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# Influence des facteurs environnementaux sur la plasticité phénotypique et le microbiote : Étude expérimentale chez des Broméliacées du genre *Aechmea*

Tristan Lafont Rapnouil

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# THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En Écologie et Biodiversité

École doctorale GAIA

Unité de recherche AMAP

## Influence des facteurs environnementaux sur la plasticité phénotypique et le microbiote : Étude expérimentale chez des Broméliacées du genre *Aechmea*

Présentée par Tristan LAFONT RAPNOUIL  
Le 14 Décembre 2023

Sous la direction de Céline LEROY  
et Régis CÉRÉGHINO

Devant le jury composé de

Alexia Stokes, Directrice de recherche, INRAE  
Marie Simonin, Chargée de recherche, INRAE  
Benoît Pujol, Directeur de recherche, CNRS  
Marc Buée, Directeur de recherche, INRAE  
Céline Leroy, Directrice de recherche, IRD  
Régis Céréghino, Professeur, Université Paul Sabatier

Présidente du Jury  
Rapporteuse  
Rapporteur  
Examineur  
Directrice  
Co-directeur



UNIVERSITÉ  
DE MONTPELLIER

Juste un petit mot concernant le présent manuscrit. Cette version de ma thèse est la version qui a été acceptée sans modification par les chercheurs ayant évalué mes travaux. Elle est cependant loin d'être exempte d'erreurs et d'imperfections. Après trois ans de travail et alors que je suis déjà en train de travailler sur de nouveaux projets je n'ai pas le temps ni la volonté pour reprendre et modifier certains aspects et notamment corriger les innombrables coquilles et fautes d'orthographe qui parsèment ces quelque 300 pages.

Je me tiens à la disposition du lecteur pour toute questions relative aux propos et résultats présent dans ce manuscrit et il y en a déjà certains avec lesquels je ne suis plus en accord (par exemple l'affirmation p15 que les organismes sessiles sont plus plastiques que les autres).

Bonne lecture et vive la science.

Tristan Lafont Rapnouil

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On me reproche souvent une tendance à dire trop de bien de tout le monde et que j'enlève par là leur valeur aux compliments. Mais que voulez-vous que je fasse d'autre puisque lorsque je regarde les gens qui m'entourent j'y vois tant de gens merveilleux.

Il ne manquera pas aux yeux du lecteur affuté que les notions d'interactions, de réseau et d'association sont aux cœurs du présent travail de recherche. J'aime voir les gens comme le résultat d'interaction multiples et complexes dont on n'aurait pas su prédire le résultat il y a 26 ans de cela. Tant d'évènement et de rencontres pour en arriver là... Quand Dooz Kawa nous dit que dans le tissu social on est fait que de soi je réponds que dans la trame de la vie on est surtout fait des autres. Je, et cette thèse pas extension, ne suis que la somme de rencontre et d'interaction avec tant et tant de belles (et de moins belles!) personnes.

Plutôt que de rajouter quinze pages de remerciement à cette thèse qui est à mes yeux fatigués de la relire bien trop longue, je vous propose une représentation graphique de mes remerciements : <https://resonant-stroopwafel-034262.netlify.app/>

A vous d'en explorer la toile et de vous amuser à y déceler des bouts de moi mais surtout des gens avec lesquels vous interagissez indéniablement à travers moi. Vertigineuse influence que la vôtre !!

Cela dit je vais tout de même expressément et noir sur blanc remercier mes encadrants Céline et Régis sans qui ces travaux n'auraient pas été possible. Céline pour sa bienveillance, son sérieux, sa disponibilité et sa rigueur qui en font un véritable idéal scientifique ; Régis pour sa bonne humeur, son regard aiguisé et son verbe affuté lui conférant des qualités rédactionnelles précieuses aux jeunes chercheurs que je suis.

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Merci à tous et toutes et bonne lecture !

À Vincent.



# Introduction

# 1 Environnement et plasticité phénotypique

## 1.1 Un environnement dynamique

L'environnement dans lequel les organismes naissent, vivent et meurent est en permanence soumis à des changements naturels. L'environnement au sens large, avec lequel les organismes interagissent, comprend deux composantes : l'environnement abiotique, ou physico-chimiques non vivant, et l'environnement biotique (*i.e.*, relatif à l'ensemble des êtres vivants présents). Les conditions environnementales définissent un espace multidimensionnel (*e.g.*, température, précipitation, pression, compétiteurs, prédateurs, disponibilité des ressources) dont chaque dimension est susceptible de changer dans le temps et/ou l'espace (Levins 1968). Ces changements peuvent survenir à différentes échelles spatiales et selon des cycles réguliers ou aléatoires. Les changements environnementaux ont des conséquences fondamentales sur la biodiversité des organismes peuplant la Terre puisqu'ils sont à l'origine des extinctions de masse (Twitchett 2006; Bond and Grasby 2017) tout comme de l'émergence de nouvelles espèces (Gavrilets 2003; Pfennig *et al.* 2010). Comprendre les liens entre les organismes et les changements environnementaux est donc essentiel puisqu'ils contribuent à façonner la biodiversité.

Les activités humaines sont responsables de changements environnementaux majeurs à l'échelle globale (*e.g.*, changement climatique) et locale (*e.g.*, pollution locale, appauvrissement des sols) à un rythme sans précédent à l'échelle géologique (Pörtner and Roberts 2022). Ces changements ont d'ores et déjà de conséquences profondes sur la biodiversité et son fonctionnement, mais également sur les sociétés humaines. Parmi les facteurs critiques se trouve l'augmentation de la température, de la fréquence et de l'intensité des sécheresses ainsi que l'appauvrissement des sols, car ils affectent directement la végétation naturelle et cultivée, menaçant ainsi les fonctions

naturelles essentielles telles que la régulation du climat, la conservation de la biodiversité, des sols et de l'eau (Jenkins and Schaap 2018) et la sécurité alimentaire (Pörtner and Roberts 2022). Comprendre comment les organismes répondront à ces changements représente donc un enjeu majeur de la recherche moderne en biologie et des expérimentations permettant de comprendre les mécanismes de réponse aux stress environnementaux sont nécessaires.

### *1.2 L'environnement : un agent de sélection et régulateur d'expression*

L'environnement joue à la fois le rôle d'agent de sélection et de régulateur d'expression du phénotype (West-Eberhard 1989; Scheiner 1993; Fig. 1). Fondamental dans le processus de sélection naturelle, l'environnement constitue le lien entre le phénotype d'un organisme et son succès reproducteur et donc sa valeur sélective (*fitness*). Ce filtrage environnemental des phénotypes, appelé pression de sélection, représente l'un des piliers de la théorie de la sélection naturelle (Darwin 1859). La sélection des organismes en fonction de leur phénotype au cours des générations entraîne une adaptation des populations aux conditions locales. En parallèle, les conditions environnementales influencent le développement des organismes et, par conséquent, les phénotypes effectivement exprimés. Ces phénotypes sont finalement soumis à la sélection naturelle (West-Eberhard 1989; Scheiner 1993).

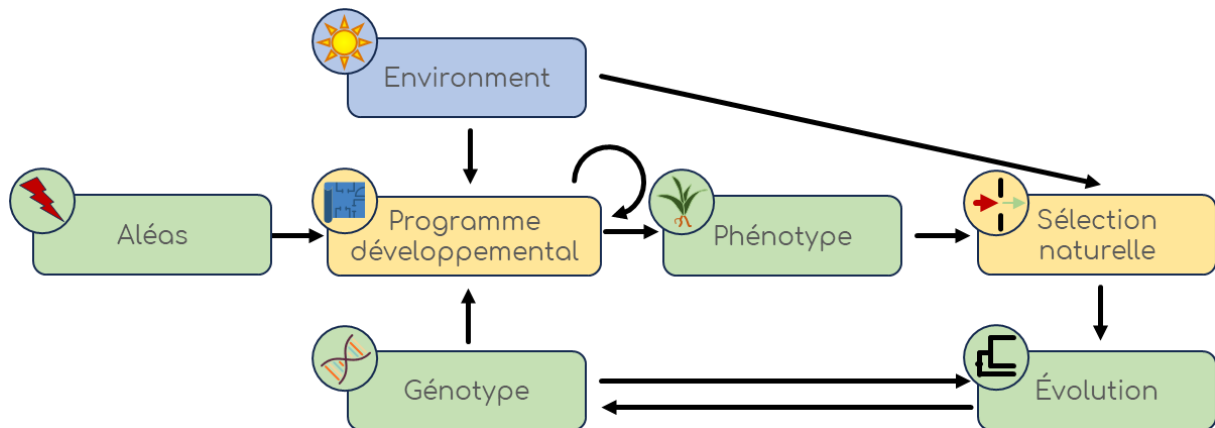


Figure 1 : L'environnement comme agent de sélection et régulateur d'expression d'après la figure 1 de Scheiner 1993.

Le rôle de l'environnement dans le développement des organismes a été longtemps négligé. La découverte du code génétique a transformé la notion de développement, qui est devenue centrée sur les gènes. Les gènes présents chez l'individu codent les protéines dont l'activité conduit au développement et la mise en place du phénotype (Trewavas and Malho 1997). Dans ce contexte, la variabilité phénotypique était souvent considérée comme un bruit dans les données, résultant de perturbations du schéma développemental normal. Cette vision qui laissait peu de place au rôle de l'environnement dans la formation du phénotype a été progressivement remise en question. Bien que les gènes contiennent l'information nécessaire au développement des organismes, l'expression de ces gènes est régulée par de nombreux mécanismes complexes (Goldberg *et al.* 1989; Trewavas and Malho 1997; Gilbert 2005; Vannier *et al.* 2015), et le phénotype observé résulte de l'interaction entre le patrimoine génétique porté par l'individu et l'environnement dans lequel ces gènes s'expriment (Gilbert 2001).

### 1.3 La plasticité phénotypique

#### 1.3.1 Définition et concepts associés

Le génotype correspond à l'ensemble des gènes d'un individu tandis que le phénotype correspond à l'expression de ceux-ci dans un environnement donné et

décrit tous les aspects de la morphologie, de la physiologie, de l'écologie et du comportement d'un individu. La plasticité phénotypique est donc la capacité d'un génotype à produire différents phénotypes en réponse à différentes conditions environnementales (Bradshaw 1965; Sultan 2000). La plasticité n'est pas définie de façon globale pour un génotype, mais elle est spécifique à un trait ou un groupe de trait (Scheiner 1993). Un génotype peut donc être plastique pour un caractère et non plastique pour un autre. La plasticité d'un trait peut d'ailleurs permettre la robustesse d'un autre vis-à-vis de certaines conditions environnementales (Richards *et al.* 2006, Fig. 2). Par exemples, des ajustements de la machinerie photosynthétique d'une plante pourraient renforcer la robustesse de son assimilation de carbone face à diverses conditions environnementales.

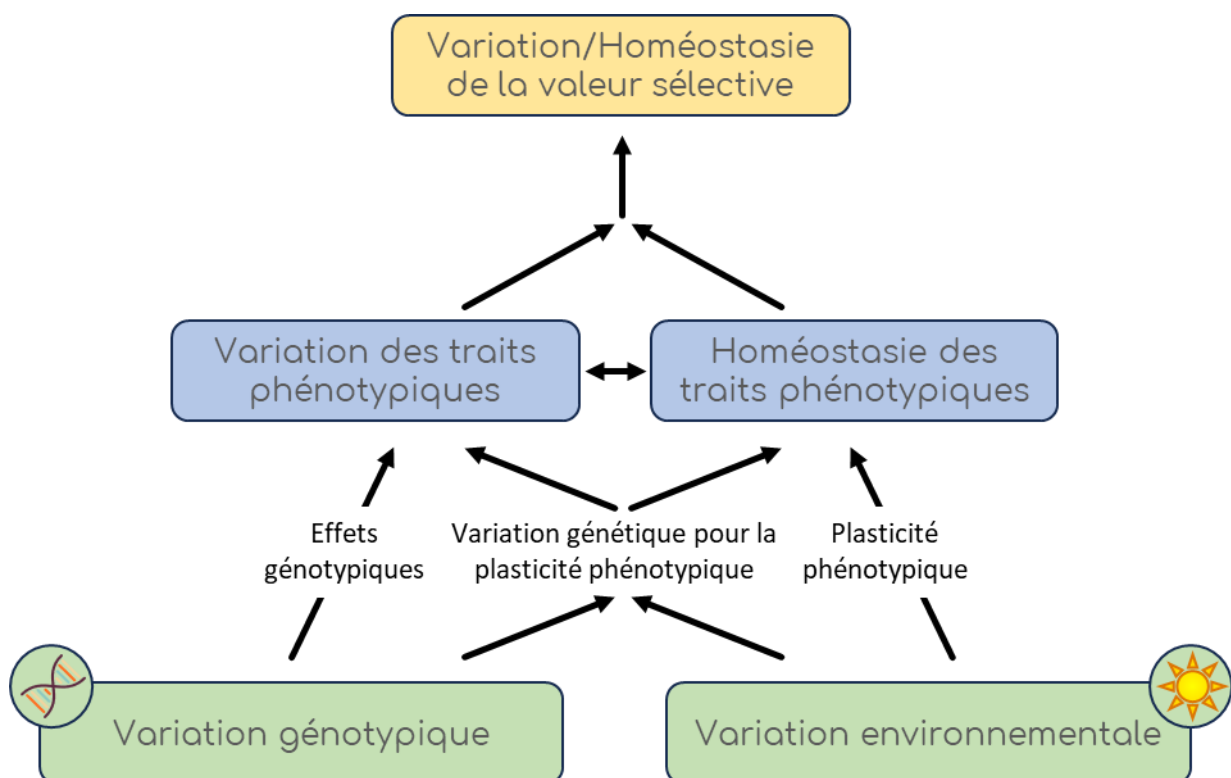


Figure 2 : Aperçu des relations entre variation génétique, environnementale, phénotypique et de la valeur sélective (d'après la figure 1 de Richards *et al.* 2006).

La caractérisation d'un phénotype n'est, en pratique, jamais exhaustive et généralement, on évalue la plasticité (ou son absence) d'un ou de quelques traits en réponse à un ou quelques facteurs environnementaux. Le phénotype d'un

individu, bien qu'ayant une définition générale, se résume en fin de compte à la mesure de certains traits d'intérêts. Ainsi, l'absence de plasticité pour ces traits ne signifie pas nécessairement l'absence de réponse aux facteurs environnementaux à l'échelle de l'individu. Parler de plasticité phénotypique sans définir clairement les traits en question demeure ainsi un concept abstrait et limité pour une application scientifique (Valladares *et al.* 2006).

La plasticité comprend à la fois les effets inévitables des contraintes environnementales sur la croissance et la physiologie (pouvant entraîner des altérations non adaptatives voir mal-adaptatives du phénotype), ainsi que les ajustements adaptatifs qui améliorent le succès de l'organisme dans l'environnement qui les induit (Sultan 2000).

### 1.3.3 Avantages et limites de la plasticité

Des modifications de l'environnement peuvent s'avérer stressantes pour les organismes qui doivent y échapper (évitement dans le temps/espace), y faire face (résistance/tolérance) ou bien y répondre plastiquement au risque de subir des altérations physiologiques léthales. La plasticité peut donc avoir d'importantes conséquences sur la *fitness* des organismes et une base génétique en faisant donc un trait soumis à sélection à part entière (Meyers and Bull 2002; Laitinen and Nikoloski 2019). Certaines conditions, telles que l'hétérogénéité temporelle et spatiale de l'environnement, peuvent ainsi favoriser l'émergence de la plasticité pour un trait par le biais de sélection naturelle (Scheiner 1993; Meyers and Bull 2002). Cependant, la plasticité phénotypique est également associées à des coûts et des contraintes (DeWitt et al. 1998; Leake et al., 2004; van der Heijden et al., 2015). La mise en place de la plasticité nécessite l'existence de système de perception de signaux de l'environnement et de voies de régulation permettant les ajustements phénotypiques (Givnish 2002), une machinerie qui a un coût pour

l'organisme. De plus, la production de structures induites par l'environnement peut représenter un coût additionnel. Enfin, des coûts liés à la stabilité du développement et des corrélations génétiques ont été envisagés, bien que leur démonstration soit complexes et leur généralisation limitée (DeWitt *et al.* 1998; Diggle 2002). La plasticité est également limitée par la fiabilité des informations environnementales (c'est-à-dire à quel point les signaux environnementaux captés par l'organisme renseignent effectivement sur l'état des conditions environnementales), la latence entre l'arrivée des changements et la mise en place du nouveau phénotype ou même l'historique de plasticité de l'individu (Valladares *et al.* 2007).

La plasticité phénotypique permet aux organismes une réponse rapide à leur environnement, parfois de manière réversible (Whitman and Agrawal 2009). Étant donné que la reproduction est l'étape clé de la sélection naturelle, le processus d'adaptation se déroule à des échelles de temps supérieures à la durée du cycle de vie des organismes. Cependant, l'adaptation par sélection naturelle ne permet pas aux organismes de faire face aux modifications environnementales ayant lieu sur des échelles de temps plus courtes que celle de leur cycle de vie. La plasticité permet ainsi aux organismes ayant des génotypes similaires (*e.g.*, des membres d'une même espèce) de faire face à une plus grande gamme de conditions environnementales contribuant à l'élargissement de leur niche écologique (Sultan 2001; González and Gianoli 2004; Saldaña *et al.* 2005).

Au sens strict, l'étude de la plasticité phénotypique nécessite une information précise sur le génotype étudié et s'intéresse donc généralement aux aspects développementaux en utilisant des lignées génétiques bien connues (Valladares *et al.* 2006). Cependant, de nombreuses études, s'intéressent à la réponse d'espèces et de populations dans leurs contextes écologiques et s'affranchissent partiellement de la composante génétique. Deux visions qui ont

amené à la distinction en plasticité *sensus stricto* et *sensus lato* (Valladares *et al.* 2006).

#### 1.3.4 Plasticité phénotypique transgénérationnelle

La plasticité est également un processus intergénérationnel (plasticité transgénérationnelle, Uller 2008). Les effets maternels, définis comme " l'influence causale du génotype et/ou du phénotype maternel sur le phénotype de sa descendance " (Wolf and Wade 2009), peuvent être considérés comme une forme de plasticité trans-générationnelle, où l'environnement de la génération précédente influence le développement de la suivante sans altération du génotype (Mousseau and Fox 1998; Uller 2008). De nombreux phénomènes peuvent être considérés comme des effets maternels (Mousseau and Fox 1998; Donohue 2009; Wolf and Wade 2009). Chez les plantes, on considère principalement la transmission maternelles des plastides, la formation de l'endosperme (triploïde, 2/3 de génotype maternel), le tégument de la graine (tissu maternel), l'approvisionnement de la graine en nutriments, hormones, protéines, transcrits, et enfin l'influence maternelle sur l'environnement de la descendance par la dispersion des graines ou la phénologie (Roach and Wulff 1987; Donohue 2009, Fig. 3). Ainsi, les plantes peuvent influencer le développement de leur descendance en réponse à un stress spécifique et ces altérations du développement peuvent favoriser la croissance et la survie de plantules confrontés à ce même stress (Herman and Sultan 2011). Cette plasticité transgénérationnelle adaptative pourrait être un facteur clef dans l'adaptation des populations à de nouveaux environnements dans l'espace (invasion, changement d'aires) et le temps (changement environnementaux graduels).



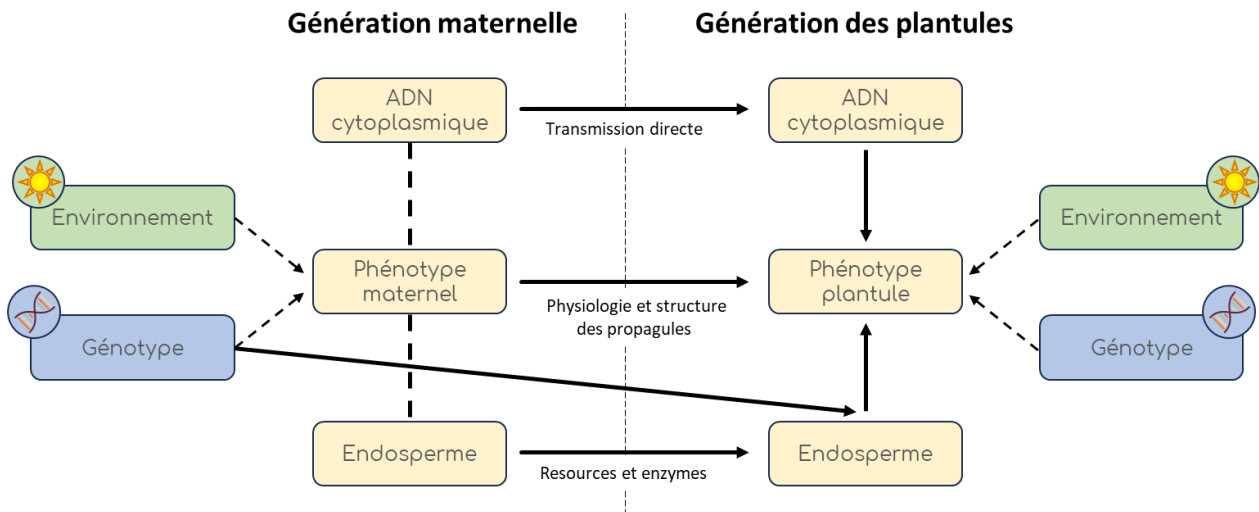


Figure 3 : Effets maternelles chez les plantes (d'après Roach et Wulff 1987).

### 1.3.5 Importance écologique de la plasticité phénotypique

Longtemps considérée comme un « bruit » dans les données biologiques, la plasticité phénotypique occupe à présent un sujet central dans la biologie des organismes, car elle entraîne d'importantes conséquences évolutives et écologiques (Bradshaw 1965; West-Eberhard 1989; DeWitt *et al.* 1998; Sultan 2000; Pigliucci 2005; Fusco and Minelli 2010). En plus d'affecter la niche écologique des organismes, la plasticité phénotypique affecte les interactions interspécifiques et les fonctions écosystémiques qui en découlent (Miner *et al.* 2005; Whitman and Agrawal 2009). Les modifications d'un nœud du réseau écologique peuvent entraîner des réactions en cascade de influencent le phénotype d'autres espèces, et par conséquent, le fonctionnement de l'écosystème. Ce rôle de la plasticité dans les interactions écologiques et leur perturbation en fait un sujet clé dans le contexte actuel des changements environnementaux à différentes échelles (Nicotra *et al.* 2010).

#### 1.4 Plasticité chez les plantes

Les organismes sessiles, tels les plantes, sont particulièrement sensibles aux modifications des paramètres environnementaux, car ils ne peuvent pas échapper à ces conditions dans l'espace (Bradshaw 1965; Sultan 2000). Les plantes ont ainsi développé des capacités plastiques qui leur permettent de répondre à leur environnement par un ajustement de leur morphologie, physiologie, anatomie, de leur histoire de vie et même de leur reproduction (Sultan 2000; Gratani 2014).

##### 1.4.1 Plasticité apparente pour un trait et relation d'allométrie

Chez les plantes, de fortes relations allométriques et de nombreux traits phénotypiques sont liés au stade de développement de la plante (*ontogenetic drift*, Evans 1972). La comparaison de plantes à différents stades peut conduire à des conclusions erronées par l'observation de variation phénotypique en lien avec le stade ontogénique et non l'environnement (Coleman *et al.* 1994; Weiner 2004; Valladares *et al.* 2006). De plus, la plupart des allométries chez les plantes sont liées à la taille plutôt qu'à l'âge. Ainsi, des plantes de tailles différentes présentant des traits différents peuvent résulter soit d'une "vraie plasticité" pour ces traits soit d'une "plasticité apparente" (Fig. 4) due à une plasticité dans le taux de croissance et à des relations allométriques fortes entre la taille et les traits en question (Weiner 2004).

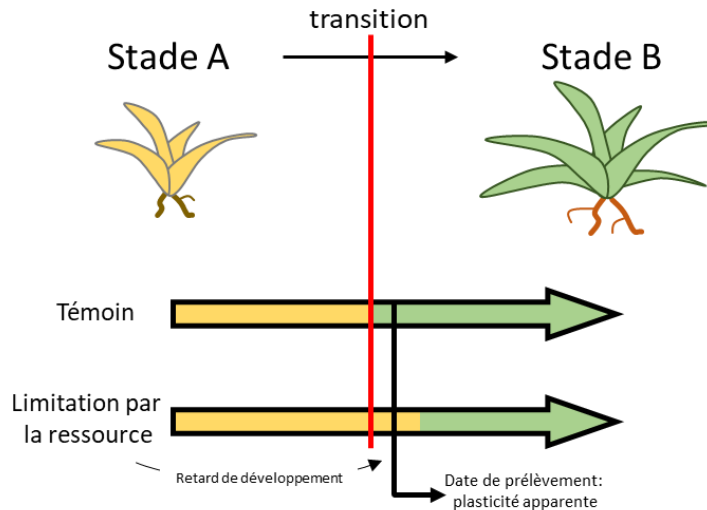


Figure 4: Illustration de la plasticité apparente résultant de trajectoire allométrique et de retard ontogénétique.

En parallèle des relations taille-trait, on trouve également de nombreuses relations entre traits et une covariation des traits phénotypiques des plantes (*e.g.*, Wright *et al.* 2004). Les traits sont organisés en réseau et différents traits sont associées à une même fonction. Ainsi, la LMA (leaf mass area, ratio entre surface et masse sèche) et la surface foliaire sont des traits liés aux allométries, au flux, à l'économie ou la tolérance au stress (He *et al.* 2020). Ces traits résultent donc de la stratégie de croissance et de survie de la plante et sont au cœur de compromis entre les différents systèmes fonctionnels de cette dernière (Fig. 5). Cette covariation rend nécessaire l'intégration de nombreux traits associés à différentes fonctions pour identifier et comprendre les similarités et les différences dans les réponses des plantes à leur environnement.

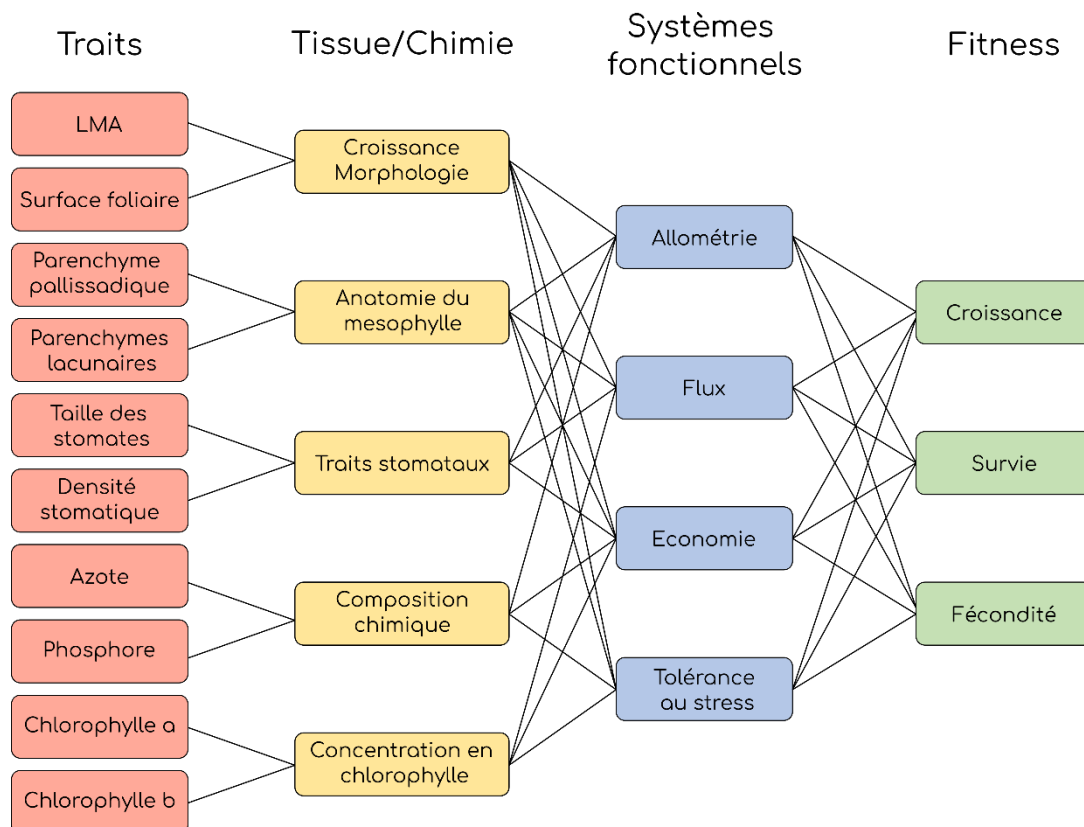


Figure 5 : Illustration des relations entre de multiples traits, au sein et entre organes et systèmes fonctionnels et leurs influences sur la fitness (d'après la Figure 1 de He *et al.* 2020).

#### 1.4.2 Plasticité intra-individuelle

Les capacités plastiques des plantes sont renforcées par la nature modulaire et itérative de leur croissance (De Kroon *et al.* 2005). La semi-autonomie des méristèmes permet en effet aux plantes des réponses plastiques à l'échelle sub-individuelle, résultant non pas en un phénotype de la plante, mais plutôt en une mosaïque phénotypique (Herrera 2009; Harder *et al.* 2019). Bien que la plupart des études se concentre sur l'échelle individuelle, cet aspect a notamment été étudié pour les traits foliaires. Les feuilles jouent un rôle clé chez les plantes car elles sont le siège de la photosynthèse et des échanges gazeux. Les variations dans les traits phénotypiques des feuilles sont associées à différentes stratégies de croissances (Wright *et al.* 2004; Díaz *et al.* 2016), et étudier la réponse des feuilles à l'environnement est donc essentiel pour comprendre l'écologie et l'évolution des plantes. Ainsi, des études ont mis en évidence une plasticité foliaire au sein d'un

même individu en fonction de la position de la feuille dans la couronne et selon un gradient de lumière intra-canopée (Niinemets *et al.* 2015; Pons 2016). Bien que la magnitude soit généralement plus faible au sein d'un même individu, on retrouve la même tendance de variation du phénotype foliaire entre les feuilles exposées à la lumière et celles situées à l'intérieur du feuillage à l'ombre qu'entre celles de conspécifiques exposés à différents niveaux de lumière.

### 1.4.3 Plasticité intra-organe

Dans l'immense majorité des études sur la plasticité, les feuilles sont implicitement considérées comme des entités uniformes sans structure spatiale. Cependant, il a été montré chez des monocotylédones et dicotylédones que les traits fonctionnels et structurels variaient au sein d'une même feuille. Par exemple, on observe un limbe plus fine avec une plus faible LMA (leaf mass area, ratio entre surface et masse sèche) à la base qu'à l'apex de la feuille (Rawson *et al.* 1987; Martre and Durand 2001; Li *et al.* 2013). La densité stomatique n'est pas non plus uniforme le long du limbe, avec une augmentation du nombre de stomates de la base à l'apex ou une diminution du centre vers le bord du limbe en fonction des espèces étudiées (Weyers and Lawson 1997; Poole *et al.* 2000; Takahashi *et al.* 2007). Le système vasculaire et les schémas de nervation peuvent également varier avec l'apex des feuilles plus riche en faisceaux vasculaires (Martre and Durand 2001; Nardini *et al.* 2008; Li *et al.* 2013) et de plus faibles distances inter-veineuse (Ocheltree *et al.* 2012). Ces différences dans la structure et la morphologie de la feuille sont associées à des différences fonctionnelles et d'importantes variations de conductance stomatique, d'efficacité photosynthétique et d'échange gazeux au sein d'une même feuille (Nardini *et al.* 2008; Li *et al.* 2013; Fanourakis *et al.* 2015). Ces variations intra-feuilles pourraient constituer un aspect clé de l'écologie des plantes, en particulier chez les espèces dont les feuilles répondent fortement aux conditions environnementales (*e.g.*, forme, taille). Cependant, rares sont les

études qui se sont intéressées aux effets des changements environnementaux à l'échelle de la feuille (mais voir Drouet and Bonhomme 1999 avec la lumière et le VPD : *vapor pressure deficit* ; Poole *et al.* 2000 avec le CO<sub>2</sub> et Amitrano *et al.* 2022 avec le VPD). Cet intérêt limité pour la plasticité à très fine échelle chez les feuilles contraste avec celui porté aux racines. En effet, malgré les difficultés à accéder et mesurer les traits racinaires, les chercheurs ont démontré leur capacité à répondre de manière très localisée à des variations environnementales (Monshausen and Gilroy 2009). Le faible nombre d'études consacrées aux réponses localisées chez les feuilles comparativement aux racines est probablement la conséquence d'une apparente homogénéité de l'atmosphère en comparaison de la pédosphère mais également la nature continue du système racinaire en opposition aux feuilles qui apparaissent naturellement comme une entité uniforme aux yeux des observateurs.

Le phénotype des plantes est donc un complexe de traits qui covarient et répondent à la fois localement (*i.e.*, intra-individu, intra-organe) et à l'échelle de l'individu. Cette intégration des signaux et les réponses qui en découlent reposent sur une importante machinerie de réception des signaux environnementaux (Scheres and van der Putten 2017) et sur une communication inter-cellulaire (Jaillais and Chory 2010; Chaiwanon *et al.* 2016). Le perceptron de la plante établit une connexion entre la plante et son environnement, permettant ainsi la coordination de ses réponses (Scheres and van der Putten 2017). Cette capacité d'intégration et de coordination est d'autant plus cruciale que les plantes vivent généralement à l'interface de deux environnements très différents en termes de conditions et d'hétérogénéité : l'atmosphère et la pédosphère.

## 2 Microbiote et plasticité phénotypique des plantes

### 2.1 Généralités

Virtuellement toutes les plantes et animaux sont habités par des communautés de microorganismes (le microbiote) qui sont cruciaux pour le bon développement et la santé de leurs hôtes (Berg *et al.* 2014; Compant *et al.* 2019; Arnault *et al.* 2022). Ces observations ont remis en question la notion d'individu en parallèle de celle de phénotype, et la notion de symbiose prend peu à peu une place centrale en biologie. La vision très compartimentée de l'individu cède progressivement le pas à une approche plus holistique de l'individu-système comme résultant d'une multitude d'interactions et de rétroactions entre hôte et microbiote (Gilbert *et al.* 2012). Au cours des deux dernières décennies les recherches sur les plantes et leurs partenaires microbiens ont explosées. Une explosion rendue possible grâce à l'émergence et la baisse des coûts des techniques moléculaires à large échelle permettant une caractérisation plus complète des communautés microbiennes par rapport aux méthodes d'observation/culture classiques. Ces recherches ont mis en lumière des communautés taxonomiquement et fonctionnellement diversifiées. D'importants efforts ont été consacrés à la description de la diversité et des effets du microbiote des plantes sur leur croissance, l'acquisition des ressources, leur résistance aux stressés et leur protection contre les pathogènes, insectes et herbivores (Santoyo *et al.* 2016; Compant *et al.* 2019). Il n'existe virtuellement pas de plantes sans micro-organismes en milieu naturel et les rares produites en laboratoire semblent systématiquement souffrir du manque de microbiote (Partida-Martinez and Heil 2011). Bien qu'il reste encore de nombreuses zones d'ombres, l'une des retombées potentielles de cette recherche concerne l'implication du microbiote dans la réponse plastique des plantes face aux modifications environnementales (Vannier *et al.* 2015). Une promesse qui place les études sur lien microbiote-phénotype des plantes au cœur des enjeux contemporains découlant des changements globaux anthropiques.

## 2.2 Le microbiote des plantes

### 2.2.1 Composition du microbiote des plantes

Le microbiote des plantes est dominé par les bactéries et les champignons, mais on y trouve également une diversité importante d'oomycètes, d'algues, d'eukaryotes unicellulaires, de nématodes ou encore de virus. Seule une fraction de l'immense diversité de champignons et de bactéries est régulièrement présente dans ces communautés (Taylor *et al.* 2014; Müller *et al.* 2016). Le microbiote bactérien est principalement composé de quelques phylums hyper-dominants que sont les *Proteobacteria*, les *Actinobacteria* et les *Bacteroidetes* (Müller *et al.* 2016; Hassani *et al.* 2018). Les deux principaux phylums fongiques colonisant les tissus des plantes sont les *Ascomycota* et les *Basidiomycota* bien que les mycorhizes arbusculaires (*Glomeromycota*) aient, avec les ectomycorhizes, été les groupes les plus étudiés au niveau des racines (Hassani *et al.* 2018).

### 2.2.2 Compartimentation du microbiote

Le microbiote des plantes comprend à la fois des microorganismes colonisant la surface et ceux présents à l'intérieur de leurs tissus. Ceux colonisant la surface de l'hôte sont appelés épiphytes, tandis que le terme endophytes désigne les microorganismes vivant à l'intérieur des tissus de la plante (Hardoim *et al.* 2015). Jusqu'à récemment, les endophytes étaient définis comme les microorganismes vivant dans la plante sans causer d'effets négatifs pour la plante hôte (Wilson 1995). Cependant, cette définition combinant à la fois le lieu de vie des organismes et leurs fonctions peut mener à confusion et a été progressivement abandonnée. Néanmoins, la distinction entre épiphytes et endophytes n'est pas une question anodine puisque différencier l'intérieur et l'extérieur de la plante peut s'avérer difficile. En effet, les feuilles présentent une cuticule constituée de plusieurs couches et percée de stomates et d'hydatodes qui connectent le milieu intérieur et l'extérieur de la plante. Par ailleurs, il existe un important chevauchement entre



les communautés d'épiphytes et d'endophytes. La majeure partie des endophytes provenant de l'environnement se retrouvent également en tant qu'épiphytes. Dans la pratique, le terme "endophytes" désigne donc généralement les microorganismes détectables à partir de tissus de plante préalablement stérilisés en surface. Différentes méthodes de stérilisation peuvent donc partiellement altérer les communautés obtenues.

Au-delà de ces considérations conceptuelles sur la limite de l'endosphère, ces deux habitats sont tout de même fondamentalement différents. Les principales différences entre les communautés endophytes et épiphytes sont l'exposition aux conditions environnementales et leur proximité avec l'hôte. Les communautés épiphytes sont confrontées à l'environnement dynamique et hétérogène qu'est l'atmosphère. La surface des feuilles est exposée aux radiations, aux variations de température et d'humidité ainsi qu'à une tendance oligotrophe (Vorholt 2012; Bringel and Couée 2015). Moins extrême, le sol est l'un des habitats microbiens les plus diversifiés sur terre (Taylor *et al.* 2014; Trivedi *et al.* 2020). On y trouve une très forte hétérogénéité spatiale, allant de l'échelle régionale à microscopique, et généralement plus de ressources avec une abondante matière organique (Vos *et al.* 2013; Prashar *et al.* 2014; Nunan 2017). En comparaison, l'endosphère apparaît plus stable, avec des interactions hôte-microbiote plus intimes et un contrôle plus fort de l'hôte (Trivedi *et al.* 2020; Compant *et al.* 2021). L'endosphère est généralement moins diversifiée et représente un sous-échantillon des communautés du sol avec un enrichissement en certains taxons. Cependant, le chevauchement entre les communautés d'endophytes racinaires et foliaires est plus important pour les bactéries que les champignons, suggérant des réservoirs autres que le sol pour les endophytes foliaires fongiques (Trivedi *et al.* 2020, Fig. 6).

En plus de la distinction entre les communautés épiphytes et endophytes, les différents tissus de l'hôte sont associés à différents microbiotes. Les organes

de l'hôte tels que les racines, les tiges, les feuilles, les fleurs, les fruits, etc., représentent autant de micro-habitats offrant des conditions environnementales particulières (e.g., pH, disponibilité en eau, et nutriments) et permettant le développement de microbiotes spécifiques (Müller *et al.* 2016; Shade *et al.* 2017; Trivedi *et al.* 2020).

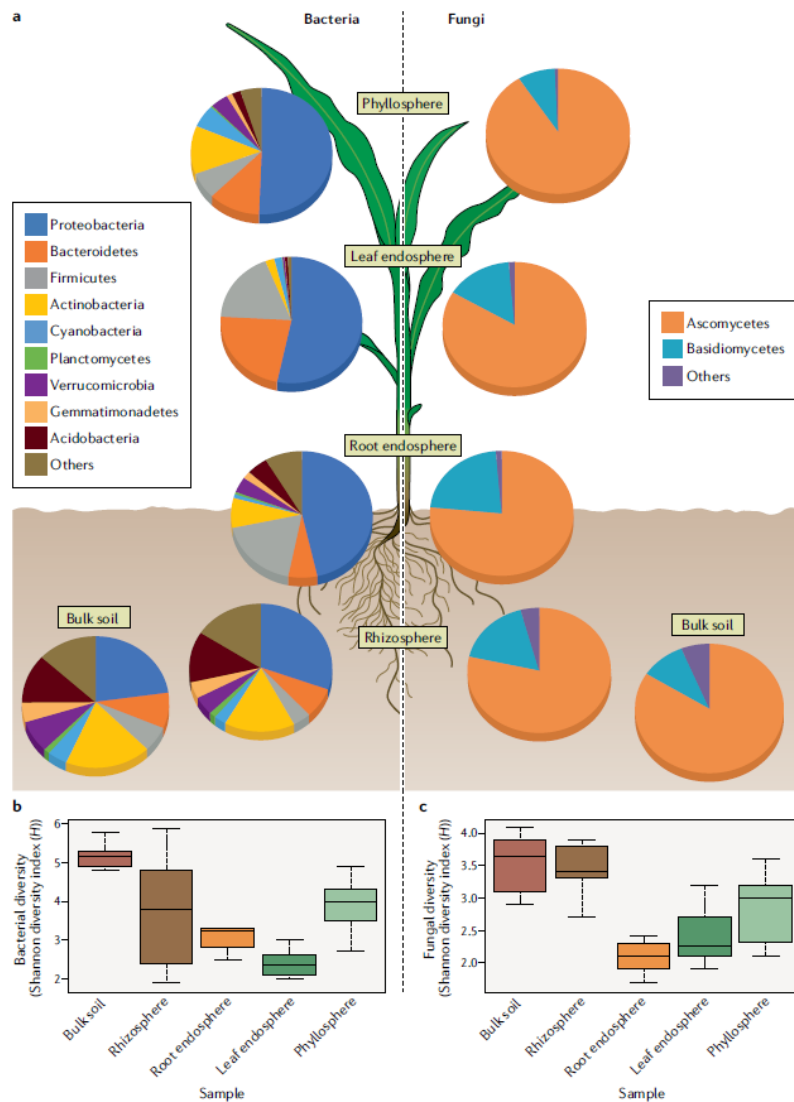


Figure 6 : Structure générale des communautés fongiques et bactériennes de différentes niches associées aux plantes. a) Illustration des variations de composition en lien avec les différents compartiments de la plante hôte. Les diagrammes circulaires montrent la moyenne des abondances relatives des principaux phyla bactériens (à gauche) et fongiques (à droite) qui sont détectés en plein sol, dans la rhizosphère, l'endosphère racinaire, l'endosphère foliaire et la phyllosphère (épiphytes foliaires) pour plusieurs espèces de plantes (canne à sucre, raisin, cactus (*Myrtillocactus geometrizans* and *Opuntia robusta*) et Agave (*Agave tequilana*, *A. salmiana* et *A. desertii*)). Les abondances ont été estimées par séquençage des gènes marqueurs 16S rRNA et ITS (internal transcribed spacer) pour les bactéries et les champignons respectivement. Seuls les phyla représentant plus de 0.5% de la population total dans au moins un échantillon sont inclus. Les études ont utilisé des méthodes de séquençage haut débit pour caractériser en une seule fois les communautés de différentes niches. b) et c) Boîtes à moustache représentant la diversité (Indice de diversité de Shannon) bactérienne (b) et fongique (c) dans le plein sol, la rhizosphère, l'endosphère racinaire, l'endosphère foliaire et la phyllosphère (d'après la Figure 1 de Trivedi *et al.* 2020).

### 2.2.2 Assemblage du microbiote

La composition des communautés microbiennes est soumise à des règles d'assemblages comparables à celles des macroorganismes, mais appliqué à l'échelle microscopique. Cela inclut des mécanismes tels que la dispersion, les filtres environnementaux et les interactions biotiques (Vorholt 2012; Bulgarelli *et al.* 2013; Hassani *et al.* 2018). Les principaux facteurs influençant les communautés microbiennes comprennent les caractéristiques du sol, le génotype/espèce de l'hôte, le système immunitaire de la plante, les traits et le stade de développement de la plante, le compartiment/organe étudié, ainsi que des dynamiques temporelles et saisonnières (Hassani *et al.* 2018).

En plus d'être "passivement" assemblées par les tissus de l'hôte, les communautés microbiennes sont activement affectées par la plante. Les plantes peuvent en réponse à certaines conditions environnementales appeler à l'aide ("Cry for help") des partenaires microbiens (Rolfe *et al.* 2019; Rolli *et al.* 2021; Rizaludin *et al.* 2021). Foster et ses collaborateurs (2017) proposent une vision du microbiote et son microenvironnement comme un écosystème en laisse (*ecosystem on a leash*). Un microbiote évoluant librement ne serait pas nécessairement bénéfique pour les plantes car la compétition entre microorganismes favoriserait typiquement l'émergence de microorganismes qui limiteraient leur dépense au bénéfice de la plante hôte en faveur de leur propre croissance (West *et al.* 2002; Foster and Wenseleers 2006). Ces microorganismes se multipliant deviendraient un coût pour la plante hôte et affecteraient négativement sa croissance et sa santé. L'hôte est donc soumis à une forte pression de sélection pour maintenir un microbiote bénéfique (Foster *et al.* 2017). Ainsi, l'hôte a développé des moyens de contrôle de son microbiote en favorisation ou en sanctionnant certains partenaires (Foster *et al.* 2017). Par exemple, la plante hôte est capable de cesser de fournir des nutriments à ses nodules racinaires si ses partenaires bactériens (ici *Bradyrhizobium japonicum*) n'assurent plus la

fixation d'azote (West *et al.* 2002; Kiers *et al.* 2003). Ce contrôle exercé par l'hôte sur son microbiote conduit, selon le principe d'Anna Karénine, à des microbiotes sains et bénéfiques pour la plante hôte qui sont plus similaires entre eux que des microbiotes dysfonctionnels (Arnault *et al.* 2022). Cette hypothèse adaptée d'une formule de Léon Tolstoï (Anna Karénine, 1877): "Toutes les familles heureuses se ressemblent, mais chaque famille malheureuse l'est à sa façon.". L'émergence d'un microbiote dysfonctionnel, ou dysbiose, suite à une perte de contrôle du microbiote par l'hôte, est supposée être régie par des processus moins déterministe et/ou plus spécifique à chaque individu. Le principe d'Anna Karénine devient dans ce cas : "Tout les microbiotes sains se ressemblent, mais chaque microbiote dysfonctionnel l'est à sa façon." (Arnault *et al.* 2022).

En parallèle des interactions hôte-microorganismes, les interactions microbiennes (*e.g.*, compétition, facilitation) participent également à la structure et au dynamisme du microbiote (Durán *et al.* 2018; Chaudhry *et al.* 2021). Non seulement les partenaires présents, mais aussi l'ordre d'arrivée de ces partenaires peuvent influencer la structure des communautés (Durán *et al.* 2018; Carlström *et al.* 2020). Cependant, l'étude de ces interactions microbiennes présente des limitations méthodologiques. En effet, établir la causalité nécessiterait la stérilisation des plantes et la construction de communautés synthétiques dont les membres sont contrôlés (Vorholt *et al.* 2017). Ainsi, malgré leur rôle dans le développement et la structure du microbiote, ces interactions microbiennes sont donc rarement identifiées et l'attention se porte davantage sur la structure taxonomique et fonctionnelle du microbiote.

### 2.2.3 Microbiote cœur : définition et enjeux

Un des enjeux majeurs de la recherche sur les microbiotes est la distinction d'un microbiote "cœur" (*core microbiota*), commun aux différents hôtes et

systematiquement présent. Ce microbiote est supposé déterminé et résultant d'interactions spécifiques, et donc potentiellement essentiel à la santé de l'hôte (Shade and Handelsman 2012; Lemanceau *et al.* 2017). Le microbiote cœur serait distingué d'une partie de la communauté plus opportuniste et non essentielle à l'hôte, qui serait donc moins contrainte et plus sujette aux spécificités environnementales et processus neutres et/ou stochastique. Cependant, la question de la définition du microbiote cœur est complexe et délicate. L'identification de ces membres se fait par une approche comparative de différentes communautés et le cœur résultant dépend donc de la résolution de l'étude, limitant ainsi sa généralisation (Shade and Handelsman 2012; Shade and Stopnisek 2019). Par exemple, il est possible de définir un cœur microbien pour une espèce dans une expérience donnée qui s'avèrera en fait propre à la population étudiée. De plus, la méthode d'identification du microbiote cœur est elle-même débattue. La méthode la plus classique consistait à l'utilisation de diagramme de Venn se basant simplement sur la présence de micro-organismes communs à tous les microbiotes étudiés. Cependant, la simple présence-absence ne donne qu'une information partielle sur les communautés microbiennes. Ainsi, des méthodes prenant en compte l'abondance relative, la stabilité temporelle ou la connectivité des membres de la communauté sont aujourd'hui recommandées, mais elles sont plus complexes à mettre en œuvre et nécessitent des données plus détaillées (Shade and Handelsman 2012, Fig. 7).

En parallèle, la pertinence d'utiliser la taxonomie comme principal moyen d'investigation du microbiote est remise en question. Les micro-organismes sont génétiquement très dynamiques et l'on observe notamment de nombreux transferts de gènes, acquisitions et perte de fonctions. Si l'étude taxonomique permet une estimation rapide de la diversité et de la structure des communautés, elle ne procure qu'une information très partielle des fonctions écologiques réalisées par le microbiote. Lemanceau *et al.* (2017) invitent donc à définir le

microbiote cœur fonctionnellement, en s'intéressant au "quoi" plutôt qu'au "qui". Bien entendu, ces deux approches sont complémentaires et, ultimement, nécessaires à notre compréhension du microbiote des plantes, de ses dynamiques et de ses conséquences pour l'hôte.

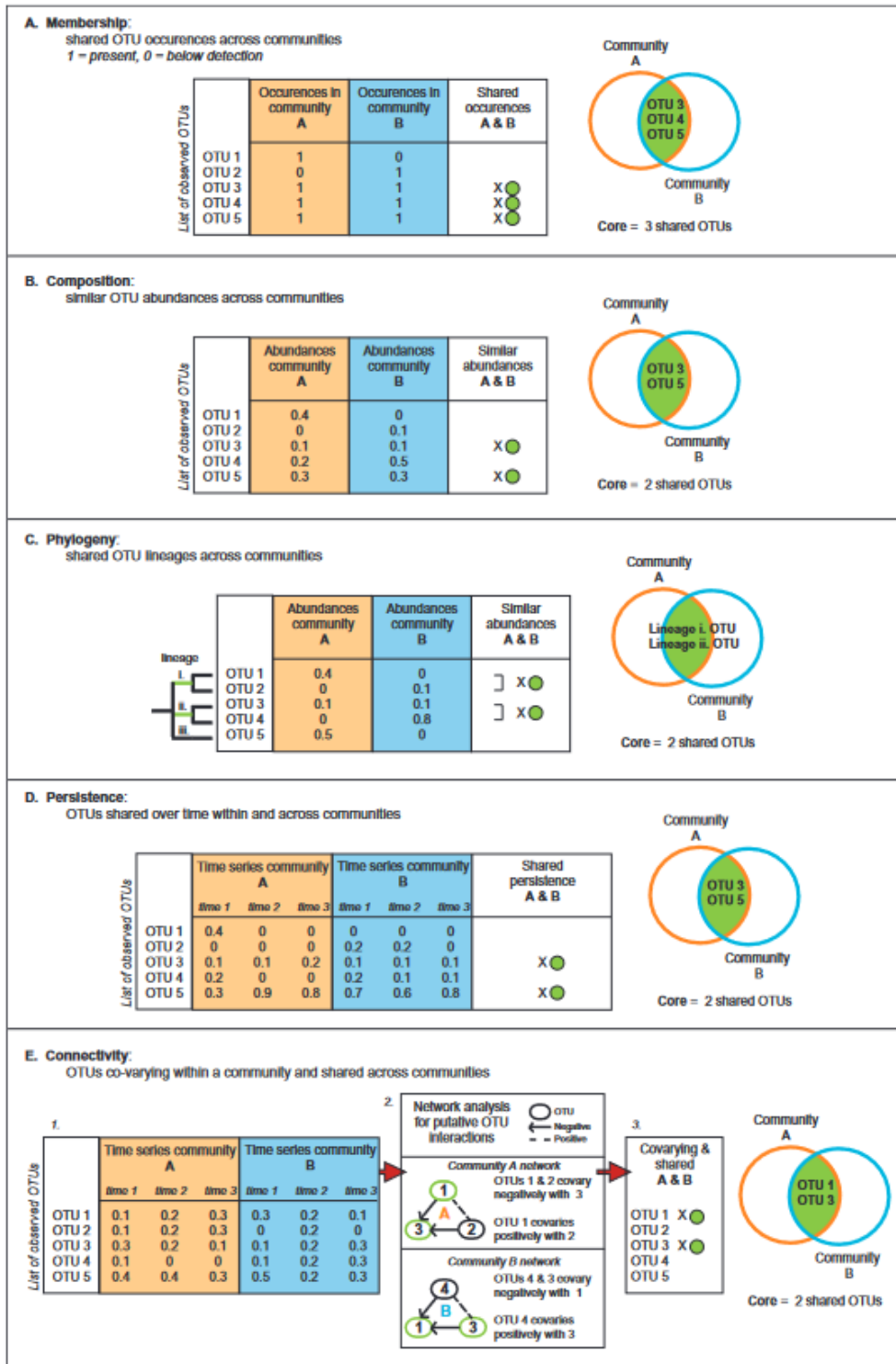


Figure 7 : Illustration de différentes définitions du microbiote cœur. A et B sont deux communautés à comparer. A. Un microbiote cœur basé sur la cooccurrence d'OTU dans des tables de présences/absences. B. Un cœur défini par des similarités de composition. Seuls les OTUs communs et d'abondance similaire sont considérés comme membre du cœur. C. Un cœur intégrant une information phylogénétique. Les OTUs d'une même lignée sont groupés dans la définition du cœur. Le niveau taxonomique peut être ajusté. D. Un cœur défini par les OTUs persistant au cours d'une série temporelle. Les OTUs retrouvés à plusieurs pas de temps sont considérés comme membre du microbiote cœur. E. Un cœur comprenant uniquement les OTUs interagissant (ou présumés interagir) avec les autres membres du microbiote. L'hypothèse d'une interaction significative est établie à partir d'une analyse de réseau sur plusieurs pas de temps (d'après la Figure 1 de Shade et Handelsman 2012).

### 2.3 Une entité hautement dynamique

Le microbiote des plantes est une entité dynamique et changeante dans le temps. On observe par exemples des changements saisonniers (Copeland *et al.* 2015; Walters *et al.* 2018) et interannuels (Zarraonaindia *et al.* 2015) dans la composition du microbiote associé aux plantes. Un autre facteur clé de la dynamique temporelle du microbiote est le développement et l'âge de l'hôte. Des plantes d'âge et/ou de stades ontogéniques différents arborent différentes communautés microbiennes (Wagner *et al.* 2016; Edwards *et al.* 2018; Arrigoni *et al.* 2018; Carper *et al.* 2018).

Les variations environnementales affectant le microbiote et l'hôte jouent également un rôle important dans la dynamique du microbiote. Des changements environnementaux peuvent affecter à la fois la composition des communautés sources du microbiote, la physiologie de l'hôte et celle des microorganismes pouvant entraîner d'importantes perturbation du fonctionnement du microbiote. Ces modifications peuvent être le résultat de processus déterministes ou bien stochastiques (Dini-Andreote *et al.* 2015). Par exemple, la sécheresse est souvent associée à un enrichissement en *Streptomyces* (Omae and Tsuda 2022) et ce de façon parfois réversible et hautement dynamique.

La nature des interactions (*e.g.*, parasitisme, mutualisme) au sein du microbiote et entre le microbiote et l'hôte est également dynamique. En effet, le microbiote comprend des microorganismes mutualistes mais également de nombreux organismes commensaux et pathogènes pouvant coloniser les tissus de la plante (Johnson *et al.* 1997; Partida-Martinez and Heil 2011). Les bénéfiques associés à la symbiose mutualiste sont contexte-dépendants et susceptibles d'être compensés voire surpassés par des coûts sous certaines conditions (Johnson *et al.* 1997; Hoeksema *et al.* 2010; Partida-Martinez and Heil 2011; Mujica *et al.* 2020). La différence entre le mutualisme et le parasitisme étant une question de bilan coût/bénéfice (Fig. 8), tout changement dans l'environnement affectant ces coûts



et/ou bénéfiques est susceptible de modifier ce bilan pour certains ou l'ensemble des partenaires. Ainsi, bien que certaines interactions soient strictement mutualistes ou parasitiques, il est plus réaliste de considérer un continuum parasitisme et mutualisme plutôt qu'une vision dichotomique des interactions symbiotiques. Si l'on se place généralement du point de vue de l'hôte, il en va de même pour les microorganismes qui lui sont associés. Par exemple, les plantes mycohétérotrophes peuvent agir en tant que parasites envers leurs champignons mycorhiziens (Selosse and Roy 2009), et ce statut de "parasite" peut varier en fonction des paramètres environnementaux tel que la lumière (Schweiger *et al.* 2019).

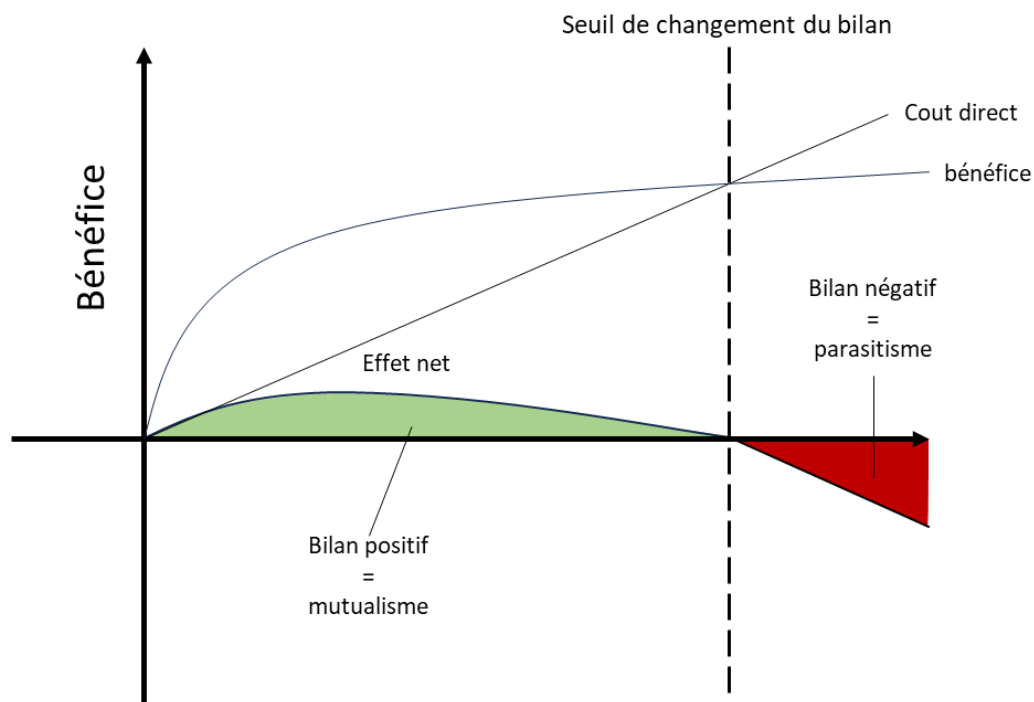


Figure 8 : Illustration du changement d'une relation mutualiste à parasitique par une approche coût/bénéfice (d'après la Figure 3 de Partida-Martinez et Heil 2011).

Enfin, le microbiote est une communauté complexe et le siège de nombreuses interactions et rétroactions entre ses différents membres (Berendsen *et al.* 2012; Bauer *et al.* 2018; Hassani *et al.* 2018) et avec l'hôte (Taulé *et al.* 2021; Middleton *et al.* 2021; Omae and Tsuda 2022). Cette complexité rend l'étude des coûts et bénéfices difficile, et il est généralement observé le bilan net des interactions pour l'hôte (Mushegian and Ebert 2016, Fig. 9).

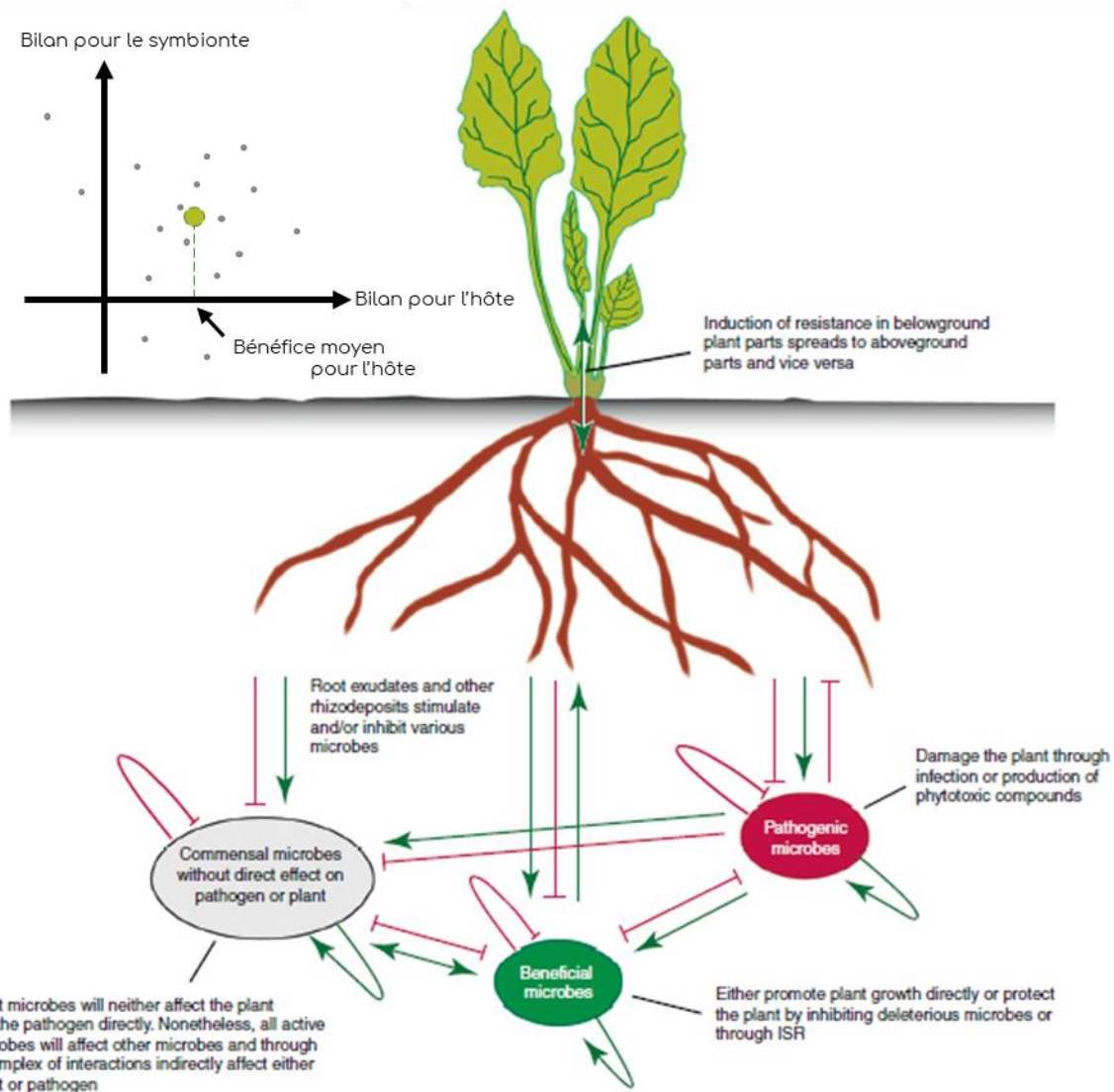


Figure 9 : Illustration des réseaux d'interactions hôte-microbiote et microbiote-microbiote rendant difficile l'isolement des effets d'une interaction spécifique au sein de la communauté (Complétée d'après la Figure 2 de Berendsen *et al.* 2012).

## 2.4 Transmission du microbiote chez les plantes

L'acquisition du cortège microbien par la plante peut se faire horizontalement (*i.e.*, recrutement à partir de l'environnement) et, dans une moindre mesure, verticalement (*i.e.*, transmission à partir des plants parents, Fig. 10) (Gundel *et al.* 2011).

Des microorganismes peuvent coloniser ou être recrutés par la plante tout au cours de la vie de cette dernière. Le microbiote est donc soumis à des changements de composition et de structure au cours du temps. La principale source de microorganismes est le sol, dont l'immense diversité microbienne constitue un réservoir d'endophytes (Trivedi *et al.* 2020; Compant *et al.* 2021). Les changements de microbiotes au cours du temps peuvent être aléatoire (dérive écologique), dépendre des modifications dans l'environnement de l'hôte (changement environnementaux) ou dépendre de l'hôte lui-même (ontogénie) (Bringel and Couée 2015; Gong and Xin 2021).

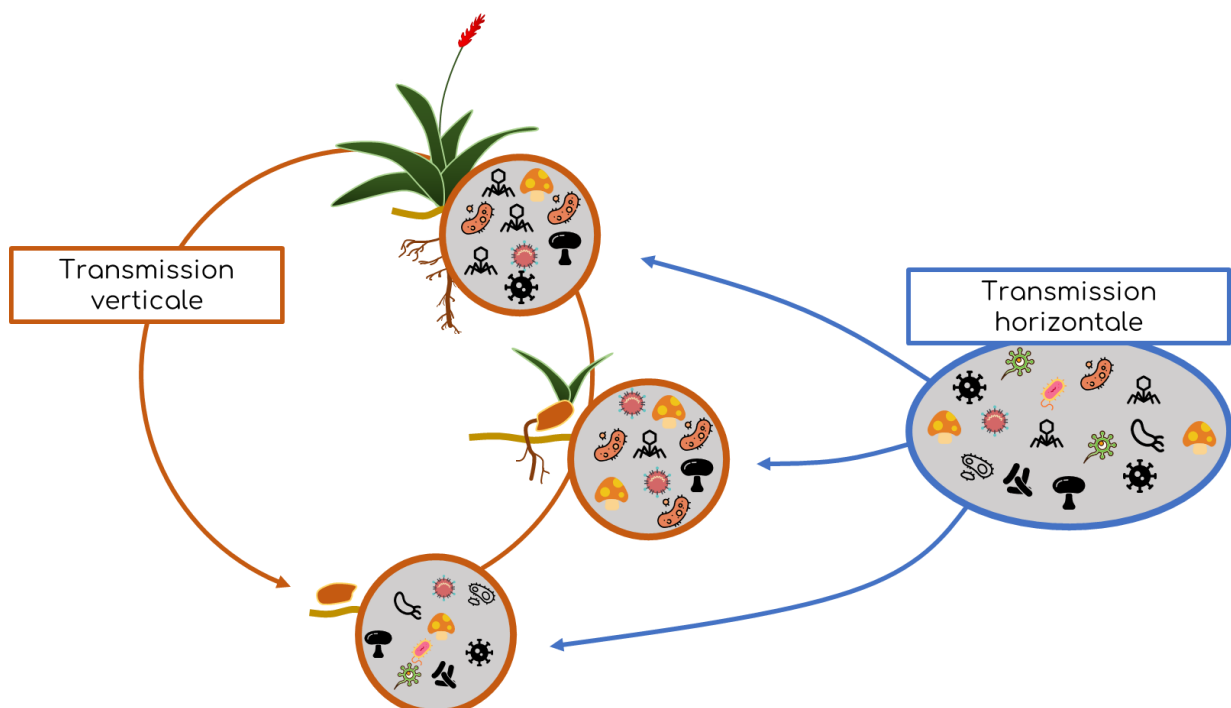


Figure 10 : Illustration de la transmission verticale et horizontale du microbiote chez les plantes. La transmission verticale n'a lieu qu'une seule fois dans le cycle de vie de la plante et se fait à partir du microbiote des plantes (orange). À l'inverse, la transmission horizontale se fait à partir du microbiote environnemental (bleu) et à potentiellement lieu continuellement au cours de la vie de la plante.

A l'inverse, la transmission verticale n'a lieu qu'au début de la vie de l'hôte. Cette transmission peut se faire via les graines (Shade *et al.* 2017; Guo *et al.* 2021; Simonin *et al.* 2022) ou la production d'organes végétatifs chez les espèces clonales (Vannier *et al.* 2018). En permettant la persistance de certains microorganismes d'une génération à l'autre, elle favorise théoriquement la mise en place d'interactions stables au cours de l'évolution tout en fournissant aux plantules un microbiote potentiellement crucial à leur croissance et survie (Shade *et al.* 2017; Vannier *et al.* 2018). La proportion du microbiote acquise verticalement chez les plantes semble très variable en fonction des taxons et des études (Shade *et al.* 2017; Newcombe *et al.* 2018; Guo *et al.* 2021), mais elle est probablement de grande importance, car elle constitue le point de départ du futur microbiote (Shade *et al.* 2017). En effet, de légères différences dans la composition du microbiote transmis peuvent entraîner d'importantes variations entre les plantules en raison de la croissance et des interactions microbiennes (par 'effet de priorité', Fukami 2015).

La question de la contribution relative de ces deux modes de transmissions à l'assemblage du microbiote chez les plantes et plus encore des facteurs environnementaux affectant cette balance reste à ce jour inexplorée mais est cruciale puisque potentiellement au centre d'une tension entre flexibilité (transmission horizontale) et fidélité (transmission verticale) du microbiote des plantes.

