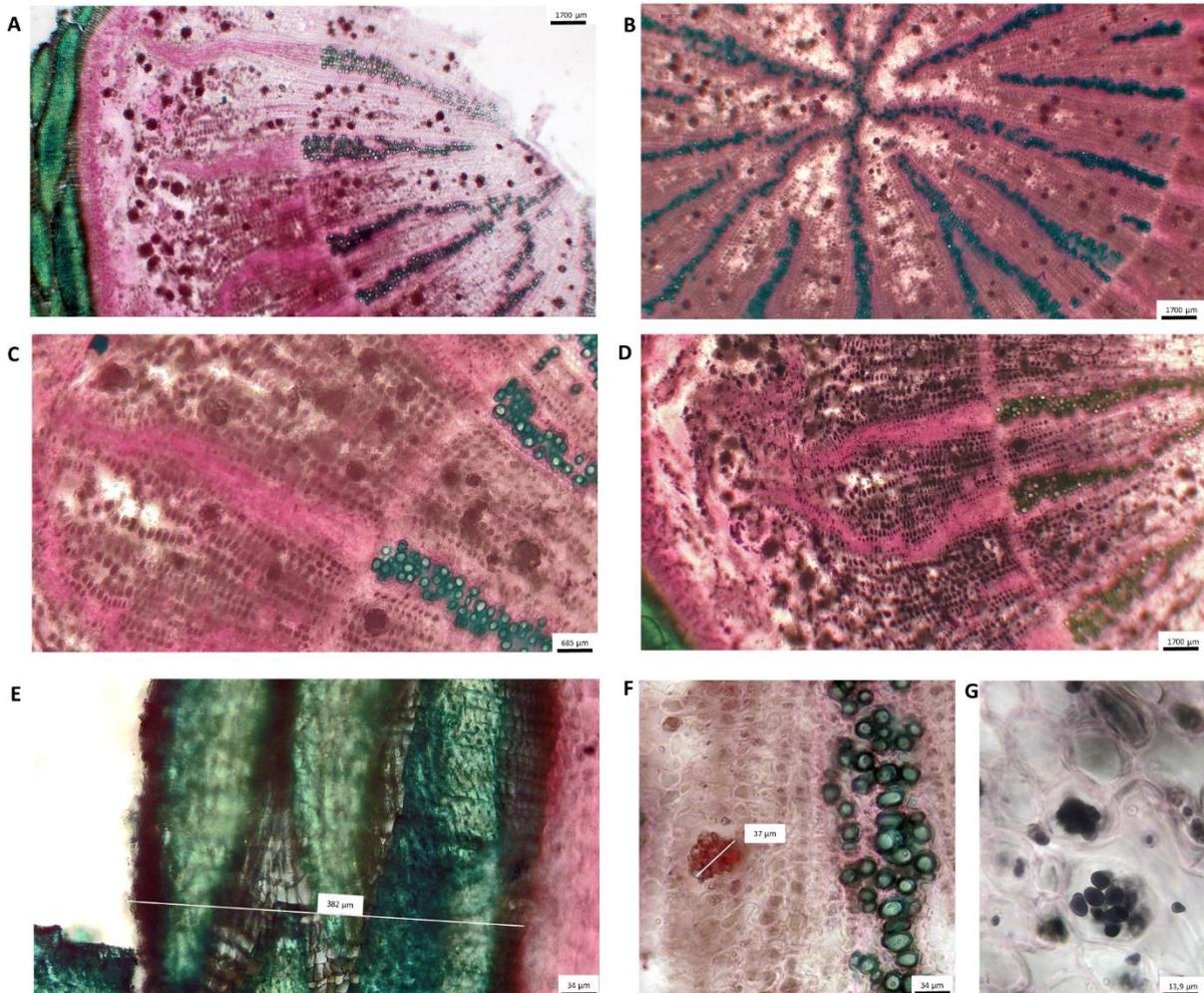


Figure S1: Anatomy of the stem of L. kerguelensis. (A) Basal stem radial section with bark, periderm, phloem, cambium, xylem and pith. Vascular bundle is discontinuous and the cambium is continuous; (B) xylem ramification and discontinuity, no pith visible at the basal part of the stem; (C) cambium, xylem and phloem; (D) the radial section with Lugol coloration, brown points are amyloplasts; (E) periderm with layers of sclerenchyma fibres and cork, length is measured; (F) calcium oxalate crystal with a star shape in the parenchyma (Behnke 1993), length is measured; (G) numerous amyloplasts in parenchyma cells with Lugol coloration.

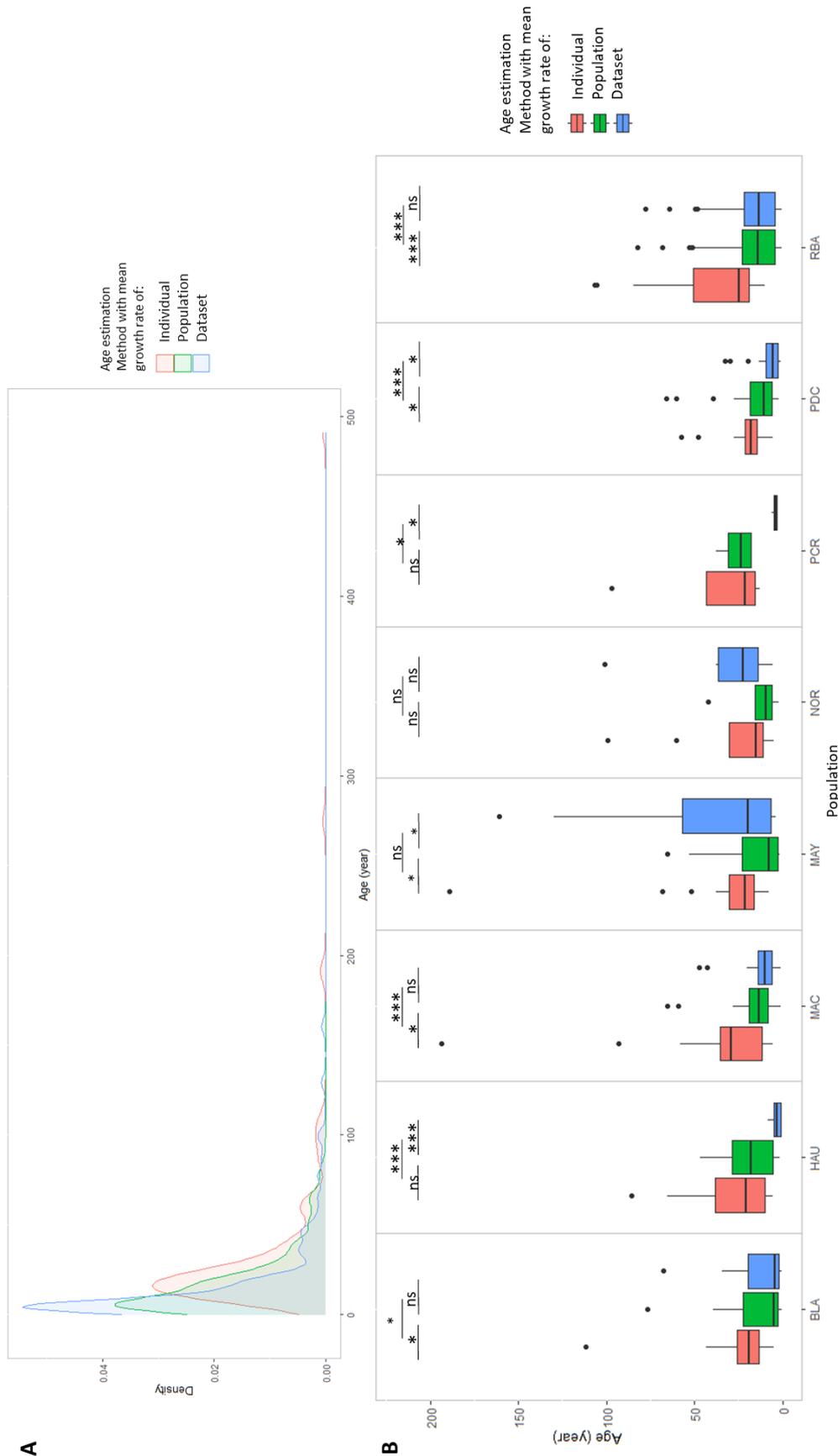


Figure S2: (A) Density plot of the age estimates for every cushion with the three methods (individual growth rate in red, mean population growth rate in green and mean dataset growth rate in blue (B) boxplot of the age per population estimated by three different methods, the same colour code is used. Different letters indicate significant differences between populations (Dunn's Kruskal-Wallis comparison).

