Université de Montréal

Structure des communautés microbiennes du sol des toits verts de l'île de Montréal

Par

Antoine Hénault

Institut de recherche en biologie végétale (IRBV)

Département de sciences biologiques, Faculté des arts et des sciences

Mémoire présenté à la Faculté des études supérieurs
en vue de l'obtention du grade de Maître ès sciences (M. Sc.)
en Sciences biologiques

Dépôt initial effectué le 6 mai 2021

Université de Montréal

Département de sciences biologiques, Faculté des arts et des sciences

Ce mémoire intitulé

Structure des communautés microbiennes du sol des toits verts de l'île de Montréal

Présenté par

Antoine Hénault

A été évalué par un jury composé des personnes suivantes

Stéphanie Pellerin
Présidente-rapporteur

Pierre-Luc Chagnon
Directeur de recherche

Isabelle Laforest-Lapointe
Évaluatrice externe

Résumé

Les toits verts sont des écosystèmes d'une grande importance pour les milieux urbains. Cependant, le microbiome du sol est peu considéré dans l'aménagement de ces habitats, alors qu'il est pourtant à la base de nombreux services écosystémique, dont le cyclage des nutriments et la productivité primaire. Il est donc nécessaire de s'intéresser davantage à l'assemblage de ce microbiome, de manière à éventuellement mieux manipuler ces communautés pour favoriser le maintien des services écosystémiques. Nous avons donc échantillonné le sol 19 toits verts en plus de cinq grands parcs urbains afin d'étudier les communautés bactériennes, fongiques et mycorhiziennes de ces habitats. Contrairement à ce que prédisent les théories classiques en écologie, les communautés microbiennes des sols des toits verts sont abondantes et diversifiées même dans les toits les plus jeunes. De plus ces communautés ne sont pas dominées par des microorganismes reconnus comme étant tolérants au stress. Ainsi, les limites à la dispersion ne semblent pas affecter ces communautés microbiennes isolées. Une grande variation dans les structures des communautés est restée non expliquée, montrant peu d'évidences d'assemblages déterministes. Ce phénomène pourrait être dû à une plus grande importance de déterminants ou processus stochastiques. Nous avons aussi observé ce phénomène chez les champignons mycorhiziens avec une plus grande abondance des espèces fréquentes régionalement et globalement. Cela montre l'importance du pool régional d'espèces pour l'assemblage des communautés des toits verts. Les toits échantillonnés ont des microbiomes uniques aux parcs environnants, avec une faible abondance de certains groupes taxonomiques, comme les Thaumarchaeota (procaryotes nitrificateurs), les actinobactéries (saprotrophes), ou les Gigasporaceae (champignons mycorhiziens produisant un important réseau d'hyphes extraracinaires). Ces profils microbiens uniques pourraient induire des conséquences biogéochimiques importantes sur les processus écosystémiques du sol des toits verts. Les recherches futures devraient évaluer les liens entre la structure du microbiome et les fonctions écosystémiques rendus par les toits verts.

Mots-clés : Écologie urbaine, microbiome, champignons mycorhiziens arbusculaire, toits verts, écologie microbienne, bactéries, champignons, assemblage des communautés, phylogénétique

Abstract

Green roofs are novel ecosystems of great importance for urban environments. However, green roof soil microbiome has received little attention, even though it supports numerous ecosystem services. It is therefore necessary to pay more attention to the green roof soil microbiome assembly and eventually better manipulate it to promote the maintenance of ecosystem services, as nutrient cycling and primary productivity. We sampled 19 green roofs in addition to five large urban parks to study the bacterial, fungal and mycorrhizal communities in these habitats. Contrary to what was expected under classic ecological theories, microbial communities were abundant and diverse, even on the youngest roofs. Moreover, green roofs soils were not dominated by microorganisms known to be particularly stress tolerant. Dispersal limitation did not appear to affect the green roof soil communities. High level of variation in community structure remained unexplained, showing little evidence of deterministic assembly. This phenomenom may be a sign of stochastic assembly in these habitats. It was partly observed for mycorrhizal fungi with greater abundance of regionally and globally frequent species. This suggests the importance of regional species pool for community assembly in green roofs. The sampled roofs showed unique microbiomes, with low abundance of some taxonomic groups, such as the Thaumarchaeota (nytrifying prokaryotes), Actinobacteria (sapotrophs), or Gigasporaceae (mycorrhizal fungi producing an important external hyphae network). This could have important biogeochemical consequences on green roofs. Much insight will be gained from future research looking at the links between microbiome composition and the ecosystem services provided by green roofs.

Keywords: Urban ecology, microbiome, arbuscular mycorrhizal fungi, green roof, microbial ecology, bacteria, fungi, community assembly, phylogenetic

Table des matières

Liste des tableaux

Liste des figures

Liste des sigles et abréviations

CMA/AMF	Champignons mycorhiziens arbusculaires
N	Azote
C	Carbone
NH_4^+	Ammonium
NO_3^-	Nitrate
PO_4^{3-}	Phosphate
$CaCl_2$	Chlorure de calcium
KOH	Hydroxyde de pottasium
KCl	Chlorure de potassium
PVGL	Lactoglycérol
M	Molaire
% mat. Org	Contenue en matière organique
% moist	Humidité gravimétrique
VTX	Taxon virtuel
ASV	Amplicon sequence variants
e.g.	Par exemple
PCR	Amplification en chaîne par polymérase
MIP	Mycorrhizal inoculum potential
Ø	Diamètre
P	Valeur de p

β	Bêta
α	Alpha
χ^2	Chi carré
PC	Coordonnées principales
PCA	Analyse en composante principale
RDA	Analyse canonique de redondance
db-RDA	Distance-based redundancy analysis
PCoA	Analyse en coordonnées principales
MNTD	Mean nearest taxon distance
PD	Diversité phylogénétique
MPD	Mean pairwise distance
PCNM	Principal coordinates of neighbor matrices
perMANOVA	Permutational multivariate analysis of variance
ANOVA	analyse de la variance

Remerciements

Je tiens tout d'abord à remercier le fond de recherche du Québec nature et technologie (FRQNT), ainsi que le conseil de recherches en sciences naturelles et en génie du Canada (CRSNG), pour les bourses qui m'ont soutenu tout le long de ma maîtrise. J'aimerais aussi remercier le centre des sciences de la biodiversité du Québec (CSBQ) pour la bourse de démarrage offert pour soutenir le projet.

J'aimerais remercier tous les stagiaires et étudiants de premiers cycles qui ont contribué à une étape ou à une autre dans l'analyse de mes échantillons. Par la suite, j'aimerais remercier tous les collaborateurs et membres de mon comité-conseil, qui m'ont laissé des commentaires et donc aidé à améliorer grandement la rigueur, la pertinence et la compréhension de mon mémoire : Geneviève Lajoie, Jacques Brisson, Danielle Dagenais, Tonia de Bellis et Stéphanie Pellerin

J'aimerais remercier tous les membres du labo Chagnon pour toute l'aide qu'ils ont pu m'apporter de près ou de loin et pour toutes nos discussions de lab meeting. J'aimerais remercier spécialement Émilie avec qui je n'aurais certainement pas pu survivre à cette étape et qui malgré qu'elle prolonge sa maîtrise. Nos maîtrises n'auront pas été comme nous le pensions et ponctuer de hauts et de bas, mais au moins je vais y avoir survécu et toi aussi peut-être éventuellement.

Maintenant pour les remerciements les plus importants. Je voudrais commencer par mon directeur de recherche, Pierre-Luc, qui a toujours cru davantage en moi et en mes compétences que moi-même. Même dans les périodes les plus difficiles et où je me sentais le moins efficace et compétent, il a toujours eu une plus grande estime de moi que moi-même je pouvais avoir. Il s'agit du meilleur support que j'aurais pu recevoir d'un directeur de maîtrise.

Maintenant pour ce qui est de mes parents. Je n'aurais jamais pu en arriver où j'en suis sans votre amour et support inconditionnel. Je n'ai jamais eu autant de difficulté que dans les deux dernières années et sans vous je n'y serais certainement jamais arrivé. Merci tout spécial de m'avoir hébergé une partie du confinement et de nos nombreux appels qui me font toujours un bien fou.

Je gardais le plus important pour la fin la personne qui m'as vu dans tous mes hauts et bas, mes découragements, mes colères, mes exaspérations et qui malgré tout continue de m'aimer. Merci Marco de continuer de me supporter tous les jours et de croire en moi lorsque moi-même, je ne crois pas en moi au point de m'exaspérer tellement qu'à ton regard il est évident que je suis le meilleur. Tu m'as littéralement suivi tout le long de ma maîtrise, alors qu'on s'est rencontré la veille de mon début, mais je n'aurais pas pu rêver d'un meilleur partenaire pour m'aider dans cette étape.

Chapitre 1 : Introduction générale

Urbanisation et toits verts

L'urbanisation transforme radicalement les écosystèmes et modifie leur écologie (Kaye et al., 2006). Le remplacement du sol et de la végétation par des surfaces imperméables cause entre autres la diminution de l'albédo, ainsi que la séquestration de la chaleur dans les rues entourées de hauts bâtiments (effet de canyon), ce qui induit l'effet d'îlots de chaleur (Rizwan, et al. 2008; Sharma et al., 2016; Sucsa et al., 2011; Oke, 1982). Avec des surfaces imperméables pouvant recouvrir jusqu'à 80% des zones urbaines (Schueler, 1994 dans Kaye et al., 2006), l'humain modifie l'hydrologie en diminuant l'évapotranspiration et le drainage et augmente le ruissèlement de surface (Kaye et al., 2006), ce qui augmente les risques d'inondation (Berndtsson 2010). Par ailleurs, en raison de la grande concentration d'activités anthropiques et des cycles biogéochimiques qui sont bouleversés (Kaye et al., 2006), les centres urbains sont souvent des points chauds de pollution de l'air et des sols (Mayer, 1999; Grimm et al., 2008; Lovett et al., 2000; Gregg et al., 2003; Kaye et al., 2006). L'ensemble de ces enjeux ont une incidence en santé publique: ils sont associés à d'importants problèmes de santé (Pope et al., 1995; Bell et al., 2004), et à une perte de productivité ce qui apporte d'importantes conséquences économiques (Dechezleprêtre , Rivers et Sadler, 2019; Quah et Boon, 2003). Avec une proportion croissante de la population mondiale qui habite maintenant les villes, le défi qui nous attend est de créer des écosystèmes urbains capables de limiter les effets négatifs de l'humain sur les services écosystémiques normalement fournis par les écosystèmes naturels, par exemple en créant de nouveaux types d'écosystèmes.

Les toits verts sont un exemple d'écosystèmes artificiels visant à rétablir certains services écosystémiques dans les milieux urbanisés (Sutton, 2015). Les toits comptent pour environ 20-25% de la surface urbaine (Akbari et Rose, 2008). Ainsi le verdissement de ces surfaces a le potentiel d'affecter considérablement les cycles biogéochimique et hydrologique, de même que le microclimat local (Besir et Cuce, 2018). En séquestrant une partie des précipitations dans le sol et les parties végétatives, et en permettant de l'évapotranspiration, les toits verts peuvent retenir entre 26% et 100% des précipitations annuelles (Carter et Rasmussen, 2006). Ces processus diminuent donc le ruissèlement de surface et permettant de diminuer les risques d'inondation (Bengtsson et al., 2005; vanWoert et al., 2005). Les toits verts s'attaquent au phénomène d'îlot de chaleur, en augmentant l'albédo et l'évapotranspiration (Gill et al., 2007; Alexandri et Jones, 2008; Susca et al., 2011; Sharma et al., 2016). Les toits verts aident aussi à améliorer la performance thermique des bâtiments par une augmentation de l'ombrage, une plus grande inertie thermique et en raison de l'évapotranspiration qui dissipe la chaleur latente (Saadatinian et al., 2013; Besir et Cuce, 2018). En diminuant le transfert de chaleur vers les bâtiments jusqu'à 96% comparativement aux toits traditionnels, les toits verts permettent de diminuer considérablement les besoins en climatisation (Wong et al., 2003; Saadatinian et al., 2013), ce qui mène inévitablement à des économies d'argent et d'énergie (Getter et al., 2011). Cette isolation thermique permet aussi d'améliorer le bien-être psychologique et physiologique, le niveau de productivité et la qualité de vie en général (Al Horr et al., 2016). En diminuant les besoins en consommations d'énergie, les toits verts permettent de diminuer indirectement la concentration de polluants atmosphériques (Sailor, 2008). Les plantes utilisées peuvent aussi capter directement les polluants atmosphériques, contribuant à la qualité de l'air dans les villes (Rowe et al., 2010; Yang et al., 2008). En protégeant les membranes des toits des rayons UV et des fluctuations thermiques, les toits verts permettent d'augmenter leur durée de vie de 10-20 ans à 40-50 ans (Rowe et al., 2010; Kosaroe et Ries, 2007). Ces environnements

peuvent servir d'habitats pour des espèces généralistes et même certaines espèces rares (Williams et al., 2014). Ceci n'est qu'une liste non exhaustive des bénéfices apportés par les toits verts, qui inclue aussi la diminution de la pollution sonore, l'amélioration de l'esthétisme du paysage et leur potentielle contribution à promouvoir l'économie circulaire via l'agriculture urbaine de proximité (Berardi et al., 2013; Shafique et al., 2018; Oberndorfer et al., 2007). Des analyses coûts-bénéfices tenant compte des aspects économiques, environnementaux et sociaux des toits verts ont soutenu que les toits verts seraient un choix durable et un investissement à faible risque et le retour net de l'investissement se ferait rapidement (Bianchini et Hewage, 2012).

Malgré ces bénéfices apparents que peuvent apporter les plantes installées sur les toits des villes, au final un nombre très restreint d'espèces végétales peuvent bien performer dans ces environnements (Oberndorfer et al., 2007; Dvorak et Volder, 2010). En raison des substrats souvent très minces, spécialement pour les toits verts extensifs (< 15 cm d'épaisseur), ces habitats sont confrontés à la sécheresse rapidement après une pluie (Bengtsson et al., 2005). Ce stress hydrique imposé aux plantes est accentué par le vent et les fortes radiations solaires en quasi-absence d'ombre (Sutton, 2015). Un des défis de l'écologie végétale appliquée est donc de sélectionner les plantes aptes à survivre et croître sur les toits (e.g., Lundholm et al. 2010; Heim et Lundhom., 2014; Lundholm 2015). Or, la vaste majorité des plantes installées sur toits verts demeurent des crassulacées du genre *Sedum*. Or, les services écosystémiques sont souvent maximisés par l'inclusion d'une plus grande diversité d'espèces de plantes, ou de plantes avec des traits différents (Ranallo et Lundholm 2008; Cook-Patton et Bauerle, 2012; Flynn et al., 2011; Maestre et al., 2012; Lundhom, 2015). On cherche donc de plus en plus à développer des mixtures d'espèces de différents groupes fonctionnels qui pourraient coexister sur les toits verts et assurer leur

multifonctionnalité (Cook-Patton and Bauerle, 2012; Dunnett et al., 2008; Franzaring et al., 2016; Johnson et al., 2016; Lundholm et al., 2010; Lundholm et al., 2015; Xie et al., 2018).