## 2.5 Holobionte : échec du concept de plante

### 2.5.1 Généralités et concepts

L'ubiquité des interactions hôte-microbiote et leurs effets sur l'écologie et l'évolution de tous les partenaires ont conduit à l'émergence du concept d'holobionte et ce que certains auteurs appelle l'échec de celui de "plante" au sens derrière toutes plantes se trouve en réalité une entité composite végétale et microbienne (Lyu *et al.* 2021). Le terme holobionte désigne l'association entre

différents individus, généralement un hôte et son microbiote, qui forment ensemble une unité anatomique, physiologique, immunologique ou évolutive (Gilbert *et al.* 2012; Bordenstein and Theis 2015; Simon *et al.* 2019). Largement attribué à Lynn Margulis (début des années 90), le concept d'holobionte trouve son origine dans les travaux du théoricien allemand Adolf Meyer-Abich qui résume ainsi sa théorie dès les années 40 :

*In a nutshell, the theory of holobiosis says that all higher and more complex organisms have developed through biontic processes, that is, parabioses, antibiosis, symbioses, and finally holobioses between simpler and lower forms of organisms. In other words, this means that the organs and the unions of organs have developed from originally independent organisms* (Meyer-Abich, 1943 d'après Baedke *et al.*, 2020).

Le regroupement du génome de l'hôte et de ceux de l'ensemble de ses partenaires constitue l'*hologénome* (Theis *et al.* 2016, Fig. 11). Le terme *microbiome* désigne quant à lui le microbiote et son environnement (molécules et paramètres physico-chimiques).

Cette approche holistique de l'individu (méta-organisme) et le concept d'holobionte a été appliqué à un large éventail d'organismes parmi lesquels les humains (Gerber 2014), les insectes (Brune and Dietrich 2015), les coraux (Rohwer *et al.* 2002; Bourne *et al.* 2016) et de manière extensive, les plantes (Vandenkoornhuyse *et al.* 2015; Sánchez-Cañizares *et al.* 2017; Hassani *et al.* 2018).

Le microbiote étant hautement dynamique sous l'influence de la dérive, la sélection, la dispersion et les interactions avec l'environnement (Vellend 2010; Nemergut *et al.* 2013; Dini-Andreote *et al.* 2015; Trivedi *et al.* 2020); l'holobionte est lui-même dynamique et sensible aux changements environnementaux (Caporaso *et al.* 2011; Gerber 2014; Rosenberg and Zilber-Rosenberg 2018; Chesneau *et al.* 2020). Ce constat justifiant la pertinence de combiner microbiote et

écophysiologie végétale pour étudier la plasticité des plantes à l'échelle de l'holobionte (Vandenkoornhuysse *et al.* 2015; Cernava *et al.* 2019).

## Holobiont

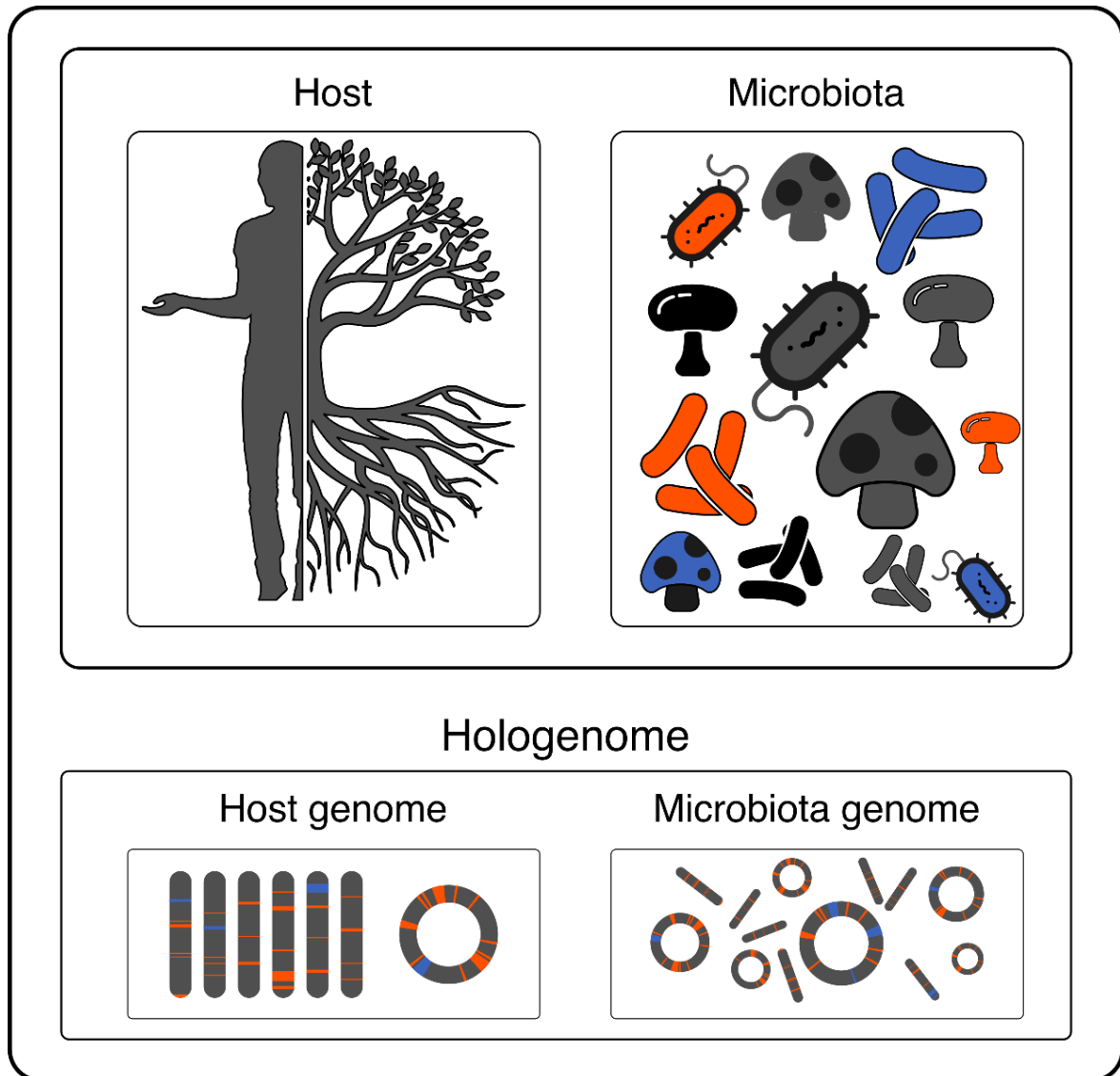


Figure 11 : Les holobiontes sont des entités constituées d'un hôte et de tous ses symbiotes microbiens. Ceci inclut à la fois les microorganismes affectant le phénotype de l'holobionte et ayant coévolués avec l'hôte (bleu), ceux affectant le phénotype sans avoir coévolués avec l'hôte (rouge) et tous ceux n'ayant pas d'effet notable sur le phénotype de l'holobionte (nuance de gris). L'hologenome est donc la somme du génome de l'hôte et de tous les membres de son microbiote à un moment donné. Les gènes et génomes constituant l'hologenome peuvent eux-mêmes être distribués dans les trois catégories : bleu, rouge et gris. Les holobiontes et les hologénomes sont des entités, des unités biologiques où la coévolution ou l'évolution d'interactions hôtes-symbiotes sont des processus (Figure d'après Theis et Bordenstein 2016).

### 2.5.2 Holobionte et phénotype

Le microbiote remplit de nombreuses fonctions liées à la croissance et la survie de leurs hôtes (Fig. 12, Bulgarelli *et al.* 2013; Lemanceau *et al.* 2017; Trivedi *et al.* 2020; Cernava and Berg 2022; Omae and Tsuda 2022). Il peut participer à la réponse et au contrôle de facteur de stress (e.g., sécheresse, pollution) par la stimulation ou la production directe d'hormones, de détoxifiants ou d'osmoprotecteurs. La production d'antibactériens, d'antifongiques et de toxines contribue directement aux défenses de la plante contre les pathogènes, herbivores et parasites tandis que des interactions entre microorganismes, telles que la compétition, peuvent indirectement empêcher le développement de bactéries et champignons nocifs (Bulgarelli *et al.* 2013; Chialva *et al.* 2022). Les microorganismes favorisent également la croissance de l'hôte en rendant les ressources du sol accessibles en les prélevant à la place de la plante (e.g., mycorhize) ou en transformant chimiquement des composés chimiques pour les rendre assimilables par l'hôte (e.g., bactéries fixatrices d'azotes). Ces fonctions assurées par le microbiote des plantes font l'objet d'une littérature dense au cours des dernières années (Bulgarelli *et al.* 2013; Bringel and Couée 2015; Müller *et al.* 2016; Lemanceau *et al.* 2017; Compant *et al.* 2019; Trivedi *et al.* 2020; Chialva *et al.* 2022). Ainsi, le phénotype des plantes est sous forte contrainte de leur microbiote (Hassani *et al.* 2018), la vie de l'hôte sans son microbiote semble impossible et ces deux entités indissociables.

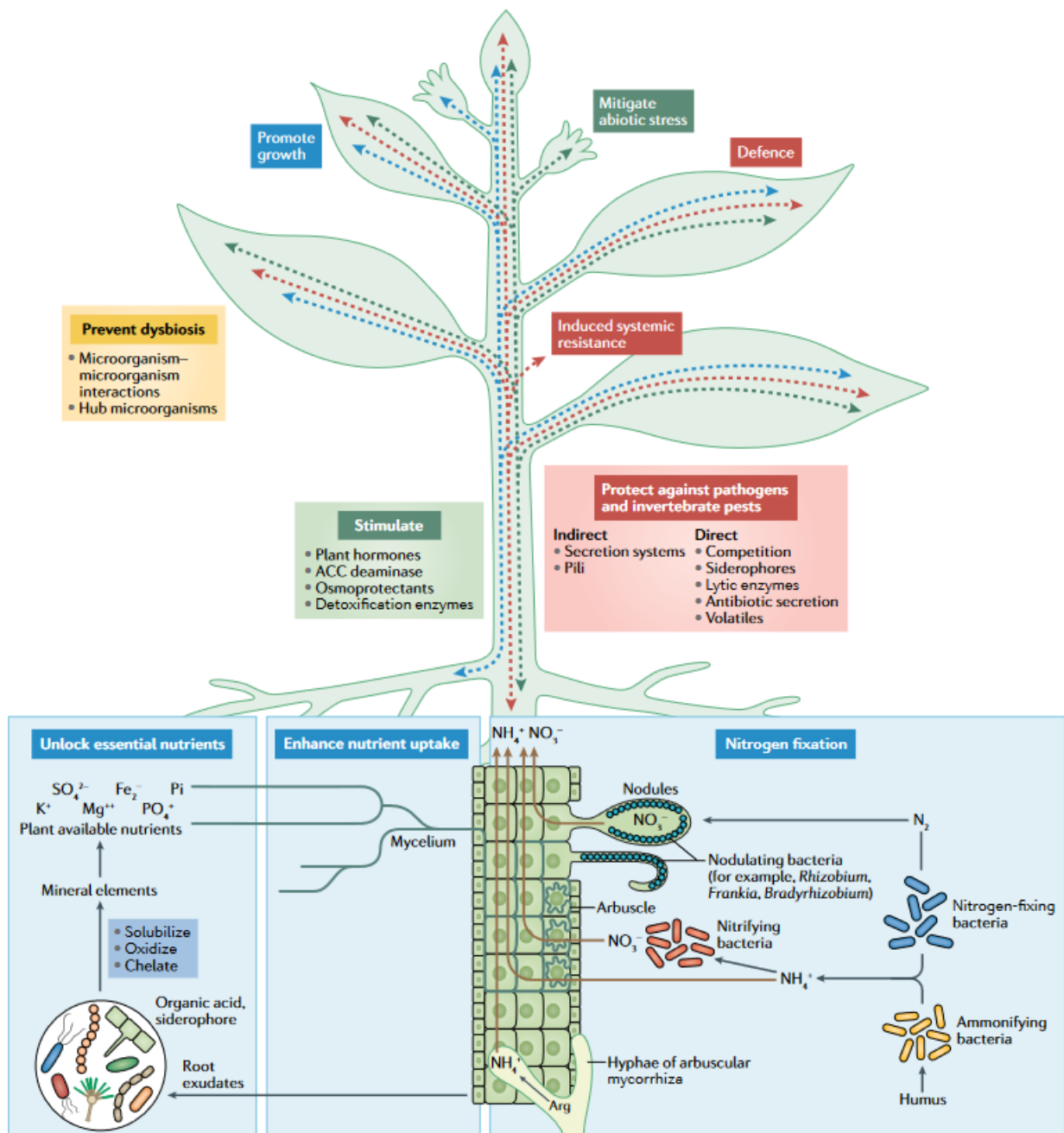


Figure 12 : Schéma récapitulatif des principaux effets bénéfiques des micro-symbiontes des plantes. Le microbiote des plantes peut procurer divers bénéfices de manière directe ou indirecte. Ces bénéfices comprennent la stimulation de la croissance (bleu), l'atténuation des stress (vert) et la défense contre les ravageurs et pathogènes (rouge). Les bénéfices du microbiote peuvent se réaliser dans n'importe quel compartiment de la plante et être transmis à d'autres organes par des signaux et mécanismes de transport assurés par l'hôte (flèche de couleur correspondant aux types de bénéfices). D'après la Figure 3 de Trivedi et al. 2020 à consulter dans l'article original pour une légende plus détaillée.



### 2.5.3 Conséquences éco-évolutives

S'il subsiste des doutes quant à la possibilité d'une sélection agissant au niveau de l'holobionte (Roughgarden 2020), il est admis que les partenaires microbiens des plantes ont participé à leur évolution. Des procaryotes sont à l'origine du chloroplaste chez les plantes (Archibald 2015), tandis que des interactions avec des champignons ont vraisemblablement facilité la conquête des continents par les premières plantes terrestre il y a 450-75Ma (Hoysted *et al.* 2018). Ces interactions existent probablement depuis l'émergence de la vie multicellulaire, et donc depuis l'apparition des plantes modernes, et de nombreux transferts de gènes depuis d'autres organismes ont également fortement impactés leur évolution (Fig. 13, Soucy *et al.* 2015). Les plantes sont aujourd'hui dépendantes de leur symbiotes (et vice et versa) pour la réalisation de certaines fonctions (Chomicki *et al.* 2020) menant à "l'échec du concept de plante" si l'on ignore son microbiote (Partida-Martinez and Heil 2011; Lyu *et al.* 2021). Cette dépendance peut être la conséquence de sous-traitance adaptative de l'hôte à son microbiote (Black Queen Hypothesis, Morris *et al.* 2012) ou la perte aléatoire de fonctions redondantes entre ces entités (Selosse *et al.* 2014) et peut être renforcée par la transmission verticale du microbiote favorisant la disparition de formes libres des microorganismes.

L'énorme et dynamique patrimoine génétique porté par ce microbiote peut être considéré comme une extension du génome de l'hôte fournissant de nouvelles fonctions (Bourne *et al.* 2016; Müller *et al.* 2016). Dès lors, le phénotype de l'hôte, et donc généralement étudié, peut être considéré comme étant en partie le phénotype étendu des organismes constituant son microbiote et le microbiote comme phénotype étendu de l'hôte (Vannier *et al.* 2015).

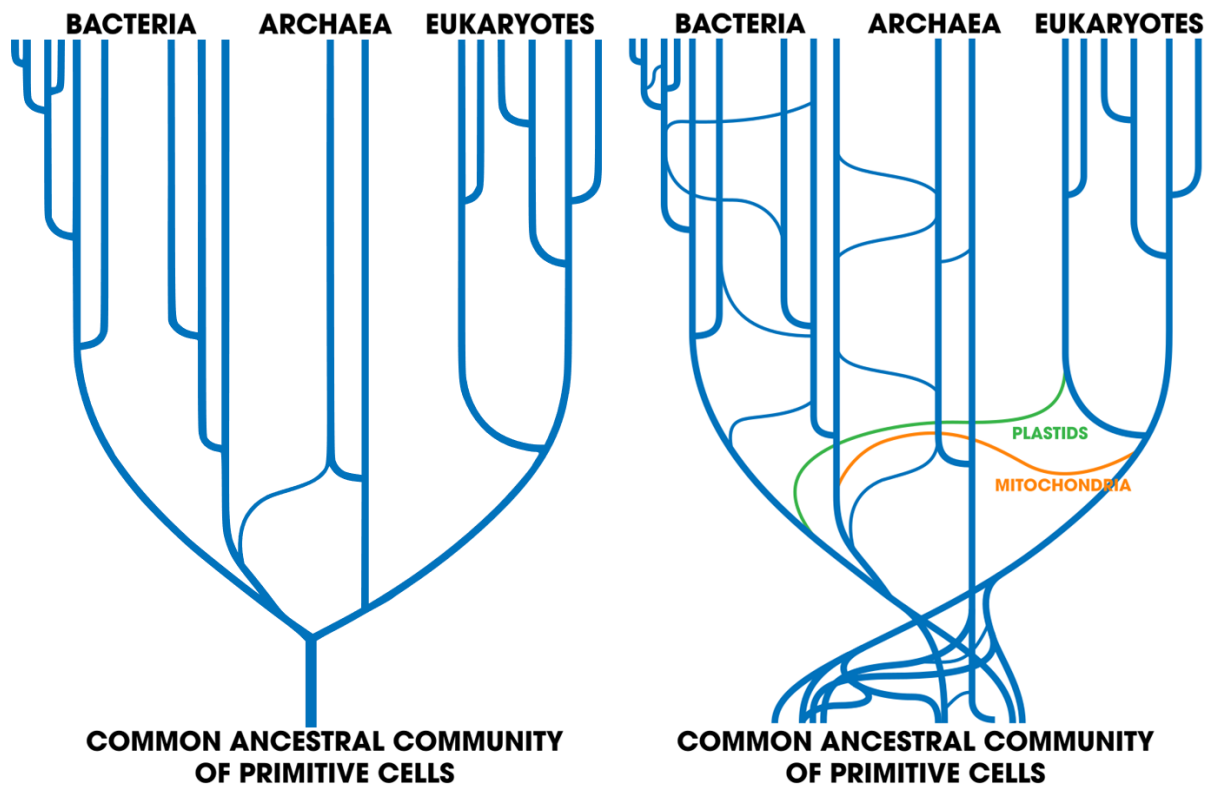
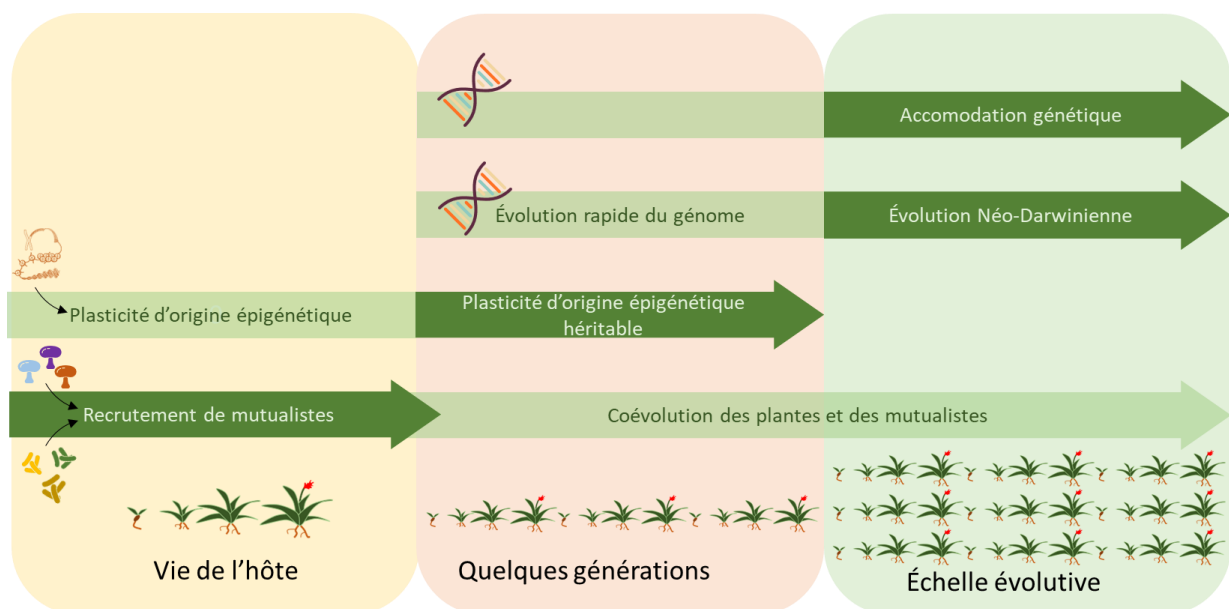


Figure 13 : Illustration de la vision classique de l'arbre du vivant (Tree Of Life, à gauche) et du concept de la Web Of Life (à droite), intégrant les transferts de gènes entre différentes lignées et donc réduisant leur indépendance évolutive à partir du dernier ancêtre commun. Illustration sous licence adaptée de Smets et Barkay 2005 et servant d'illustration du concept de transfert horizontal de gènes sur wikipédia ([https://fr.wikipedia.org/wiki/Transfert\\_horizontal\\_de\\_g%C3%A8nes](https://fr.wikipedia.org/wiki/Transfert_horizontal_de_g%C3%A8nes)). Le concept de Web Of Life est exposé par Soucy et al. 2018.

Cette vision de l'organisme modifie notre conception de sa plasticité. En effet la définition de Sultan (2000) n'est pas compatible avec un phénotype résultant de l'interaction d'une multitude de génotypes en interaction. Le temps de génération très court des microorganismes et leur importante diversité permet des ajustements rapides aux conditions environnementales (Fig. 14, une forme de « sous-traitance » de certaines fonctions nécessaire à l'hôte à son microbiote (Partida-Martinez and Heil 2011; Gilbert *et al.* 2012; Vannier *et al.* 2015; Voolstra and Ziegler 2020). La réponse plastique à l'environnement peut se faire par des ajustements du microbiote et non plus seulement de l'hôte. N'étant plus confronté à un génotype mais un hologénotype nous n'observons plus une plasticité phénotypique de l'hôte mais holophénotypique (*sensus* Roughgarden 2020). Par souci de simplicité et parce que ce terme n'est pas utilisé par la communauté

scientifique et fait également référence au phénotype complet (et donc non mesurable, Chitwood and Topp 2015) nous utiliserons *phénotype* de manière indifférenciée dans les différents chapitres.

Le phénotype étant l'objet des interactions organismes-environnement et organismes-organismes, cette conception du phénotype a donc d'importantes conséquences écologiques. Le rôle potentiel du microbiote dans la réponse face aux variations environnementales en fait une question cruciale dans le contexte de changements globaux actuels. Comprendre l'holobionte chez les plantes est un des enjeux majeurs de la biologie moderne avec des retombées à la fois fondamentales et appliquées. Le rôle des plantes dans les écosystèmes naturels et leurs importances pour les sociétés humaines est fondamentale et la manipulation de leur microbiome (microbiome engineering) est une solution envisagée contre les nuisibles, maladies et conséquences des changements climatiques (Foo *et al.* 2017).



Échelle de temps à laquelle ont lieu les changements environnementaux

Figure 14 : Illustrations des principaux mécanismes permettant l'héritabilité de la variation phénotypique chez les plantes et les échelles de temps auxquelles ils ont lieu. Cette variation n'est pas forcément associée à une variation génétique et des processus épigénétique et/ou le recrutement de microorganismes peuvent permettre une réponse rapide du phénotype. Ces deux processus peuvent être suivi à plus long terme par une accomodation génétique et l'adaptation des organismes (adaptée de la Figure 2 de Vannier et al. 2015).

## 4 Un modèle pour comprendre la réponse phénotypique et microbiotique de l'holobionte aux facteurs environnementaux

### 4.1 Les broméliacées

Les Broméliacées constituent une famille d'angiospermes monocotylédones extrêmement diversifiée et spécifiquement présente dans les Néotropiques, à l'exception de *Pitcairnia felicitiana*. Avec plus de 3700 espèces réparties dans 82 genres, les broméliacées présentent une extraordinaire diversité tant sur le plan taxonomique qu'écologique (Benzing 2000; Butcher and Gouda 2023). Originaire du bouclier des Guyanes (Givnish *et al.* 2011), les Broméliacées sont naturellement présentes de la pointe de la Floride au nord de l'Argentine. On les trouve dans une grande variété d'habitats, depuis les plateaux andins jusqu'aux caatingas brésiliennes en passant par le bassin amazonien. Près de la moitié des espèces sont épiphytes (c'est le cas des *Tillandsia*), tandis que d'autres sont lithophytes ou terrestres (comme le genre *Ananas*). Les broméliacées représentent la deuxième famille d'épiphytes après les orchidées (Benzing 2000, silvestro 2013).

Les Broméliacées se caractérisent par leurs feuilles alternes disposées en rosette. Le succès écologique et évolutif de cette famille est notamment attribué à la mise en place d'innovations morphologiques et fonctionnelles telles que l'épiphytisme, les trichomes foliaires absorbants l'eau et les minéraux, les réservoirs d'eau, ainsi qu'une photosynthèse à métabolisme acide crassulacéen (CAM) (Givnish *et al.* 2014; Males 2016). En se basant sur ces innovations, les broméliacées ont été classées en 5 types écologiques (Fig. 15, Benzing 2000; Leroy *et al.* 2016) :

- Le type I regroupe des broméliacées terrestres appartenant aux sous-familles des Pitcairnioideae (CAM et C<sub>3</sub>) et des Bromelioideae (CAM) qui

absorbent l'eau et les minéraux par leurs racines; les trichomes (s'ils sont présents) ne sont pas absorbants.

- Le type II est constitué d'espèces terrestres appartenant à la sous-famille des Bromelioideae ayant un fonctionnement photosynthétique CAM. Ces plantes forment des réservoirs très rudimentaires et absorbent l'eau et les minéraux par leurs racines.
- Le type III comprend des broméliacées à réservoir d'eau, de la sous-famille des Bromelioideae, pouvant être épiphytes, terrestres ou lithophytes. Ces espèces ont un fonctionnement photosynthétique CAM et leurs trichomes foliaires absorbent l'eau et les minéraux contenus dans le réservoir d'eau. Le système racinaire joue principalement un rôle mécanique pour l'accrochage.

Les broméliacées de type IV appartiennent à la sous famille des Tillandsioideae (et dans une moindre mesure au genre *Brocchinia* de la sous famille des Pitcairnioideae). Majoritairement avec un fonctionnement photosynthétique C3, elles présentent une densité élevée de trichomes absorbants qui assurent la nutrition depuis le réservoir. Le système racinaire joue un rôle mécanique pour l'accrochage.

Le type V regroupe des espèces de la sous-famille des Tillandsioideae, appelées plantes atmosphériques. Qu'elles soient épiphytes ou lithophytes, ces plantes, ne possédant pas de réservoir d'eau, absorbent l'eau et les minéraux présents dans l'humidité de l'air. Les racines servent uniquement à l'ancrage sur le substrat, voire sont simplement absentes chez certaines espèces.

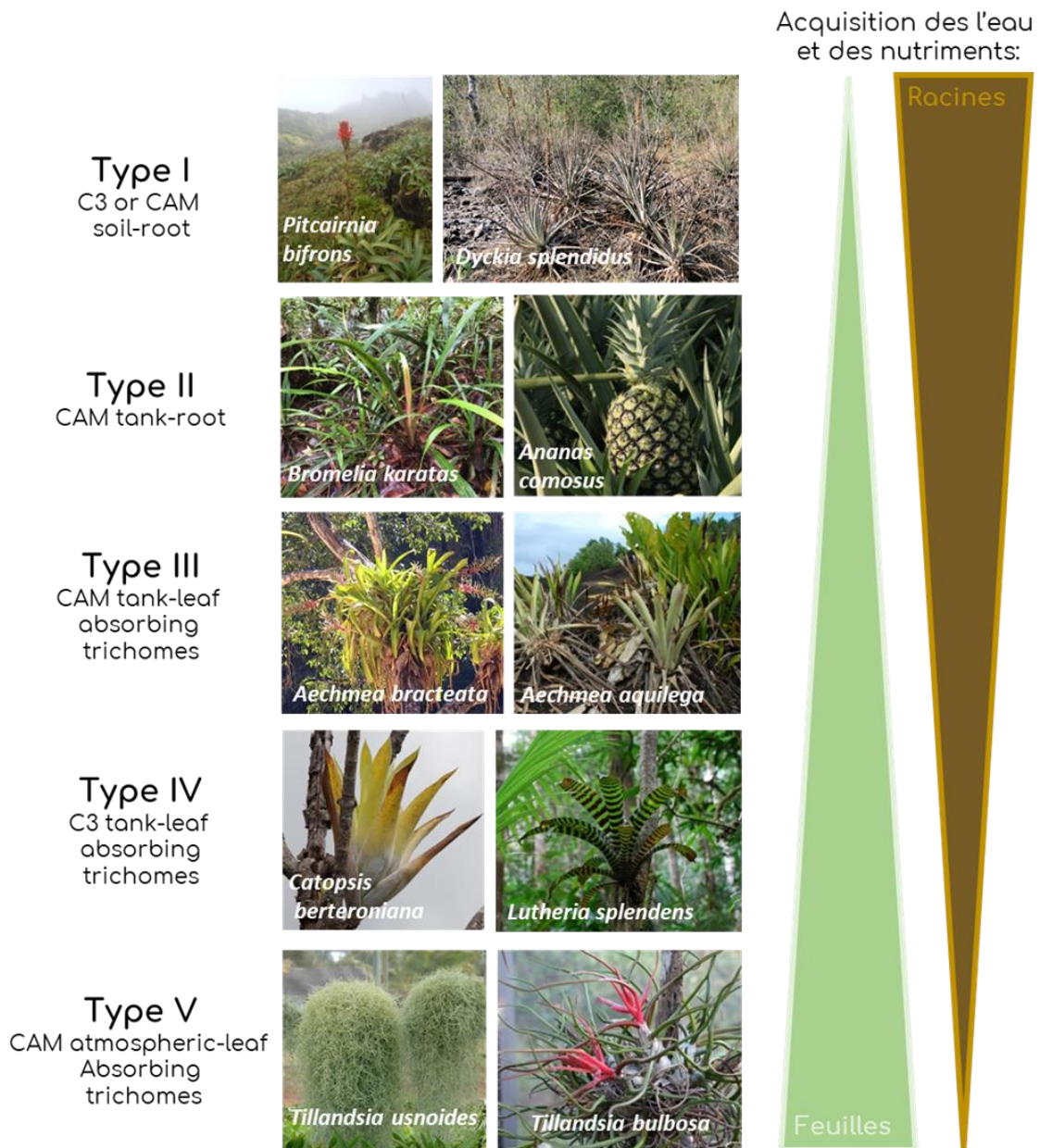


Figure 15 : Récapitulatif des principaux types écologiques définis par Benzing (2000) en présentant deux espèces représentatives de chaque type et illustrant le gradient de dépendance vis-à-vis de la nutrition racinaire ou foliaire en fonction des types écologiques.

#### 4.2 Des plantes-écosystèmes complexes : les broméliacées à réservoir d'eau

Chez près de la moitié des espèces de broméliacées, la base élargie de chaque feuille forme un réservoir permettant l'accumulation d'eau de pluie et de litière provenant la canopée (Types III et IV, Zotz 2016). Ainsi, elles fournissent un habitat pour de nombreux macro- et microorganismes organisés en un réseau trophique aquatique (Brouard *et al.* 2012b; Dézerald *et al.* 2013; Leroy *et al.* 2016). La litière

collectée constitue la principale source d'énergie et de nutriments à la base du réseau trophique.

Les microcosmes aquatiques abrités par les broméliacées sont considérés par les écologues comme des systèmes modèles pertinents pour mener des expérimentations et tester des prédictions (Srivastava *et al.* 2004). En effet, ils forment des micro-écosystèmes naturels où les communautés peuvent être quasi exhaustivement échantillonnées. Ils sont facilement manipulables et transportables, et se trouvent en grand nombre sur une large échelle géographique, permettant d'avoir de nombreux réplicats. Par exemple, ces micro-écosystèmes ont permis de caractériser les effets des perturbations environnementales (*e.g.*, extrêmes de précipitation, disponibilité en nutriment) sur la structure des communautés aquatiques et les fonctions écosystémiques associées (Carrias *et al.* 2012; Dézerald *et al.* 2015; Bonhomme *et al.* 2020; Srivastava *et al.* 2020).

Chez les broméliacées, ce sont principalement les organismes aquatiques présents dans les réservoirs d'eau qui font l'objet d'études approfondies, laissant ainsi les microorganismes présents dans les tissus de ces plantes sous-étudiés. Quelques études ont néanmoins mis en évidence la présence de bactéries et de champignons endophytes et mycorhiziens dans les racines de diverses espèces de broméliacées par des méthodes de caractérisation microscopiques et de mise en culture (Rabatin *et al.* 1993; Grippa *et al.* 2007; Lugo *et al.* 2009, 2015; Giongo *et al.* 2013; Viana *et al.* 2020; Félix *et al.* 2022). Plus récemment, l'étude de la diversité des champignons a été abordée à l'aide d'approches de barcoding (Tellez *et al.* 2020) et de métabarcoding (Leroy *et al.* 2021, 2022) pour les feuilles et les racines, respectivement.

#### 4.3 Stratégies d'acquisition des ressources chez les broméliacées à réservoir d'eau

Les broméliacées à réservoir d'eau ont développé des stratégies particulières pour l'acquisition de l'eau et des minéraux, leur permettant de s'adapter à leur environnement souvent pauvre en nutriments. Le réservoir d'eau formé par les feuilles en rosette joue un rôle crucial dans ce processus. Les macro- et micro-organismes présents dans l'eau décomposent la matière organique et libèrent des minéraux, qui sont ensuite absorbés par les trichomes foliaires situés à la base des feuilles en contact avec l'eau (Benzing 2000; Inselsbacher *et al.* 2007; Leroy *et al.* 2016). Les feuilles des broméliacées à réservoir d'eau présentent donc une dualité fonctionnelle avec un gradient allant de l'apex à la base (Fig. 16). L'apex assure un rôle de feuille "classique", c'est le siège de la photosynthèse et des échanges gazeux. En revanche, à la base, la feuille en contact avec l'eau assure une fonction d'absorption des minéraux, se comportant ainsi comme un analogue des racines.

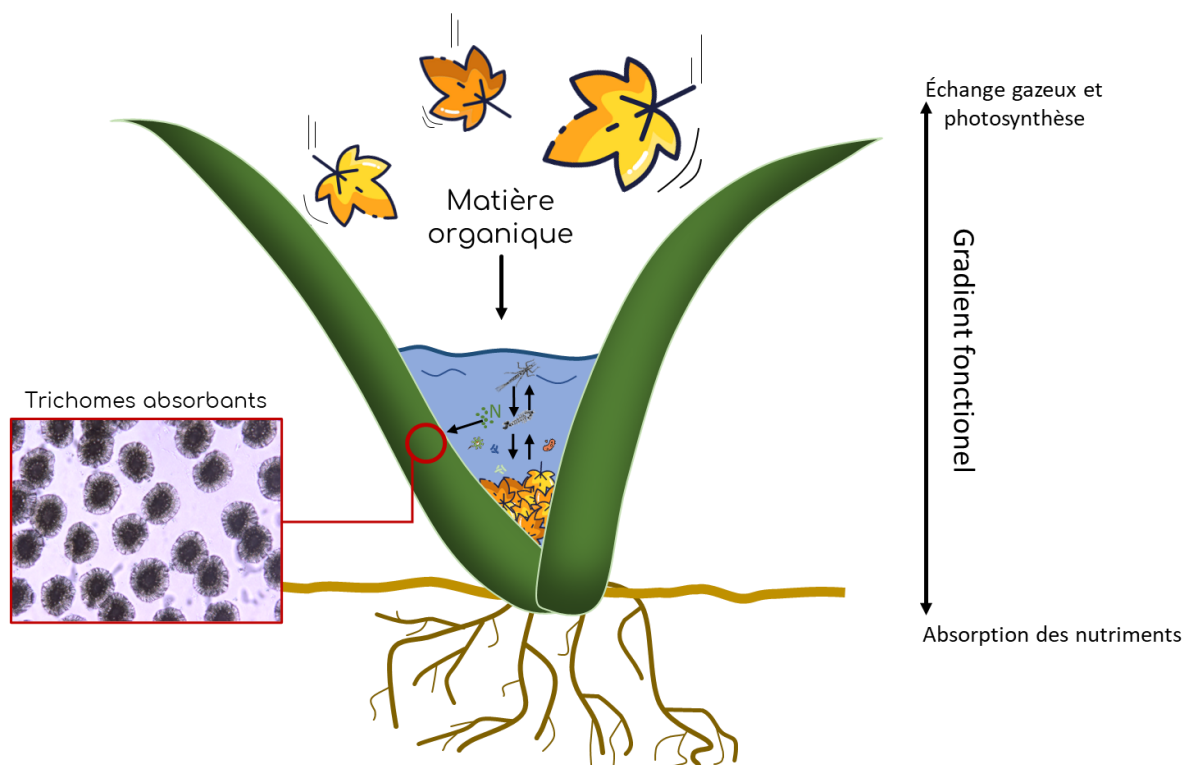


Figure 16 : Schéma illustrant le mode de nutrition des broméliacées à réservoir d'eau et le gradient fonctionnel au sein des feuilles (photosynthèse à l'apex et absorption des nutriments à la base). L'accumulation de matière organique dans le réservoir alimente un écosystème aquatique dont les nutriments dérivés sont absorbés à la base des feuilles par des trichomes absorbants.



Cette dualité fonctionnelle se traduit par des différences morpho-anatomiques, biochimiques et physiologiques entre les différentes parties d'une même feuille (Takahashi *et al.* 2007; Takahashi and Mercier 2011; Hermes *et al.* 2018; Mercier *et al.* 2019; Gonçalves *et al.* 2020). Ces plantes constituent un modèle idéal pour étudier la plasticité intra-organe, car les différentes régions de la feuille sont exposées à des environnements contrastés. De plus, cette dualité offre l'opportunité de s'interroger sur les liens entre la diversité et la structure des communautés microbiennes entre les « organes » : base et apex des feuilles et racines.

Étant donné que la base des feuilles joue un rôle essentiel pour l'acquisition de l'eau et des minéraux, les racines ont longtemps été considérées comme ayant principalement une fonction mécanique d'ancrage. Cependant, des études récentes ont révélé que les racines de certaines espèces sont capables d'absorber l'eau et les minéraux (Carvalho *et al.* 2018; Silva *et al.* 2018; Leroy, Gril, *et al.* 2019; Gomes *et al.* 2021). Chez les juvéniles de ces espèces, l'absence de trichomes absorbants et d'un réservoir d'eau implique une acquisition de l'eau et des minéraux par les racines (Petit *et al.* 2014) et dès la mise en place du réservoir d'eau et des trichomes foliaires absorbants, la fonction d'acquisition des ressources bascule en partie vers les feuilles (Leroy, Gril, *et al.* 2019). Cependant, les conséquences à long terme de la nutrition racinaire sur la croissance et les performances des broméliacées à réservoir adultes restent largement méconnues. La contribution des racines à la nutrition des broméliacées à réservoir est encore mal comprise, en particulier ce qui concerne les implications écologiques liées à la forte hétérogénéité des substrats, et donc de la disponibilité en minéraux, sur lesquelles ces plantes poussent.

#### 4.4. Un modèle pertinent : le genre *Aechmea*

Le genre *Aechmea* comprend 244 espèces (Butcher and Gouda, 2023) qui sont majoritairement épiphytes et lithophytes. Ce genre fait partie de la sous-famille des *Bromelioideae* et les espèces ont un fonctionnement photosynthétique CAM. Les *Aechmea* peuvent se développer dans les forêts humides et ombragées, sur des zones rocheuses en plein soleil, ou encore dans des régions plus arides. Ces plantes sont faciles à faire germer et à entretenir, ce qui permet de les utiliser expérimentalement pour tester l'effet de divers stress environnementaux sur le taux de germination, la croissance, la plasticité, et les associations microbiennes.

##### 4.4.1 *Aechmea aquilega*

*Aechmea aquilega* est présente dans la moitié Est de l'Amérique du Sud (Fig. 17 A) et se retrouve dans une grande diversité d'habitats. En Guyane Française, cette espèce se rencontre en milieu ouvert de savane roche, en milieu forestier ainsi que dans les milieux urbanisés. Elle peut vivre sur des substrats très différents, à même le sol (*i.e.*, formes terrestres et lithophytes) ou dans la couronne des arbres (*i.e.*, forme épiphyte). Cette espèce représente donc un modèle d'étude pertinent pour aborder la plasticité phénotypique en fonction de la fertilité du substrat (Chapitre I) et des gradients naturels de lumière à l'échelle de la rosette et des différentes régions foliaires (Chapitre II).

##### 4.4.2 *Aechmea mertensii*

*Aechmea mertensii* est présente dans la moitié Nord de l'Amérique du Sud (Fig.17 B). Elle a la particularité d'avoir un cycle de vie rapide par rapport aux autres espèces de broméliacées. Alors que les broméliacées fleurissent en général après 5 à 7 ans depuis la germination de la graine, *A. mertensii* met en place son inflorescence en seulement une année. De plus, il a été mis en évidence de

l'importance des endophytes fongiques racinaires pour le développement et la survie des plantules d'*A. mertensii* (Leroy, Maes, *et al.* 2019). La présence d'endophytes dans les parties végétatives et les graines (Leroy, Maes, *et al.* 2019) en fait un modèle pertinent pour l'étude du microbiote (Chapitre III), notamment sa transmission entre les générations (Chapitre IV) puisque la production d'une nouvelle génération se fait dans un délai raisonnable. Finalement, malgré les innombrables services écosystémiques qu'elles assurent dans les forêts néotropicales, le microbiote des broméliacées reste aujourd'hui largement méconnu.

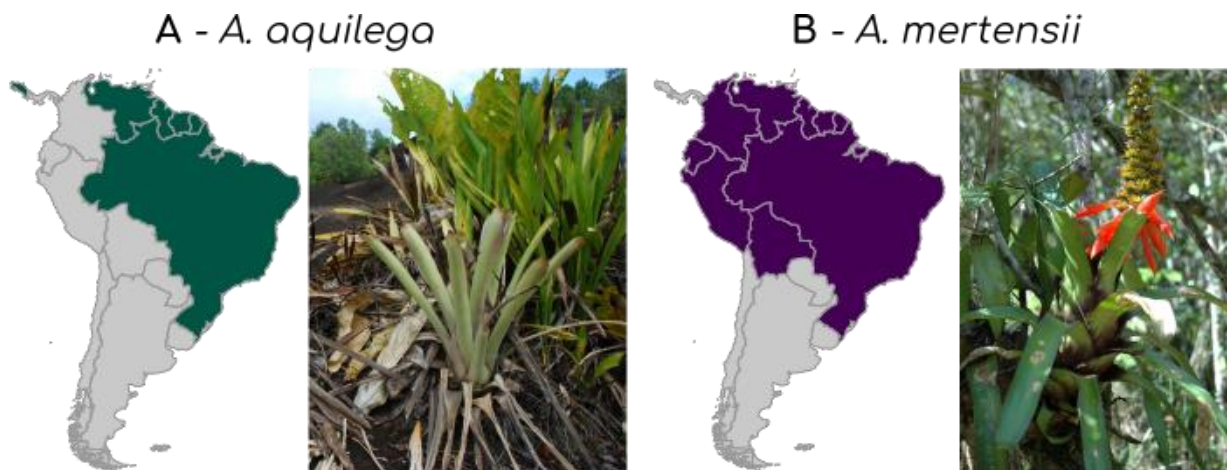


Figure 17 : Cartes de répartition et photos de **A)** *Aechmea aquilega* et **B)** *Aechmea mertensii*, les deux espèces de broméliacées étudiées dans cette thèse. Photos de Céline Leroy et les cartes de répartition ont été réalisées à partir du package R bromeliad (<https://github.com/idiv-biodiversity/bromeliad>).

## Les objectifs de la thèse

Les enjeux découlant des changements globaux ont amené à la production d'une littérature abondante sur la réponse des plantes aux paramètres environnementaux.

L'acquisition de ressources est l'un des enjeux clés de la croissance et la *fitness* des plantes qui ont mis en place de nombreuses stratégies pour assurer un apport suffisant en eau, nutriment et lumière. Cette acquisition se fait par la coordination des deux principaux organes végétatifs : les feuilles et les racines. Cependant, de nombreuses zones d'ombres persistent sur la coordination de ces organes en réponse à l'environnement, notamment chez des plantes aux stratégies écologiques diverses comme le sont les broméliacées à réservoir d'eau.

En parallèle, le passage d'une vision de la « plante individu » à la « plante écosystème » ou holobionte est un des grands enjeux de la biologie moderne, mais il souffre encore de nombreuses lacunes sur notre compréhension de cette entité et de sa réponse face aux changements environnementaux. Les variations phénotypiques des plantes résultent des interactions entre le génome de l'hôte, son microbiote et de leur environnement. L'objectif général de cette thèse est d'explorer la plasticité des plantes à différents niveaux d'intégration. Cette thèse a deux objectifs principaux :

Caractériser la plasticité phénotypique de broméliacées le long de gradients environnementaux naturels. Dans cette partie, nous avons adopté une vision classique du phénotype des plantes en mesurant des traits morpho-anatomiques et physiologiques dans le but de caractériser l'étendue et le type de réponse des plantes en fonction des facteurs environnementaux. Nous avons abordé cette plasticité phénotypique aux échelles inter-individuelles et intra-organes. Ici, nous avons utilisé *Aechmea aquilega* comme modèle car cette espèce est naturellement

présente dans un très large gradient de conditions environnementales, notamment d'ouverture de la canopée.

Caractériser la plasticité phénotypique de l'holobionte face à un environnement variable. Le phénotype des plantes est également fortement influencé par leurs microbiotes, qui font partie intégrante du phénotype étendu de la plante. Dans cette seconde partie, nous élargissons l'étude des traits des plantes en intégrant les réponses des communautés microbiennes afin de mieux comprendre le système holobionte sous contrainte environnementale. De plus, nous cherchons à mieux comprendre l'effet de l'environnement maternel sur la plasticité phénotypique des descendants via l'étude de certains processus transgénérationnels (plasticité transgénérationnelle des traits et flux microbien). Pour traiter cette question, nous avons utilisé *Aechmea mertensii* dont le temps de génération court (floraison annuelle) permet la mise en place d'expérimentations transgénérationnelles dans un délai raisonnable.

Pour répondre à ces objectifs, nous avons combiné des approches morpho-anatomiques et écophysiologiques (mesures de traits) afin de caractériser la plasticité phénotypique et les performances des plantes avec des approches de métabarcoding pour caractériser les communautés d'endophytes bactériennes et fongiques et l'interaction entre ces composantes. Ces différents objectifs ont été abordés par des observations en milieu naturel et par la mise en place d'expérimentations en serre.

## **Chapitre I** - Effect of substrate fertility on tank-bromeliad performances (Lafont Rapnouil *et al.* 2023, *Plant and Soil*)

Tout d'abord, nous explorons les effets de la richesse du substrat sur la croissance d'*A. aquilega*. De travaux récents ont montré que les broméliacées à réservoir d'eau sont capables d'obtenir des nutriments par leurs racines et en sont même dépendantes avant la mise en place d'un réservoir fonctionnel (Takahashi *et al.*

2022). Cependant, une fois adultes, les racines sont souvent considérées comme jouant uniquement un rôle d'ancrage et la contribution de la nutrition racinaire à la croissance de la plante, ainsi que ses conséquences à long terme, restent méconnues. Nous avons donc étudié l'effet de la richesse du substrat sur les performances de plantes âgées de 15 mois par des mesures de traits foliaires et racinaires. Des graines ont été mises à germer en serre sur 3 substrats différents (riche, pauvre et fertilité intermédiaire). Nous supposons que si les racines contribuent effectivement à la nutrition, nous devrions observer des différences phénotypiques majeures entre les plantes des différents traitements.

**Chapitre II** - Light intensity mediates phenotypic plasticity and leaf trait regionalisation in a tank bromeliad (Lafont Rapnouil *et al.* 2023, *Annals of Botany*)

Dans ce chapitre, nous examinons la variation phénotypique des feuilles le long d'un gradient naturel de lumière. Les feuilles d'*A. aquilega* assurent à la fois la photosynthèse (à l'apex) et la nutrition minérale (à la base). Le lien entre traits et fonctions prédit donc des valeurs de traits différentes entre ces deux régions. De plus, la disposition en rosette des feuilles entraîne une exposition plus importante de l'apex des feuilles aux variations de lumière, tandis que la base est plus ombragée. Étant donné que la base de la feuille remplit une fonction qui n'est pas liée à la lumière et qu'elle est moins exposée, elle devrait être moins sensible aux changements d'exposition à la lumière que l'apex. La question est ici double, *A. aquilega* est-elle capable d'une plasticité au niveau de l'organe et, si oui, a-t-on effectivement une plus forte sensibilité de l'apex au gradient lumineux comme prédit?

**Chapitre III** - Water and nutrient resources shape plant traits, leaf endophytic microbiota, and their relationships (Lafont Rapnouil *et al.*, *en préparation*)

Dans ce chapitre, nous ajoutons un niveau d'intégration en considérant le microbiote en plus des traits phénotypiques d'*A. mertensii* poussant dans différentes conditions environnementales. Nous avons donc caractérisé les effets de la disponibilité en eau et en nutriment sur le phénotype des plantes et sur les communautés bactériennes et fongiques dans les feuilles. Notre principal objectif était de caractériser les réponses écophysologiques et du microbiote de l'holobionte et leur éventuelle coordination. Pour cela, nous avons mis en place une expérimentation en serre où les graines issues d'une même plante-mère ont été mises à germer sur 3 substrats différents (riche, pauvre et fertilité intermédiaire) et deux conditions d'arrosage (bien arrosé et condition sèche). Les mesures des traits foliaires et la composition du microbiote bactérien et fongique ont été réalisées sur des plantes matures au stade de floraison. Nous avons examiné les relations entre les traits foliaires et la composition des communautés d'endophytes et finalement nous avons identifié les microorganismes associés de manière positive ou négative à certains traits foliaires. Nous avons ici deux hypothèses alternatives; 1) l'holobionte s'adapte aux différentes conditions environnementale ce qui se traduit par un maintien de l'appareil photosynthétique et des microbiotes similaires aux seins de chaque traitements mais différents entre traitements suite au "cry for help" de la plante hôte ou 2) les conditions environnementales sont trop dures (sécheresse et sol pauvre) et font sauter la "laisse" de l'hôte sur son microbiote résultant en des microbiotes hétérogènes au sein d'un même traitement pour les traitements les plus durs (Anna Karenina Principle).

#### **Chapitre IV** – Phenotypic and microbiotic maternal effects in a tank-bromeliad (Lafont Rapnouil *et al.*, *en préparation*)

L'acquisition et la transmission des endophytes microbiens jouent un rôle fondamental dans le développement et la croissance des plantes. Des études sur

des espèces cultivées suggèrent des effets maternels, mais ces effets restent peu connus dans les écosystèmes naturels, par ailleurs soumis à de forts gradients environnementaux. L'objectif de ce chapitre est de comprendre quelle part de la communauté microbienne est héritée de la plante mère via les graines, dans quelle mesure les flux microbiens sont influencés par les conditions environnementales locales, et comment ces microorganismes régulent la germination et la croissance des plantules. Ce chapitre s'inscrit donc dans la continuité du chapitre précédent. Ici, nous caractérisons l'influence de l'environnement des plantes-mères (Chapitre III) sur la transmission des communautés bactériennes et fongiques via les graines ainsi que sur le phénotype et le microbiote de jeunes plantules. Nous supposons que les conditions environnementales des plantes mères ont induit la production de graines avec des communautés endophytes différentes et exercent une influence sur la performance des plantules et leur microbiote. L'importante contribution du sol sur la composition du microbiote racinaire devrait résulter en une plus grande similarité entre les communautés des graines et de feuilles qu'avec celles racinaires. Finalement, si les effets maternels sont spécifiques aux conditions environnementales nous devrions avoir une meilleure performance des plantules poussant dans des conditions similaires à celle de leur mère.



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# Chapitre I

*Effects of substrate fertility on tank-bromeliad  
performances*

Cet article porte sur l'étude de l'effet de la richesse du substrat sur les performances d'*Aechmea aquilega*. Il s'agit d'une expérimentation en serre visant à déterminer l'importance de la nutrition racinaire pour *A. aquilega*.

Cette étude dévoile d'importante conséquence de la richesse du substrat sur la croissance et le phénotype de cette espèce puisque les plantes poussant sur le substrat avait jusqu'à 1.5 fois plus de feuilles. La réponse d'*A. aquilega* à la disponibilité en nutriment combine une réponse morphologique, physiologique et chimique.

Ces travaux sont les premiers à démontrer une importance de la fertilité du substrat pour la croissance et les performances à long terme d'*A. aquilega* et que ce facteur pourrait avoir de cruciales conséquences écologiques en milieu naturel.

Cet article est l'aboutissement de travaux initiés durant mon stage de Master 2 (Janvier – Mai 2020) sous la direction de Céline Leroy et Sabrina Coste. L'expérimentation avait été mis en place en amont de mon stage qui a consisté en la mesure des différents traits écophysologiques avec l'aide de Jean-Yves Goret puis au traitement des données. J'ai mené la rédaction du manuscrit en collaboration avec Sabrina Coste, Clément Stahl et Céline Leroy. Les analyses chimiques ont été réalisées à Toulouse par Frédéric Julien.

# Effects of substrate fertility on tank-bromeliad performances

Tristan Lafont Rapnouil<sup>1,2,\*</sup> <http://orcid.org/0000-0002-5411-3479>, Sabrina Coste<sup>2</sup> <http://orcid.org/0000-0003-3948-4375>, Jean-Yves Goret<sup>2</sup> <http://orcid.org/0000-0003-3604-5756>, Frédéric Julien<sup>3</sup> <http://orcid.org/0000-0001-5411-1169>, Clément Stahl<sup>2</sup> <http://orcid.org/0000-0001-5411-1169>, Céline Leroy<sup>1,2</sup> <http://orcid.org/0000-0003-4859-8040>

<sup>1</sup>AMAP, Univ. Montpellier, CIRAD, CNRS, INRAE, IRD, France

<sup>2</sup>EcoFoG, AgroParisTech, CIRAD, CNRS, INRAE, Université des Antilles, Université de Guyane, Campus agronomique, Kourou, France

<sup>3</sup>Laboratoire Écologie Fonctionnelle et Environnement, Université Paul Sabatier Toulouse 3, CNRS, Toulouse, France

**\*Corresponding author** Tristan Lafont Rapnouil,  
[tristan.lafontrapnouil@gmail.com](mailto:tristan.lafontrapnouil@gmail.com)



## Abstract

- *Purpose* Members of the plant family Bromeliaceae can uptake nutrients directly from their leaves via leaf absorbing trichomes and their roots have long been reduced to anchorage function, thus overlooked. Recently, evidence has accumulated for a significant role for the roots of some species of tank bromeliads in both water and nutrients absorption. However, to date, little attention has been paid to the importance of the substrate fertility for the structure of the roots and the growth and performances of tank bromeliads.

- *Methods* This study investigated the effect of substrate fertility on *Aechmea aquilega* regarding leaf and root traits, nutrient content, and growth. Seeds of this tank bromeliad were sowed in a greenhouse in French Guiana in three different substrates: a nutrient-poor, a nutrient-rich and a mixed substrate. The performances of 15-month-old *A. aquilega* were assessed by measuring leaf and root traits related to nutrient acquisition and resources capture.

- *Results* We show that plants growing in nutrient-poor substrate grew twice slower and were smaller than plants grown on the nutrient-rich substrate with fewer leaves and roots, lower total dry mass, and smaller leaves and root length. Overall, 70% of measured traits responded significantly to the experimental treatments indicating that the response of *A. aquilega* to nutrient availability is a combination of physiological processes, leaf and root structure, and chemistry.

- *Conclusion* This study is the first to show that the fertility of the substrate on which the bromeliad *A. aquilega* grows has a strong and lasting effect on the plant performances and may be a relevant factor for bromeliad ecology.

**Keywords:** Leaf traits, Plant performance, Root traits, Root uptake, Substrate fertility, Tank bromeliad

## Introduction

One of the key challenges faced by sessile plants is ensuring their uptake of sufficient nutrients and water for their growth and survival. Soil is the main source of water and nutrients for most plants, but as nutrients are unevenly spatially distributed, the availability of nutrients for plants also varies (Cain *et al.*, 1999) as do the plants response strategies (Bloom *et al.*, 1985; Kraiser *et al.*, 2011). Ecologists use the functional traits of leaves, stems and roots to obtain information on the resource (*i.e.*, light, water, nutrients) acquisition strategies of species (Sterck *et al.*, 2011). Cross-species comparisons have revealed major axes of trait variations reflecting strategies (*i.e.* leaves and roots economic spectra) which range from rapid acquisition to conservation of resources (Roumet *et al.*, 2016; Wright *et al.*, 2004). The same range can also be found within a single plant species across environmental gradients (Buchanan *et al.*, 2019; Delpiano *et al.*, 2020; Fajardo & Siefert, 2018; Hajek *et al.*, 2013; Hayes *et al.*, 2019; Isaac *et al.*, 2017). Plants facing harsh conditions (*i.e.* low resources availability) are expected to have more conservative traits than plants growing in nutrient-rich environments (Lambers & Poorter, 1992; Wright *et al.*, 2004). Functional trait approaches have mainly been used in trees and herbaceous species (*e.g.*, Fort *et al.*, 2013; Freschet *et al.*, 2013) while such studies of epiphytes are rare (de Paula Oliveira *et al.*, 2021; Richards & Damschen, 2021).

Epiphytes, plants that live non-parasitically on trees, have to cope with heterogeneous and intermittent nutrient and water availability (Zotz, 2016). These plants have consequently developed numerous morphological adaptations (*e.g.*, leaf-absorbing trichomes, *velamen radicum*, water-storage tanks, pseudo-bulbs) to optimise water and nutrient uptake and conservation (Males, 2016; Zotz, 2016). Epiphytic plant species can be obligate or facultative and can be found growing on different substrates including bark, rocks, canopy soil, or the ground (Wu *et al.*, 2020; Zhang *et al.*, 2021). Such different substrates can modify the morphological,

anatomical, physiological and stoichiometric traits of conspecific individuals (Chen *et al.*, 2019; Lu *et al.*, 2015; Wu *et al.*, 2020; Zhang *et al.*, 2021). Hoeber & Zotz (2021) recently found that accidental epiphytic individuals performed better than terrestrial conspecifics due to the beneficial growth conditions in the forks of branches filled with organic-rich arboreal soil. The nutrient use strategies of facultative epiphytes have been shown to be flexible, thus enabling facultative epiphytes to exploit different substrate interchangeably (Wu *et al.*, 2020; Zhang *et al.*, 2021).

Plants belonging to the *Bromeliaceae* family display many remarkable morphological, anatomical and physiological adaptations to facilitate nutrient uptake and conservation (Givnish *et al.*, 2014; Leroy *et al.*, 2016; Males, 2016). Bromeliads grow on different substrates (terrestrial, lithophyte or epiphyte) and differ in their ability to retain water and nutrients (*i.e.*, tank-forming, or tankless) and in the photosynthetic pathway (*i.e.*, C<sub>3</sub> or CAM). The leaves of tankless *Tillandsioideae*, of tank-forming *Bromelioideae*, and of some *Brocchinioideae* are the most important vegetative organ because they perform essential physiological functions including photosynthesis, water and nutrient assimilation and water conservation (Benzing 2000). In tank bromeliads, the basal part of the leaf is devoted to water and nutrient absorption through absorbing trichomes and to nutrient uptake thanks to transporters, plus nitrate reductase activity similar to that found in the roots of terrestrial plants (Gonçalves *et al.*, 2020b; Kleingesinds *et al.*, 2018). The middle and apical portions of the leaf are devoted to photosynthesis and glutamine synthetase activity (Gonçalves *et al.*, 2020b).

The functional importance of leaves in epiphytic tank-bromeliad nutrition led scientists to overlook the roots, which were thought to only be used for anchorage (Benzing, 2000; Takahashi *et al.*, 2022). However, a few recent studies showed that the roots of some facultative epiphytic tank bromeliads were involved in nutrient and water absorption (Carvalho *et al.*, 2018; Gomes *et al.*, 2021; Leroy

*et al.*, 2019a; Silva *et al.*, 2018; Vanhoutte *et al.*, 2017). These recent studies investigated mature tank bromeliads, either collected in the field or from commercial nurseries and relied on short-term experimental approaches. Because they used fully grown plants, these studies are unable to identify the real quantitative role of the roots and the consequences for plant growth and performances. In addition, at the seedling stage, some tank-forming bromeliads belonging to the genus *Aechmea* were shown to be totally devoid of leaf absorbing trichomes and thus depended entirely on their roots for water and nutrient absorption (Leroy *et al.*, 2019b, 2017; Petit *et al.*, 2014). In that case, the seedlings performed better when growing on an organic-rich substrate, pointing to a significant nutritional role for the roots (Leroy *et al.*, 2017, 2019b). Despite this increasing evidence for a significant role for roots in bromeliad nutrition, little is known about the root structure and the importance of the nutrient content of the substrate for the development and growth of tank-bromeliads. The lasting effects of substrate fertility on tank-bromeliad development (from the seedling to the mature stage) may have a key ecological impact on their performances.

The two objectives of this study were thus to (i) assess the extent to which the fertility of the substrate affects the growth and overall performances of the tank-bromeliad *Aechmea aquilega* and (ii) to identify specific physiological and morpho-anatomical leaf and root responses to substrate fertility. *Aechmea aquilega* (salisb.) Griseb is a tank-bromeliad which belongs to the subfamily *Bromelioideae* and has crassulacean acid metabolism (CAM) photosynthesis (Crayn *et al.*, 2004). The root system of *A. aquilega* contributes significantly to both nutrient uptake (Leroy *et al.*, 2019a) and seedling performance (Leroy *et al.*, 2019b). Furthermore, in nature, this species can be found growing on terrestrial, lithophytic and epiphytic supports (Leroy *et al.*, 2013). Because leaf absorbing trichomes appeared several weeks after germination of *A. aquilega* (Leroy *et al.*, 2017, 2019b), we hypothesised that the substrate on which the bromeliad grows

since germination plays a key role and lasting effect on trait expression (*i.e.*, growth and overall performance of plants growing on nutrient-rich substrates are better than that of plants growing on nutrient-poor substrates). In addition, because environmental pressures have profound impacts on leaf and root trait values (Wang *et al.*, 2021) and because *A. aquilega* individuals are found in different habitats (Talaga *et al.*, 2017), we hypothesised that leaf and root trait values vary widely across different substrates allowing high capacity for adaptive phenotypic plasticity. To test these hypotheses, we grew *A. aquilega* for fifteen months in different substrate fertilities (a nutrient-poor, a nutrient-rich and a mixed substrate) and we measured leaf and root traits related to nutrient acquisition and resources capture.

## Material and methods

### *Plant material and growth conditions*

In October 2018, seeds from one mother epiphytic plant growing in natural environment were sowed in a greenhouse at the *campus agronomique* in Kourou (French Guiana) in horticultural seedling trays in three different substrate: 100% white sand (nutrient-poor substrate, WS), 100% potting soil (nutrient-rich substrate, P) and an equal volume of white sand and potting soil (v:v 50:50, intermediate substrate, WSP). The sand was collected from a location close to Paracou research station (5°16'26"N, 52°55'26"W), while the potting soil was purchased. Physico-chemical analyses were performed on triplicate samples of each type of substrates to determine the mean particle size and carbon, nitrogen, and phosphorus contents (PAPC, Toulouse). Results showed significant differences of mean particle sizes with WSP being intermediate between WS and P. Substrate

CNP contents were also significantly different between the three treatments but with WSP closer to WS (Fig. S1).

During their development, individual plants were transplanted in their corresponding substrate into 0.5-l, 1-l and then 2-l pots as the plants grew bigger. The pots were placed randomly on horticultural tables in similar environmental conditions. Plants were irrigated with tap water for ten minutes twice a day at 8:00 am and 6:00 pm to maintain soil moisture at field capacity. Greenhouse temperature, light intensity and relative humidity were monitored with HOBO probes (model UA-002-64, HOBO Pendant Temp/Light – 64k and model U23-001, HOBO Pro V2 Temp/RH Data logger, Amanvillers, France). The mean relative humidity was  $82.9 \pm 0.1\%$ , mean air temperature was  $27.8 \pm 0.1 \text{ }^\circ\text{C}$ , and light intensity was  $21016.7 \pm 580.6 \text{ lux}$  (*ca.* 30% of full external irradiance, corresponding to a mean PAR (photosynthetically active radiation) of  $496.5 \pm 34.4 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ).

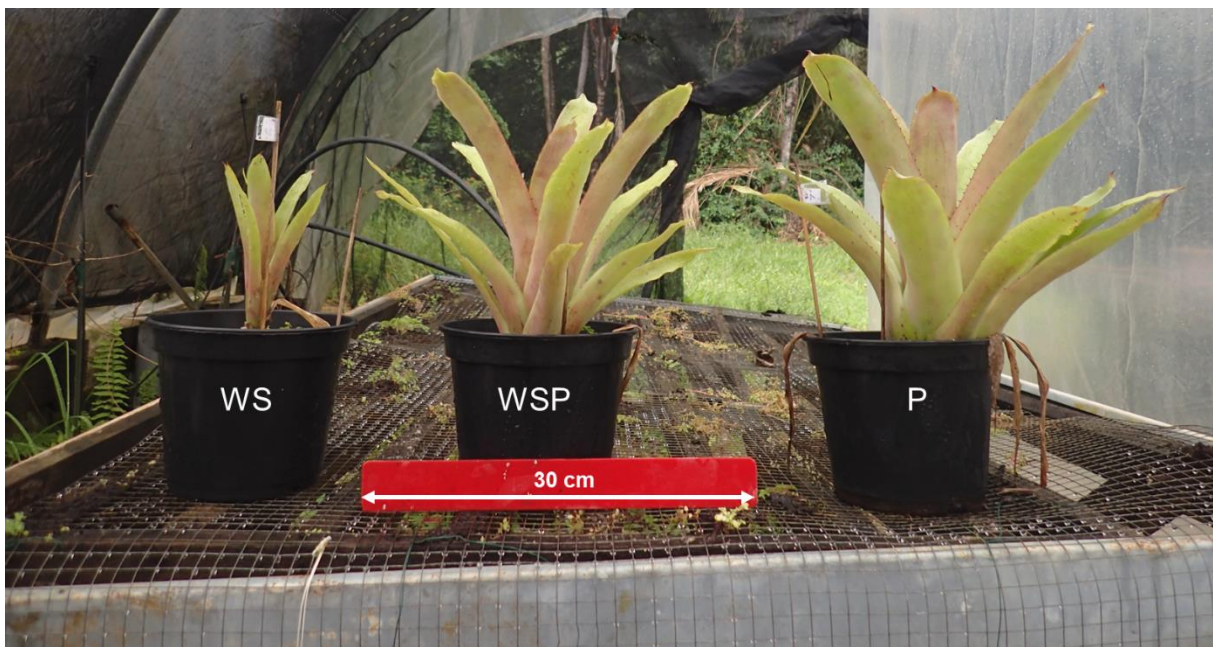


Figure 18: Picture of representative plants of the three treatments from the poorest to the richest from left to right. WS, white sand; WSP, white sand/potting soil; P, potting soil. The red ruler is 30cm long.

### *Sampling*

Measurements and samplings were performed on 15-month-old plants (Fig. 18). Ten plants per treatment were randomly selected and all measurements and sampling were performed on three to four mature leaves, depending on plant size and the amount of plant material required for chemical analysis.

### *Carbon metabolism traits*

Chlorophyll fluorescence— Two chlorophyll fluorescence parameters, the maximum quantum yield of photosystem II ( $F_v/F_m$ ) and the maximum electron transport rate ( $ETR_{max}$ ,  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) were measured with a portable fluorometer (MINI-PAM II, Walz, Effeltrich, Germany). Measurements were made on the adaxial surface of the leaf between 8:00 am and 12:00 am.

To measure  $F_v/F_m$ , a portion of the leaf was dark acclimated for 30 minutes with a dark leaf clip (DLC-8, Walz). The minimal fluorescence  $F_0$  was measured by exposing the leaf to a low intensity light ( $<0.1 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), then a 0.8 second saturating pulse ( $5000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) was produced to assess maximal fluorescence  $F_m$ .  $F_v/F_m$  was calculated as:  $F_v/F_m = \frac{F_m - F_0}{F_m}$

$ETR_{max}$  was calculated using the rapid light curve (Manzi *et al.*, 2022; Rascher *et al.*, 2000) procedure on the same leaf as that used for  $F_v/F_m$ . For quasi-dark acclimation, the leaf was placed in an opaque plastic bag for 30 seconds (Manzi *et al.*, 2022; Rascher *et al.*, 2000) and left in the bag for the rapid light curve. The leaf was then gradually exposed to increasing PAR values in 12 steps from 50 to 3000  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  each lasting for 30 seconds. The ETR was calculated using the fluorometer and the WinControl-3 software (Walz, Effeltrich, Germany) according to the photoinhibition REG1 function of Platt *et al.* (1980).  $ETR_{max}$  was then extracted from the resulting curves as the highest measured ETR.