General discussion

One of the greatest challenges of this century is to understand the possible impact of climate change on species and ecosystems. We currently have a limited understanding of the adaptive capacity of plant species under climate change. It is essential because many plant species may not be able to shift geographically to more suitable environments, leading to local and eventually overall extinction. Knowledge is even more limited for species in the remote sub-Antarctic Islands, while these islands face one of the most rapid climate changes on Earth. One of them, the Kerguelen Islands, harbour the long-lived endemic species, *Lyallia kerguelensis*, which might seem ill-equipped to face drastic climate change. Its strict endemism to these islands, its relatively scarce distribution, its low reproductive capacity and occurrence of necrosis led to questioning its adaptive capacity. We hypothesized that the pool of variability (for morphology, transcriptome and soil rhizomicrobiome) of *L. kerguelensis* across contrasted environments might provide insights into its adaptation to harsh environments and its possible response to rapid climate change.

Pool of variability of *L. kerguelensis*

This study revealed (Fig. 1) that *L. kerguelensis* showed strong allometry in morphological and architectural traits (chapters 1 and 5). Morphological trait variability was observed across populations (Chapters 1, 4 and 5) and was related to environmental variables such as soil water content and wind exposure (chapter 1). Across regions, *L. kerguelensis* gene expression profiles were specific, within region expression profiles showed low individual and population variabilities (Chapter 2). Differential gene expression between regions seemed to be related to abiotic and biotic responses (Chapter 2). Fellfield soil microbiome compositions of *L. kerguelensis* showed differences across populations in relation to soil nutrient contents (Chapter 3). *L. kerguelensis* rhizomicrobiome was as diverse as the bulk soil, nevertheless with a partly different composition (Chapter 3). Across 5-year time, under the *Lyallia kerguelensis* Observatory, we found that species growth rate was not significant, the number of cushions that grew was offset by the number of cushions that shrunk (Chapter 4). When taking into account the subsample of significantly grown cushions, growth rate and age were population-specific (Chapter 5). Necrosis extent was related to soil sodium content (Chapter 1), other plant morphological traits (chapter 4) and soil microbiome composition and function (Chapter 3), while its increase was more strongly related to fast drying and warming climate (Chapter 4).

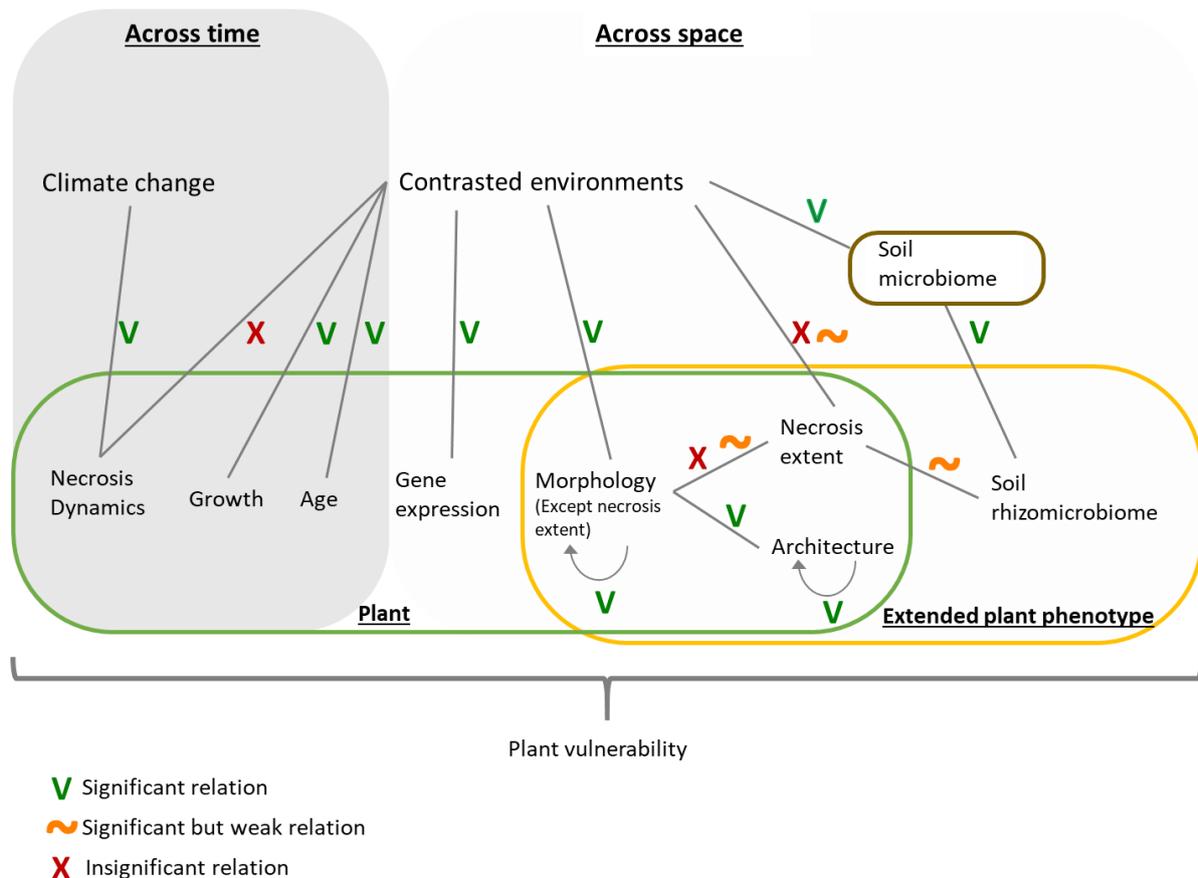


Figure 1: schematics of the results obtained in this Ph.D. for: the variability across time of plants' necrosis dynamics, growth and age in relation (or not) with environmental variables and climate change; the variability across space of plant gene expression, morphology, necrosis extent and soil rhizomicrobiome in relation (or not) to contrasted environments. The relation between soil rhizomicrobiome and contrasted environments has been studied indirectly with the bare soil microbiome. Relation between morphological traits has been studied (curve arrow). Traits indicated the relation studied between two variables. Green "V" indicates significant relation, while red "X" indicates insignificant relation and orange "~" indicates significant but weak relation. Grey shade indicates the study across time, while white shade indicates the study across space.

Towards an estimation of the adaptive capacity of *L. kerguelensis*

Inter-population or region variability

Among populations in contrasted environments, variability of plant morphology across space (Chapter 1) and across time (Chapter 4) was observed. Gene expression profiles and differential expression showed greater variability among regions than among populations within each region. Variability of age, growth (Chapter 5) and of rhizomicrobiome communities (Chapter 3) were observed between populations.

L. kerguelensis' cushion surface area, shape, and compactness were well explained by environmental variables related to soil water content and/or wind exposure, without clear

geographical structuration (Chapter 1). These three traits are essential elements of cushion morphology to maintain vigour under strong mechanic stresses, as observed in other cushion species (Hausmann et al. 2009; Kleier et al. 2015; Zhao et al. 2018; Combrinck et al. 2020). This suggests that the pool of morphological variability of *L. kerguelensis* exists and responds to specific environmental variables studied. We might hypothesize that its range of morphological variability across space and time is limited when the change is too intense due to the strong correlation among traits, (i.e. cushion diameter and high) as phenotypic integration occurring in this species (Hermant et al. 2013). Loss of vigour was recently observed in several cushion plant species in relation to a modification of the morphology (Zotz et al. 2000; Combrinck et al. 2020). The growth rate variability was insignificant over time when studying all cushions (Chapter 4). Indeed at the species level the number of cushions that grew was offset by the number of cushions that shrunk. However, when focusing only on grown ones, variability was observable between two populations (Chapter 5). Similarly, cushion age differences were visible between two populations but not among other populations (Chapter 5). Therefore, environmental variables seem to interact little with plant life span and growth rate which are major drivers of plant morphology in general. We conclude that extreme slow growth can hardly be captured with our methods and would require other techniques such as fix camera. Improved accuracy in growth measurements may better reveal the implication of environmental variables in growth variability. Indeed, the growth rate of *Azorella selago* in Marion Island was well explained by environmental variables at macro and micro scales (le Roux and McGeoch 2004). Therefore, the variability of morphological traits, usually in response to the local environment, might reveal a response capacity of *L. kerguelensis* to change in environmental conditions if the intensity or speed of change is rather low.

Understanding the patterns of variability that underlie morphological traits was addressed by studying gene expression profiles. Indeed, the transcriptome is known to be correlated to a certain extent to the proteome (Voelckel et al. 2017). This approach was even more meaningful for *L. kerguelensis* as inter and intra-population genetic variation is extremely low (Robert et al. 2021). Populations within a region showed little contrasting gene expression while differences among regions were larger (Chapter 2). The gene expression pattern of populations was linked to the population environmental variables (Chapter 2). For the two most contrasting regions, the genes differentially expressed were related to abiotic and biotic stress responses (Chapter 2). Such environmental responses might be expected if *L. kerguelensis* is adapted to its local conditions or micro-environment through plastic expression of its adaptive genetic variation. This first study indicates directions for future analyses that will aim at comparing transcription profiles from other regions, test results with regional characteristics (such as climatic data used in Chapter 4) or other environmental data.