Microbiome du sol des toits verts : de grands oubliés

Alors que la vaste majorité de nos efforts pour modeler les écosystèmes sur les toits ont gravité autour des plantes, les microorganismes du sol et colonisant les racines des plantes sont demeurés des grands oubliés de cette histoire. Pourtant, par leurs rôles, ils affectent la majorité des fonctions écosystémiques fournies par les toits, par exemple la séquestration de carbone et de polluants (e.g. Wall et al., 2010; Kibblewhite et al., 2008; Barrios, 2007; Van der Heijden, 2008; Bardgett et van der Putten, 2014). On commence à peine à mieux connaître la composition de ces communautés dans ces habitats et leur abondance (McGuire et al., 2013; Rumble et Gange, 2013; Molineux et al., 2015; Chaudhary et al., 2018; Rumble, Finch et Gange, 2018; Hoch et al., 2019; Gill et al., 2020). Ces études ont entre autres permis de constater que les toits verts abritaient une grande diversité taxonomique et phylogénétique et que les microorganismes avaient la capacité de bien coloniser naturellement ces habitats. Cependant, on en connaît encore très peu sur les facteurs contrôlant l'assemblage de ce microbiome. Plusieurs auteurs ont souligné le rôle potentiellement prédominant du microbiome du sol pour l'efficacité des toits verts (e.g. Chagnon et Brisson, 2017; Fulthorpe et al., 2018). Ces communautés sont essentielles pour de nombreuses fonctions écosystémiques, comme le cyclage des nutriments (Bardgett & van der Putten, 2014), la décomposition de la litière (Setälä & McLean, 2004) , la séquestration du carbone (Six et al., 2006; Clemmensen et al., 2013; Wilson et al., 2009), la prévention de l'érosion (Six et al., 2002), la régulation des pathogènes (Sikes et al., 2009; Schlatter et al., 2017), la décontamination et bioremédiation (Marin et al., 2005; Kavamura et Esposito, 2010), la pédogenèse (Jongmans et al., 1997) et la productivité primaire (van der Heijden et al., 1998, 2008; Wagg et al., 2011). La perte

de biodiversité dans le sol et la simplification des communautés microbiennes entraînent généralement des effets négatifs sur de nombreuses fonctions écosystémiques (Wagg et al., 2014; Li et al., 2019; Delgado-Baquerizo et al., 2017; Hooper et al., 2012). En conditions hostiles comme les toits verts, la présence des communautés microbiennes serait davantage nécessaire en raison de leurs fortes résistances aux perturbations comme les cycles de sécheresse. Cette plus grande résistance permettrait du même coup d'offrir une résilience plus grande aux écosystèmes (Barnard et al., 2013). De plus, les communautés microbiennes du sol sont souvent reliées à une meilleure tolérance des plantes aux stress environnementaux, comme la sécheresse, via leurs effets directs et indirects (e.g. via leur effet sur les propriétés du sol) (Lau et Lennon, 2012; Jaynes et Quigley, 2014; Ortiz et al., 2015). Par leurs rôles et les molécules qu'elles produisent, ces communautés affectent aussi les traits des plantes, tels que la tolérance aux stress et la longueur spécifique des racines (Friesen et al., 2011). Cet effet a un effet direct sur l'efficacité des écosystèmes à supporter certaines fonctions (Diaz et al., 2007; Luck et al., 2009; de Bello et al., 2010; Friesen et al., 2011).

Parmi les organismes qui composent le microbiome du sol, nous porterons un intérêt particulier aux champignons mycorhiziens arbusculaires (CMA) dans ce projet. Ces champignons forment avec les plantes une symbiose ancestrale qui aurait permis au règne végétal de conquérir la terre ferme (Feijen et al., 2018). Puisque les plantes se sont diversifiées avec des champignons en symbiose dans leurs racines, il semble naturel que ces champignons soient connus pour influencer une panoplie de processus physiologiques chez la plante comme sa nutrition, sa tolérance aux herbivores ou encore sa résistance au stress comme la sécheresse (Smith & Read, 2008; Delavaux et al., 2017). Cette contribution des CMA à la tolérance à la sécheresse des plantes n'est pas anodine pour des écosystèmes secs comme les toits verts et s'effectue via une variété de mécanismes (voir tableau 1.1) (Augé 2001; Augé 2004; Allen 2007; Ruth et al., 2011; Ruiz-Lazano et al., 2012; Jayne

et Quigley, 2014). Ils offriraient aussi une protection contre les pathogènes et les maladies (Newsham et al., 1995; Sikes et al., 2009), une meilleure agrégation et structure du sol (Rillig et Mummey., 2006) et une plus grande tolérance aux métaux lourds (Hildebrandt et al. 2007). Ils permettent aussi une meilleure acquisition de nutriments par les plantes hôtes, surtout du phosphore et potentiellement de l'azote (Smith et Read, 2008). Ces organismes affectent aussi l'ensemble de la communauté végétale. En effet, les exsudats des CMA ainsi que la force physique appliquée par leurs hyphes sur les agrégats du sol affectent la structure du sol et améliorent la tolérance à la sécheresse des plantes environnantes (Augé et al., 2004; Rillig et Mummey, 2006).

Tableau 1.1 : Mécanismes par lesquels les champignons mycorhiziens arbusculaires contribuent à la tolérance des plantes à la sécheresse

Mécanisme	Effet	Articles
Augmentation de l'agrégation du sol par l'effet physique des hyphes et les exsudats excrétés.	Augmentation de la capacité de rétention d'eau du sol	Augé et al., 2004; Rillig et Mummey, 2006
Acquisition directe d'eau par les hyphes	Accès à de l'eau présente dans des pores inaccessibles aux racines	Augé, 2001; Allen, 2007; Ruiz-Lozano et al., 2012
Transfert de composés à la plante hôte (K+, Ca2+, carbone non structurel)	Diminution du potentiel osmotique des tissus	Wu et Xia., 2006
Régulation des aquaporines	Meilleure absorption d'eau par les racines	Ruiz-Lozano et Aroca., 2010
Augmentation de l'activité antioxydante	Réduction des effets négatifs des formes oxydatives de l'oxygène produites en période de stress hydrique	Ruiz-Lozano et Aroca., 2012
Modulation de la balance hormonale de l'hôte, surtout l'acide abscissique (ABA)	Augmentation des échanges gazeux et amélioration de l'efficacité de l'utilisation de l'eau par l'hôte	Augé 2001; Ruiz-Lozano, 2003
Augmentation de l'apport en phosphore	Maintien de la conductivité hydraulique des racines et des cellules corticales	Smith et al., 2010
Modification de l'architecture racinaire	Affecte la tolérance à la sécheresse et augmente la capacité d'aller chercher des nutriments	Wu et al., 2013

Bénéfices de mieux comprendre l'assemblage des communautés

Comme présenté, alors que le microbiome du sol joue des rôles essentiels pour les écosystèmes, comprendre les filtres en place dans les différents écosystèmes nous permet de mieux prédire

l'assemblage des communautés microbiennes et du même coup de prédire quelles fonctions pourraient être affectées. Identifier les filtres d'assemblage des microbiomes des toits verts peut donc aider à mieux comprendre l'impact des décisions lié à l'aménagement des « novels ecosystems » (sensu Hobbs, 2006), dont les toits verts, sur leurs fonctionnalités. Cette approche visant une meilleure compréhension de l'assemblage des communautés microbiennes pour améliorer la gestion des habitats n'est pas nouvelle, alors que par exemple, dans les agroécosystèmes, le rôle des microorganismes pour la santé du sol est de plus en plus reconnu (Kennedy et Stubbs, 2006). Une meilleure connaissance de l'assemblage des communautés dans ces écosystèmes permet entre autres de cibler quelles pratiques, telles que l'ajout de fertilisant, le type d'amendement, le labour et le type de plante, affectent le plus les communautés microbiennes (e.g. Zhou et Fong, 2021, Kelper et al., 2020; Silva et al., 2012) et du même coup la santé des sols et la capacité des terres à soutenir la demande en nourriture. Une étude récente de Zhou et Fong (2012) a permis en autre de déterminer que les pratiques d'aménagement agricoles étaient le facteur le plus important pour la structure des communautés bactériennes et fongiques, comparativement à d'autres variables, comme le choix de plantes ou des variables du sol (Siva et al., 2012).

Pour les toits verts spécifiquement, la découverte des filtres d'importance pourrait aussi nous guider dans leur gestion. Par exemple, une sélection basée sur les capacités de tolérance aux stress, tels que la sécheresse, pourrait compromettre la capacité de ces milieux à maintenir les cycles biogéochimiques en raison de compromis fonctionnels chez les microorganismes (e.g. Ferenci, 2016; Garcia et al., 2020). D'autre part, si la capacité de dispersion aérienne et donc la limitation à la dispersion joue un rôle prédominant sur les toits vers, certains taxons pourraient être désavantagé encore une fois en raison de compromis fonctionnels. Par exemple, chez les CMA, les Gigasporaceae qui ont généralement des spores plus larges que d'autres familles (Aguilar-

Trigueros et al., 2019), pourraient être désavantagés (voir Chaudhary et al., 2020). Cela pourrait affecter certaines fonctions des toits, comme l'agrégation du sol et la capacité de rétention, alors que les taxons de cette famille sont reconnus pour avoir un réseau d'hyphes extraracinaires plus développé que d'autres familles (Hart et Reader, 2002; Powell et al., 2009). S'attarder aux mécanismes écologiques qui déterminent l'assemblage des communautés microbiennes sur les toits verts est primordial afin de bien comprendre éventuellement les fonctions qui pourraient être compromises par la sélection de certains traits au détriment d'autres. Une telle compréhension pourrait aussi nous informer sur la nécessité de procéder à des inoculations exogènes et les conditions qui seraient favorables à de telles pratiques.

L'assemblage des communautés microbiennes

Certaines études ont regardé la composition des communautés microbiennes sur des toits verts et ont démontré qu'elles étaient très diversifiées (McGuire et al., 2013, Gill et al., 2020; Hoch et al., 2019; Rumble, Finch et Gange, 2018; Molineux et al., 2015; John et al., 2014; Deep et al., 2018) et abondantes (Chaudhary et al., 2018), contrairement à ce qu'on aurait pu croire en raison de leur isolation spatiale (hauteur élevée faible aire) ainsi que leur haut niveau de stress (MacArthur et Wilson, 1967; Grime, 1973). Cependant, la structure des communautés microbiennes des toits verts était généralement distincte de celles retrouvées dans d'autres habitats urbains, tels que les parcs (McGuire et al.,2013; Gill et al., 2020), les friches industrielles (Molineux et al., 2015) et les fosses d'arbres (Gill et al., 2020). McGuire et al., (2013) ont d'ailleurs observé sur les toits verts une dominance de certains taxons tolérants aux stress et associés aux milieux anthropiques, dont *Pseudallescheria* et *Peyronellaea*, deux taxons de champignons. Des études ont aussi étudié l'effet d'inoculations exogènes, surtout de mycorhizes, sur la performance des plantes (Molineux et al., 2014; Young et al., 2015; Rumble et Gange, 2017; Molineux et al., 2017; Schröder et al., 2019).

Ces études ont généralement observé une augmentation de la diversité suite à des inoculations, une plus grande colonisation racinaire et une meilleure performance des plantes (mais voir Young et al., 2015).

Cependant, on s'est très peu attardé jusqu'à présent aux facteurs qui pourraient sélectionner des groupes microbiens distincts dans ces environnements. La sélection de taxons spécifiques pourrait potentiellement affecter la capacité des toits verts à soutenir certaines fonctions directement ou indirectement via leurs relations intimes avec les communautés végétales (e.g. Delgado-Baquerizo et al., 2017). L'effet sur les communautés végétales pourrait être déterminant alors que par exemple, Farrell et al. (2013) soutenaient que la performance des plantes déterminerait ultimement la capacité des toits verts à restaurer les fonctions écologiques. Par exemple, Speak et al (2013) a observé une diminution de l'effet de l'isolation thermique par les toits verts en raison de dommages à la végétation. En raison de leurs nombreux bénéfices pour la performance des plantes, les communautés microbiennes pourraient empêcher de tels dommages.

Les connaissances concernant la structure des communautés microbiennes du sol sont déjà bien avancées (e.g. Kaiser et al., 2016; Nemergut et al., 2013; Hanson et al., 2012; Powell et al., 2015; Fierer et al., 2017; Lauber et al., 2008). Le pH, le statut nutritionnel et certains facteurs climatiques sont souvent reconnus pour être des variables clés pour l'assemblage des communautés microbiennes du sol (Fierer et al., 2017; Lauber et al., 2009). Cependant, même en procédant à des mesures extensives des variables environnementales, une importante variation dans la structure des communautés microbiennes du sol reste souvent inexpliquée (Zhou et Ning, 2017; Evans et al., 2017). Cela pourrait être expliqué par l'importance des processus stochastiques (ex. : effet de priorité, dérive écologique et limitation à la dispersion) pour l'assemblage de ces communautés.

L'assemblage des communautés microbiennes du sol en contexte urbain

Les organismes font face à une multitude de stress dans les villes, comme la chaleur, la pollution, les perturbations et la fragmentation de l'habitat (Niemelä et al., 2011), ce qui impose des pressions uniques pour les communautés des sols (voir Yan et al., 2016). Il pourrait donc être difficile de transposer les connaissances des milieux ruraux pour l'écologie urbaine (Guilland et al., 2018). Certaines études ont justement observé des interactions complexes et contradictoires dans les milieux urbains (McDonnell et al., 1997). Un récent exemple de cette dichotomie entre l'écologie urbaine et les connaissances antérieures en milieux naturels provient de Lin et al., (2021). Cette étude a observé une diminution de la sélectivité des plantes hôtes envers les CMA dans les milieux urbains, en plus d'une réduction des espèces spécialistes aux dépens des généralistes. Cette diminution de la sélectivité et des espèces spécialistes a mené à un réseau non niché pour les associations plantes-CMA, contrairement à ce qui est généralement observé en milieux naturels (Chagnon et al., 2012; Montesinos-Navarro et al., 2012). Les communautés urbaines sont confrontées à une modification des forces de sélection. Par exemple, les variations temporelles de nutriments et la prédation sont moins importantes qu'en milieux naturels (Shochat et al., 2006), et d'autres forces prennent de l'importance, modifiant la morphologie, la phénologie et la physiologie des organismes (Grimm et al., 2008 ; Shochat et al., 2006). Bien que le microbiome du sol dans les milieux ruraux réponde aussi fortement au pH (e.g. Yan et al., 2016; Hui et al., 2017, Guilland et al., 2018), des études ont trouvé des différences dans la réponse des communautés (Guilland et al., 2018; Xu et al., 2013; Yan et al., 2016). Ils ont entre autres trouvé que l'assemblage de ces communautés pouvait aussi être expliqué par des facteurs spécifiques aux milieux urbains, tels que le niveau de stress anthropique et le niveau d'urbanisation (Yan et al., 2016; Voir Laforest-Lapointe et al., 2017 pour communautés microbiennes foliaires).

Pour ce qui des toits verts spécifiquement, certaines forces de sélection uniques pourraient affecter considérablement l'assemblage des communautés microbiennes. Par exemple, l'isolement de ces écosystèmes, principalement dû à leur élévation comparativement à la matrice environnante d'habitats pourrait imposer un filtrage lié à la dispersion et sélectionner pour les taxons avec une meilleure capacité de dispersion. Contrairement, à ce qui a été longtemps cru par l'hypothèse de Baas-Becking (1934) concernant l'assemblage des communautés microbiennes qui supposait que « everything is everywhere, the environment select », de nombreuses études ont démontré que ces communautés font face à des limitations à la dispersion (Telford & al., 2006; Peay & al., 2010; Adams & al., 2013; Talbot & al., 2014). Cette limitation à la dispersion devrait mener à une importance de la stochasticité dans l'assemblage de ces communautés (Powell et Bennet, 2016; Evans et al., 2017; Cline et Zak, 2014; Zhou et Ning, 2017, mais voir Gill et al., 2020). Dans le même ordre d'idée, des évènements stochastiques de dispersion pourraient jouer un rôle et affecter considérablement les communautés, par exemple via l'effet de priorité (Fukami et al., 2010; Dumbrell et al., 2010; Mummey et al., 2009). L'effet de priorité serait aussi plus important dans les habitats moins connectés (Zhou et Ning, 2017), comme le sont les toits verts.