Chlorophyll content— Leaf chlorophyll a and b content (CHL, mg g<sup>-1</sup>) were estimated with a SPAD-502 chlorophyll meter (KONICA MINOLTA, U.S.A). Eight measurements were taken on the apical and median leaf portion and averaged to obtain the average SPAD value of the leaf. SPAD values were then converted into µg cm<sup>-2</sup> according to Coste et al. (2010):  $Chloro (\mu\text{g cm}^{-2}) = \frac{(117.1 * SPAD)}{(148.84 - SPAD)}$ , then converted into mg g<sup>-1</sup> as follows:  $CHL = (Chloro * 10^{-3}) * (\frac{1}{LMA * 10^{-4}})$

Gas exchange— Net photosynthesis assimilation (A, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) and stomatal conductance (Gs, µmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured on 18 additional plants (N = 6 per treatment) with a CIRAS-3 analyser (PP Systems, Amesbury, U.S.A). All the measurements were made at ten-minute intervals throughout the night from 6:00 pm to 9:00 am the following morning. The CO<sub>2</sub> concentration in the leaf chamber was set to 400 ppm, the temperature to 27 °C, and the air flow to 250 µmol s<sup>-1</sup> while relative humidity and light were left at ambient conditions. To compare treatments, we calculated the maximum net photosynthesis assimilation (A<sub>max</sub>, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) by averaging the five highest consecutive values and the corresponding G<sub>s</sub> values were averaged to obtain the maximum stomatal conductance (G<sub>smax</sub>, µmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). In addition, we quantified integrated net photosynthesis assimilation (A<sub>int</sub>, mmol CO<sub>2</sub> m<sup>-2</sup> over a 15 h period) by integrating the area under the assimilation curve for the whole night (15 h) using the AUC function in the DescTools R package (Andri Signorell et mult. al., 2021) (Fig. S2).

#### *Plant size and growth*

Prior to all measurements, leaves were counted (Nb leaves) and the water volume in the tank (Tank capacity, mL) was measured. The total leaf and root dry mass (see below) were used to assess the root-to-shoot ratio as follows: *Root – to – Shoot ratio* =  $\frac{DM_{root}}{DM_{shoot}}$ . We calculated the relative growth rate (RGR, mg g<sup>-1</sup> month<sup>-1</sup>) as the increase of dry mass of the plants relative to the initial dry mass over 15



months. RGR of the plant biomass was calculated as follow:  $RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1)$ , where  $W_2$  and  $W_1$  are the dry mass of final and initial plant biomass, and  $t_2$  and  $t_1$  are time. 85 mature seeds that were oven dry at 60°C for 48h were the initial plant biomass ( $W_1$ , *i.e.*, 0.987mg) and the total leaf and root dry mass after 15-month growth was the final plant biomass ( $W_2$ ).

### *Leaf structural traits*

The youngest mature leaf was sampled, and its length measured (Leaf length, cm). Eight 10-mm diameter disks were collected with a cork borer from the apical portion of the leaf of plants growing on nutrient-rich and intermediate substrates (P and WSP) while four 8-mm diameter disks were collected from bromeliads growing on nutrient-poor substrate (WS). The thickness of each disk was measured with a micrometric calliper (Digit Outside Micrometre 193-101, Mitutoyo, Japan) and averaged to determine leaf thickness (Leaf thickness, mm). Fresh mass (FM, g) was determined by weighing on an electronic balance (AB 204-S Mettler Toledo, Switzerland), while to obtain the turgid mass (TM, g), the disks were stored in distilled water at 4 °C in the dark for 48 to 72 h. To determine the dry mass (DM, g), the disks were then dried at 60 °C for 72 h. The leaf mass area (LMA,  $g\ m^{-2}$ ) was calculated as  $DM / \text{sum of leaf disk area}$ , while the leaf dry matter content (LDMC,  $g\ g^{-1}$ ) was calculated as  $DM / FM$ .

A 1x4 cm portion of each leaf was collected from both the apical and basal part of the leaf and fixed in FAA (5% formalin, 5% glacial acetic acid, 70% ethanol and 20% water) for two weeks and then stored in 70° ethanol. Stomatal density (Nb stomata  $mm^{-2}$ ) was measured on the adaxial face of the apical portion and trichome density (Nb trichomes  $mm^{-2}$ ) and diameter (mm) were measured on both sides of the basal part of the leaf. Stomata and trichomes were observed from imprints made using transparent nail varnish. The imprints were observed with an inverted microscope (Olympus BX51). Four pictures per imprint were acquired

with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ software (Schneider *et al.*, 2012). The number of stomata and trichomes per mm<sup>2</sup> were recorded for each imprint and averaged. The diameter of 20 trichomes (5 per picture) was measured on ImageJ and averaged to estimate the mean trichome diameter. A trichome area index (TAI, %), giving a proxy for leaf trichome coverage, was calculated as:

$$TAI (\%) = \frac{(trichome\ diameter \times 0.5)^2 \times \pi \times trichome\ density}{leaf\ area}$$

### *Root structural traits*

Once all the measurements of the aerial part were completed, the bromeliads were gently unpotted. The root system was carefully washed with tap water and any remaining soil particles were very carefully removed from each adventitious root. The roots were dried with a paper towel and weighed on an electronic balance to get the fresh mass (FM<sub>root</sub>, g). The root system was scanned using an office scanner (Xerox DocuMate 4700 5.1) and the basic WinRHIZO software (Instrument Regent, Quebec City, QC, Canada). The roots were then placed in distilled water at 4 °C for 48-72 h to get the turgid mass (TM<sub>root</sub>, g) and oven dried at 60 °C for another 48 h to get the dry mass (DM<sub>root</sub>, g). From the scanned images we obtained the following morphological root traits: total root length (TRL, cm), number of adventitious roots (Nb roots), number of root tips (Nb tips), average root diameter (ARD, mm), root volume (cm<sup>3</sup>), and the total surface area of the root system (cm<sup>2</sup>). The root tissue density (RTD, g cm<sup>-3</sup>) was calculated by dividing DM<sub>root</sub> by its volume, specific root length (SRL, m g<sup>-1</sup>) TRL by DM<sub>root</sub> and specific tip root average (STRA, tips g<sup>-1</sup>) Nb tips by DM<sub>root</sub>.

### *Leaf and root chemical traits*

Carbon, nitrogen and phosphorus were quantified in both leaf tips and roots. The leaf tips and roots were dried in an oven at 60 °C for 72 h and ground into fine powder in a mill. About 9–11 mg of leaf and root powder were used to quantify carbon (C, mg g<sub>DM</sub><sup>-1</sup>) and nitrogen (N, mg g<sub>DM</sub><sup>-1</sup>) contents (elemental analyser, Flash 2000 ThermoFisher, NFISO 10694, NF ISO 13878, NF EN 13137). 3–4 mg of leaf and root powder were used to quantify total phosphorus content (P, mg g<sub>DM</sub><sup>-1</sup>; spectrometer, Uvi Light XT5 Secomam, spectrometric method with ammonium molybdate at 880 nm after H<sub>2</sub>SO<sub>4</sub> acid hydrolysis and persulfate oxidation, adapted NFEN 6878).

### *Statistical analysis*

All statistical analyses were carried out in R version 4.0.4 (R Core Team, 2021). Graphs were produced using the R package ggplot2 (Wickham, 2016). In order to estimate multivariate differences in *A. aquilega* leaf and root trait coordination, we used standardised multiple factor analysis (MFA, Escofier & Pages, 1990). The MFA method enables examination of common structures in datasets with many variables that can be separated into different groups of variables (*i.e.*, leaf and root traits). MFA was performed with the Factominer package (Lê *et al.*, 2008) on two set of variables: leaf traits (LMA, LDMC, Stomatal density, TAI, C, N, P, Chl, F<sub>v</sub>/F<sub>m</sub>, ETR<sub>max</sub>) and root traits (STRA, SRL, ARD, RTD, C, N, P). We then plotted all individuals and variables on the two first MFA dimensions and showed the 95% confidence ellipses for all three treatments. To test whether bromeliad traits were significantly affected by the fertility of the substrate, we performed permutational multivariate analysis of variance (perMANOVA) with the adonis function of the vegan package (Oksanen *et al.*, 2022) using Euclidean distances and 10,000 permutations. A post-hoc test was then conducted with the pairwise.adonis wrapper function in the

pairwiseAdonis package (Martinez Arbizu, 2022) with p-values adjusted with Holm's method.

In addition, we examined differences in all traits in the three treatments with the Kruskal-Wallis rank test, which is a non-parametric alternative to one-way analysis of variance (ANOVA) when the assumptions of homogeneity of variance and normality are not met. The Kruskal-Wallis test was performed with the `kruskal.test` function in base R. When the Kruskal-Wallis associated p-value was significant ( $P < 0.05$ ) the post-hoc pairwise Wilcoxon test was used to identify the effects of the treatments on each trait. P-values were corrected for multiple comparisons using Holm's procedure. Finally, we calculated the coefficients of variation (CV, %) of each trait among treatments as  $CV = \frac{SD}{mean} \times 100$  to describe the extent of phenotypic plasticity of each trait. A summary table with mean  $\pm$  SD, CV and statistics values and significance is available in the supplementary (Tab. S1)

## Results

### *Effects of substrate fertility on A. aquilega size and growth*

Plants growing on nutrient-rich substrate (P) had approximately twice as many leaves, three times more adventitious roots, 16-fold higher total biomass, 35 times greater tank water capacity, *ca.* 1.5-fold higher RGR, a five times lower root-to-shoot ratio, longer and thicker leaves, and total root length was up to six times greater than that of plants growing on nutrient-poor substrate (WS; Fig. 19). Plants grown on the intermediate substrate (WSP) had either intermediate numbers of roots, total dry mass, tank capacity, growth rate, leaf thickness and total root length compared to plants grown on the two other substrates (Fig. 19 B-E, H, I) or the number of leaves, root-to-shoot ratio and leaf length did not significantly differ from those of plants grown on the nutrient-rich substrate (Fig. 19 A, F, G). Number

of roots, total dry mass, tank capacity, root-to-shoot ratio and root length were the traits with the largest coefficient of variation (Fig. 19 B, C, D, F, I).

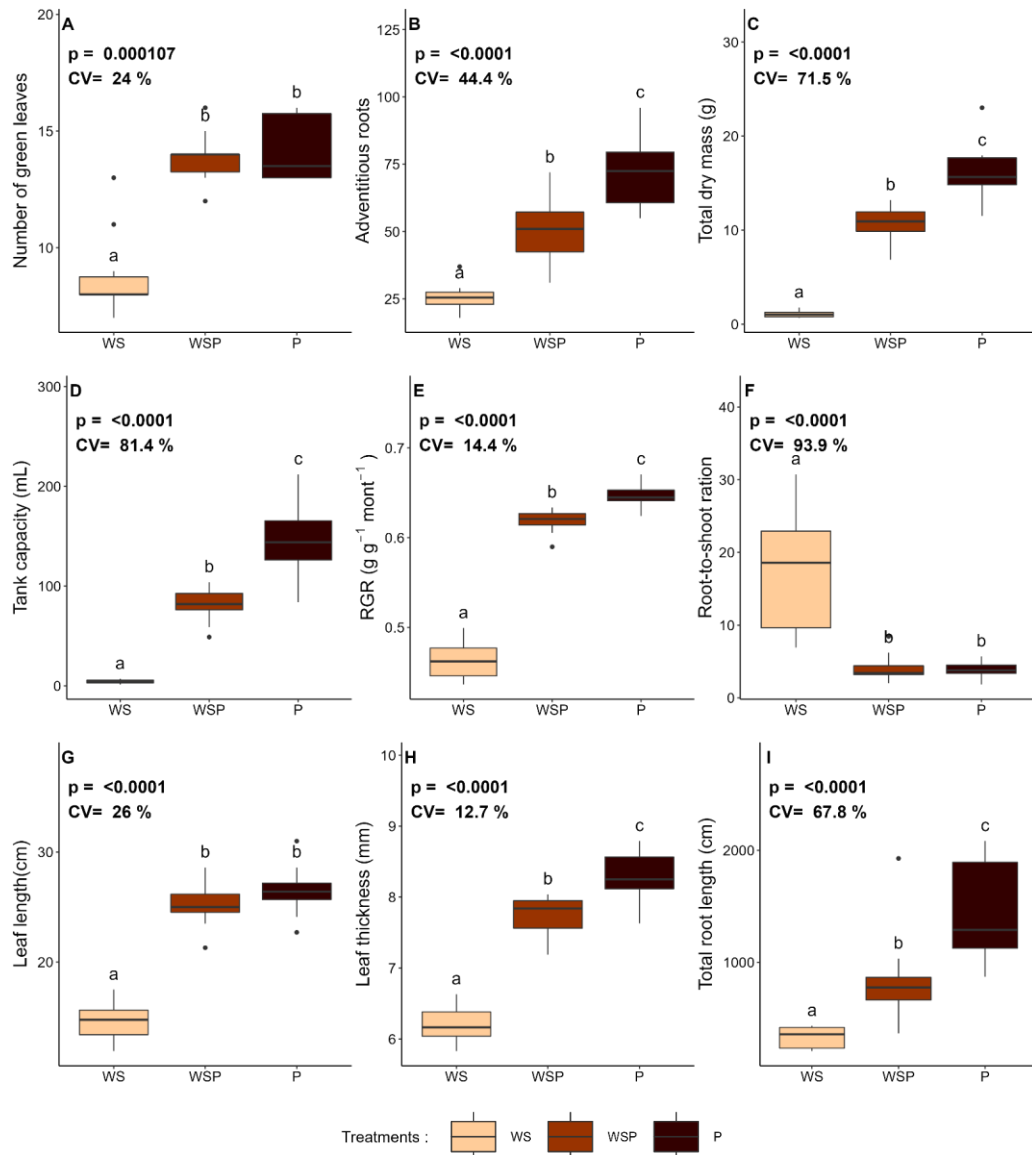


Figure 19: Effects of substrate fertility on (A) number of green leaves, (B) number of adventitious roots, (C) total dry mass (g), (D) tank water capacity (mL), (E) RGR ( $\text{mg g}^{-1} \text{month}^{-1}$ ), (F) Root-to-shoot ratio, (G) leaf length (cm), (H) leaf thickness (mm), and (I) total root length (cm). Different letters indicate significant differences between treatments based on pairwise Wilcoxon tests ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ). CV, coefficients of variation of each trait among treatment; WS, white sand; WSP, white sand/potting soil; P, potting soil.

### *Trait correlations and overall effect of substrate fertility on A. aquilega*

The two first dimensions of the MFA explained 51.9% of the variability of the data (PC1 explained 31.2% and PC2 explained 20.7%). The MFA showed two orthogonal

groups of traits (Fig. 20A). Most of the traits were correlated with PC1 while RTD, STRA and SRL were correlated with PC2. Multivariate analysis of trait correlations showed that root structural traits were orthogonal to leaf structural and chemical traits. Projection of all individuals separated the three treatments along PC1 whereas no segregation appeared on PC2 (Fig. 20B). PerMANOVA revealed a significant effect of the substrate on the trait values ( $F = 7.2195$ ,  $Df = 2$ ,  $p < 0.0001$ ). Specifically, all individuals grown on each of the three substrates differed significantly from one another (Pairwise.adonis, Holm adjusted  $P < 0.01$  in all cases).

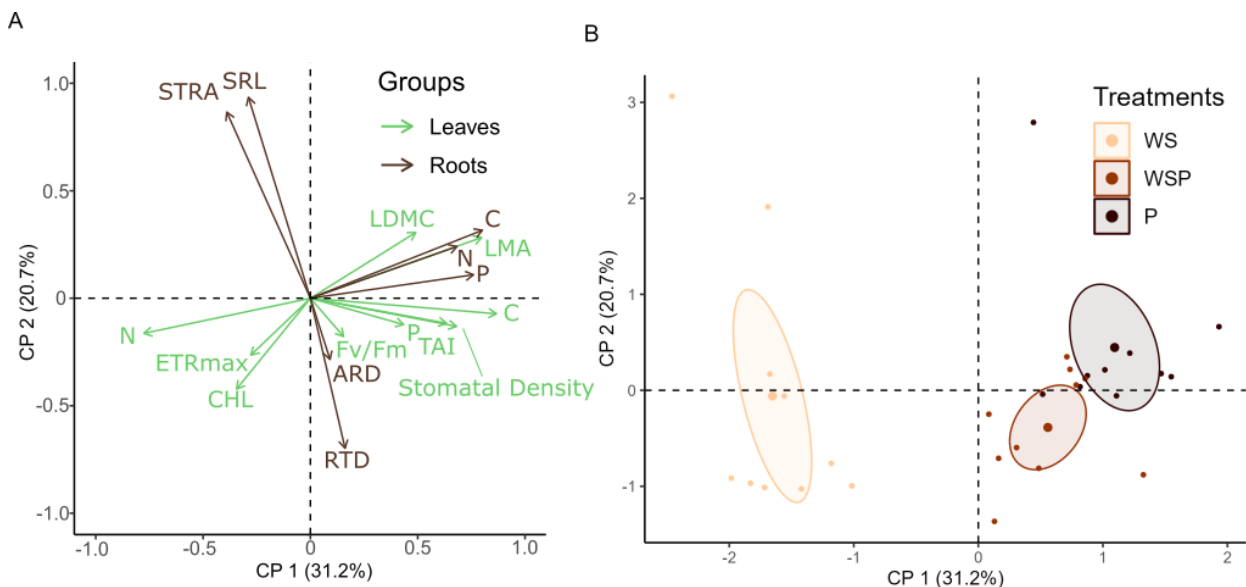


Figure 20: (A) Correlation circle of variables to the first two dimensions of the MFA. Variables are coloured by groups, green for leaves and brown for roots. (B) Projections of individuals in the first two PCs plan. Individuals are coloured by treatments. Small dots are individuals' projections, big dots the centroid for each treatment. The 95% confidence ellipse is shown for each treatment. Substrates are WS, white sand; WSP, white sand/potting soil; P, potting soil. Leaf traits are C, carbon content ( $\text{mg g}^{-1}$ ); CHL, Chlorophyll content ( $\text{mg g}^{-1}$ );  $\text{ETR}_{\text{max}}$ , maximum electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $F_v/F_m$ , PSII maximum quantum efficiency; LDMC, leaf dry mass content ( $\text{g}_{\text{DM}} \text{g}_{\text{FM}}^{-1}$ ); LMA, leaf mass area ( $\text{g m}^{-2}$ ); N, nitrogen content ( $\text{mg g}^{-1}$ ); P, phosphorus content ( $\text{mg g}^{-1}$ ); stomatal density ( $\text{Nb mm}^{-2}$ ), and TAI, trichome area index (%). Root traits are ARD, average root diameter (mm); C, carbon content ( $\text{mg g}^{-1}$ ); N, nitrogen content ( $\text{mg g}^{-1}$ ); P, phosphorus content ( $\text{mg g}^{-1}$ ); RTD, root tissue density ( $\text{g m}^{-3}$ ); SRL, specific root length ( $\text{m g}^{-1}$ ), and STRA, specific tip root average (root tips  $\text{g}^{-1}$ ).

### *Effects of substrate fertility on plant functioning*

Substrate fertility had no significant effect on  $G_{s_{\text{max}}}$  (Fig. 21A). Net photosynthesis assimilation ( $A_{\text{max}}$  and  $A_{\text{int}}$ ) differed significantly between the three substrates with higher values for plants growing on nutrient-rich (P) and intermediate (WSP)

substrates than for plants growing on nutrient-poor (WS) substrate (but with marginal non-significant differences for  $A_{max}$ , Fig. 21B-C). There was no significant effect of treatment on chlorophyll fluorescence as  $F_v/F_m$  and  $ETR_{max}$  values were similar (Fig. 21D-E). The latter was very stable with a CV of 2.74%. Chlorophyll content was similar in the three treatments (Fig. 21F).

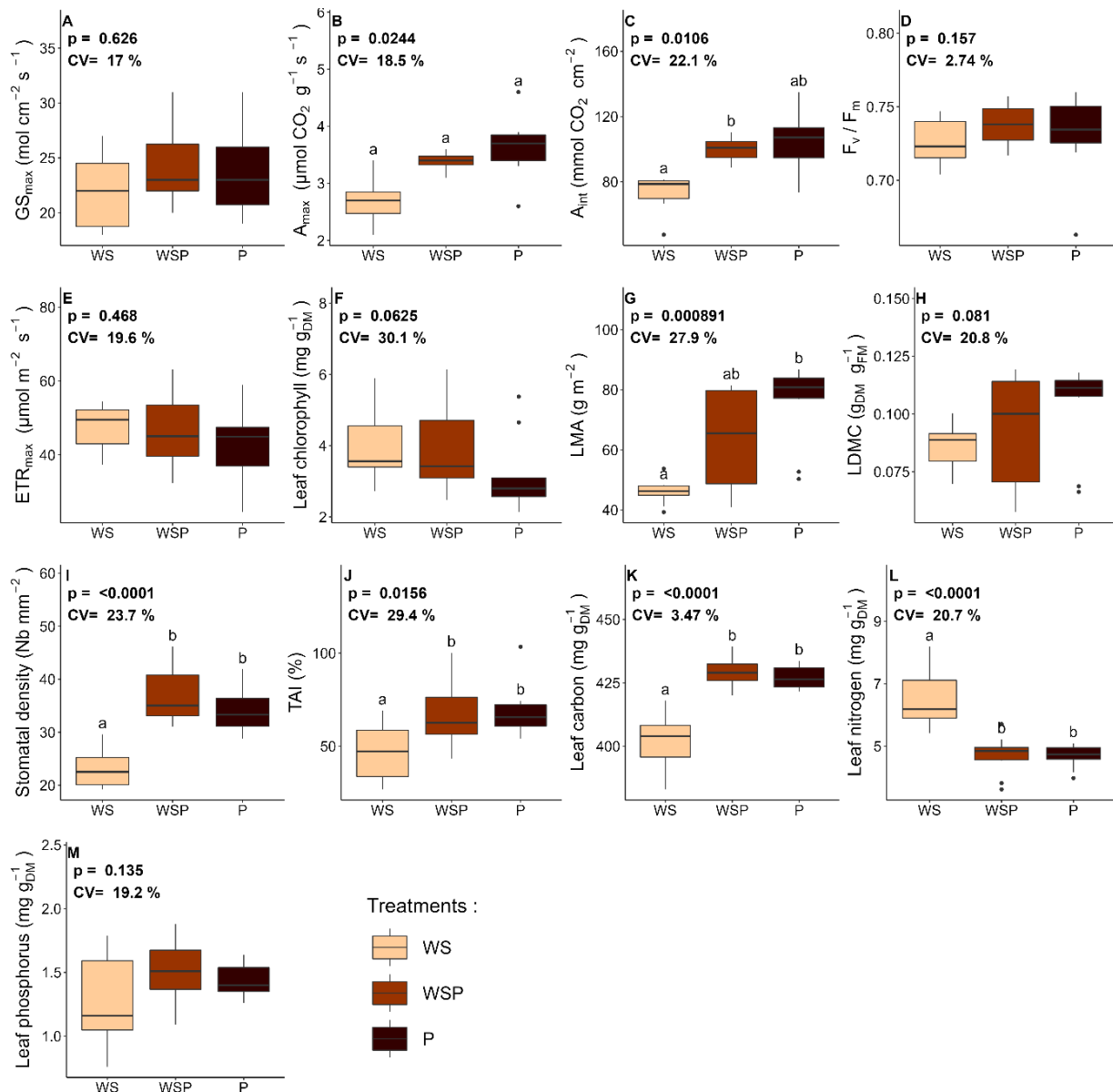


Figure 21: Effects of substrate fertility on (A) maximal stomatal conductance ( $GS_{max}$   $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), (B) maximal  $\text{CO}_2$  assimilation ( $A_{max}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), (C) integrated overnight  $\text{CO}_2$  assimilation ( $A_{int}$ ,  $\text{mol m}^{-2}$ ), (D)  $F_v/F_m$ , (E)  $ETR_{max}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), (F) leaf chlorophyll content ( $\mu\text{g g}^{-1}$ ), (G) leaf mass area (LMA,  $\text{g} \cdot \text{m}^{-2}$ ), (H) leaf dry mass content (LDMC,  $\text{g}_{DM} \cdot \text{g}_{FM}^{-1}$ ), (I) stomatal density ( $\text{Nb mm}^{-2}$ ), (J) trichomes area index (TAI, %), (K) leaf carbon content ( $\text{mg g}^{-1}_{DM}$ ), (L) leaf N content ( $\text{mg g}^{-1}_{DM}$ ), and (M) leaf P content ( $\text{mg g}^{-1}_{DM}$ ). Different letters indicate significant differences between treatments based on pairwise Wilcoxon tests ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ). The Kruskal-Wallis p-value and coefficient of variation (CV, %) are indicated for each variable within the plot. WS, white sand; WSP, white sand/potting soil; P, potting soil.

### *Effects of substrate fertility on leaf structural and chemical traits*

Substrate fertility had significant effects on LMA but not on LDMC (Fig. 21G-C). Plants growing on nutrient-rich substrate (P) had significantly higher LMA than plants growing on nutrient-poor substrate (WS), whereas there was no significant difference between the plants growing on intermediate substrate (WSP) and those growing on the two other substrates (Fig. 21G). Stomatal density and TAI were *ca.* 1.5 and 0.5 times higher in plants growing on nutrient-rich substrate than in plants growing on nutrient-poor substrate, respectively (Fig. 21I-J). Higher TAI was mainly due to change in trichomes density rather than size (Fig. S3). Plants growing on the intermediate substrate had similar stomatal density and significantly lower trichome leaf coverage than plants growing on rich substrate. Finally, substrate fertility had significant effects on leaf C and N contents but not on the leaf P contents (Fig. 21K-M). The leaves of plants growing on the nutrient-rich substrate were characterised by higher leaf C but lower leaf N contents than the leaves of plants growing on the nutrient-poor substrate (Fig. 21K and L, respectively). The C and N contents of the leaves of plants growing on the intermediate substrate were similar to those of the leaves of plants growing on the nutrient-rich substrate. Overall, all the leaf traits displayed moderate variation (12.7%-30.1%), except for leaf C content, which had a particularly low coefficient of variation (3.47%).

### *Effects of substrate fertility on root structural and chemical traits*

Substrate fertility did not affect STRA, SRL or RTD (Fig. 22A, B and D respectively). Substrate fertility had a significant effect on the average root diameter with plants growing on the intermediate substrate had significantly larger roots than plants growing on the nutrient-rich substrate (Fig. 22C). Root C, N, and P contents were significantly higher in plants growing on the nutrient-rich substrate than in plants growing on the nutrient-poor substrate (Fig. 22E-G). Plants growing on the intermediate substrate had intermediate values compared to the two other



substrates. STRA and SRL had high coefficients of variation (around 50%) while other root structural (RTD, and ARD) and chemical traits had lower CVs.

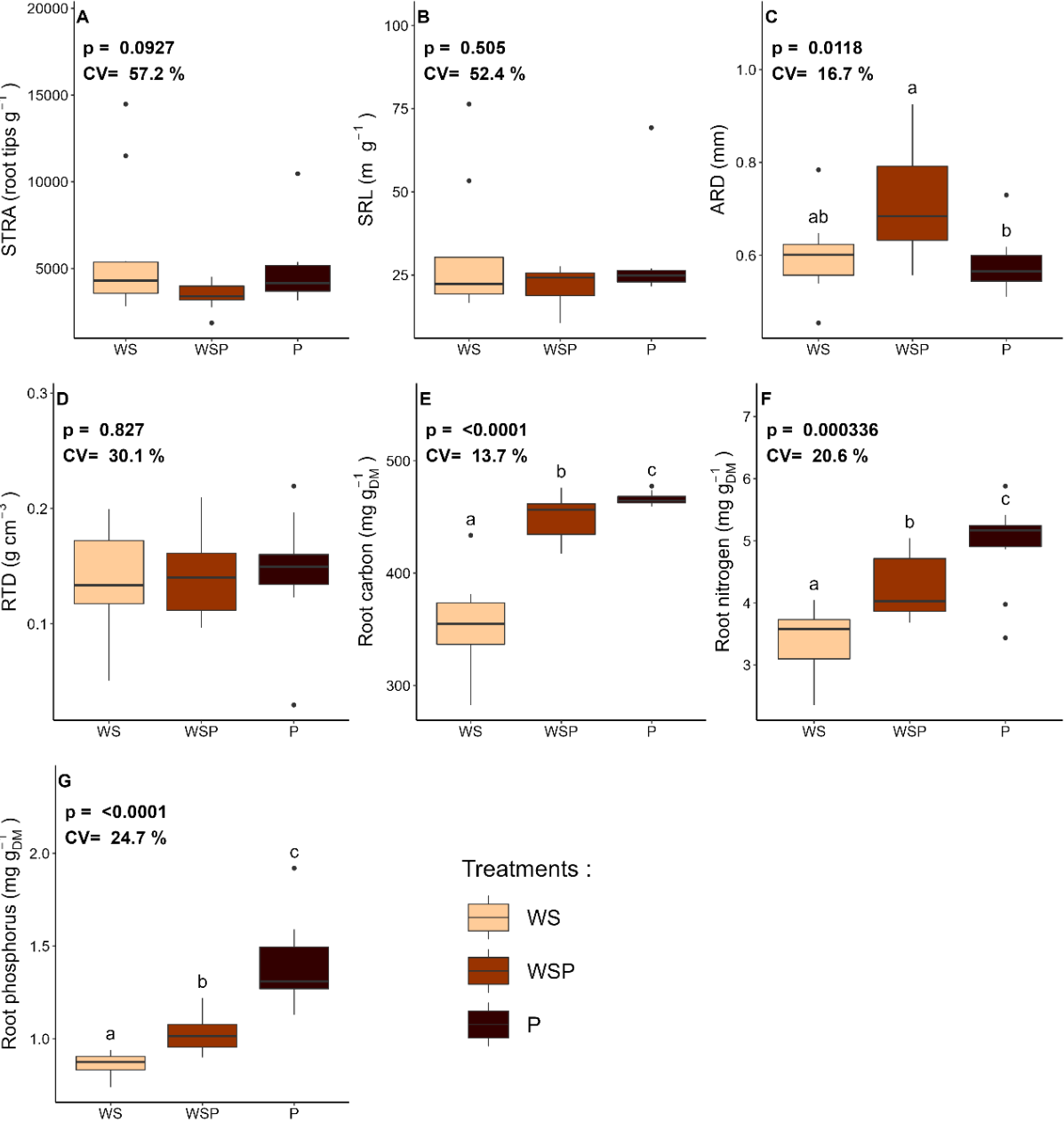


Figure 22: Effects of substrate fertility on (A) specific tip root average (STRA, tips g<sup>-1</sup>), (B) specific root length (SRL, cm g<sup>-1</sup>), (C) average root diameter (ARD, mm), (D) root tissue density (RTD, g cm<sup>-3</sup>), (E) root carbon, (F) root nitrogen, and (G) root phosphorus contents (mg g<sup>-1</sup>). Different letters indicate significant differences between treatments based on pairwise Wilcoxon test ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ). The Kruskal-Wallis p-value and coefficient of variation (CV, %) are indicated for each variable within the plot. WS, white sand; WSP, white sand/potting soil; P, potting soil.

## Discussion

Substrate fertility had a strong effect on the size and growth of *A. aquilega*. Plants growing on the nutrient-poor substrate (white sand, WS) had a 50% lower RGR and were 16 times smaller than plants growing on the nutrient-rich substrate (potting soil, P) had fewer leaves and roots, less total dry mass, smaller leaves, and shorter total root length. This overall smaller size reduced the capacity of the tank. Biomass partitioning was also strongly affected by substrate fertility. The root-to-shoot ratio was *ca.* 4 times higher in *A. aquilega* growing on the nutrient-poor substrate, indicating a higher proportion of biomass in the roots, thus enhancing foraging. Such biomass partitioning is a well-known mechanism by which plants of a wide range of growth forms cope in nutrient-poor environments (*e.g.*, Hermans et al. 2006; Mašková and Herben 2018; Sainju et al. 2017). In addition to differences between rich and poor substrates, plants growing on the nutrient-rich substrate (P) also out-performed the plants growing on the intermediary substrate (WSP) with larger tank capacity, higher total biomass, higher RGR, and higher and longer roots. The fertility of the substrate on which *A. aquilega* was grown from seed to 15 months strongly affected both the morphology of the rosette and biomass allocation. Such phenotypic plasticity is relatively frequent in bromeliads, which grow in a broad range of light and water regimes, and different nutrient availability conditions (de Freitas *et al.*, 2003; González *et al.*, 2011; Scarano *et al.*, 2002; Zotz & Asshoff, 2010).

Most of the structural and chemical leaf traits were related to substrate fertility. Plants growing on the nutrient-rich substrate were characterised by higher LMA, thicker leaves, higher stomatal density and trichome coverage, along with higher leaf C and Chl and lower leaf N contents. Concerning LMA, our results disagree with those generally reported in the literature. Indeed, plants growing in nutrient-poor habitats often display higher LMA (Givnish 1979; Poorter et al. 2009). Givnish (1979) suggested that change in LMA was related to change in leaf

thickness while Poorter et al. (2009) reported that most nutrient-driven change in LMA are due to alteration in leaf tissue density. Moreover, as LDMC is closely related to leaf tissue density (Shipley & Vu, 2002), a variation in LMA with no variation in LDMC is likely to be the result of variations in leaf thickness (Vile *et al.*, 2005). Hence, in our study, higher LMA in the nutrient-rich substrate is likely to result from leaf thickening because we did not find significant variation in LDMC. As leaves of bromeliads show strong allometric relationships between plant size and leaf thickness (Zotz *et al.*, 2004; Meisner *et al.*, 2013), we assume that the increase of LMA with substrate fertility is mainly the consequence of the strong treatment effect on plant size.

In general, epiphytes have lower N and P content in their tissues than ground-rooted herbaceous plants and trees (Hietz *et al.*, 2021; Reich & Oleksyn, 2004). Epiphytes are considered slow growing plants whose traits are associated with “slow” species, because of their low foliar nutrient concentrations and long leaf lifespan (Zotz, 2016; Hietz *et al.*, 2021). In our study, *A. aquilega* had overall low leaf N values compared to most of other plants, which is consistent with the literature (Hietz *et al.*, 2021; Wagner *et al.*, 2021; Wanek & Zotz, 2011). We showed that plants growing on the nutrient-poor substrate had the highest leaf N content, but similar leaf P contents as compared to plants growing on the nutrient-rich substrate. These findings are highly surprising as in nutrient-poor habitats, species are generally known to have low leaf nutrient concentrations (Lambers & Poorter, 1992; Wright *et al.*, 2004) making the high leaf nitrogen concentration of nutrient-poor substrate plants confusing. Given that bromeliads from the nutrient-poor substrate had similar chlorophyll content as compared to the two other treatments, it is likely that nitrogen was allocated to other parts of the photosynthetic apparatus (e.g., RuBisCo), or to defence compounds and other non-photosynthetic processes (Evans, 1989; Takashima *et al.*, 2004). Additionally, we found similar  $ETR_{max}$  and  $F_v/F_m$  across treatments. This result suggests that the

substrate fertility did not affect photosystem functioning in *A. aquilega*. Nutrient deficiency has been shown to strongly influence the structure and functions of the photosynthetic apparatus with some damage to PSII, resulting in a reduction in  $F_v/F_m$  and ETR (Kalaji *et al.*, 2014, 2018; Wu *et al.*, 2008). Conversely, in other studies, N supply was reported to have no effect on photochemical efficiency (Cruz *et al.*, 2003; Shrestha *et al.*, 2012). These divergent results could be due to several factors (*e.g.*, plant form and species, age of the plant, growing conditions, N recycling mechanisms) but Gonçalves *et al.* (2020a) showed that water and nutrient deprived *Guzmania monostachia* were able to maintain  $F_v/F_m$  similar to those of well-watered and nourished plants confirming that, at least at short terms, bromeliads can cope with low resource conditions via physiological adjustments. Nevertheless, concerning carbon assimilation, we found higher net photosynthesis assimilation ( $A_{int}$ ) in *A. aquilega* growing on the nutrient-rich substrate than in plants growing on the nutrient-poor substrate. Our results show that the light harvesting (photosystem and electron transport) and CO<sub>2</sub> fixation (Calvin cycle and Rubisco) processes of photosynthesis responded differently to substrate fertility. The light harvesting process was not sensitive to our treatments while it did affect CO<sub>2</sub> fixation. Such contrasting responses may result from a trade-off in N allocation to different components (*e.g.*, thylakoids which are important for the electron transport capacity or soluble proteins which are important for the Calvin cycle, Evans, 1989). Additionally, bromeliads from nutrient-rich substrate with more and larger leaves may likely had higher overall carbon assimilation which is in line with the *ca.* 16-fold size biomass differences observed between rich and poor substrate.

Concerning P content, *A. aquilega* had a higher concentration than that usually reported for other bromeliad species (*e.g.*, Wanek and Zotz 2011; González *et al.* 2011). In addition, root P content was linked to substrate fertility with higher P storage in plants growing on nutrient-rich substrate. The increase in root but not

leaf P contents as the substrate got richer can result from a preferential allocation of P to leaves rather than roots in nutrient-poor bromeliads. However, the fact that leaf P did not increase with substrate richness is surprising, especially considering that leaf N decreased as well. Indeed, as the substrate got richer the leaf N:P ratio dropped from 5.39 (nutrient-poor substrate) to 3.16/3.29 (intermediate and nutrient-rich substrates, respectively, Fig. S4). This points to a severe N limitation (Güsewell, 2004; Zotz & Asshoff, 2010; Wanek & Zotz, 2011) and makes even more confusing the low leaf N content. However, leaf and root P, N, and N:P ratio do not always respond to substrate fertility and can be affected by plant functional groups (Hong *et al.*, 2015), size and/or leaf age (Schreeg *et al.*, 2014), RGR or ammonium *versus* nitrate availability making interpretation less straightforward (Güsewell, 2004). Further studies with bromeliads and epiphytes in general are needed to disentangle the respective contribution of N and P to the efficiency of photosynthesis and plant growth as well as to identify their specific critical threshold if we are to understand our results and the nature of nutrient-growth relationship in epiphytes.

Quantitative data on structural and chemical root traits in bromeliads and in epiphytes in general are only very rarely reported in the literature. In our study, we found that chemical root traits were linked to the fertility of the substrate, whereas this was not the case for structural root traits. Plants growing on the nutrient-rich substrate had higher C, N, and P contents in their roots than plants growing on the nutrient-poor substrate. In ground-rooted species, specific tip root average, root length and root tissue density are expected to be linked with soil resources and properties (Freschet *et al.*, 2021). The second axis of the MFA showed clear co-variation between root structural traits (SRL, STRA, ARD and, RTD) but failed to distinguish between our three treatments, which was confirmed by the non-significance of the pairwise comparisons. The marked variability explained by this axis (21%) seems to result from soil properties or other variables

that were not accounted for in our study rather than substrate fertility. The negative relationships between SRL and ARD was observed in another bromeliad species (*Lutheria splendens*, Leroy *et al.*, 2022) and such negative relationships have been widely observed in the context of interspecific variations of mature plants (Bergmann *et al.*, 2020; Kong *et al.*, 2014; Roumet *et al.*, 2016; Spitzer *et al.*, 2021; Wagner *et al.*, 2021). While not different between our treatments, SRL and RTD were negatively correlated in our study as shown by the second axis of the MFA. This results were in contrast to what has been reported in vascular epiphytes (Wagner *et al.*, 2021) and in *L. splendens* regarding ontogenetic effects (Leroy *et al.*, 2022). SRL and RTD co-variation across species is not clear, as some studies found a negative correlation (Bergmann *et al.*, 2020; Garbowski *et al.*, 2021), some found a positive correlation (Holdaway *et al.*, 2011; Kong *et al.*, 2014) while still others found no correlation (Kramer-Walter *et al.*, 2016; Valverde-Barrantes & Blackwood, 2016). Such variability in root trait combinations may be due to trade-offs between different root functions. Indeed, roots fulfil a wide range of functions comprising physical anchoring, resource storage, and resource capture via the interface with soil bacterial and fungal symbionts (Freschet *et al.*, 2021). Anchorage is likely to be a dominant function in the roots of epiphytes (Wagner *et al.*, 2021) which could mask the structural root trait response to substrate fertility. In addition, in our study, *A. aquilega* were potted in different substrates and this might have affected the structural root traits even if the root systems were not constrained by the pot (pers. obs.).

While the role of the roots in nutrient absorption has already been demonstrated for some bromeliad species (e.g., Gomes *et al.* 2021; Leroy *et al.* 2019a), this study is the first to show that the fertility of the substrate on which the bromeliad *A. aquilega* grows following germination has a strong and lasting effect on overall plant morphology and performance. Because of the lack of leaf-absorbing trichomes and of a water tank, *A. aquilega* seedlings can only rely on the

root system for nutrient absorption (Leroy *et al.*, 2017, 2019b). In a close congeneric species (*A. mertensii*), leaves became larger to form wells and the density of leaf-absorbing trichomes increased as the plant grew (Petit *et al.*, 2014). In *A. aquilega*, leaf-absorbing trichomes and wells appeared at an age of 4-6 months (pers. obs.). With broader leaves, higher absorbing trichome leaf coverage at the base of the lamina, and a 30-fold higher tank capacity, *A. aquilega* plants growing on a nutrient-rich substrate may benefit from higher nutrient supply compared to plants growing on a nutrient-poor substrate. We have shown that the fertility of the substrate during the germination and establishment stages in *A. aquilega* is of the utmost importance as it also affects performances at later ontogenetic stages. Given the high diversity of substrate (soil, bark or rocks) used by wild *A. aquilega*, this could have important ecological repercussions. Tank bromeliads provide a habitat for aquatic, semi-aquatic and terrestrial organisms and contribute to many ecosystem services such as maintenance of biodiversity, nutrient cycling, and the provisioning of food and water (Ladino *et al.*, 2019). Large plants with a high tank capacity were characterised by a greater diversity and abundance of aquatic organisms that echo the bromeliad nutrition (Leroy *et al.*, 2016) and a higher external water storage to resist drought stress (Males, 2016). Hence, when growing in nutrient-rich substrate tank bromeliads may sustain higher biodiversity and resistance to climatic change, resulting in ecological advantages.

Overall, we found that 70% of measured traits linked to plant performance responded significantly to the experimental treatments, indicating that *A. aquilega* response to nutrient availability is a combination of physiological processes and leaf and root structure and chemistry. The strong effect of substrate fertility on overall plant size and performance confirmed the importance of the root system in the establishment and growth of *A. aquilega*. This study and others showed that the roots of some bromeliad species are able to absorb nutrients in addition to

the leaf trichomes. In our study, we further demonstrate that the nutrients absorbed by the roots determine the growth, size and performance of the plants which likely reflects bromeliad ecology. It thus appears that we need to reconsider the functional role of roots in bromeliad nutrition and the existence of different degrees of dependence on the substrate in the bromeliad family with probably significant variations both between and within species along ontogeny. Given the extraordinary diversity of bromeliads, future studies need to cover a much wider range of species, spanning both phylogenetic and ecological diversity.

## **Supplementary Information**

Fig. S1 Treatment granulometry and CNP content.

Fig. S2 Overnight CO<sub>2</sub> assimilation and stomatal conductance curves.

Tab. S1 Summary table for all variables (mean  $\pm$  sd, CV and statistics)

Fig. S3 Effect of substrate fertility on trichome density and diameter.

Fig. S4 Effect of substrate fertility on N:P ratio.

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# Chapitre II

*Light intensity mediates phenotypic plasticity and leaf trait regionalisation in a tank bromeliad*

Cette étude porte sur l'effet de l'exposition à la lumière sur le phénotype d'*Aechmea aquilega* et la régionalisation apex-base de ses feuilles. Ces travaux tirent profit d'une population d'*A. aquilega* poussant naturellement le long d'un gradient d'intensité lumineuse en lisière de forêt.

Les résultats montrent une forte influence de l'intensité lumineuse reçue par la plante sur sa morphologie et ses traits foliaires. Elle est notamment la première à mettre en évidence un effet de la lumière sur la différenciation entre les valeurs de traits mesurés à l'apex et à la base des feuilles avec une réduction de la spécialisation de ces régions à mesure que l'intensité lumineuse augmente.

Les données présentées ici sont issues d'un échantillonnage réalisée en Octobre 2021 avec l'aide d'un stagiaire de Master 2 (Matthieu Gallant-Canguilhem) avec qui nous avons réalisé l'ensemble des mesures des traits. Les analyses chimiques ont été réalisées par Frédéric Julien à Toulouse. J'ai ensuite pris en charge le traitement des données et la rédaction de l'article avec la collaboration de Régis Céréghinon, Céline Leroy et Matthieu Gallant Canguilhem.



# **Light intensity mediates phenotypic plasticity and leaf trait regionalisation in a tank bromeliad**

Tristan Lafont Rapnouil<sup>1,2,\*</sup> <http://orcid.org/0000-0002-5411-3479>, Matthieu Gallant Canguilhem<sup>1,2</sup> <https://orcid.org/0009-0004-8137-4342>, Frédéric Julien<sup>3</sup> <http://orcid.org/0000-0001-5411-1169>, Régis Céréghino<sup>3</sup> <http://orcid.org/0000-0003-3981-3159>, Céline Leroy<sup>1,2</sup> <http://orcid.org/0000-0003-4859-8040>

<sup>1</sup>AMAP, Univ. Montpellier, CIRAD, CNRS, INRAE, IRD, France

<sup>2</sup>EcoFoG, AgroParisTech, CIRAD, CNRS, INRAE, Université des Antilles, Université de Guyane, Campus agricole, Kourou, France

<sup>3</sup>Laboratoire Écologie Fonctionnelle et Environnement, Université Paul Sabatier Toulouse 3, CNRS, Toulouse, France

**\*Corresponding author:** Tristan Lafont Rapnouil,  
[tristan.lafontrapnouil@gmail.com](mailto:tristan.lafontrapnouil@gmail.com)

## Abstract

- *Purpose* Phenotypic plasticity allows plants to cope with environmental variability. Plastic responses to the environment have mostly been investigated at the level of individuals (plants) but can also occur within leaves. Yet, the latter has been underexplored, as leaves are often treated as functional units with no spatial structure. We investigated the effect of a strong light gradient on plant and leaf traits and examined whether different portions of a leaf show similar or differential responses to light intensity.
- *Methods* We measured variation in 27 morpho-anatomical and physiological traits at the rosette and leaf portions (*i.e.*, base and apex) of the tank-bromeliad *Aechmea aquilega* (Bromeliaceae), when naturally exposed to a marked gradient of light intensity.
- *Results* The light intensity received by *A. aquilega* had a strong effect on the structural, biochemical, and physiological traits of the entire rosette. Plants exposed to high-light intensity were smaller and had wider, shorter, more rigid, and more vertical leaves. They also had lower photosynthetic performance and nutrient levels. We found significant differences between the apex and basal portions of the leaf under low light conditions, and the differences declined or disappeared for most of the traits as light intensity increased (*i.e.*, leaf thickness, adaxial trichome density, abaxial and adaxial trichome surface, and vascular bundle surface and density).
- *Conclusion* Our results reveal a strong phenotypic plasticity in *A. aquilega*, particularly in the form of a steep functional gradient within the leaf under low-light conditions. Under high-light conditions, trait values were relatively uniform along the leaf. This study sheds interesting new light on the functional complexity of tank-bromeliad leaves, and on the role of environmental conditions on leaf trait regionalisation.

**Key words** (6-12): *Aechmea aquilega*, functional traits, leaf anatomy, leaf morphology, leaf regionalisation, light intensity gradient, phenotypic plasticity, tank bromeliad

## Introduction

Phenotypic plasticity, *sensu stricto*, is the ability of a genotype to display different phenotypes according to the environment while the responses of different species and populations in their ecological context is referred to as phenotypic plasticity *sensu lato* (Valladares *et al.*, 2006). Sessile organisms, such as plants, cannot escape adverse environmental conditions and thus heavily rely on phenotypic plasticity *sensu lato* to cope with variability in light, temperature, water and nutrient availability, wind, or pollutant exposure (*e.g.*, Audet and Charest 2008; Bossdorf and Pigliucci 2009; Fromm 2019). Among these factors, light has a major influence on plant life because it provides energy for photosynthesis and controls individual growth and development. The heterogeneous light environment in many ecosystems requires the acclimation to different light regimes, achieved through adjustments at both the whole-plant and the leaf level (Givnish, 1988). However, phenotypic plasticity is not necessarily a whole-plant response (De Kroon *et al.*, 2005), but rather a property of individual meristems, leaves, branches, and roots, triggered by local environmental conditions.

Leaf traits display extraordinary plasticity in response to varying environmental conditions (Sultan, 2000; Gratani, 2014). Hence, leaves play important roles in plant life strategies (Wright *et al.*, 2004; Shi *et al.*, 2020) especially because they are directly involved in photosynthetic processes (Mathur *et al.*, 2018). This has led to numerous studies on leaf traits response to light and researchers distinguished sun-exposed from shaded species based on well documented leaf traits in a wide range of plant taxa (*e.g.*, Niinemets and Valladares 2004; Rozendaal *et al.* 2006). This holds at the individual scale with the modification of morpho-anatomical and physiological leaf traits in response to the light gradient formed as light penetrates the foliage. According to their position and exposure to light, leaves of the same plant can display shade or sun associated traits (Niinemets, 2010; Niinemets *et al.*, 2015). Sun and shade leaves differ predictably

in several functional traits (Popma & Bongers, 1988). Typically, sun leaves are smaller and thicker, have higher leaf dry mass per surface area (LMA), have a thicker palisade parenchyma, epidermis, and cuticle, and may also have more stomata density as compared to shade leaves from the same individual (Givnish, 1988; Terashima *et al.*, 2001; Dörken & Lepetit, 2018). Furthermore, in sun leaves the photosynthetic rate and the total nitrogen are higher, and the chlorophyll content is lower than in the shade leaves (Gratani *et al.*, 2006; Niinemets, 2010).

Bromeliads (Bromeliaceae) are a family of flowering plants native to the Neotropics and representing some 3742 species (Gouda & Butcher, 2023). Bromeliads are either epiphytes, lithophytes or are rooted on the soil. The leaves of tank-forming bromeliads are tightly interlocking, forming wells that collect rainwater and detritus. Tank-bromeliads span a broad range of habitat and understory conditions, leading to a wide range of structural and functional adaptations (Benzing, 2000). They are therefore relevant model species to evaluate the effects of multivariate environmental change on phenotypic plasticity. Some bromeliad species are found either in sun-exposed or shaded areas under sun or shade plants while others experience very heterogeneous light environments (see Barberis *et al.* 2017). Light exposure can affect whole-plants characteristics with variations in the shape of the rosette, leaf colour, and size of leaf indicative of more compact rosettes in high light as compared to low light level (Cavallero *et al.*, 2009, 2011; Rodrigues Pereira *et al.*, 2013; Barberis *et al.*, 2017). Light can also affect morpho-anatomical, biochemical, and physiological leaf traits (*e.g.*, Medina *et al.* 1986; Ceusters *et al.* 2011; Rodrigues Pereira *et al.* 2013; North *et al.* 2016). Rodrigues Pereira *et al.* (2013) found that trichome density was higher in both *Billbergia elegans* and *Neoregelia mucugensis* when exposed to high light. These authors also found that the thickness of the leaf blade (along with water-storage parenchyma and chlorenchyma) and stomatal density were higher in individuals exposed to low light whereas Leroy *et al.* (2019) found the opposite for

*Aechmea mertensii*. Previous investigations have established for *Guzmania monostachia* a strong photoinhibition and a decrease by around 50 % of the total chlorophyll with increasing light condition (Maxwell *et al.*, 1995). Finally, North *et al.* (2016), found that the effects of different light conditions on leaf hydraulic conductances were divergent for *G. monostachia* and *G. lingulata*.

Most of the above-mentioned studies have examined trait variation by sampling and comparing the intermediate portion of leaves across environments. Indeed, leaves are often treated as single functional units with no spatial structure. However, it has been shown for both dicotyledonous and monocotyledonous species that structural and functional traits are heterogeneous and can vary across leaf portions (Nardini *et al.*, 2008; Li *et al.*, 2013). This is particularly true of tank bromeliads, where the basal leaf portions are in direct contact with the water and organic debris that accumulates in the tank, whereas the apical area is aerial and receives higher amounts of light. The plant obtains nutrients through the absorbing trichomes on the surface of the basal part of the leaf (Leroy *et al.*, 2016; Kleingesinds *et al.*, 2018). The intermediate and apical portions of a leaf capture light and ensure photosynthesis (Pikart *et al.*, 2018). This functional duality implies morpho-anatomical, biochemical, or physiological differences between the different portions of the same leaf. Trichomes and stomata densities show a clear inverse relationship with higher stomata in the apical portion and higher trichomes in the basal portion (Freschi *et al.*, 2010; Rodrigues *et al.*, 2016; Kleingesinds *et al.*, 2018). The nitrogen metabolism is also partitioned along the leaf lamina. The basal portion is preferentially involved in nitrogen absorption (*e.g.*, nitrate, ammonium, urea), nitrate reduction and urea hydrolysis, while the apical portion could be the main area responsible for ammonium assimilation into glutamine through the action of glutamine synthetase (GS) and glutamate dehydrogenase (GDH) activities (Takahashi *et al.*, 2007; Takahashi & Mercier, 2011; Gonçalves *et al.*, 2020)

The objective of this study was to investigate whether different portions of the leaf have a similar or differential response to the environment for tank bromeliads experiencing a strong light gradient. We evaluated differences in several morpho-anatomical and physiological leaf traits of the tank bromeliad *Aechmea aquilega* along a marked gradient of light intensity. We focused on the whole-rosette and leaf portion levels. We hypothesised a gradual change in both structural and functional leaf traits at both rosette and leaf levels in response to the gradient of light intensity. We also compared trait plasticity between the apex and basal portions of the leaf. Assuming that the basal portion is less exposed and functionally decoupled from light (*i.e.*, nutrient acquisition), we expected higher plasticity in the apical portion of the leaf in response to the light intensity gradient due to the structural and functional relationship with light (*i.e.*, photosynthesis) and its higher exposure.



Figure 23: Pictures of *Aechmea aquilega* plants representative of high (on the left) and low (on the right) exposition to light. These plants were naturally growing at the two end of a light intensity gradient from the understorey to a clearing.

## Material and methods

### *Study area and plant material*

This study was conducted in October 2021 in a tropical rainforest near the Petit Saut hydroelectric dam, Sinnamary, French Guiana (05° 04 '39" N, 052° 59' 11" W). The climate is moist tropical, with 3000 mm of yearly precipitation in the study area. The dry season extends between September and November, and there is another shorter and more irregular dry period in March. The study site is located on the edge of the forest with a shift from a closed rainforest to an open area. Environmental HOBO sensors were used to measure the air relative humidity, air temperature and light intensity (model UA-002-64, HOBO Pendant Tem Light – 64 k and model U23-001, HOBO Pro V2 Temp/RH Data logger) from the forest to the open area.

From the low to the high-light gradient, mean daily (from 7 am to 7 pm) relative humidity (RH) ranged from  $97.55 \pm 6.58$  to  $90.83 \pm 12.07$  %, mean daily temperature ( $T^\circ$ ) ranged from  $25.87 \pm 1.99$  to  $27.24 \pm 3.42$  °C, mean daily vapor pressure deficit (VPD) ranged from  $0.01 \pm 0.27$  to  $0.42 \pm 0.66$  kPa and the light ranged from  $4388 \pm 8204$  and  $37114 \pm 515547$  Lux which represent a range from  $100.92 \pm 188.69$  to  $853.62 \pm 1185.58$   $\mu\text{mol s}^{-1} \text{m}^{-2}$  in term of photon flux density.

*Aechmea aquilega* (Salisb.) Griseb, Bromelioideae, is a tank bromeliad with classical Crassulacean Acid Metabolism (CAM) photosynthesis that can be found as epiphytic, lithophytic or terrestrial life forms (Leroy *et al.*, 2013). In the study site, this species has a terrestrial life form and was found in different incident light conditions from closed rainforest area to full sun-exposed area (Fig. 23). We selected 20 mature, non-flowering *A. aquilega* with non-damaged young leaves along this incident light gradient. We quantified the percentage of light intensity reaching each tank-bromeliad rosette with a digital camera (Nikon Coolpix 4500) equipped with a Nikon Fisheye Converted lens (FC-E8) that provide a 180° canopy



view. Hemispherical images were analysed using Gap Light Analyzer software v.2.0 (Frazer *et al.* 1999) to determine the amount of total incident radiation transmitted through gaps (hereafter light intensity). The light intensity ranged from 10.41 to 60.64% from the closed rainforest to the open area.

### *Plant morphology*

For all individuals, we counted the number of green leaves (Leaves) and we measured plant height (from the base of the tank to highest leaf tip, Height, cm) and plant diameter as the maximum distance between the tips of the leaves (two measurements at 90 °, Diam, cm). The length (Length, cm) and mid leaf width (Width, cm) of the second youngest fully expanded leaf were measured.

### *Carbon metabolism traits*

Chlorophyll fluorescence— The maximum quantum yield of photosystem II ( $F_v/F_m$ ) and the maximum electron transport rate ( $ETR_{max}$ ,  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) were measured with a portable fluorometer (MINI-PAM II, Walz, Effeltrich, Germany). Measurements were done between 9:00 am and 12:00 am on the adaxial surface of the first youngest fully expanded leaf.

To measure  $F_v/F_m$  (*i.e.*, potential maximum photosynthetic capacity of plants) a portion of the leaf was acclimated to dark for 30 minutes with a dark leaf clip (DLC-8, Walz). The minimal leaf fluorescence  $F_0$  was measured at low intensity light ( $< 0.1 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), then a 0.8 second saturating pulse ( $5000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) was produced to assess maximal fluorescence.  $F_v/F_m$  was calculated as:  $(F_m - F_0) / F_m$ .

The same leaf was placed in an opaque plastic bag for quasi-dark acclimation for 30 seconds and maintained in the bag during a rapid light curve procedure (Rascher *et al.*, 2000; Manzi *et al.*, 2022). The leaf was exposed to 12

gradually increasing PAR values ranging from 50 to 3000  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  for 30 seconds each. The ETR was calculated by the fluorometer and the WinControl-3 software (Walz, Effeltrich, Germany) according to the photoinhibition REG1 function of Platt *et al.* (1980).  $\text{ETR}_{\text{max}}$  was then extracted from the obtained curves as the highest measured ETR.

Chlorophyll content— The leaf chlorophyll (a and b) content ( $C_{\text{chl}}$ ,  $\mu\text{g cm}^{-2}$ ) was estimated on the same leaf with a chlorophyll metre SPAD-502 (KONICA MINOLTA, U.S.A). Ten measurements (distributed equally on the apical leaf portion) were averaged to obtain a SPAD ( $U_{\text{SPAD}}$ ) value. Using Coste *et al.* (2010) equation:  $C_{\text{chl}} = (117.1 \times U_{\text{SPAD}}) / (148.84 - U_{\text{SPAD}})$  and the leaf mass area (see mass-related traits), we obtained the chlorophyll content per unit of dry mass (Chlorophyll,  $\text{mg g}^{-1}$ ).

#### *Leaf biochemistry*

The upper part of the first youngest fully expanded leaf of each tank bromeliad was harvested at dusk, corresponding to maximum storage of carbohydrates, to quantify metabolite content. The samples were immediately frozen in liquid nitrogen for 15 min and stored in a freezer until they were freeze-dried (Alpha 1-2 LD; Christ). Each sample was then ground to a fine powder in a Retsch MM301 Mixer Mill and stored in airtight vials in the dark until quantification of C, N and P and non-structural carbohydrates (soluble sugars and starch).

C, N, and P content— About 9–11 mg of leaf powder was used to quantify carbon (C,  $\text{mg cm}^{-2}$ ) and nitrogen (N,  $\text{mg cm}^{-2}$ ) contents (elemental analyzer, Flash 2000 ThermoFisher, NFISO 10694, NF ISO 13878, NF EN 13137). 3–4 mg of leaf powder was used to quantify total phosphorus (P,  $\text{mg cm}^{-2}$ ) contents (spectrometer, Uvi Light XT5 Secomam, spectrometric method with ammonium molybdate at 880 nm after  $\text{H}_2\text{SO}_4$  acid hydrolysis and persulfate oxidation, adapted NFEN 6878).

Non-structural carbohydrate extraction— Soluble sugars ( $\text{mg g}^{-1}$ ) were extracted from 10 to 15 mg powder mixed in 0.5 mL 80 % ethanol (v/v) and incubated for 20 min at 80 °C. Extraction was repeated twice and all three supernatants were collected in a tube and dried (Refrigerated CentriVap Vacuum Concentrators, Labconco). The resulting soluble sugar extract was solubilised in 1.5 mL ultrapure water by sonication and agitation and then stored at -20 °C. Starch was extracted from the dried pellets in 1.5 ml of 0.2 M KOH solution incubated for 20 min at 80 °C and then stored at 4 °C. Total soluble sugar contents were determined by spectrophotometry at 620 nm (spectrophotometer UV-visible: UVmc2, SAFAS, Monaco) after heating at 100 °C for 10 min, using anthrone reagent (0.15 % (w / v) dissolved in 70 %  $\text{H}_2\text{SO}_4$ ) and glucose as standard (Yemm and Willis, 1954); analyses were carried out in triplicate. Starch contents were determined after hydrolysis into glucose by amyloglucosidase (from *Aspergillus niger*, Sigma, EC 3.2.1.3) for 2 h at 50 °C. The obtained glucose was determined colorimetrically using an enzymatic reagent containing glucose oxidase (Type II from *Aspergillus niger*, Sigma, EC 1.1.3.4) / peroxidase (Type I from horseradish, Sigma, EC 1.11.1.7) and o-dianisidine dihydrochloride (Sigma, EC 243-737-5). After adding 6N hydrochloric acid, absorbance was measured at 530 nm, using glucose as a standard (Chow and Landhausser, 2004). Soluble sugars and starch contents were expressed as mg (equivalent glucose)  $\text{g}^{-1} \text{M}_D$  (Dry Mass).

#### *Leaf apex and base measurements*

The second youngest fully expanded leaf was cautiously stripped off the plant and brought back to the lab in a cool box for subsequent measurements.

Mass-related traits— 8 discs of 10mm diameter were sampled in a 2.5 x 6 cm leaf rectangle in both basal and apical regions. The discs were immediately weighed using an electronic balance (AB204-S, Metler-Toledo) to determine the fresh mass ( $M_F$ , g), stored in distilled water at 4 °C for 48 h to get the turgid mass ( $M_T$ , g) and

then dried at 60 °C for 48 h to get the dry mass ( $M_D$ , g). Leaf mass area (LMA,  $\text{g m}^{-2}$ ) was calculated as:  $M_D / \text{sum of leaf discs surface}$ , leaf dry mass content (LDMC,  $\text{mg g}^{-1}$ ) as:  $M_D / M_F$ , relative water content (RWC, %) as  $(M_F - M_D / M_T - M_D) \times 100$  and leaf succulence (LS,  $\text{g}_{\text{H}_2\text{O m}^{-2}}$ ) as:  $(M_F - M_D) / \text{sum of leaf discs surface}$ .

Stomatal and trichome density and size— Two 1 x 4 cm leaf portions from the middle part of the apex and the base were fixed in FAA (5 % formalin, 5 % acetic acid and 90 % ethanol at 70%) for 2 weeks, and then transferred to 70 % ethanol for storage. Then, the fixed leaf portions were removed from the 70% ethanol and allowed to dry for few minutes to eliminate the ethanol from the leaf surface. Stomata and trichomes were observed from imprints on the surface-dried fixed leaf samples using a thin layer of transparent nail varnish, followed by examination under an inverted microscope (Olympus BX51). Three pictures per imprint were made with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ (US NIH, Schneider *et al.* 2012). The number of stomata and trichomes were counted and divided by the picture area ( $1.27 \text{ mm}^2$ ) to estimate their density per  $\text{mm}^2$  (average of the three pictures). As *A. aquilega* is hypostomatous and presents no stomata at leaf base, stomatal density ( $\text{Nb mm}^{-2}$ ) is only available for the abaxial face of the leaf apex. Trichome density ( $\text{Nb mm}^{-2}$ ) and trichome area ( $\text{mm}^2$ ) were measured on both abaxial and adaxial faces for both leaf apex and base. The average area of the trichome was measured for 45 trichomes for each leaf portion using the FreeHandTools from ImageJ

Anatomical structure and measurements— Handmade transversal sections ( $< 0.5 \text{ mm}$  thick) from the second 1 x 4 cm portion were performed using a razor blade. One picture per location was taken using an inverted microscope (Olympus BX51-TF, Tokyo, Japan). Images were acquired with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and measurements were made using the “straight line” (thickness) and “freehand” (surface) tools from the ImageJ software. The total leaf thickness (mm, mean of 4 measurements), abaxial and adaxial epidermal wall

and cuticle thickness (mm, 6 measurements) and hydrenchyma thickness (mm, 6 measurements) were measured. Additionally, the density of fibres (Nb mm<sup>-2</sup>), the density of vascular bundles (Nb mm<sup>-2</sup>), and the mean surface of the vascular bundles (mm<sup>2</sup>) were measured. The interveinal distance (IVD, mm) was measured as the distance between the centre of two adjacent vascular bundles, and the vein-epidermis distance (VED, mm) as the distance from the centre of a vascular bundle to the stomatiferous abaxial epidermis to get the IVD/VED ratio.

### *Statistical analysis*

All statistical analyses were carried out in R version 4.1.1 (R Core Team, 2021). Graphs were produced using the R package ggplot2 (Wickham, 2016). To study the response of leaf traits to light intensity, we fitted simple linear models with the light intensity (%) as the predictor (lm function, stats package, R Core Team 2021). For traits measured at different positions on the leaf blade ("Apex" or "Base") the position was added as a predictor with an interaction term with Light. All variables were log-transformed to match conditions of linear models while all figures display raw data and back transformed models estimates. Complete model outputs are available as supplementary data (supplementary Table S2 and S3).

## **Results**

### *Effect of light intensity gradient on whole-rosette characteristics*

The light intensity had a significant effect on the whole-rosette structure, photosynthesis, and chemical contents with the exception of leaf starch content (Fig. 24). As light intensity increased, the number of leaves were significantly reduced, and the diameter and height of the rosette decreased by two-fold (Fig. 24A, C). The leaves were two to three times shorter and *ca.* two times wider at the highest light intensity as compared to those at the lowest (Fig. 24D, E). Stomatal

density, chlorophyll content, and photosynthetic performance ( $ETR_{max}$  and  $F_v/F_m$ ) significantly decreased as light intensity increased (Fig. 24F, I). On the other hand, leaf C and soluble sugars were significantly reduced with light intensity while leaf starch content was not affected by the light (Fig. 24J and K, L). Finally, leaf N and P contents were halved with increased light exposure (Fig. 24M, N).

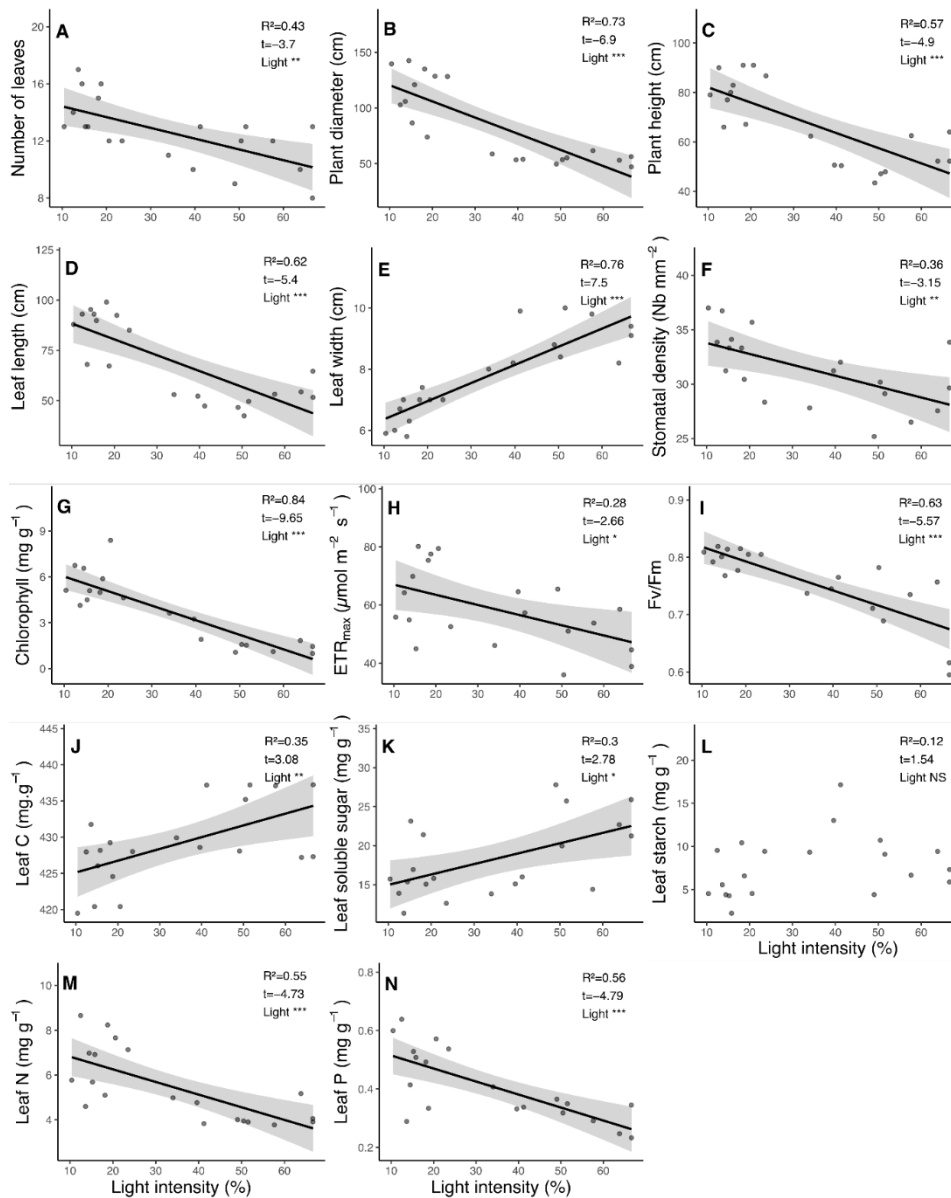


Figure 24: Effect of light intensity gradient on whole-rosette characteristics. Effects of light intensity (%) on (A) the number of leaves, (B) plant diameter (cm), (C) plant height (cm), (D) leaf length (cm), (E) leaf width (cm), (F) stomatal density (Nb. mm<sup>-2</sup>), (G) leaf chlorophyll content (mg g<sup>-1</sup>), (H) maximum electron transport rate ( $ETR_{max}$ ,  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), (I) maximum quantum yield of photosystem II ( $F_v/F_m$ ), (J) leaf carbon content (mg g<sup>-1</sup>), (K) leaf soluble sugars content (mg g<sup>-1</sup>), (L) leaf starch content (mg g<sup>-1</sup>), (M) leaf nitrogen content (mg g<sup>-1</sup>) and, (N) leaf phosphorous content (mg g<sup>-1</sup>). Dots are observations. Solid lines represent the regression line of significant ( $\alpha < 0.05$ ) linear models and shaded in grey is the 0.95 confidence interval. At the top right corner of each plot is given the regression  $R^2$ , t value, and the significance of the light effect. \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ ; NS, not significant.