The extended phenotype of *L. kerguelensis* with its rhizomicrobiome was studied during the Ph.D. and showed variability across populations (Chapter 3). Several filters characterised its variability, first, the soil microbial communities were spatially structured according to ammonium and phosphorus contents of the soil, which induced site specificity (Chapter 3). Both elements are known to limit organism development in sub-Antarctic and Antarctic regions (Smith 1979; Yergeau 2014). Second, rhizospheres shaped the rhizomicrobiome compositions mainly at population scale for bacteria, while it was more complex for fungi, notably because of numerous unknown ones (Chapter 3). Plant filtering should be toward an increase of beneficial microorganisms for its functioning (Berendsen et al. 2012) in relation to contrasted environments and might participate to its adaptive capacity. The tight relation between cushion plant rhizomicrobial composition and environment was highlighted in a few other studies (Roy et al. 2013; Wang et al. 2020) and might be seen as an essential component to help the plant to adapt to its environment, especially under rapid climate change.

When combining the results of morphological variability, gene expression and rhizomicrobiome composition, we observed that they all responded to environmental variables that structured populations or regions. Thus, *L. kerguelensis* and its extended phenotype seem to respond to a certain range of environmental variables. This might be an essential element for species to keep up with the speed of climate change and local adaptation (Moritz and Agudo 2013; Cotto et al. 2017). However, the plant necrosis (*i.e.* possible abiotic stress response) visible in numerous plants might indicate that *L. kerguelensis* adaptive capacity is limited.

When focusing on the cushion necrosis extent, we observed variability across populations (Chapter 1) and across time (Chapter 4) and that might be partly related to environmental variables (Chapter 1 and 4) and soil rhizomicrobiome (Chapter 3). The lack of relation between necrosis extent and plant morphology is further discussed in the section below. When linking necrosis extent to environmental variables, soil sodium content was positively associated with the increase of necrosis in cushions with low levels of necrosis (Chapter 1). In contrast, cushions with a higher level of necrosis (Chapter 1) or randomly sampled (Chapter 4) were not related to any environmental variables. Osmotic potential is an essential element for plant functioning and stress induced by more saline conditions might lead to modification of water and nutrient absorption (Pardo and Quintero 2002; Shabala 2013). Such might be the case for *L. kerguelensis*. We only highlight that the soil sodium content was positively correlated to necrosis extent, however, the origin of necrosis might rely on the interaction between several factors. The clearest link between necrosis extent and environments comes from the temporal study (Chapter 4). Within five years, the increase of necrosis was related to climate change, a faster increase of necrosis occurred in the warmest and driest locations in winter (Chapter 4). Furthermore, during

the study, only a few cushions showed either re-greening or death (Chapter 4), suggesting that necrosis does not lead necessarily to death within a short timeframe, but re-greening is rather rare. It should be noted that *L. kerguelensis* is a long life-span species, therefore, the necrosis extent across populations might be related to previous events (stochasticity) which might partly explain why necrosis extent could not be clearly related to the environmental variables recorded in this study. The possible origin of the necrosis is visible also at the “omic” level. The Gene expression in vigorous plants were related to various abiotic stresses, possibly drought, in the driest and warmest regions (Chapter 2). Relation between rhizomicrobiome composition and necrosis extent was raised. No shared microorganisms were related to necrosis extent across populations, but a shift of microbial functions (decomposition, soil phosphorus content and nitrogen assimilation) might occur (Chapter 3). Furthermore, a study performed by collaborators and targeting leaf bacteria in the *L. kerguelensis* apices could not evidence any pathogens (Portier 2019). These results support an abiotic origin of the necrosis in *L. kerguelensis* and possible relation to the recent climate change that may impact plant (and its extended phenotype) functioning, particularly drought stress. The impact of drought stress is already visible in numerous species such as trees, in various ecosystems, hydraulic failure and damping carbon storage were highlighted as main mechanisms leading to plant death (Choat et al. 2018; Tixier et al. 2019; McDowell et al. 2020). This was one of our mechanistical hypotheses for explaining necrosis in *L. kerguelensis* shoots and we tried to approach the hydric potential of cushions in summer to estimate its hydraulic functioning (Annex 1). The results showed contrasting patterns, with variability of the intensity of the hydric potential values and daily patterns at population and individual scales (Annex 1). The results suggested that demonstrating the impact of drought on *L. kerguelensis* cushions might be extremely challenging. Variability of intensity might be a limit of this method in harsh environments, while daily patterns might suggest a possible complex hydraulic functioning. This result raised the question about *L. kerguelensis* photosynthetic pathway such as C3-CAM pathway, already highlighted in its family (Montiaceae) in species considered adapted to dry conditions (Wang et al. 2019). The difficulties in capturing the origin of necrosis in *L. kerguelensis* are reminiscent of those raised for other plant species in the sub-Antarctic. While this topic has been the subject of several studies, the impact of climate change was highlighted in every one of them (Whinam et al. 2014; Bergstrom et al. 2015; Dickson et al. 2019, 2020).

Overall, *L. kerguelensis* seems to be able to buffer environmental stress to a certain extent with its morphological traits locally adapted to its environment, gene expression in relation to stress responses and rhizomicrobiome shaped by the plant. Nevertheless, necrosis extent might be a sign of limit of adaptation to rapid climate change. Every approach points out the relation between necrosis and abiotic stresses with a possible threshold above which necrosis might spread. Finally, the necrosis might not directly lead to death faith. Indeed, its increase was vast, but the record of plant death was

limited. This might be seen as a possibility for *L. kerguelensis* to survive, to a certain extent, in unsuitable environments and raises the question about population regeneration capacity, further addressed below.

Inter-individual variability

Within populations, morphological (cushion surface area, growth rate and age; Chapters 1, 4 and 5) and rhizomicrobiome (diversity and composition; Chapter 3) variabilities were observed while the transcriptome was conserved (Chapter 2). The presence of variability between individuals within a population is important as it increases the probability that a phenotypic trait value matches the novel environment and allows plant survival and population persistence (Bolnick et al. 2011). Within a population, *L. kerguelensis*' morphological variability might be related, to age or micro-environment. We highlighted that age and growth varied within populations (Chapter 5). It should be noted that younger cushions might react differently to older ones, as metabolism might be more active in young ones as generally observed in long-lived plants (Thomas 2013). Furthermore, younger/smaller plants might have a lower potential to buffer surrounding conditions and, due to a smaller and less extensive root system, less resistance to frost-heaving during soil freezing and less ability to reliably access resources in heterogeneous soils (Frenot et al. 1998). Morphological variability might be related to micro-environmental variables (not always captured) as observed for other cushion plant species (Kleier and Rundel 2004; le Roux and McGeoch 2004). Cushion evolutionary convergence in harsh environments might suggest that this form is adapted to the mechanical stresses occurring in such environments (*i.e.* wind, soil water content, freeze-thaw cycles and large temperature fluctuations) (Hausmann et al. 2009; Körner 2016; Combrinck et al. 2020). Strong correlations between morphological traits to sustain cushion shape (Niklas 1994; Zotz et al. 2000; Combrinck et al. 2020) might limit phenotypic variability. For *L. kerguelensis*, we observed strong allometry of morphological and architectural traits (Chapters 1 and 5), strengthening the conserved phenotype of this plant. The rhizomicrobiome diversity and composition were partly individual-specific for bacteria and mostly individual-specific for fungi (Chapter 3). The fungal rhizomicrobiome composition might be sensitive to both soil composition and host-plant genotype, as observed in the case of *Silene acaulis* (Roy et al. 2018). Finally, the transcriptome within populations is conserved for *L. kerguelensis* (Chapter 2). The extremely low genetic diversity might explain this observation within *L. kerguelensis* populations (Robert et al. 2021). Variability of necrosis extent across individuals within populations was observed (Chapters 1 and 4) and might be related weakly to inter-individual variability of cushion surface area and neighbouring plant community (Chapter 4). Necrosis extent in vigorous and necrotic cushions was not related to morphological traits (Chapter 1). When applying random sampling, the extent of

necrosis was barely explained by cushion surface area and neighbouring plant amount (Chapter 4). Necrosis extent seems to be independent of cushion morphology and plant age. Therefore, necrosis extent might not exclusively result from aging of the plant (Thomas 2013), where older cushions exhibit larger necrosis (Molau 1996; Kleier and Rundel 2004). The majority of the variability remains unexplained and might be related to micro-environmental scale or stochastic events that occurred within the population as necrosis was not related to cushion allometry, in the section detailed below. Therefore, more research is needed to better understand the intra-population necrosis dynamics in relation to micro-climate, micro-topography (such as wind pattern) and biotic relations (such as distance with the first neighbouring plant). All these elements were partly involved in necrosis extent in other cushion plant species in the sub-Antarctic islands (le Roux and McGeoch 2004; Le Roux et al. 2005; Haussmann et al. 2009; Bergstrom et al. 2015; Combrinck et al. 2020). Overall, the variability within a population, which we could not fully capture and explain, might be advantageous for the plant to cope with climate change as it increases the possibility of a trait to be appropriate to the new environment. These measure of variability likely need to be complemented by demographic (next section) Allometry of morphology and architecture was observed and the morphological variability was related to environmental variables. Necrosis variability would need to be more studied and better understood to have more insight into the vulnerability of individuals within a population. *In-natura*, an experiment of environmental modifications at plant scale (shading, sheltering, rain exclusion, warming) with climate monitoring and regular plant pictures could be valuable approaches to identify environmental conditions that increase or decrease the necrosis extent. Such approaches were performed for *A. selago* and allowed to identify drought as likely contributing to necrosis (Le Roux et al. 2005).