Les conditions récurrentes de sécheresse des toits verts pourraient aussi imposer un important filtre sur les microbes capables de persister dans ces habitats (Meisner et al., 2018; Hartmann et al., 2017; Manzoni et al., 2012; Bastida et al., 2017), et ce même si ces communautés se sont montrées à plusieurs reprises résistantes et résilientes (Barnard et al., 2013; de Vries et al., 2018; Kaisermann et al., 2015). Il y a des résultats contraires concernant l'effet de la sécheresse sur des taxons spécifiques. Certains semblent présenter des effets relativement constants, tels que les Actinobacteria dont leur abondance relative en conditions de sécheresse augmente (Hartmann et al., 2017; Barnard et al., 2013; Von Rein et al., 2016), tandis que les Planctomycetes présenteraient

une réponse négative face à une diminution de la disponibilité en eau (Hartmann et al., 2017; Von Rein et al., 2016). Ces modifications dans l'abondance relative de taxons pourraient être expliquées par les stratégies d'histoire de vie. Par exemple, les Actinobacteria auraient la capacité de dégrader le carbone récalcitrant (Ventura et al. 2007; Rosenberg et al. 2014), sont plus abondants en conditions de sécheresse et feraient partie du clade des Terrabacteria qui possèderaient des adaptations pour la résistance à des stress environnementaux (Battistuzzi et Hedges, 2009).

Concernant les communautés mycorhiziennes spécifiquement, des études montrent que certains taxons perdent l'habilité de coloniser les racines en condition de sécheresse (Ruiz-Lozano et al., 1995; Schellenbaum et al., 1998; Staddon et al., 2004). Certains résultats suggèrent que Glomus serait peu adapté à ces stress et seraient remplacés par d'autres taxons, tel que les Diversispora (Zhang et al., 2016; Yang et al., 2010). Ce stress reconnu des toits et affectant potentiellement les communautés microbiennes pourrait s'additionner aux stress de chaleur et de CO_2, qu'on retrouve dans les villes et étant reconnu comme des facteurs modifiants les communautés microbiennes (Compant et al., 2010; Von Rein et al., 2016). Finalement, la sélection restreinte d'espèces végétales pourrait aussi imposer un important filtre. Cet effet ne se limiterait pas seulement aux mycorhizes qui ont majoritairement une spécificité dans la sélection de leur hôte et vice versa (e.g. Van der Heijden et al., 1998 Husband et al., 2002; Helgason et al., 2002; Oehl et al., 2003; Zobel et Öpik, 2014; Helgason et al., 2007; Chagnon et al., 2015; Sepp et al., 2019; Lekberg et Waller, 2016; Öpik et al., 2009), mais affecterait aussi l'ensemble de la communauté microbienne (e.g. Berg et Smalla, 2009; Prober et al., 2015; Hoch et al., 2019; Vannier et al., 2020). Ainsi, l'utilisation disproportionnée d'espèces comme les *Sedum* sp qui sont généralement faiblement colonisés par les CMA, pourrait affecter considérablement les espèces présentes sur les toits verts.

Ces filtres uniques pourraient affecter de nombreux processus, dont le cyclage des nutriments (e.g. Wall et al., 2012).

Dans l'optique que les toits verts sont construits en suivant relativement les mêmes directives, par exemple dans le choix de substrat (Shafique et al., 2018) selon les recommandations du FLL allemand (FLL, 2002), les conditions abiotiques d'un toit à l'autre ont le potentiel d'être relativement similaires. Cette absence d'importants gradients environnementaux, tels que de pH qui est souvent considéré comme le facteur biotique le plus importants pour les communautés microbiennes (Davison et al., 2021; Fierer et al., 2017; Lauber et al., 2009), pourrait induire un faible effet de ces variables. Cette hypothèse est soutenue par Powell et Bennet (2016) qui ont observés que dans des environnements similaires, les communautés de CMA étaient grandement imprédictibles par les conditions environnementales. Cette homogénéisation des conditions environnementales peut aussi se refléter comme présenté précédemment dans la sélection d'un nombre restreint d'espèces de plantes.

Une autre stratégie afin d'aborder la question d'assemblage des communautés microbiennes des toits verts est d'étudier la structure phylogénétique de ces communautés. Elle peut nous permettre de nous informer sur l'importance potentielle de la compétition biologique et du filtrage abiotique pour l'assemblage des communautés (Webb et al., 2002; Kraft et al., 2007). Ces analyses reposent principalement sur la présomption que les taxons proches phylogénétiques possèdent des traits similaires (Webb et al., 2002; Kraft et al., 2007). Ainsi, un « cluster » phylogénétique est généralement reconnu comme étant un proxy d'un assemblage abiotique et donc de l'importance des filtres environnementaux, alors qu'une surdispersion est reconnue comme étant un proxy d'un assemblage biotique, surtout via la compétition (mais voir Gerhold et al., 2015). Ces conclusions

liées à la structure phylogénétique sont davantage supportées pour les CMA, alors que leurs traits et leur niche sont relativement bien conservés au niveau de la famille (Hart et Reader, 2002; Powell et al., 2009; Davison et al., 2021), ce qui signifie que la phylogénie représente un bon proxy des traits d'histoire de vie (Chagnon et al., 2013). Ainsi la structure phylogénétique des communautés peut nous permettre d'avoir une bonne idée si les filtres environnementaux d'un habitat donné sélectionnent pour des traits particuliers (e.g. Saks et al., 2014). Ce conservatisme phylogénétique au sein de ce groupe d'organisme, le rend particulièrement intéressant afin d'étudier l'assemblage écologique des communautés. Cependant, les autres communautés microbiennes n'auraient pas un aussi bon conservatisme phylogénétique, ce qui rend difficile d'inférer les processus en jeux pour l'assemblage de ces communautés en connaissance de leur structure phylogénétique.

Finalement, en raison de la présence de nombreux stress sur les toits verts et de leur isolation spatiale, ces écosystèmes sont un modèle idéal pour étudier l'effet combiné de ces facteurs sur l'assemblage des communautés microbiennes. Autant l'isolation physique (MacArthur et Wilson, 1967) que les conditions stressantes des toits verts (Grime, 1973) devraient restreindre la richesse en espèces. L'étude de l'assemblage des communautés microbiennes en milieux urbains est un avancement pour une meilleure compréhension de l'écologie microbienne dans son ensemble. Avec l'importance des milieux urbains dans le paysage, l'écologie se doit d'étudier comment les mécanismes d'assemblage des communautés sont affectés dans ces milieux. Une meilleure compréhension de cette unicité dans les toits verts permettra aussi potentiellement de transposer une partie de ces connaissances dans d'autres milieux anthropiques, comme les bordures de rues, les cours résidentielles, les parcs urbains, etc. (Reese et al., 2016). Se pencher sur ces questions liées à l'assemblage des communautés microbiennes est essentiel à la compréhension des fonctions et les services et comment ils divergent de ce qu'on peut observer dans les habitats naturels. De

telle sorte, on sera en mesure d'éventuellement prédire le rôle de ces organismes pour affronter les problématiques liées à l'urbanisation.

Chapitre 2

Stressful, isolated, yet diverse: green roofs have rich microbiomes that are not dominated by oligotrophic taxa

***L'article a été soumis à Ecology**

Hénault, A.[1], Brisson, J.[1], Dagenais[2], D., De Bellis[3], T., Chagnon, P.L.[1*]

1. Université de Montréal, Faculté des Arts et Sciences, Département des Sciences Biologiques, Montréal (QC) Canada
2. Université de Montréal, Faculté d'Aménagement, Montréal (QC) Canada
3. Dawson College, Montréal (QC) Canada

Avant-propos

Antoine Hénault est l'auteur principal de l'article en plus d'avoir procédé à la majorité des analyses statistiques, à l'exception de certaines figures produites par Pierre-Luc Chagnon. Jacques Brisson, Danielle Dagenais et Tonia De Bellis ont participés à la révision de l'article, alors que Pierre-Luc Chagnon a apporté un apport plus important avec un suivi à toutes les étapes de la rédaction.

Abstract

Green roofs are unique ecosystems combining two major community assembly filters, namely stress and spatial isolation. As such, they represent an interesting model ecosystem in community ecology. As we increasingly recognize the pivotal role of the green roof soil microbiome in driving urban ecosystem services, the logical next step is to better understand how this microbiome assembles. Only through such understanding will we be able to engineer it and predictively influence downstream ecosystem services. In this study, we characterized the microbiome structure on 19 green roofs and 5 urban parks as a benchmark comparison (i.e., non-isolated, non-stressful habitats). Green roofs were not species depauperate, showing similar α-diversity compared to surrounding parks. This was true even for roofs that had been installed recently. We also did not find an overrepresentation of microbial phyla typically recognized as oligotrophs, which overall calls into question the notion of green roofs as highly stressful habitats. The geographical position of a roof, or its degree of spatial isolation (assessed through its height and area) were not important predictors of microbiome structure, suggesting that dispersal limitations impose little constraints on green roof microbiome assembly. Finally, community structure differed between roofs and parks, with key microbial groups (e.g., archaeal nitrifiers, Actinobacteria) being much less frequent and/or abundant on green roofs, which may have important implications for nutrient cycling and urban biogeochemistry. More work will be required to phenotype the microorganisms overrepresented on green roofs and specifically measure key soil processes in these unique urban ecosystems.

Keywords: Microbiome, community assembly, urban ecology, stress, life history strategies, trait-based ecology, microbial ecology, bacteria, fungi

Introduction

A key goal of microbial ecology is to determine assembly rules for microbial communities and how this may translate into altered ecosystem functioning. Soil microorganisms drive important ecosystem functions such as primary production (van der Heijden et al., 1998, 2008; Wagg et al., 2011), litter decomposition (Setälä & McLean, 2004), pedogenesis (Jongmans et al., 1997) and nutrient cycling (Bardgett & van der Putten, 2014). Soil microorganisms often respond differently to environmental gradients compared to larger organisms (e.g. Bryant et al., 2008) and may be more resistant and/or resilient to various stressing agents (Battistuzzi & Heedges, 2009; Manzoni et al., 2012; Griffith & Philippot, 2013; Kaisermann et al., 2015; Barnard et al., 2013). They also seem to face less dispersal limitations (e.g., Finlay et al. 2001; Finlay, 2002). Such ability for long-distance microbial dispersal typically translates into low endemism (Moyersoen et al., 2003; Davison et al., 2015, 2018), which affect the importance of environment conditions on community assembly and how communities are interconnected. Although others have found quite a different picture (Telford et al., 2006; Peay et al., 2010; Adams et al., 2013; Talbot et al., 2014). There remains a large uncertainty around the distance over which microbial cells/propagules can travel. With urbanization changing drastically the landscape (e.g. Cadenasso et al., 2007) and because urban ecosystems often develop as both fragmented and stressful (e.g., trace contaminants, deicing salts) green patches, it is critical that we better understand how stress and spatial isolation jointly shape microbial communities.

Green roofs are particularly well suited to study both the effect of isolation and stress on microbial community assembly. Their small size and physical isolation should constrain species richness and α -diversity (MacArthur & Wilson, 1967) and this should be further amplified by the harsh abiotic conditions they impose (Grime, 1973). Extensive green roofs (substrate depth less than 15cm) are

usually expected to be even more stressful, due to their very high propensity to drought. Yet, these small, isolated and stressful islands can host a surprisingly high microbial diversity (e.g., McGuire et al., 2013; Molineux et al., 2015). However, there is evidence that soil green roof microbial community structure differs from surrounding habitats, like urban parks and post-industrial sites (McGuire et al., 2013; Molineux et al., 2015). What remains to be disentangled is whether this is due to an overrepresentation of stress tolerating microorganisms, likely to cope with frequent droughts on roofs (e.g., Manzoni et la., 2012), or of ruderal microorganisms producing massive amounts of efficiently dispersing propagules (Grime, 1977), more likely to land on small, isolated islands through source-sink dynamics (Peay et al., 2007). This has implications for green roof ecosystem functioning because if green roof microbiomes are dominated by ruderals that are maladapted to the stressful environment they land on, even high microbial α-diversity is unlikely to translate into improved ecosystem services on green roofs. A major research goal should thus be to not only compare microbial community composition between green roofs and other natural/semi-natural surrounding habitats, but to specifically identify the taxa that are over/under-represented in green roofs and what drive their presence/absence. Attention should also be paid to spatio-temporal dynamics of green roof microbial community assembly, to evaluate how rapidly can green roof substrates gain microbial diversity, as artificial growing substrates used for green roof construction are expected to display low microbial activity and diversity (John et al., 2014).

Here, we sampled 19 green roof ecosystems in the city of Montreal (Québec, Canada). These sites were selected to encompass the broadest gradient of green roof types as possible, with sampling sites varying in substrate depth, fertilizer application, presence of an irrigation system, vegetation diversity and age, to name a few. We also sampled 5 public parks as urban green spaces to serve as benchmark comparisons representing semi-natural, mesic (less stressful) sites. Microbial

communities (prokaryotes and fungi) were characterized using next-generation sequencing (Illumina MiSeq) to look at α- and β-diversity patterns to elucidate the main drivers of green roof microbiome assembly. Specifically, we hypothesized that:

1- Young, small and/or spatially isolated roofs would display lower microbial α-diversity;
2- Given their lower propensity to drought, roofs with deeper substrates (i.e., "intensive" green roofs with >15cm substrate thickness) would be intermediate between xeric extensive roofs (<15cm substrate thickness) and mesic city parks, both in terms of α-diversity- and community structure;
3- Because of the high dispersal efficiency of microorganisms, even in urban landscapes (e.g., Chaudhary et al., 2020), microbiomes community structure should be strongly correlated with soil abiotic properties. Accordingly, spatial autocorrelation at the landscape level should be a poor predictor of community structure;
4- As stressful, dry ecosystems, green roofs should be enriched in clades of slow-growing, oligotrophic microorganisms and host few fungi with a motile zoosporic life stage (e.g., chytrids).

Materials and Methods

Study sites

Samplings were conducted on 19 green roofs ecosystems: 6 intensive roofs (substrate thickness >15cm) and 13 extensive roofs (<15cm). We also included 5 large city parks as benchmark habitats exposed to a similar regional microbial species pool, but without the distinctive characteristics of

green roofs (i.e., substrate nature, propensity to drought, etc.). These sites were distributed across the island of Montreal (45.4-45.6 N and 73.5-73.8 W) (fig S2.1) and were sampled at the end of June 2019. Mean annual precipitation in this region is approximately 1000 mm, with an average temperature of 16.8C in June (Environment Canada, 2020). Green roofs were selected to encompass the broadest range of roof characteristics possible (e.g., height [2.5-33m], age [2-20 years], substrate thickness [5-30 cm], etc). Green roofs age ranged between 2 and 20 years.

Soil sampling and chemical analyses

For each roof, we measured substrate thickness and visually estimated the light exposure of the roof (a value of 100% meaning that all of the roof surface is free of shade from surrounding buildings at any time of the day). We then randomly established five $1m^2$ quadrats (10 for city parks, to take into account their greater size and better ensure a representative characterization of soil properties and vegetation structure). For each quadrat, we estimated the percent cover for each plant species present and took 5 randomly placed soil cores (3cm diameter, ~15 cm deep). All soil cores per site (25 for green roofs and 50 for parks) were combined into one composite sample per site. These composite samples were placed in sterile plastic bags, sealed and kept on ice upon arrival to the laboratory, within less than 4 hours.

In the laboratory, soils were sieved (ø 2mm) and mixed thoroughly. Subsamples were air-dried and used to measure soil abiotic properties. A 2mL subsample was kept at -20°C for downstream molecular analyses (see below). Soil pH was measured by soaking 10g of soil into 20mL of 0.01M $CaCl_2$ (Gregorich and Carter, 2007). Available NO_3^- and NH_4^+ were extracted from 2g of soil using

20mL of 2M KCl and quantified colorimetrically using Berthelot reaction (NO_3^-) and a persulfate oxidation followed by an acidic Griess reaction (NH_4^+) (Hood-Nowotny et al., 2010). Mehlich-III extractible P was quantified using 3g of soil and 30mL of Mehlich-III solution (Gregorich & Carter, 2007). Inorganic PO_4^{3-} concentration was determined colorimetrically using the Murphy-Riley method (Murphy & Riley, 1962). Soil gravimetric moisture was measured by weighing fresh soil and drying at 105℃ to calculate water content per g dry soil. Soil organic matter content was measured using loss on ignition in a muffle furnace (Hoogstenn et al., 2015).