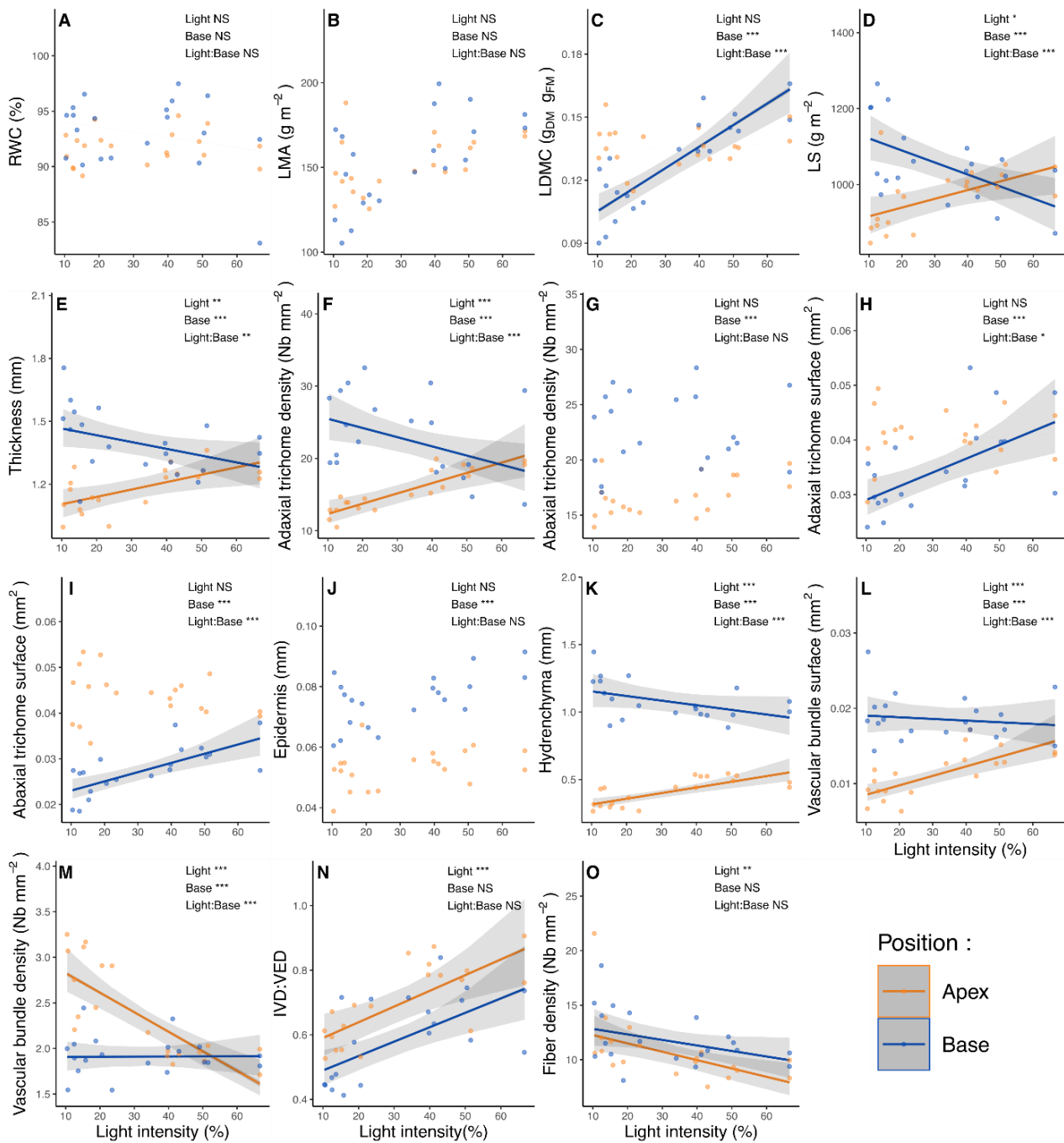


Figure 25: Effect of light intensity gradient on apex and basal leaf portion characteristics. Effects of light intensity (%) according to the leaf portion (apex and base) on (A) the relative water content (RWC, %), (B) leaf mass area (LMA,  $\text{g m}^{-2}$ ), (C) leaf dry mass content (LDMC,  $\text{mg g}^{-1}$ ), (D) leaf succulence (LS,  $\text{g m}^{-2}$ ), (E) leaf thickness (mm), (F) adaxial trichome density ( $\text{Nb mm}^{-2}$ ), (G) abaxial trichome density ( $\text{Nb mm}^{-2}$ ), (H) adaxial trichome surface ( $\text{mm}^2$ ), (I) abaxial trichome surface ( $\text{mm}^2$ ), (J) epidermis and cuticle thickness (mm), (K) hydrenchyma thickness (mm), (L) vascular bundle surface ( $\text{mm}^2$ ), (M) vascular bundle density ( $\text{Nb mm}^{-2}$ ), (N) interveinal distance : vein-epidermis distance ratio (IVD:VED), and (O) fibre density ( $\text{Nb mm}^{-2}$ ). Dots are observations, orange and blue colours correspond to the apex and basal portion of the leaf, respectively. Solid lines represent the regression line of significant ( $\alpha < 0.05$ ) linear models and shaded in grey is the 0.95 confidence interval. At the top right corner of each plot is given the significance of the effects of Light, position and their interactions. \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ ; NS, not significant.

### *Effect of light intensity gradient on apex and basal leaf portion characteristics*

RWC and LMA were not affected by either the light intensity or portion of the leaf (Fig. 25A, B). As light intensity increased, LDMC increased at the base while it remained constant at the apex portion of the leaf (Fig. 25C). Plants under low light had thus leaves with high LDMC at the apex and low LDMC at the base, whereas it was the opposite for plants exposed to higher light. Leaf succulence and thickness decreased at the base and increased at the apex portion of the leaf with increasing light intensity (Fig. 25D, E). Hence, plants under low light had higher leaf succulence and thickness at the base compared to the apical portion of the leaf, a pattern that tends to disappear at higher light intensities.

Adaxial trichome densities increased at the apex and decreased at the basal portion of the leaf with increasing light, while the density of abaxial trichomes remained unchanged (Fig. 25F, G). The density of abaxial trichomes was higher at the base as compared to the apex portion of the leaf, regardless of the light intensity. For adaxial trichomes, the density was similar for both leaf portions at high light. The surfaces of adaxial and abaxial trichomes were significantly affected by the leaf portion and the interaction of light intensity and leaf portion (Fig. 25H, I). In both cases, the surface of the trichomes was significantly higher at the apex compared to the basal portion of the leaf for plants under low light intensity, while the surface of the trichomes remained similar for both leaf portions in the most exposed plants.

Epidermis and cuticle were thicker at the base as compared to the apex portion of the leaf, regardless of the light intensity and remained unchanged with increasing light (Fig. 25J). Hydrenchyma significantly increased at the apex and decreased at the basal portion of the leaf with increasing light intensity but remained significantly thicker at the base compared to the apical portion of the leaf, regardless of the light intensity (Fig. 25K). Vascular bundle surface significantly increased with increasing light intensity at the apex portion of the leaf while it



remained unchanged at the basal portion (Fig. 25L). Hence, at low light intensity, the surface of vascular bundles was twofold higher at the base compared to the apex portion of the leaf. Conversely, the density of vascular bundles significantly decreased with increasing light at the apex portion of the leaf, while it remained constant for the basal portion. At the lowest light intensity, the density of vascular bundles was thus higher in the apex portion of the leaf compared to the base (Fig. 25M). The IVD:VED ratio and the density of fibres significantly increased and decreased, respectively, with increasing light intensity but were not affected by the position on the leaf (Fig. 25N, O).

## Discussion

### *Plasticity of the rosette in response to light intensity*

Light intensity had a strong effect on the rosette shape, size, and leaf colour. Plants in the high-light end of the gradient were smaller, and characterised by wider, shorter, and more vertical leaves. Similar responses to light intensity were reported for other bromeliad species (e.g., Cavallero *et al.* 2009, 2011; Leroy *et al.* 2009; Rodrigues Pereira *et al.* 2013; Barberis *et al.* 2017). These architectural differences enable higher exposure to light by limiting self-shading due to narrower and longer leaves in shaded areas, and lower exposure to excessive radiation through wider and smaller leaves in sun-exposed areas. The linear shape of the leaves under low-light intensity suggests a trade-off between carbon gain and mechanical support (Read & Stokes, 2006; Cavallero *et al.*, 2011). In addition, we found that shaded leaves had a higher density of fibres compared to sun-exposed leaves, which would increase their flexural stiffness and reduce bending loads (Oliveira *et al.*, 2008; Onoda *et al.*, 2011; Cavallero *et al.*, 2011). The stomatal density was lower in the leaves of *A. aquilega* exposed to high-light intensity which contrast with what was usually found in the literature. Exposure to high light levels cause higher stomatal density in other bromeliad species (e.g., Scarano *et al.* 2002;

Cavallero *et al.* 2011; Petit *et al.* 2014; Leroy *et al.* 2017 but see Oliveira *et al.* 2008) and more generally in dicotyledonous plants (Bertolino *et al.*, 2019). Species with higher stomatal density under high light have greater photosynthetic capacity (Tanaka *et al.*, 2013) and transpiration rates, contributing to a cooling effect of the leaves (Rozendaal *et al.*, 2006). However, a reduction of stomatal density constrains both stomatal conductance and transpiration, allowing for a more conservative use of water (Bertolino *et al.*, 2019) in line with the CAM habit.

*Aechmea aquilega* is a classical CAM species that permits the net uptake of CO<sub>2</sub> at night, thus improving the water-used efficiency of carbon assimilation under high solar radiation (Pierce *et al.*, 2002). In our study, the light intensity did not modify the type of CAM photosynthesis (*e.g.*, from classical to CAM idling) as gas exchange and stomatal conductance occurred in both high and low light environments (Fig. S5). Instead, we observed differences in metabolic and biochemical processes that may underlie the response to varying levels of light intensity. The higher chlorophyll concentration under low light compared to high-light intensity generally confers better light-capturing ability to *A. aquilega* leaves (Lee *et al.*, 1989; Fetene *et al.*, 1990). In parallel, low chlorophyll content under high-light intensity can also result from damage caused by reactive oxygen species (ROS) (Nishiyama *et al.*, 2006; Pospíšil, 2016). Reductions in  $F_v/F_m$  (below 0.8) and  $ETR_{max}$  with increasing light intensity are common in sun-exposed plants (*e.g.*, He *et al.* 1996; Valladares and Pearcy 1997) and can result from multiple processes, such as the degradation of the photosynthetic apparatus (Takahashi & Badger, 2011) or investment in photoprotective pigments (Young, 1991; Kumar & Pal, 2022). Nutrient deficiency, as suggested by the lower leaf N and P contents in high light-exposed *A. aquilega*, might also contribute to the lower  $F_v/F_m$  (Wu *et al.*, 2008; Kalaji *et al.*, 2014, 2018) and chlorophyll content (Evans, 1989). Despite having low leaf N content,  $F_v/F_m$  and  $ETR_{max}$  in sun-exposed *A. aquilega*, we found higher leaf C and soluble sugar contents than in the shaded plants. This indicates that

photosynthetic C fixation was sufficient for carbohydrates reserve production (*i.e.*, starch) and did not differ from that of plants in low light intensity. A higher leaf C/N ratio can result from both increasing light and N scarcity (Grechi *et al.* 2007) and is associated with higher nitrogen use efficiency (Zhang *et al.* 2020). On the other hand, differences in C/N ratio might also be due preferential allocation of N to the leaf in order to maximize growth under low-light conditions (Makino *et al.*, 1997).

*Structural and anatomical modifications of the apex and basal portions of the leaf in response to light intensity*

LMA and LDMC are two structural traits that indicate adjustments to light conditions (Poorter *et al.*, 2009, 2010). By increasing the surface area relative to the leaf biomass (*i.e.*, low LMA), the interception of light is increased under low-light conditions (Poorter *et al.* 2009). LDMC is often correlated with LMA and is considered as an alternative predictor of plant resource capture (Wilson *et al.*, 1999). In *A. aquilega*, only LDMC was affected by light, however, unexpectedly, this effect was observed only for the basal portion of the leaf. As we found significant differences in leaf thickness and anatomical traits with increasing light intensity, our unexpected results for LMA and LDMC may be due to other factors, such as soil fertility (Hodgson *et al.*, 2011), air temperature (Zhu *et al.*, 2020) or soil nutrient levels (Zheng *et al.*, 2017). Regardless of the light intensity, the leaf water status (*i.e.*, RWC) remained constant for both leaf portions, suggesting that *A. aquilega* was well supplied with water and did not experience drought under high-light condition. Conversely, leaf succulence increased at the apex and decreased at the basal portion of the leaf with increasing light intensity. Leaf succulence reflects the water storage capacity of the leaf and is mainly attributed to hydrenchyma tissue (Winter *et al.*, 1983). In *A. aquilega*, we also observed an increase in hydrenchyma tissue thickness at the apical part of the leaf, which likely contributes to the overall increase in leaf succulence with higher light intensity. This increased succulence at

the apical portion of the leaf under higher light intensity may provide a greater capacity for photoprotection (Graham & Andrade, 2004).

Water and nutrient absorption are mainly mediated by absorbing trichomes that cover both sides of the leaf surface. The higher trichome density in the basal portion of the leaf in *A. aquilega* supports previous studies conducted on various tank-bromeliad species (e.g., Freschi *et al.* 2010; Rodrigues *et al.* 2016; Kleingesinds *et al.* 2018). However, when considering the adaxial and abaxial sides of the leaf, no consistent pattern of trichome density was observed across different bromeliad species (Adams & Martin, 1986; Cach-Pérez *et al.*, 2016; Kleingesinds *et al.*, 2018). Under low-light intensity, we observed smaller trichomes occurring in higher density at the base of the leaf and larger trichomes occurring in lower density at the apex, potentially facilitating enhanced resource uptake at the basal portion of the leaf (Benzing, 2000). Conversely, under high-light intensity, both leaf portions exhibited approximately similar trichome density and size, similar to findings in different species of atmospheric tillandsioids (Cach-Pérez *et al.*, 2016).

In contrast, leaf thickness, and surface and density of vascular bundles showed a different response of the two leaf portions to light. These results may be due to the different functional role of the two leaf portions. Additionally, we found that the vascular bundle density at the apex portion of the leaf decreased with light intensity, along with a decrease in stomatal density, suggesting a coordinated response between water demand (e.g., leaf gas exchange) and water supply (transport) capacity (Sack & Scoffoni, 2013). Across diverse angiosperm species, IVD and VED are approximately equal, indicating an optimal arrangement of the vascular bundles for hydraulic efficiency (Zwieniecki & Boyce, 2014). In our study, we found an IVD:VED ratio  $< 1$  for both leaf portions with no significant difference between them. With  $IVD < VED$ , *A. aquilega* is a species that “overinvests” in veins, a feature that is physiologically disadvantageous since it involves the replacement of photosynthetic mesophyll cells with hydraulically redundant

vascular bundles (Zwieniecki & Boyce, 2014; Males, 2017). Similar vascular overinvestment has been observed in other succulent and CAM taxa and was associated to thicker leaves in water-limited environments (Males, 2017; Leverett *et al.*, 2022). This low ratio can facilitate hydraulic recharge to efficiently provide water to the mesophyll and resistance to transpirational water loss (de Boer *et al.*, 2016; Males, 2017; Leverett *et al.*, 2022).

*Attenuation of the longitudinal degree of differences between the apex and basal portions of the leaf with increasing light intensity*

Overall, we found strong structural and anatomical differences along the leaf of *A. aquilega* growing in low light compared to individuals growing in a high-light environment. Most of the measured traits showed high longitudinal degree of difference between the apex and basal portions of the leaf in low light, and the differences were reduced or disappeared with increasing light intensity for most of the traits (*i.e.*, thickness, adaxial trichome density, abaxial and adaxial trichome surface, vascular bundle surface and density). In a few other traits (*i.e.*, LDMC, LS), we found a reverse pattern. These results suggest that the structural and functional regionalisation were reduced in leaves from high light compared to leaves from low-light intensity. The positive or negative co-variations of the two leaf portions may be due to mechanical and/or physiological adjustments at the leaf scale in response to light intensity and/or other strongly linked confounding factors, such as gradient of leaf litter/nutrient supplies. However, it is unlikely that drought is the driving factor behind these leaf adjustments, as the tanks in high-light intensity were consistently filled with rainwater throughout the year (pers. obs.). Regardless of the underlying mechanism, the loss of longitudinal differentiation between the apical and basal portions of the leaf in sun-exposed environments may have strong ecological and physiological implications, particularly in terms of nitrogen acquisition and photosynthetic performance.

The greater structural and anatomical differences between the two leaf portions under low-light intensity, compared to leaf in high-light environments, may be attributed to the leaf base-to-tip developmental gradient. In monocotyledonous plants, cell divisions primarily occur in the basal meristem of the leaf, follow by cell elongation, and finally, the oldest and most mature cells are present at the tip (Fournier *et al.*, 2005). Consequently, a positional gradient of cell ages is formed along the leaves during their growth, with the youngest cells located at the base and the oldest at the apex of the leaf (Sharman, 1942). Thus, it is likely that the cell age of the apical portion of the leaf under low-light intensity is higher than that of the leaf under high-light intensity, due to a greater number of cell divisions occurring in the longer leaf. It has been shown that the shade can facilitate higher levels of cell division, cell elongation, or both, depending on the species (Rahim and Fordham 1991). Nevertheless, if cell age was the only factor explaining the differences between leaves in low light and high-light environments, it should have only affected the values of traits in the apical portion of the leaves.

## **Conclusion**

We found significant morphological and functional trait differences in *A. aquilega* bromeliads growing along a light intensity gradient. Interestingly, we showed that the light intensity had contrasting effects on the morpho-anatomy of leaf apical and basal portions. When growing in low-light conditions, leaves of *A. aquilega* exhibit a particularly steep within-leaf functional gradient, while in high-light conditions leaf traits become homogeneous throughout the length of the leaf. This contrasting pattern might be due to a combination of apparent plasticity and true plasticity that would be essential to disentangle. These results reinforce the remarkable complexity of tank bromeliad leaves and show how structural and functional traits within a single leaf can be either heterogeneous or uniform, depending on the light environment. Overall, this study highlights the significance

of considering different portions of the leaf in order to gain a better understanding of how plants respond to environmental changes.

## **Supplementary Information**

Tab. S2 Table summary of linear models output for plant traits according to the light intensity (%).

Tab. S3 Table summary of linear models output for plant traits according to light intensity (%) and position on the leaf blade.

Fig. S5 Overnight CO<sub>2</sub> assimilation and stomatal conductance curves for 8 plants taken along the light intensity gradient (%).

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# Chapitre III

*Water and nutrient resources shape plant traits, their leaf endophytic microbiota, and their relationships*



Cette étude porte sur l'influence de la disponibilité en eau et nutriments sur les traits et communauté d'endophytes foliaires d'*A. mertensii*. Pour cela, des plantes ont été cultivées dans 2 niveaux d'irrigation et 3 substrats de richesses différentes résultant en 6 traitements jusqu'à floraison.

Nos résultats montrent une réponse écophysiological et également du microbiote de l'holobionte *A. mertensii* à ces facteurs. Les endophytes bactériens apparaissent notamment liés aux contenus en eau et nutriments des feuilles tandis que les champignons sont plus fortement affectés par les conditions environnementales dans lesquelles la plante se développe. Nous avons également identifié des associations de certains taxa microbiens avec certains traits foliaires parmi lesquels la LMA et le contenu en azote.

Cette étude s'inscrit dans le cadre du projet HOLOBROM du méta-programme HOLOFLUX de L'INRAE coordonnée par Céline Leroy avec l'appui d'Heidy Schimann, Lucie Zinger, Corinne Vacher, Eliane Louisana et Sandrine Étienne.

Cette expérience avait été initiée en novembre 2019 avant le début de mon contrat doctoral. J'ai réalisé l'essentiel des mesures écophysiologicals. Le travail de laboratoire (extraction, PCR) a été réalisé en collaboration avec Eliane Louisana, Sandrine Etienne, Heidy Schimann et Céline Leroy. J'ai ensuite mené les analyses bioinformatiques et le traitement des données. La version actuelle du manuscrit est le fruit de ma collaboration avec Régis Céréghino et Céline Leroy.

# **Water and nutrient resources shape plant traits, their leaf endophytic microbiota, and their relationships**

Lafont Rapnouil Tristan<sup>1-2</sup>, Heidy Schimann<sup>3</sup>, Eliane Louisana<sup>2</sup>, Sandrine Etienne<sup>2</sup>, Frédéric Julien<sup>4</sup>, Lucie Zinger<sup>5</sup>, Corinne Vacher<sup>3</sup>, Céréghino Régis<sup>4</sup>, Céline Leroy<sup>1-2</sup>

<sup>1</sup>AMAP, Univ. Montpellier, CIRAD, CNRS, INRAE, IRD, Montpellier, France

<sup>2</sup>EcoFoG, AgroParisTech, CIRAD, CNRS, INRAE, Université des Antilles, Université de Guyane, Campus agronomique, Kourou, France

<sup>3</sup>BIOGECO, Univ. Bordeaux, INRAE, Bordeaux, France

<sup>4</sup>Laboratoire Écologie Fonctionnelle et Environnement, Université Paul Sabatier Toulouse 3, CNRS, Toulouse, France

<sup>5</sup>IBENS, École Normale Supérieure, Paris, France.

## Abstract

- *Purpose* Plants and their microbiota constitute an entity known as the holobiont. The diversified microbiota harboured by the host plant has been shown to be involved in their plastic response to environmental factors. This should result in coordination between host leaf ecophysiological traits and leaf microbial endophytic communities the underlying mechanisms and microbial taxa involved remain largely unknown. We explored these issues in a greenhouse experiment using the tank bromeliad *Aechmea mertensii*. In this study we aimed to investigate the effects of water and nutrient supply on both ecophysiological traits and microbial communities, their coordination and identify specific OTUs-traits correlations.

- *Methods* Seeds from one *A. mertensii* (Bromeliaceae) plants were sowed in a full factorial design in three substrates of different nutrient content (P: poor; I: intermediate and, R: rich) and two watering conditions (W: wet and D: Dry) and grown until flowering. Then leaf ecophysiological traits were measured and tissues sampled for metabarcoding analysis of bacterial (16s rRNA gene) and fungal (ITS) endophytic communities.

- *Results* Our results show that *A. mertensii* response to resource availability involved both ecophysiological and microbiotic response. We found no microbiotic response in terms of  $\alpha$ -diversity but a strong effect of treatments on  $\beta$ -diversity. Additionally, we found different microbiota-traits relationships between bacterial and fungal communities. Bacterial communities' variation appeared to be linked to leaf water and nutrient status while fungi were mainly driven by the host growing conditions. However, we found both bacterial and fungal taxa positively and negatively associated with specific host traits.

- *Conclusion* Taken together our results shed interesting light on holobiont scale plasticity with variation of both host ecophysiological traits and microbiota composition. Noteworthy, fungal and bacterial endophytes showed different

responses to our treatments suggesting that they might have different functions and roles in the holobiont adjustment to specific environmental factors. Studies combining fungal and bacterial communities' investigation as well as various environmental factors will be required to fully understand the holobiont scale response to environmental conditions.

**Key words** Holobiont plasticity - Microbiota - Ecophysiological traits - Environmental factors - *Aechmea mertensii* - Bromeliads.

## Introduction

In sessile organisms, phenotypic plasticity represents an important strategy for dealing with environment variability. Phenotypic plasticity is the ability of a single genotype to produce multiple distinct phenotypes in different environmental conditions (Bradshaw, 1965; Sultan, 2000). Phenotypic plasticity gives a plant the ability to adjust its performance by altering morphology and/or physiology in response to varying environmental conditions throughout its lifespan. Functional traits that reflect characteristics related to life history and ecological strategies (*e.g.*, Violle *et al.* 2012) should be related to the ability of a species to survive and reproduce in a given set of environmental conditions (Funk *et al.*, 2017) and thus might be associated with plant phenotypic plasticity. To date, phenotypic plasticity is primarily viewed as a genotype-by-environment interaction (El-Soda *et al.*, 2014; Sultan, 2021). However, all eukaryotes, including plants and animals, are not autonomous entities (Gilbert *et al.*, 2012; Bordenstein & Theis, 2015) but, instead, are holobionts that comprise a host and consortium of associated microbiota (McFall-Ngai *et al.*, 2013; Bordenstein & Theis, 2015; Theis *et al.*, 2016). The holobiont encompasses not only the host and its obligate symbionts but also its facultative symbionts and the emergent properties arising from these multivarious and dynamic associations (Theis *et al.*, 2016).

Plants are intimately associated with an extreme diversity of taxonomically structured communities of microorganisms. The plant microbiota includes bacteria, archaea, fungi, protists, nematodes, and viruses that associate with the plant (Trivedi *et al.*, 2020) and inhabit different plant compartments, including the rhizosphere, phyllosphere, and endosphere (Compant *et al.*, 2019). The endophytic microbial communities are in part heritable and affect the plant fitness (Bordenstein & Theis, 2015; Theis *et al.*, 2016). Colonization by microbiota can offer significant benefits to their host plants by producing various metabolites that promote plants health and crop yields (Berendsen *et al.*, 2012; Berg *et al.*, 2014;

Trivedi *et al.*, 2020). Microbiota can also promote mineral solubilization, nutrients and water acquisition, phytohormones production, and provide protection against abiotic stresses (*e.g.*, drought, salinity, temperature). Furthermore, they offer protection against biotic stresses, including phytopathogens, insects, and herbivores (Bulgarelli *et al.*, 2013; Trivedi *et al.*, 2020; Chialva *et al.*, 2022). Hence, endophytic microbiota can affect various aspects of the physiology, metabolism, and ecological interaction of the plants (Zilber-Rosenberg & Rosenberg, 2008), and are thus an important part of the plant phenotype (Theis *et al.*, 2016). While endophytic microorganisms are often ignored, several studies have shown that microbial biodiversity is a trait forming part of the extended phenotype of the host plant (Whitham *et al.*, 2003; Agler *et al.*, 2016; Petipas *et al.*, 2020) with important effects on plant health and fitness (Berendsen *et al.*, 2012; Petipas *et al.*, 2020).

Endophytes, which refer to microorganisms capable of colonizing internal tissues of plants, including pathogens (Hardoim *et al.*, 2015), are particularly suitable for studying the relationship between microbiota and plant traits. They are thought to have more intricate relationships with the host, particularly with its immune system (Trivedi *et al.*, 2020; Compant *et al.*, 2021). The host plant genotype is an important determinant of the leaf-associated microbiota community composition (Bálint *et al.*, 2013; Qian *et al.*, 2018). Endophytes are not randomly distributed among plant species, and it has been proposed that host plant properties play a key role in endophyte colonization (Saunders *et al.*, 2010; Van Bael *et al.*, 2017). Since the host tissues effectively serve as habitats for these microorganisms, it is expected that they are strongly controlled by their host traits and potentially play a significant role in the host's plastic response to environmental factors (Bernard *et al.*, 2021). Recent studies showed that leaf traits related to leaf/root economic spectrum, drought stress, nutrient supply, and resistance against pathogens and/or herbivores can influence the composition of endophytic bacterial and/or fungal communities (Kembel *et al.*, 2014; Li *et al.*, 2018;

González-Teuber *et al.*, 2020; Oono *et al.*, 2020; Cambon *et al.*, 2022). However, while these studies focused on various host plant species or genotypes, this may introduce biases in understanding the true relationship between plant phenotypic characteristics and endophytic microbiota composition. The importance of intraspecific phenotypic trait variation in microbial communities and how environmental stressors affect both plant phenotypic traits and endophytic microbiota have been overlooked.

Environmental stressors are increasing worldwide, including abiotic stress such as changes in water and nutrient availability (limitation or excess) or exposure to extreme temperatures, as well as biotic stress such as pathogen infection and herbivory. Due to the close relationship between plants and their associated microbiomes, both can be affected by external factors that, in turn affect each other. Therefore, plants, microbiota, and the environment are highly intertwined (Omae & Tsuda, 2022). Currently, in the face of environmental change, research interest in plant phenotypic plasticity has accelerated to understand its potential role for population persistence and adaptation (*e.g.*, Chevin *et al.* 2010, 2013; Arnold *et al.* 2019), but without considering plant microbiota. Plant-associated microbiota have gained interest from researchers for their applications in agriculture because they influence plant physiological parameters under abiotic stress conditions and promote plant development and tolerance to abiotic stresses (*e.g.*, Rodriguez and Durán 2020; Fadiji *et al.* 2022; Omae and Tsuda 2022). However, research on plant-microbiota interactions is mainly restricted to model plants and crop species, which have distinct characteristics compared to wild species. Understanding the intricate relationships between the host plant and its microbial associates in response to a changing environment is crucial for predicting the dynamics of plant species in natura and ultimately the regeneration of natural ecosystems.

In this study, we investigated the potential effects of substrate fertility and water availability on the interplay between plant phenotype and the composition and structure of foliar microbiota of *Aechmea mertensii*, Bromeliaceae. Bromeliads represents one of the most species rich and ecologically important plant families found of the Neotropics, with 3754 described species (Gouda et al. 2023). Bromeliads have lanceolate leaves arranged spirally, with tightly overlapping basal sheathes that form water tanks or can be tankless, and both blades and sheathes often bear scale-like water-absorbing trichomes (Benzing, 2000). Most bromeliad species have a wide geographic distribution and show large phenotypical plasticity due to their exposition to a wide range of environmental heterogeneity, including light (Barberis et al., 2017; Lafont Rapnouil et al., 2023b), substrate (Lafont Rapnouil et al., 2023a), and precipitation (Cach-Pérez et al., 2018). However, Zizka et al. (2020) showed that 81% of bromeliad species are potentially threatened by pervasive anthropogenic and environmental impacts, according to IUCN Red List criteria. While some studies have focused on the response of bromeliads to individual environmental stressors, such as drought (Svensk et al., 2020), temperatures (Duarte et al., 2018), substrate fertility (Lafont Rapnouil et al., 2023a), metal contamination (Rodrigues Martins et al., 2021) or air pollutant (Giampaoli et al., 2021), only a few have investigated the effects of combined environmental stressors (Medina et al., 1986; Scarano et al., 2002; Gonçalves et al., 2020). Yet, no study has examined the link between plant traits and endophytic microbiota. Our main objectives were to (i) characterise the effects of substrate fertility and water supply on plant phenotypes and on the leaf bacterial and fungal diversities, (ii) test the relationship between plant phenotypes and the composition of leaf fungal and bacterial communities, and (iii) identify leaf microbial taxa positively or negatively associated with leaf traits. We hypothesized that *A. mertensii* would respond differently to changes in the abiotic environment because of morphological, physiological, and biochemical adjustments. We expected plants to have higher



performance in optimal conditions (high substrate fertility and water supply) and lower performance in non-optimal conditions (low substrate fertility and water supply). According to the Anna Karenina principle (Arnault *et al.*, 2022), we anticipated more dissimilar microbiota communities in plants with lower performance because the host plants would lose the capacity to regulate its microbiota. Considering the expected differences in trait expression related to nutrient and water supply, we hypothesised that leaf microbiota communities would differ, and some microbial taxa would be positively or negatively associated with specific leaf traits.

## **Material and methods**

### *Experimental procedure and growth conditions*

We conducted an experiment in a greenhouse at the Campus agronomique Kourou (French Guiana) as a full factorial design with two factors. The first factor had three levels: rich (R), intermediate (I), and poor (P) substrate fertility; and the second factor had two levels: well-watered (Wet) and water deficit (Dry). The substrates used in the experiment consisted in 100 % commercial potting soil (rich substrate, R), a mixture of 70 % potting soil and 30 % (v:v) white sand (intermediate substrate, I), and a mixture of 30 % of potting soil and 70 % (v:v) white sand (poor substrate, M). Physico-chemical analyses of the three substrates were conducted on triplicate samples to determine their carbon, nitrogen, and phosphorus contents (PAPC, Toulouse). The CNP contents of the substrates decreased from rich to poor soils, with the poor and intermediate substrates being closer in composition (Fig. S6).

Greenhouse temperature, light intensity and relative humidity were monitored with HOBO probes (model UA-002-64, HOBO Pendant Temp/Light - 64 k and model U23-001, HOBO Pro V2 Temp/RH Data logger, Amanvillers, France). Over

the course of the experiment, mean temperature, relative humidity, and light intensity were recorded as follow: for the wet treatments, the values were  $27.93 \pm 0.89$  °C,  $89.57 \pm 5.43\%$ , and  $10052 \pm 3381.72$  Lux, respectively and for the drought treatments, the values were  $29.75 \pm 1.58$  °C,  $76.02 \pm 6.82\%$ , and  $12058 \pm 4148.19$  Lux, respectively.

Seeds derived from a single *A. mertensii* mother plant were germinated in the three substrates in horticultural seedling trays in November 2019 to ensure genetic homogeneity of experimental plants. After the germination period (ca. 20 days), half of the plants from each substrate were moved either to a well-watered (Wet) or a water deficit (Dry) part of the greenhouse. Thus, a total of six treatments was set up: Rich-Wet, Intermediate-Wet, Poor-Wet, Rich-Dry, Intermediate-Dry, and Poor-Dry (Fig. 26).

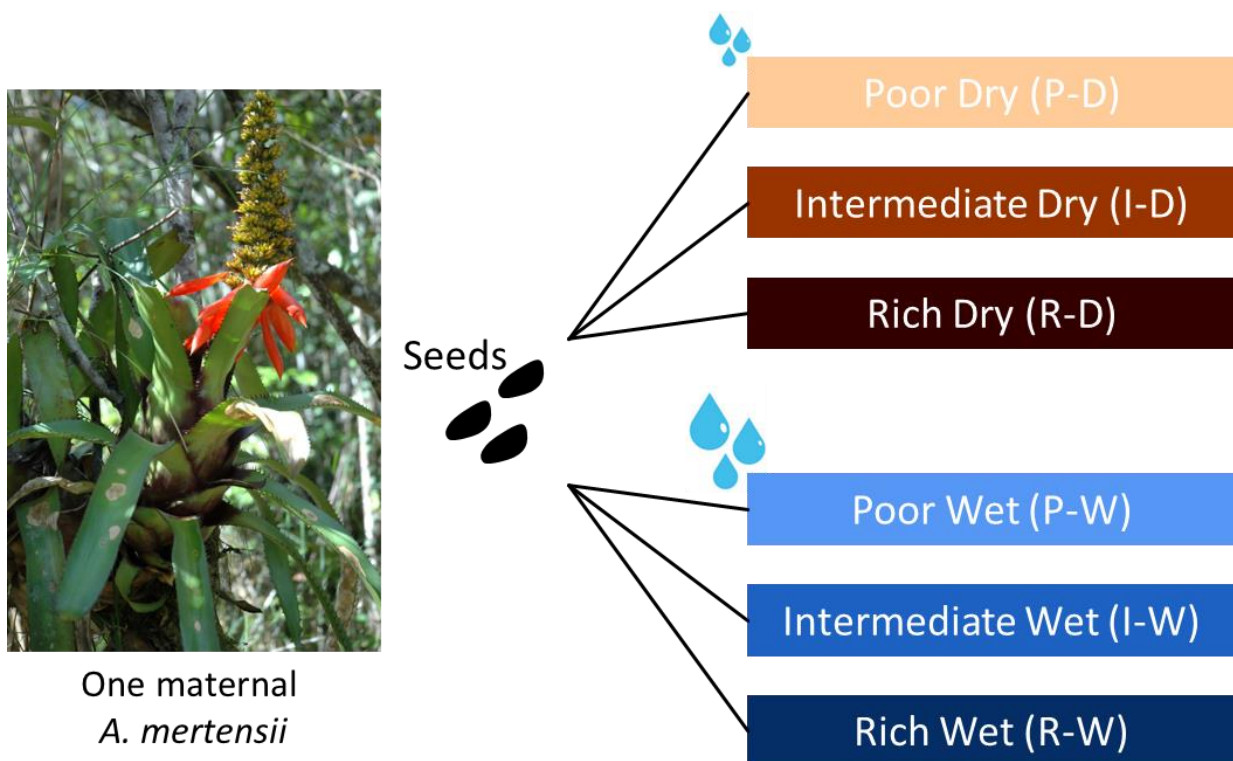


Figure 26: Experimental design and name of the 6 treatments resulting from the factorial combination of 2 water levels (Dry and Wet) and 3 nutrient levels (Poor, Intermediate and Rich). The 6 treatments are named P-D (Poor-Dry), I-D (Intermediate-Dry), R-D (Rich-Dry), P-W (Poor-Wet), I-W (Intermediate-Wet) and R-W (Rich-Wet).

Plants in the wet treatment were irrigated with tap water for ten minutes twice a day at 8:00 am and 6:00 pm, while plants in the drought treatment were irrigated twice a week. As plants grew larger, they were transplanted into 2-litre pots in March 2020, according to their respective substrates. The pots were randomly placed on horticultural tables for each water treatment, and the plant were allowed to grow until they reached the flowering stage, that occurred between October 2020 and February 2021 depending on the treatment. Measurements and samplings were conducted on fifteen plants per treatment, except for Rich-Dry treatment were only 7 plants survived. Plant heights, number of leaves, and the length and width of the longest mature leaf were recorded. Then, measurements and samplings were taken on the two youngest mature leaves of the rosette.

#### *Photosynthetic-related traits*

Chlorophyll content – Leaf chlorophyll a and b content were estimated using a SPAD502 chlorophyll meter (KONICA MINOLTA, U.S.A) and the relation from Coste et al. (2010) linking the SPAD value with the chlorophyll concentration ( $\mu\text{g cm}^{-2}$ ). Eight measurements were made on the leaf blade apical region and averaged before conversion from SPAD value to  $\mu\text{g cm}^{-2}$  using Coste et al. relation and finally to  $\text{mg g}^{-1}$  using leaf mass area (LMA, see below).

Chlorophyll fluorescence – The maximum quantum yield of photosystem II ( $F_v/F_m$ ) and the maximum electron transport rate ( $\text{ETR}_{\text{max}}$ ,  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) were measured on the adaxial face of the youngest mature leaf with a portable fluorometer (MINI-PAM II, Walz, Effeltrich, Germany). To measure  $F_v/F_m$ , the leaf was dark acclimated for 30 using a dark leaf clip (DLC-8, Walz). The minimal fluorescence  $F_0$  was measured by exposing the leaf to a low intensity light ( $< 0.1 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ), then a 0.8 s saturating pulse ( $5000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) was produced to assess maximal fluorescence  $F_m$ .  $F_v/F_m$  was calculated as  $(F_m - F_0)/F_m$ .

To measure  $ETR_{max}$ , the same leaf was quasi-dark acclimated for 150s in an opaque plastic bag before gradual exposition to increasing PAR values in twelve 30s steps ranging from 50 to 3000  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ .  $ETR_{max}$  was extracted as the highest ETR obtained using the WinControl-3 software (Walz, Effeltrich, Germany) and the photoinhibition accounting function REG1 from Platt et al. (1980).

#### *Leaf structural traits*

Eight 8-mm diameter disks were collected with a cork borer from the apical portion of the first youngest leaf. Leaf thickness (mm) was estimated as the average disk thickness measured with a micrometric calliper (Digit Outside Micrometre 193–101, Mitutoyo, Japan). Disks were then weighed using an electronic balance (AB 204-S Mettler Toledo, Switzerland) to determine Fresh mass (FM, g) and stored in distilled water at 4 C in the dark for 48 to 72 h to get the turgid mass (TM, g). The dry mass (DM, g) was obtained from oven dried disks weighed after 72h at 60°C. The leaf mass area (LMA,  $\text{gm}^{-2}$ ) was calculated as DM/sum of leaf disk area, while the leaf dry matter content (LDMC,  $\text{g g}^{-1}$ ) was calculated as DM/FM.

Stomatal density (Nb stomata  $\text{mm}^{-2}$ ) was measured on the adaxial face. A 1 × 4 cm portion of the second leaf apical region was collected and fixed in FAA (5% formalin, 5% glacial acetic acid, 70% ethanol and 20% water) for two weeks and transferred to 70° ethanol for long-term storage. Stomata were observed from imprints made using transparent nail varnish. A thin coat of transparent nail polish was applied, dried, removed with double-sided tape, and placed on a glass slide to be observed under the microscope (Olympus BX51). Three pictures per imprint were acquired with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ software (Schneider *et al.*, 2012). The number of stomata per  $\text{mm}^2$  were recorded for each picture and averaged.

### *Water-related leaf traits*

The leaf relative water content (RWC, %) was calculated as  $(FM - DM / TM - DM) \times 100$ . Midday leaf water potential ( $\psi_{md}$ , MPa) was measured with thermocouple psychrometers (76-1VC leaf cutter thermocouple psychrometer, Merrill Specialty Equipment) connected to a PsyPro water potential data-logger (Psypro; Wescor Inc.). Each psychrometer was calibrated with NaCl solutions to obtain a calibration equation, determined by linear regression relating  $\mu V$  readings to known water potential values (see Campbell and McInnes 1999 for more details). For each leaf, triplicate leaf discs (diameter 6.4 mm) were sampled at midday on the upper part of the leaf with the leaf cutter thermocouple psychrometer and sealed inside. After sampling, the psychrometers were placed in a waterproof plastic bag and in a water bath at a constant temperature of 25°C to equilibrate overnight. Water potential was then calculated from the initial slope of the psychrometric response curve (*i.e.*, plot of thermocouple output in  $\mu V$  with time), previously calibrated with NaCl solutions. Each individual  $\psi_{md}$  corresponds to the mean of three samples.

### *Nutrient-related leaf traits*

The tip of the first leaf was harvested at dusk to quantify chemical and metabolite content. The samples were immediately frozen in liquid nitrogen for 15 min and stored in a freezer until they were freeze-dried (Alpha 1-2 LD; Christ). Each sample was then ground to a fine powder in a Retsch MM301 Mixer Mill and stored in airtight vials until quantification of C, N and P and soluble sugars.

C, N, and P content— About 9–11 mg of leaf powder were used to quantify carbon (C, mg cm<sup>-2</sup>) and nitrogen (N, mg cm<sup>-2</sup>) contents (elemental analyzer, Flash 2000 ThermoFisher, NFISO 10694, NF ISO 13878, NF EN 13137). 3–4 mg of leaf powder were used to quantify total phosphorus (P, mg cm<sup>-2</sup>) contents (spectrometer, Uvi Light XT5 Secomam, spectrometric method with ammonium molybdate at 880 nm

after H<sub>2</sub>SO<sub>4</sub> acid hydrolysis and persulfate oxidation, adapted NFEN 6878). C and N content were also used to get the leaf C/N ratio.

Soluble sugars extraction— Soluble sugars (mg g<sup>-1</sup>) were extracted from 10 to 15 mg powder mixed in 0.5 mL 80 % ethanol (v/v) and incubated for 20 min at 80 °C. Extraction was repeated twice and all three supernatants were collected in a tube and dried (Refrigerated CentriVap Vacuum Concentrators, Labconco). The resulting soluble sugar extract was solubilised in 1.5 mL ultrapure water by sonication and agitation and then stored at -20 °C. Soluble sugars contents were expressed as mg (equivalent glucose) g<sup>-1</sup> DM (Dry Mass).

#### *DNA extraction and sequencing*

Two 1 cm<sup>2</sup> pieces were sampled from the middle part of the youngest mature leaf and surface sterilized by dipping the leaf portions successively into 70% ethanol, ultra-pure water, 9% sodium hypochlorite for 1 min each. Then, the leaf portions were washed twice 1 min each in ultra-pure water and finally immersed 1 min in a CTAB buffer (2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone, 100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM Na<sub>2</sub> EDTA). The sterilized samples were subsequently stored at -80 °C in 2mL Eppendorf sterile tubes for downstream DNA extractions.

DNA was extracted using a CTAB extraction method (Carrell & Frank, 2014). The samples were ground using two sterile beads (2 and 4 mm) and a grinder (type Tissue Lyser II RETSCH). We added 1400 µL of hot (60 °C) extraction buffer (Trisma base 100 mM, NaCl 1.4 M, EDTA Na<sub>2</sub>/H<sub>2</sub>O 20 mM, 2 % CTAB, 2 % PolyVinyl Pyrrolidone, Ascorbic acid 0.1% and β-mercapto-ethanol.) and incubated the mixture for 30 min at 60°C in an incubator shaker (New Brunswick I26 Incubator Shaker). A first deproteinization was performed by adding 600 µL of Dichloromethane/Chloroform/Isoamylalcohol (25:24:1) before centrifugation at

9600 rpm for 30 min at 20 °C (Sigma 4-16KS, Sigma, Germany). Supernatant was transferred to new 2mL Eppendorf tubes for a second deproteinization with 500µL of Chloroform/Isoamylalcohol (24:1) and centrifugating as for the first deproteinization. Supernatant was transferred to new 2mL Eppendorf tubes. Nucleic acids were precipitated by adding 120 µl of sodium acetate and 1 mL of cold Isopropanol (-20°C). Tubes were stored at -20°C for 12 h and then centrifugated at 4°C at 9600 rpm for 30 minutes. The supernatant was discarded, and a final wash was performed using 1mL of 70% cold ethanol (-20°C) and centrifugated at 9600 rpm at 4°C for 20 min. Ethanol was discarded, and DNA resuspended in 100 µL of TE buffer (1.0 M Tris-HCL and 0.1 M EDTA) and stored at -20°C.

To characterise fungal communities, ITS2 nuclear rDNA gene was amplified using the ITS86F forward (GTGAATCATCGAATCTTTGAA, Turenne *et al.* 1999) and the ITS4R reverse (TCCTCCGCTTATTGATATGC, Op De Beeck *et al.* 2014). For bacteria, the V5-V6 region of the bacterial 16s RNA gene was amplified using the chloroplast-excluding forward primer 799F (AACMGGATTAGATACCKG, Chelius and Triplett 2001) and the reverse primer 1115R (AGGGTTGCGCTCGTTG, Redford *et al.* 2010). Forward and reverse primers were tagged using eight nucleotide tags for post-sequencing discrimination. Polymerase Chained Reaction (PCR) was performed twice in a total volume of 25 µL (resulting in 50 µL of amplicons) comprising 5µL of 5X Blend Master Mix, 12µL of ultra-pure water, 1µL of Bovine Serum Albumin (BSA), 2µL of each primer and 3µL of DNA. PCR were performed with a Tetrad2 thermocycler (Tetrad2, BioRad, USA) under the following parameters for both primers: 15 min polymerase reactivation at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 58 °C, 30 s at 72 °C followed by a final step of 7 min at 72 °C. Amplicons were purified with magnetic beads according to the manufacturer (CleanNGS beads, CleanNA), quantified with a fluorescence-based method (Quant-it PicoGreen dsDNA assay kit; Thermo Fisher Scientific), and pooled in equimolar

conditions. The library was built using the Fasteris MetaFast protocol (FASTERIS SA, Plan-les-Ouates, Switzerland) to minimize the amounts of tag-switches (Esling *et al.*, 2015) and sequenced on one MiSeq Illumina run (FASTERIS SA,) for each primer using the paired-end sequencing technology (Metafast protocol, FASTERIS SA).

### *Bioinformatics*

Sequencing output was then processed using R (R Core Team, 2021), the OBITools (Boyer *et al.*, 2016), and SUMAclust (Mercier *et al.*, 2013) wrapped in a Snakemake workflow (Mölder *et al.*, 2021). The snakemake pipeline template is publicly hosted on github ([https://github.com/LafontRapnouilTristan/OBIholo\\_pipeline](https://github.com/LafontRapnouilTristan/OBIholo_pipeline)).

Using the OBITools v-1.2.13, sequences were aligned and filtered based on alignment quality score (threshold of 40), demultiplexed, filtered on length (> 80 pb) and sequencing quality (> 20) and dereplicated. OTUs were clustered using SUMAclust v-1.0.31 (default parameters) and Taxonomic assignments were performed using the RDP Naive Bayesian Classifier algorithm (Wang *et al.*, 2007) implemented in the assignTaxonomy function of the R package dada2 v-1.26 (Callahan *et al.*, 2016), with an 80 % confidence threshold. The classifier was trained with the SILVA database v138.1 and the UNITE database for bacteria and fungi, respectively. Used releases were UNITE database sh\_general\_release\_dynamic\_16.10.2022 (Abarenkov *et al.*, 2022) and the bacterial silva\_nr\_v138.1\_train\_set (Quast *et al.*, 2013). Although the use of the OBITools allowed the removal of erroneous sequences introduced during PCR and Sequencing, further processing based on controls and read counts using the metabar R package (Zinger *et al.*, 2021) were used to filter spurious OTUs and contaminants. Putative contaminants OTUs, present in high abundance in negative controls were removed using 'contaslayer' function. Additionally, abundance of OTUs rare in some samples while abundant in other were considered to results from tag jumps (Schnell *et al.*, 2015) and set to 0



('tagjumpslayer' function, threshold = 0.05). This assumes that if otherwise abundant OTUs became rare in one sample, this would likely indicate an artefactual presence in this sample. Finally, OTUs assigned to chloroplasts, mitochondria and non-fungal or bacterial DNA as well as singletons were removed from the dataset.

### *Data analyses*

All data analyses were conducted with R (v4.3.0, R Core Team, 2023) and R studio (Posit team, 2023).

To investigate the effects of the treatments on the phenotype of *A. mertensii*, we performed a PCA on all measured traits with Factominer package (Lê *et al.*, 2008). Permutational multivariate analysis of variance (PERMANOVA) were performed with the adonis2 function in vegan using 1000 permutations (Oksanen *et al.*, 2022). Post-hoc multilevel pairwise analysis was performed using the pairwise.adonis function with Holm-adjusted P values (Martinez Arbizu, 2022). Then, kruskall wallis tests were performed to compare trait values between treatments. When the kruskall wallis were significant, pairwise Wilcoxon tests were performed to test for pairwise differences.

Microbial  $\alpha$ -diversity was assessed using the Hill number framework for different q values corresponding to the observed richness (q = 0) and the exponential of the Shannon index (q = 1). The Shannon index is particularly relevant in the case of under-sampling as it gives less weight to rare species and thus to deal with the high heterogeneity in sampling depth (Haegeman *et al.*, 2013; Calderón-Sanou *et al.*, 2020) (Fig. S7). To examine patterns of microbial community dissimilarity within treatment, we used pairwise  $\beta$ -diversity as computed by the hill\_taxa\_parti\_pairwise function of the hillR package with q = 1 (Li, 2018). Then for each sample its pairwise  $\beta$ -diversity (q = 1) with members of the same treatment

was averaged to evaluate its mean divergence to the communities harboured by plants growing in similar conditions. Higher average  $\beta$ -diversity values within treatment indicate higher microbial species turnover, and consequently higher community heterogeneity.

In order to visualize the effect of treatments and plant traits on bacterial and fungal microbiota, we performed distance-based redundancy analyses (dbRDA) using the `dbrda` function of the `vegan` package (Oksanen *et al.* 2022). We calculated Morisita-Horn distance on Hellinger transformed communities to limit the weight of rare OTUs. To reduce its effect on the analysis the sequencing depth (*i.e.*, number of reads) of each sample was partialled out in the `dbrda` model using the “Condition” option available in `vegan`. We performed a model selection with the `ordiR2step` function in `vegan` to keep relevant variables based on significance and  $R^2$ . To identify treatment induced differences, PERMANOVA on the `dbrda` scores were performed as for traits PCA. Post-hoc multilevel pairwise analysis was performed on the sites scores using the `pairwise.adonis` function with Holm-adjusted P values.

To identify microbial OTUs that covaried with leaf traits, we performed Threshold Indicator Taxa Analysis (TITAN) using the TITAN2 package v2.4.1 (Baker *et al.*, 2020). We considered the full range of values for each leaf trait, irrespective of treatments, in order to have the complete gradient of trait values. To meet the analysis requirements, OTUs present in fewer than three samples or with fewer than 100 reads were removed from the dataset. For each of the 14 leaf traits (PCA-derived traits excluding size-related ones, *i.e.*, number of leaves, leaf width, length, and plant height), the analysis quantified the change in OTUs relative abundance related to leaf trait values. The analysis computed a z-score, representing the strength and the direction (*i.e.*, positive, or negative) of the relationship between each OTU and the leaf traits. Associations between an OTU and a trait in 95% of 500 bootstraps were considered significant.

## Results

### *Effects of soil fertility and water deficit on the plant phenotypes*

The treatments induced significant phenotypic differences among the plants. The two dimensions of the PCA explained 54.2% of the data variability (36.2% and 18% for PC1 and PC2 respectively, Fig. 27). The first dimension was associated with plant size and leaf structural traits such as leaf thickness, number of leaves, leaf width, plant height, leaf length as well as RWC, LMA, and water potential that all contributed more than expected to this dimension (Fig. S8). The first dimension mainly distinguished the well-watered and water-deficit treatments with the former on the left side and the latter on the right side of the plot (Fig. 27). The second dimension was characterised by leaf chemistry, including leaf nitrogen and phosphorous contents, C/N ratio, and chlorophyll content (Fig. S8), and it distinguished the substrate fertility, with the rich substrates located at the top and the poor substrates located at the bottom of the plot. PerMANOVA analysis revealed a significant effect of the substrate fertility and water supply on plant traits ( $F= 15.22$ ,  $Df= 5$ ,  $p < 0.001$ ). Specifically, the phenotypes of the plants were significantly distinct between all treatments but for Rich-Dry and Intermediate-Dry (Pairwise.adonis, Holm adjusted  $P < 0.02$  in all cases, Table S4). All traits were influenced by either substrate fertility, water supply, or both as shown by kruskal-walis tests followed if significant by pairwise wilcoxon test whit holm corrected p-values but  $F_v/F_m$  pairwise tests were not significant (Table S5).

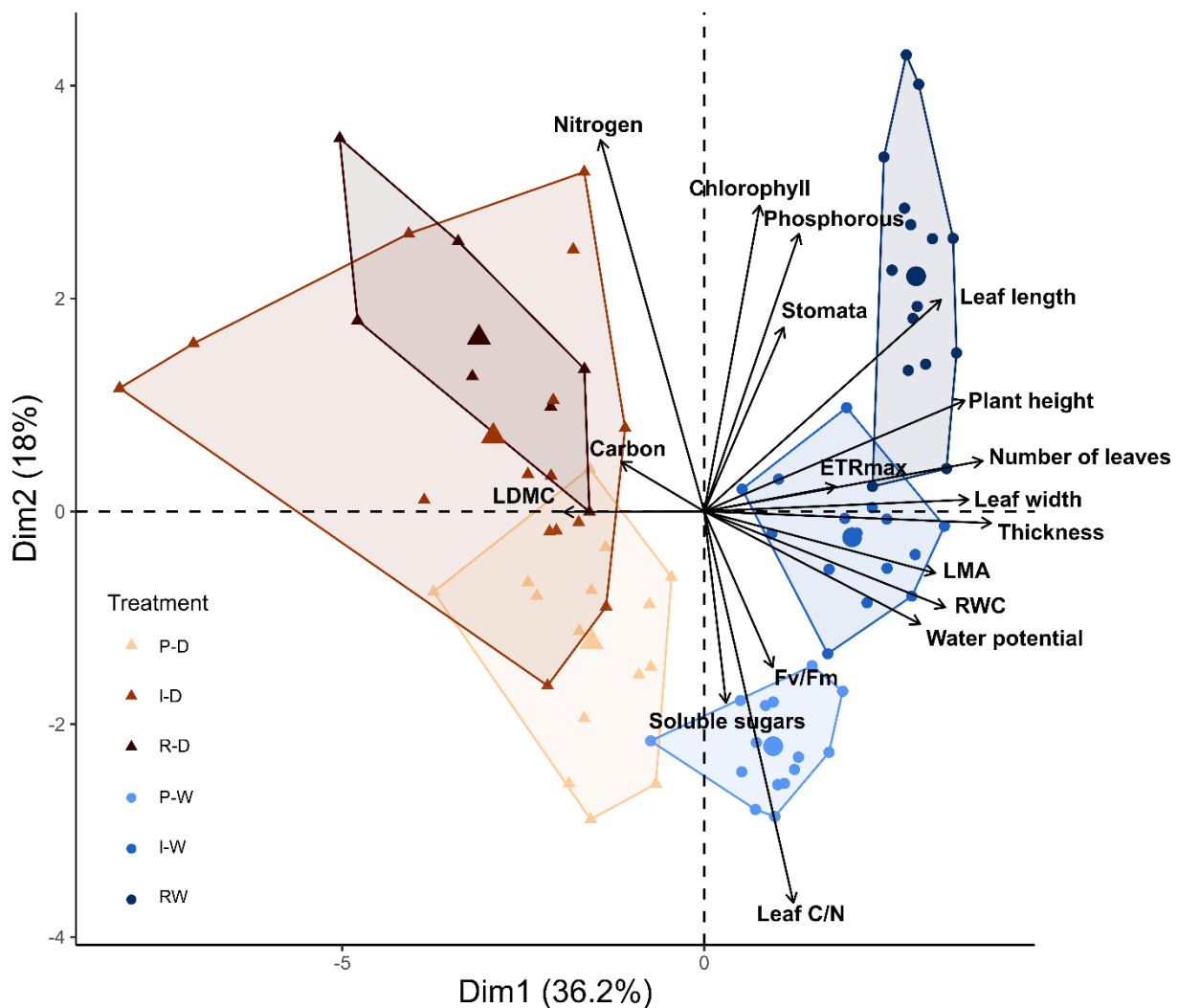


Figure 27: A) Biplot of the two first dimensions of the PCA on plant traits. Arrows represent the variable projection on the first dimensions and dots the individuals projections grouped by treatments by convex hulls. Dots and hulls are coloured according to treatments and bigger dots represent the groups centroids. Treatments are P-D (Poor-Dry), Dry Sand/Potting soil; I-D (Intermediate-Dry), Dry Potting soil/Sand; R-D (Rich-Dry), Dry Potting soil; P-W (Poor-Wet), Wet Sand/Potting soil; I-W (Intermediate-Wet), Wet Potting soil/Sand and, R-W (Rich-Wet), Wet Potting soil. Triangles indicates individuals from Dry treatments and dots those from the Wet treatments. Leaf traits are C/N, leaf carbon and nitrogen content ratio; C, leaf carbon content ( $\text{mg g}^{-1}$ ); Chlorophyll, leaf chlorophyll content ( $\mu\text{g cm}^{-2}$ );  $\text{ETR}_{\text{max}}$ , maximum electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $F_v/F_m$ , PSII maximum quantum efficiency; LDMC, leaf dry mass content ( $\text{g}_{\text{DM}} \text{g}_{\text{FM}}^{-1}$ ); Leaf length, longest leaf length (cm); Leaf width, longest leaf width (cm); LMA, leaf mass area ( $\text{g m}^{-2}$ ); Nitrogen, leaf nitrogen content ( $\text{mg g}^{-1}$ ); Number of leaves, number of green leaves; Phosphorous, leaf phosphorous content ( $\text{mg g}^{-1}$ ); Plant height (cm); RWC, leaf Relative Water Content (%); Soluble sugar, leaf soluble sugars content ( $\mu\text{g mg}^{-1}$ ); Stomata, leaf stomatal density ( $\text{Nb mm}^{-2}$ ); Thickness, leaf blade thickness (mm); Water potential (MPa).

### *Composition of the bacterial and fungal communities*

For bacteria, a total of 1281 non-singletons OTUs were found, with an average of 74 OTUs per sample (ranging from 19 to 256). The average number of reads per sample was 4682, ranging from 253 to 14556. Bacterial communities were

dominated by six classes including *Alphaproteobacteria* (20.5% of total reads across treatments), *Gammaproteobacteria* (13.1%), *Actinobacteria* (11.6%), *Bacteroida* (10%), *Bacilli* (6.7%), *Clostridia* (4.6%) and, *Polyangia* (2.1%) (for more details see Table S6 and Fig. S9). For fungi, a total of 1592 non-singletons OTUs were found, with an average of 153 OTUs per sample (ranging from 17 to 288). The average number of reads per sample was 35414 (ranging from 3832 to 79371). Leaf fungal communities consisted in seven major classes with *Sordariomycetes* (29.6%), *Dothideomycetes* (17.6%), *Agaricomycetes* (13.9%), *Eurotiomycetes* (5.5%), *Leotiomycetes* (2.3%), *Saccharomycetes* (1.8%) and, *Tremellomycetes* (1.6%) (Table S6 and Fig. S9).

For bacteria, we did not find significant differences in OTU richness among treatments when considering both observed richness and Shannon index (Fig 28A, B). However, a large variation in richness and Shannon index was observed within the Rich-Dry treatment. For fungi, we found slight differences in observed richness between some treatments (Fig. 328). In particular, leaves from the Intermediate-Wet treatment had lower fungal richness compared to those from Rich-Dry and Intermediate-Dry and Rich-Wet treatments. The Shannon index of fungal communities were similar across treatments, but with large variations within the Rich-Dry and Intermediate-Wet treatments (Fig. 28D).

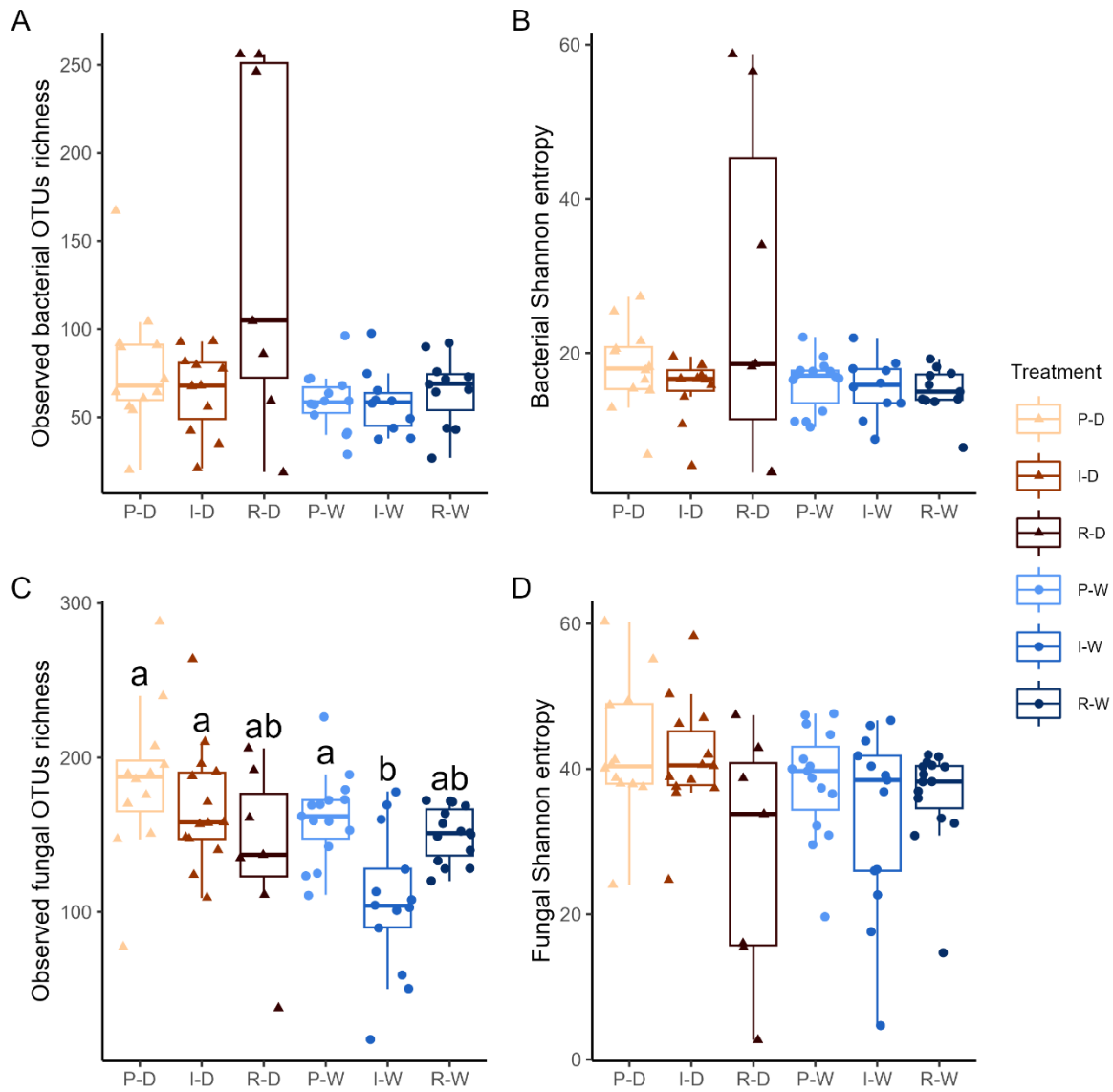


Figure 28: A) Observed bacterial OTU richness, B) Bacterial Shannon entropy, C) Observed fungal OTU richness and, D) Fungal Shannon entropy. Treatments are P-D (Poor-Dry), Dry Sand/Potting soil; I-D (Intermediate-Dry), Dry Potting soil/Sand; R-D (Rich-Dry), Dry Potting soil; P-W (Poor-Wet), Wet Sand/Potting soil; I-W (Intermediate-Wet), Wet Potting soil/Sand and, R-W (Rich-Wet), Wet Potting soil. Triangles indicates individuals from Dry treatments. Different letters indicate significant differences between treatments based on pairwise Wilcoxon test ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ). No letters are displayed when the Kruskal-Wallis was not significant.

### *Within treatment heterogeneity*

The average within treatment pairwise  $\beta$ -diversity differed between treatments for both bacterial and fungal communities (Fig. 29A and B). For bacteria, well-watered treatment showed an increase in microbial community heterogeneity as the substrate got poorer with the Intermediate-Wet treatment having intermediary

values (Fig. 29A). There was no difference in microbial community heterogeneity between the nutrient poor treatment from both dry and wet conditions. The Rich-Dry treatment had the highest microbial community heterogeneity whilst not significantly different from the Intermediate-Dry treatment, likely due to outliers in the latter. Fungal communities in the Dry treatments showed similar pattern as bacteria with an increase in heterogeneity as soil got richer (Fig. 29B). However, for wet treatments, intermediate substrate fertility had significantly higher microbial community heterogeneity than rich substrate but not from poor substrate.

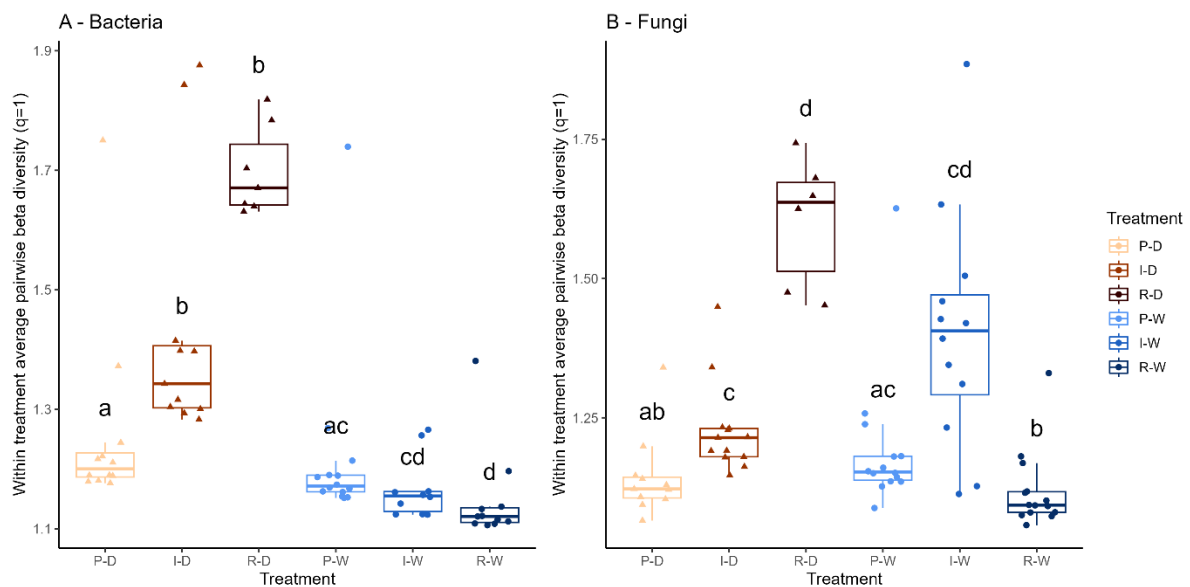


Figure 29: Mean within treatment pairwise beta-diversity with hill numbers ( $q = 1$ ) for A) Bacteria and B) Fungal communities. Treatments are P-D (Poor-Dry), Dry Sand/Potting soil; I-D (Intermediate-Dry), Dry Potting soil/Sand; R-D (Rich-Dry), Dry Potting soil; P-W (Poor-Wet), Wet Sand/Potting soil; I-W (Intermediate-Wet), Wet Potting soil/Sand and, R-W (Rich-Wet), Wet Potting soil. Triangles indicates individuals from Dry treatments. Different letters indicate significant differences between treatments based on pairwise Wilcoxon test ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ).