Long life-span and regeneration

Endemic long-lived plant species might persist longer than expected under climate change in unsuitable habitats thanks to individual plasticity and due to adult plants having possibly greater tolerance for altered environmental conditions. Hence, species abundance might better reflect their response to climate change than their distribution range (Dullinger et al. 2012; Cotto et al. 2017). This trade-off was highlighted for four endemic long-lived alpine plant species when comparing models of prediction and field surveys (Cotto et al. 2017). A direct consequence is the production of maladapted seeds leading to a reduction of population size. An issue is that small population sizes can fall beyond a threshold where stochasticity may overrule even favourable deterministic trends (Cotto et al. 2017; Hoffmann et al. 2017). This framework might occur for long-lived endemic species in the sub-Antarctic Islands, facing harsh environments and climate change. For *L. kerguelensis*, life span seems to be at

least several decades and for some plants, possibly around a hundred years (chapter 5). Cushions of *L. kerguelensis* might persist, to a certain extent, in response to the climate change that started to be recorded in the 1960's at the Kerguelen Islands. To this regard, it should be noted that *L. kerguelensis* possibly originated in Kerguelen Islands during the cooling-rewarming phases in the Antarctic Miocene-Neogene (Wagstaff & Hennion, 2007; Winkworth et al. 2015), suggesting that this species has already experience drastic climatic changes during its evolutionary history. Paleo-records highlight the occurrence of *L. kerguelensis* in "Peninsule Courbet" in the periods 13.600 until 12.920 cal yr BP (Van der Putten et al. 2015). Nowadays, no population, only a few relict individuals are recorded in this vast peninsula, strengthening a possible niche contraction of this species and local extinction which the rabbit (introduced in 1874; Chapuis et al. 2004) most likely worsened in the last two centuries. However, relative to these past changes, the speed of current climate change is incredibly fast in the sub-Antarctic (Verfaillie et al. 2021). Regeneration is another parameter important to the renewal of populations and their maintenance. In *L. kerguelensis* populations, several cushions (usually at least 10 cm wide) show fruits in late summer (Hennion and Walton 1997b). However, in recent surveys we observed that some cushions did not produce any fruits and/or flowers during summer (F. Hennion and L. Marchand pers. obs). Only a few germinations and young seedlings are visible (Hennion and Walton 1997b and annex 2). In harsh environments, breaking the dormancy of seeds and then survival of young seedlings might be challenging (Hennion and Walton 1997a; Kellmann-Sopyła and Giełwanowska 2015). *L. kerguelensis* seeds are affected by a deep dormancy, the highest germination rate earlier obtained in the lab was 34% with gibberellic acid whereas seed coat scarification raised the percentage from 4% to 20% (Hennion and Walton 1997a). During the Ph.D., a side experiment was led to try several other treatments to break seed dormancy (Annex 2). Only cold stratification was able to break the dormancy of numerous seeds (80%) in one population (Annex 2). The population specificities might be related to the collection of partly immature seeds in the population with a low germination rate, as already observed for other sub-Antarctic species (Hennion and Walton 1997a; Kellmann-Sopyła and Giełwanowska 2015). Furthermore, every seedling died within a few days/weeks, suggesting the need for particular conditions for the seedling to establish (Hennion and Martin-Tanguy 1999). Similar negative results were observed for seedling establishment in Artic tundra with increase of soil temperature (Graae et al. 2009). Requirement of cold stratification might be complicated with warmer and drier winters recorded under climate change, this might limit population regeneration even more in the future. Thus, *L. kerguelensis*' current distribution might not reflect its optimal niche. Populations with low numbers of seedlings might be seen as populations at the limit or even outside the optimal niche of the species. Studying the demography, *i.e.* abundance and size class of cushions

in various populations might give insight into the new optimal niche of the species under current climate change.

A scenario under strong climate change

With the new expectation of the IPCC report, the prediction of climate change is worse than expected in the previous one (Ranasinghe et al. 2021). A vegetation shift is occurring with climate change in numerous environments (Román-Palacios and Wiens 2020). However long-lived native plants, including some long-lived cushion plants, seem not to follow this path due to multiple reasons and might get locally extinct (Fosaa et al. 2004; Ferreira et al. 2016; Cotto et al. 2017). This interpretation might be applicable for other long-lived cushion plants in the sub-Antarctic. Indeed, climate change is recorded in every sub-Antarctic Island and both the persistence and local decline of long-lived species were observed (Le Roux et al. 2005; Bergstrom et al. 2015; Dickson et al. 2019; Hennion et al. 2006; Harter et al. 2015 among others). Indeed, the environmental change expected in the Kerguelen Islands is an increase in minimum temperatures of about + 1 or + 2.3 °C before the end of the current century, along with up to 11% or 23% change in precipitation (Verfaillie et al. 2021), which may cause considerable stress for plants restricted to cold habitats. However, the combination of warming and drier or wetter trends will have different impacts on plants. For *L. kerguelensis*, the five-year population monitoring revealed a fast increase of necrosis in cushions in the warmest and driest locations (Chapter 4). In contrast, the differential gene expression tends to indicate abiotic stress response in the warmest/driest site and biotic response in the wettest site (Chapter 2). Furthermore, salt stress might worsen the necrosis extent (Chapter 1). We may wonder whether the species variability in phenotypic traits, gene expression with the help of microorganisms will be sufficient in the presence of rapid change.

Under warmer and wetter climates, *L. kerguelensis* might increase its growing season, which will be advantageous for the plant productivity as observed in the Arctic tundra (Ernakovich et al. 2014). However, this mild environment might induce pathogens' proliferation, which might worsen plant necrosis as observed for *A. macquariensis* in Macquarie Island (Bergstrom et al. 2015). This is plausible as fungus stress response genes ontology terms were found in the wetter summer site for *L. kerguelensis* (Chapter 2). In contrast, under warmer and drier climates, *L. kerguelensis* might experience a decrease in its growing season due to more frequent and/or intense drought in summer and winter as observed in alpine tundra (Ernakovich et al. 2014). Regeneration might be challenged as breaking dormancy will be harder under warmer and drier conditions and drought might easily damage seedlings as wilting of seedlings was already observed in summer in this species (Wagstaff and Hennion 2007) as well as in *Pringlea antiscorbutica* (Hummel et al. 2004). Furthermore, the results from our

several approaches suggest that *L. kerguelensis* cushion necrosis may be accelerated in the driest and most saline environments.

General conclusion

The interlinking of approaches used towards estimating the adaptive capacity of *L. kerguelensis* under climate change reveals the extent of the scientific challenges we currently face. *L. kerguelensis* morphology reveals allometry and responses to soil water content and wind intensity. Its transcriptome in vigorous plants is region-specific with differential expression of genes related to abiotic or biotic stress responses. *L. kerguelensis* growth is very slow and population-specific and its life span is of several decades at least. The microbiome of the Kerguelen Islands fellfield soils is specific and varies in relation to the soil nutrient content. The rhizomicrobiome showed a similar variation while its composition was under the influence of *L. kerguelensis*. Finally, necrosis extent variability might be related to cushion morphology and environmental variables. More precisely, necrosis might be drought stress damages related to warming and drying winter trends and might be worsened by salt stress and a shift in rhizomicrobiome composition. Under current climate change trends in the Kerguelen Islands, *L. kerguelensis* may have the capacity to cope and change to adjust to environmental variations to a certain extent.

Perspectives of the thesis work

At the end of this PhD, it is possible to identify some research topics on the adaptive capacity of *L. kerguelensis* that are currently being explored by the scientific community on other plant species and that would deserve special attention in the near future.