Molecular methods and bioinformatics

DNA was extracted from 0.5g of soil sample using Qiagen PowerSoil Kit following manufacturer instructions. Extraction yields were estimated using a nanodrop spectrophotometer. Extracted DNA was then PCR amplified. For prokaryotes, the V4 region of the 16S rRNA gene was amplified using the primers 515F (5'- GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011). For fungi, the ITS rRNA gene region (more specifically the ITS1 subregion) was amplified using the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (Gardes & Burnes, 1993; Bellemain et al., 2010). The PCR conditions used for the 16S rRNA gene were a 2 min 94℃ hot start, 33 cycles of 30 s at 94℃, 30 s at 58℃ and 30 s at 72, followed by a final 7 min elongation at 72℃. The PCR conditions used for the ITS rRNA gene were 15 min at 96℃, 33 cycles of 30 s at 96℃, 30 s at 52℃ and 30 s at 72C℃ followed by 10 min elongation at 72℃. Amplicons were sequenced using the Illumina Miseq technology at Genome Québec facilities (Montréal, Canada).

Raw sequences were analysed using the dada2 pipeline (Callahan et al., 2016) to identify amplicon sequence variants (ASVs). Reads were first quality-filtered (q threshold = 2) and trimmed at 250bp length prior to paired-ends merging. All other pipeline parameters were kept at their default values. Chimeras were removed using the "pooled method" (Callahan et al., 2016). Taxonomy was assigned using the curated reference databases Silva for prokaryotes (Quast et al., 2013) and UNITE for fungi (Abarenkov et al., 2010). To avoid focusing overly on the large quantity of very rare microbial taxa that could have arose form biases during amplification and bioinformatic pipeline, ASVs with less than 10 reads in the dataset were kept out of downstream analyses. An ASV was considered absent from a site if it had less than 3 reads in this sample. Each sample was then rarefied at 19533 reads for prokaryotes and 9786 reads for fungi, using the function *rrarefy* of the R package *vegan* (Oksanen et al. 2020).

Statistical analysis

1) α-diversity in green roofs

We compared microbial diversity of green roofs (extensive or intensive) and city parks using ASV richness as a surrogate, conducting separate analyses for prokaryotes and fungi. We used ANOVA to compare prokaryotic and fungal richness among habitats, after verifying assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett tests, respectively. For green roofs specifically, we identified drivers of ASV richness using Poisson regression, selecting predictors (i.e., soil chemistry, roof properties and *Sedum* percent cover, see table 1) using AIC-based backward selection as implemented in the R package *MASS* (Ripley et al., 2013). Following the island biogeography paradigm (MacArthur & Wilson 1967), we hypothesized that young, small

and isolated roofs (height was used here as a proxy for isolation) would display lower ASV richness. We controlled for spatial autocorrelation by analyzing principal coordinates of neighbour matrices (PCNMs) (Borcard and Legendre, 2002) and including PCNMs associated with positive eigenvalues as predictor variables in the Poisson regression models.

We also specifically looked at ASV accumulation over time in our set of green roofs, which ranged between 2 and 20 years old. To do so, we partitioned β-diversity between roofs in its turnover *vs* nestedness components using ASV presence-absence data (Podani and Schmera 2011). This approach disentangles variation in microbial community structure that is due to different roofs having (1) distinct ASVs (i.e., turnover) or (2) a different number of ASVs (i.e., nestedness). We were interested into the nestedness component more specifically, hypothesizing that microbial ASVs could accumulate over time such that old roofs would harbour more ASVs and the ASVs in young roofs would be a nested subset of ASVs in older roofs. To test this hypothesis, we correlated (using Spearman's ρ) the nestedness component of β-diversity between pairs of roofs to the age difference between these roofs (expecting a positive correlation).

2) Shifts in microbial community structure among habitat types

We compared microbial community structure between extensive green roofs, intensive green roofs and city parks using model-based analysis of multivariate abundance data (Warton et al. 2012). This approach has higher power than traditional distance-based approaches such as perMANOVA (Warton et al., 2012). We used the R package *mvabund* (Wang et al. 2012) to regress microbial community structure against habitat type (extensive roofs, intensive roofs or city parks), with a post-hoc test determining whether intensive roofs, with their deeper substrates and lower propensity to drought, would be intermediate between extensive roofs and city parks. To visualize

results, we conducted a principal coordinate analysis (PCoA) using the Bray-Curtis distance as a site pairwise dissimilarity metric, using the R package *vegan* (Oksanen et al., 2020).

3) Drivers of microbial community structure on green roofs

To identify the drivers of microbiome structure on green roofs, we conducted redundancy analyses (RDAs) regressing microbial abundance against roof characteristics. The latter included soil chemical properties, as well as roof properties such as height, surface area, age, substrate depth and state-variables like the presence of an irrigation system or the use of fertilizers. To include vegetation as a predictor in RDAs, we conducted a principal component analysis (PCA) on Hellinger-transformed plant community data and used the site scores along the axes. We included spatial distribution of our sites as predictors of microbial community structure using the PCNMs generated as describe above. We used automated backward model selection (α=0.05) to identify the best predictors of microbial community structure.

4) Distribution of specific microbial groups among green roofs and parks

We identified ASVs preferentially found in specific habitats (our categories being extensive roofs, intensive roofs and city parks) using indicator species analysis. We did so using the R package *indicspecies* (De Cáceres et al., 2020). We then merged indicator ASVs by phylum to determine whether some phyla were preferentially found in one of the three habitats, using a χ^2- test.

Results

Microbial α-diversity

We did not find green roofs to be species depauperate (fig 2.1). Alpha diversity (ASV richness) ranged between 1794 and 3585 for prokaryotes and between 120 and 433 for fungi, with no significant difference between parks and green roofs ($F_{2,21} = 0.15$, $P = 0.86$) ($F_{2,21} = 0.033$, $P = 0.97$) for prokaryotes and fungi respectively. Microbial richness was rather driven by roof properties affecting the likelihood of microbial colonization (i.e., height and area), or by soil chemical properties (Table 2.1). More specifically, prokaryotic and fungal richness were especially responsive to nitrogen availability, organic matter content and pH. The latter influenced prokaryotes and fungi in opposite direction, with acid substrates promoting higher fungal richness and lower prokaryotic richness. The roof age came out as the strongest predictor of prokaryotic richness, with more ASVs in older roofs. On the other hand, fungal richness did not change according to roof age. This result was confirmed by β-diversity partitioning analyses. Indeed, the nestedness component of β-diversity increased as pairs of sites compared differed in age (fig S2.2), showing that as roofs age, they accumulate prokaryotic ASVs. No PCNM was retained as a predictor of microbial α-diversity, meaning that microbiomes were not spatially autocorrelated at the geographical scale investigated.

Table 2.1. Poisson regression linking microbial richness with roof properties. See methods for description of predictors' measurements. % Org mat = percent organic matter of the substrate. Watering = presence, or not, of an irrigation system. % Sedum = estimated percent ground cover by Sedum spp. Values presented are model coefficients per predictor and their associated P-values. Blank (-) spaces indicates that the predictor was not retained through backward, AIC-based model selection.

	FUNGI		**PROKARYOTES**	
	Coef.	***P*-val**	**Coef.**	***P*-val**
Height	-8.43	<0.001	9.54	<0.001
Area	-6.54	<0.001	8.72	<0.001
Thickness	4.27	<0.001	-11.59	<0.001
Age	-	-	26.35	<0.001
Light	2.89	<0.01	-	-
NH_4^+	3.67	<0.001	17.1	<0.001
NO_3^-	4.89	<0. 001	9.56	<0.001
PO_4^{3-}		n.s		n.s
pH	-9.31	<0.001	10.88	<0.001
% Org mat	-6.13	<0.001	14.07	<0.001
Watering	3.64	<0.001	-	-
% *Sedum*	7.85	<0.001	-13.6	<0.001

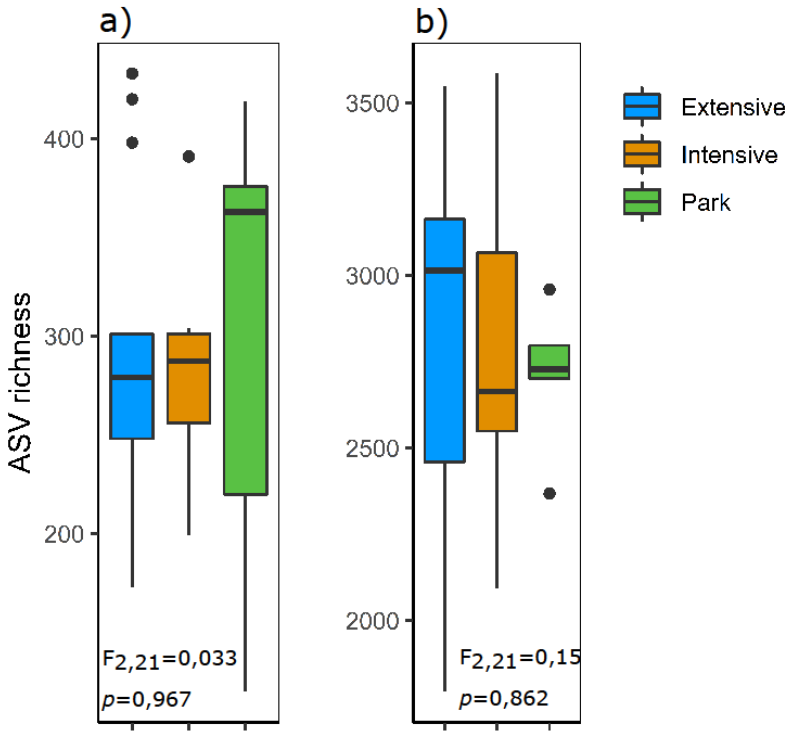

Figure 2.1. ASV richness per site for a) fungi and b) prokaryotes. *F* and *P* values come from one-way ANOVAs.

Microbial β-diversity

Although ASV richness did not vary between parks and extensive green roofs, the microbial community structure did (P = 0.002 and P = 0.007 for bacteria and fungi respectively) (fig 2.2). Intensive roofs were less distinct from urban parks (P = 0.013 and 0.055 for bacteria and fungi, respectively). This suggests that substrate depth can at least partly explain shifts in microbiome in green roofs vs parks, with intensive roofs with thick substrates being intermediates between thin roofs and parks. The only outlier to this trend was the Brébeuf intensive roof, the only roof with mature trees on it, which clustered near parks in the ordination. The tendency of fungal and bacterial communities between intensive roofs and parks was slightly different. Indeed, fungal

communities were less distinct between these habitats which suggest that the impact of depth was more important on them than on bacterial communities. When comparing green roofs among themselves, we note that the split between extensive and intensive roofs is not as clearly defined as the one between roofs and parks (fig 2.2). These analyses suggest that green roofs have distinct microbiomes from parks and therefore high level of turnover and that the type of roof investigated (intensive vs extensive) has relatively little influence on microbial community structure.

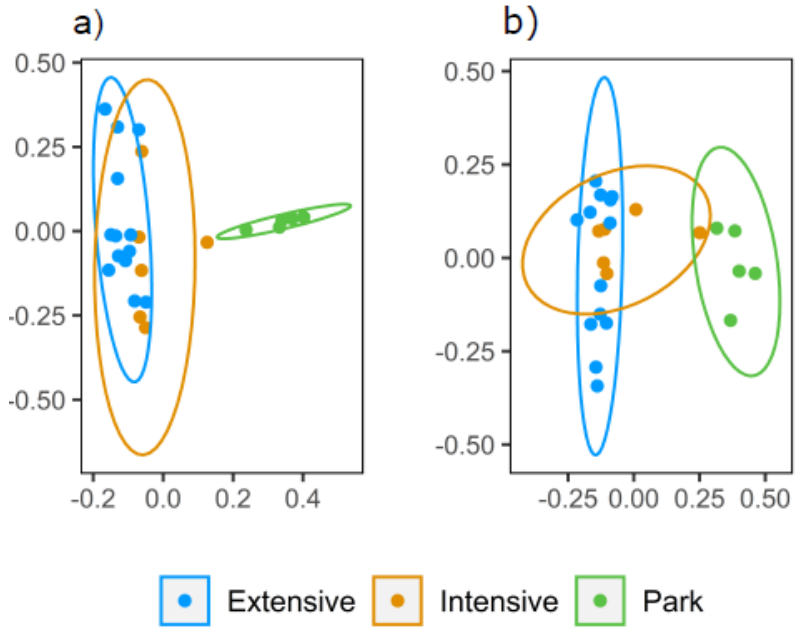

Figure 2.2. Principal coordinates analysis (PCoA) of a) fungal communities and b) prokaryotic communities. Bray-Curtis dissimilarity (computed on Hellinger-transformed microbial abundance data) was used as a distance metric for pairwise site comparisons.

Indicator species analysis revealed a large number of indicator ASVs for each habitat type (fig 2.3a,b). In line with the results presented above (fig 2.2), parks shared much more indicator taxa with intensive roofs than with extensive ones (fig 2.3c,d). Intensive roofs had a higher number of indicator taxa belonging to the Chytridiomycota and Kickxellomycota (fig 2.3a). Regarding prokaryotes, the majority of green roof indicator taxa belonged to Proteobacteria,

Armatimonadetes, Plantomycetes and Chloroflexi, while the majority of park indicator taxa were from the Thaumarchaeota, Verrucomicrobia, Actinobacteria and Rokubacteria (fig 2.3c). In line with the results presented above, there was no obvious distinctions between intensive and extensive roofs, certainly not as clear as the one between roofs and parks (fig 2.3b,d).

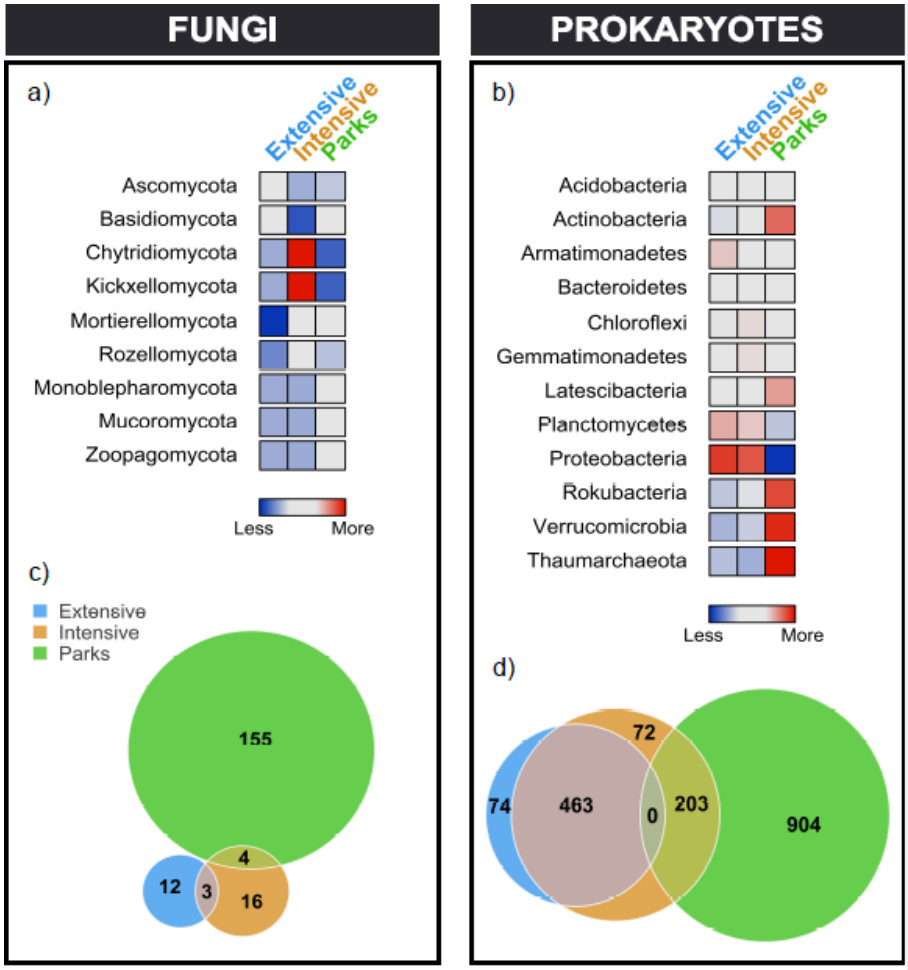

Figure 2.3. Non-random distribution of ASVs and phyla across habitat types. In a) and c) (fungi and prokaryotes, respectively), Pearson residuals from a χ^2-analysis are plotted to show whether each microbial phylum had more (red) or less (blue) indicator ASV in any given habitat type than by chance. Venn's diagrams in b) (fungi) and d) (prokaryotes) shows the number of indicator ASVs shared by the different habitat types.