## Drivers of the bacterial and fungal communities

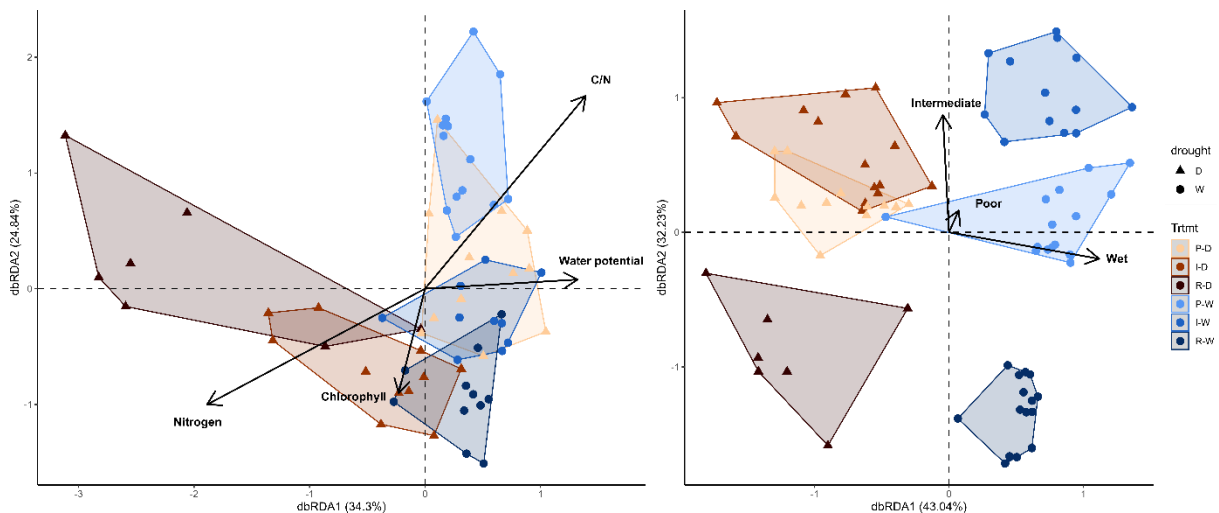


Figure 30: dbRDA biplots for A) bacterial and, B) fungal communities. Arrows are the projections of variables that were kept in the model after forward selection. Dots are the individuals' projection in the dbRDA plan, grouped in convex hulls and coloured according to treatments. Treatments are P-D (Poor-Dry), Dry Sand/Potting soil; I-D (Intermediate-Dry), Dry Potting soil/Sand; R-D (Rich-Dry), Dry Potting soil; P-W (Poor-Wet), Wet Sand/Potting soil; I-W (Intermediate-Wet), Wet Potting soil/Sand and, R-W (Rich-Wet), Wet Potting soil. Triangles indicates individuals from Dry treatments. Variables kept in the dbRDA models are, for bacteria, chlorophyll, leaf chlorophyll content ( $\mu\text{g cm}^{-2}$ ); C/N, leaf carbon and nitrogen content ratio; Nitrogen, leaf nitrogen content ( $\text{mg g}^{-1}$ ); Water potential (MPa). For fungi, were kept, drought, the irrigation status of the plants (W or D for wet and dry, respectively) and, Substrate, the soil treatments of the plants (P, PS and SP for peat, 2/3 peat-1/3 white-sand mix and 2/3 white-sand-1/3 peat, respectively). For drought and Substrate, the arrows correspond to the differential effect of the current level compared to reference (*i.e.*, D for drought and P for Substrate).

The dbRDA-based variable forward selection procedure for bacterial and fungal communities resulted in two distinct models (Fig. 30A-B). Treatments were associated with different location of bacterial communities in the dbRDA space (PERMANOVA,  $F = 10.208$ ,  $R^2 = 0.464$ ,  $p = 0.001$ ). Among the six treatments, most of the pairwise comparisons were significantly different ( $p < 0.02$ ), with the exception of Rich-Wet and Intermediate-Wet treatments ( $p = 0.099$ ; Fig. 30A, Table S7). We found that leaf traits related to water status (*i.e.*, water potential), nutrients (*i.e.*, leaf C/N ratio, nitrogen), and chlorophyll contents played a significant role in structuring bacterial communities ( $p < 0.05$ , Table S8). The position of fungal communities in the dbRDA space differed according to treatments (PERMANOVA,  $F = 74.142$ ,  $R^2 = 0.84$ ,  $p = 0.001$ ). Among the six treatments, all the pairwise



comparisons were significantly different ( $p < 0.02$ , Fig. 30B, Table S9). No leaf trait was selected during the forward selection, only the treatment conditions (*i.e.*, drought and substrate) played a significant role in structuring fungal communities (Table S10).

#### *Linking microbial taxa with leaf traits*

TITAN analysis identified 58 bacterial OTUs whose relative abundance changed significantly with the values of at least one of the leaf traits (Fig. 31). Among these OTUs, 31 increased in abundance with certain traits, 5 decreased in abundance, and 22 showed both increased and decreased depending on the leaf traits. Structural traits (*i.e.*, LMA, lamina thickness, and stomatal density) were associated with 34 OTUs, nutrient status traits (leaf N and P content, soluble sugars, and C/N ratio) were associated with 54 OTUs, photosynthetic status traits (*i.e.*,  $ETR_{max}$ ,  $F_v/F_m$ , and chlorophyll content) were associated with 11 OTUs, and water status traits (*i.e.*, RWC and water potential) were associated with 1 OTUs. Specifically, LMA was associated with 28 bacterial OTUs, followed by nitrogen with 24 OTUs, C/N ratio with 18 OTUs, and soluble sugar was associated with 8 OTUs. All other traits were associated with fewer than five OTUs. Bacteria from *Gammaproteobacteria*, *Actinobacteria*, and *Bacili* classes were the most represented.

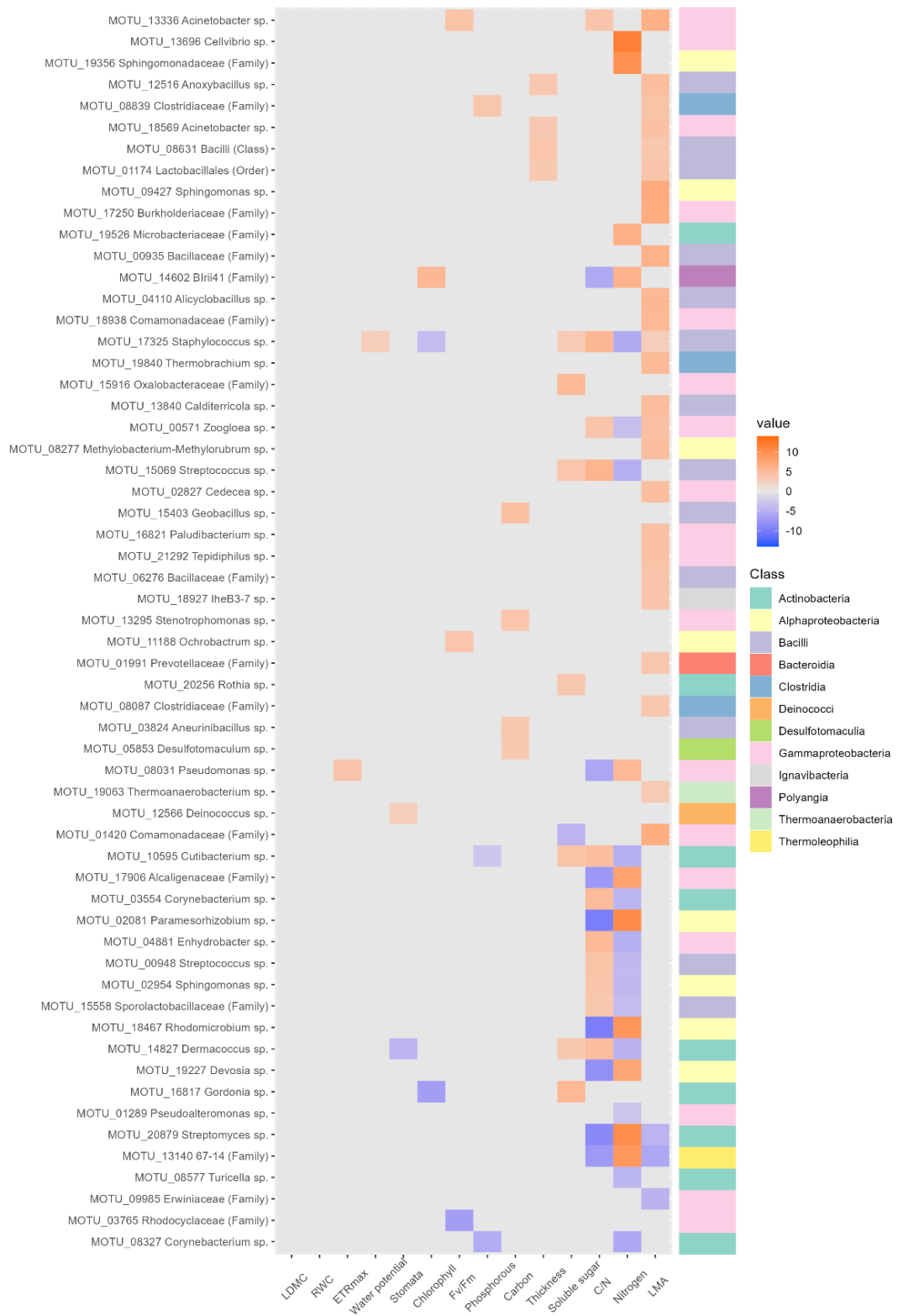


Figure 31: Bacterial MOTUs associated with *Aechmea mertensii* traits across treatments according to TITAN2 analysis. Positive association z-scores (value) between the relative abundance of the MOTUs and the value of the traits are represented in orange and negative in blue. Only MOTUs significantly associated to at least one of the traits are shown. OTUs have been ordered according to their mean z-score, and drought tolerance traits have been ordered according to their number of associated MOTUs. For each MOTU is given its lowest known taxonomic assignment (On the left) and its bacterial class on the right column.

For fungi, 137 OTUs significantly changed their relative abundances according to leaf traits (Fig. 32). Among these OTUs, 44 increased in abundance with certain traits, 52 decreased in abundance, and 41 showed both increased and decreased depending on the leaf traits. Structural traits were associated with 123 OTUs, nutrient status traits were associated with 64 OTUs, photosynthetic status traits were associated with 70 OTUs, and water status traits were associated with 39 OTUs. Leaf thickness, LMA and leaf carbon content were associated with the highest number of OTUs, with 48, 33 and 33 OTUs, respectively. All other traits were associated with between 8 and 29 OTUS. Fungi from the *Sordariomycetes* and *Dothideomycetes* classes were the most represented.

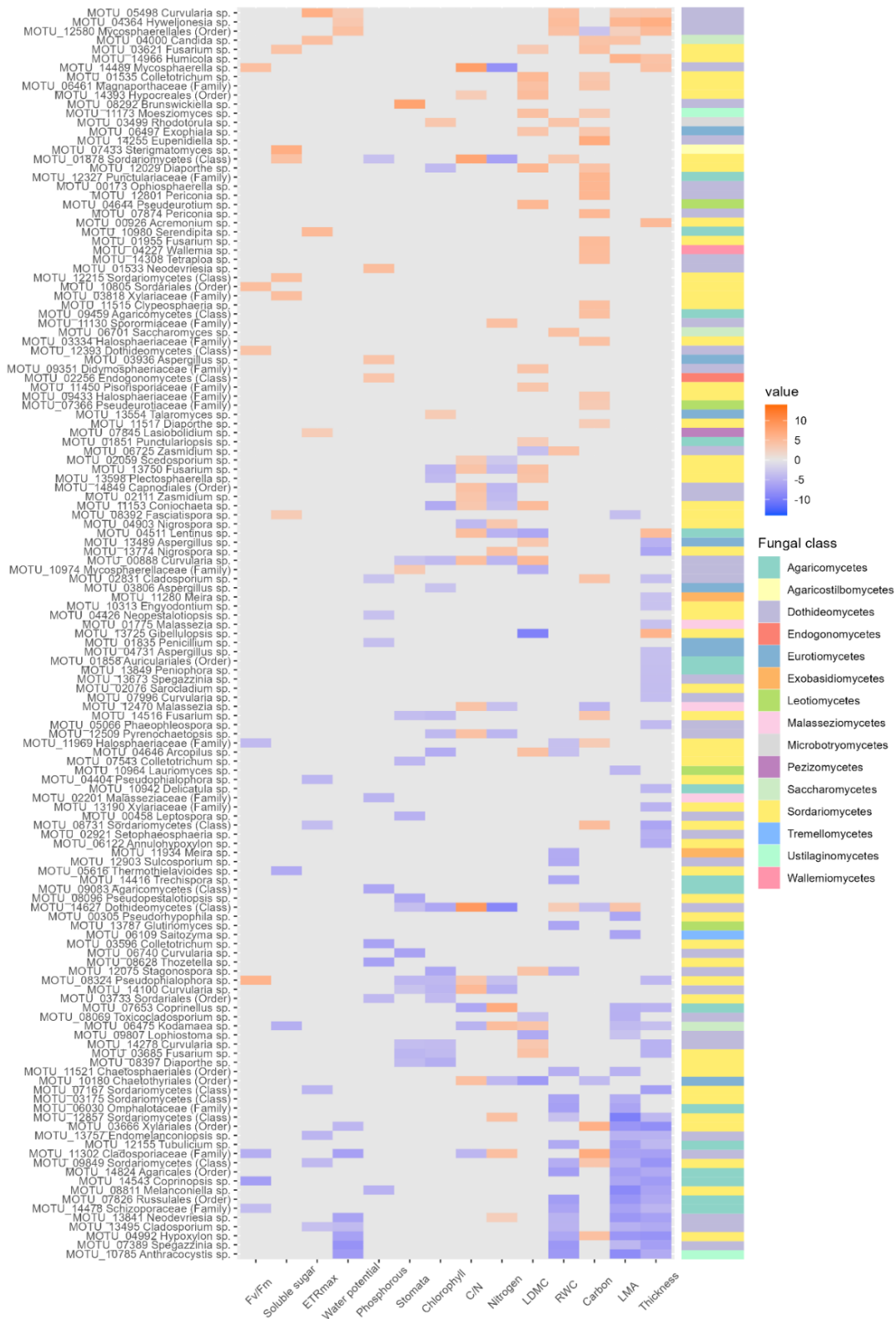


Figure 32: Fungal MOTUs associated with *Aechmea mertensii* traits across treatments according to TITAN2 analysis. Positive association z-scores (value) between the relative abundance of the MOTUs and the value of the traits are represented in orange and negative in blue. Only MOTUs significantly associated to at least one of the traits are shown. OTUs have been ordered according to their mean z-score, and traits have been ordered according to their number of associated MOTUs. For each MOTU is given its lowest known taxonomic assignment and class.

## Discussion

### *Aechmea mertensii* response to substrate fertility and water supply

The availability of mineral nutrients and water are important determinants of plant growth. As predicted, the experimental variations in substrate fertility and water supply had significant effects on the development of *A. mertensii*. This plant species showed a strong phenotypic plasticity in terms of structural, nutrient, water, and photosynthetic status leaf traits. The fertility of the substrate, on which *A. mertensii* was grown from seed to the flowering stage, affected both the size and the morphology of the rosette. This pattern aligns with findings observed in the closely related species *A. aquilega* (Lafont Rapnouil *et al.*, 2023a). Water scarcity negatively influenced *A. mertensii* water status by reducing significantly both the water potential and the relative water content compared to the well-watered plants. Plant size and morphology were significantly hampered by water scarcity, leading to a subsequent decline in growth and development. However, the distinctions between substrate treatments were less pronounced. The reduction in plant size due to drought stress has been commonly observed in various plant species (*e.g.*, Khatun *et al.* 2021; Zhai *et al.* 2023; Batool *et al.* 2023), and it is regarded as an adaptation to reduce the water demand at the whole-plant scale.

Under the combined effect of substrate fertility and water deficit stressors, the plants were able to maintain a certain level of photosynthetic performance, as evidenced by  $F_v/F_m$  values remaining close to the optimal value ( $\geq 0.75$ ). *Aechmea mertensii* is a plant species with crassulacean acid metabolism (CAM), enhancing water and nutrient use efficiencies by absorbing atmospheric  $CO_2$  during the night, thus minimizing transpiration cost (Borland *et al.*, 2014). In *A. aquilega*,  $F_v/F_m$  values started to decrease significantly when the leaf relative water content dropped below 75% (Svensk *et al.*, 2020), which correspond to the average values we found for *A. mertensii* in the dry treatments. Gonçalves *et al.* (2020) showed that *Guzmania monostachia*, when deprived of water and nutrients, was able to

maintain  $F_v/F_m$  levels comparable to those of well-watered and nourished plants. These findings, along with our study, support that bromeliads can adapt to conditions of limited resources through physiological adjustments. Plants grown in the nutrient-rich substrate with limited water supply seemed to experience the most severe treatment, as it caused the death of 8 plants out of 15. Plants from this treatment had the lowest water potential and RWC values, comparable to plants that suffer 10-21 days of drought stress (Svensk *et al.*, 2020). This result might potentially be attributed to the interplay between soil texture and water availability, which could have exacerbated the impact of the drought treatment. Indeed, the size of soil particles is known to influence water retention and the distribution and continuity of pores (Mosaddeghi *et al.*, 2007; Deepagoda *et al.*, 2011).

One notable result from this study is the significantly higher mass-based leaf N content observed in the dry compared to the well-watered treatments. Most of the time, water deficit inhibits the uptake of nitrogen, phosphorus and potassium within the plant (Shangguan *et al.*, 2000; Kirnak *et al.*, 2001; Ciríaco da Silva *et al.*, 2010; Saud *et al.*, 2017). In our study, leaf P content was only slightly reduced in the nutrient-rich substrate when water deficit occurred. For leaf N, all three substrate treatments had significantly higher values during water deficit compared to well-watered treatments. Plants from the dry treatments have not received a continuous supply of water and nutrients like those from the wet treatments and are therefore considered as pulse supplied. *Aechmea mertensii* might have adapted to such pulse-supplied water and nutrient conditions by developing the ability to accumulate large amounts of nutrients in tissues by luxury nutrient uptake (de Mazancourt & Schwartz, 2012; Ohtake *et al.*, 2021) as observed in other bromeliad species (Winkler & Zotz, 2010). Since N is a vital component required for the synthesis of chlorophyll and photosynthetic enzymes in plants (Mu & Chen, 2021), the observed higher leaf N content in the dry

treatments may explain the maintenance of the photosynthetic machinery, with comparable chlorophyll content,  $F_v/F_m$  and  $ETR_{max}$ , compared to the wet treatments.

#### *Effects of substrate fertility and water supply on the microbiota $\alpha$ - and $\beta$ -diversities*

Our study showed that the substrate fertility and water supply did not affect the bacterial richness or the bacterial and fungal Shannon entropy. Only fungal richness exhibited a slight reduction in the Intermediate-Wet treatment. Exposure of microbiota to different biotic or abiotic stressors leads to either significant decrease (Koskella *et al.*, 2017; Rocca *et al.*, 2019; Bhandari *et al.*, 2022; Flemer *et al.*, 2022) or increase in  $\alpha$ -diversity (Yang *et al.*, 2016; Madigan *et al.*, 2019), depending on the type of microbiota considered, the types of stressors and the intensity/duration of the stress. In contrast, we observed an increased  $\beta$ -diversity dispersion in bacterial and fungal composition in response to substrate fertility and water deficit. Specifically, bacterial, and fungal  $\beta$ -diversity dispersion showed a minor yet significant increase with reduced substrate fertility. Nevertheless, an unexplained presence of highly divergent and heterogeneous fungal microbiota was observed in the Intermediate substrate. Intriguingly, under water deficit, we found the opposite pattern, with a strong significant increase in both bacterial and fungal  $\beta$ -diversity dispersion associated with enriched substrate fertility.

Such increase in  $\beta$ -diversity dispersion indicate more dissimilar microbial composition that can be explained by a loss of control of microbiota composition by the host and/or by modification of microbe–microbe interactions and a related cascading effect (Arnault *et al.*, 2022).  $\beta$ -diversity dispersion metrics have been applied in microbiome studies to show destabilization of the microbiome in stressed and diseased corals (Zaneveld *et al.*, 2016). In plants, it has been found higher  $\beta$ -diversity in diseased chili pepper for both fungal and bacterial

communities (Gao *et al.*, 2021). Dysfunctions of the microbiota can lead to dysbiosis, implying a loss of function that leads to a reduction of the host's fitness (Petersen & Round, 2014; Arnault *et al.*, 2022). One of the hypotheses that predicts the differences between healthy and disease-associated microbiota is the Anna Karenina principle, which was first coined by Zaneveld *et al.* (2017) for animal microbiomes and recently introduced by Arnault *et al.* (2022) for plant microbiota. It postulates that disease-associated microbiota are more dissimilar than healthy ones. Further analysis including functional assignments of the OTUs should allow us to investigate for an enrichment in pathogenic microbes under harsh conditions. Furthermore, although plants from water deficit treatments had lower size and growth, they were able to sustain a certain level of photosynthetic and nutrient metabolism and did not present any leaf necrosis. Therefore, changes in fungal and bacterial communities between substrate fertility and water supply treatments might relate to adaptative response.

Plants can employ the “cry for help” strategy to attract distinct microbial communities that enhance their ability to cope with both biotic (Rolfe *et al.*, 2019) and abiotic stresses (Rolli *et al.*, 2021). This strategy is crucial for the survival of plants, as the plant microbiome can be considered as a complementary functional repertoire that expands the capacity of the plants to mitigate the adverse effects of stresses on plant growth and functioning (Mueller & Sachs, 2015; de Vries *et al.*, 2020). In recent years, there has been increasing evidence for such strategy in the rhizosphere and root region, where attraction of microorganism during stress is mediated by root exudates (Rolfe *et al.*, 2019; Rizaludin *et al.*, 2021; Sharma *et al.*, 2023). In addition, root exudates might contribute to nutrient mobilization and their enhanced uptake by roots (Dissanayaka *et al.*, 2017; Rizaludin *et al.*, 2021; Hao *et al.*, 2022), a strategy that could potentially explain, in our study, the relatively high level of N contents in the leaves of plants subjected to water deficit as compared to well-watered plants. However, so far, it remains unknown if the



cry for help strategy exists in the aboveground plant parts (Liu *et al.*, 2020). Less is known about the drivers of microbiome variation in the leaves as compared to the root system (Firrincieli *et al.*, 2020).

#### *Microbial community and taxa linked with host traits and treatments*

We observed significant differences in the leaf bacterial and fungal communities that were influenced by the abiotic environmental conditions in which the plants grew from seed to flowering stage. For bacteria, chlorophyll contents, water potential and nutrient status (leaf nitrogen, leaf C/N ratio) were the primary drivers of the difference in the community structure. Leaf resource uptake strategies would impact local leaf nutrients and water availability that may influence the composition of phyllosphere bacterial communities (Kembel *et al.*, 2014; Laforest-Lapointe *et al.*, 2016; Schimann *et al.*, 2023). Other previous studies have demonstrated that leaf anatomical characters (De Costa *et al.*, 2006), wood density and leaf nutrient content (Kembel *et al.*, 2014; Laforest-Lapointe *et al.*, 2016), and the levels of leaf soluble carbohydrates, calcium, and phenolic compounds (Hunter *et al.*, 2010), all significantly influence bacterial community structure on the leaf surfaces. Li *et al.* (2018) found that host phenotypic traits including leaf length, leaf water content, leaf water storage capacity, leaf dry mass per area, leaf nitrogen content, leaf phosphorous content, leaf potassium content, leaf  $\delta^{13}\text{C}$  values, stomatal conductance, net photosynthetic rate, intercellular carbon dioxide concentration, and transpiration rate were significantly correlated with the diversity and composition of the epiphytic and endophytic bacterial and fungal communities. Traits such as leaf thickness, LMA, and tissue density are also known to influence both leaf bacterial and fungal composition (Kembel *et al.*, 2014; Laforest-Lapointe *et al.*, 2016; González-Teuber *et al.*, 2020). However, in our study, such structural traits did not influence the microbial communities. Changes in the fungal communities were mostly related to the treatments in which the plant

growth and not to any leaf traits. One possible explanation is that other leaf traits, not considered in this study, may have a “hidden” contribution to such changes in microbial communities. Indeed, recent studies have shown that fungal endophytes communities can be impacted by leaf biochemical properties such as leaf chlorophylls and flavonoids (Unterseher *et al.*, 2016), foliar nutrients or levels of toxic compounds (Oono *et al.*, 2020), and foliar content of cell wall polysaccharides, flavonoids, anthocyanins and terpenoids (González-Teuber *et al.*, 2020).

In our study, *Proteobacteria*, *Firmicutes*, and *Actinobacteria* accounted for more than 55% of all samples. These bacterial phyla were typical of the aboveground parts and the belowground parts of different plant host species (Bodenhausen *et al.*, 2013; Jin *et al.*, 2014; Zhang *et al.*, 2019). TITAN indicated that 58 bacterial OTUs (5% of the total OTUs) covaried with plant traits. From these OTUs, 48 % and 41 % were significantly associated with LMA and leaf nitrogen content, respectively. These OTUs were thus particularly related to change in plant traits due to the modifications of environmental conditions. Bacteria from *Proteobacteria* (49%), *Firmicutes* (25%), and *Actinobacteria* (15%) were the three main phyla that covaried with plant traits. It has been demonstrated that the *Proteobacteria* (including *Gammaproteobacteria* and *Alphaproteobacteria*), *Firmicutes* and *Actinobacteria* may protect their plant hosts from disease or promote growth (Taghavi *et al.*, 2009; Kumar *et al.*, 2012; Saikia *et al.*, 2022; Borah *et al.*, 2022). Concerning fungi, *Sordariomycetes*, *Dothideomycetes*, and *Agaricomycetes* classes collectively represented more than 60% of all samples. The five more abundant genus were *Fusarium*, *Curvularia*, *Nigrospora*, *Diaporthe*, and *Exophiala*, that accounted for 35-55 % of the total genus depending on the treatments. While *Diaporthe* was classified as pathotroph-saprotroph-symbiotroph (Gomes *et al.*, 2013) all four others genus were symbiotroph with *Nigrospora* and *Exophiala* known to be dark septate endophytes (Bonfim *et al.*,

2016). A total of 137 fungal OTUs (8% of the total OTUs) covaried with plant traits. Structural traits such as leaf thickness and LMA were the traits that drive 35 and 24 %, respectively, of the OTUs. Fungi from *Sordariomycetes* (53 OTUs, 38.6%) and *Dothideomycetes* (39 OTUs, 28.4%) were the two main classes that covaried with plant traits. This study revealed statistical correlation between the relative abundances of several bacterial and fungal OTUs and leaf traits in *A. mertensii*. Because some traits are positively and negatively correlated with some OTUs, and the way round, we have to go deeper in the interpretation of the functional role of each OTU.

### *Conclusion*

In summary, our study provides evidence that environmental changes affect the plant holobiont. Firstly, *A. mertensii* responded differently to changes in the abiotic environment because of morphological, physiological, and biochemical adjustments. Plants performed better under conditions of high substrate fertility and high-water supply whereas their performance declined under conditions of low water supply conditions. Secondly, while the  $\alpha$ -diversity of both bacteria and fungi did not differ between treatments, we found significant  $\beta$ -diversity differences among treatments. Changes in microbiota community composition may result from more dissimilar microbiota communities in plants with lower performance, possibly indicating a lower ability of the host plants to regulate its microbiota (*cf.* Anna Karenina principle). Alternatively, plants may attract distinct microbial communities that enhance their capacity to cope with changes in nutrient and water supply (*cf.* "cry for help" theory). Thirdly, we identified positive and negative correlations between the relative abundances of several bacterial and fungal OTUs and functional trait expressions related to nutrient and water supply. Generally, most of the studies have focused on either bacterial or fungal communities of the plant holobiont, but their co-occurrence and interactions are

likely key to the holobiont we found different response of fungal and bacterial endophytes to environmental conditions. More data integrating bacteria and fungi within a single system are needed. Host-microbiota coordination is a key hypothesis of the holobiont theory, but further experimental data is required to explore the holobiont response to the environment. We particularly emphasize the importance of using non-crop and model species to better understand the diversity of responses, which is likely to mirror the diversity of host-microbiota systems *in natura*.

## Supplementary Information

Fig. S6 Substrate C, N and P content.

Fig. S7 Number of reads per sample.

Fig. S8 Variable contribution to the first and second PCA axes.

Tab. S4 Result table for pairwise perMANOVA performed on leaf ecophysiological traits.

Tab. S5 Statistical summary table of ecophysiological traits. Mean  $\pm$  sd and significance.

Tab. S6 Relative abundance of the most abundant bacterial and fungal taxa.

Fig. S9 Leaf endophytes relative abundance according to treatments at the order rank.

Tab. S7 Result table for pairwise perMANOVA performed on bacterial communities' coordinates in the dbRDA space.

Tab. S8 Result table of the Anova performed on the forward selected bacterial dbRDA model.

Tab. S9 Result table pairwise perMANOVA performed on fungal communities' coordinates in the dbRDA space.

Tab. S10 Result table of the Anova performed on the forward selected fungal dbRDA model.

Fig. S10 Leaf endophytes genus relative abundance according to treatments.

Fig. S11 Leaf endophytes class relative abundance according to treatments.

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# Chapitre IV

*Maternal effects and current environmental conditions affect the phenotype and the microbiota assemblage in Aechmea mertensii seedlings*

Dans ce chapitre nous étudions les effets de l'environnement maternel et actuel sur le phénotype et les communautés endophytes de plantules d'*A. mertensii* âgés de 2 mois.

Nous mettons en évidence l'existence d'effets maternels de type 'silver spoon' sur le phénotype d'*A. mertensii*. C'est-à-dire que les plantules aux meilleures performances ne sont pas ceux dont les conditions actuelles sont similaires aux conditions maternelles (effet "home-site") mais systématiquement ceux issus de mères "riches".

En parallèle cette étude montre une influence de l'environnement maternel sur les communautés d'endophytes fongiques et bactériennes des plantules. Ces résultats sont d'autant plus intéressants que les communautés bactériennes contenues dans les graines ne sont pas affectées par l'environnement là ou celles fongiques le sont. Ces résultats suggèrent l'existence de médiateurs indirects (chimie de la graine, interactions bactéries-champignons) des effets maternels sur le microbiote des plantes.

Cette étude fait partie de la seconde expérience du projet HOLOBROM du méta-programme HOLOFLUX de L'INRAE coordonnée par Céline Leroy avec l'appui d'Heidy Schimann, Lucie Zinger, Corinne Vacher, Eliane Louisana et Sandrine Étienne. J'ai réalisé avec Céline Leroy et Mélodie Schmidt (stagiaire de Master 1) la mise en place, le suivi, l'échantillonnage et les mesures de traits des plantules. Le travail de laboratoire (extraction, PCR) a été réalisé en collaboration avec Eliane Louisana, Sandrine Etienne, Heidy Schimann et Céline Leroy. J'ai ensuite mené les analyses bioinformatiques et le traitement des données. La version actuelle du manuscrit est le fruit de ma collaboration avec Régis Céréghino et Céline Leroy.

**Maternal effects and current environmental conditions affect the phenotype and the microbiota assemblage in *Aechmea mertensii* seedlings.**

Lafont Rapnouil Tristan<sup>1-2</sup>, Heidy Schimann<sup>3</sup>, Eliane Louisana<sup>2</sup>, Sandrine Etienne<sup>2</sup>, Frédéric Julien<sup>4</sup>, Lucie Zinger<sup>5</sup>, Corinne Vacher<sup>3</sup>, Céréghino Régis<sup>4</sup>, Céline Leroy<sup>1-2</sup>

<sup>1</sup>AMAP, Univ. Montpellier, CIRAD, CNRS, INRAE, IRD, Montpellier, France

<sup>2</sup>EcoFoG, AgroParisTech, CIRAD, CNRS, INRAE, Université des Antilles, Université de Guyane, Campus agronomique, Kourou, France

<sup>3</sup>BIOGECO, Univ. Bordeaux, INRAE, Bordeaux, France

<sup>4</sup>Laboratoire Écologie Fonctionnelle et Environnement, Université Paul Sabatier Toulouse 3, CNRS, Toulouse, France

<sup>5</sup>Institut de Biologie de l'ENS (IBENS), Département de Biologie, École Normale Supérieure, CNRS, INSERM, Université PSL, 75005 Paris, France



## Abstract

- *Purpose* Maternal effects allow for rapid response of plant population to local conditions. The vertical transmission of seed-borne microbiota to seedling potentially enables microbiota associated maternal effects. However, little is known about the influence of maternal environment on seed and seedling microbiota. This study aimed to investigate the influence of substrate fertility on seed microbiota and to assess the influence of current and maternal growth condition on seedling performances and roots and leaves fungal and bacterial endophytic communities.
- *Methods* Seeds from *A. mertensii* (Bromeliaceae) plants grown in three substrates of various fertility (P: Poor, I: Intermediate and R: rich) were sowed in a full factorial design in the same three substrates and grown for two months. Then leaf and root ecophysiological traits were measured and tissues sampled for metabarcoding analysis of bacterial (16s rRNA gene) and fungal (ITS) endophytic communities.
- *Results* Our results revealed a strong influence of maternal seed on *A. mertensii* seedling performance as well as leaves and roots microbiota. We provide evidence that direct vertical transmission of microbiota is not the sole mechanism mediating maternal influence over offspring microbiota and that this influence affects both the quantity and quality of microbial symbionts harboured by the progeny. More importantly, we showed that this maternal influence is long lasting (1/6<sup>th</sup> of the plant life cycle).
- *Conclusion* Given the significant contribution of plant microbiota to their health, ecology, and evolution, these results contribute to the growing interest in microbiota-associated maternal effects.

## Introduction

Phenotypic plasticity, the ability of a genotype to produce multiple phenotypes according to different environments (Bradshaw, 1965; Sultan, 2000), is a fundamental strategy used by plants to cope with environmental variability. Phenotypic plasticity includes all the mechanisms linking the expression of the genotype (*i.e.*, the development) and the environment, making it a key aspect of plants ecological development (Sultan, 2003). Plasticity may also occur across generations when the phenotype of the offspring is influenced by the past environments experienced by the previous generation (Agrawal *et al.*, 1999; Galloway & Etterson, 2007; Salinas *et al.*, 2013). Such maternal effects represent a transgenerational form of phenotypic plasticity transmitted to the offspring without involving changes in the DNA sequence (Roach & Wulff, 1987; Mousseau & Fox, 1998; Uller, 2008; Herman & Sultan, 2011). The maternal environment can influence the development and fitness of the offspring, as well as the subsequent development of the mature plant (Agrawal, 2002; Blödner *et al.*, 2007; Vivas *et al.*, 2019, p. 201). Transgenerational plasticity may enable plants to cope with fast-changing environments because it refines the offspring phenotype in anticipation of the environmental conditions they are likely to experience (Herman & Sultan, 2011; Vivas *et al.*, 2020) (Herman & Sultan, 2011; Vivas *et al.* 2020).

The transmission of the maternal effects to the offspring is achieved through two non-exclusive mechanisms: seed provisioning or epigenetic mechanisms (Herman & Sultan, 2011). In seed provisioning, the maternal plants contribute the seeds with biomolecules (*e.g.*, hormones, secondary metabolites, proteins, lipids, RNA) and nutrients (*e.g.* carbohydrates, mineral nutrients) that are transmitted to the offspring (Roach & Wulff, 1987). These substances are allocated by the mother plant to the seed and are used by the germinating seedling to develop the initial stages of the plant (Herman & Sultan, 2011). However, this is an ephemeral mechanism that persists for only a single generation (Elwell *et al.*,

2011). In contrast, epigenetic processes can modify the gene expression (*e.g.*, DNA methylation, histone modification, and small RNA interference) in the parental plants and can be transmitted via the seeds to the offspring (Holeski *et al.*, 2012), potentially influencing the phenotype of the offspring across multiple generations (Herman *et al.*, 2012). One aspect of the biotic maternal environment that may have significant consequences for offspring's phenotype is the impact of associated microbial communities.

Plants host diverse microbial communities that can be considered as part of the extended phenotype of the plants (Zilber-Rosenberg & Rosenberg, 2008; Partida-Martinez & Heil, 2011). This microbiota can affect various aspect of the plant physiology, metabolisms, ecology, and, ultimately, plant fitness (Bulgarelli *et al.*, 2013; Chialva *et al.*, 2022). While the majority of these microbiota are horizontally acquired (*i.e.*, from the environment) over the plant life, a small fraction is vertically transmitted by the mother plants to the offspring through the seeds (Shade *et al.*, 2017; Vannier *et al.*, 2018; Chesneau *et al.*, 2020, 2022; Rochefort *et al.*, 2021). The microbial communities associated with the mother plant aboveground tissues can directly affect the phenotype and fitness of the seedlings (Truyens *et al.*, 2015). Such effects can be considered as part of maternal effects (Vivas *et al.*, 2015; Gundel *et al.*, 2017; Murphy *et al.*, 2023).

Seed microbiota have been shown to play a crucial role in seedling performance, affecting germination success (Mitra *et al.*, 2014; Zahoranová *et al.*, 2016; Bakhshandeh *et al.*, 2020) as well as seedling survival or growth (Clay, 1987; Novas *et al.*, 2003; Pickens *et al.*, 2003; Shearin *et al.*, 2018; White *et al.*, 2018; Leroy *et al.*, 2019; Chesneau *et al.*, 2022). While the number of taxa in the seed microbiota is significantly lower compared to other maternal tissues, it still constitutes a diverse community (Nelson, 2018). This microbial community represents the starting point for the future seedling microbiota and can have important consequences in the microbiota assembly through priority effects (Shade *et al.*,

2017). The initial microbiota can favour or prevent the establishment and proliferation of other microorganisms, thereby exerting a major effects on the microbiota assembly and, consequently, on the host phenotype (Vivas *et al.*, 2015; Shade *et al.*, 2017; Murphy *et al.*, 2023). Vertical transmission of microbiota from mother to offspring is particularly important for plant population dynamics and evolution because it can provide offspring with beneficial microbiota that facilitate adaptation to local biotic and abiotic conditions (Shade *et al.*, 2017). As a result, the microbiota serves functions that enhance the plant ability to respond to various environmental conditions (Vannier *et al.*, 2015).

Plants growing in different environments often display distinct microbiota due to variations in both the microbial reservoir and host recruitment under specific conditions (Vandenkoornhuys *et al.* 2015; Trivedi *et al.* 2020, Chapter III). Transmitting microorganisms adapted to the experienced environmental conditions could thus be a way for the maternal plants to provide a critical advantage to their offspring (Vannier *et al.*, 2018), corresponding to adaptive maternal effects (Mousseau & Fox, 1998). This type of maternal effects would result in an advantage for seedlings growing in an environment that matches the maternal one (home-site advantage). The environmental conditions experienced by the maternal plants can influence the composition of microbial communities in their offspring (Vivas *et al.*, 2015, p. 20, 2017). However, the effect of maternal environment on the offspring's phenotype through the microbiota transmitted to the seed is still overlooked. Therefore, understanding how much of the microbiota is inherited from the mother plant through the seeds, to what extent these vertically-transmitted microbiota are influenced by changes in the abiotic maternal environment, and how these microbiota regulate seed germination and plant growth is crucial to our ability to predict plant species dynamics.

To date, many studies have investigated plant transgenerational plasticity under various environmental conditions (Sultan *et al.*, 2009; Latzel *et al.*, 2014;

Münzbergová & Hadincová, 2017; Groot *et al.*, 2017). However, studies linking transgenerational phenotypic plasticity to the maternal effects of associated microbial communities are scarce. In the present study, we investigated the contributions of the maternal environment and the current abiotic environment of the offspring to the performance and composition of microbial communities in the seedlings. To examine such maternal effects, we conducted a reciprocal transplant experiment in a full factorial design. This experiment allowed us to quantify maternal effects of the offspring microbiota and phenotype, as well as to tease apart the effects of abiotic environment from maternal effects on the offspring microbiota and phenotype. We hypothesised that maternal environmental conditions (substrate fertility) would improve offspring performance when seeds were planted under the same type of environmental condition of the maternal plant. Likewise, we hypothesised that a more beneficial microbiota would benefit the offspring growing in the same abiotic environmental conditions as the maternal plants.

## Material and methods

### *Studied species*

*Aechmea mertensii* Schult.f. (Bromeliaceae) is a tank-forming bromeliad belonging to the subfamily Bromelioideae with CAM photosynthesis (Griffiths & Smith, 1983). This bromeliad forms a rosette with spirally arranged lanceolate leaves. This species was selected as our model system because (i) we recently provided the first evidence of endophytic fungi in both the seeds and seedlings that were most likely transmitted vertically (Leroy *et al.*, 2019), (ii) fructification occurs *ca.* 10-12 months after sowing (C. Leroy, pers. obs.) allowing to study several generations of the plant in a reasonable amount of time, and (iii) terminal inflorescence form

numerous non-dormant seeds that can germinate only 3-5 days after sowing, a convenient feature for the proposed experimental approach (Leroy *et al.*, 2017).

#### *Experimental procedure and growth conditions*

To investigate the maternal and environmental effects on seedling phenotypes and their endophytic microbiota, we conducted a two-step experiment in a greenhouse at the Campus agronomique in Kourou, French Guiana. The first step consisted in growing *A. mertensii*, from seeds collected from a single plant, in three different substrates (see Chapter III): 100% commercial potting soil (rich substrate, R), a mixture of 70 % potting soil and 30 % (v:v) white sand (Intermediate substrate, I), and a mixture of 30 % of potting soil and 70 % (v:v) white sand (Poor substrate, P). At fructification, 27 seeds were collected from each of the 30 mother plants (10 per substrate treatment) and sowed in a common garden (*i.e.*, seeds of different treatments in the same environmental condition) and a reciprocal transplantation design (*i.e.*, seeds of the same treatment in different environmental conditions) to obtain generation N+2 seedlings. A total of 9 treatments was obtained (3 substrate treatments of the mother plants x 3 substrate treatments of the seedlings, Fig. 33). For each mother plant, 9 seeds were sowed per substrate (9 x 3 substrates = 27 seeds), resulting in a total of 1215 seeds. The seeds were germinated in the three substrates in horticultural seedling trays until they reached the age of 2 months. They were irrigated with tap water twice a day at 8:00 am and 6:00 pm.

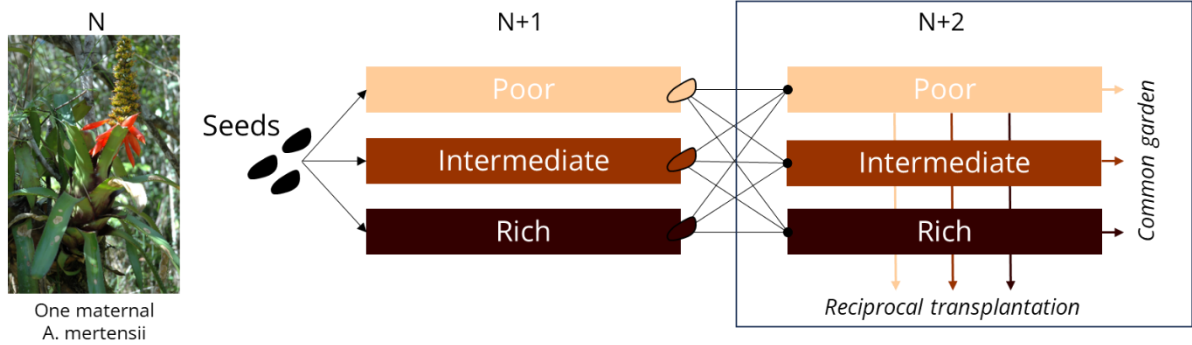


Figure 33: Diagram summary of the experimental design. Seeds from plants grown in three different substrates were sowed in a full factorial design in the same three substrates to tease apart the effect of current and maternal environmental conditions on seedling performances and microbiota.

Chemical analyses of the three substrates were conducted on triplicate samples to determine the carbon, nitrogen, and phosphorus contents (PAPC, Toulouse). The CNP contents of the substrates decreased from rich to poor soils, with the poor and intermediate substrates being closer in composition (supplementary information, Fig. S12). Greenhouse temperature, light intensity and relative humidity were monitored with HOBO probes (model UA-002-64, HOBO Pendant Temp/Light - 64 k and model U23-001, HOBO Pro V2 Temp/RH Data logger, Amanvillers, France). Over the course of the experiment, mean temperature, relative humidity, and light intensity were recorded as follow: for the wet treatments, the values were  $27.93 \pm 0.89$  °C,  $89.57 \pm 5.43$  %, and  $10052 \pm 3381.72$  Lux and  $29.75 \pm 1.58$  °C, respectively.

Germination success and timing was monitored daily for ten days, with no difference in final germination success among the 9 treatments (98% of germination success, see Fig. S13). Then, for each of the 9 treatments, two-month-old seedlings were randomly collected and divided into two parts: one for trait measurements and the other for molecular processes (see Table S11 for sample sizes for traits and molecular approaches).

### *Structural trait measurements*

*Leaf traits*— The leaves were counted (Number of leaves), gently rinsed to remove dirt, and weighed using an electronic balance (AB 204-S Mettler Toledo, Switzerland) to determine the fresh mass (FM, g). Then they were scanned using an office scanner (Xerox DocuMate 4700 5.1) to determine the total leaf area (LA, cm<sup>2</sup>) using an automated ImageJ (Schneider *et al.*, 2012) script. The leaves were stored in distilled water at 4°C in the dark for 72 h to get the turgid mass (TM, g). The leaves were oven dried at 60°C for 72 hours and finally weighed to get the dry mass (DM, g). The leaf mass area (LMA, gm<sup>-2</sup>) was calculated as DM/LA and the leaf dry matter content (LDMC, g g<sup>-1</sup>) was calculated as DM/FM. The leaf relative water content (RWC, %) was calculated as ((FM-DM)/(TM-DM)) x100. Additionally, leaf imprints were made on the abaxial face using transparent nail varnish to estimate the stomatal density (Nb stomata mm<sup>-2</sup>). A thin coat of transparent nail polish was applied, dried, removed with double-sided tape, and placed on a glass slide to be observed under the microscope (Olympus BX51). Three pictures per imprint were acquired with a digital camera (Lumenera LW1135C-IO, Ottawa, Canada) and processed using ImageJ. The number of stomata per mm<sup>2</sup> were recorded for each picture and averaged.

*Root traits*— The whole root system was carefully separated from the shoot and rinsed with tap water. Using a brush and a water-filled Petri dish, adventitious roots were cleaned of all small-sized particles and counted (Number of roots). Subsequently, they were dried using paper towels to remove excess water and weighed to determine the fresh mass (FM, g). The whole root system was scanned using an office scanner (Xerox DocuMate 4700 5.1) and then oven dried at 60°C for 48 h and weighed to obtain the dry mass, (DM, g). We used WinRHIZO software (Basic version 2016, Regent Instruments, Canada) to obtain the average root diameter (ARD, mm), root volume (RV) and root length (RL, cm). RL and DM were



then used to compute the specific roots length (SRL,  $\text{mg g}^{-1}$ ) and DM and RV were used to get the root tissue density (RTD,  $\text{g cm}^{-3}$ ).

*Relative growth rate*— The relative growth rate (RGR,  $\text{mg g}^{-1} \text{month}^{-1}$ ) was calculated as the increase of dry mass of the plants relative to the initial dry mass over 2 months. RGR of the plant biomass was calculated as follow:  $\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$ , where  $W_2$  and  $W_1$  are the dry mass of final and initial plant biomass, and  $t_2$  and  $t_1$  are time. Seeds from each mother plants were oven dry at  $60^\circ \text{C}$  for 48 h and their weight was averaged to serve as the initial plant biomass ( $W_1$ , *i.e.* 30 values, one per mother plant) and the total leaf and root dry mass after 15-month growth was the final plant biomass ( $W_2$ ).

Finally, using both roots and leaves DM the root to shoot biomass ratio was computed as  $\text{DM}_{\text{root}} / \text{DM}_{\text{leaves}}$  (RS ratio).

#### *Microbial communities sampling*

The seeds of the 30 mother plants were collected as the fruits matured and stored in  $-80^\circ \text{C}$  before surface sterilization. A total of 383 two-month-old seedlings were collected. All leaves and roots were separated and carefully rinsed using tap water to remove any substrate debris. Leaves and roots were cut in pieces that would fit in the sterilization pits (tissue culture plates 24 well, Z707791, SIGMA). Surface sterilization was performed by dipping the seeds, leaves and roots portions successively into 70% Ethanol, ultra-pure water, 9% sodium hypochlorite for 1 min each. Then they were washed twice 1 min in ultra-pure water and finally immersed 1 min in a CTAB buffer (100 mM Tris-HCl (pH 8), 1.4 M NaCl, 20 mM Na<sub>2</sub> EDTA, 2% CTAB). Sterilized samples were then stored at  $-80^\circ \text{C}$  in 2mL Eppendorf sterile tubes for downstream DNA extractions.

### *DNA extraction and sequencing*

DNA was extracted using a CTAB extraction method (Carrell & Frank, 2014). The samples were ground using two sterile beads (2 and 4 mm) and a grinder (type Tissue Lyser II RETSCH). We added 1400  $\mu$ L of hot (60 °C) extraction buffer (Trisma base 100 mM, NaCl 1.4 M, EDTA Na<sub>2</sub>/H<sub>2</sub>O 20 mM, 2 % CTAB, 2 % PolyVinyl Pyrrolidone, Ascorbic acid 0.1% and  $\beta$ -mercapto-ethanol.) and incubated the mixture for 30 min at 60°C in an incubator shaker (New Brunswick I26 Incubator Shaker). A first deproteinization was performed by adding 600  $\mu$ L of Dichloromethane/Chloroform/Isoamylalcohol (25:24:1) before centrifugation at 9600 rpm for 30 min at 20 °C (Sigma 4-16KS, Sigma, Germany). Supernatant was transferred to new 2mL Eppendorf tubes for a second deproteinization with 500 $\mu$ L of Chloroform/Isoamylalcohol (24:1) and centrifugating as for the first deproteinization. Supernatant was transferred to new 2mL Eppendorf tubes. Nucleic acids were precipitated by adding 120  $\mu$ L of sodium acetate and 1 mL of cold Isopropanol (-20°C). Tubes were stored at -20°C for 12 h and then centrifugated at 4°C at 9600 rpm for 30 minutes. The supernatant was discarded, and a final wash was performed using 1mL of 70% cold ethanol (-20°C) and centrifugated at 9600 rpm at 4°C for 20 min. Ethanol was discarded, and DNA resuspended in 100  $\mu$ L of TE buffer (1.0 M Tris-HCL and 0.1 M EDTA) and stored at -20°C.

To characterise fungal communities, ITS2 nuclear rDNA gene was amplified by Polymerase Chained Reaction (PCR) using the ITS86F and ITS4R (GTGAATCATCGAATCTTTGAA, Turenne *et al.* 1999) and the ITS4R reverse (TCCTCCGCTTATTGATATGC, Op De Beeck *et al.* 2014). For bacteria, the V5-V6 region of the bacterial 16s RNA gene was amplified using the chloroplast-excluding forward primer 799F (AACMGGATTAGATACCKG, Chelius and Triplett 2001) and the reverse primer 1115R (AGGGTTGCGCTCGTTG, Redford *et al.* 2010). Primers were tagged using eight nucleotide tags for post-sequencing demultiplexing. PCR

was performed twice in a total volume of 25  $\mu$ L (resulting in 50  $\mu$ L of amplicons) comprising 5 $\mu$ L of 5X Blend Master Mix, 12 $\mu$ L of ultra-pure water, 1 $\mu$ L of Bovine Serum Albumin, 2 $\mu$ L of each primer and 3 $\mu$ L of DNA. PCR were performed with a Tetrad2 thermocycler (Tetrad2, BioRad, USA) under the following parameters for both primers: 15 min polymerase reactivation at 94 °C, 30 cycles of 30 s at 94 °C, 30 s at 58 °C, 30 s at 72 °C followed by a final step of 7 min at 72 °C. Amplicons were purified with magnetic beads according to the manufacturer (CleanNGS beads, CleanNA), quantified with a fluorescence-based method (Quant-it PicoGreen dsDNA assay kit; Thermo Fisher Scientific), and pooled in equimolar conditions. The library was built using the Fasteris MetaFast protocol (FASTERIS SA, Plan-les-Ouates, Switzerland) to minimize the amounts of tag-switches (Esling *et al.*, 2015) and sequenced on one MiSeq Illumina run (FASTERIS SA,) for each primer using the paired-end sequencing technology (Metafast protocol, FASTERIS SA).

### *Bioinformatics*

Sequencing output was then processed using R (R Core Team, 2021), the OBITools (Boyer *et al.*, 2016), and SUMAclust (Mercier *et al.*, 2013) wrapped in a Snakemake workflow (Mölder *et al.*, 2021). The snakemake pipeline template is publicly hosted on github ([https://github.com/LafontRapnouilTristan/OBIholo\\_pipeline](https://github.com/LafontRapnouilTristan/OBIholo_pipeline)).

Using the OBITools v-1.2.13, sequences were aligned and filtered based on alignment quality score (threshold of 40), demultiplexed, filtered on length (> 80 pb) and sequencing quality (> 20) and dereplicated. At this stage, libraries from seeds and seedling were merged and OTUs clustered using SUMAclust v-1.0.31 (default parameters) before taxonomic assignments were performed using the RDP Naive Bayesian Classifier algorithm (Wang *et al.*, 2007) implemented in the assignTaxonomy function of the R package dada2 v-1.26 (Callahan *et al.*, 2016), with an 80 % confidence threshold. The classifier was trained with the SILVA

database v138.1 and the UNITE database for bacteria and fungi, respectively. Used releases were UNITE database sh\_general\_release\_dynamic\_16.10.2022 (Abarenkov *et al.*, 2022) and the bacterial silva\_nr\_v138.1\_train\_set (Quast *et al.*, 2013).

Although the use of the OBITools allowed the removal of erroneous sequences introduced during PCR and Sequencing, further processing based on controls and read counts using the metabar R package (Zinger *et al.*, 2021) were used to filter spurious OTUs and contaminants.

Putative contaminants OTUs, present in high abundance in negative controls were removed using 'contaslayer' function. Additionally, abundance of OTUs rare in some samples while abundant in other were considered to results from tag jumps (Schnell *et al.*, 2015) and set to 0 ('tagjumpslayer' function, threshold = 0.05). This assumes that if otherwise abundant OTUs became rare in one sample, this would likely indicate an artefactual presence in this sample. OTUs assigned to chloroplasts, mitochondria, non-fungal or bacterial DNA and with no assignment at the Class level as well as singletons were removed from the dataset. Finally, the samples with less than 1000 reads at the end of the cleaning process or without sufficient sequencing depth as identified after examination of the rarefaction curves were removed. This cleaning resulted in 30 seeds samples for 16s and ITS, respectively, 346 and 311 leaf samples for 16s and ITS2, respectively, and 298 and 293 root samples for 16s and ITS2, respectively.

### *Data analysis*

All statistical analyses were carried out with R version 4.3.0 (R Core Team, 2021), data manipulated with dplyr-v1.1.2 and graph produced with ggplot2-v3.4.2. Many other packages have been used for data handling, visualisation, and statistical tests. All R session information (as returned by sessionInfo() ) and R scripts are

publicly hosted on github and accessible at the following address:  
[https://github.com/LafontRapnouilTristan/Greenhouse\\_Holobrom/tree/main/articlez/gen12](https://github.com/LafontRapnouilTristan/Greenhouse_Holobrom/tree/main/articlez/gen12)

To visualize the effects of seedling and maternal treatments on seedlings phenotype we performed a PCA on seedling traits (LDMC, LMA, Number of roots and leaves, RGR, ARD, RS ratio, RTD, RWC, SRL and, Stomatal density). PCA was performed using the Factominer R package (Lê *et al.*, 2008). Individuals and traits were plotted on the two first PCA dimensions and 95% confidence ellipses of the group centroids estimation for all 9 treatments were displayed. To test for the influence of seedling and maternal treatments on seedling phenotype, we performed permutational multivariate analysis of variance (perMANOVA) with the `adonis2` function of the `vegan` package (Oksanen *et al.*, 2022) using Euclidean distance and 999 permutations. A post-hoc test was then performed using the `pairwise.adonis2` function of the `pairwiseAdonis` package (Martinez Arbizu, 2022) with Holm corrected p values.

The effect of treatments on seeds, leaves and roots alpha-diversity was assessed using both OTU count and the Effective Number of Species index of general order  $q=1$  (Jost<sub>1</sub> index) based on Hill number (Chao *et al.*, 2010). The effect of maternal treatments on seeds and on seedling and maternal treatments for leaves and roots, were tested with the non-parametric Kruskal-Wallis test. When significant (pvalue < 0.05) this test was followed by a post-hoc pairwise wilcoxon test with Holm corrected pvalues and the result displayed on boxplots.

To visualize seeds communities, we conducted PCoA associated with a perMANOVA to test for differences in both bacterial and fungal seed endophytic microbiota. These analyses were performed on Hellinger transformed communities with the Morisita-Horn distance. To test to what extent seed microbiota was similar to both leaf and root communities we computed average

pairwise  $\beta$ -diversity between each seed and seedling leaf and root microbiota for each treatment combination for both fungal and bacterial communities. To test for the effects of organ, maternal and seedling treatment on seed to sample pairwise  $\beta$ -diversity we fitted a linear mixed model with seed ID as a random effect. Additionally, we examined the number of shared OTUs between seeds, leaves and roots for each treatment combination and organs.

Finally, PCoA were performed to visualize seedling leaves and roots microbiota for each marker (*i.e.*, 16s and ITS2). A first global perMANOVA was conducted on the whole dataset to test for treatments and organs effect on endophytic communities. Then separate perMANOVAS were performed on leaves and roots to test the effect of treatments (Maternal and Seedling), their interaction and of the mother identity were performed corresponding to the following model formulation  $\sim$ Maternal\_treat\*Seedling\_treat+motherID.

## **Results**

### *Effects of maternal and current treatments on seedling phenotype*

The two first dimensions of the PCA explained 48.54% of the total variance (31.36% for PC1 and 17.18% for PC2, Fig.34). The first dimension was mainly defined by the RGR, number of leaves and roots as well as LMA, all of which contributed more than expected to this dimension. Root tissue density was the main variable defining PC2 followed by LDMC, SRL and RS ratio (Fig. 34B). The phenotypes of the seedlings were affected by both the pure and interactive effects of maternal and current treatments (perMANOVA,  $p = 0.001$  in all cases, see Table S12 for details). There was an overlap of the confidence ellipses of group centroids for seedlings originating from Poor and Intermediate maternal substrates, while those from Rich maternal substrates did not overlap. Additionally, there were differences based on the current seedling treatment, with Poor, Intermediate, and Rich

current seedling treatments positioned from left to right on the PCA plot (Fig. 34A). When the seeds came from the same maternal treatment (reciprocal transplant), the current seedling treatments had significant effects on all of the seedling phenotypes (see pairwise perMANOVA, Table S13 for more details). When considering the same current seedling treatments (common garden), the maternal treatments had significant effects on all of the seedling phenotypes except for the Poor current treatment when the seeds originated from Poor and Intermediate maternal treatments (Table S13). Overall, regardless of the maternal treatment, seedlings growing in the Rich current treatment had higher performance, while those growing in the Poor current treatment had the lowest performance (see Table S14).

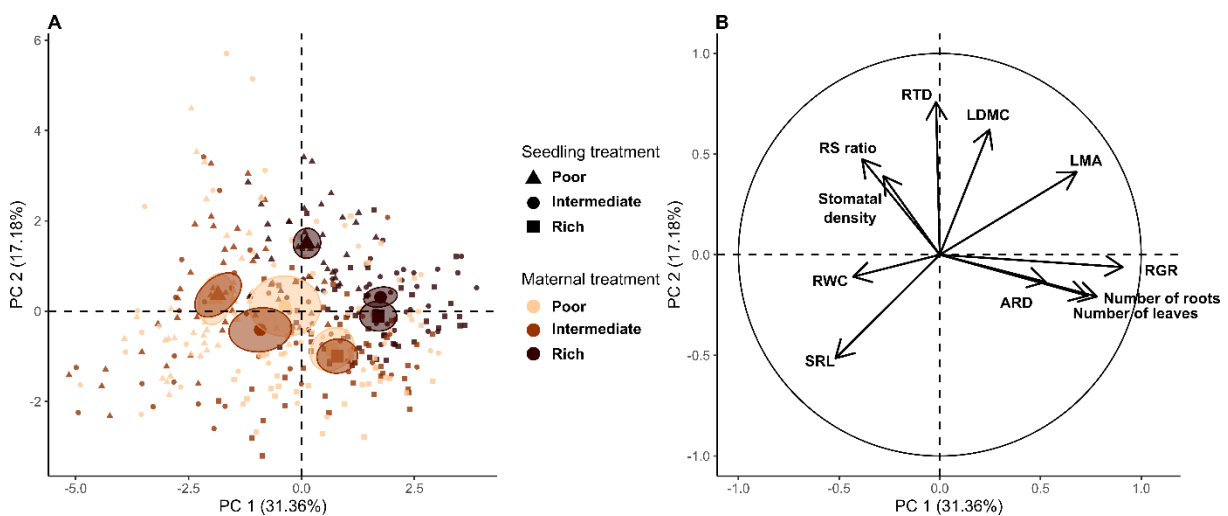


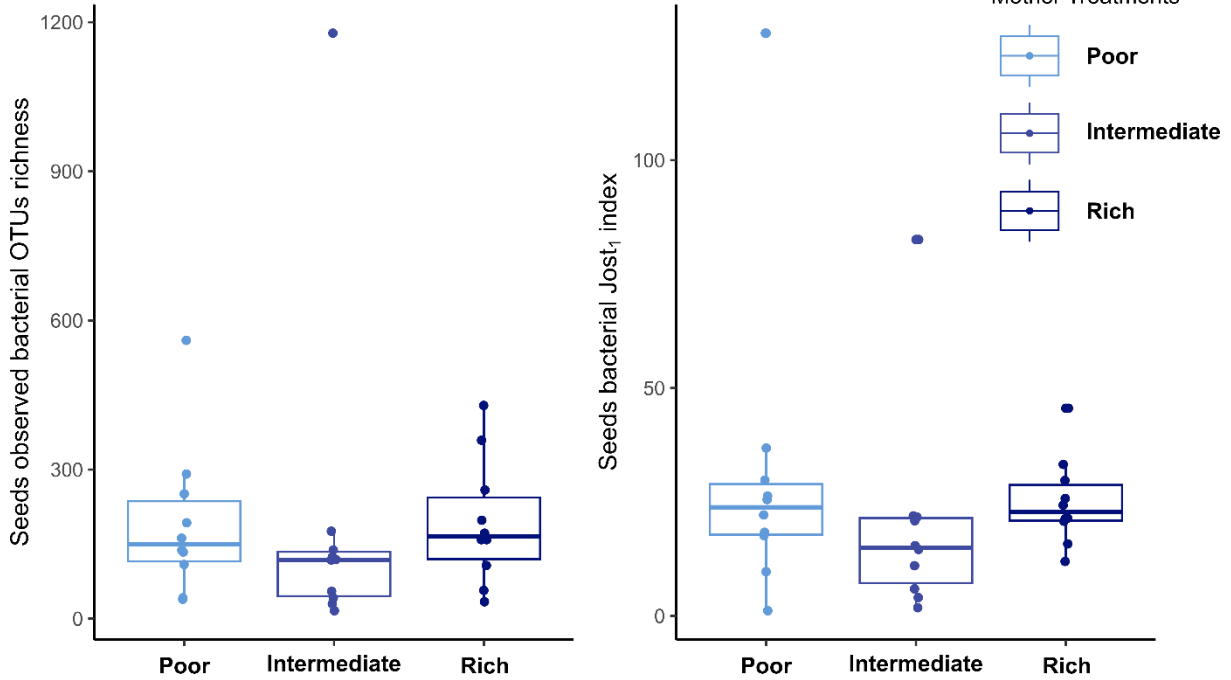
Figure 34: A) Projections of individuals in the first two PCs plan. Individuals are coloured by maternal treatments and shapes indicate the seedling treatment. Small dots are individuals' projections, big dots the centroid for each treatment. The 95% confidence ellipse of the group centroid estimation is shown for each treatment. Substrates are S, white sand; SP, white sand/potting soil; P, potting soil. B) Correlation circle of variables to the first two dimensions of the PCA. Plant traits LDMC, leaf dry mass content ( $\text{gDMg FM}^{-1}$ ); LMA, leaf mass area ( $\text{g m}^{-2}$ ); stomatal density ( $\text{Nb mm}^{-2}$ ); ARD, average root diameter (mm); RTD, root tissue density ( $\text{g m}^{-3}$ ); SRL, specific root length ( $\text{m g}^{-1}$ ); the number of leaves and roots; the root-to-shoot ratio (RS ratio) and, the relative growth rate (RGR,  $\text{mg g}^{-1} \text{month}^{-1}$ ).

### *Effects of maternal environments on seed microbiota*

The substrate on which the mother plants were grown did not affect bacterial  $\alpha$ -diversity when considering OTU richness and Jost<sub>1</sub> index (Fig. 35A). However, for fungi, we found a slightly significant higher fungal OTU richness for the Poor substrate compared to the Intermediate substrate (Fig. 35B). The differences were not significant when comparing Jost<sub>1</sub> index (Fig. 35B). The two first axes of PCoAs explained 12.7% and 11.1% for bacteria and fungi, respectively (Fig. 36A and B). Maternal treatments did have significant effect on seed fungal community composition (perMANOVA,  $F = 1.291$ ,  $p = 0.001$ ), but not on bacterial community composition (perMANOVA,  $F = 1.073$ ,  $p = 0.085$ ). Specifically, mother plants from the Poor substrate produced seeds with significantly different fungal communities compared to the two other treatments (pairwise perMANOVA,  $p = 0.003$ , see Table S15). *Alphaproteobacteria*, *Gammaproteobacteria*, *Bacillii*, *Actinobacteria* and *Bacteroida* were the dominant bacterial classes found in seeds (>90% relative abundance, Fig. S14A) while the fungal communities were dominated by *Sordariomycetes*, *Dothideomycetes* (~75%, Fig. S14B).



### A - Bacteria



### B - Fungi

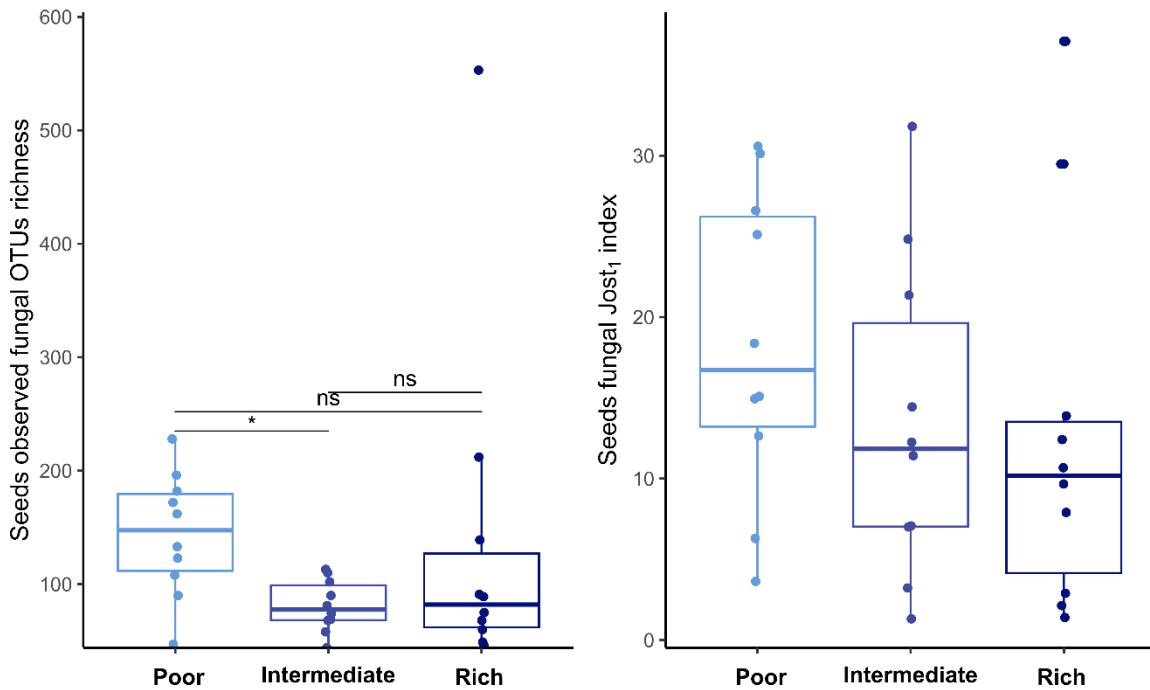


Figure 35:  $\alpha$ -diversity of seeds microbiota according to the three maternal treatments. A show  $\alpha$ -diversity as observed OTUs count and Jost<sub>1</sub> index ( $q = 1$ ), left and right panels respectively. B show the same for fungi. Stars indicate significant differences between groups from a pairwise Wilcoxon test following a significant ( $p < 0.05$ ) Kruskal-Wallis nonparametric test (\* $< 0.05$ ; \*\* $< 0.01$ ., \*\*\* $< 0.001$ , ns $> 0.05$ ).