Towards a better understanding of the cushion entity

One topic that should be developed in the future is the understanding of the genotype diversity within a cushion. Indeed, we hypothesized that several genotypes might be present in the large or bumpy cushions as germinations of *L. kerguelensis* offspring *in situ* in the cushion were observed (Hennion and Walton 1997a). We eliminated such cushions from our analyses, but they deserve a proper study to better understand both their possible different dynamics and key role in population persistence. If a cushion comprises several genotypes, it might ease maintaining an optimal micro-environment within the cushion entity and might facilitate nutrient and water absorption. Moreover, when necrosis occurs, re-greening by another genotype (already within the cushion or the establishment of a seedling) might be advantageous for restoring the cushion entity shape. Furthermore, genotypes might

react differently to environmental variables and microbiome. Therefore, one genotype of the cushion entity might better adapt to the changing environment than the others, which could be advantageous for the cushion entity. Different rhizomicrobiomes might be associated with different genotypes, as observed for *Silene acaulis* (Roy et al. 2018), thus allowing the cushion entity to influence strongly toward beneficial microorganisms. Cushion plants are known to modify their micro-environment (buffered temperature, higher humidity and nutrient-richer soil). This might ease plant germination and the survival of seedlings. It might help the renewal and persistence of populations. The cushion entity diversity was genetically highlighted for cushions with irregular canopy or long cushion mats of *Azorella selago* in Marion Island (Cerfonteyn et al. 2011). If this applies to *L. kerguelensis*, it would change our understanding and expectation of cushion persistence and responses over time. The cushion entity (possibly with several genotypes) might indeed be more important than a genotype to respond to fast changes in the case of *L. kerguelensis*.

Shift of fellfield functioning with L. kerguelensis as a model plant

Under climate change, the seasonality of the plants' functioning, microbiota, and nutrient cycles might shift. This PhD gives a broad knowledge of *L. kerguelensis* adaptive capacity and highlights the particularities of fellfield environments and microorganisms they harbour. First, fellfield soil microorganisms are characterized by cosmopolitan and endemic microorganisms, which might be extremely important for soil functioning (Donhauser and Frey 2018; Cox et al. 2019). Nutrient cycles depend mainly on microorganisms' activity in fellfields and it might be the case for the Kerguelen Islands fellfields. A shift in the composition or diversity of soil microorganisms might negatively impact the fellfield plant functioning. Modification of bacterial and fungal soil composition due to climate change was highlighted in various Arctic and alpine ecosystems (Ernakovich et al. 2014). Second numerous species showed a shift in their growing season in the Arctic and Alpine regions (Ernakovich et al. 2014). Nevertheless, the modification of the rhizomicrobiome in relation to climate change is not well understood yet (Botnen et al. 2020). *L. kerguelensis* responds to its environment and might shift its growing season and functioning in response to climate change and its impact on soil microbiome functioning. Therefore, *L. kerguelensis* might be an interesting model plant to study the possible shift of seasonality and its impact on plant functioning and its relation with its rhizomicrobiome. This integrative framework could give insights into how climate change might impact fellfield ecosystems' functioning in the Kerguelen Islands. As knowledge of other cushion plants functioning is available in the sub-Antarctic, we could propose to model fellfield functioning with their respective model cushion plants in relation to sub-Antarctic Islands climate change.

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Annex 1: Hydric potential of *Lyallia kerguelensis*

Context

In the context of climate change, more severe droughts are recorded in several places on earth and have impacted numerous species (Allen et al. 2010; IPCC 2014). One important impact is the drought stress for plants due to extremely low soil water content. Water is transported through the plant xylem under tension (*i.e.*, under negative absolute pressure). As tension increases due to low soil water capacity and high evapotranspiration demand, vessels become increasingly vulnerable to hydraulic failure (*i.e.*, the loss of the ability to transport water due to xylem cavitation) (Choat et al. 2012, 2018). Hydraulic failure has already been associated with numerous forest diebacks (Mayr et al. 2002; McDowell et al. 2008, 2020; Allen et al. 2010; Voltas et al. 2013). In this Ph.D., we aimed to investigate cavitation as a possible mechanism at the origin of necrosis in *L. kerguelensis*.

Various methodologies exist to study plant hydric potential and cavitation (Cochard et al. 2013). However, most of them are destructive or require heavy equipment not compatible with fieldwork in harsh environments. Our understanding of hydric potential, cavitation and relation with occurrence of dieback is extremely limited in the whole sub-Antarctic, although this region harbours diebacks in several keystone species (Le Roux et al. 2005; Bergstrom et al. 2015; Dickson et al. 2020). Rolland et al. (2015) collected living cushions of *Azorella macquariensis* and *Colobanthus muscoides* and maintained them in controlled conditions with the ambition to use destructive and non-destructive methods on their shoots to study the possible refilling of embolised vessels. They concluded that physical properties of the vessels (membrane porosity, hydrophilic walls, vessel dimension and helical thickening) allow water capillarity action to refill embolised vessels with both methods in few minutes. This result suggests that both species seem adapted to highly variable environments in terms of soil water storage. In order to evaluate the hydraulic potential of *Lyallia kerguelensis* during the summer (driest season of the year), we recorded the hydraulic flow in the principal stem of cushion plants harbouring various degrees of necrosis during 24h. In summer in Kerguelen Islands, the sun rises around 4 AM and sets around 9 PM and fluctuation of stem water potential should follow this schedule. Mean temperature varies between 4.6 °C and 11.6 °C in January at Port-aux-Francais (Météo France 2021). We expected a more negative value of the water potential of the plant during the day when the stomata are open and a lower negative value during the night when the stomata are closed, which is typical for C3 plants without drought stress.

Materials and Methods

Eight small (<10cm diameter) individuals from Ile Australia, four in AUS30 and four in AUS32 were selected. We chose small individuals as they have more accessible stems, which would ease the setup of the sensor and avoid causing damage to the plant. The choice of populations was motivated by their proximity one to each other.

Stem water potential was monitored for around 24 hours (one day and one night) with a stem psychrometer (PSY1 stem psychrometer, ICT International, Germany). This equipment measures stem water potential following the Peltier theory: “The measurement is performed by passing a Peltier cooling pulse through the Chamber Thermocouple to generate a Psychrometric Wet Bulb Depression (WBD). This Wet Bulb depression is automatically corrected for temperature and processed using the slope and intercept of the specific calibration to obtain the stem water potential (MPa)” (Manual guide, ICT International, Germany). Calibration of the sensor was done at Rennes before being shipped to Kerguelen Islands. In order to install it, the stem bark was removed with a razor blade of 1cm length (Fig. 1A). Then the sensor was delicately put around the stem (Fig. 1 B and C). Perfect sealing of the stem cuts with the sensor was done by applying vacuum grease (Dow Corning, USA). The sensor was connected to a recorder connected itself to an external battery to have enough power for several days at low temperatures. The sensor was cleaned and dried before installing it on another stem.

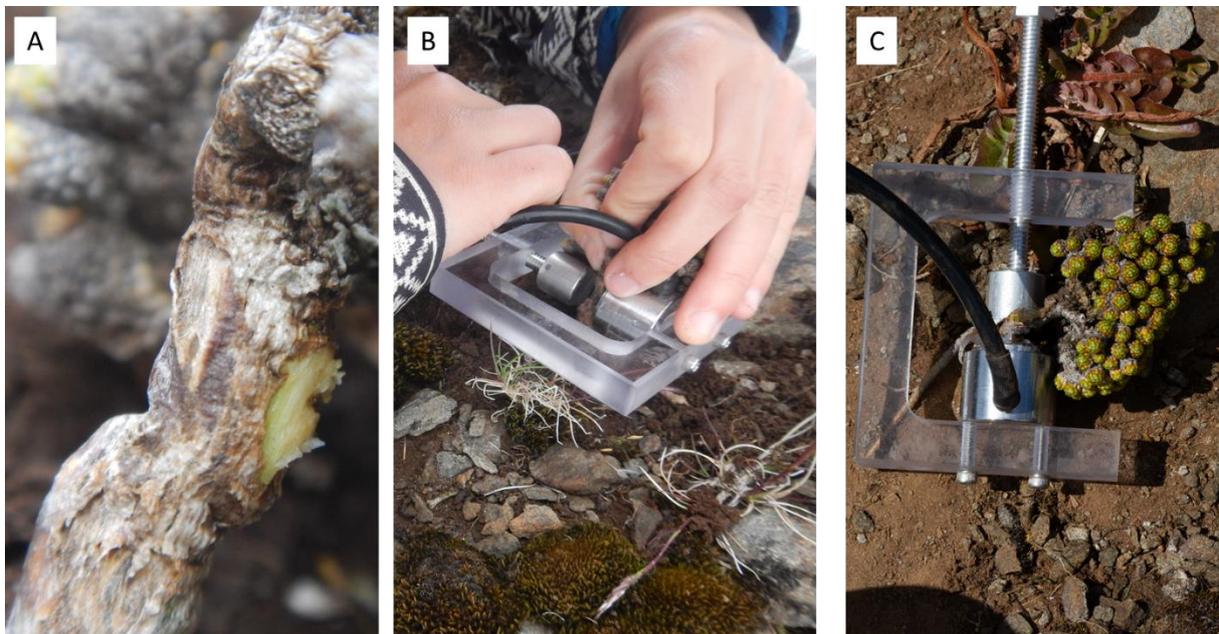


Figure 1: installation of the stem psychrometer, (A) bark removed from the base of the main stem of cushion, (B) adjustment of the sensor precisely on the stem where we removed the bark; (C) sensor ready to measure stem water potential on *L. kerguelensis* stem.