Drivers of microbial β-diversity

Canonical ordinations revealed distinct drivers of prokaryotic vs fungal β-diversity patterns among our set of green roofs (fig 2.4). The only common driver between these microbial groups was the presence of an irrigation system, suggesting that drought stress is an important factor shaping the microbiome on green roofs. However, prokaryotes were more responsive to pH and roof age, while fungi covaried more strongly with roof fertilization and exposure to sunlight (fig 2.4). Also, the impact of vegetation on the microbiome was distinct when comparing prokaryotes and fungi, with the two groups covarying with different ordination axes of the vegetation principal component analysis.

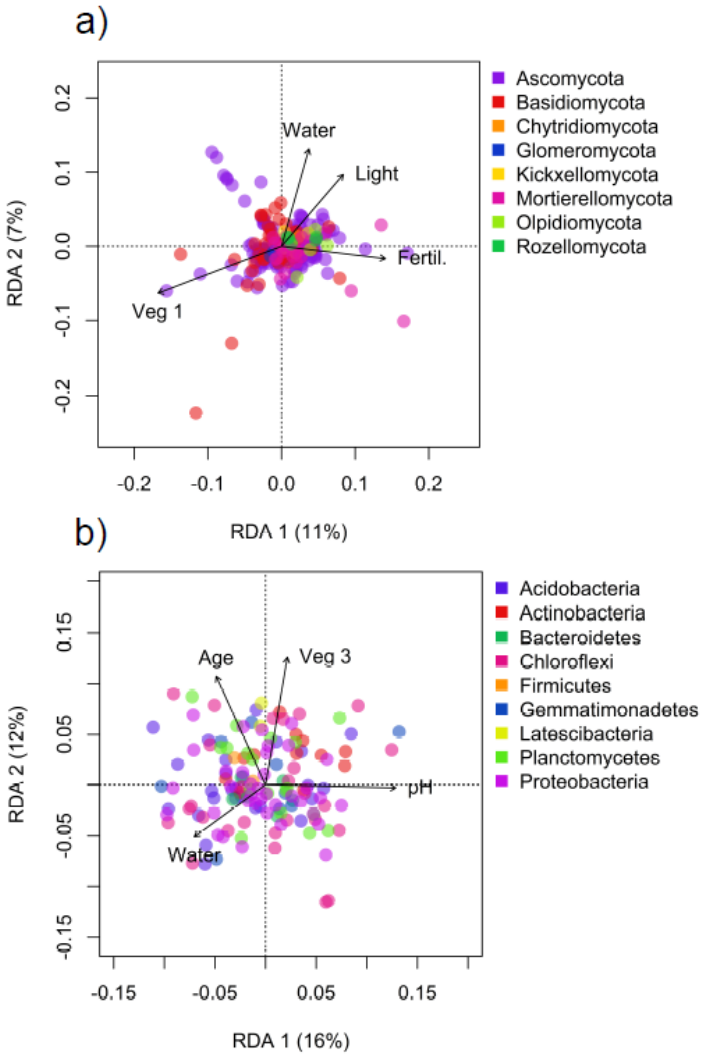

Figure 2.4. Redundancy analysis (RDA) of a) fungal and b) prokaryotic community structure, in relation to roof characteristics. Vectors show the predictors kept through backward model selection. Each symbol represents a microbial ASV.Fertil= Presence of any form of fertilisation, veg 1 and veg 3 represent the first and third axis of PCA on Hellinger-transformed plant community data.

We identified phylum-dependent responses to environmental filters among prokaryotes and fungi. For example, Actinobacteria and Thaumarchaeota were the only prokaryotic phyla responsive to fertilizer applications and Olpidiomycota (typically pathogens) were the only fungal phylum responding only to vegetation structure and not roof abiotic properties (fig S2.3).

Discussion

Green roof microbiomes are diverse

In line with other investigations (McGuire et al. 2013; Molineux et al. 2015) green roofs were not species depauperate (fig 2.1). This goes against classic paradigms of community assembly, predicting that spatial isolation (MacArthur & Wilson 1967) and stress (Grime 1973) should limit species richness. In island biogeography, nested species distributions are typical, with large and accessible islands being the most speciose (Matthews et al., 2015). In this study, we found no evidence for strong dispersal limitation. The effect of roof height or area on microbial α-diversity was not consistent among prokaryotes and fungi and sometimes counterintuitive (e.g., more fungal ASVs on small roofs, or prokaryotic ASVs on the top of higher buildings) (Table 2.1).These results contrast with the idea of green roofs being physically isolated islands in a fragmented urban landscape but is in line with Li et al. (2020), who found that island isolation was a poor predictor of microbial diversity in an archipelago. The small size of microbial propagules could explain why dispersal limitation does not limit site colonization (e.g., Diamond 1969), yet unlike previously assumed (Baas Becking 1934, Finlay 2002), many recent studies have shown that microorganisms do face dispersal limitation driving their ability to colonize suitable habitats (e.g., Peay et al 2007, 2010, Kivlin et al., 2011; Hanson et al., 2012; Adams et al. 2013; Cline & Zak, 2014). We did find lower bacterial richness in young roofs, which could indicate some temporal dispersal constraint over site colonization. However, this constraint appears to vanish as roofs age (fig S2.2) and could not be osberved for fungi. Overall, green roof microbiomes may be more connected to the surrounding habitat matrix than previously assumed. Future research will be need to assess microbial community composition right at the time of roof installation, as substrates and particularly plants are certainly not completely sterile at this stage (Rumble et al., 2018, but see

John et al., 2014). This could induce historical effects on community assembly that cannot be anticipated from island biogeography alone (Fukami, 2015).

Green roofs may also be less stressful to microorganisms than previously thought. Our definition of green roofs as being stressful habitats is largely a phytocentric one, since only drought tolerant plants tend to survive on green roofs (Vanuytrecht et al. 2014). When looking more carefully at the soil chemical parameters measured in this study, we do not find evidence for unusually stressful conditions: pH near neutrality, Mehlich-III phosphorus and KCl-extractible mineral nitrogen low but still comparable to values that can be observed in natural landscapes such as grasslands (e.g., Chagnon et al. 2018) and gravimetric moisture ranging between 12 and 72% of soil dry weight. So even if green roofs can be prone to drought relatively soon after rain events (Berreta et al., 2014; Stovin et al., 2013), here we argue that the soil conditions faced by the green roof microbiome is not orders of magnitude more stressful than what would be faced in natural soils. Drought stress on green roofs could be more of a pulse disturbance (Baryla et al., 2019) to which microbes are more resilient than plants (Barnard et al. 2013, but see Maestre et al., 2015). In this view, temporal dynamics in water availability (as opposed to snapshot measurements of gravimetric moisture as taken here) is clearly an overlooked environmental parameter of interest for green roofs, that deserves further attention in future studies.

What drives green roof microbiome?

Our hypothesis that green roof microbiomes would be assembled mostly through species sorting received mixed support. While microbial communities were responsive to substrate pH and N availability, which is common in natural soils (Fierer et al., 2009; Lauber et al. 2009; Delgado-Baquerizo et al., 2016), our constrained ordinations left more than 70% of the variation

unexplained. This was particularly striking for fungi, with 82% of unexplained variation, which is in line with recent studies showing that fungal community assembly was more stochastic than bacterial community assembly (Powell et al., 2015; Wang et al., 2020). A stronger dispersal limitation for fungi (Peay et al., 2012; Adams et al., 2013) could drive a poor correlation between their community structure and environmental filters but does not reconcile well with the fact that even very young roofs showed high α-diversity. Alternatively, unexplained variation in RDAs could arise through hidden niches, i.e., unmeasured yet ecologically relevant environmental filters for green roof microbiomes. Our sampling design included some roof-specific variables (e.g., age, substrate thickness, etc.), but many other particularities of these "novel" ecosystems (sensu Hobbs 2006) might play important roles in urban microbial community assembly. We mentioned above temporal dynamics of water availability, but this could include other aspects of green roofs such as microclimatic variables (reviewed in Lundholm & Heim, 2020) related to heat islands, UV exposure or atmospheric concentrations of trace contaminants. Alternatively, priority effects could contribute to truly make microbial community assembly more stochastic in green roofs. Since initial green roof substrates tend to have low numbers of microbial propagules (e.g., John et al. 2014), chance colonization by opportunistic microbes during early roof development may contribute to community divergence over time, in a way that is unrelated to deterministic environmental filters. Such priority effects in microbial community assembly has received support in a wide range of study systems (Fukami et al., 2010; Mummey, Antunes & Rillig, 2009; Chase, 2007, Dickie et al., 2012). This would be in line with the fact that microbial community β-diversity was higher among roofs than among parks, with the latter showing more convergent microbiomes (fig 2.2).

Who are the green roof specialists?

Although stress was not to a level where it constrained microbial richness (fig 2.1), we did observe contrasts in the soil microbiome of green roofs vs city parks (fig 2.2). Interestingly, there was not as much of a contrast when comparing extensive roofs to intensive ones, suggesting that this distinction could be rather artificial when it turns to how it may drive the microbiome. This point is further supported by the lack of clear differences in intensive vs extensive roofs regarding vegetation structure and soil chemical properties (fig S2.4). Moreover, intensive roof microbiomes were indeed intermediate between extensive roofs and parks, but much closer to the former than the latter (fig 2.2). This trend is further supported by our indicator species analysis. While extensive roofs share no (or very few) bacterial and fungal indicator species with parks, intensive roofs share a high proportion of indicator species both with parks and extensive roofs (fig 2.3). Moreover, the very high number of indicator microbial taxa found in roofs but not in parks (fig S2.5) highlight the need to identify alternative sources of microbial propagules in urban ecosystems (e.g., private gardens, medians strips). This stresses the idea that human-modified urban landscape may have unique ecological value by contributing to the regional species pool differently as compared with more pristine ecosystems (Reese et al. 2016). Alternatively, suburban natural ecosystems may contribute to city microbiomes more than previously expected and here may have provided microbial colonizers for green roofs.

When conceiving this study, we anticipated that as stressful and isolated habitats, green roofs would be enriched in oligotrophic, slow-growing microorganisms known to have a resource conservative strategy. Many have tried to define ecological strategies that would be relatively conserved at the phylum level for prokaryotes (e.g., Fierer et al., 2007; Philippot et al., 2010; Evans et al., 2014;

Nemergut et al., 2010; Fierer et al., 2012; Ramirez et al., 2012). For example, Acidobacteria tend to be considered an oligotrophic phylum, with slow growth (Davis et al., 2011) and the ability to use a variety of carbon compounds (Ward et al., 2009). In resource-poor environments, oligotrophs outcompete fast growing taxa (copiotrophs) that rely on high concentrations of simple, energy-rich carbon substrates. Yet, the distribution of our indicator prokaryotes did not support current literature. With the exception of Actinobacteria (assumed copiotrophs, e.g. Fierer et al., 2012; Ramirez et al., 2012) and Gemmatimonadetes (assumed oligotrophs, e.g. Hanada & Sekiguchi, 2014), the distribution of all major phyla was opposite to our expectations, with assumed copiotrophs being more prevalent in green roofs and vice versa for oligotrophs (fig 2.3). This may highlight the overly simple nature of the oligotrophic-copiotrophic continuum (Ho et al., 2017), which is largely based on population-level dynamics under a single growth-limiting resource, as an extension of the *r-K* selection theory (Pianka et al. 1970). Just like for plants, the ability of microorganisms to acquire and conserve resource and to cope with abiotic stresses, may be highly multidimensional (Grime, 1977; Wright et al., 2004; Reich, 2014; Weemstra et al., 2016; Laliberté, 2017) and the challenge of microbial ecophysiology is now to pinpoint the microbial traits that specifically mediate such multidimensional trade-offs (Chagnon et al., 2013; Krause et al. 2014; Zanne et al. 2020). This might further highlight the complexity of stress tolerance as a microbial trait. For example, Acidobacteria tend to resist low pH but are susceptible to drought (Barnard et al. 2013), indicating that there is no single trait syndrome for "stress tolerance" (Chapin, 1991; Pierce et al., 2005). Our results also do not support a phylum-level conservatism of microbial life-history strategies, as would be reflected by their distributions across habitat types. The vast majority of phyla harboured at the same time many green roof specialists and many park specialists (fig S2.5).

We also expected to see few fungi with a motile zoosporic life stage, as these require water to move in the soil. This was again a surprise, then, to find more chytrids in roofs than in the mesic soils of parks (fig 2.3). Chytrids, however, were associated with intensive roofs, which may impose less of a drought filter on the fungal community.

The use of short marker sequencing could have affected our results. Indeed, this sequencing method can detect living, dead and dormant cells and therefore could reduce our statistical power and could be another explanation of the high level of unexplained variance observed in this study. However, as shown by Chen et al., (2019), the use of PFLA method that make the distinction between living and dead cells give results in line with the ones obtained with DNA sequencing. To refine the sequencing and amelioration taxonomic assignation, further research would benefit to use more recent technologies, like real-time sequencing (SMRT). This sequencing method can use longer sequences. It has been progressively more used for 16S and ITS rRNA gene to study fungal, bacterial and mycorrhizal communities because of its increased specificity and resolution (e.g. Schlaeppi et al., 2016; Schloss et al., 2016). Moreover, fungal marker used for fungal sequencing have some bias, for example against Micosporidia and Tulasnellaceae (Tedersoo et Lindhal, 2016). Therefore, using another marker could have led to slightly different results.

One of the upcoming challenges in green roof microbial ecology will be to uncover the functional consequences of their unique microbiomes. For example, our roofs were largely depauperate of archaeal nitrifiers from the Thaumarchaeota. Does it translate into reduced nitrification rates (Pester et al., 2011) and altered relative importance of NH_4-N and NO_3-N in green roof substrates? Green roof microbiomes were also enriched in Proteobacteria, which also tend to dominate C-rich rhizospheres (Philippot et al., 2013). Is it because overall, plant root-derived C is more available in

green roofs, where stressed plants tend to produce more exudates, crying for microbial help (e.g., Rolfe et al. 2019; de Vries 2019; Williams et de Vries, 2020)? Green roofs may thus select for stress-tolerant plants that, in turn, promote microorganisms that capitalize on massive inputs of labile C sources (i.e., copiotrophs). How do these rhizosphere colonists (Paterson et al., 2007) influence, in turn, plant performance and ecosystem delivery? Finally, Actinobacteria, known to be involved in lignocellulose degradation (Zak et al. 2011), were much less abundant in roofs. This could impact organic matter residence time in the substrate. Further work exploring litter breakdown in green roofs is needed in this regard. Overall, despite similar richness in roofs vs parks, it remains unclear whether the rates of biogeochemical cycles in green roofs should be similar to those seen in more pristine ecosystems. This urgently requires further investigation, as it will determine the rate at which mineral nutrients are made available to plants, as well as the propensity of the mineral matrix of green roof substrates to stabilize organic C over long timescales (Cotrufo et al., 2013). In this regard, much research is needed to phenotype microbial strains isolated from green roofs, in order to characterize their drought tolerance and avoidance strategies (Kaisermann et al., 2015; Barnard et al., 2013; de Vries et al., 2018), their C use efficiency (Manzoni et al., 2012) and their ability to secrete various enzymes involved in nutrient cycling and mobilization (Janusz et al., 2017).