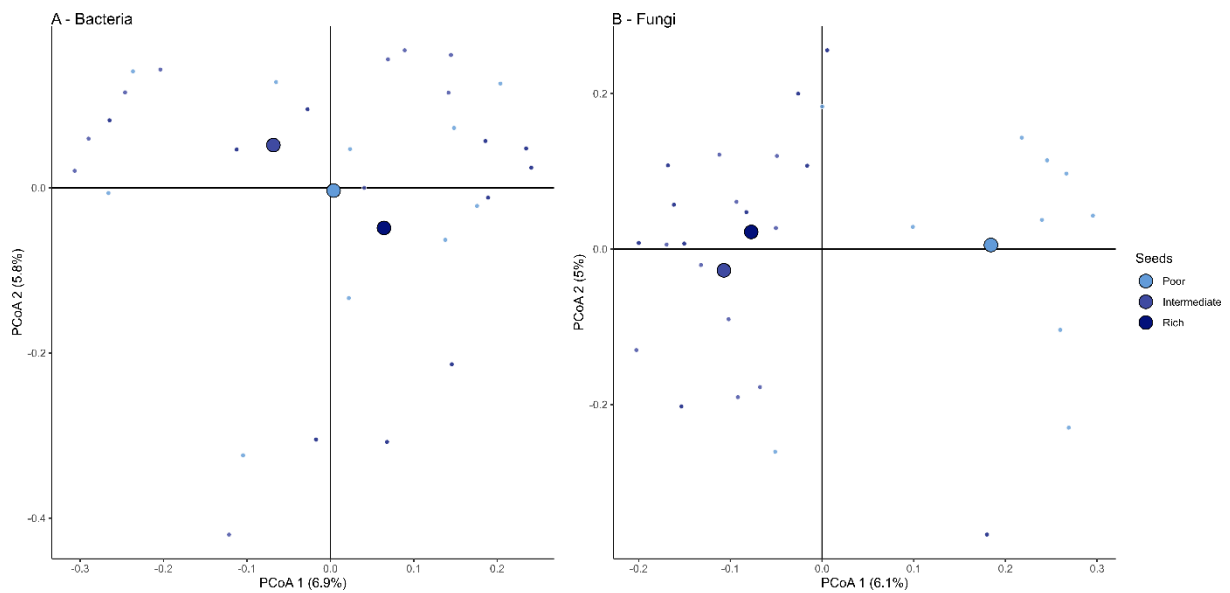
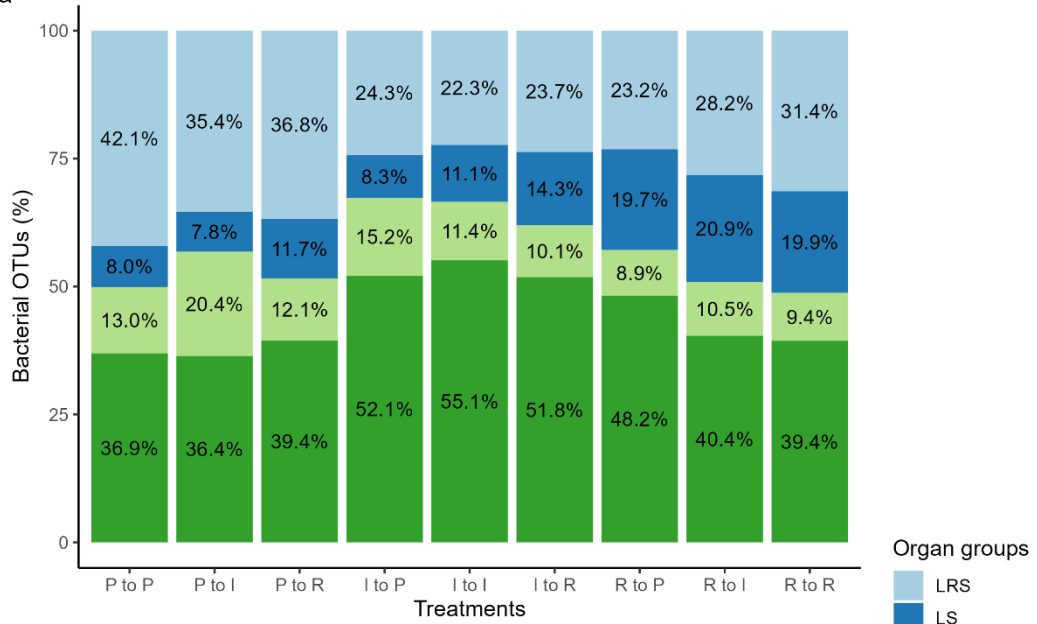


Figure 36 : Individuals projections on the two first dimensions of PCoA of the seeds bacterial (A) and Fungal (B) microbiota. Small dots represent individuals while big circles are the groups centroid. Shapes are colored according to the treatments of the plant that produced the seeds.

### *Seeds and seedling leaves and roots bacterial and fungal communities*

Approximately 45-64% of the bacterial and 50-63% of the fungal OTUs found in the seeds were also found in the leaves or roots or both leaves and roots of the seedlings (Fig. 37 A-B). Specifically, *ca.* 10-20 % of the bacterial and fungal OTUs in the seeds were also found in the leaves and roots, with some variation depending on the treatment. For example, seeds from Rich-substrate mother plants shared two times more fungal OTUs with the roots than with the leaves. When considering the overall communities, seed-associated bacteria and fungi represented 0.95 and 1.01% in the roots and leaves and 3.4 and 3.6% in the roots and leaves, respectively (Fig. S15). The bacterial communities in the seeds were more similar to the leaf communities than to the root communities.

A - Bacteria



B - Fungi

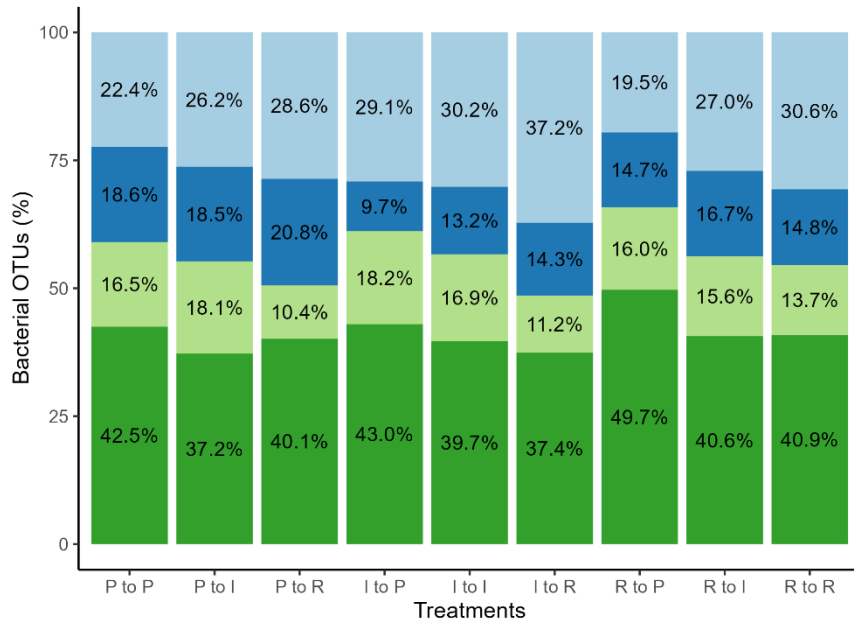


Figure 37: Proportion of OTUs found in seeds that are exclusive to the seeds (S), also found in seedling leaves (LS) or roots (SR) or both (LRS) for bacteria (A) and fungi (B) according to the 9 different treatments. P – Poor, I – Intermediate en R – Rich. Treatments are labelled as ‘Maternal treatment’ to ‘Seedling treatment’.

The current treatment of the seedlings had no significant effect on pairwise  $\beta$ -diversity (Fig. 38A, Table S16). For fungal communities, we found higher dissimilarity between seeds and roots compared to seeds and leaves, except when the seeds came from Poor-substrate plants (Fig. 38B, Table S16). In addition, seed microbiota from Intermediate-substrate plants appeared to be more divergent from the corresponding seedling microbiota (Fig. 38B, Table S16).

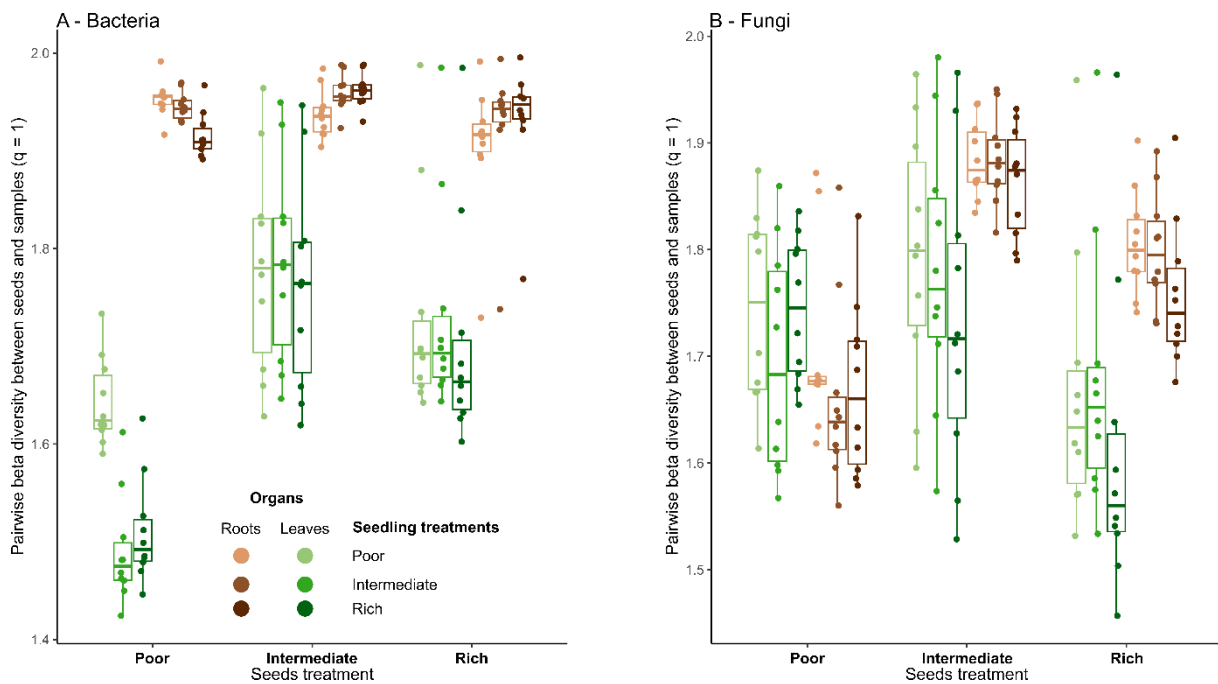


Figure 38: Average  $\beta$ -diversity between each seed microbiota and those of seedling from mother of the same treatment as seeds for leaves (greens) and roots (browns) and A) bacteria and B) fungi. Each dot represent correspond to the microbiota associated with the seeds of a maternal plant.

*Alphaproteobacteria*, *Gammaproteobacteria*, *Bacilli*, *Actinobacteria* and *Bacteroidia* were the five major classes of bacteria present in the seeds, which were also present in the leaves, representing more than 90% (Fig. 39A). Roots could be distinguished from the leaves because, in addition to the five major taxa, non-abundant taxa from the seeds were also present in the roots, such as *Dehalococcoidia*, *OLB14*, and *Polyangia*. For fungi, two main classes (*Dothideomycetes* and *Sordariomycetes*) were commonly found in the seeds, and to the leaves and roots of the developing seedlings (Fig. 39B).

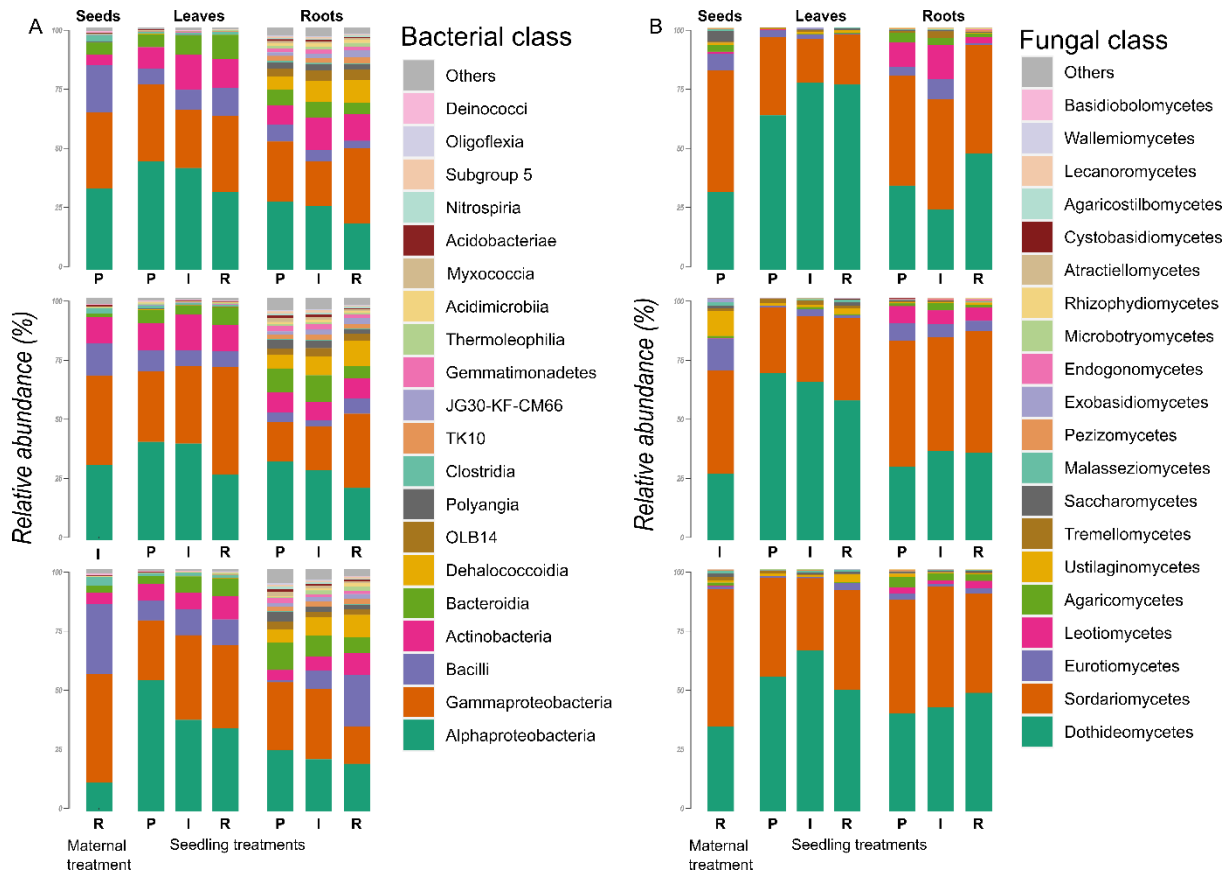


Fig. 39: Class assignment and relative abundance of the OTUs found in the seeds and those from the seeds that are retrieved in leaves and roots according to seed and seedling treatments for (A) Bacteria and (B) Fungi. The 'seed' bar represent the seed community for the three maternal treatments while the leaves and roots correspond to the OTUs that are also found in the corresponding seeds according to the three seedling treatments. The proportion of these OTUs compared to the global dataset (including OTUs not present in the seeds) are available in Fig. S4. P – Poor, I – Intermediate en R – Rich

### *Effects of maternal and current treatments on leaf and root microbiota of the seedlings*

For bacteria, we observed higher diversity in the roots compared to the leaves, with an average of 1200 and 300 OTUs, respectively (Fig. 40A and B), while for fungi, the diversity was similar in both plant compartments, with an average of 100 OTUs (Fig. 41A and B). Bacterial  $\alpha$ -diversity (OTU richness and Jost<sub>1</sub> index) was not affected by maternal or current seedling treatments. On the contrary, for fungi, we did find significant maternal effects on both OTU richness and Jost<sub>1</sub> index in the leaves of the seedlings, but there was no effect of the current seedling treatments (Fig. 41A). Specifically,  $\alpha$ -diversities were significantly higher in the leaves of seedlings originating from mother plants that had grown in Poor substrate and significantly reduced in the leaves of seedlings originating from

mother plants that had grown in Rich substrate. For root fungal endophytes, we found significant maternal effects on OTU richness but not on Jost1 index (Fig. 41B), and the current seedling treatments did not affect  $\alpha$ -diversity. Contrary to the leaves, fungal OTU richness was significantly lower in the roots of seedlings originating from mother plants that had grown in Poor substrate.

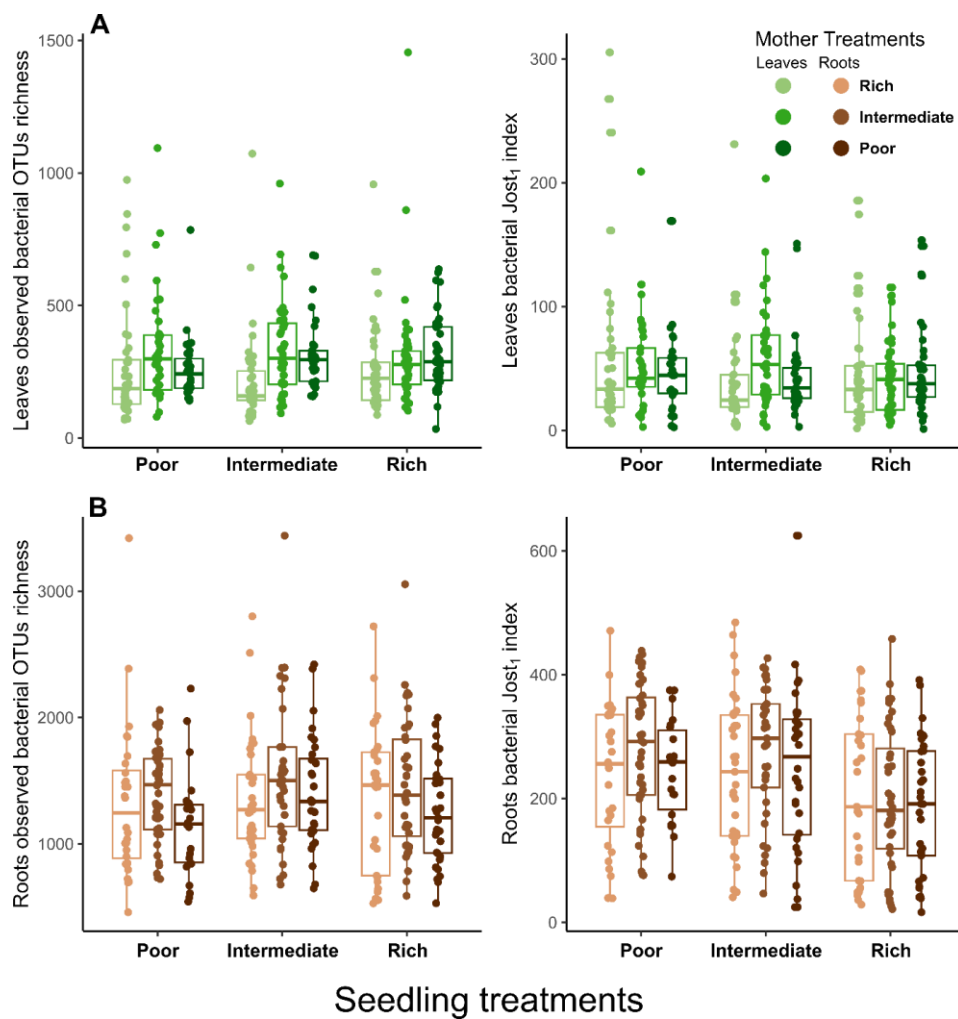


Figure 40: Bacterial endophytes alpha-diversity for leaves (A) and roots (B) with on the left panel the observed OUT richness and on the right the Jost<sub>1</sub> index. Samples are grouped according to seedling treatments and colored according to maternal treatments. Green hue indicates leaf samples and Brown indicates roots.

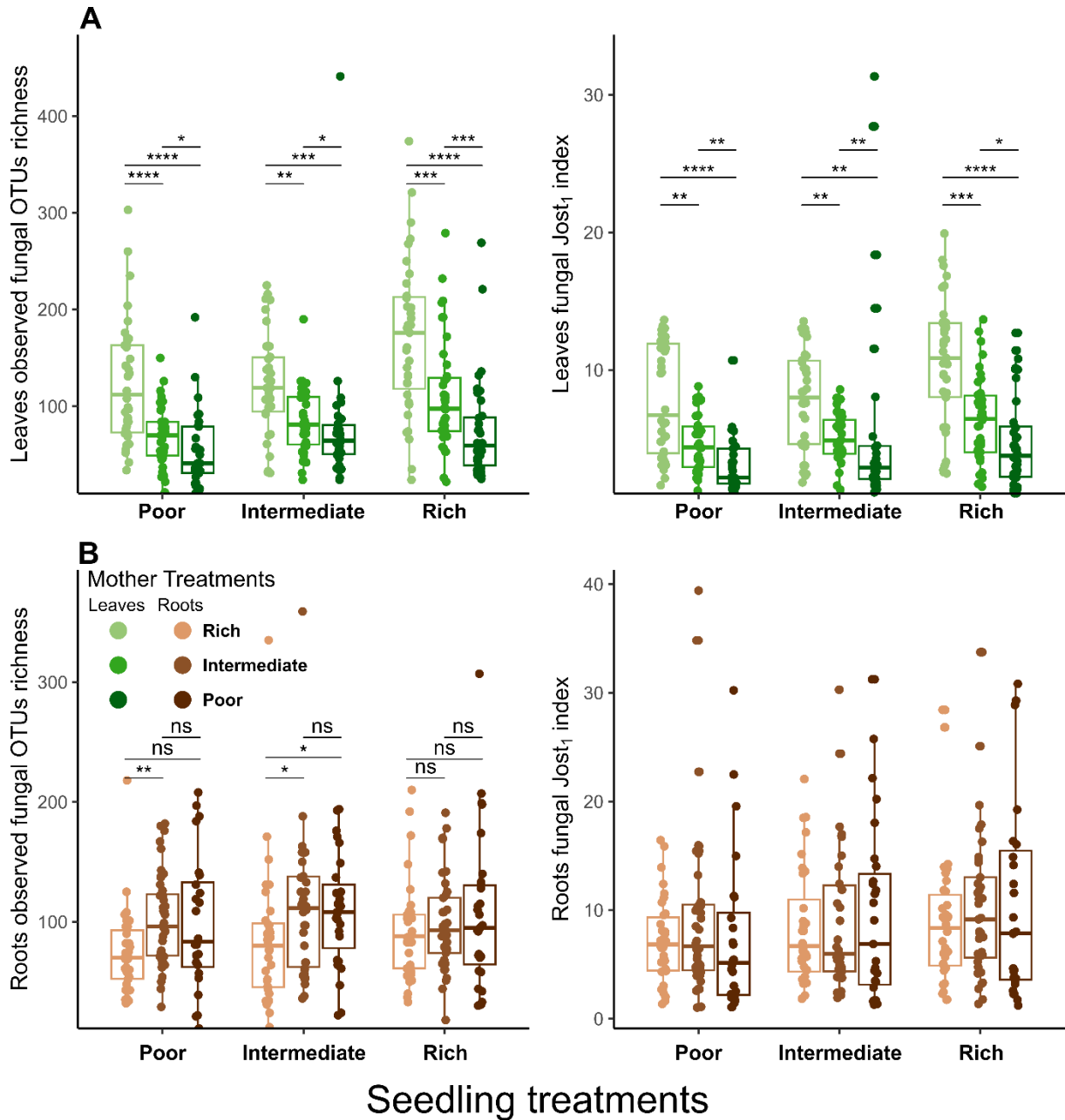


Figure 41: Fungal endophytes alpha-diversity for leaves (A) and roots (B) with on the left panel the observed OUT richness and on the right the Jost<sub>1</sub> index. Samples are grouped according to seedling treatments and colored according to maternal treatments. Green hue indicates leaf samples and Brown indicates roots. For each seedling treatments, the effect of maternal treatments on fungal diversity was tested with a non parametric kruskall-wallis test. If significant, the test was followed by a post-hoc pairwise wilcoxon test. Stars indicates significant difference in alpha-diversity metric between maternal treatments ( \*<0.05; \*\*<0.01., \*\*\*<0.001, ns>0.05).

The bacterial community in the leaves was mainly composed of an average of 70% of Proteobacteria (*Alphaproteobacteria* and *Gammaproteobacteria*), while *Bacteroida*, *Actinobacteria*, and *Bacillii* accounted for 20-30%. In the roots, *Alphaproteobacteria* and *Gammaproteobacteria* accounted for 40-50%, and other

classes such as *Polyangia*, *Chloroflexia*, *Dehalococcoidia*, and *Fibrobacteria*, which were not present in the leaves and the seeds, accounted for another 30-40% (Fig. S16 A and B). Regarding the fungal community, *Sordariomycetes* and *Dothideomycetes* were the two dominant classes in both leaves and roots (Fig. S16 A end B). The first axes of PCoAs performed on seedling samples (roots and leaves) explained little variation, with 2.3% and 0.6% for bacteria and 1.5% and 0.9% for fungi (Fig. 42). PerMANOVA showed significant effect of both maternal and current seedling treatments, as well as a significant effect of the mother identity on roots and leaves bacterial and fungal communities ( $p < 0.01$  in all case, Table S17).

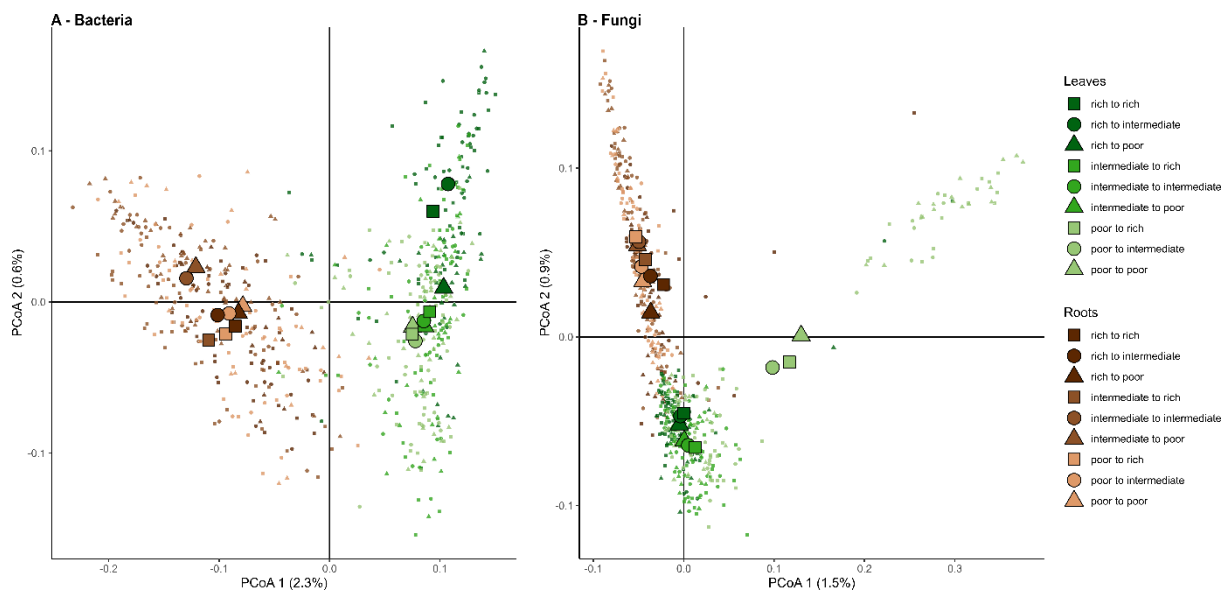


Figure 42: Individuals projections on the two first dimensions of PCoA of the bacterial (A) and fungal (B) microbiota. Small dots represent individuals while big circles are the groups centroid. Dots in green and brown scales respectively correspond to leaves and roots samples. Shapes indicate the maternal treatments: square for rich; circle for intermediate; triangle for poor. Seedling treatment was indicated by the color value with the dots going darker as the substrate got richer.



## Discussion

### *Seedling performance variation*

The abiotic conditions of the maternal environment and current seedling environment had both independent and interactive effects on the phenotype of 2-month-old *A. mertensii*. Seedlings grown from rich-substrate maternal seeds had higher performance compared to seedlings grown from seeds of Intermediate or Poor substrate maternal plants. However, we did not observe a home-site advantage because seedlings from Intermediate or Poor substrate maternal seeds, when grown in the current Intermediate or Poor substrate, did not outperform seedlings from other current treatments. In nutrient-limited environments, the offspring did not have the abilities to cope with the same nutritional stress to which maternal plants were exposed. Thus, our results suggest non-adaptive transgenerational plasticity. While numerous experimental studies reported adaptive transgenerational effects across plant taxa (e.g., Herman and Sultan 2011; Latzel *et al.* 2014; González *et al.* 2017; Groot *et al.* 2017; Baker *et al.* 2019), there is also ample evidence of non-adaptive or even maladaptive effects of parental stress on offspring fitness (e.g., Sultan *et al.* 2009; Uller *et al.* 2013; González *et al.* 2017; Münzbergová and Hadincová 2017). Transgenerational effects in plants, both positive and negative, can be mediated by differences in seed provisioning (Herman & Sultan, 2011). In our study, maternal plants from the rich-substrate environment may provide their offspring with more valuable resources because seedlings performed better even in current resource-poor substrate. Our results align with the “silver spoon” effect, where offspring of resource-rich maternal plants receive higher provisioning compared to those grown in resource-poor conditions, and therefore, are expected to have higher performance (Engqvist & Reinhold, 2016; Portela *et al.*, 2020). Although offspring phenotypic adjustment due to “silver spoon” effects is certainly a

transgenerational effect, it does not represent adaptive plasticity (Engqvist & Reinhold, 2016).

### *Seed microbiota composition*

We revealed rich endophytic microbiota in the seeds of *A. mertensii*. However, we have to be cautious because we used bulk samples (several pooled seeds per mother plants) which are known to inflate microbial diversity (Newcombe *et al.*, 2018). A large-scale meta-analysis including 50 plant species all around the world revealed that seed microbiota are diverse and highly variable, with taxa richness varying from one to up to several hundred and sometimes thousands of taxa if considering bacteria (Simonin *et al.*, 2022). In our study, we found in average 200 bacterial taxa and 100 fungal taxa. In general, seed microbiota presented a variable proportion of bacterial and fungal taxa in which, seed microbiota can be dominated either by bacterial or fungal taxa in terms of diversity depending on the considered species (Simonin *et al.*, 2022). Specifically, Simonin *et al.* (2022) found that seeds from Poaceae had higher proportion of bacteria compared to fungi, our study on *A. mertensii* (Bromeliaceae, Poales order) is another case of such domination of bacteria. In *A. mertensii*, seed communities appeared largely dominated by *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidota* bacterial Phyla as well as Pleosporales and Hypocreales fungal order. This bacterial and fungal composition was in line with what have been reported in other studies (Truyens *et al.*, 2015; Fort *et al.*, 2021; Abdullaeva *et al.*, 2021; Gao *et al.*, 2022; Simonin *et al.*, 2022). In general, seed microbiota was influenced by the plant genotype and the abiotic environmental conditions (Rocheffort *et al.*, 2019; Fort *et al.*, 2021; Bziuk *et al.*, 2021; Gao *et al.*, 2022). In our study, the substrate fertility on which the mother plants were grown did not affect the bacterial diversity neither the  $\beta$ -diversity but did affect the fungal OTU richness and  $\beta$ -diversity. Specifically, seeds from poor-substrate maternal environments had significantly higher fungal

OTU richness and different community composition compared to the seeds of two other substrate conditions. These results may indicate either a higher sensitivity of fungal endophytes to the seed characteristic or reflect differences in the source of fungal communities from which seed microbiota was assembled.

#### *“Transmission” of seed-associated microbiota*

We found that 45-64 % of the seed-associated bacterial and 50-63 % of the seed-associated fungal OTUs were also found in the leaves and roots of the developing seedlings (Table S9). These seed-associated microorganisms, which persist into the next generation, are categorized as persistent or transmitted members of the plant-associated microbiota (Shade *et al.*, 2017). However, the similarity between the microbial communities found in seeds and plant tissues does not clearly demonstrate microbial inheritance (Abdelfattah *et al.*, 2021). Further studies, including data on the soil microbiota, which is known to be the main source of plant microbial diversity, will be necessary to assess the actual level of out transmission from seeds to seedlings. In contrast, 36-55 % of the seed-associated bacteria and 37-50 % of the seed-associated fungi were categorized as transient because they were not found in either the leaves or the roots of two-month-old *A. mertensii* (Shade *et al.* 2017). Overall, the potential contribution of seed-associated microbiota to the assembly of bacterial and fungal OTUs accounted for *ca.* 1 % and 3.5 %, respectively, in the roots and leaves of two-month-old *A. mertensii*. Recent studies have shown that soil contributes a larger fraction to the diversity of the seedling-assembled community compared to inherited microorganisms (Wolfgang *et al.*, 2020; Rochefort *et al.*, 2021; Walsh *et al.*, 2021). For example, in *Brassica napus*, Rochefort *et al.* (2021) found that transmission of seed-borne microorganisms to roots and stems average 2 % of bacterial ASVs and 12 % of fungal ASVs detected in 14-day-old seedlings. The persistence of at least a subset of the seed-associated community is crucial to ensure the continuity of the plant

microbiota for future plant generations (Abdelfattah *et al.*, 2021). We observed a higher similarity between seed and leaf microbial communities compared to seed and root microbial communities. This difference may likely be due to the fact that root microbiota is strongly influenced by soil communities (Trivedi *et al.*, 2020) or could be driven by the overall higher OTU richness in the roots compared to the leaves, rather than a higher contribution of seed microbiota to the leaf communities, as the proportion of shared OTUs between seeds and leaves and seeds and roots was roughly the same across all treatments for both bacteria and fungi.

#### *Roots and leaves microbiota*

With higher bacterial diversity in the roots than in the leaves and fewer differences between these organs regarding fungi, our findings in *A. mertensii* align with what is known about the general distribution of microbial diversity in the plants compartments (Trivedi *et al.*, 2020). Microbial load and diversity is also regulated by the host (Foster *et al.*, 2017; Compant *et al.*, 2021) to control the costs associated with harbouring microbial symbionts. The lower diversity of fungal leaf endophytes might reflect resource allocation strategy. Indeed, plants growing in conditions of high nutrient availability conditions have been shown to reduce their investments in microorganisms, as they were capable of self-reliance for nutrient acquisition (Mujica *et al.*, 2020), and the benefits of symbiosis were reduced under these conditions (Hoeksema *et al.*, 2010). Thus, the lower fungal diversity in seedlings from nutrient-rich mother plants might result from increased independence from microbial symbionts due to higher seed quality, reducing the need for abundant microbial partners. However, this explanation is not entirely satisfactory, as we would have expected to observe similar patterns in root microbial communities and an effect of the substrate currently experienced by the seedlings. This is especially true when considering that most of the fungi that assist

with nutrient acquisition are likely to reside in the roots (Trivedi *et al.*, 2020). Understand the factors that drive fungal and bacterial diversity patterns in different ecological context and plant compartments will require much more work and a more thorough characterisation of the microbial habitats (*e.g.*, chemistry, cell density). On a compositional basis, we identified all the major group known to compose plant microbiota, with major differences in dominant bacterial phyla and fungal classes between root and leaf communities. Plant compartment is well-known to be one of the main factors shaping endophytic microbial communities (Coleman-Derr *et al.*, 2016; Hassani *et al.*, 2018; Trivedi *et al.*, 2020; Félix *et al.*, 2022) and this is confirmed in *A. mertensii*.

#### *Effects of maternal and current environmental conditions on root and leaf microbiota*

We found an influence of both maternal and current environmental conditions on all microbial communities (*i.e.*, leaves/roots and bacteria/fungi). Differential vertical transmission of the microbiota via the seed could explain maternal influence over the seedling microbiota (Vivas *et al.*, 2015; Murphy *et al.*, 2023). However, we did not find any differences in the seed bacterial microbiota or in its representation in seedling microbiota between treatments. Thus, this suggests that other factors affecting seed germination, seedling development, or microbiota recruitment have influenced bacterial microbiota assembly. The seed provisioning (*e.g.*, secondary metabolites, nutrients, or hormones content) might have directly favoured the recruitment and development of different bacteria, thereby influencing the microbiota assembly. Moreover, as seed provisioning affects seedling phenotype, differences in seedling growth and traits might have indirectly affected its microbiota by creating different habitats and niches that suit the needs of different bacterial groups (Compant *et al.*, 2021). In addition, cross-kingdom microbial interactions might have mediated maternal influence over bacterial microbiota. We indeed found that seeds from mother plants with poor-

nutrient substrate harboured distinct fungal endophytes. Differences in fungal communities could, in turn, affect the assembly of bacterial communities through cross-kingdom facilitation or competition (Chaudhry *et al.*, 2021). Finally, priority effects have been shown to have major consequences on microbiota assembly (Ridout *et al.*, 2019; Carlström *et al.*, 2020), and minor differences in communities and presence/absence of key microbial taxa can have long-term effects on microbiota trajectory. Minor differences in inherited microbiota, as well as seed provisioning and structure between mother plants, regardless of their treatment, might also have influenced seedling microbiota assembly.

Despite having a significant effect, treatments explained very little of the variation in our microbial communities. Moreover, variance partitioning consistently showed a stronger influence of maternal identity rather than treatments on seedling microbiota, supporting the existence of strong individual variation. Combined with the relatively low effects of treatments, this suggests that the microbiota of *A. mertensii* was assembled from microorganisms ubiquitous across treatments and was not highly responsive to soil characteristics. Therefore, it appears that maternal effects on *A. mertensii* microbiota are more related to maternal identity than environment, which may have consequences under natural conditions. It is unlikely that these microorganisms provide benefits based on specific environmental conditions, but stochastic processes may also play an important role in the transmission and development of *A. mertensii* seedling microbiota. However, the absence of strong evidence for consistent associations of specific taxa with different environments does not imply the absence of consistent associations of microbial functions. Diverse taxa share common functions, and it has been suggested that transitioning from taxonomic to functional approaches would aid in understanding the patterns and processes of the plant holobiont (Lemanceau *et al.*, 2017).

Finally, if early stages are supposed to be highly dynamic, the plant microbiota undergoes changes throughout the entire life cycle of its host (Williams *et al.*, 2013; Wagner *et al.*, 2016; Edwards *et al.*, 2018; Zhang *et al.*, 2018). The leaf microbiota of *A. mertensii* seedling was quite different from what was observed in the maternal leaves (see Chapter III). For instance, seedling microbiota appeared to be enriched in *alphaproteobacteria* compared to mature plants (*ca.* 40% vs 10%, respectively), suggesting that the microbiota assemblages observed here are likely to undergo significant changes over the plant development. In our study, maternal influence was still detectable in two-month-old seedlings (about 1/6<sup>th</sup> of *A. mertensii* lifecycle), and this effect may persist throughout the plant life. Monitoring *A. mertensii* over its entire lifecycle will be necessary to assess the effect of plant growth and aging on its microbial partners.

### *Conclusion*

Our results revealed a strong influence of maternal seed on *A. mertensii* seedling performance. Additionally, this study shed new light on maternal influence on offspring microbiota, making it, to the best of our knowledge, the first records of maternal effects and assessment of seed, leaf, and root bacterial and fungal microbiota in Bromeliads. We provide evidence that direct vertical transmission of microbiota is not the sole mechanism mediating maternal influence over offspring microbiota and that this influence affects both the quantity and quality of microbial symbionts harboured by the progeny. More importantly, we showed that this maternal influence is long lasting and potentially affects the microbiota trajectory throughout the entire lifecycle of the plants. Given the significant contribution of plant microbiota to their health, ecology, and evolution, these results contribute to the growing interest in microbiota-associated maternal effects.

## Supplementary Information

Fig. S12 Treatment CNP content.

Fig. S13 % of germinated seeds according to maternal and seedling treatment.

Tab. S11 Summary table for all sample size of ecophysiological measurements and microbiota investigation.

Tab. S12 Result table of the perMANOVA on seedling traits.

Tab. S13 Result table of the pairwise perMANOVA on seedling traits.

Tab. S14 Summary table of ecophysiological traits measured on seedlings (mean  $\pm$  sd) from the nine treatments and statistical tests results.

Tab. S15 Result table of pairwise perMANOVA on seed fungal communities.

Fig. S14 Compositional plot of seeds bacterial and fungal communities at the class taxonomical rank.

Fig. S15 Bacterial and fungal OTUs repartition in the different organs according to treatments.

Tab. S16 Result table of LMM on seed to seedling pairwise  $\beta$ -diversity for bacterial and fungal communities.

Fig. S16 Compositional plot of bacterial and fungal leaves and roots communities at the class taxonomical rank.

Tab. S17 Results of perMANOVAs on leaves and roots bacterial and fungal communities.

Fig. S17 Relative abundance of dominant root fungal orders.

Fig. S18 Relative abundance of dominant bacterial and fungal class in seedling leaves.



Fig. S19 Relative abundance of dominant root bacterial and fungal genus.

Fig. S20 Relative abundance of dominant leaf bacterial and fungal genus.

Fig. S21 Relative abundance of dominant seed bacterial and fungal genus.

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# Discussion générale



# 1 Synthèse et principaux résultats

Les travaux de l'IPCC prédisent d'importantes modifications des régimes climatiques, notamment une augmentation des températures et une augmentation de la fréquence, de la durée et de la sévérité des épisodes de sécheresse dans de nombreuses régions du globe (Pörtner & Roberts, 2022), parmi lesquelles se trouvent des réservoirs de biodiversité tel le bassin amazonien. L'élévation de la température et la sécheresse affectent les communautés végétales, impactant tous les aspects de la biologie des populations de plantes. Les effets exacts que ces changements globaux auront sur les populations naturelles sont difficiles à prédire, mais ont un potentiel dévastateur pour la biodiversité et les sociétés humaines qui en dépendent.

L'objectif de ma thèse était d'explorer la réponse plastique des plantes à leur environnement à différents niveaux. Nous avons abordé cette question sous deux angles, en nous concentrant d'abord sur une vision classique du phénotype, c'est-à-dire la mesure de traits morphologiques et physiologique de la plante, puis en intégrant le microbiote comme faisant partie du phénotype étendu et donc soumis à une forme de plasticité. Pour cela, nous avons tiré profit du modèle biologique que représentent les broméliacées à réservoir d'eau. Nous avons combiné des expérimentations en serre (Chapitres I, III et IV) et des observations en milieu naturel (Chapitre II) pour caractériser la réponse des broméliacées à différents facteurs environnementaux cruciaux pour leur croissance, tels que la disponibilité en nutriments, en eau et en lumière.

Nous avons mis en évidence la contribution significative de la nutrition racinaire à la croissance d'*Aechmea aquilega*, invitant à une reconsidération du rôle de ces organes chez les broméliacées à réservoir d'eau. Probablement parce qu'elles sont souvent épiphytes, de nombreux chercheurs n'ont attribué aux racines des broméliacées qu'un rôle mécanique et une contribution faible à la nutrition de la plante au regard du rôle des feuilles. Nous démontrons que la

nutrition racinaire est fondamentale pour ces plantes, puisque la qualité du substrat a des effets importants et durables sur leur développement.

Le deuxième chapitre avait pour objectif de déterminer si l'intensité lumineuse affecte la différenciation morphologique entre les régions apicales et basales des feuilles de broméliacées à réservoir d'eau. Cette différenciation est associée à des différences fonctionnelles, avec une région apicale assurant la photosynthèse et les échanges gazeux, tandis que la base assure l'acquisition de l'eau et des nutriments présents dans le réservoir d'eau à l'aide de trichomes foliaires absorbants (Benzing, 2000; Mercier *et al.*, 2019). Nous mettons en évidence une réduction de la différenciation morphologique entre ces deux régions en réponse à l'exposition à la lumière le long d'un gradient naturel. Étant donné que les traits morfo-anatomiques que nous avons mesurés sont vraisemblablement associés au fonctionnement des feuilles, ces résultats suggèrent une altération des modes d'acquisition des ressources (racines vs feuilles) par les conditions de luminosité. *Aechmea aquilega*, comme d'autres broméliacées à réservoir d'eau, se développe naturellement sous une gamme importante de luminosité allant des sous-bois à une exposition en pleine lumière. Par conséquent, nos résultats ont potentiellement d'importantes implications sur les stratégies d'acquisition des ressources. Ce chapitre met également en évidence l'importance de considérer, en particulier chez les plantes à grandes feuilles ou dont la taille varie en fonction de l'environnement, l'existence de variations intra-feuilles et de ne pas toujours considérer la feuille comme une entité uniforme.

Les résultats des deux premiers chapitres révèlent une possible sous-appréciation de la complexité des stratégies d'acquisition des ressources chez ces plantes et offrent de belles perspectives de recherche pour approfondir notre compréhension de l'émergence de l'hyperdiversité des broméliacées, ainsi que leur écologie et leur évolution.

Dans la seconde partie de cette thèse, nous avons intégré l'étude du microbiote, encore largement méconnu chez les broméliacées, dans leur réponse à l'environnement (Chapitre III) et dans son potentiel rôle dans la plasticité transgénérationnelle (Chapitre IV).

Le troisième chapitre visait à identifier les relations entre les traits écophysiologiques mesurés et les communautés d'endophytes foliaires bactériens et fongiques le long d'un gradient de stress hydrique et nutritif. En combinant deux niveaux d'irrigation et trois substrats de richesse différente, nous avons créé 6 conditions environnementales allant de la plus sèche et pauvre en nutriments à la plus riche et irriguée. L'objectif était donc d'observer si la réponse morpho-physiologique des plantes était couplée à une réponse du microbiote, en testant notamment les prédictions du principe Anna Karénine (AKP) (Arnault *et al.*, 2022). Nous avons montré que les plantes semblent maintenir un microbiote stable et sous contrôle bien que légèrement différent entre chaque condition environnementale, excepté pour les environnements les plus stressants où, conformément au principe d'Anna Karénine, nous observons une importante variation dans la composition du microbiote entre chaque individu, suggérant une perte de contrôle du microbiote par la plante hôte et donc une libre prolifération de ce dernier.

Enfin, dans le dernier chapitre (chapitre IV) nous avons testé l'existence et les mécanismes sous-jacents des effets maternels sur le phénotype et le microbiote de plantules âgées de deux mois. Les effets maternels sont une forme de plasticité transgénérationnelle qui peut jouer un rôle important dans la réponse locale des populations de plantes aux modifications environnementales. Dans ce chapitre, nous mettons en évidence l'existence d'effets maternels qui influencent les performances et la composition du microbiote des plantules. En somme, ces deux derniers chapitres apportent de nouvelles preuves que l'intégration du microbiote au phénotype des plantes est pertinente et a le

potentiel d'améliorer notre compréhension des processus biologiques actuels (interactions avec l'environnement), passés (histoire évolutive des plantes) et futurs (réponse aux changements globaux). Parmi les résultats notables, nous avons mis en évidence des réponses différentes entre les endophytes fongiques et bactériens. Notamment, il est intéressant de souligner l'existence d'une influence maternelle sur les communautés bactériennes des plantules en l'absence de différences dans les communautés bactériennes des graines mais avec des différences de communautés fongiques des graines. Ceci suggère que d'autres facteurs comme la chimie de la graine ou des interactions bactéries-champignons pourraient servir de médiateurs de l'influence maternelle sur le microbiote. Tout comme pour les champignons (Brundrett & Tedersoo, 2018), il est vraisemblable que la diversité des symbiontes bactériens colonisant les plantes soit structurée phylogénétiquement, géographiquement et écologiquement et que différentes familles de plantes (*e.g.*, les Fabaceae et *Rhizobium*) ait développé un degré de dépendance plus ou moins fort envers les bactéries ou les champignons. Comprendre les facteurs qui régissent la richesse et la composition des communautés de ces deux groupes, à la fois conjointement et indépendamment, sera une prochaine étape importante dans notre compréhension des interactions hôte-microbiote-environnement.

Les résultats de ces travaux à l'interface entre microbiologie et écophysiologie végétale permettent donc d'acquérir une meilleure compréhension du système broméliacée, d'aboutir à des conclusions plus générales concernant les concepts d'holobionte et de plasticité chez les plantes, tout en soulevant de nombreuses questions qui restent à explorer.

## 2 Plasticité et stratégies d'acquisition des ressources

### 2.1 Forte plasticité en lien avec l'acquisition des ressources

Nos résultats mettent en évidence les capacités plastiques d'*Aechmea aquilega* et d'*A. mertensii* en ce qui concerne l'acquisition des ressources : la disponibilité en lumière (Chapitre II), les éléments nutritifs (Chapitre I, III, et IV), et l'eau (Chapitre III). D'autres études portant sur diverses espèces de broméliacées ont également rapporté une forte plasticité en réponse à ces facteurs environnementaux (Cavallero *et al.*, 2009, 2011; Rodrigues Pereira *et al.*, 2013; Barberis *et al.*, 2017). La lumière, l'eau et les minéraux sont des éléments cruciaux pour le développement des plantes en général, et tout particulièrement pour les épiphytes. La canopée est un habitat hétérogène avec d'importants gradients environnementaux et un accès difficile aux ressources (Zotz, 2016). L'un des principaux aspects est l'existence d'un important gradient lumineux présent du sous-bois jusqu'à la cime des arbres qui structurent les communautés d'épiphytes selon une distribution verticale (Petter *et al.*, 2016). De plus, les conditions lumineuses dans la canopée sont fortement variables dans le temps et dans l'espace (Rascher *et al.*, 2012). La plasticité phénotypique des broméliacées face au gradient lumineux semble particulièrement adaptative, leur permettant de croître dans des environnements variés et changeants. De même, l'eau et les nutriments sont considérés comme des facteurs limitant pour la colonisation de la canopée, étant donné que les plantes sont privées des réserves du sol (Zotz, 2016). Cette contrainte a vraisemblablement favorisé l'apparition répétée du métabolisme CAM et du stockage externe (réservoir d'eau) et interne (feuilles épaisses avec un hydrenchyme) chez les broméliacées (Givnish *et al.* 2014 mais voir Hietz *et al.* 2021 questionnant l'importance du métabolisme CAM pour les épiphytes à l'échelle globale).

## *2.2 Stratégies d'acquisition de l'eau et des minéraux : racines versus trichomes foliaires absorbants*

L'évolution des broméliacées est intimement liée à leurs stratégies d'acquisition, de stockage et d'utilisation des ressources. En s'affranchissant des ressources du sol, ces stratégies ont entraîné chez certaines espèces une forte diminution voire une perte de la nutrition racinaire (Benzing, 2000), un constat qui a longtemps été généralisé aux broméliacées des Types III et IV. Cependant, nos résultats (Chapitres I, III et IV) démontrent l'importance de cette nutrition minérale sur la croissance, les performances à long terme, ainsi que sur la fitness des deux espèces de broméliacées à réservoir d'eau. En effet, bien que cela ne soit pas présenté dans les chapitres, nous avons montré qu'en plus d'affecter les traits fonctionnels et la croissance de ces plantes, la richesse du substrat affectait également la production de graines. D'une part, les plantes poussant sur un substrat riche produisaient plus de graines avec un approvisionnement en éléments nutritifs et en phytohormones différent (Fig. 43). D'autre part, nous montrons dans le Chapitre IV que l'origine de la graine affecte les performances des plantules, ce qui laisse penser que ces graines sont également plus "viables". Ainsi, la richesse du substrat pourrait avoir une grande influence sur le succès reproducteur des broméliacées à réservoir d'eau.

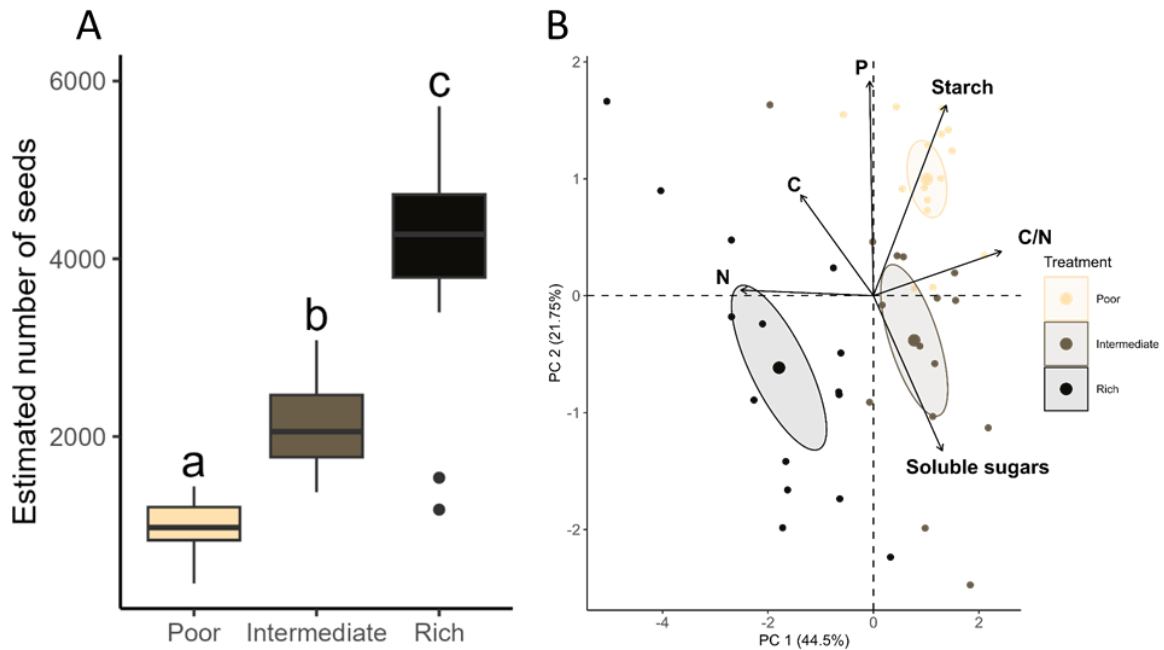


Figure 43 : (A) Estimation du nombre de graines produites par les plantes du traitement Wet du Chapitre III (comprenant les mères du Chapitre IV) en fonction du traitement. Différentes lettres indiquent une différence significative ( $\alpha < 0.05$ ) d'après un test de wilcoxon par paire après un test de Kruskal-Wallis significatif. (B) Projection des individus sur le plan formé par les deux premières dimensions d'une PCA réalisée sur les caractéristiques chimiques des graines produites par ces mêmes plantes. C, N et P correspondent respectivement au contenu en carbone, azote et phosphore; C/N au ratio carbone/azote; Starch au contenu en amidon et Soluble sugars à celui en sucre soluble.

Ces résultats viennent s'ajouter à une littérature suggérant la coexistence de ces deux modes de nutrition chez les broméliacées à réservoir (Vanhoutte *et al.*, 2017; Carvalho *et al.*, 2018; Leroy *et al.*, 2019). La classification des broméliacées en 5 types écologiques par Benzing (2000) ne semble donc pas capturer la diversité des modes de nutritons utilisés par les broméliacées à réservoir d'eau. En effet, la mise en place des structures nécessaires à l'absorption des nutriments par les feuilles se fait progressivement avec la mise en place des petits réservoirs d'eau. Ainsi, âgées de 2 mois, les plantes ne possèdent pas encore de trichomes foliaires absorbant (chapitre IV, Petit *et al.* 2014). Les broméliacées opèrent donc une transition d'une nutrition racinaire à foliaire au cours de leur développement (Takahashi *et al.*, 2022), mais la dynamique et leur degré de dépendance à ces modes restent inconnus. De plus, les effets de l'environnement sur les traits foliaires que nous avons mis en évidence (Chapitres I, II et III), notamment sur la

densité et la taille des trichomes, suggèrent que la transition entre ces modes pourrait également être liée aux conditions environnementales.

### *2.3 Potentiels effets interactifs de la disponibilité en lumière et de la nutrition minérale*

Nous avons montré que la disponibilité en lumière avait le potentiel d'affecter, chez *A. aquilega*, la nutrition par les feuilles en altérant la différenciation entre les régions apicales et basales des feuilles (Chapitre II). Il existe une forte différenciation physiologique entre ces deux régions (Takahashi & Mercier, 2011; Abreu *et al.*, 2018; Mercier *et al.*, 2019), et il est donc fort probable que l'altération du gradient morpho-anatomique que nous avons observée soit associée à une modification du gradient physiologique même si une confirmation plus formelle (*e.g.*, approche transcriptomique) reste nécessaire. Ainsi, nos résultats semblent indiquer l'existence d'un lien, et potentiellement de compromis, entre l'acquisition des minéraux et l'énergie lumineuse nécessaire à la photosynthèse. A partir de là, nous pouvons envisager des stratégies découlant de ces compromis où la plante aurait plus ou moins intérêt à investir dans l'un ou l'autre mode de nutrition. Par exemple, des broméliacées exposées à un fort ensoleillement pourraient privilégier une nutrition racinaire plutôt que foliaire. Les communautés aquatiques présentes dans des réservoirs exposés au soleil ont tendance à être dominées par des algues (Brouard *et al.*, 2012) qui sont potentiellement en concurrence avec la plante pour l'acquisition des ressources minérales (Rogy & Srivastava, 2023). Dans ce contexte, la nutrition par le réservoir d'eau pourrait s'avérer moins rentable que l'acquisition racinaire des ressources. Ces hypothèses pourraient être testées par des expérimentations en serres manipulant le degré d'ensoleillement et les qualités nutritives du réservoir d'eau et du substrat. En mesurant les valeurs isotopiques du N et du P contenus dans les tissus de la plante au cours de son développement, nous devrions pouvoir estimer la contribution des différentes sources (sol vs réservoir) à la nutrition (Hietz & Wanek,



2003; Reich *et al.*, 2003; Q. Romero *et al.*, 2010). Ces hypothèses devront être confirmées par des observations de terrains, mais si elles devaient être vérifiées, elles pourraient avoir d'importantes conséquences sur la dynamique des populations naturelles. En effet, nos résultats suggèrent que l'importante hétérogénéité à petite échelle caractérisant l'habitat des broméliacées (Woods *et al.*, 2015; Petter *et al.*, 2016) pourrait complètement déséquilibrer le succès reproducteur au sein de la population.

#### *2.4 Histoire évolutive et conservation*

L'histoire évolutive des broméliacées est marquée par de nombreuses innovations morphologiques et physiologiques (Silvestro *et al.*, 2014; Givnish *et al.*, 2014) associées à une importante diversification qui a conduit à plus de 3700 espèces actuelles (Butcher and Gouda, 2023). On trouve notamment l'apparition répétée et indépendante du mode de vie épiphyte à des degrés plus ou moins stricts (Givnish *et al.*, 2014). Les stratégies d'acquisition et de conservation des ressources sont cruciales dans la conquête de la canopée, où la disponibilité en ressources est fortement variable constituant un environnement très hétérogène (Woods *et al.*, 2015). Nos résultats viennent affiner notre compréhension de l'éventail des modes d'acquisition des nutriments adoptés par les broméliacées à réservoir d'eau et mettent en avant d'importantes implications pour leur *fitness* en milieu naturel.

En plus de leur importance fondamentale, nos résultats ont des implications pour la conservation des broméliacées. D'après l'IUCN (2023), 181 espèces de broméliacées sont en danger dont 60 de manière critique (+47 depuis Ladino *et al.* 2017). Or les broméliacées à réservoir d'eau jouent un rôle essentiels des forêts néotropicales, où elles assurent de nombreuses fonctions écologiques (Coxson & Nadkarni, 1995; Goffredi *et al.*, 2011a; Ladino *et al.*, 2019). Elles sont notamment le support d'une importante diversité d'insectes (et de vertébrés) qui dépendent

de leur réservoir d'eau pour la réalisation de toute ou une partie de leur cycle de vie (e.g., larves de diptères, coléoptères, d'odonates). Le fonctionnement des écosystèmes hyperdiversifiés, telles que le sont les forêts néotropicales, est donc intimement lié aux dynamiques des populations de broméliacées à réservoir d'eau. Malgré cela, et malgré les efforts fournis par la communauté scientifique pour les étudier, nous continuons à découvrir des spécificités et finesses fonctionnelles, ce qui confirme la nécessité de maintenir une recherche active sur ce modèle biologique.

### **3 Le microbiote des Broméliacées**

Nos travaux apportent des connaissances et des résultats inédits concernant le microbiote des broméliacées. Les chapitres III et IV ont permis de mettre en évidence un microbiote diversifié, comprenant les Phyla qui dominent généralement le microbiote des plantes (Trivedi *et al.*, 2020). Nous avons également observé certains patrons globaux, tels que des communautés racinaires plus diversifiées que celles des feuilles, ainsi qu'une plus forte diversité de bactéries par rapport aux endophytes fongiques (Trivedi *et al.*, 2020). Cependant, nous disposons de très peu d'études sur le microbiote des broméliacées (Tellez *et al.* 2020; Leroy *et al.* 2019, 2021, 2022) en tant que point de comparaison pour mettre nos résultats en perspective. De plus, ces quelques rares travaux se concentrent exclusivement sur l'étude des associations fongiques.

#### *3.1 Les associations fongiques*

Nous avons constaté que les feuilles d'*A. mertensii* étaient principalement associées aux champignons des ordres *Hypocreales*, *Pleosporales*, *Trichosphaeriales*, *Sordariales* et *Diaporthales*. Les cinq genres les plus abondants étaient *Fusarium*, *Curvularia*, *Nigrospora*, *Diaporthe*, and *Exophiala*. Seule deux

études ont porté sur les communautés d'endophytes fongiques chez 6 espèces de broméliacées des genres *Aechmea*, *Guzmania* et *Tillandsia* (Tellez et al. 2020) et chez trois espèces du genre *Tillandsia* (Unterseher et al. 2013). Ces auteurs ont trouvé une quantité importante de *Xylariales*, qui, avec les *Sordariales* et les *Hypocreales* dominaient ces microbiotes. Ces résultats contrastent avec nos propres observations, car les *Xylariales* n'ont pas été identifiés comme taxons dominants dans le microbiote d'*A. mertensii* dans nos expériences en serre. Les *Xylariales* constitue un groupe diversifié qui domine le microbiote de nombreuses plantes tropicales, incluant des arbres (Donald et al., 2020; Cambon et al., 2022), des herbes (Higgins et al., 2014), des fougères (Del Olmo-Ruiz & Arnold, 2014) et des broméliacées (Unterseher et al., 2013; Tellez et al., 2020). Cette disparité avec la littérature pourrait résulter de nos conditions expérimentales en serres, qui pourraient ne pas favoriser une colonisation importante des plantes par cet ordre. Cette hypothèse devra être confirmée par une étude *in natura* du microbiote des feuilles d'*A. mertensii*. De plus, les travaux de Tellez (2020) ont mis en évidence un enrichissement en *Hypocreales* dans les broméliacées à photosynthèse C3 et un appauvrissement en *Sordariales* comparées aux broméliacées CAM.

Les racines des plantules d'*A. mertensii* étaient associées avec des *Pleosporales*, *Hypocreales*, *Sordariales*, *Diaporthales* et *Thelebolales* (>60% de la communauté, Chap IV, Fig. S17). Les principaux genres étaient *Curvularia*, *Fusarium*, *Diaporthe* et *Pseudeurotium* (Chap IV, Fig. S19). Concernant la diversité fongique des communautés racinaires nos résultats contrastent avec ceux de Leroy et al. (2022a) qui ont trouvé chez des *A. mertensii* adultes une dominance d'*Hypocreales*, *Glomerales*, *Chaetothyriales*, *Capnodiales*, *Agaricales* et *Eurotiales*. L'utilisation d'un marqueur ciblant spécifiquement les *Glomeromycota* en plus du marqueur ITS ciblant *Ascomycota* et *Basidiomycota* par Leroy et al. explique vraisemblablement les différences concernant les *Glomerales* mais pas les autres groupes. Leroy et al. ont également retiré de leur jeux de données les OTUs

n'étant pas désignés comme symbiotrophes suite à une assignation par la base de données FUNGuild (Nguyen *et al.*, 2016) qui pourrait expliquer nos différences d'abondance relative rendant la comparaison difficile. Finalement, leur étude était réalisée chez des adultes en milieu naturel là où nous avons étudiés des plantules en serres, facteur pouvant également amener à des différences dans le microbiote d'*A. mertensii* entre ces deux études. Des études plus poussées et à plus large échelles taxonomiques et géographiques seront nécessaires pour caractériser la diversité fongiques associées aux broméliacées.

### 3.2 Les associations bactériennes

A notre connaissance, aucune étude s'est penchée sur le microbiote bactérien chez les broméliacées. Aux niveaux taxonomiques supérieurs (Phyla et Classes), nous avons globalement retrouvé les groupes dominants du microbiote des plantes, avec une forte abondance relative de *Proteobacteria*, *Firmicutes* et *Actinobacteria* (Bulgarelli *et al.*, 2013). De manière remarquable, nous avons observé une forte abondance du genre *Tepidiphilus* dans les feuilles des plantes matures (Chapitre III, Fig. S10) et, dans une moindre mesure, dans celle des plantules (Chapitre IV, Fig. S20). Les *Burkholderiales*, auxquelles appartient *Tepidiphilus spp.*, sont un ordre important du microbiote des plantes, mais nous n'avons pas trouvé de mention de ce genre en tant qu'endophytes foliaires dans la littérature. Il convient cependant de noter que les études utilisant des approches métabarcoding ne présentent que rarement leurs résultats à la résolution du genre pour les bactéries, et un examen minutieux des bases de données sera nécessaire pour confirmer si ces observations sont propres aux broméliacées ou non. Jusqu'à présent, ce genre a été retrouvé dans des puits et réservoirs de pétrole (Wang *et al.*, 2020; Zhang *et al.*, 2020; Bedoya *et al.*, 2021), de stations de traitement des eaux usées (Manaia *et al.*, 2003) et des sources chaudes (Poddar *et al.*, 2016; Narsing Rao *et al.*, 2020). Les *Tepidiphilus* semblent capables

d'utiliser des acides organiques et des amino-acides comme sources de carbones et de réduire le nitrate (Manaia *et al.*, 2019). Ces résultats sont hautement préliminaires, mais ils sont tout aussi intéressants, étant donné l'importance du métabolisme du nitrate réalisé dans les feuilles des broméliacées à réservoir d'eau (Takahashi & Mercier, 2011; Gonçalves *et al.*, 2020). Un lien fonctionnel entre le métabolisme de l'azote chez les broméliacées et la présence de *Tepidiphilus* pourrait notamment expliquer la dominance de ce genre chez les adultes (Chapitre III) plutôt que chez les plantules (Chapitre IV), qui n'ont pas encore développé de réservoir. De plus, *Tepidiphilus* était aussi le genre dominant dans les graines d'*A. mertensii* (Chapitre IV, Fig. S21) et donc potentiellement transmis de génération en génération. Alternativement, ces bactéries pourraient être acquises dans l'environnement. Les *Tepidiphilus* mentionnés dans la littérature semblent se développer dans des milieux aquatiques et chauds, ce qui pourrait correspondre aux conditions rencontrées dans le réservoir d'eau des broméliacées. De nombreux travaux rapportent la présence d'*Hydrogenophilaceae* (Herrera-García *et al.* 2022) ou de *Burkholderiales* (Goffredi *et al.* 2011b; Carrias *et al.* 2020) dans les réservoirs d'eau des broméliacées, et une étude à une résolution taxonomique plus fine devrait confirmer ou infirmer le rôle potentiel de ces derniers comme source de *Tepidiphilus*. En effet, le réservoir d'eau des broméliacées pourrait représenter une source importante pour le microbiote de ces plantes. Ces réservoirs d'eau abritent une importante diversité de bactéries et de champignons (Brouard *et al.*, 2012; Carrias *et al.*, 2014; Simão *et al.*, 2020) qui sont en contact avec la base des feuilles des broméliacées, ce qui pourrait constituer un point d'entrée pour certains endophytes.

### 3.3 Distinction du microbiote entre apex et base des feuilles

Tout comme les variations phénotypiques à fine échelle sont généralement ignorées, la plupart des études n'échantillonnent que le microbiote d'une petite

partie de l'hôte (e.g., Chapitre III) ou, au contraire, agrègent des microorganismes prélevés sur différentes feuilles ou graines (e.g., Chapitre IV). Or, par nature, les microorganismes sont sensibles à des gradients micro-environnementaux et l'on peut observer des variations de communautés très locales à des échelles de l'ordre du centimètre (Vos *et al.*, 2013; Petrolli *et al.*, 2021). A l'échelle microbienne, les tissus de la plante ne sont pas uniformes et constituent des habitats aux conditions environnementales variées et variables (e.g., température, disponibilité en eau et nutriments). Ainsi, les différents organes de la plante représentent des habitats fondamentalement différents et, malgré un recouvrement important, on trouve des microorganismes fonctionnellement différents entre les feuilles et les racines (Bai *et al.*, 2015). Chez les broméliacées, nous avons observé une forte différenciation morpho-anatomique et physiologique entre la partie apicale et la partie basale de la feuille, en lien avec des fonctions différentes (photosynthèse dans la partie aérienne et nutrition minérale dans la partie basale en contact avec l'eau du réservoir). En plus des mesures écophysiologicalues à l'apex et à la base des feuilles d'*A. aquilega* (Chapitre II), nous avons prélevé des morceaux de limbes dans les parties apicales et basales, ainsi qu'une partie du système racinaire, pour en étudier les microorganismes bactériens et fongiques associées. L'analyse de ces données permettra d'identifier le degré de variation des communautés microbiennes au sein d'une même feuille, dont les régions assurent des fonctions différentes et sont exposées à des environnements différents (voir hypothèses Fig. 44). De plus, nous serons attentifs à la présence ou à l'absence du genre *Tepidiphilus*. L'étude du microbiote des feuilles et des racines permettra de mettre en perspective les résultats du Chapitre II concernant la réponse différentielle de l'apex et de la base des feuilles.