Results and Discussion

Four individual stem water potential patterns were conform to our expectations (Fig 2). The plant had higher negative water potential during the day than during the night. This pattern was recorded for cushions with low and high necrosis extents. While for four other cushions, the pattern was not consistent with our expectations (Fig 3). Indeed, three of them (AUS30 – 23, AUS30 – 21, AUS32 – 23) showed a more negative value during the night than in the day. This pattern is known for CAM plants when stomata are open during the night rather than during the day. Finally, the last plant (AUS30 – 19) showed low stem water potential during both the day and night. Plants facing drought stress keep stomata closed all day long. However, both patterns might result from an artifact of measurement, for example, incomplete sealing of the sensor on the plant or temperature fluctuation beyond the correct range of the equipment. Furthermore, the difference of negativity of the stem water potential between individuals might be due to an artifact. The system might be more sensitive than expected to low temperatures. Therefore, the value of stem water potential will not be interpreted and these results will not be analysed in more detail.

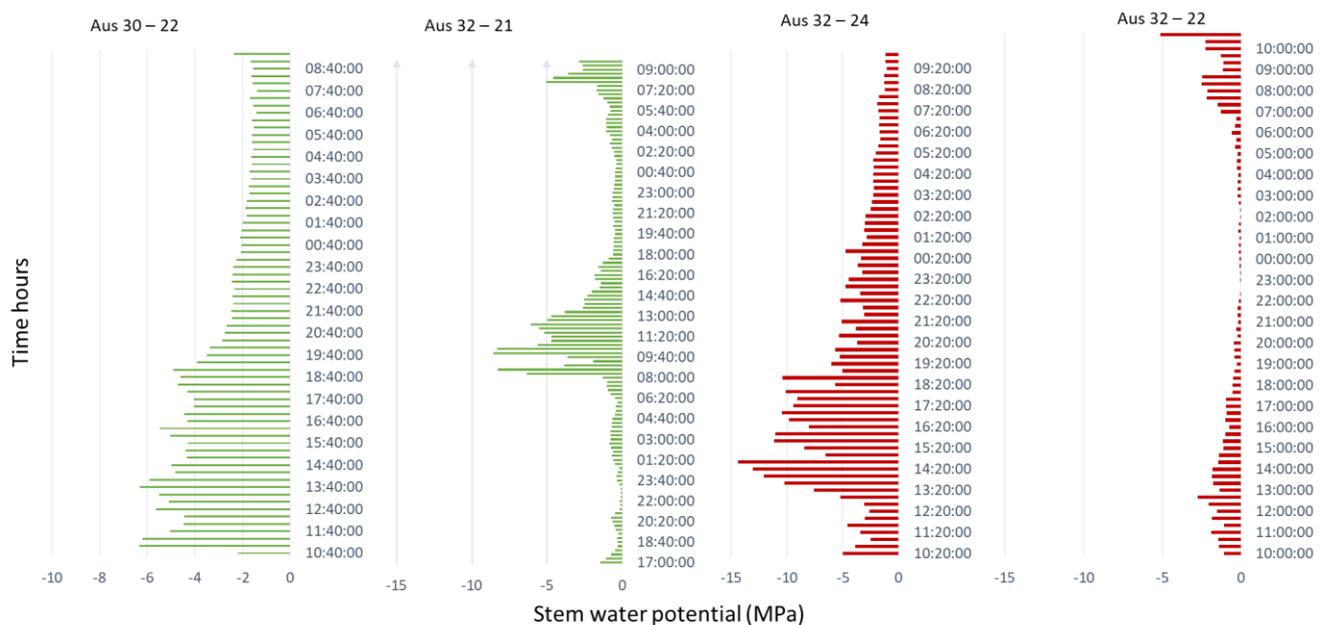


Figure 2: stem water potential measured for four *L. kerguelensis* plants that showed a pattern of C3 plants. The green color indicates an individual with less than 10% of necrosis, while the red indicates more than 30% of necrosis.

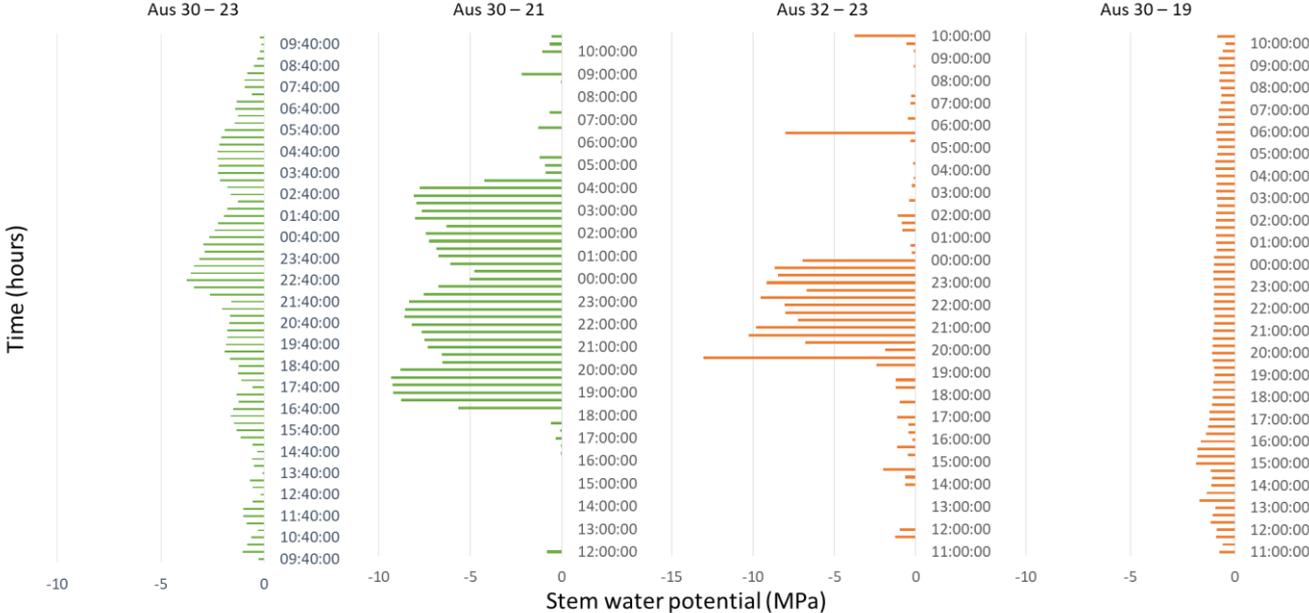


Figure 3: stem water potential measured for four plants that showed particular patterns. The green color indicates individuals with less than 10% of necrosis, while the red indicates individuals with necrosis extent around 20%.

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Annex 2: Germination experiments

Context

Population regeneration might be an essential element to study for estimating the adaptive capacity of a species. Indeed it has been highlighted that long-lived alpine species, persisting in changing environments, might produce a higher rate of maladapted seeds (Cotto et al. 2017). Maintaining a sufficient population size is an essential parameter to maintain a certain diversity of traits (Hoffmann et al. 2017). Small populations should contain fewer alleles selected by the selection, carry a higher risk of losing favourable alleles due to genetic drift, and harbour relatively fewer novel mutations (Willi et al. 2006; Hoffmann et al. 2017). Therefore, the size of the population can fall beyond a threshold where stochasticity may overrule even favourable deterministic trends (Cotto et al. 2017; Hoffmann et al. 2017). For *L. kerguelensis*, few seedlings are observed in the field (Hennion and Walton 1997b). At the same time, in some populations, most plants (above 10 cm of diameter) showed numerous fruits *i.e.* indehiscent capsules (Fig. 1). Fruits contain from one to two seeds (about one third of the capsules for the latter case in Hennion and Walton 1997b). Seeds are small 1.53 ± 0.07 mm in length and 1.24 ± 0.08 in width (Hennion and Walton 1997b). *L. kerguelensis* germination was studied in several populations by Hennion and Walton (1997a). They reported an extremely low germination rate (0.3% and 4% in 9 weeks tests), with non-vernalized or vernalized (7 months) seeds. A higher rate of germination (20%) was obtained when seeds were previously scarified. Finally, the induction of germination with gibberellin treatments (5/10000 gibberellin) gave 34% of germination (Hennion and Walton 1997a). When the authors dissected non germinated seeds, they observed mature embryos and concluded there was no apparent reason for this apparently complex dormancy response. To try and solve the deep seed dormancy in *L. kerguelensis*, we designed a protocol mimicking climatic conditions of Kerguelen Islands, such as freeze-thaw cycles, severe cold and a combination of cool and wet climates.