Conclusion

Contrary to expectations, green roofs harbour diverse and in many ways unique, microbial assemblages. This calls into question the notion of spatial isolation and high stress levels that up to now have been so central to the definition of green roofs (which has tended to be phytocentric) (John et al. 2017). We argue that from a microbial perspective, green roofs may not be unusually

isolated or stressful. We point to the need to incorporate trait-based ecology in the green roof microbiome research. Phenotyping microbial strains from green roofs will be pivotal to better understand how they may respond to environmental filters, how they may influence nutrient cycling and C storage in green roof substrates and how they may help plants coping with the chronic drought stress characteristic of these urban ecosystems.

Acknowledgements

The authors acknowledge the support of the Canada National Science and Engineering Research Council (NSERC) for a Discovery Grant to PLC and a Master's degree fellowship to AH. We also acknowledge the financial support of the Fonds de Recherche du Québec sur la Nature et les Technologies (FRQNT) through a fellowship to AH and a seed grant provided by the Québec Center for Biodiversity Science (QCBS).

Chapitre 3

Green roof islands are dominated by cosmopolitan, generalist arbuscular mycorrhizal fungi

***L'article seras soumis très bientôt à New phytologist**

Hénault, A.[1], Brisson, J.[1], Dagenais[2], D., De Bellis[3], T., Lajoie[1], G., Chagnon, P.L.[1*]

1. Université de Montréal, Faculté des Arts et Sciences, Département des Sciences Biologiques, Montréal (QC) Canada
2. Université de Montréal, Faculté d'Aménagement, Montréal (QC) Canada
3. Dawson College, Montréal (QC) Canada

Avant-propos

Antoine Hénault est l'auteur principal de l'article en plus d'avoir procédé à toutes les analyses statistiques. Geneviève Lajoie a produit la phylogénie utilisée pour certaines des analyses statistiques. Pierre-Luc Chagnon, Jacques Brisson, Danielle Dagenais et Tonia De Bellis ont participés à la révision de l'article, mais avec un apport plus important de Pierre-Luc Chagnon qui a fait un suivi à toutes les étapes de la rédaction.

Abstract

The relative contribution of environmental filtering and dispersal to the biogeography of arbuscular mycorrhizal (AM) fungi remains elusive. Urban green roofs, as spatially isolated patches presenting a variety of environmental conditions (vegetation and substrate types) are ideal habitats to tackle this question. We characterized AM fungal communities from 19 green roofs and 5 urban parks (as putative AM fungal propagule sources) across an urban island. We evaluated the taxonomic and phylogenetic structure of local AM fungal communities and correlated these with environmental properties and space. There was no evidence of strong dispersal limitation and spatial autocorrelation in AM fungal communities across the island. Most of the variation in AM fungal community beta-diversity was left unexplained by environmental filters (i.e., vegetation structure, soil physico-chemistry and roof characteristics). However, we found stronger phylogenetic dispersion in local AM fungal communities than predicted by a regional null model, which could suggest that phylogenetic affiliation of resident and colonists AM fungi constrains community invasion. Interestingly, the AM fungi dominating local communities tended to be frequent in the regional pool of taxa. These generalist, locally dominant taxa were found in other studies to be cosmopolitan. We argue that green roof AM fungal communities were assembled following two successive, hierarchical filters. First, sites may be colonized by ruderal, cosmopolitan AM fungi producing massive amounts of well-dispersed propagules, which dominate the regional species pool. Second, further invasion by later colonists is constrained by limiting similarity to resident, already established AM fungi.

Keywords: Microbiome, community assembly, urban ecology, stress, green roof, arbuscular mycorrhiza fungi, phylogenetic community structure, microbial ecology, competition

Introduction

Understanding the drivers of community assembly has held a central place in the ecology research agenda for decades (Gause, 1934; MacArthur & Wilson, 1967; Tilman, 1982; Chesson, 2000; Chase & Myers, 2011; Guzman et al., 2019). However, the focus has traditionally been placed on pristine ecosystems or agroecosystems (Yan et al., 2016; De Kimpe and Morel 2000), with comparatively little attention paid to urban communities. This is now changing rapidly (e.g. Yan et al., 2016; Hui et al., 2017; Guilland et al., 2018; Laforest-Lapointe et al., 2017; Xu et al., 2013; Bainard, Klironomos et Gordon, 2011), with the growing recognition that cities are transforming the landscape in a unique way (Cadenasso et al., 2007). These novel ecosystems (Hobbs et al., 2006) present species with a unique combination of environmental filters with little or no parallel in nature. Green roofs are a good example of such novel ecosystems, with a joint selection pressure for both high colonization/dispersal and stress tolerance, due to their spatial isolation and their shallow, drought-prone substrate (Oberndorfer et al., 2007). New sets of environmental filters may imply selection for unique life-history strategies (Gill et al., 2020), new species associations (Lin et al., 2021) and cascading impacts on ecosystem functioning (McGuire et al., 2015; Guilland et al., 2018; Gill et al., 2020).

Given the growing recognition of the soil microbiome's contribution to ecosystem functioning (van der Heijden et al., 2008; Wagg et al., 2011; Clemmensen et al., 2013), there is a rising interest in characterizing the microbiomes of green infrastructures in cities (McGuire et al., 2013; Molineux et al., 2015; Deeb et al., 2018; Rumble, Finch et Gange, 2018; Hoch et al., 2019; Joyner et al.,

2019; Gill et al., 2020). However, we know little about the mechanisms governing the assembly of these communities. This is especially true for arbuscular mycorrhizal (AM) fungi. The unique nature of green roofs, as spatially isolated, stressful habitats, may serve as an interesting model system to advance our knowledge on AM fungal biogeography. AM fungal biogeography has had a checkered development. Traditionally, the high local diversity and low global diversity has led to the assumption that generalism was high, not only in terms of host species that could be colonized (Law & Lewis, 1983), but also of abiotic niches that could be occupied (Chaudhary et al., 2008). However, the development of molecular tools to characterize AM fungal communities has led many to cast doubt on this, based on the idea that morphological descriptions of AM fungal species may mask hidden functional diversity and merge together morphologically similar, yet functionally divergent fungi (Fitter, 2005). The rise of next-generation sequencing was thus seen as a promise to overcome this "linnean shortfall" of inadequate AM fungal species description (but see Bruns & Taylor, 2016). Yet, using next-generation sequencing, Davison et al. (2015) have shown that the traditional premise of low endemism and high generalism seems to hold true, which could be caused by early land colonization and divergence (before the break up of Pangea) (Pirozynski & Dalpé, 1989; Chaudhary et al., 2008; Dotzler et al., 2009) and to more efficient dispersal than previously thought (Davison et al., 2018; Chaudhary et al., 2020, but see Egan et al., 2014). As a result, we still have a very poor understanding of the relative roles of dispersal and various environmental filters (e.g., plants vs soil, Johnson et al., 1992; Helgason & Fitter, 2009) as drivers of local AM fungal community assembly. We know even less about the relative importance of patch dynamics, environmental filtering and drift (Leibold et al., 2004; Vellend, 2010) as structuring agents of AM fungal metacommunities across spatial scales (Fitzsimmons et al., 2008; Chaudhary et al., 2008; Powell & Bennett, 2016; Vályi et al., 2016). There is thus a need to jointly

investigate these drivers, especially in urban infrastructures where dispersal limitations and stress may strongly constrain community assembly (Sutton et al., 2008).

In this study, we sampled 19 green roof ecosystems and five urban parks in Montreal city (Québec, Canada). These sites were selected to encompass the broadest gradient of green roof types as possible, with sampling sites varying in substrate depth, fertilizer application, presence of an irrigation system, vegetation diversity and age, to name a few. Arbuscular mycorrhiza fungal communities were characterized using Illumina sequencing (MiSeq) of the 18S rRNA gene and we looked at the alpha- and beta-diversity patterns to elucidate the main drivers of green roof microbiome. We also evaluate the abundance of AMF in the sampled site using a bioassay with the collected soil and *Zea mays* as a host.

Materials and Methods

Study sites

We characterized AM fungal communities from 19 green roofs across the island of Montreal (45.4-45.6 N and 73.5-73.8 W; fig S2.1). Six roofs were classified as "intensive" (i.e., substrate thickness >15cm) and 13 were classified as "extensive" (thickness <15cm). Roof age ranged between 2 and 20 years. We also sampled 5 large urban parks, which served as benchmark mesic habitats in this regional landscape (fig S2.1). Samples were taken at the end of June 2019. Mean annual precipitation in Montreal is approximately 1000 mm and mean temperature in this time of the year is 16.8°C (Environment Canada, 2020)

Soil sampling and chemical analyses

For all roofs, we measured substrate thickness and visually estimated the light exposure of the roof (a value of 100% meaning that all of the roof surface is free of shade from surrounding buildings at any time of the day). We then sampled five $1m^2$ quadrats in green roofs and 10 in city parks (to account for their greater size and to encompass their greater range of soil proprieties and vegetation structure). In each quadrat, we evaluated percent cover for each plant species present and sampled 5 random soil cores (3cm diameter, ~15 cm deep). These cores were pooled together in a sterile plastic bag to produce one composite soil sample per site. Soil was kept on ice upon arrival to the laboratory within less than 4 hours.

In the laboratory, soils were sieved (ø 2mm) and mixed thoroughly. Subsamples were air-dried for chemical analyses. A random field root subsample was immediately stored at -20°C for DNA extractions (see below). Soil pH was measured by soaking 10g of soil into 20mL of 0.01M CaCl2 (Gregorich and Carter, 2007). Available NO_3^- and NH_4^+ were extracted from 2g of soil using 20mL of 2M KCl and quantified colorimetrically using Berthelot reaction (NO_3^-) and a persulfate oxidation followed by an acidic Griess reaction (NH_4^+) (Hood-Nowotny et al., 2010). Mehlich-III extractible P was quantified using 3g of soil and 30mL of Mehlich-III solution (Gregorich & Carter, 2007). Inorganic PO_4^{3-} concentration was determined colorimetrically using the Murphy-Riley method (Murphy & Riley, 1962). Soil gravimetric moisture was measured by weighing fresh soil and drying at 105°C to calculate water content per g dry soil. Soil organic matter content was measured using loss on ignition in a muffle furnace (Hoogstenn et al., 2015).

Measurement of mycorrhizal inoculum potential (MIP)

For each site, we used a mycorrhizal inoculum potential bioassay with maize (Moorman & Reeves, 1979; Chaudhary et al., 2019) to estimate the density of viable infective AM propagules. *Zea mays* seeds were surface sterilized (ethanol 70% v/v) and planted in 150 ml field soil from each site in duplicates, for a total of 48 pots. Seedlings were thinned to one plant per container, watered daily and grown in a growth chamber (Conviron Gen-1000) at a constant temperature (22°C) and a 16h-8h day:night photoperiod. After 40 days, maize shoots were removed and roots were gently washed to removed soil particles. From each root system, a random subsample of roots was cleared in KOH, stained with a 5% ink-in-vinegar solution, destained in PVGL for 24 h and root fragments were mounted on a microscope slide in glycerol for observations at G400X (Vierheilig et al., 1998; Dalpé et Séguin, 2013). The presence of fungal structures was scored using the grid-line intercept method (McGonigle et al., 1990) on 100 intersections per sample.

Molecular methods and bioinformatics

Prior to DNA extraction, 1.5g of roots that were frozen at the arrival to the laboratory was frozen in liquid nitrogen, then ground with zirconium beads in a ball mill (Retsch Mixer Mill 301, Germany). DNA was then extracted from 0.5g ground roots using Qiagen PowerSoil Kit following manufacturer instructions. Extraction yields were estimated using a nanodrop spectrophotometer. Extracted DNA was then PCR amplified using the WANDA and AML2 primer couple (Lee et al., 2008; Dumbrell et al., 2011). The PCR conditions were a 5 min 95°C hot start, 35 cycles of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C, followed by a final 10 min elongation at 72°C. Amplicons were sequenced using the Illumina Miseq technology at Genome Québec facilities (Montréal, Canada).

Raw sequences were analysed using the dada2 pipeline (Callahan et al., 2016) to identify amplicon sequence variants (ASVs). Reads were first quality-filtered (*q* threshold = 2). Because of insufficient overlap between forward and reverse sequences, we conducted downstream processing on the forward reads as in Morgan et al, (2017), since this part of the fragment offers a very good interspecific discrimination power (Davison et al., 2012), totally comparable to what is generally possible using longer reads. All other pipeline parameters were kept at their default values. Chimeras were removed using the "pooled method" (Callahan et al., 2016) and taxonomy was assigned using the reference database MaarjAM (Öpik et al., 2010). ASV with less than 5 reads in the overall database were kept out for further analyses to avoid putting emphasis on very rare taxa, then taxon were considered absent from a site if it had only one read in this sample. Each site was rarefied to 51 464 reads (lowest number of reads across samples), using the function *rrarefy* of the R package

AMF phylogeny construction

We generated a maximum likelihood phylogeny for the ASVs identified. We first generated an alignment for the 18S query sequences using the SINA aligner (v.1.2.11), which aligns query sequences according to the SILVA alignment of rRNA genes (Pruesse et al. 2012). For each query sequence, we searched for 15 neighbour sequences from the non-redundant SILVA Ref database with at least 95% identity and used this pool of sequences as the alignment template using default parameters. We used the alignment of neighbor sequences to generate a reference tree under a GTR GAMMA nucleotide substitution model using RAXML (v.8.2.12) (Stamatakis 2014). We then performed a maximum likelihood tree search including the query sequences using the reference tree as a backbone using the same model. To obtain support for the branches of the best-scoring

tree, we lastly conducted a bootstrap analysis using the autoMRE bootstrap convergence criterion to find the optimal number of bootstraps.

Statistical analysis

To elucidate the potential drivers of AM fungal metacommunity assembly among our green roofs, we evaluated different aspects of this metacommunity: community density (i.e., MIP), local (alpha) diversity, drivers of phylogenetic beta-diversity and mean relatedness of co-occurring taxa (i.e., phylogenetic community structure).

1) AM fungi abundance and diversity in green roofs

We used Faith's phylogenetic diversity (PD) as our alpha-diversity metric, as it has been shown by Miller et al. (2017) to have desirable statistical properties: low type I error and high statistical power. We compared community alpha-diversity (PD) and density (MIP) across habitat types (extensive green roofs, intensive green roofs and urban parks) using ANOVA with Tukey post-hoc tests for pairwise comparisons. Normality and homoscedasticity were validated using Shapiro-Wilk and Bartlett tests, respectively. For green roof specifically, we assessed the drivers of phylogenetic diversity using multiple linear regression (gaussian family). We did not include urban parks for this analysis, as we were interested in evaluating the role of roof-specific variable (e.g., height, area) that could not be compiled for parks. We selected predictors (i.e. soil chemistry, roof properties, vegetation community structure), using AIC-based stepwise selection as implemented in the R package *MASS* (Ripley et al., 2013). To include vegetation as predictor, we conducted a principal component analysis (PCA) on Hellinger-transformed plant community data and used the site scores for the 5 first axes as 5 explanatory variables in the regression. We also controlled for

spatial autocorrelation by analyzing principal coordinates of neighbour matrices (PCNMs) (Borcard and Legendre, 2002) and including PCNMs associated with positive eigenvalues as predictor variables in the regression model.