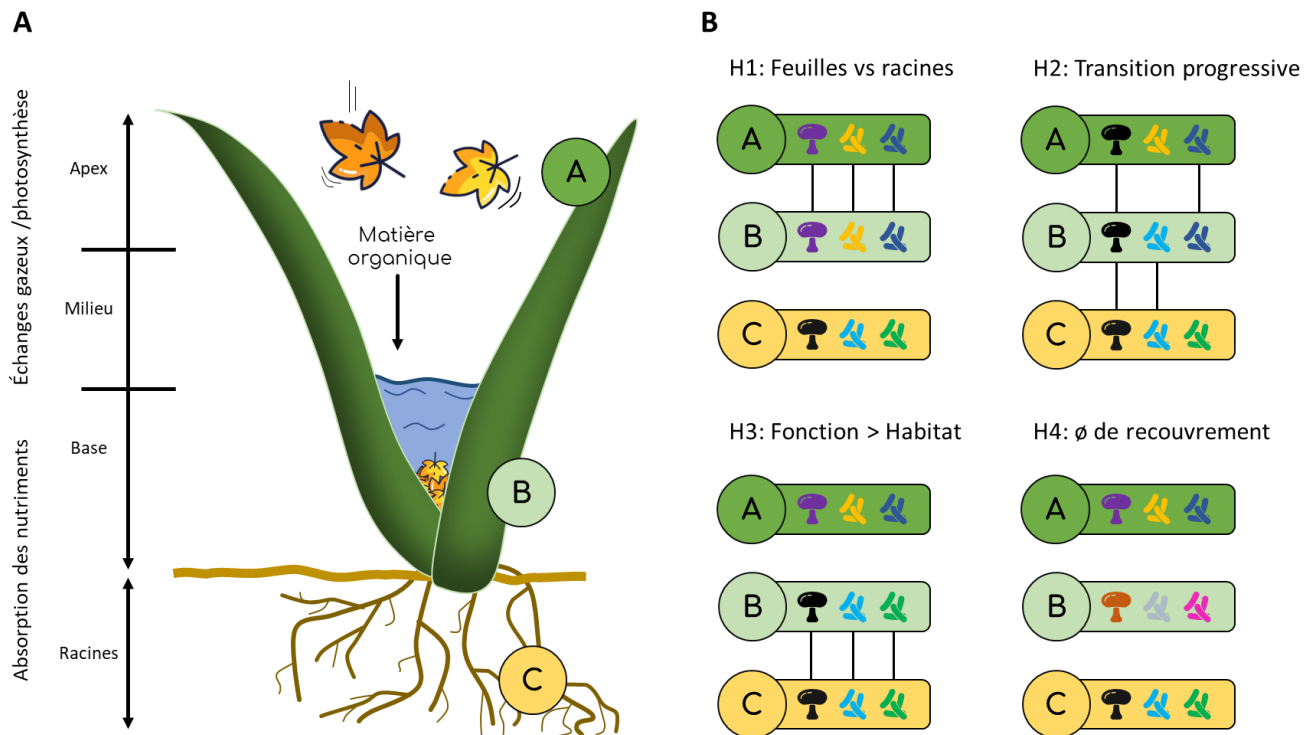


Figure 44 : Schéma récapitulatif du plan d'échantillonnage (A) et des principales hypothèses (B) d'une étude visant à caractériser les variations du microbiote en fonction du type de tissus végétal (Racinaires/Foliaires) et de la fonction assurée par l'apex (photosynthèse) ou la base de la feuille (absorption eau et minéraux). Les lettres majuscules correspondent aux différentes zones échantillonnées. A : Région apicale de la feuille, B : Région basale de la feuille et, C : Racines. Sont formulés 4 hypothèses (B, H1 à 4). H1 : Le type de tissus est le facteur dominant l'assemblage du microbiote, H2 : Le type de tissus et la fonction de l'organe sont également dominant résultant une transition progressive du microbiote entre Apex et racines, H3 : La fonction est le facteur dominant et, H4 : Les trois communautés sont complètement différentes.

### 3.4 Rôle des microorganismes présent dans le réservoir d'eau ?

Le réservoir d'eau pourrait lui-même être inclut dans le phénotype étendu des broméliacées. Le mode de nutrition des broméliacées à réservoir d'eau, en particulier de certaines espèces (e.g., *Brocchinia reducta*, *B. hechtioides* et *Catopsis berteroniana*), est parfois désigné comme une forme de proto-carnivorie (Leroy *et al.*, 2016). Ces plantes ont les feuilles recouvertes d'une substance favorisant la chute d'invertébrés dans le réservoir (piège passif) mais ne sécrètent pas d'enzymes permettant la digestion, qui est donc assurée par les communautés d'organismes aquatiques du réservoir d'eau (Leroy *et al.*, 2016). Cette relation est donc considérée comme un mutualisme digestif entre les communautés aquatiques et la plante, pouvant justifier l'intégration des résidents du réservoir d'eau dans le phénotype étendu de ces broméliacées. Récemment, Vergne *et al.*

(2021) ont apporté des preuves suggérant que les communautés bactériennes phototrophes anoxygéniques du réservoir d'eau étaient structurées par l'espèce de la plante hôte et ont émis l'hypothèse d'une dynamique co-évolutive entre la plante et les communautés bactériennes aquatiques.

## **4 Approches dynamique de l'holobionte**

### *4.1 Dynamique de l'holobionte entre génération*

Au cours de cette thèse, nous avons principalement adopté une approche statique de la plasticité en observant à un moment donné le phénotype de plantes exposées à différents environnements. Au moment de l'observation, le phénotype résulte de l'intégration des aspects invariants (non plastiques), de tous les ajustements plastiques non réversibles survenus au cours de la vie de l'individu, mais également du reflet de son état actuel. En effet, la plasticité peut être réversible et le phénotype s'ajuste en permanence aux conditions environnementales. Le phénotype est donc une entité dynamique, d'autant plus si l'on considère le microbiote, qui est lui-même hautement dynamique.

Dans le Chapitre IV, nous explorons l'aspect temporel de la plasticité en étudiant les effets maternels tant sur le microbiote que sur les traits fonctionnels des plantules. Nous y mettons en évidence l'existence d'effets maternels, car les plantules issues de plantes mères ayant poussées sur un substrat riche ont tendance à mieux performer que celles exposés aux mêmes conditions environnementales mais issus de plantes mères ayant poussées sur un substrat pauvre. Ce type d'effets maternels est bien connu chez les plantes et peut résulter de différents mécanismes non exclusifs (Roach & Wulff, 1987). En revanche, les effets maternels sur le microbiote sont bien moins connus, et nos résultats font partie des rares études s'y intéressant, bien que la littérature sur le sujet se densifie rapidement (Funkhouser & Bordenstein, 2013; Vivas *et al.*, 2015; Fort *et al.*, 2021; Murphy *et al.*, 2023).



Si l'on peut retracer l'émergence du concept d'holobionte dans les années 40 (Baedke *et al.*, 2020), ce n'est que depuis quelques années qu'il reçoit une attention importante de la communauté scientifique et, malgré ses efforts, nous n'en sommes encore qu'aux prémices de notre compréhension de cette entité. Nos résultats restent principalement exploratoires et descriptifs, mais ils posent les bases d'intéressantes perspectives. Tout d'abord, ils révèlent la présence au sein des graines d'*A. mertensii* d'une importante diversité de microorganismes. L'agrégation d'une cinquantaine de graines pour chaque individu ne nous permet pas de déterminer la diversité par graine, mais celle-ci est potentiellement assez élevée et variable, puisque nous avons retrouvé entre 15 et 1011 OTUs bactériens et entre 41 et 461 OTUs fongiques. Ceci et l'observation de la composition des communautés microbiennes, suggère également une probable importante hétérogénéité entre les graines d'une même mère, ce qui serait cohérent avec les résultats de Chesneau *et al.* (2022). Par ailleurs, les analyses multivariées réalisées sur les communautés des graines n'ont pas permis d'identifier un effet fort et cohérent des différents traitements sur le microbiote des graines. Ceci supporte un effet maternel individu-dépendant, plutôt qu'en lien avec l'environnement, sur la transmission de microorganismes par la graine et potentiellement un triage aléatoire des potentiels organismes colonisant la graine plutôt qu'un processus activement régulé par la plante hôte et coordonné en réponse aux conditions environnementales.

Malgré cela, nous avons mis en évidence une influence maternelle sur le microbiote de la descendance. Ces résultats semblent indiquer que des effets maternels sur le microbiote pourraient en fait être médiés par d'autres facteurs que la transmission directe de symbiotes microbiens. A ce stade et avec ces données, nous ne pouvons pas proposer un facteur privilégié, mais potentiellement tous les processus susceptibles d'influencer le développement du microbiote et d'être affecté par les conditions environnementales des plantes

mères pourraient jouer le rôle de régulateur d'effets maternels sur le microbiote. Par exemple, l'approvisionnement de la graine en différentes molécules et ressources, déjà identifié comme un acteur des effets maternels chez les plantes (Roach & Wulff, 1987), pourrait également favoriser la croissance de certains microorganismes chez la jeune plantule. Nous avons réalisé des analyses chimiques et métabolomiques révélant que la fertilité du substrat a affecté l'approvisionnement des graines (Fig. 43) et que notamment les graines de plantes mères poussant sur un substrat riche avaient un profil très différent des autres. Une analyse plus poussée sera nécessaire pour identifier les différences en contenu de certaines molécules, mais ces caractéristiques ont pu directement affecter le développement du microbiote.

En parallèle, ces différences ont vraisemblablement influencé le développement de la plante (habitat du microbiote) et les caractéristique physico-chimique (effets maternels du Chapitre IV) des tissus de la plante, ayant un effet indirect sur les communautés susceptibles de s'y développer. Les nombreuses boucles de rétroaction entre le microbiote et le phénotype de la plante rendent difficile de déterminer qui influence qui, car chaque modification de l'hôte ou du microbiote a le potentiel de déclencher des modifications de l'autre de partenaire (Sánchez-Cañizares *et al.*, 2017).

#### *4.2 Dynamique de l'holobionte au sein d'une génération*

Nous avons caractérisé le microbiote de plantes matures au stade de floraison, de graines et de plantules âgés de deux mois. Si l'on se replace dans le cycle de vie de l'holobionte, nous avons donc une idée de l'influence de l'environnement sur son état final et initial, puisque la reproduction et la germination représentent les deux extrémités de ce cycle. Cependant, malgré des conditions expérimentales similaires (même serre et substrats), nous avons observé des différences entre les microbiotes de ces deux stades développementaux. Ainsi, les plantes matures

(Chapitre III) ont un microbiote foliaire fortement enrichi en *Bacilli*, *Actinobacteria* et *Leotiomycetes* en comparaison à celui retrouvé chez des plantules (Chapitre IV) (Chapitre III, Fig. S11 & Chapitre IV, Fig. S16). Ces différences peuvent être partiellement imputable à des effets environnementaux ou saisonniers (Copeland *et al.*, 2015), mais il est plutôt vraisemblable qu'elles soient également dues à l'ontogénie, c'est-à-dire une succession écologique au sein du microbiote au cours de la croissance de l'hôte (Liu *et al.*, 2013; Edwards *et al.*, 2018).

Au cours de son développement, la plante est exposée à la colonisation de nouveaux microorganismes depuis l'environnement, mais subit également de fortes modifications morphologiques et physiologiques associées à sa croissance. Or, les conditions environnementales affectent le développement d'*A. mertensii* et le microbiote environnementale servant de réservoir d'endophytes. La succession écologique au sein du microbiote résulte probablement à la fois de l'ontogénie de l'hôte, de l'environnement et des éventuelles rétroactions entre ces deux facteurs. Comprendre la dynamique du microbiote au cours du développement de l'holobionte est une question cruciale puisqu'un important turnover dans la composition du microbiote au cours de la vie de l'hôte entraîne d'importantes différences entre le microbiote reçu verticalement et ultimement transmis. Comprendre les successions microbiennes et les facteurs environnementaux qui les affectent est une question majeure, étant donné l'importance de ces interactions biotiques sur la *fitness* et la dynamique des populations de leurs hôtes.

Pour étudier ces questions, j'ai obtenu, au cours de ma thèse, un financement dans le cadre de l'appel à projets annuels du LabEx CEBA (Centre d'Étude de la Biodiversité Amazonienne). Le projet ENDOSHIFT a pour objectif de suivre l'évolution du microbiote au cours du développement, depuis la graine jusqu'à des plantes matures, pour mieux caractériser les trajectoires des communautés d'endophytes au cours de l'ontogénie de la plante hôte et leur lien

avec l'environnement. Pour cela, nous avons réalisé une expérience en serre ou nous avons échantillonné à intervalles réguliers (à T0 jour, T1 jour, T3 jours, T6 jours, T10 jours jusqu'à T120 jours, Fig. 44) des individus poussant dans différents substrats (riche et pauvre).

Ces résultats devraient nous permettre de mieux comprendre comment se déroule la succession écologique du microbiote. Est-elle lente et graduelle ou une succession de modifications rapides et importantes ? Est-elle synchronisée avec les changements phénotypiques de la plante ? Quelle est l'importance relative de l'ontogénie et de l'environnement sur la trajectoire des communautés endophytes racinaires et foliaires ? Retrouve-t-on la succession de même groupes taxonomique dans différents environnements ou alors un fort effet environnemental ? Enfin, cette succession est-elle différente dans sa dynamique et les taxa concernés en fonction des compartiments de la plante (*i.e.*, racines et feuilles).

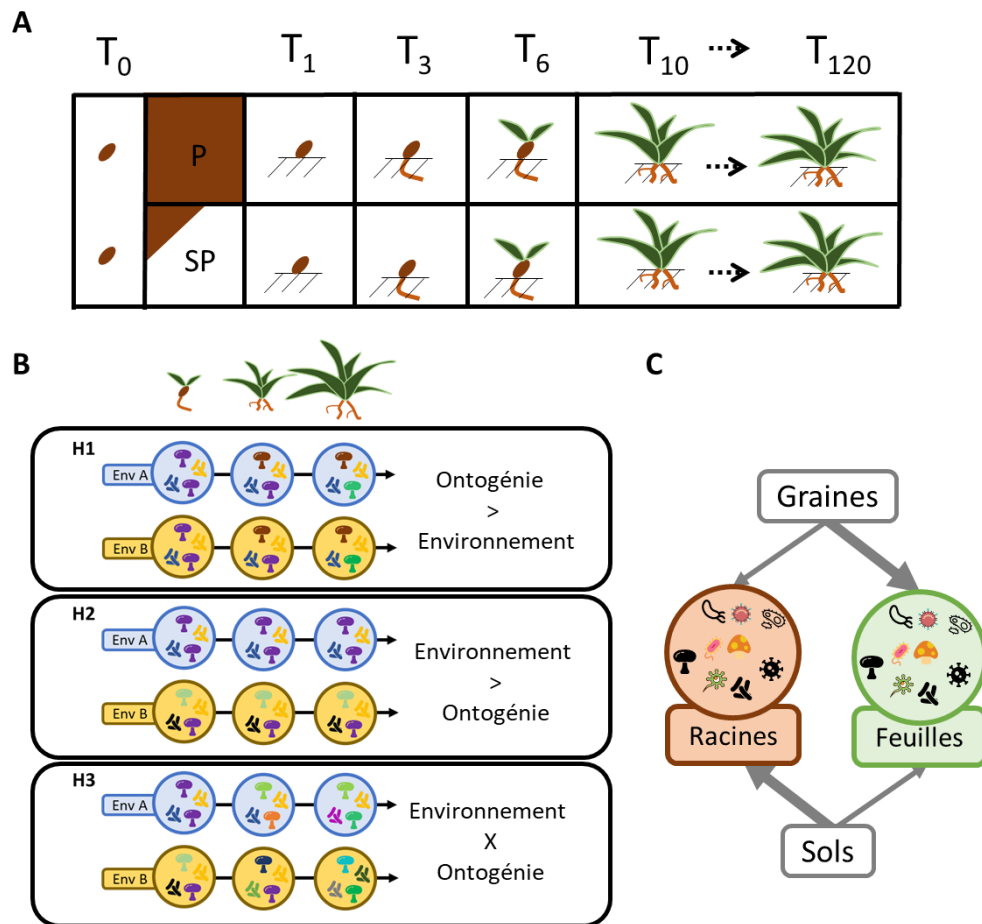


Figure 44: Schéma récapitulatif du design expérimental du projet ENDOSHIFT (A) et des principales hypothèses (B et C). Des graines issues d'une même plante mère ont été semées dans deux types de substrats (P : Terreau et SP : 2/3 sable blanc et 1/3 terreau) puis des individus ont été régulièrement prélevés pendant 120 jours pour des mesures de traits et une caractérisation du microbiote. Nous illustrons les hypothèses alternatives d'une plus grande importance de l'ontogénie par rapport à l'environnement dans la détermination du microbiote (B, H1), résultant à une trajectoire commune aux deux environnements, ou d'une contrainte plus forte de l'environnement caractérisée par des microbiotes plus différents entre les environnements que les stades de développement (B, H2) et enfin, une combinaison de ces deux facteurs résultants en des microbiotes bien distincts entre environnements et à chaque stade du développement (B, H3). Enfin, nous prédisons également une plus grande influence du microbiote hérité des graines sur les celui des feuilles que celui des racines (C).

#### 4.3 Dynamique de l'holobionte en fonction d'un environnement changeant

Si la dynamique de l'holobionte suscite autant d'intérêt, c'est en grande partie parce que l'environnement lui-même est hautement dynamique, et que la composante microbienne de l'holobionte pourrait permettre un ajustement rapide à de nouvelles conditions changeantes (Vannier *et al.*, 2015). Cependant, dans ce travail de thèse, nous avons uniquement étudié la réponse de l'holobionte

à différentes conditions environnementales maintenues constantes au cours du temps.

Dans le but d'étudier la réponse de l'holobionte à un changement environnemental, nous avons mis en place une expérimentation en milieu naturel. Nous avons tiré profit de la transportabilité des broméliacées à réservoir d'eau pour simuler une ouverture de la canopée et décrire les réponses phénotypiques et microbiennes mises en place par l'holobionte *A. aquilega*. La disponibilité en lumière n'est pas forcément constante au cours de la vie d'une broméliacées puisque des dynamiques de trouées (naturelles ou non) viennent régulièrement ouvrir la canopée des forêts tropicales (Hubbell *et al.*, 1999) ou que des broméliacées ayant grandi en canopée peuvent tomber à terre et continuer leur croissance suite à des coups de vent ou chablis (Toledo-Aceves *et al.*, 2014). Pour simuler ce genre d'évènement, nous avons prélevé des broméliacées de sous-bois puis les avons déplacées vers une zone exposée. Au bout 6 mois, nous avons caractérisé les différences écophysologiques entre des individus vivant en pleine lumière et dans le sous-bois, ainsi que ceux ayant été déplacés (plantes « déforestés »). Des prélèvements de morceaux de feuilles (parties apicales et basales) ont été réalisés pour obtenir les communautés microbiennes associées. Les résultats écophysologiques montrent les plantes "déforestées" ont un phénotype intermédiaire entre celles de forêt et celles de milieu ouvert (Fig. 45A). Ces résultats suggèrent un ajustement de la plante se rapprochant du phénotype des individus poussant naturellement en milieu ouvert. Cependant, la transition n'est pas complète et il faudrait renouveler les mesures à un troisième pas de temps pour vérifier si les plantes "finissent" leur acclimatation (plasticité complètement réversible) ou si elles conservent des caractéristiques intermédiaires.

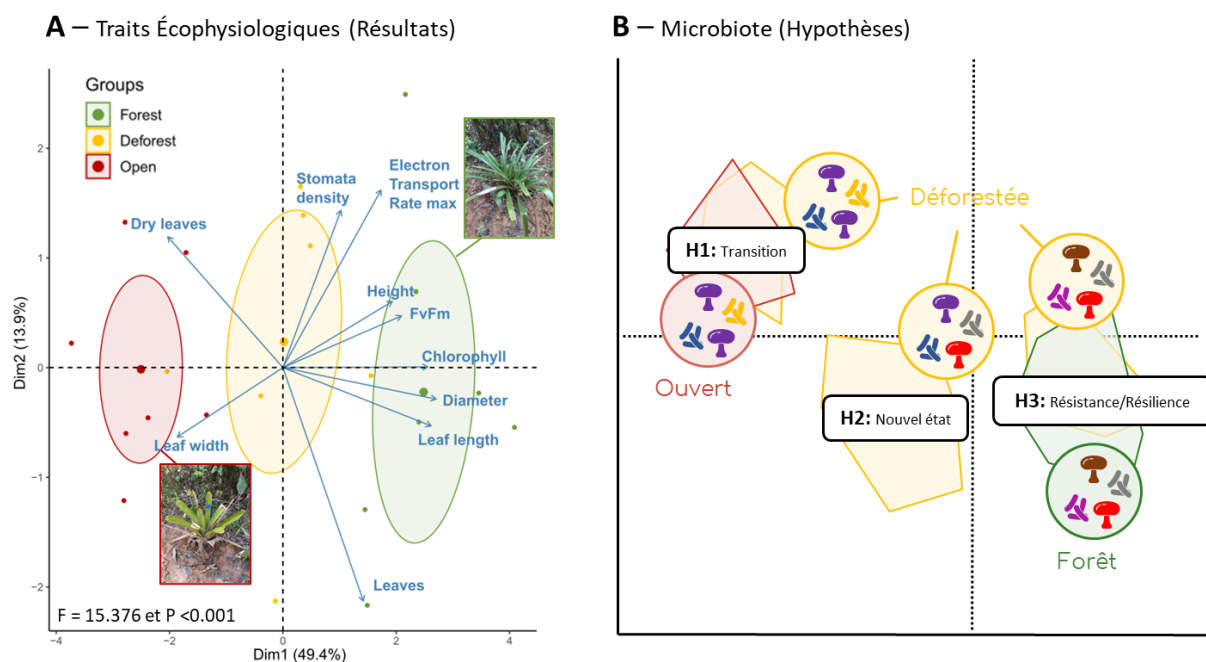


Figure 45: (A) ACP mettant en évidence la réponse phénotypique d'*A. aquilega* à une simulation de "déforestation" (déplacement d'un milieu fermé à ouvert). Les petits points représentent la projection des individus sur le plan formé par les deux premières composantes principales. Les gros points indiquent les centroïdes de chaque groupe et les ellipses les intervalles de confiance à 95% de l'estimation de ces centroïdes. Les flèches bleues représentent la projection de traits mesurés sur ces deux mêmes axes. (B) Résultat de PCoA théorique sur le microbiote des feuilles sur la base de trois hypothèses. H1 : Le microbiote a complètement transitionné d'une communauté retrouvée dans les feuilles des plantes de forêt à celle du milieu ouvert, H2 : Le microbiote des plantes "déforestées" est différent de celui des autres plantes présentant un nouvel état de la communauté ou, H3 : Le microbiote des plantes "déforestées" a été parfaitement résistant/résiliant à la perturbation et est indiscernable de celui des plantes de forêt. Pour les deux graphiques sont représentées en rouge les plantes de milieu ouvert, en vert celles de forêt et en jaune celles ayant subi une "déforestation".

En ce qui concerne le microbiote, nous avons trois hypothèses alternatives (Fig. 45B). Soit le microbiote a résisté ou fait preuve d'une résilience parfaite résultant en notre incapacité à distinguer le microbiote des plantes "déforestées" de celui des plantes restées en forêt. Soit le microbiote a accompli une transition complète et parfaite vers celui présent chez les plantes de milieux ouverts Soit le microbiote des plantes « déforestées » est distinct de celui des autres plantes, suggérant alternativement un état transitoire ou un nouvel état. Cette dernière hypothèse est cependant la plus vraisemblable étant donné l'effet de l'environnement sur le microbiote (excluant une résistance/résilience totale) et l'influence de la composition initiale sur les trajectoires des communautés (excluant une transition complète).

## 5 Vers un holobionte “Ecologique”

L'un des écueils majeurs de la recherche sur la question est indéniablement la difficulté à établir des liens causalité généralisables entre les différentes facettes de l'holobionte. La plupart des études permettant une forme de causalité sont réalisées dans des environnements hautement contrôlés sur des espèces de plantes modèles et/ou d'intérêt économique (Bai *et al.*, 2015; Vorholt *et al.*, 2017). Nous manquons de connaissances sur l'importance de ce concept dans la nature et à quelle point ces interactions contribuent à ce que nous observons, des organes aux paysages. Jusqu'à présent, l'holobionte a été un concept débattu principalement pour des questions d'évolution (Roughgarden, 2020) mais placer l'écologie au centre du débat et questionner la diversité des partenaires microbiens des plantes au regard de l'écologie de leurs hôtes semble être la prochaine étape à notre compréhension de cette entité. Parmi les voies se dessinant, on trouve des approches permettant une caractérisation fonctionnelle du microbiote plutôt que taxonomique (Lemanceau *et al.*, 2017). Ces méthodes devraient nous permettre de mieux comprendre la relation entre fonctionnement de l'hôte et de son microbiote tout en augmentant notre capacité à généraliser nos résultats.

Une caractérisation plus systématique et complète des fonctions assurées par le microbiote pourrait également permettre d'éclaircir les différences de réponses observées entre symbiontes bactériens et fongiques. Ces deux groupes sont les représentants majeurs du microbiote et sont essentiels au fonctionnement de l'holobionte. Bien qu'ayant identifié de nombreuses fonctions associées à ces taxa (Bulgarelli *et al.*, 2013; Müller *et al.*, 2016; Trivedi *et al.*, 2020), leur sensibilité à certains facteurs environnementaux et pas à d'autres est encore bien souvent mystérieuse. Par exemples, dans les Chapitres III et IV nous avons constaté des réponses différentes entre ces groupes à la richesse de sol et à la disponibilité en eau. Ces différences sont probablement dues en partie à des



différences biologiques et de préférences écologiques mais il est également envisageable que les facteurs environnementaux affectent différenciellement les relations hôtes-champignons et hôte-bactéries.

Par ailleurs, la constitution de base de données toujours plus complètes et intégrant différentes approches, groupes phylogénétiques, environnements et stades de développement devrait permettre la réalisation d'études globales visant à dégager des patrons à l'échelle mondiale. Par exemple, Simonin et al. (2022) ont récemment compilé les données de plusieurs études et ont ainsi révélé l'existence d'un microbiote cœur au sein des graines. Parmi les 50 espèces considérées 18 sont des espèces cultivées. Or on sait aujourd'hui que la domestication des plantes a également eu un effet notable sur leur microbiote (Martínez-Romero *et al.*, 2020; Soldan *et al.*, 2021) limitant notre capacité à réellement généraliser.

Allant dans le même sens, des travaux à l'échelle globale ont permis la mise en évidence de liens entre les fonctions réalisées par les communautés microbiennes et leur habitats (Lajoie & Kembel, 2023). Ce type d'approches chez les plantes pourrait mener à la définition de ce que nous pourrions appeler un *Microbiota Economic Spectrum*. L'objectif de ces travaux serait de mettre en évidence, à l'échelle globale, des associations systématiques et donc prédictibles de certains microorganismes (taxa/fonctions) avec des stratégies écologiques des plantes (traits et/ou formes de vie).

Les broméliacées à réservoir d'eau et les épiphytes en général représentent une opportunité de questionner ces aspects en raison de leur diversité et leur écologie particulières. Tout comme elles occupent une place à part dans le *Leaf Economic Spectrum* global (Hietz *et al.*, 2021), les épiphytes sont vraisemblablement associée à des microorganismes différents en fonction de la phylogénie et géographie mais également des conditions micro-environnementales (*e.g.*, sol vs canopée) et des stratégies écologiques (Leroy *et al.*, 2021). En effet, on trouve chez de nombreuses épiphytes des stratégies d'acquisition de nutriments alternatives

à la nutrition racinaire qui sont potentiellement associées à différents microbiotes. Les broméliacées et leurs réservoirs d'eau, mais également les pitcher-plants (*Nepenthes*), les plantes mycohétérotrophes ou mixotrophes (certaines *Orchidaceae*, *Monotropaceae*, *Ericaceae*) sont autant de modèles dont nous pourrions tirer une compréhension de l'importance du microbiote dans les stratégies des partenaires végétaux.

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# Annexes

**Annexes Chapitre I : Light intensity mediates phenotypic  
plasticity and leaf trait regionalisation in a tank  
bromeliad**

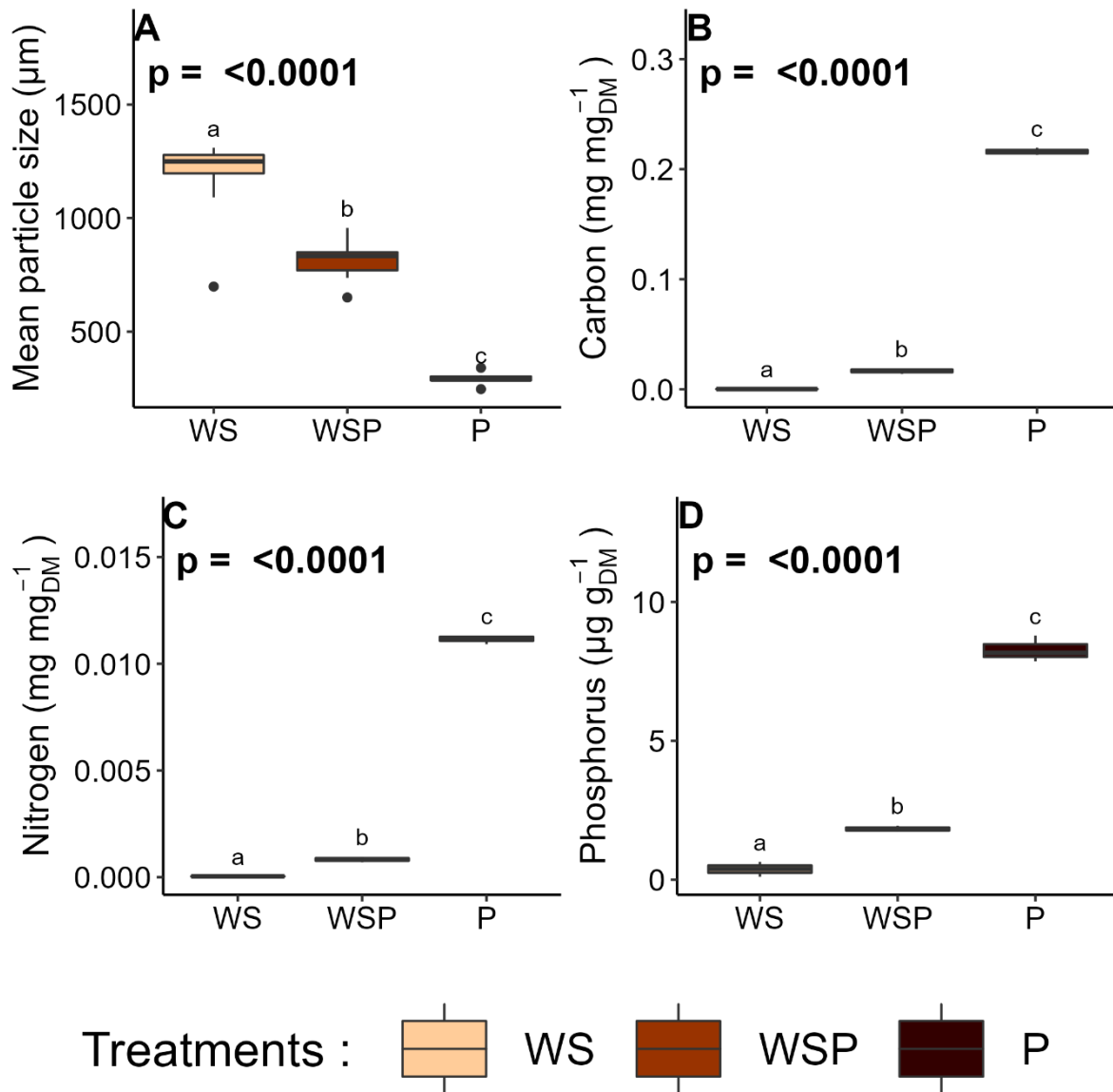


Figure S1 Treatment granulometry and CNP content. Boxplots for (A) substrate mean particle diameter ( $\mu\text{m}^{-1}$ ), (B) substrate N ( $\text{mg g}_{\text{DM}}^{-1}$ ), (C) substrate C ( $\text{mg g}_{\text{DM}}^{-1}$ ), and (D) substrate P ( $\mu\text{g g}_{\text{DM}}^{-1}$ ) contents. Different letters indicate significant differences between treatments based on pairwise t test ( $\alpha < 0.05$ ) after significant Anova ( $\alpha < 0.05$ ). p is the p value of the Anova test. WS, white sand; WSP, white sand/potting soil; P, potting soil

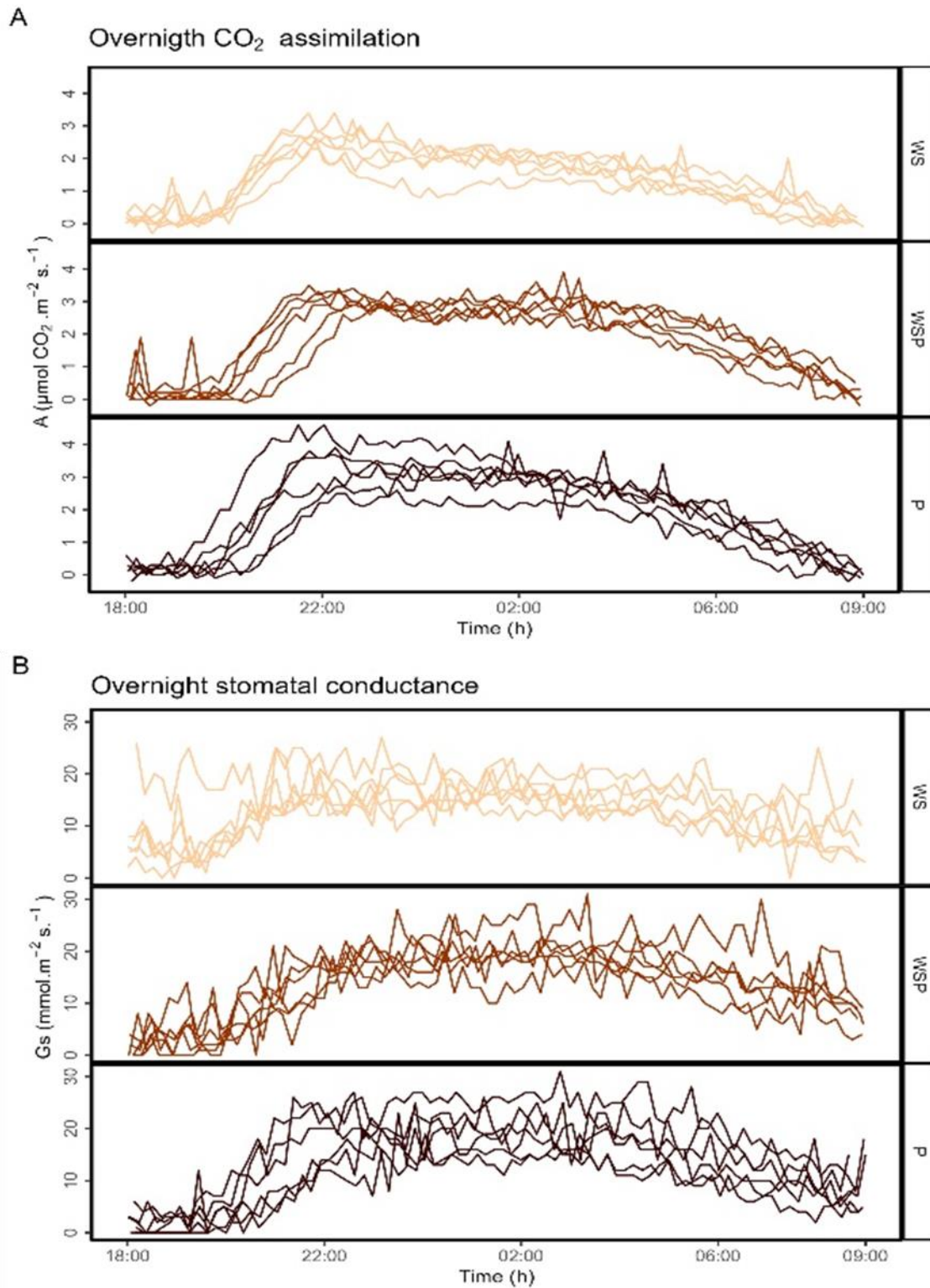


Figure S2: Overnight CO<sub>2</sub> assimilation and stomatal conductance curves. Curves of overnight (A) CO<sub>2</sub> assimilation ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and (B) stomatal conductance ( $G_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ). These measurements were conducted on 6 extra plants per treatments. One plant per day with one of each treatment every three days over a month. WS, white sand; WSP, white sand/potting soil; P, potting soil.

Table S1: Statistical summary table: Mean and standard deviation (SD) of each trait are displayed for the three treatments (WS, white sand; WSP, white sand/potting soil; P, potting soil). The global coefficient of variation (CV) is given in %. The associated Kruskal Wallis Chi<sup>2</sup> and P-values are shown. Significant P-values (<0.05) are in bold. Letters indicates significant pairwise differences based on Wilcoxon pairwise test ( $\alpha < 0.05$ ). Growth,  $A_{max}$ ,  $GS_{max}$  and  $A_{int}$  have been measured on 6 extra bromeliads. Abbreviations: relative growth rate (RGR); total root length (TRL); leaf mass area (LMA); leaf dry mass content (LDMC); trichome area index (TAI); leaf chlorophyll content (CHL); PSII maximum quantum efficiency ( $F_v/F_m$ ); maximum electron transport rate ( $ETR_{max}$ ); maximal assimilation ( $A_{max}$ ); stomatal conductance ( $GS_{max}$ ); overnight integrated assimilation ( $A_{int}$ ); specific tip root average (STRA); specific root length (SRL); average root diameter (ARD); root tissue density (RTD).

Traits	Mean $\pm$ SD			CV	Significance	
	WS	WSP	P	%	KW.chi	P.val
<b>Overall plant performance</b>						
Number of leaves	8.7 $\pm$ 1.89 (a)	13.9 $\pm$ 1.1 (b)	14.2 $\pm$ 1.4 (b)	24.00	18.300	<b>0.000107</b>
Number of roots	25.7 $\pm$ 5.08 (a)	51.5 $\pm$ 12.7 (b)	72.3 $\pm$ 13.3 (c)	44.40	23.100	<b>&lt;0.0001</b>
Total DM (g)	1.09 $\pm$ 0.372 (a)	10.7 $\pm$ 1.97 (b)	16.3 $\pm$ 3.08 (c)	71.50	25.100	<b>&lt;0.0001</b>
Tank capacity (ml)	4.15 $\pm$ 1.83 (a)	81 $\pm$ 17.2 (b)	145 $\pm$ 34.7 (c)	81.40	25.100	<b>&lt;0.0001</b>
RGR (mg month <sup>-1</sup> )	0.464 $\pm$ 0.02 (a)	0.618 $\pm$ 0.013 (b)	0.646 $\pm$ 0.012 (c)	14.4	25.055	<b>&lt;0.0001</b>
Root-to-shoot-ratio	17.1 $\pm$ 8.18 (a)	4.16 $\pm$ 1.87 (b)	3.84 $\pm$ 1.22 (b)	93.90	18.600	<b>&lt;0.0001</b>
Leaf length (cm)	14.6 $\pm$ 1.84 (a)	25.1 $\pm$ 1.95 (b)	26.5 $\pm$ 2.28 (b)	26.00	20.300	<b>&lt;0.0001</b>
Leaf thickness (mm)	6.21 $\pm$ 0.262 (a)	7.75 $\pm$ 0.272 (b)	8.3 $\pm$ 0.356 (c)	12.70	23.600	<b>&lt;0.0001</b>
TRL (cm)	328 $\pm$ 96.5 (a)	832 $\pm$ 436 (b)	1460 $\pm$ 464 (c)	67.80	20.900	<b>&lt;0.0001</b>
<b>Leaf traits</b>						
LMA (g m <sup>-2</sup> )	46.1 $\pm$ 3.95 (a)	64.1 $\pm$ 16.4 (ab)	76 $\pm$ 13.3 (b)	27.90	14.000	<b>0.000891</b>
LDMC (g <sub>DM</sub> g <sub>FM</sub> <sup>-1</sup> )	0.0859 $\pm$ 0.0105	0.0938 $\pm$ 0.0243	0.104 $\pm$ 0.0193	20.80	5.030	0.081
Stomata density (Nb mm <sup>-2</sup> )	23.2 $\pm$ 3.56 (a)	37.1 $\pm$ 5.61 (b)	34 $\pm$ 4.04 (b)	23.70	19.200	<b>&lt;0.0001</b>
TAI (%)	46.68 $\pm$ 15.62 (a)	66.66 $\pm$ 16.13 (b)	68.62 $\pm$ 13.9 (b)	29.37	8.322	<b>0.015</b>
Leaf C (mg g <sub>DM</sub> <sup>-1</sup> )	402 $\pm$ 11.3 (a)	429 $\pm$ 6.2 (b)	427 $\pm$ 4.27 (b)	3.47	19.500	<b>&lt;0.0001</b>
Leaf N (mg g <sub>DM</sub> <sup>-1</sup> )	6.48 $\pm$ 0.982 (a)	4.71 $\pm$ 0.62 (b)	4.68 $\pm$ 0.371 (b)	20.70	18.600	<b>&lt;0.0001</b>
Leaf P (mg g <sub>DM</sub> <sup>-1</sup> )	1.27 $\pm$ 0.351	1.53 $\pm$ 0.246	1.44 $\pm$ 0.122	19.20	4.000	0.135
CHL ( $\mu$ g g <sub>DM</sub> <sup>-1</sup> )	3.96 $\pm$ 0.98	3.91 $\pm$ 1.2	3.14 $\pm$ 1.04	30.1	5.546	0.0624
$F_v/F_m$	0.726 $\pm$ 0.0151	0.738 $\pm$ 0.0144	0.732 $\pm$ 0.028	2.74	3.700	0.157
$ETR_{max}$ ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )	47.3 $\pm$ 6.45	46.1 $\pm$ 10.2	42.7 $\pm$ 9.94	19.60	1.520	0.468
$A_{max}$ ( $\mu$ mol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	2.7 $\pm$ 0.443 (a)	3.38 $\pm$ 0.172 (a)	3.63 $\pm$ 0.662 (a)	18.50	7.420	<b>0.0244</b>
$GS_{max}$ ( $\mu$ mol m <sup>-2</sup> s <sup>-1</sup> )	22 $\pm$ 3.69	24.3 $\pm$ 4.03	23.8 $\pm$ 4.49	17.00	0.936	0.626
$A_{int}$ (mmol CO <sub>2</sub> m <sup>-2</sup> )	12.7 $\pm$ 2.33 (a)	17.5 $\pm$ 1.41 (b)	18.3 $\pm$ 3.66 (ab)	22.10	9.090	<b>0.0106</b>
<b>Root traits</b>						
STRA (root tips g <sup>-1</sup> )	5880 $\pm$ 3890	3460 $\pm$ 803	4840 $\pm$ 2120	57.20	4.760	0.0927
SRL (m g <sup>-1</sup> )	0.6 $\pm$ 0.085 (ab)	0.706 $\pm$ 0.119 (a)	0.58 $\pm$ 0.0625 (b)	16.70	8.880	<b>0.0118</b>
ARD (mm)	30.9 $\pm$ 19.2	21.9 $\pm$ 5.64	28.9 $\pm$ 14.3	52.40	1.370	0.505
RTD (g cm <sup>-3</sup> )	0.139 $\pm$ 0.0451	0.142 $\pm$ 0.0368	0.145 $\pm$ 0.0502	30.10	0.379	0.827
Root C (mg g <sub>DM</sub> <sup>-1</sup> )	352 $\pm$ 44.1 (a)	450 $\pm$ 18.4 (b)	466 $\pm$ 5.94 (c)	13.70	21.100	<b>&lt;0.0001</b>
Root N (mg g <sub>DM</sub> <sup>-1</sup> )	3.4 $\pm$ 0.53 (a)	4.26 $\pm$ 0.536 (b)	4.94 $\pm$ 0.715 (c)	20.60	16.000	<b>0.000336</b>
Root P (mg g <sub>DM</sub> <sup>-1</sup> )	0.856 $\pm$ 0.067 (a)	1.03 $\pm$ 0.106 (b)	1.39 $\pm$ 0.231 (c)	24.70	24.400	<b>&lt;0.0001</b>

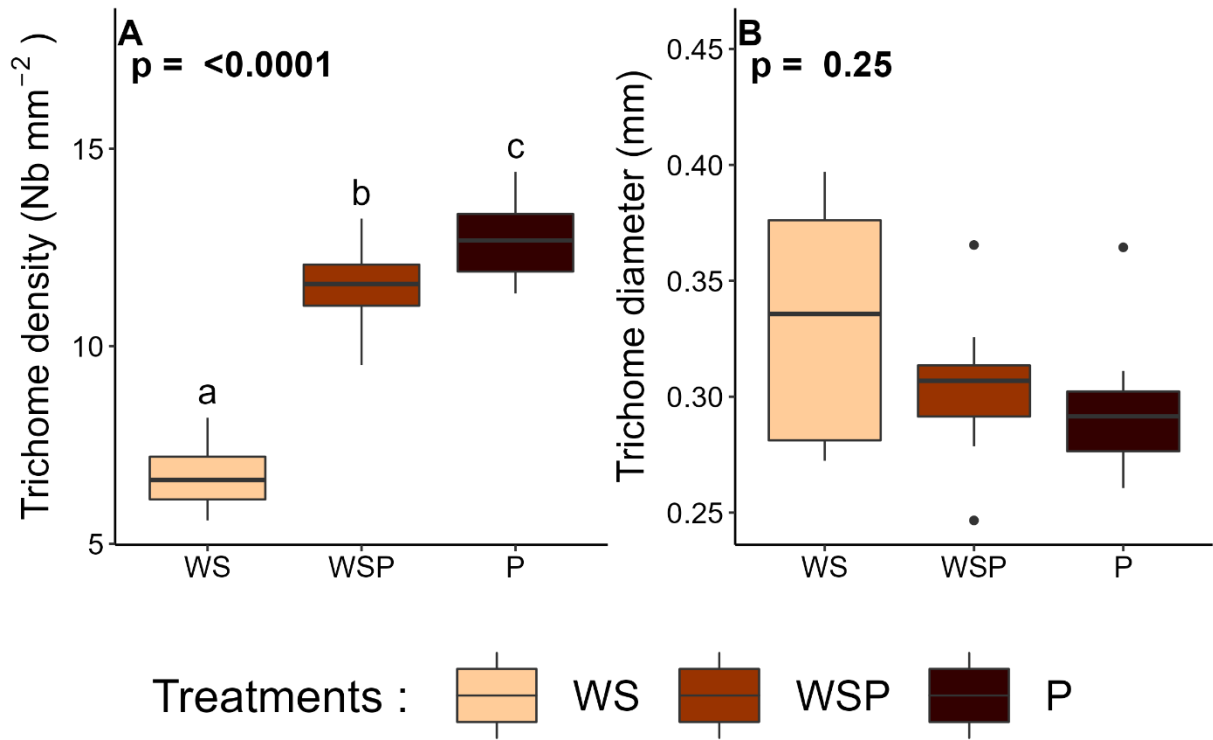


Figure S3: Effect of substrate fertility on leaf trichomes. Boxplots for (A) trichomes density ( $\text{Nb mm}^{-2}$ ) and (B) trichome diameter (mm). Different letters indicate significant differences between treatments based on pairwise Wilcoxon test ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ).  $p$  is the  $p$  value of the Kruskal-Wallis test. WS, white sand; WSP, white sand/potting soil; P potting soil.

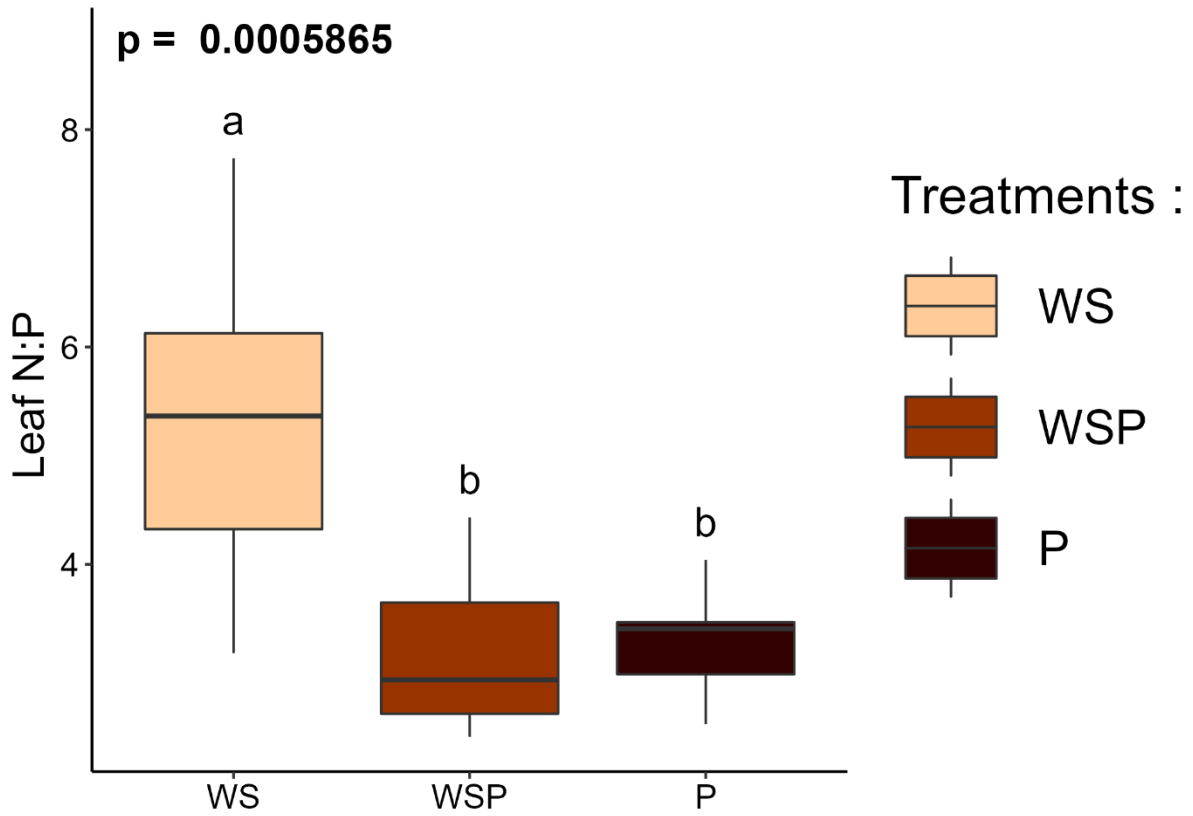


Figure S4: Effect of substrate fertility on leaf N:P ratio. Boxplots for N:P ratio. Different letters indicate significant differences between treatments based on pairwise Wilcoxon test ( $\alpha < 0.05$ ) after significant Kruskal-Wallis ( $\alpha < 0.05$ ). p is the p value of the Kruskal-Wallis test. WS, white sand; WSP, white sand/potting soil; P, potting soil.

**Annexes Chapitre II : Light intensity mediates  
phenotypic plasticity and leaf trait regionalisation in a  
tank bromeliad**



Table S2: Table summary of linear models output for plant traits according to the transmitted light (%). Are presented for each traits the estimates (mean and standard deviation) of the linear models' coefficients, t-statistic, R<sup>2</sup>, and associated p-value. Models were performed on log-transformed variables and estimates must be back-transformed to get biological values. Bold p-values indicate significance (p < 0.05).

<b>Variables</b>		<b>Estimates: mean ± se</b>	<b>t Statistic</b>	<b>R<sup>2</sup></b>	<b>p value</b>
<b>Number of leaves</b>	Intercept	2.7302 ± 0.0667	40.96		<b>&lt;0.001</b>
	Light	-0.0062 ± 0.0017	-3.70	0.43	<b>0.002</b>
<b>Plant diameter</b>	Intercept	4.9620 ± 0.1000	49.60		<b>&lt;0.001</b>
	Light	-0.0175 ± 0.0025	-6.90	0.73	<b>&lt;0.001</b>
<b>Plant Height</b>	Intercept	4.4964 ± 0.0749	60.03		<b>&lt;0.001</b>
	Light	-0.0093 ± 0.0019	-4.90	0.57	<b>&lt;0.001</b>
<b>Leaf length</b>	Intercept	4.5892 ± 0.0838	54.76		<b>&lt;0.001</b>
	Light	-0.0115 ± 0.0021	-5.40	0.62	<b>&lt;0.001</b>
<b>Leaf width</b>	Intercept	1.7759 ± 0.0404	43.94		<b>&lt;0.001</b>
	Light	0.0077 ± 0.0010	7.50	0.76	<b>&lt;0.001</b>
<b>Stomatal density</b>	Intercept	3.5494 ± 0.0401	88.42		<b>&lt;0.001</b>
	Light	-0.0032 ± 0.0010	-3.15	0.36	<b>0.006</b>
<b>Chlorophyll</b>	Intercept	2.1876 ± 0.1292	16.93		<b>&lt;0.001</b>
	Light	-0.0316 ± 0.0033	-9.65	0.84	<b>&lt;0.001</b>
<b>ETRmax</b>	Intercept	4.2548 ± 0.0911	46.69		<b>&lt;0.001</b>
	Light	-0.0061 ± 0.0023	-2.66	0.28	<b>0.016</b>
<b>FvFm</b>	Intercept	-0.1621 ± 0.0249	-6.51		<b>&lt;0.001</b>
	Light	-0.0035 ± 0.0006	-5.57	0.63	<b>&lt;0.001</b>
<b>Leaf C</b>	Intercept	6.0485 ± 0.0048	1248.51		<b>&lt;0.001</b>
	Light	0.0004 ± 0.0001	3.08	0.35	<b>0.006</b>
<b>Leaf soluble sugars</b>	Intercept	2.6268 ± 0.1004	26.15		<b>&lt;0.001</b>
	Light	0.0071 ± 0.0025	2.78	0.3	<b>0.012</b>
<b>Leaf starch</b>	Intercept	1.6642 ± 0.2079	8.00		<b>&lt;0.001</b>
	Light	0.0081 ± 0.0053	1.54	0.12	0.142
<b>Leaf N</b>	Intercept	2.0113 ± 0.0862	23.32		<b>&lt;0.001</b>
	Light	-0.0103 ± 0.0022	-4.73	0.55	<b>&lt;0.001</b>
<b>Leaf P</b>	Intercept	-0.5629 ± 0.0911	-6.18		<b>&lt;0.001</b>
	Light	-0.0111 ± 0.0023	-4.79	0.56	<b>&lt;0.001</b>

Table S3: Table summary of linear models output for plant traits according to transmitted light (%) and position on the leaf blade. Are presented the estimates (mean and standard deviation) of the linear models' coefficients, t-statistic, R<sup>2</sup>, and associated p-value. Models were performed on log-transformed variables and estimates must be back-transformed to get biological values. Bold p-values indicate significance (p < 0.05).

<b>Variables</b>		<b>Estimates: mean ± se</b>	<b>t Statistic</b>	<b>R<sup>2</sup></b>	<b>p value</b>
<b>RWC</b>	Intercept	4.5154 ± 0.0125	360.19	0.12	<b>&lt;0.001</b>
	Light	0.0001 ± 0.0003	0.28		0.784
	Position Base	0.0332 ± 0.0177	1.87		0.069
	Light:Position Base	-0.0006 ± 0.0005	-1.22		0.23
<b>LMA</b>	Intercept	4.9283 ± 0.0554	88.96	0.34	<b>&lt;0.001</b>
	Light	0.0029 ± 0.0015	1.89		0.067
	Position Base	-0.0914 ± 0.0783	-1.17		0.251
	Light:Position Base	0.0031 ± 0.0021	1.43		0.161
<b>LDMC</b>	Intercept	-2.0134 ± 0.0399	-50.44	0.62	<b>&lt;0.001</b>
	Light	0.0004 ± 0.0011	0.40		0.693
	Position Base	-0.3051 ± 0.0565	-5.40		<b>&lt;0.001</b>
	Light:Position Base	0.0074 ± 0.0015	4.80		<b>&lt;0.001</b>
<b>LS</b>	Intercept	6.7976 ± 0.0337	201.47	0.46	<b>&lt;0.001</b>
	Light	0.0024 ± 0.0009	2.57		<b>0.015</b>
	Position Base	0.2566 ± 0.0477	5.38		<b>&lt;0.001</b>
	Light:Position Base	-0.0054 ± 0.0013	-4.16		<b>&lt;0.001</b>
<b>Thickness</b>	Intercept	0.0714 ± 0.0392	1.82	0.58	0.076
	Light	0.0029 ± 0.0011	2.76		<b>0.009</b>
	Position Base	0.3354 ± 0.0554	6.05		<b>&lt;0.001</b>
	Light:Position Base	-0.0053 ± 0.0015	-3.50		<b>0.001</b>
<b>Adaxial trichome density</b>	Intercept	2.4383 ± 0.0808	30.20	0.67	<b>&lt;0.001</b>
	Light	0.0089 ± 0.0022	4.05		<b>&lt;0.001</b>
	Position Base	0.8620 ± 0.1142	7.55		<b>&lt;0.001</b>
	Light:Position Base	-0.0147 ± 0.0031	-4.71		<b>&lt;0.001</b>
<b>Abaxial trichome density</b>	Intercept	2.7241 ± 0.0573	47.57	0.62	<b>&lt;0.001</b>
	Light	0.0027 ± 0.0016	1.74		0.09
	Position Base	0.3745 ± 0.0810	4.62		<b>&lt;0.001</b>
	Light:Position Base	-0.0024 ± 0.0022	-1.07		0.291
<b>Adaxial trichome surface</b>	Intercept	-3.2348 ± 0.0709	-45.62	0.4	<b>&lt;0.001</b>
	Light	0.0006 ± 0.0019	0.33		0.741
	Position Base	-0.3662 ± 0.1003	-3.65		<b>&lt;0.001</b>
	Light:Position Base	0.0065 ± 0.0027	2.36		<b>0.024</b>
<b>Abaxial trichome surface</b>	Intercept	-3.0963 ± 0.0594	-52.16	0.8	<b>&lt;0.001</b>
	Light	-0.0012 ± 0.0016	-0.71		0.48
	Position Base	-0.7358 ± 0.0839	-8.77		<b>&lt;0.001</b>
	Light:Position Base	0.0083 ± 0.0023	3.63		<b>&lt;0.001</b>
<b>Epidermis</b>	Intercept	-3.0204 ± 0.0486	-62.16	0.77	<b>&lt;0.001</b>
	Light	0.0024 ± 0.0013	1.85		0.073
	Position Base	0.3188 ± 0.0687	4.64		<b>&lt;0.001</b>

Variables		Estimates: mean $\pm$ se	t Statistic	R <sup>2</sup>	p value
<b>Hydrenchyma</b>	Light:Position Base	0.0012 $\pm$ 0.0019	0.65	0.93	0.523
	Intercept	-1.2323 $\pm$ 0.0681	-18.10		<0.001
	Light	0.0099 $\pm$ 0.0019	5.35		<0.001
	Position Base	1.4085 $\pm$ 0.0963	14.63		<0.001
<b>Vascular bundle surface</b>	Light:Position Base	-0.0132 $\pm$ 0.0026	-5.01	0.75	<0.001
	Intercept	-4.8494 $\pm$ 0.0811	-59.81		<0.001
	Light	0.0108 $\pm$ 0.0022	4.87		<0.001
	Position Base	0.9000 $\pm$ 0.1147	7.85		<0.001
<b>Vascular bundle density</b>	Light:Position Base	-0.0120 $\pm$ 0.0031	-3.82	0.66	<0.001
	Intercept	1.1456 $\pm$ 0.0535	21.43		<0.001
	Light	-0.0095 $\pm$ 0.0015	-6.51		<0.001
	Position Base	-0.4998 $\pm$ 0.0756	-6.61		<0.001
<b>IVD:VED</b>	Light:Position Base	0.0095 $\pm$ 0.0021	4.63	0.53	<0.001
	Intercept	-0.5864 $\pm$ 0.0696	-8.43		<0.001
	Light	0.0068 $\pm$ 0.0019	3.59		<0.001
	Position Base	-0.1907 $\pm$ 0.0984	-1.94		0.06
<b>Fiber density</b>	Light:Position Base	0.0006 $\pm$ 0.0027	0.22	0.33	0.829
	Intercept	2.5892 $\pm$ 0.0837	30.94		<0.001
	Light	-0.0075 $\pm$ 0.0023	-3.28		0.002
	Position Base	0.0093 $\pm$ 0.1183	0.08		0.938
	Light:Position Base	0.0031 $\pm$ 0.0032	0.96		0.346

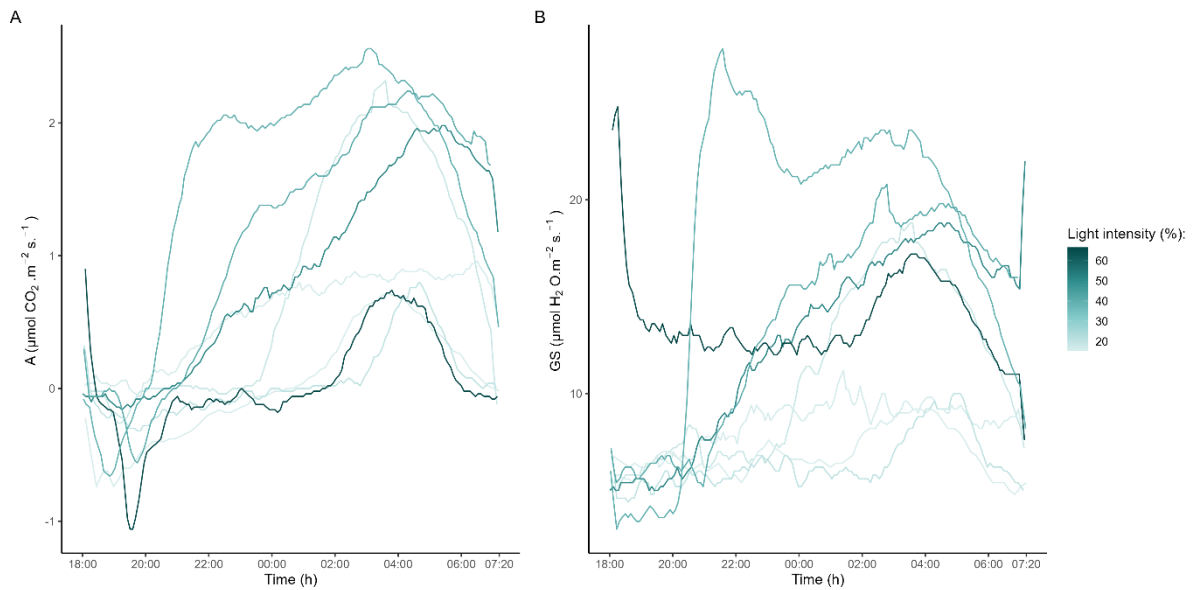


Figure S5: Overnight CO<sub>2</sub> assimilation and stomatal conductance curves. Curves of overnight (A) CO<sub>2</sub> assimilation ( $A$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) and (B) stomatal conductance ( $G_s$ ,  $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ). Values are rolled means with a window of 5 (zoo R package, rollmean function). These measurements were conducted on 8 plants (light intensity from 15.24 to 66.64%) using a Ciras-3 analyser (PP Systems, Amesbury, U.S.A). All measurements were made at ten-minute intervals throughout the night from 6:00 pm to 7:20 am the following morning. The CO<sub>2</sub> concentration in the leaf chamber was set to 400 ppm, the temperature to 27 °C, and the air flow to 250  $\mu\text{mol s}^{-1}$  while relative humidity and light were left at ambient conditions. Plants were gently unpotted and brought to the laboratory for these measurements.

**Annexes Chapitre III : Water and nutrient resources  
shape plant traits, their leaf endophytic microbiota, and  
their relationships**

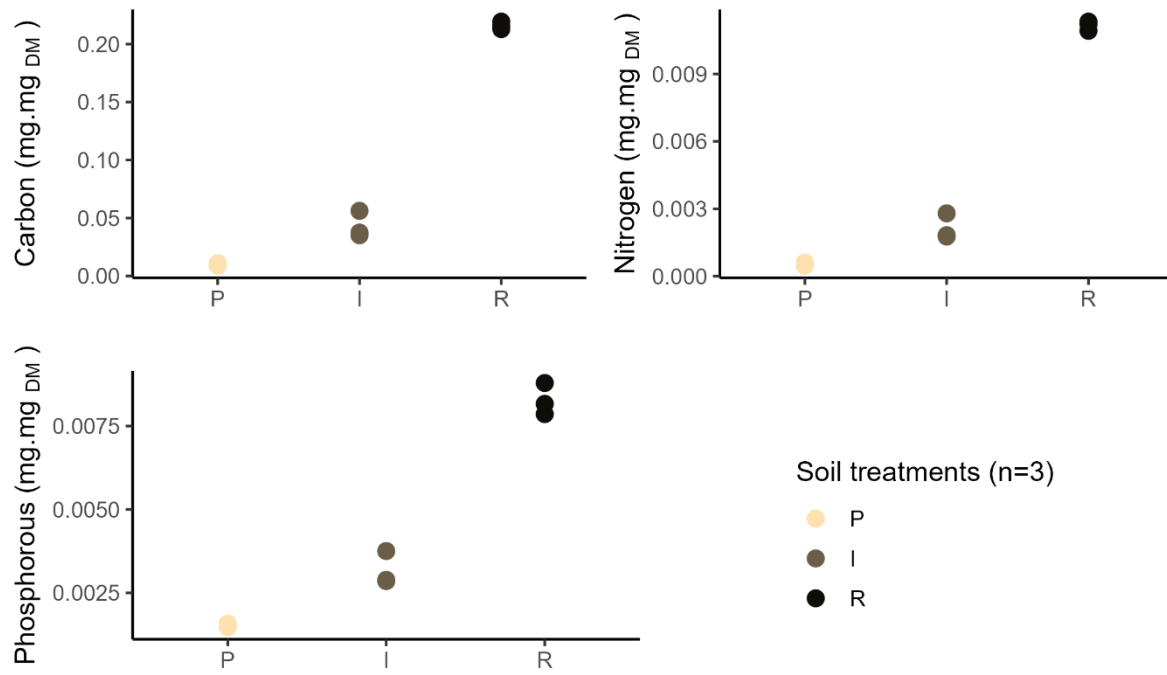


Figure S6: C, N and P content of the three substrates. P – Poor, I – Intermediate and R – Rich.

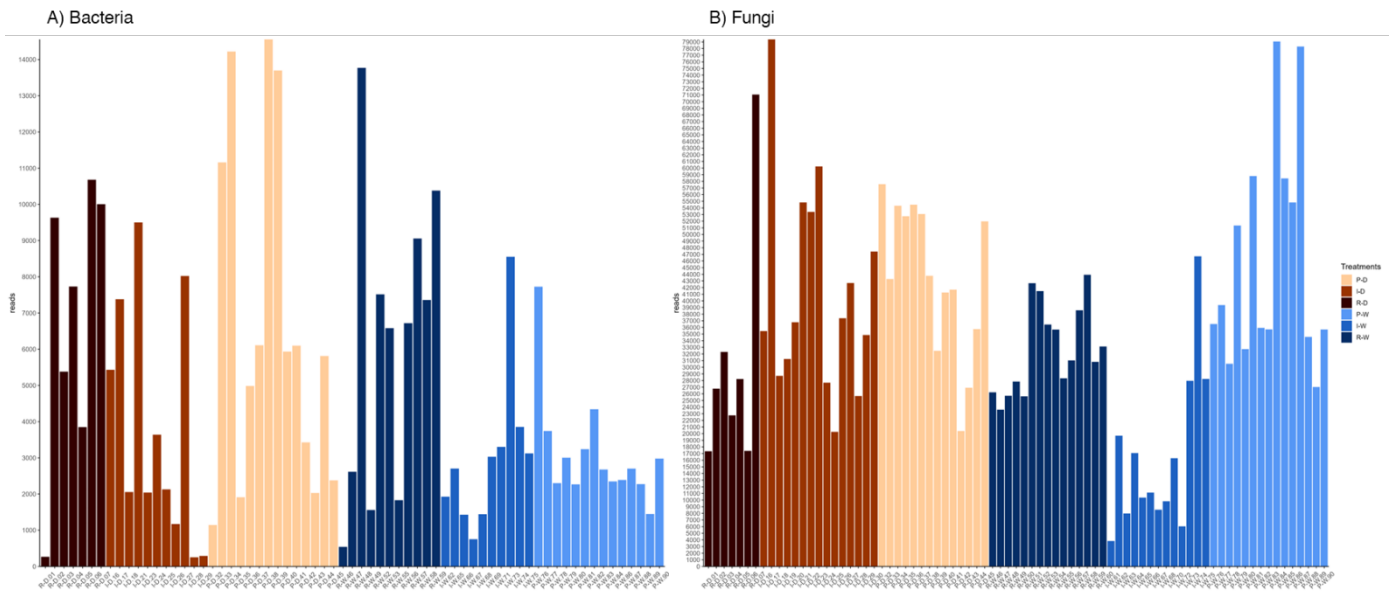


Figure S7: Number of reads per sample colored according to treatments.