Material and methods

Mature capsules were collected at the end of summer 2019 in Ile Australia from three populations (AUS25, AUS30 and AUS32). Twelve cushions showed mature seeds in AUS25, while only seven in AUS32 and five in AUS30. Per plant, around 10% of mature fruits were collected in paper bags. A few days later, they were dried for several days at room temperature before being stored at 4°C with silica gel to avoid moisture (Hennion and Walton 1997a) and shipped to the laboratory in Rennes. Several months later, fruits were dissected and seeds were collected. Fruits contained around two to three

seeds (Marchand pers. obs.) and a total of 940 seeds were collected with 484 in AUS25, 329 in AUS30 and only 127 in AUS32.

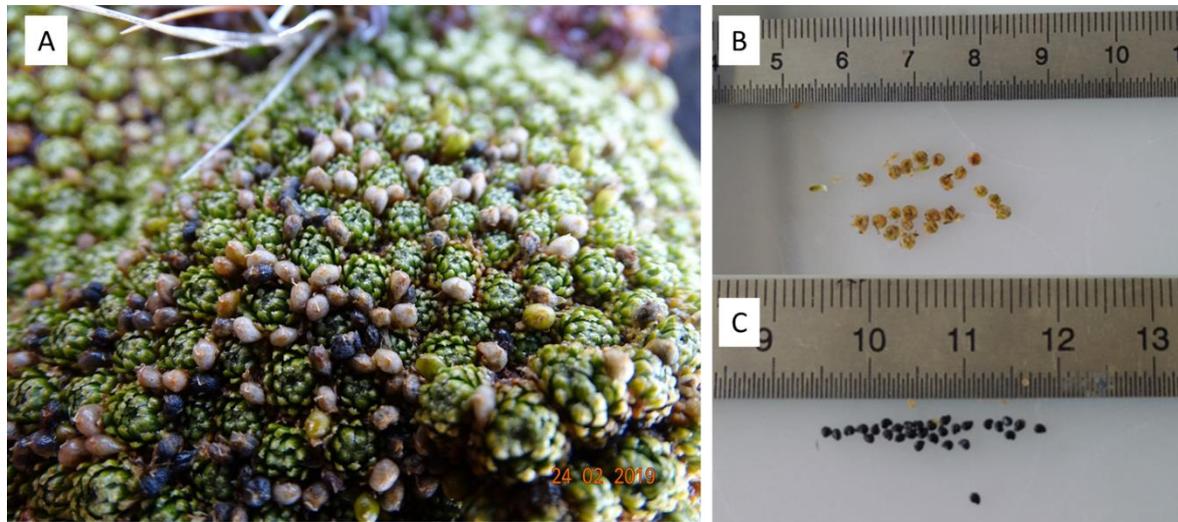


Figure 1: (A) *L. kerguelensis* cushion with numerous fruits at different stages of maturation. (B) Dried fruits and (C) mature seeds.

Germination experiments were done with the two populations with the highest numbers of seeds, AUS25 and AUS30. Seeds were disinfected with a solution of methylated spirits for 1 minute followed by 2h in sodium hypochloride solution (Hennion and Walton 1997a). Three treatments were tested, each time on 30 seeds for each population, seeds were put on a substrate (Whatman filters) in a glass Petri dish filled with small glass balls. This technique allows maintaining the filter wet for several days when necessary. Experiment 1 consisted of a dry cold treatment of -20°C for 24h, to try to break the seed tegument without killing the embryo (Fig. 2). For the other treatment as well as the control, seeds were soaked in distilled water for 4h and then water was poured in the Petri dish to maintain humidity of the substrate (Fig. 2). Treatment 2 consisted of mimicking a freeze-thaw cycle with -2°C during 2h followed by 0°C for 2h (Fig. 2). Treatment 3 consisted of stratification at 4°C on a wet substrate for 10 weeks (Fig. 2). Afterwards, every treatment and the control were put into an incubator (SANYO MIR-154 cooled incubator, Panasonic, Japan) for several weeks (Fig. 2). Sixteen hours of light were programmed and daily variation of temperature from 5°C to 20°C was set up to mimic summer temperature at Kerguelen Islands (Table I).

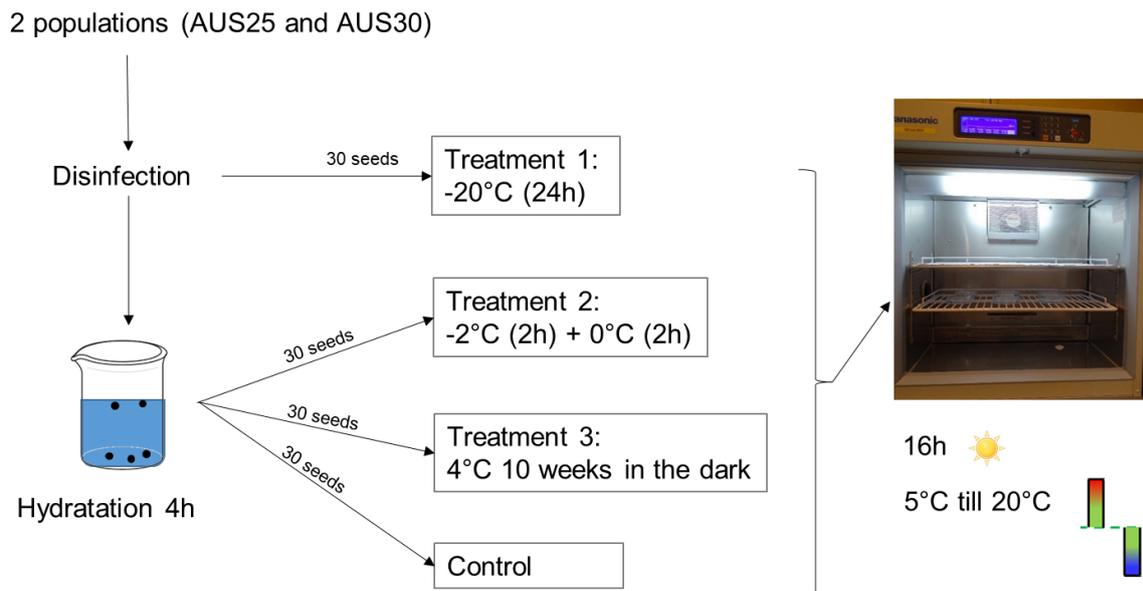


Figure 2: schematic view of the germination experiment, with the three treatments, the control and the germination set up in the incubator.

Table I: temperature and light set up in the incubator for the germination experiment.

TIME (h)	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Temperature	10		5										10		15		20		15		10			
light	Light off										Light on													

Results and discussion

Results of the germination experiment can be found in Table II.

Each treatment led to germination of some seeds, indicating that seed maturation was reached in the field for each population. The control treatment had a rate of 4% to 10% germination, which was low but consistent with previous results obtained by Hennion and Walton (1997a). Treatment 1 and 2 gave roughly similar results to the control with 3 to 13% germination for both populations. Extremely cold temperature (-20°C for 2 hours) did not kill the embryo as germination occurred and one freeze-thaw cycle was probably insufficient to modify the outcome of germination. These results may be explained by hardiness of the seed, being adapted to survive harsh conditions such as high frequency of freeze-thawing events in the Kerguelen Island fellfields (Lebouvier et al. 2011). Finally, treatment 3 provided higher germination rates. Indeed, AUS25 had 20% of germinated seeds and AUS30 87%. For AUS30 such a high germination rate was never obtained before. The low germination in AUS25 population

may be due to incomplete maturation of fruits. It should be noted than all seedlings died within couples of days after removing from the incubators. They were placed in a growing chamber mimicking Kerguelen summer light and thermoperiod and allowing growth of small *L. kerguelensis* cushions, but seedlings of this species might require different condition to establish.

Table II: results of the germination experiment for the three treatments and the control for both populations.

	AUS25		AUS30	
	Date of the 1st germination	Germination (%)	Date of the 1st germination	Germination (%)
Treatment 1	34	13%	35	7%
Treatment 2	15	3%	13	13%
Treatment 3	10	20%	9	87%
Control	15	4%	21	10%

Conclusion

We made progress toward estimating the germination conditions required to release dormancy in the seed of *L. kerguelensis*. This species needs a long wet, cold period before it can germinate and possibly helped by freeze-thawing cycles.