2) Drivers of phylogenetic beta-diversity in green roofs

To identify the drivers of AM fungi phylogenetic beta-diversity on green roofs, we conducted a distance based redundancy analysis (dbRDAs) regressing phylogenetic beta-diversity (pairwise site MPD index calculated using the *comdist* function from *picante,* Kembel et al., 2010) against roof characteristics. The dbRDA was computed using *capscale* from the *vegan* R package (Oksanen et al. 2020). Roof characteristics included soil chemical properties, as well as roof properties such as height, surface area, age, substrate depth and state-variables like the presence of an irrigation system or the use of fertilizers. We included vegetation structure and spatial autocorrelation in the model as outlined in the previous section. We selected explanatory variables using automated stepwise model selection ($\alpha = 0.05$) to identify the best predictors of microbial community phylogenetic structure. As this procedure is heuristic, we computed 50 independent runs and retained the constrained ordination with the highest proportion of constrained inertia.

We also specifically looked at spatial autocorrelation across sites using a Mantel correlogram of phylogenetic beta-diversity. This was done using the function *mantel.correlog* from *vegan*. *P*-value alpha threshold across distance classes using a progressive Bonferroni approach following Legendre & Legendre (2012).

3)Phylogenetic community structure

We calculated the mean pairwise distance (MPD) of AM fungi co-occurring in every local community and compared it to 1000 random local communities drawn from the regional pool (Lessard et al., 2012; Miller et al., 2017). We thus computed MPD *z*-scores to determine whether each local community was more phylogenetically clustered or overdispersed than expected by chance.

4)Frequency-abundance relationship and generalist AM fungi

As we found high asymmetry in local abundance (with few abundant AM taxa and many rare ones, see Dumbrell et al., 2010), we investigated the relationship between frequency and local abundance (Brown, 1984; Gaston et al., 1997). This allowed us to identify generalist AM taxa (defined here operationally as the AM taxa present in at least 10 roofs and having a local abundance of more than 1% of the reads). To determine whether these generalists were clustered in the AM phylogeny, we calculated the MPD among these generalists and compared it to 999 random scenarios where MPD was calculated for sets of the same number of AM taxa randomly drawn from the regional pool (by weighing the probability of drawing every single AM taxon according to its regional abundance).

We also compared the regional frequency of our generalists with their global frequency observed in Davison et al. (2021). We also did 999 permutations by sampling randomly from the global dataset the same number of generalists than previously found, to see if our generalists were more frequent globally than what we could expected by chance.

Results

We obtained 1,933,111 filtered reads from all our samples, belonging to 732 ASV, of which 82 could be associated to a known VTX in the MaarjAM database. These taxa belonged to 8 different families: Acaulasporaceae, Ambisporaceae, Archaeosporaceae, Diversisporaceae, Gigasporaceae, Glomeraceae and Paraglomeraceae. The Glomeraceae was the most abundant family in our sites with 79.3% of all the reads.

Green roof showed high AM fungal densities, with bioassay plants colonized up to 80% after 5 weeks (fig 3.1). Extensive green roof had significantly higher MIPs ($F_{2,21}$= 4.714, P=0.02) (fig 3.1). Phylogenetic diversity, calculated as Faith's PD, was similar across roofs and parks ($F_{2,21}$=0.251, P=0.78) (fig 3.1). PD on roofs was mostly driven by plant community structure, soil properties and irrigation. More specifically, AM PD was positively related to organic matter content, pH, light and negatively to nitrate and irrigation (table 3.1). AM PD was also positively correlated to the second and fifth axis of the vegetation community structure and negatively to the first (fig S3.2). Contrary to our expectations, PD was not lower for young or isolated (small and high roofs) sites as age wasn't kept in the best model (table 3.1).

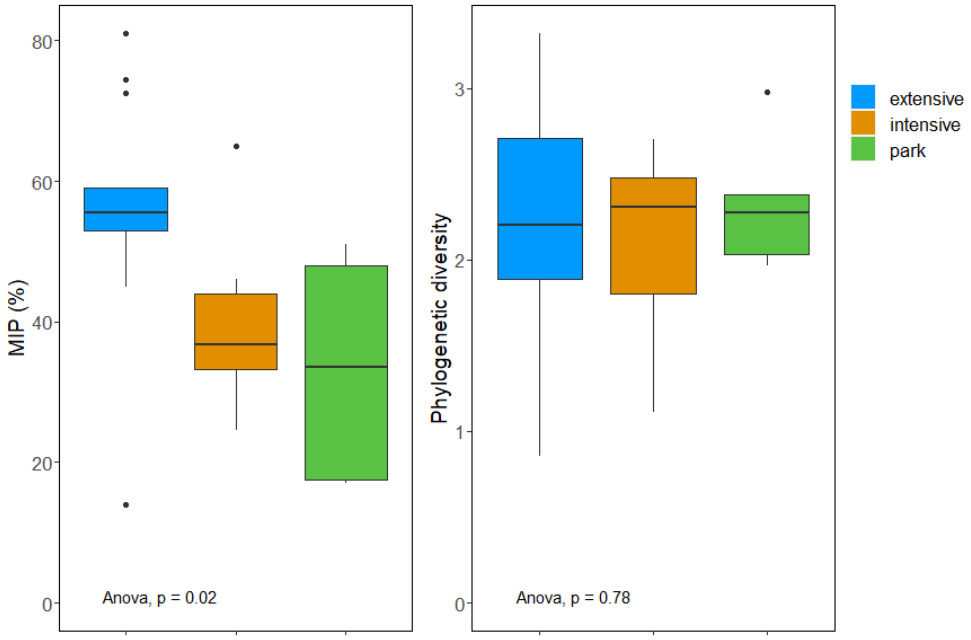

Figure 3.1: a) Density of viable infective AM measured through a mycorrhizal inoculum potential bioassay with maize and b) Faith's phylogenetic diversity. F and P values come from one-way ANOVAs

Table 3.1: Linear Gaussian regression linking Faith's phylogenetic diversity with roof properties. See methods for description of predictors measurement. % Org mat = percent organic matter of the substrate, luminosity= % of light exposure of the roof, Irrigation = presence, or not, of an irrigation system and PC1, PC2 and PC5= first, second and fifth axis of PCA on Hellinger-transformed plant community data. Values presented are model coefficients per predictor and their associated P-values.

	Coeficient	**P-value**
% org mat	2.917	0.01
pH	3.953	0.002
Light	2.919	0.01
Veg2	1.321	0.21
Veg5	2.911	0.015
NO_3^-	-3.847	0.003
Irrigation	-1.940	0.08
Veg1	-4.30	0.002

The beta-diversity of the green roof calculated with the MPD metric was driven only by the fifth axis of the vegetation community structure and explained 11% of the variation of the beta-diversity in de distance-based RDA. We included the PCNM to control for potential spatial autocorrelation,

but none of them were kept in the best model in the db-RDA. This absence of effect of spatial autocorrelation was further supported by a Mantel correlogram (fig 3.2).

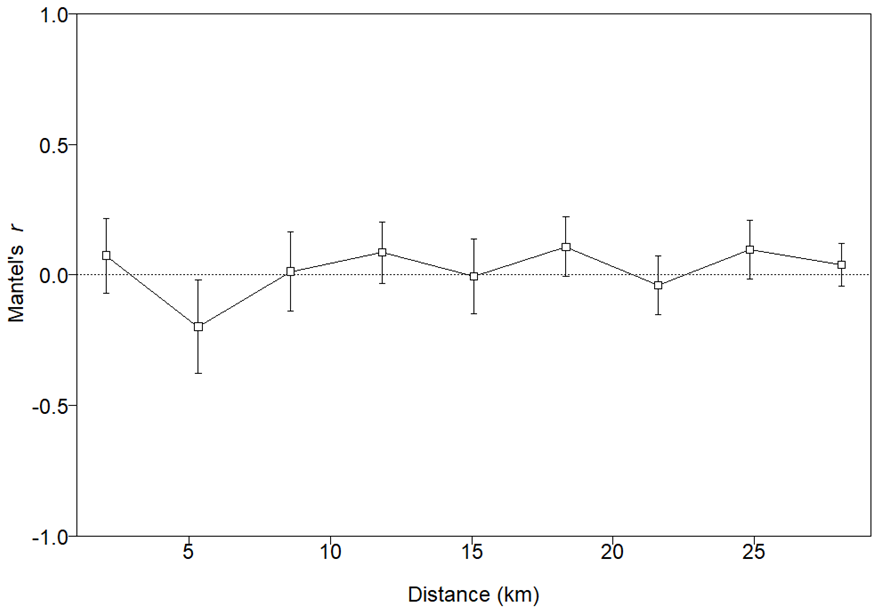

Figure 3.2: Spatial autocorrelation in phylogenetic mycorrhizal communities. Empty symbols indicate a correlation index not significantly different from 0.

We found a positive linear correlation (gaussian familly) between logarithm transformed regional frequency and mean local relative abundance ($P < 0.001$, fig 3.3a). We operationally defined "generalists" here as AM fungi occurring in more than 10 sites and with more than 1% of the reads per site on average. This identified 16 generalist taxa (table S3.1): 13 Glomeraceae, 2 Diversisporaceae and 1 Claroideoglomeraceae. Because of the core of generalist Glomeraceae, we found these generalists to be clustered in the AM fungal phylogeny (fig 3.4). By comparing our dataset with global occurrence data from Davison et al. (2021), we found a strong correlation between regional frequency across our sites and global frequency ($P = <0,001$, fig 3b). Moreover, our generalist VTX were more frequent in the global dataset than what we would expect by chance.

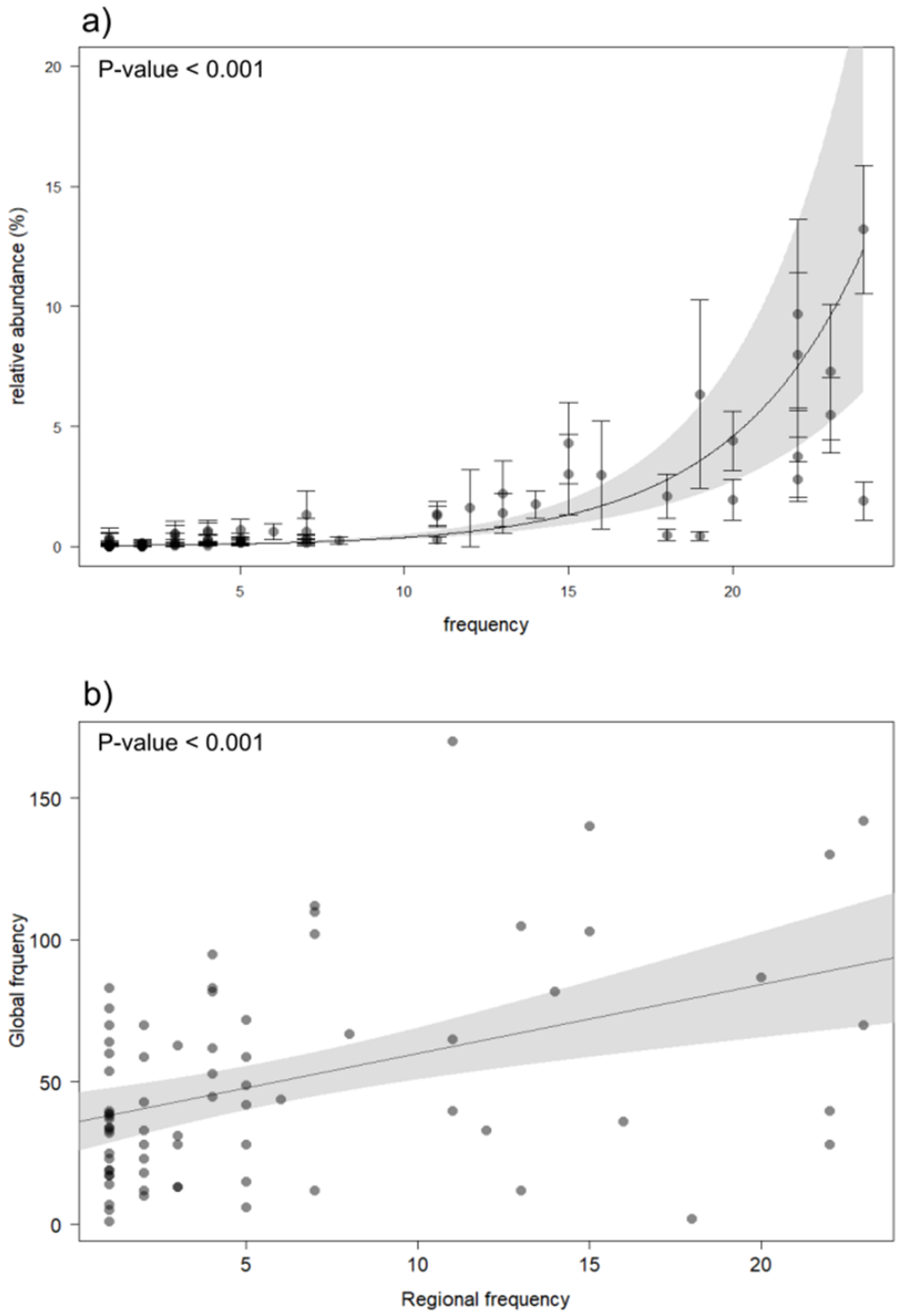

Figure 3.3: a) Relationship between the mean relative abundance of VTX and their frequency in the regional pool with a 90% confidence interval b)relationship between the frequency of VTX in the regional pool and in their global frequency observed in Davison et al. (2021) with a 95% confidence interval

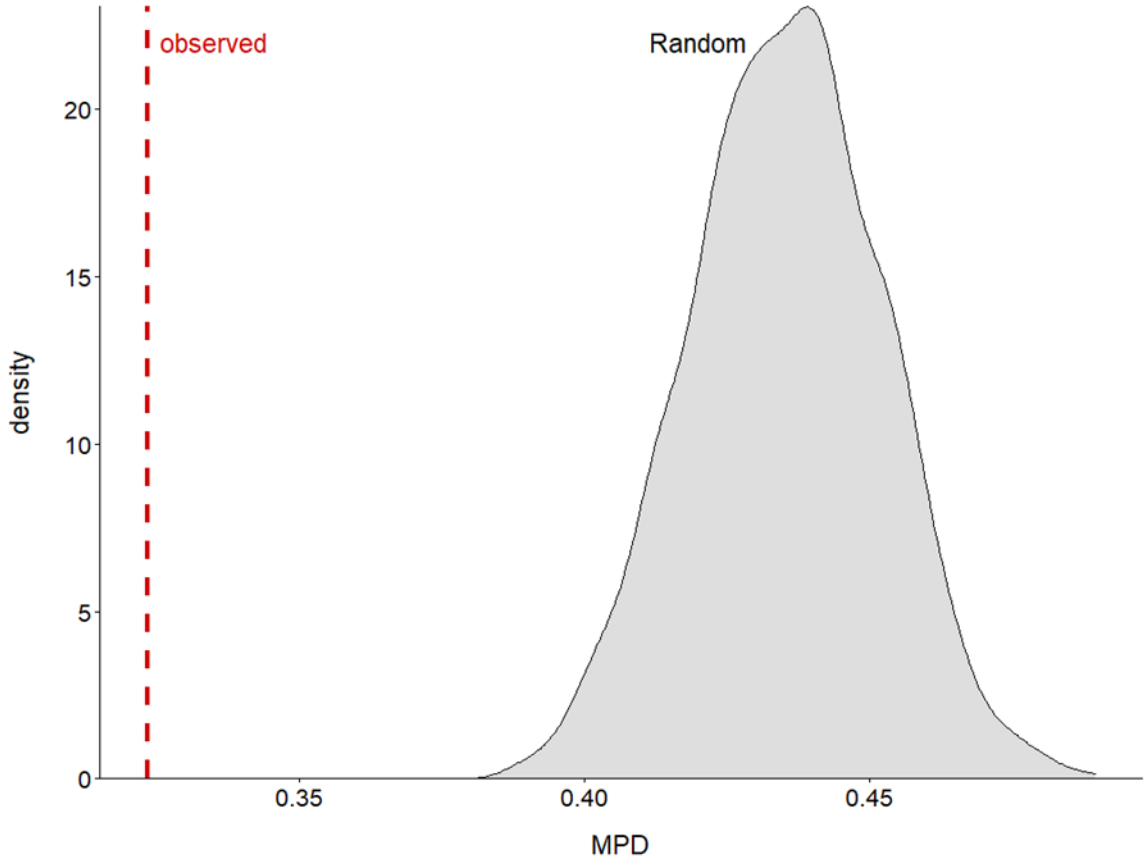

Figure 3.4: Density of the mean pairwise distance (MPD) from 999 scenarios with the same number of AM taxa randomly sampled from the regional pool and the observed MPD from the generalist of the regional pool.