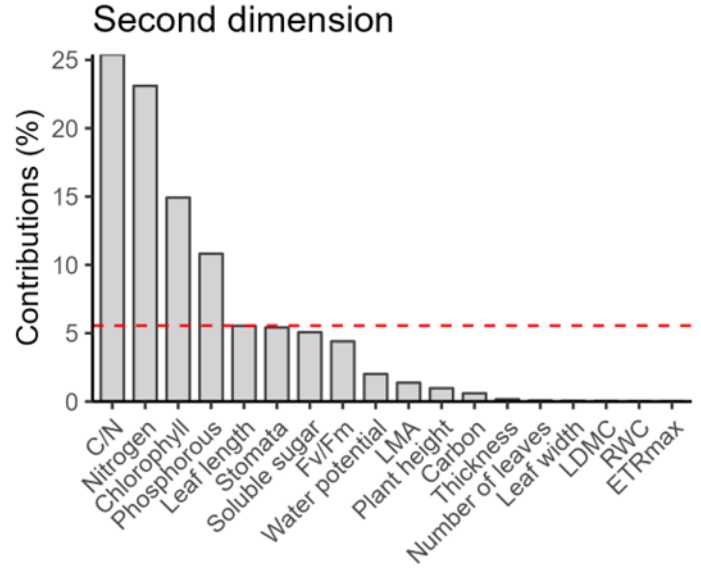
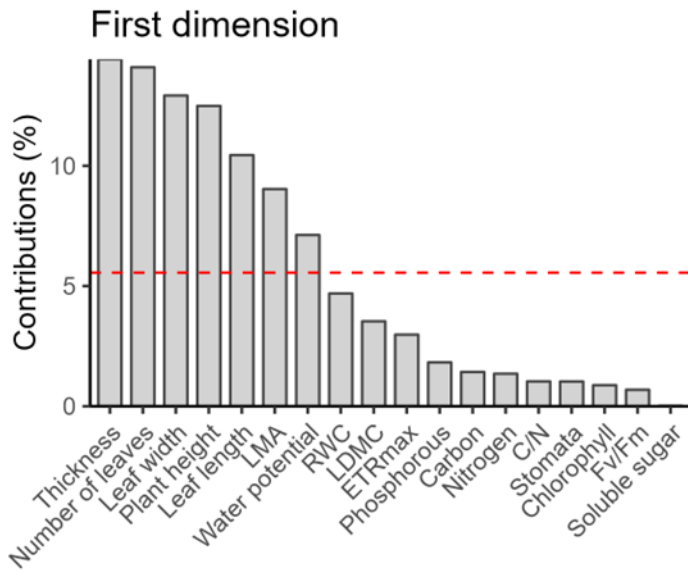


Figure S8: Contribution of the measured traits to the first (left panel) and second (right panel) dimensions of the PCA performed on leaf traits. The red line indicates the expected equal contribution of all variables (*i.e.*,  $100\%/18=5.5$ ).



Table S4: Result table of the pairwise perMANOVA performed on plant traits. Each row is the comparison of a pair of treatments. P.adjusted corresponds to holm's corrected p.values for multiple comparisons and are in bold when significant (<0.05).

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
R-D vs I-D	1	38.933	2.089	0.095	0.057	0.057
R-D vs P-D	1	72.509	7.636	0.276	0.001	<b>0.015</b>
R-D vs R-W	1	199.650	20.495	0.506	0.001	<b>0.015</b>
R-D vs I-W	1	171.030	23.320	0.538	0.001	<b>0.015</b>
R-D vs P-W	1	170.126	22.511	0.530	0.001	<b>0.015</b>
I-D vs P-D	1	52.514	3.683	0.116	0.001	<b>0.015</b>
I-D vs R-W	1	282.037	19.538	0.411	0.001	<b>0.015</b>
I-D vs I-W	1	207.468	16.316	0.368	0.001	<b>0.015</b>
I-D vs P-W	1	193.206	15.006	0.349	0.001	<b>0.015</b>
P-D vs R-W	1	252.172	31.901	0.533	0.001	<b>0.015</b>
P-D vs I-W	1	119.653	19.345	0.409	0.001	<b>0.015</b>
P-D vs P-W	1	78.670	12.399	0.307	0.001	<b>0.015</b>
R-W vs I-W	1	79.487	12.496	0.309	0.001	<b>0.015</b>
R-W vs P-W	1	181.765	27.875	0.499	0.001	<b>0.015</b>
I-W vs P-W	1	68.593	14.287	0.338	0.001	<b>0.015</b>

Table S5: Statistical summary table. For each trait the mean  $\pm$  standard deviation (SD) is displayed for the 6 treatments. The associated Kruskal Wallis  $\chi^2$  and P-values are shown. Significant P-values ( $<0.05$ ) are in bold. Letters within brackets indicates significant pairwise differences, Wilcoxon pairwise test ( $\alpha < 0.05$ ). Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet. Traits are the Number of leaves; Plant height, leaf length and width are in cm; LMA ( $\text{g}\cdot\text{m}^{-2}$ ); LDMC ( $\text{g}\cdot\text{g}^{-1}$ ); Thickness (mm); Chlorophyll ( $\text{mg}\cdot\text{g}^{-1}$ ); PSII quantum yield:  $F_v/F_m$ ; maximal electron transfer rate:  $\text{ETR}_{\text{max}}$  ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); Water potential (MPa); RWC (%); C/N ratio; Soluble sugars ( $\mu\text{g}\cdot\text{mg}^{-1}$ ); Carbon, Nitrogen and Phosphorous ( $\text{mg}\cdot\text{g}^{-1}$ ).

Traits	Mean $\pm$ SD						Significance
	P-D	I-D	R-D	P-W	I-W	R-W	
Number of leaves	9.6 $\pm$ 1.18 (a)	8.47 $\pm$ 2.29 (a)	8.86 $\pm$ 1.35 (a)	13.1 $\pm$ 1.13 (b)	15.9 $\pm$ 1.85 (c)	16.1 $\pm$ 0.884 (c)	<b>&lt;0.0001</b>
Plant height	9.9 $\pm$ 1.67 (ab)	10.2 $\pm$ 2.13 (a)	7.79 $\pm$ 1.73 (b)	21.9 $\pm$ 2.55 (c)	23.6 $\pm$ 3.04 (c)	34.3 $\pm$ 6.1 (d)	<b>&lt;0.0001</b>
Leaf length	29.1 $\pm$ 3.66 (ab)	28.7 $\pm$ 3.36 (ab)	25 $\pm$ 4.1 (a)	30.4 $\pm$ 2.44 (b)	43.5 $\pm$ 3.47 (c)	54.5 $\pm$ 6.65 (d)	<b>&lt;0.0001</b>
Leaf width	3.01 $\pm$ 0.338 (a)	2.99 $\pm$ 0.348 (a)	3.27 $\pm$ 0.419 (a)	4.26 $\pm$ 0.28 (b)	4.3 $\pm$ 0.484 (b)	4.47 $\pm$ 0.244 (b)	<b>&lt;0.0001</b>
LMA	84 $\pm$ 15.9 (a)	76.8 $\pm$ 10 (a)	64 $\pm$ 21.4 (a)	107 $\pm$ 8.82 (b)	101 $\pm$ 13 (b)	120 $\pm$ 24.6 (b)	<b>&lt;0.0001</b>
LDMC	0.0984 $\pm$ 0.0165 (ab)	0.126 $\pm$ 0.0571 (a)	0.0866 $\pm$ 0.0297 (ab)	0.103 $\pm$ 0.0112 (a)	0.0843 $\pm$ 0.0084 (b)	0.0977 $\pm$ 0.0161 (ab)	<b>0.00216</b>
Thickness	0.935 $\pm$ 0.16 (a)	0.762 $\pm$ 0.23 (a)	0.819 $\pm$ 0.191 (a)	1.15 $\pm$ 0.125 (b)	1.32 $\pm$ 0.109 (c)	1.37 $\pm$ 0.108 (c)	<b>&lt;0.0001</b>
Stomata	79 $\pm$ 13.9 (ab)	93.5 $\pm$ 19 (ac)	79.6 $\pm$ 17.5 (abc)	71.7 $\pm$ 9.08 (b)	98.8 $\pm$ 12.1 (c)	94.4 $\pm$ 13.3 (ac)	<b>&lt;0.0001</b>
Chlorophyll	2.4 $\pm$ 0.784 (ab)	2.84 $\pm$ 0.856 (ac)	3.19 $\pm$ 1.43 (abc)	1.96 $\pm$ 0.42 (b)	3.25 $\pm$ 0.596 (c)	4.05 $\pm$ 1.66 (c)	<b>&lt;0.0001</b>
$F_v/F_m$	0.788 $\pm$ 0.0683 (a)	0.732 $\pm$ 0.128 (a)	0.726 $\pm$ 0.0685 (a)	0.795 $\pm$ 0.0275 (a)	0.799 $\pm$ 0.0194 (a)	0.775 $\pm$ 0.0236 (a)	<b>0.00161</b>
$\text{ETR}_{\text{max}}$	49.2 $\pm$ 9.85 (a)	47.7 $\pm$ 8.15 (a)	53.6 $\pm$ 13.3 (ab)	66 $\pm$ 26.1 (ab)	49.2 $\pm$ 13.5 (a)	71.7 $\pm$ 12.6 (b)	<b>0.0001</b>
Water potential	-0.129 $\pm$ 0.306 (ab)	-0.526 $\pm$ 0.717 (a)	-0.623 $\pm$ 0.653 (a)	-0.0441 $\pm$ 0.0894 (bc)	-0.00485 $\pm$ 0.0188 (c)	-0.0021 $\pm$ 0.00813 (c)	<b>&lt;0.0001</b>
RWC	83.4 $\pm$ 4.45 (a)	73.1 $\pm$ 21.4 (ab)	72.8 $\pm$ 12.6 (a)	89 $\pm$ 2.71 (bc)	91.2 $\pm$ 2.71 (c)	91.4 $\pm$ 2.74 (c)	<b>&lt;0.0001</b>
C/N	58.3 $\pm$ 10.5 (a)	40.6 $\pm$ 6.45 (b)	26.4 $\pm$ 6.38 (c)	80.7 $\pm$ 4.77 (d)	62.6 $\pm$ 5.57 (a)	37.2 $\pm$ 5.91 (b)	<b>&lt;0.0001</b>
Soluble sugar	90.4 $\pm$ 37.6 (a)	76.1 $\pm$ 25.9 (a)	60.4 $\pm$ 19.3 (a)	83.6 $\pm$ 17.6 (a)	85.9 $\pm$ 24.3 (a)	63 $\pm$ 27.3 (a)	<b>0.02062</b>
Carbon	410 $\pm$ 7.74 (ab)	416 $\pm$ 11.4 (a)	406 $\pm$ 12 (abc)	406 $\pm$ 6.41 (bc)	402 $\pm$ 3.46 (c)	408 $\pm$ 5.99 (bc)	<b>0.00052</b>
Nitrogen	7.26 $\pm$ 1.41 (a)	10.5 $\pm$ 1.76 (b)	16 $\pm$ 3.29 (c)	5.05 $\pm$ 0.33 (d)	6.48 $\pm$ 0.562 (a)	11.2 $\pm$ 1.64 (b)	<b>&lt;0.0001</b>
Phosphorous	1.32 $\pm$ 0.139 (a)	1.46 $\pm$ 0.432 (ab)	1.32 $\pm$ 0.145 (a)	1.31 $\pm$ 0.172 (a)	1.52 $\pm$ 0.266 (ab)	1.77 $\pm$ 0.27 (b)	<b>0.00032</b>

Table S6: Relative abundance of the most abundant taxa at the Phylum, Class and Order rank for fungi and bacteria.

Phylum		Class		Order	
Name	Otu count	Name	Otu count	Name	Otu count
<b>Fungi</b>					
Aphelidiomycota	1 (0.06%)	Agaricomycetes	222 (13.94%)	Capnodiales	36 (2.26%)
Ascomycota	1034 (64.95%)	Dothideomycetes	281 (17.65%)	Chaetothyriales	24 (1.51%)
Basidiobolomycota	1 (0.06%)	Eurotiomycetes	87 (5.46%)	Diaporthales	36 (2.26%)
Basidiomycota	317 (19.91%)	Leotiomycetes	36 (2.26%)	Glomerellales	21 (1.32%)
Chytridiomycota	16 (1.01%)	Malasseziomycetes	14 (0.88%)	Hypocreales	105 (6.6%)
Entomophthoromycota	1 (0.06%)	Pezizomycetes	13 (0.82%)	Mycosphaerellales	34 (2.14%)
Glomeromycota	7 (0.44%)	Saccharomycetes	28 (1.76%)	Pleosporales	161 (10.11%)
Mortierellomycota	2 (0.13%)	Sordariomycetes	471 (29.59%)	Sordariales	62 (3.89%)
Mucoromycota	12 (0.75%)	Tremellomycetes	25 (1.57%)	Thelebolales	11 (0.69%)
		Ustilaginomycetes	9 (0.57%)	Trichosphaeriales	18 (1.13%)
		others	73 (4.59%)	others	629 (39.51%)
unknown	201 (12.63%)	unknown	333 (20.92%)	unknown	455 (28.58%)
<b>Bacteria</b>					
Acidobacteriota	19 (1.48%)	Actinobacteria	148 (11.55%)	Acetobacterales	18 (1.41%)
Actinobacteriota	206 (16.08%)	Alphaproteobacteria	264 (20.61%)	Bacillales	25 (1.95%)
Bacteroidota	136 (10.62%)	Bacilli	85 (6.64%)	Burkholderiales	74 (5.78%)
Chloroflexi	18 (1.41%)	Bacteroidia	129 (10.07%)	Clostridiales	17 (1.33%)
Deinococcota	9 (0.7%)	Clostridia	59 (4.61%)	Enterobacteriales	17 (1.33%)
Fibrobacterota	8 (0.62%)	Deinococci	9 (0.7%)	Lactobacillales	20 (1.56%)
Firmicutes	161 (12.57%)	Gammaproteobacteria	167 (13.04%)	Propionibacteriales	24 (1.87%)
Myxococcota	32 (2.5%)	Ignavibacteria	3 (0.23%)	Pseudomonadales	31 (2.42%)
Patescibacteria	13 (1.01%)	Polyangia	27 (2.11%)	Rhizobiales	101 (7.88%)
Proteobacteria	439 (34.27%)	Thermoanaerobacteria	3 (0.23%)	Sphingomonadales	31 (2.42%)
others	93 (7.26%)	others	217 (16.94%)	others	668 (52.15%)
unknown	147 (11.48%)	unknown	170 (13.27%)	unknown	255 (19.91%)

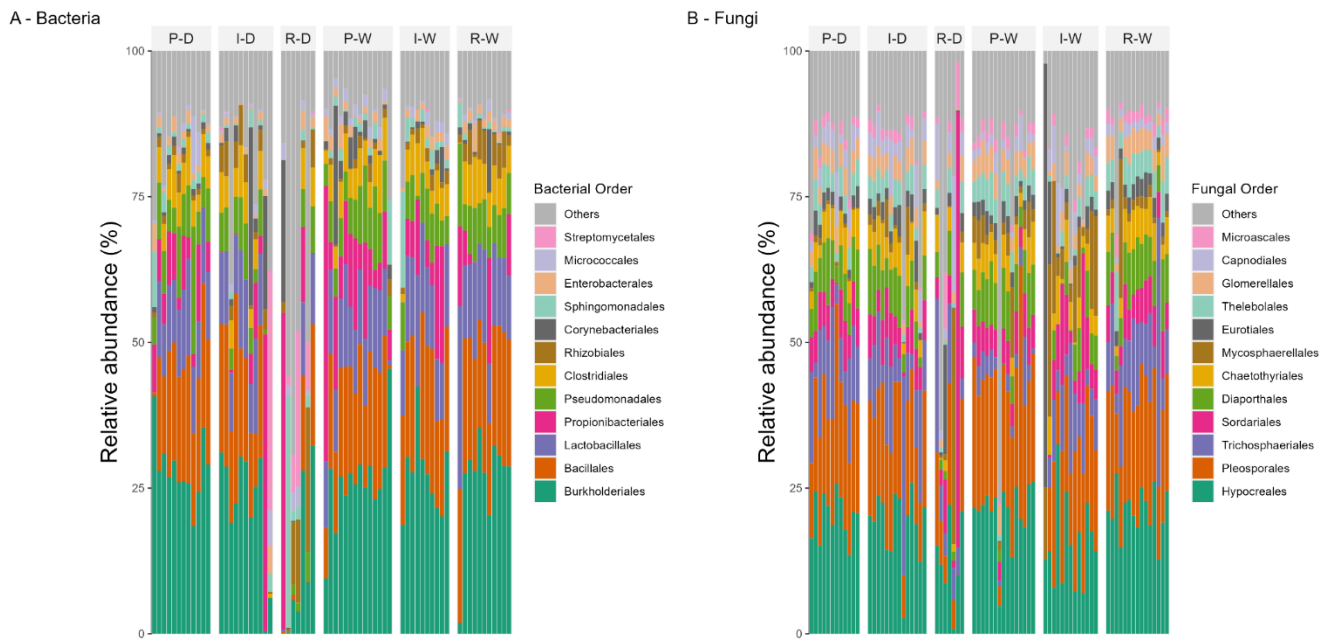


Figure S9: Relative abundance of bacterial (A) and fungal (B) orders in the 6 treatments. Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet.

Table S7: Result table of the pairwise perMANOVA performed on the position of bacterial communities in the dbRDA space according to the different treatments. The perMANOVA was conducted on individuals coordinates in the multivariate spaces retrieved from the dbRDA. P.adjusted corresponds to holm's corrected p.values for multiple comparisons and are in bold when significant (<0.05). Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
R-D vs I-D	1	22.043164	6.507155	0.28911493	0.001	<b>0.015</b>
R-D vs P-D	1	30.250818	13.285273	0.43867106	0.001	<b>0.015</b>
R-D vs R-W	1	28.817413	10.665847	0.39998155	0.001	<b>0.015</b>
R-D vs I-W	1	26.566445	13.548725	0.47458249	0.001	<b>0.015</b>
R-D vs P-W	1	31.206621	18.718147	0.49626370	0.001	<b>0.015</b>
I-D vs P-D	1	10.640009	4.839587	0.18729350	0.006	<b>0.015</b>
I-D vs R-W	1	12.718617	5.018256	0.20058376	0.001	<b>0.015</b>
I-D vs I-W	1	12.331108	6.354162	0.25061612	0.001	<b>0.015</b>
I-D vs P-W	1	31.051111	18.248338	0.44240177	0.001	<b>0.015</b>
P-D vs R-W	1	11.102162	6.623773	0.23978525	0.001	<b>0.015</b>
P-D vs I-W	1	4.016283	3.756880	0.15813861	0.004	<b>0.015</b>
P-D vs P-W	1	7.825354	7.942722	0.24865515	0.001	<b>0.015</b>
R-W vs I-W	1	2.862094	2.099519	0.09950553	0.099	0.099
R-W vs P-W	1	29.404178	24.011527	0.51075829	0.001	<b>0.015</b>
I-W vs P-W	1	13.924215	21.349983	0.49250268	0.001	<b>0.015</b>

Table S8: Result table of the marginal significance Anova performed on the dbRDA model after variable forward selection for bacterial communities. Bold p value indicates significance of the variable effect (<0.05). Marginal means a different test was performed for each variable in a model containing all the variables.

Variables	Df	SumOfSqs	F	p value
Nitrogen	1	0.651	1.474	<b>0.001</b>
C/N	1	0.587	1.328	<b>0.001</b>
Water potential	1	0.493	1.116	<b>0.049</b>
Chlorophyll	1	0.496	1.122	<b>0.044</b>
Residual	59	26.082		

Table S9: Result table of the pairwise perMANOVA performed on the position of fungal communities in the dbRDA space according to the different treatments. The perMANOVA was conducted on individuals coordinates in the multivariate spaces retrieved from the dbRDA. P.adjusted corresponds to holm's corrected p.values for multiple comparisons and are in bold when significant (<0.05). Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet.

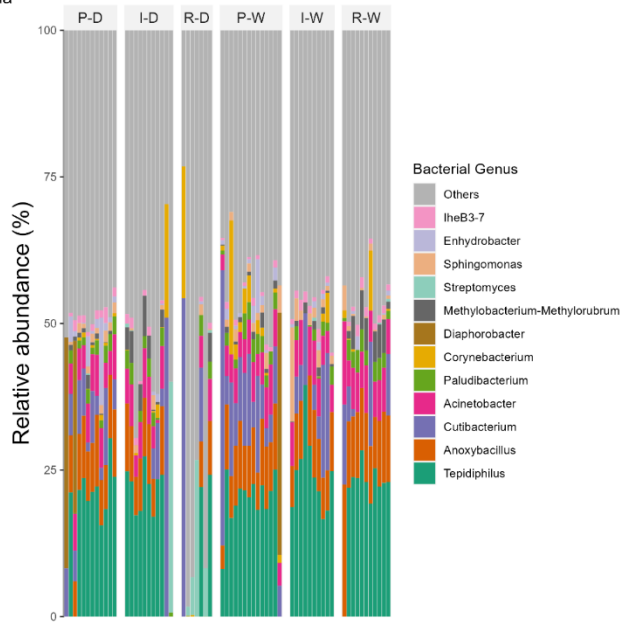
pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
R-D vs I-D	1	20.74934	8.528524	0.3098068	0.001	<b>0.015</b>
R-D vs P-D	1	24.90178	12.665446	0.4269427	0.001	<b>0.015</b>
R-D vs R-W	1	26.85748	22.315397	0.5273588	0.001	<b>0.015</b>
R-D vs I-W	1	46.30076	30.650964	0.6300176	0.001	<b>0.015</b>
R-D vs P-W	1	44.40278	19.365529	0.4919413	0.002	<b>0.015</b>
I-D vs P-D	1	32.02626	18.266755	0.4321778	0.001	<b>0.015</b>
I-D vs R-W	1	52.55523	43.361253	0.6162661	0.001	<b>0.015</b>
I-D vs I-W	1	24.88511	17.356285	0.4097688	0.001	<b>0.015</b>
I-D vs P-W	1	57.26582	28.364187	0.5123201	0.001	<b>0.015</b>
P-D vs R-W	1	58.39346	73.273367	0.7456076	0.001	<b>0.015</b>
P-D vs I-W	1	47.55351	47.465177	0.6735976	0.001	<b>0.015</b>
P-D vs P-W	1	24.48065	14.673170	0.3698512	0.001	<b>0.015</b>
R-W vs I-W	1	54.66178	103.816681	0.7997176	0.001	<b>0.015</b>
R-W vs P-W	1	41.40997	35.834331	0.5613646	0.001	<b>0.015</b>
I-W vs P-W	1	28.04338	20.552529	0.4414911	0.001	<b>0.015</b>

Table S10: Result table of the marginal significance Anova performed on the dbRDA model after variable forward selection for fungal communities. Bold p value indicates significance of the variable effect (<0.05). Marginal means a different test was performed for each variable in a model containing all the variables.

Variables	Df	SumOfSqs	F	p value
Drought	1	0.704	1.695	<b>0.001</b>
Substrate	2	0.939	1.131	<b>0.012</b>
Residual	71	29.482		



A - Bacteria



B - Fungi

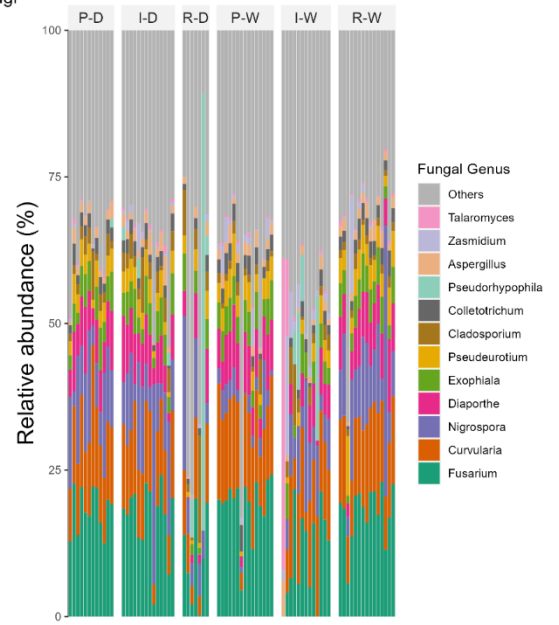


Figure S10: Relative abundance of bacterial (A) and fungal (B) genus in the 6 treatments. Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet.

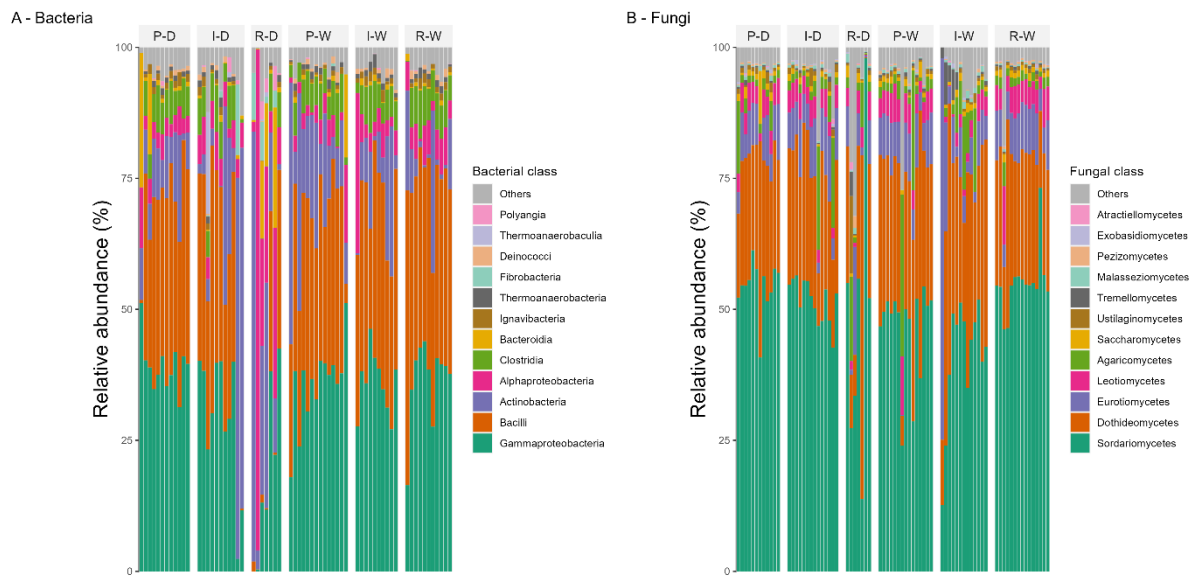


Figure S11: Relative abundance of bacterial (A) and fungal (B) class in the 6 treatments. Treatments are P-D, Poor-Dry; I-D, Intermediate-Dry; R-D, Rich-Dry; P-W, Poor-Wet; I-W, Intermediate-Wet and R-W, Rich-Wet.

**Annexes chapitre IV : Maternal effects and current environmental conditions affect the phenotype and the microbiota assemblage in *Aechmea mertensii* seedlings**

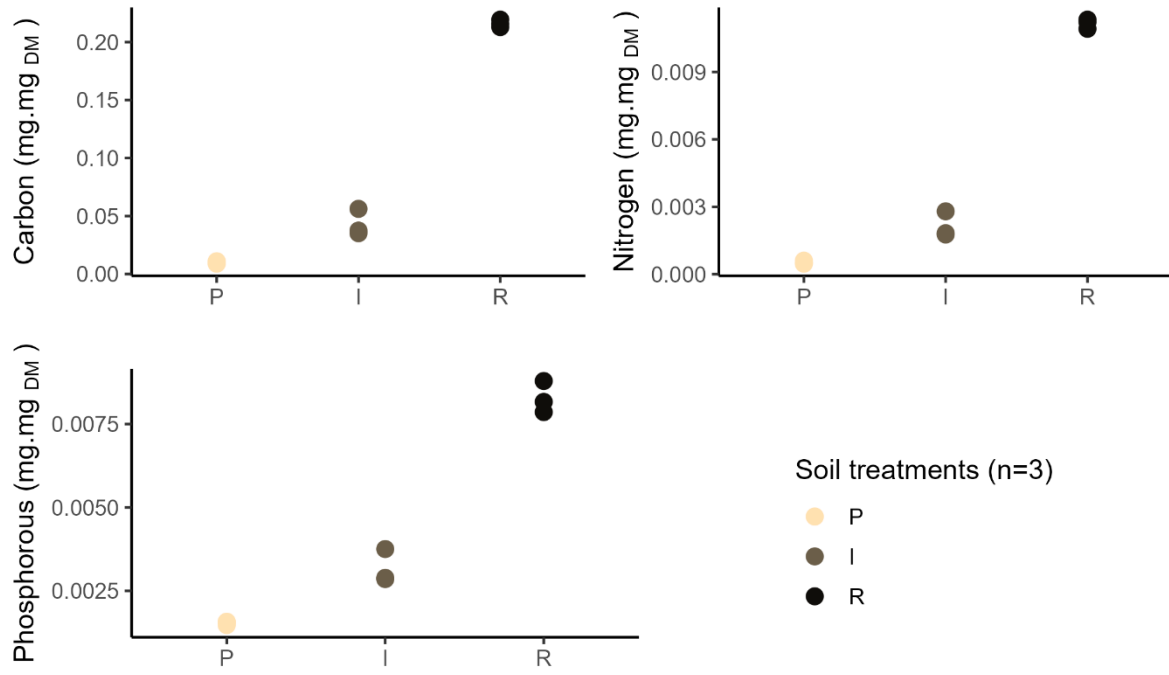


Figure S12: C, N and P content of the three substrates. P – Poor, I – Intermediate and R – Rich.

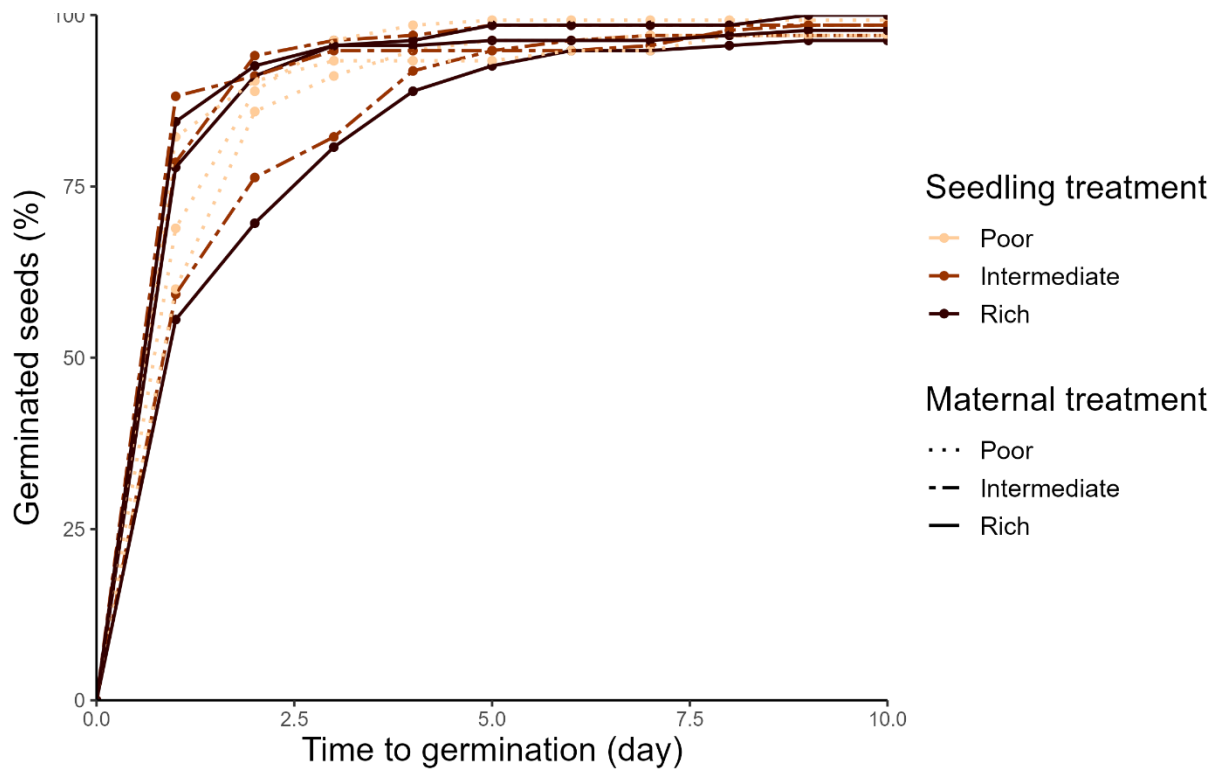


Figure S13: % of germinated seeds of the 10 first day according to maternal and seedling treatment (colour and line type respectively).

Table S11: Sample size for the 9 treatments for ecophysiological measurements and microbiota investigation.

Seedling \ Mother	Poor	Intermediate	Rich	Total
<b>Ecophysiology sampling</b>				
Poor	41	41	37	119
Intermediate	43	45	42	130
Rich	44	43	43	130
Total	128	129	122	379
<b>Microbiota sampling</b>				
Poor	45	44	38	127
Intermediate	41	43	42	126
Rich	43	43	44	130
Total	129	130	124	383

Table S12: Results of the perMANOVA performed on seedling traits to test the effects of maternal and seedling treatment as well as their interaction. Bold p-value indicates significance of the tested effect (<0.05).

	<b>Df</b>	<b>Sum of squares</b>	<b>R2</b>	<b>F</b>	<b>p value</b>
<b>Maternal treatment</b>	2	470.676	0.116	28.262	<b>0.001</b>
<b>Seedling treatment</b>	2	473.968	0.117	28.460	<b>0.001</b>
<b>Interaction</b>	4	105.674	0.026	3.173	<b>0.001</b>
<b>Residual</b>	360	2,997.681	0.741		
<b>Total</b>	368	4,048.000	1.000		

Table S13: Results of the pairwise permanova performed on Seedling traits to test for pairwise differences between all 9 treatment combinations resulting in 36 comparisons. P-values are Holm's adjusted and displayed in bold when significant (<0.05).

Pairs		Df	Sum of squares	R2	F	Adjusted p value
R to R - R to I	Treatment	1	15.431	0.031	2.596	0.051
	Residual	80	475.542	0.969		
	Total	81	490.972	1.000		
R to R - R to P	Treatment	1	129.321	0.217	21.015	<b>0.036</b>
	Residual	76	467.693	0.783		
	Total	77	597.014	1.000		
R to R - I to R	Treatment	1	91.816	0.146	13.897	<b>0.036</b>
	Residual	81	535.172	0.854		
	Total	82	626.988	1.000		
R to R - I to I	Treatment	1	163.184	0.210	21.469	<b>0.036</b>
	Residual	81	615.667	0.790		
	Total	82	778.851	1.000		
R to R - I to P	Treatment	1	295.318	0.340	41.135	<b>0.036</b>
	Residual	80	574.337	0.660		
	Total	81	869.655	1.000		
R to R - P to R	Treatment	1	76.463	0.105	9.523	<b>0.036</b>
	Residual	81	650.396	0.895		
	Total	82	726.859	1.000		
R to R - P to I	Treatment	1	130.566	0.138	12.932	<b>0.036</b>
	Residual	81	817.813	0.862		
	Total	82	948.378	1.000		
R to R - P to P	Treatment	1	299.827	0.321	37.902	<b>0.036</b>
	Residual	80	632.852	0.679		
	Total	81	932.680	1.000		
R to I - R to P	Treatment	1	89.987	0.171	15.650	<b>0.036</b>
	Residual	76	437.009	0.829		
	Total	77	526.996	1.000		
R to I - I to R	Treatment	1	97.424	0.162	15.642	<b>0.036</b>
	Residual	81	504.488	0.838		
	Total	82	601.912	1.000		
R to I - I to I	Treatment	1	186.244	0.241	25.788	<b>0.036</b>
	Residual	81	584.983	0.759		
	Total	82	771.227	1.000		
R to I - I to P	Treatment	1	283.282	0.343	41.686	<b>0.036</b>
	Residual	80	543.652	0.657		
	Total	81	826.934	1.000		
R to I - P to R	Treatment	1	97.612	0.136	12.758	<b>0.036</b>
	Residual	81	619.712	0.864		
	Total	82	717.324	1.000		
R to I - P to I	Treatment	1	141.936	0.153	14.606	<b>0.036</b>
	Residual	81	787.128	0.847		
	Total	82	929.064	1.000		
R to I - P to P	Treatment	1	299.196	0.332	39.749	<b>0.036</b>
	Residual	80	602.168	0.668		
	Total	81	901.364	1.000		
R to P - I to R	Treatment	1	203.585	0.291	31.564	<b>0.036</b>
	Residual	77	496.639	0.709		
	Total	78	700.225	1.000		
R to P - I to I	Treatment	1	154.676	0.211	20.637	<b>0.036</b>
	Residual	77	577.134	0.789		
	Total	78	731.811	1.000		
R to P - I to P	Treatment	1	139.252	0.206	19.752	<b>0.036</b>
	Residual	76	535.804	0.794		
	Total	77	675.056	1.000		
R to P - P to R	Treatment	1	194.947	0.242	24.533	<b>0.036</b>
	Residual	77	611.863	0.758		
	Total	78	806.810	1.000		



Pairs		Df	Sum of squares	R2	F	Adjusted p value
R to P - P to I	Treatment	1	126.373	0.140	12.487	<b>0.036</b>
	Residual	77	779.280	0.860		
	Total	78	905.652	1.000		
R to P - P to P	Treatment	1	165.030	0.217	21.104	<b>0.036</b>
	Residual	76	594.319	0.783		
	Total	77	759.349	1.000		
I to R - I to I	Treatment	1	99.374	0.134	12.641	<b>0.036</b>
	Residual	82	644.614	0.866		
	Total	83	743.988	1.000		
I to R - I to P	Treatment	1	213.991	0.262	28.732	<b>0.036</b>
	Residual	81	603.283	0.738		
	Total	82	817.275	1.000		
I to R - P to R	Treatment	1	26.274	0.037	3.171	<b>0.044</b>
	Residual	82	679.343	0.963		
	Total	83	705.616	1.000		
I to R - P to I	Treatment	1	97.550	0.103	9.447	<b>0.036</b>
	Residual	82	846.759	0.897		
	Total	83	944.309	1.000		
I to R - P to P	Treatment	1	201.907	0.234	24.712	<b>0.036</b>
	Residual	81	661.799	0.766		
	Total	82	863.706	1.000		
I to I - I to P	Treatment	1	45.352	0.062	5.372	<b>0.036</b>
	Residual	81	683.778	0.938		
	Total	82	729.130	1.000		
I to I - P to R	Treatment	1	73.081	0.088	7.887	<b>0.036</b>
	Residual	82	759.838	0.912		
	Total	83	832.919	1.000		
I to I - P to I	Treatment	1	26.025	0.027	2.301	0.116
	Residual	82	927.254	0.973		
	Total	83	953.279	1.000		
I to I - P to P	Treatment	1	38.292	0.049	4.178	<b>0.036</b>
	Residual	81	742.294	0.951		
	Total	82	780.586	1.000		
I to P - P to R	Treatment	1	183.817	0.204	20.722	<b>0.036</b>
	Residual	81	718.507	0.796		
	Total	82	902.325	1.000		
I to P - P to I	Treatment	1	67.267	0.071	6.150	<b>0.036</b>
	Residual	81	885.923	0.929		
	Total	82	953.191	1.000		
I to P - P to P	Treatment	1	9.446	0.013	1.078	0.357
	Residual	80	700.963	0.987		
	Total	81	710.410	1.000		
P to R - P to I	Treatment	1	47.228	0.047	4.026	<b>0.036</b>
	Residual	82	961.983	0.953		
	Total	83	1,009.211	1.000		
P to R - P to P	Treatment	1	170.694	0.180	17.794	<b>0.036</b>
	Residual	81	777.023	0.820		
	Total	82	947.717	1.000		
P to I - P to P	Treatment	1	62.047	0.062	5.322	<b>0.036</b>
	Residual	81	944.439	0.938		
	Total	82	1,006.486	1.000		

Table S14: Statistical summary table. For each trait the mean  $\pm$  standard deviation (SD) is displayed for the nine treatments combination (three maternal to three seedling). The associated Kruskal Wallis  $\chi^2$  and P-values are shown. Significant P-values ( $<0.05$ ) are in bold. Letters within brackets indicates significant pairwise differences, Wilcoxon pairwise test ( $\alpha < 0.05$ ). P – poor substrates, I – intermediate, R – rich. Traits are Number of leaves, Stomatal density (Nb mm<sup>-2</sup>); Number of roots; Average root diameter (ARD, mm); Leaf Mass Area (LMA, g m<sup>-2</sup>); Leaf Dry Matter Content (LDMC, g g<sup>-1</sup>); Relative Water Content (RWC, %); Specific Root Length (SRL, m g<sup>-1</sup>); Root Tissue Density (RTD, g cm<sup>-3</sup>); Root to Shoot ratio (RS ratio) and, Relative Growth Rate (RGR, g g<sup>-1</sup> month<sup>-1</sup>).

Traits	Mean $\pm$ SD									Significance	
	Mother			Seedling			Rich			KW-chi	P-val
	Poor	Intermediate	Poor	Intermediate	Poor	Intermediate	Poor	Intermediate	Rich		
Number of leaves	5.5 $\pm$ 0.9 (a)	6.3 $\pm$ 1.5 (ab)	6.9 $\pm$ 1.5 (bc)	6.6 $\pm$ 1.4 (ab)	5.6 $\pm$ 1.3 (a)	7.5 $\pm$ 1.3 (cd)	6.7 $\pm$ 1.2 (b)	8.1 $\pm$ 1.2 (d)	8.2 $\pm$ 1.1 (d)	137.298	<b>&lt;0.0001</b>
Stomatal density	70 $\pm$ 10 (abc)	66 $\pm$ 9.8 (abde)	63 $\pm$ 8.7 (d)	69 $\pm$ 9.1 (abde)	73 $\pm$ 9.3 (c)	66 $\pm$ 8.7 (ade)	72 $\pm$ 7.5 (bc)	66 $\pm$ 9.1 (ade)	64 $\pm$ 6.8 (de)	55.435	<b>&lt;0.0001</b>
Number of roots	3 $\pm$ 1.1 (a)	4.8 $\pm$ 2.1 (bc)	5.8 $\pm$ 1.6 (b)	3.9 $\pm$ 2 (bc)	3.1 $\pm$ 1.3 (a)	5.9 $\pm$ 2.1 (b)	3.7 $\pm$ 1.2 (ac)	5.1 $\pm$ 1.6 (b)	5.1 $\pm$ 1.5 (b)	115.936	<b>&lt;0.0001</b>
ARD	1.2 $\pm$ 0.22 (a)	1.4 $\pm$ 0.26 (abcd)	1.4 $\pm$ 0.27 (bcd)	1.3 $\pm$ 0.18 (abcd)	1.3 $\pm$ 0.14 (ab)	1.3 $\pm$ 0.23 (abc)	1.3 $\pm$ 0.16 (abcd)	1.4 $\pm$ 0.22 (cd)	1.5 $\pm$ 0.24 (d)	43.610	<b>&lt;0.0001</b>
LMA	16 $\pm$ 2.9 (ab)	17 $\pm$ 4 (ab)	18 $\pm$ 3.9 (ac)	15 $\pm$ 3 (ab)	16 $\pm$ 3 (ab)	19 $\pm$ 2.4 (cd)	21 $\pm$ 2.7 (de)	22 $\pm$ 2.9 (e)	20 $\pm$ 3.4 (cd)	125.485	<b>&lt;0.0001</b>
LDMC	0.035 $\pm$ 0.0059 (ab)	0.034 $\pm$ 0.0061 (ab)	0.033 $\pm$ 0.0068 (ab)	0.033 $\pm$ 0.0041 (ab)	0.035 $\pm$ 0.0049 (a)	0.034 $\pm$ 0.0034 (ab)	0.043 $\pm$ 0.0044 (c)	0.038 $\pm$ 0.0036 (d)	0.036 $\pm$ 0.0039 (a)	104.521	<b>&lt;0.0001</b>
RWC	96 $\pm$ 2.8 (a)	94 $\pm$ 3.2 (ab)	94 $\pm$ 2.3 (b)	94 $\pm$ 2.7 (ab)	95 $\pm$ 2.4 (ab)	94 $\pm$ 3.5 (b)	88 $\pm$ 3.6 (c)	88 $\pm$ 3.1 (c)	88 $\pm$ 4 (c)	188.990	<b>&lt;0.0001</b>
SRL	19 $\pm$ 7.7 (ab)	15 $\pm$ 8 (acd)	16 $\pm$ 8.8 (abcd)	17 $\pm$ 7 (acd)	19 $\pm$ 7.9 (abc)	20 $\pm$ 7.3 (b)	15 $\pm$ 4.5 (acd)	14 $\pm$ 4.1 (cd)	13 $\pm$ 3.8 (d)	46.447	<b>&lt;0.0001</b>
RTD	0.0054 $\pm$ 0.0025 (ab)	0.0057 $\pm$ 0.0029 (ab)	0.0047 $\pm$ 0.0015 (ab)	0.005 $\pm$ 0.0023 (ab)	0.0049 $\pm$ 0.0019 (ab)	0.0042 $\pm$ 0.0012 (a)	0.0055 $\pm$ 0.0017 (b)	0.0049 $\pm$ 0.00071 (b)	0.0052 $\pm$ 0.0016 (b)	21.148	<b>0.00676</b>
RS ratio	0.33 $\pm$ 0.16 (ab)	0.33 $\pm$ 0.19 (ab)	0.21 $\pm$ 0.12 (cde)	0.26 $\pm$ 0.12 (ab)	0.38 $\pm$ 0.14 (a)	0.17 $\pm$ 0.052 (e)	0.36 $\pm$ 0.12 (a)	0.25 $\pm$ 0.063 (bc)	0.2 $\pm$ 0.086 (de)	123.029	<b>&lt;0.0001</b>
RGR	0.55 $\pm$ 0.37 (a)	0.95 $\pm$ 0.57 (bc)	1.3 $\pm$ 0.26 (bd)	0.78 $\pm$ 0.5 (bc)	0.54 $\pm$ 0.4 (a)	1.4 $\pm$ 0.24 (d)	0.86 $\pm$ 0.23 (c)	1.4 $\pm$ 0.24 (d)	1.4 $\pm$ 0.24 (d)	188.984	<b>&lt;0.0001</b>

Table S15: Results of pairwise perMANOVA on seeds fungal communities. P values are Holm's adjusted and in bold when significant (<0.05).

Pairs		Df	Sum of squares	R2	F	Adjusted p value
P - PS	Maternal treatment	1	0.482	0.040	1.050	0.141
	Residual	25	11.471	0.960		
	Total	26	11.953	1.000		
P - SP	Maternal treatment	1	0.606	0.053	1.351	<b>0.003</b>
	Residual	24	10.766	0.947		
	Total	25	11.372	1.000		
PS - SP	Maternal treatment	1	0.641	0.059	1.433	<b>0.003</b>
	Residual	23	10.279	0.941		
	Total	24	10.919	1.000		

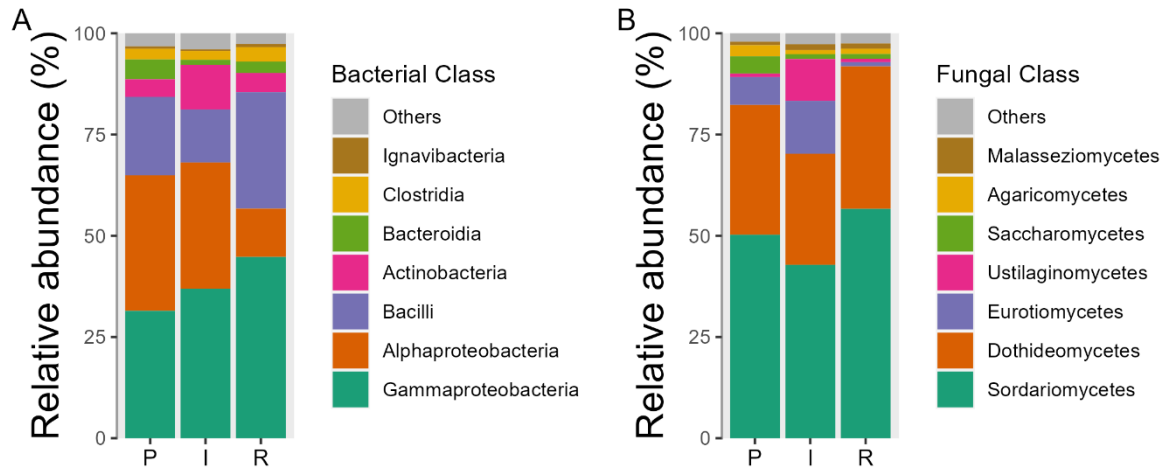
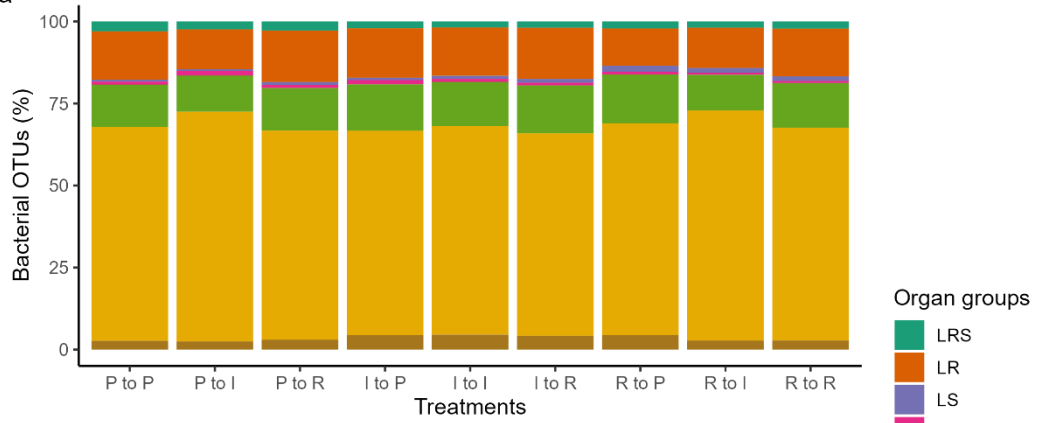


Figure S14: Bacterial (A) and fungal (B) class relative abundance in seed microbiota according to treatments. Only the 7 most abundant classes are displayed. P – Poor, I – Intermediate, R – Rich.

A - Bacteria



B - Fungi

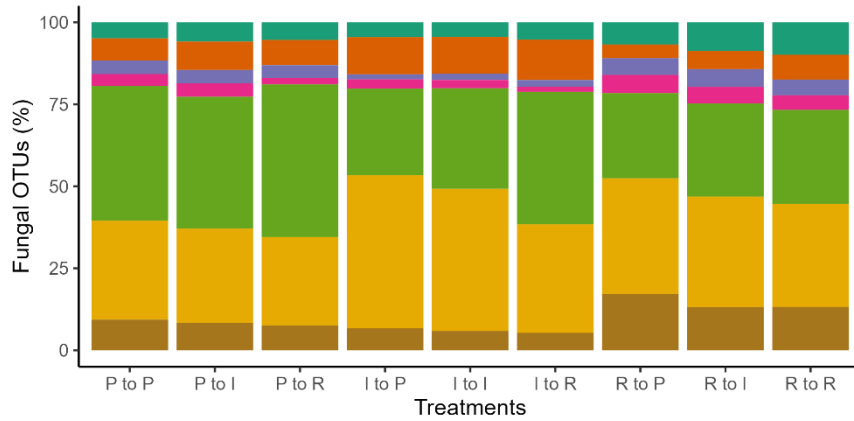


Figure S15: Overall OTUs repartition for each treatment combination between the different organs for (A) bacteria and (B) fungi. L, R and S are the OTUs exclusively found in seedling leaves, seedling roots, or seeds respectively. LRS those found in the three organs and LR, LS and SR those either found in Leaves and Roots, Leaves and Seeds or Seeds and Roots, respectively. P – Poor, I – Intermediate and R – Rich. Treatments are named as ‘Maternal treatment’ to ‘Seedling treatment’.

Table S16: Output of the mix linear model testing the effects of seed treatment, seedling treatment and seedling tissue on the average pairwise  $\beta$  diversity between seed and seedling microbiota.

<b>Bacteria</b>	<b>Estimate</b>	<b>Std. Error</b>	<b>df</b>	<b>t value</b>	<b>p value</b>
Intercept	1.683	0.021	43.517	79.635	<b>&lt;0.001</b>
Seed Intermediate	0.043	0.026	27.000	1.621	0.117
Seed Poor	-0.079	0.026	27.000	-2.998	<b>0.006</b>
Seedling Intermediate	0.008	0.014	147.000	0.599	0.55
Seedling Poor	0.027	0.014	147.000	1.925	0.056
Roots	0.255	0.011	147.000	22.374	<b>&lt;0.001</b>
<b>Fungi</b>					
Intercept	1.669	0.028	34.505	60.589	<b>&lt;0.001</b>
Seed Intermediate	0.103	0.037	27.000	2.815	<b>0.009</b>
Seed Poor	-0.017	0.037	27.000	-0.455	0.653
Seedling Intermediate	0.020	0.013	147.000	1.489	0.139
Seedling Poor	0.037	0.013	147.000	2.745	<b>0.007</b>
Roots	0.064	0.011	147.000	5.929	<b>&lt;0.001</b>

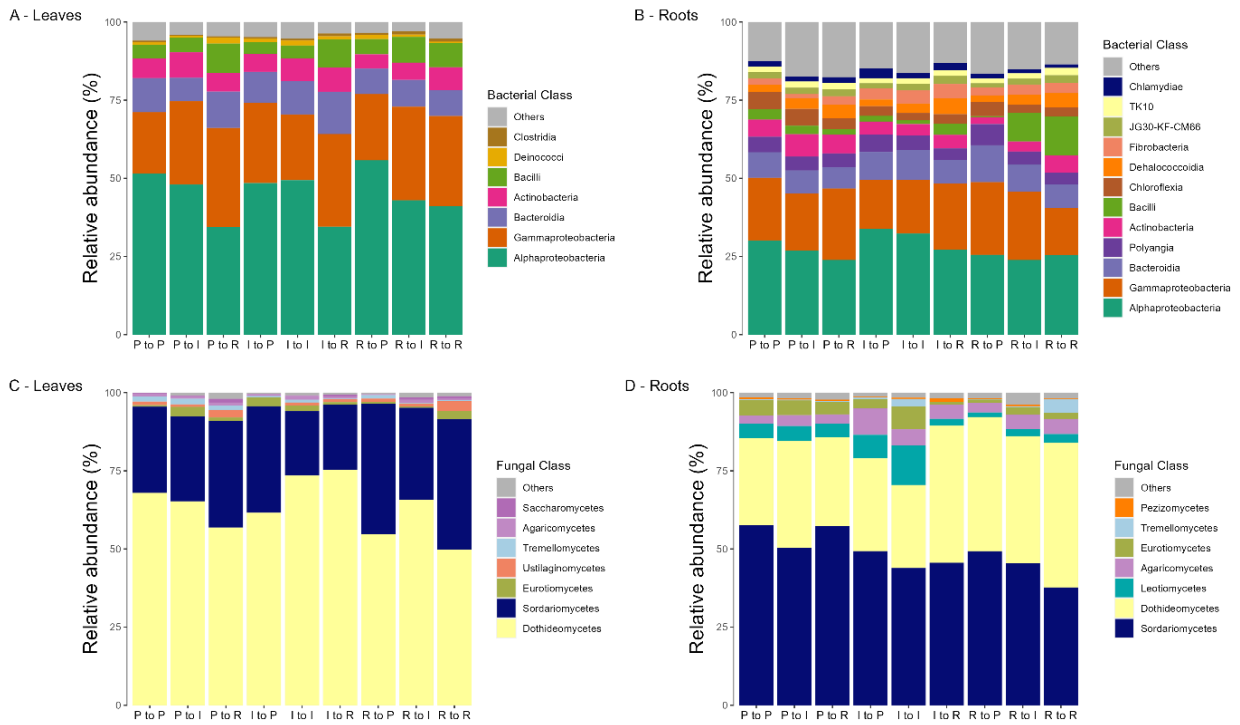


Figure S16: Relative abundance of the dominant bacterial and fungal taxa in leaves (A and C respectively) and roots (B and D). P – Poor, I – Intermediate and R – Rich. Treatments are named as ‘Maternal treatment’ to ‘Seedling treatment’.

Table S17: Results of the perMANOVAs performed on leaves and roots bacterial and fungal communities. PerMANOVAs tested for the effect of the mother and seedling treatments as well as their interaction and the mother plant identity (Mother ID). P values in bold indicates a significant effect ( $p < 0.05$ ).

			<b>Df</b>	<b>Sum of squares</b>	<b>R2</b>	<b>F</b>	<b>p value</b>	
<b>Leaves</b>	<b>Bacteria</b>	Mother treatment	2	1.684	0.010	1.758	<b>0.001</b>	
		Seedling treatment	2	1.226	0.007	1.279	<b>0.001</b>	
		Mother ID	27	14.203	0.085	1.098	<b>0.001</b>	
		Treatments interaction	4	2.121	0.013	1.107	<b>0.001</b>	
		Residual	310	148.474	0.885			
		Total	345	167.708	1.000			
		<b>Fungi</b>	Mother treatment	2	2.271	0.015	2.387	<b>0.001</b>
	Seedling treatment		2	1.170	0.008	1.229	<b>0.001</b>	
	Mother ID		26	14.764	0.097	1.194	<b>0.001</b>	
	Treatments interaction		4	2.081	0.014	1.094	<b>0.003</b>	
	Residual		276	131.297	0.866			
	Total		310	151.582	1.000			
	<b>Roots</b>		<b>Bacteria</b>	Mother treatment	2	1.896	0.014	2.087
		Seedling treatment		2	1.646	0.012	1.812	<b>0.001</b>
Mother ID		27		14.358	0.103	1.171	<b>0.001</b>	
Treatments interaction		4		2.227	0.016	1.226	<b>0.001</b>	
Residual		262		119.023	0.855			
Total		297		139.151	1.000			
<b>Fungi</b>		Mother treatment		2	1.808	0.013	1.886	<b>0.001</b>
		Seedling treatment	2	1.247	0.009	1.301	<b>0.001</b>	
		Mother ID	27	14.548	0.102	1.124	<b>0.001</b>	
		Treatments interaction	4	2.091	0.015	1.091	<b>0.001</b>	
		Residual	257	123.163	0.862			
		Total	292	142.857	1.000			



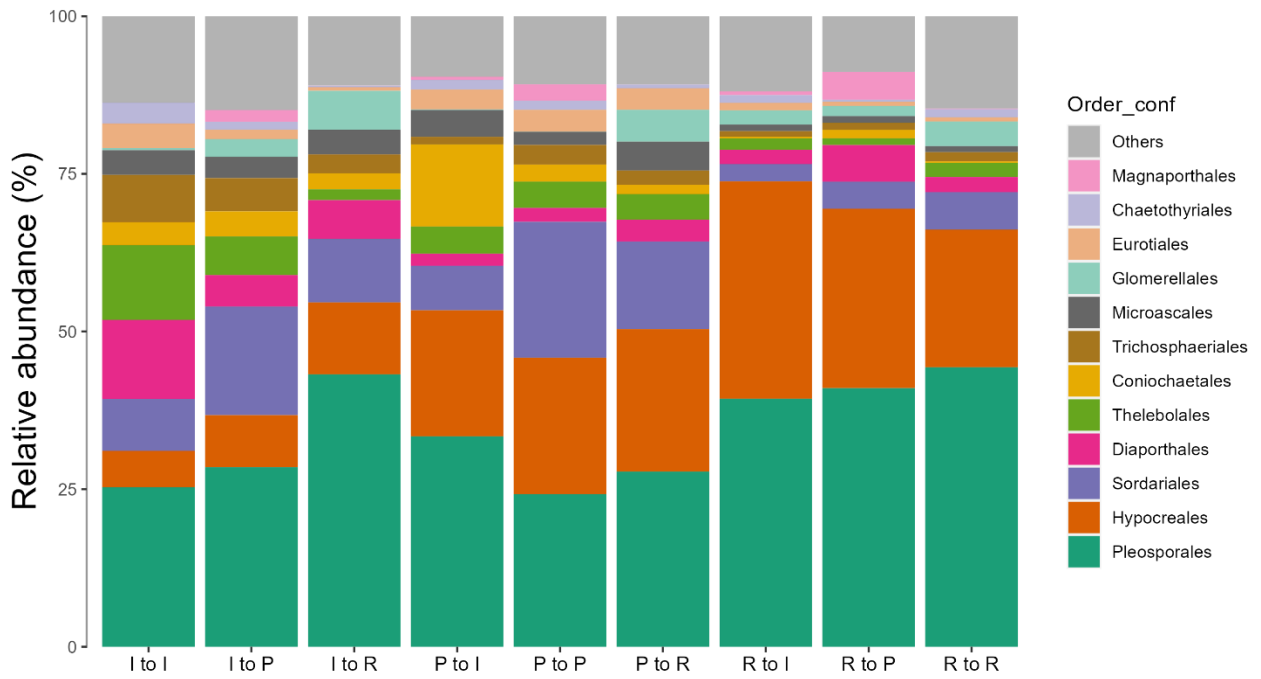


Figure S17: Relative abundance of the dominant fungal orders roots. P – Poor, I – Intermediate and R – Rich. . Treatments are named as 'Maternal treatment' to 'Seedling treatment'.

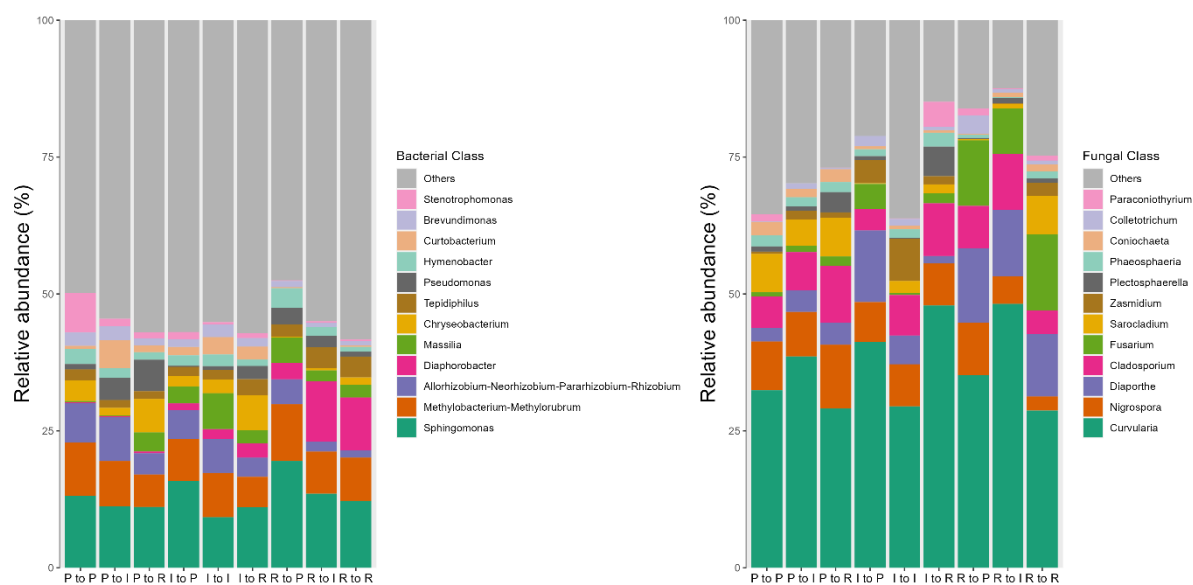


Figure S18: Relative abundance of the dominant bacterial (left panel) and fungal (right panel) class in the leaves of seedlings. P – Poor, I – Intermediate and R – Rich. Treatments are named as ‘Maternal treatment’ to ‘Seedling treatment’.

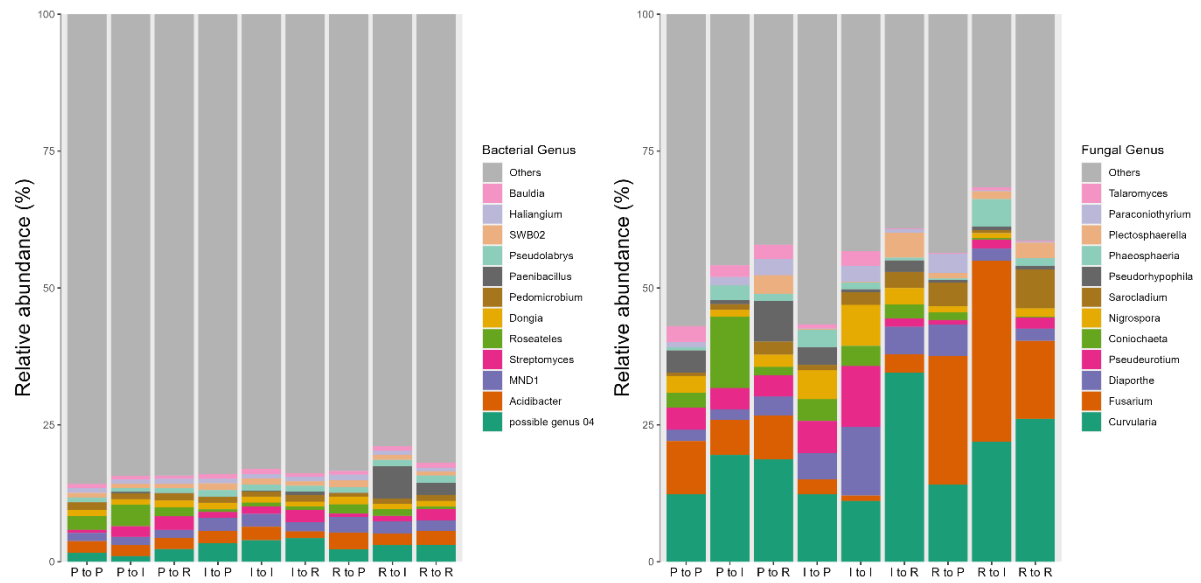


Figure S19: Relative abundance of the dominant bacterial (left panel) and fungal (right panel) genus in the roots of seedlings. P – Poor, I – Intermediate and R – Rich. Treatments are named as ‘Maternal treatment’ to ‘Seedling treatment’.

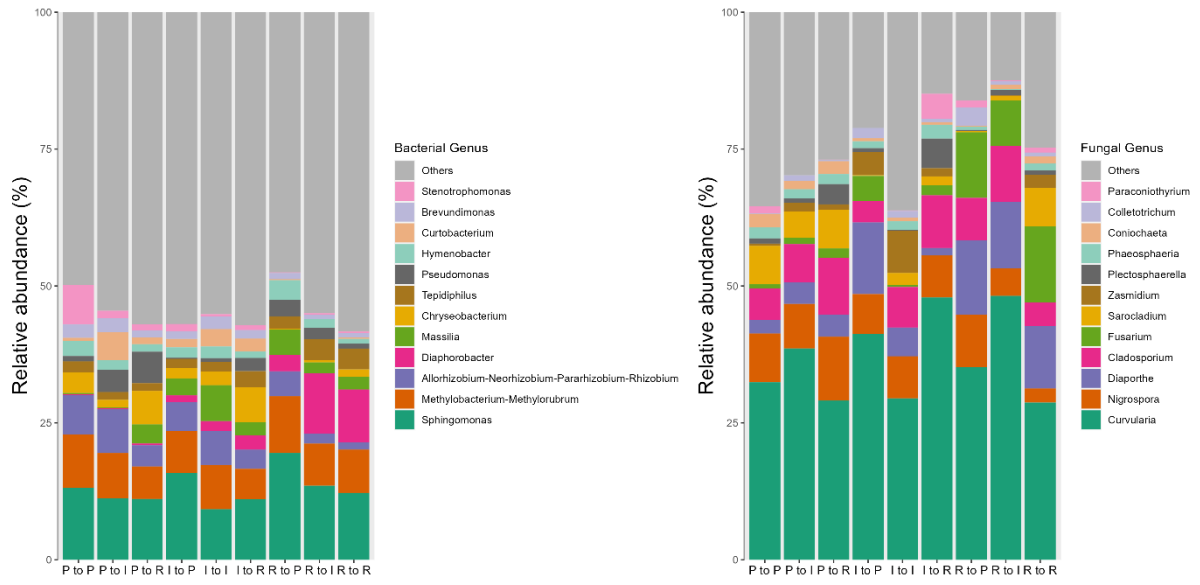


Figure S20: Relative abundance of the dominant bacterial (left panel) and fungal (right panel) genus in the leaves of seedlings. P – Poor, I – Intermediate and R – Rich. Treatments are named as ‘Maternal treatment’ to ‘Seedling treatment’.

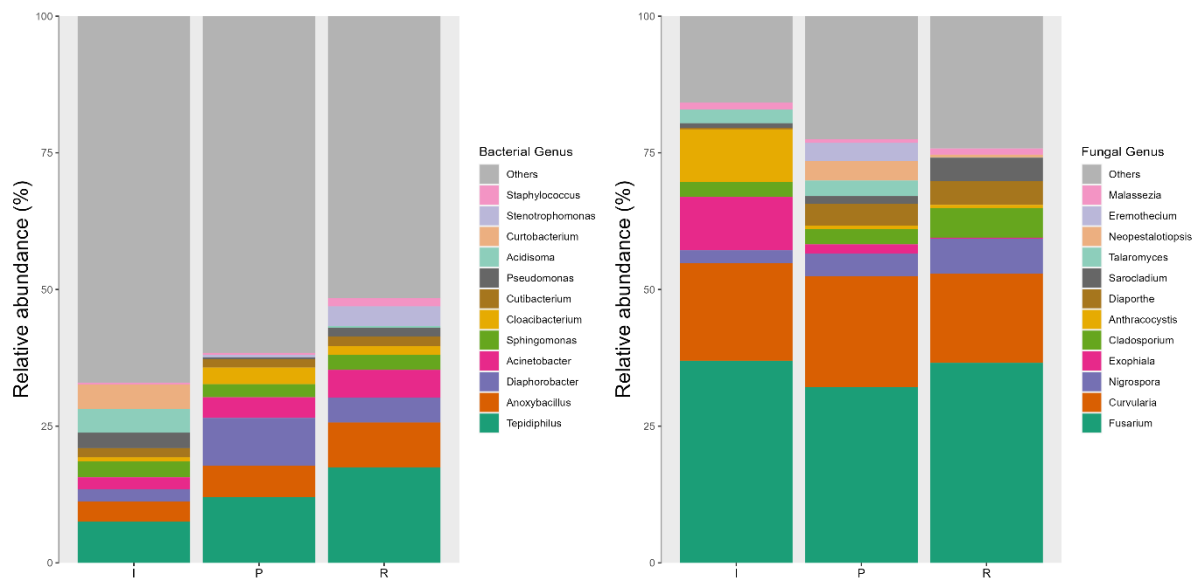


Figure S21: Relative abundance of the dominant bacterial (left panel) and fungal (right panel) genus in the seeds. P – Poor, I – Intermediate and R – Rich.