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Annex 3: Scientific outreach

PhD Publications

2021 Marchand L.J., Tarayre M., Dorey T., Rantier Y., Hennion F. Morphological variability of *Lyallia kerguelensis* in relation to environmental conditions and geographical distribution in the Kerguelen Islands. *Polar biology*, DOI:10.1007/s00300-020-02768-2

In prep Marchand L.J., Tarayre M., Chambrin L., Verfaillie D., Favier V., Jomelli F., Hennion F.. Slow growth and rapid necrosis increase in the endemic species *Lyallia kerguelensis* across Kerguelen Islands: implications of climate change. *Will be submitted to Global Change Biology in November 2021*

In prep Marchand L. J., Hennion F., Tarayre M., Martin M.-C., Martins B. R., Monard C.. Fellfields of the Kerguelen Islands harbour specific soil microbiome and rhizomicrobiomes of a long-lived endemic cushion plant facing necrosis. *Will be submitted to The ISME Journal in November 2021*

Other Publications

2021 Marchand L.J., Dox I., Gričar J., Prislan P., van den Bulcke J., Fonti P., Campioli M. Timing of spring xylogenesis in temperate deciduous tree species relates to tree growth characteristics and previous autumn phenology. *Tree Physiology*, DOI:10.1093/treephys/tpaa171

2020 Marchand L.J., Dox I., Gričar J., Prislan P., Sebastien Leys, Van den Bulcke J., Fonti P., Lange H., Matthysen E., Peñuelas J., Zuccarini P., Campioli M. Inter-individual variability in spring phenology of temperate deciduous trees depends on species, tree size and previous year autumn phenology *Agricultural and Forest Meteorology*. DOI:10.1016/j.agrformet.2020.108031

2020 Dox I., Gričar J., Marchand L.J., Sebastien Leys, Zuccarini P., Geron C., Prislan P., Mariën B., Fonti P., Lange H., Peñuelas J., Van den Bulcke J., Campioli M.. Timeline of autumn phenology in temperate deciduous trees. *Tree Physiology*. DOI:10.1093/treephys/tpaa058

2019 Mariën B., Balzarolo M., Dox I., Leys S., Marchand L.J., Geron C., Portillo-Estrada M., AbdElgawad H, Asard H, Campioli M.. Detecting the onset of autumn leaf senescence in deciduous forest trees of the temperate zone. *New Phytologist*. DOI: 10.1111/nph.15991

Communications

2021 A specific rhizomicrobiome selected by a long-lived plant in the sub-Antarctic Kerguelen Islands. L.J. Marchand, F. Hennion, M. Tarayre, M.-C. Martin, B. R. Martins and C. Monard. World Microbe Forum 20th of June (i-poster)

2020 Necrosis dynamics over time and space of *Lyallia kerguelensis* from individual to population scale. L.J. Marchand, M. Tarayre, L. Chambrin, D. Verfaillie, V. Favier and F. Hennion. CNFRA 22-23th September (oral presentation)

2019 Morphological variability in relation to environmental conditions in the endemic cushion plant *Lyallia kerguelensis* from Kerguelen Islands. L.J. Marchand, M. Tarayre, T. Dorey, Y. Rantier and F. Hennion. GDR plasphen: 28-29th November 2019 Paris, France (oral presentation)

Scientific vulgarisation

2021 Vulgarisation for the University of Rennes 1 of the paper: Marchand. et al. 2021 (Polar Biology)

2020 Interview for the University of Rennes 1: PhD during the lockdown and afterward

2020 Ma Thèse en 180 secondes: Final Bretagne et Pays de Loire

<https://www.youtube.com/watch?v=xFVOqZ6BngM&feature=youtu.be/>

Teaching experiences

In 2019 (64 hours) and 2020 (24h)

1st year of bachelor

Field excursions in the forest: forest ecology and botany (18 hours)

Tutorials: algae, phylogeny, biology and ecology (6 hours online)

Practical works: plant anatomy and adaptation (algae, moss, fern and angiosperm) (25 hours)

2nd year of bachelor

Practical works: angiosperm anatomy and reproduction (29 hours partly online)

3rd year of bachelor

Practical works: anatomy of plant adaptation to drought stress (10 hours)

Classes followed during the PhD

2021, « Analyse de données statistique: niveau 3 », (30 hours) Ecole Pratique des Hautes Etudes (EPHE), Paris, France

2020, « Recherche reproductible : principes méthodologiques pour une science transparente » (MOOC, 25 hours) France Université Numérique, France

2020, « Ma thèse en 180 secondes », (14 hours) Université de Rennes 1, Rennes, France

2020, « Sensibilisation à l'éthique de la recherche et à l'intégrité scientifique » (3 hours)

2019, « Technique de mesure de l'activité photosynthétique des organismes photosynthétiques », (7,5 hours) Université de Le Mans, Le Mans, France

Title: Adaptive capacity to climate change in the long-lived endemic cushion plant species *Lyallia kerguelensis* from the sub-Antarctic Kerguelen Islands

Keywords: cushion plant, morphology, gene expression, rhizomicrobiome, necrosis extent, climate change

Abstract: The adaptive capacity of plants to climate change is defined as the ability of a species or population to cope with new environmental conditions and persist in surviving and reproducing. The Kerguelen Islands, in the sub-Antarctic region, harbour the long-lived endemic species, *Lyallia kerguelensis* Hook.f. (Montiaceae). The plant's distribution is restricted to the Island fellfields, regarded as a form of tundra ecosystem of rock with cold climate and strong winds. The plant might seem ill-equipped to face drastic climate change. Its strict endemism, relatively scarce distribution, cushion form and the occurrence of necrosis led to questioning its adaptive capacity. We hypothesized that the pool of variability (of morphology, transcriptome and soil rhizomicrobiome) of *L. kerguelensis* across contrasted environments might provide insights into its adaptation to harsh environments and its possible response to rapid climate change. In addition, we used data from long-term monitoring

of the fate of populations and their morphological dynamics. *L. kerguelensis*' morphology reveals allometry and responses to soil water content and wind intensity. Its transcriptome is region-specific with differential expression of genes related to abiotic or biotic stress responses. The microbiome of the Kerguelen Islands' fellfield soils is specific and varies in relation to the soil nutrient content, and the rhizomicrobiome shows a similar variation while its composition is under the influence of the plant. Necrosis might be drought stress damages strongly related to fast drying and warming climate and might be worsened by salt stress and a shift in rhizomicrobiome composition. Finally, *L. kerguelensis* growth is very slow and population-specific and its life span is estimated at least several decades. Under current climate change trends in the Kerguelen Islands, *L. kerguelensis* may have the capacity to cope and change to adjust to environmental variation to a certain extent.

Titre : Capacité d'adaptation au changement climatique chez l'espèce en coussin à longue durée de vie *Lyallia kerguelensis*, endémique des îles subantarctiques de Kerguelen

Mots clés : plante en coussin, morphologie, expression des gènes, rhizomicrobiome, étendue de nécrose, changement climatique

Résumé : La capacité d'adaptation des plantes au changement climatique est définie comme l'aptitude d'une espèce ou d'une population à faire face à de nouvelles conditions environnementales et à persister en survivant et en se reproduisant. Les îles Kerguelen, dans la région subantarctique, abritent une espèce endémique à longue durée de vie, *Lyallia kerguelensis* Hook.f. (Montiaceae). La distribution de la plante est limitée aux fellfields considérés comme une forme d'écosystème de toundra rocheux au climat froid et aux vents forts. Cette plante semblerait mal équipée pour faire face à un changement climatique drastique. Son endémisme strict, sa distribution relativement éparse, sa forme en coussin et l'apparition de nécroses ont conduit à s'interroger sur sa capacité d'adaptation. Nous avons émis l'hypothèse que la réserve de variabilité (de la morphologie, du transcriptome et du rhizomicrobiome du sol) de *L. kerguelensis* dans des environnements contrastés pourrait fournir des informations sur son adaptation aux environnements hostiles et sur sa réponse possible au changement climatique rapide. En outre, nous avons utilisé des données provenant d'un suivi à long terme du devenir des populations et de leur dynamique morphologique. La

morphologie de *L. kerguelensis* révèle une allométrie et des réponses à la teneur en eau du sol et à l'intensité du vent. Son transcriptome est spécifique à la région avec une expression différentielle des gènes liés aux réponses aux stress abiotiques ou biotiques. Le microbiome des sols des fellfields des îles Kerguelen est spécifique et varie en fonction de la teneur en nutriments du sol. Le rhizomicrobiome a montré une variation similaire alors que sa composition est sous l'influence de *L. kerguelensis*. La nécrose pourrait être un dommage dû au stress de la sécheresse fortement liée aux rapides réductions de précipitations et au réchauffement climatique et pourrait être aggravée par le stress salin et un changement dans la composition du rhizomicrobiome. Enfin, la croissance de *L. kerguelensis* est très lente et spécifique de chaque population et sa durée de vie est estimée à plusieurs décennies au moins. Dans le cadre des tendances actuelles du changement climatique aux îles Kerguelen, *L. kerguelensis* pourrait avoir la capacité de faire face et de s'adapter aux variations environnementales dans une certaine mesure.