Most local AM fungal assemblages were phylogenetically overdispersed (MPD P-value>0,95) (fig 3.5). Qualitatively similar patterns were found using mean nearest taxon distance (MNTD, Hardy, 2008) as the phylogenetic distance metric (fig S3.3).

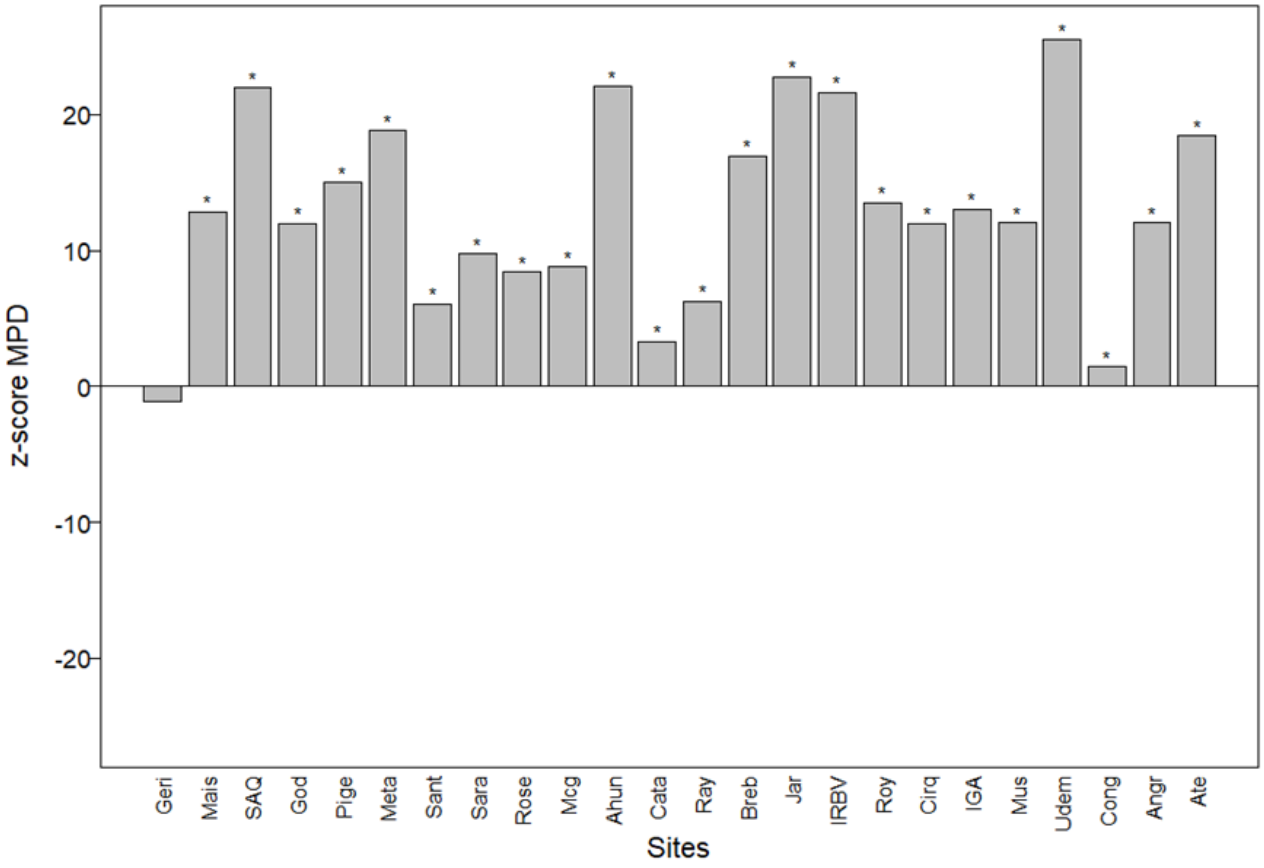

Figure 3.5 : Z-score of the mean pairwise distance (MPD) of the sampled sites compared to 1000 random local communities drawn from the regional pool (Lessard et al., 2012; Miller et al., 2017). "*" mean a significative value.

Discussion

This study provides interesting insights on the assembly of AM fungal communities on small, isolated urban islands. First, contrary to classic paradigms of island biogeography (MacArthur & Wilson 1967) and life-history strategies (Grime 1973), even the youngest and smallest roofs showed high alpha diversity. We also found high mycorrhizal densities on green roofs (fig 3.1), even more so on extensive roofs compared to urban parks. Collectively, these results suggest that the stressful conditions of drought-prone roofs promote plant investment into AM symbionts. This

is in line with the high diversity (McGuire et al., 2013) and abundance (Chaudhary et al., 2019; Gill et al., 2020) of AM fungi found on other roofs. Although this stands in contrast with data showing lower abundance of AM fungi in dry ecosystems (Staddon et al., 2004; Gai et al., 2009). Yet, stressed plants are known to adjust their exudates and potentially recruit beneficial microbes (including AM fungi) under drought (DeVries et al., 2019; Williams & DeVries, 2020). Here, it should be noted that green roofs are not arid ecosystems per se, as the defining feature of green roofs is not overall precipitation received, but rather temporal dynamics in water availability, with rapid return of drought conditions after a precipitation event.

Despite the high density and phylogenetic diversity of roof AM fungal communities, we found little evidence for deterministic drivers of community structure through environmental filtering. Phylogenetic beta-diversity was only constrained by one vegetation axis (highly related to the abundance of grass, *Solidago* sp., *Sedum* sp., *Fragaria virginiana*, *Rubus alleghensis* and *Matricaria chamomilla*) and most inertia remained unconstrained, potentially mirroring the stochastic nature of AM fungal community assembly (Dumbrell et al., 2010; Powell & Bennett, 2016; Lekberg et Waller., 2016; Bouffaud et al., 2017). The fact that only plants were retained as a predictor for AM fungal community structure may revive the debate as to whether AM fungi are more responsive to soil conditions vs. host plant communities (e.g., Helgason & Fitter 2009; Dumbrell et al., 2011). Here, as green roof substrates tend to be fairly devoid of mycorrhizal propagules (John et al., 2014) and most plants on our roofs are introduced during roof installation, we could see our green roof metacommunity as a field experiment supporting AM fungi as passengers responding to plant community structure (Hart et al., 2001; Hausmann & Hawkes, 2011; Koziol & Bever, 2016; Smilauer et al., 2020). This is in line with Hoch et al. (2019) that found an impact of plant communities on microbial communities in green roofs.

Although our trends in beta-diversity were hard to predict, alpha diversity (PD) was significantly correlated with a number of ecological variables. We find of particular interest that irrigation seemed to reduce alpha diversity. This brings focus to an underrated driver of AM fungal communities in natural systems: temporal variability. While it is well established in community ecology that temporal fluctuations can promote species coexistence (Menge & Sutherland, 1976; Warner & Chesson, 1985), we often fail to incorporate this in studies investigating patterns of mycorrhizal diversity and conceptual models predicting such diversity (e.g., Allen et al., 1995). Yet, this is a defining feature of green roofs and potentially thin soils in general, to show wide fluctuations in water potential, given the rapid return of drought following precipitations. We contend that irrigation may bring more temporal homogeneity in terms of water availability, which may reduce AM alpha diversity.

Our analyses show little evidence for strong dispersal limitations of AM fungi in this urban metacommunity. Spatial predictors were never retained in our model selection procedures and even very young (as young as 2 years since installation) showed high density and alpha diversity although initial substrates tend to harbour few (if any) mycorrhizal propagules (John et al., 2014). Collectively, this supports the assertion made by Davison et al. (2015) that we tend to underestimate the role of long-distance dispersal in AM fungal biogeography. The finding that small mammals (Janos et al., 1995; Mangan & Adler, 2004) or birds (Correia et al., 2019) can disperse AM spores have raised the potential for rare long-distance dispersal events to significantly influence AM fungal distributions (Davison et al., 2018). Here, we show that efficient, long-distance dispersal events need not be considered rare events. Young, isolated green roof islands were rapidly colonized by AM fungi, which is in line with Chaudhary et al. (2020) who recently

showed large number of AM spores dispersed to rooftops in Chicago, from a wide array of AM families. This is also in line with high densities (Dodd et al., 2002) and diversity (Kawahara et al., 2016) of AM fungi appearing early during primary succession. Collectively, these evidences point to a much more efficient dispersal by AM fungi than previously appreciated (Peay et al., 2010, Lekberg et al., 2011; Hazard et al., 2013; Egan et al., 2014; Kivlin et al., 2014).

Moreover, our results show that the AM fungi facing little or no dispersal limitation come from a non-random subset of the AM phylogeny (fig 3.4). Interestingly, some AM taxa were both regionally frequent and locally dominant, which is a common pattern in biogeography (e.g., Brown, 1984; Gaston & Lawton, 1990). As these were not "roof specialists" with the absence of indicator species in green roofs, this suggests that these generalists are not frequent and dominant because of the high availability of their preferred habitat (Gaston et al., 1997). The fact that the generalists found here are also cosmopolitan species in Davison et al.'s (2021) global dataset rather suggest that some traits allow AM fungi to disperse efficiently to remote sites and to colonize a broad range of habitat types. This is supported by Kawahara et al. (2016) who found that AM fungi with high regional frequency were habitat (i.e., pH) generalists. In other words, some "jack-of-all-trades" taxa do not show evidence for niche breadth and local competitive ability (Strau et al., 2011). Chaudhary et al. (2020) suggested that efficiently dispersing taxa were those having smaller spores with wall ornamentations. Here, we argue more broadly that these generalist, cosmopolitan taxa are likely ruderals investing massively in constitutive propagule production. The fact that most of them come from a clustered clade in the Glomeraceae (Chagnon et al., 2013) and that very rapidly in green roofs dense communities of AM fungi established following roof installation (fig 3.1) further support the idea that ruderals are efficient colonizers of this empty niche. In comparison to other AM fungal families, Glomeraceae colonize more intensively roots (Hart et Reader, 2002),

grow faster (Powell et al., 2009), fuse more easily hyphae (De La Providencia et al., 2005), invest more energy and faster in spore formation (Oehl et al., 2009) and form cross-walls that enable root pieces and severed hyphal fragment to heal and recolonize host roots (Klironomos et Hart, 2002; De La Providencia et al., 2005).

One could then ask why cosmopolitan AM fungi (Davison et al., 2021) are ruderals. Covariance between generalism and efficient dispersal is expected on evolutionary grounds (Brown & Pavlovic, 1992). But if there is a trade-offs between competitive vs colonization ability exists for AM fungi (Bennett & Bever, 2009), then what explains the broad success of cosmopolitan, putatively ruderal AM fungi, even in undisturbed habitats (e.g., Öpik et al., 2009; Chagnon et al., 2015)? Colonizers should always be favored by local disturbances, which represent colonization opportunities. Even in stable habitats, where vegetation is not severely disturbed, we could speculate that annual root flush and new root growth provide such opportunities for colonization, maintaining the abundance of ruderals high. While these assertions linking the cosmopolitan distribution of AM fungi to the possession of traits characteristic of a ruderal strategy will require further testing, our results show that the regional pool of available species can be a major driver of AM metacommunity assembly, in line with many other studies (e.g., Torrecillas et al., 2013; Smilauer et al., 2020).

While we found a core of cosmopolitan typically dominating roofs, we found that overall, phylogenetic structure of local roofs tended towards overdispersion. We suggest that during local community assembly, later colonists (i.e., subordinate taxa) can only persist if they are sufficiently phylogenetically distant from the pool of resident AM fungi already in place. This is consistent with studies showing intense competition among AM fungi (e.g., Wilson, 1984; Cano & Bago.,

2005; Engelmoer et al., 2014) that promotes coexistence of distant lineages (Maherali & Klironomosc, 2007, 2012). Such competition is expected to be especially intense when the system is already colonized by ruderal AM fungi (Mummey et al., 2009). We could thus imagine a metacommunity assembly going through two successive phases, with (1) a neutral site colonization by regionally abundant ruderals, showing the important of the regional pool in local community assembly (Pärtel et al., 1996), followed by (2) progressive community invasion by later colonizers which would be constrained by competition with resident taxa. Such hierarchical community assembly is in line with what has been suggested for the establishment of preferential plant-AM partnerships (Chagnon et al., 2012, 2015).

Further research would benefit to use sequencing technologies that can use longer sequences like real-time sequencing (SMRT). It would help to refine the phylogenetic tree of species observed in sampled sites. A study of Schlaeppi et al., (2016) used this technology to study mycorrhizal communities and has found that it improved specificity and enhanced resolution. This sequencing method could also improve statistical power, which could increase the rate of explained variation.

A major frontier for future research will be to determine whether cosmopolitan taxa provide plants with as much benefits as would less generalist taxa and if they support a high ecosystem functionnality. For example, it remains to be disentangled whether opportunistic ruderal colonizers gain their plant-derived carbon from reward mechanisms vs scavenging from the apoplast (Bever, 2015). Linking AM fungal traits with benefits provided to hosts is much needed (Chagnon et al., 2013).

Chapitre 4 – Conclusion générale

Les mécanismes d'assemblage des communautés microbiennes ont le potentiel d'affecter la fonctionnalité des écosystèmes. Être capable de prédire l'assemblage de ces communautés nous donne le pouvoir de bien cerner si des actions sont possibles et nécessaires pour contrôler leur structure et leur composition dans le but de supporter les services écosystémiques. Dans le cadre d'habitats d'origine entièrement anthropique, comme les toits verts, le maintien de certaines fonctions spécifiques est clé pour la santé des populations humaines urbaines. Ainsi, l'étude de l'assemblage des communautés microbiennes est primordiale. De meilleures connaissances de ces mécanismes dans ces habitats peut nous permettre d'élargir notre compréhension de l'assemblage des communautés microbiennes et surtout dans les « novel ecosystems » (sensu Hobbs, 2006).

Les résultats de ces projets ont permis de mieux cerner les mécanismes en jeu dans l'assemblage des communautés microbiennes et de remettre en question certaines théories classiques en écologie dans un contexte de toits verts. Par exemple, les théories de MacArthur et Wilson (1967) et Grime (1973) qui prédisent une plus faible richesse dans les habitats isolés géographiquement et stressés ne semblent pas être applicables dans le contexte de toits verts. Ces habitats pourraient être moins isolés et moins stressants que préalablement crus. De plus, les résultats sur les champignons et les bactéries remettent en perspective la classification oligotrophe-copiotrophe chez les microorganismes (e.g. Ho et al., 2017) et mettent de l'avant sa simplicité. En effet, alors que cette classification intègre les compromis liés à la tolérance au stress surtout par les nutriments, la tolérance aux stress serait beaucoup plus multidimensionnelle et aurait avantage à intégrer d'autres dimensions, dont les conditions hydriques.

Même si les analyses portant sur les communautés de bactéries et de champignons qui se basaient sur la taxonomie divergeaient de celles des communautés de CMA qui se basaient sur la phylogénie, nous avons pu observer de nombreuses similarités. Tout d'abord, la limitation à la dispersion ne semblait pas jouer un rôle prédominant pour l'assemblage des communautés. De plus, les toits, même les plus jeunes, présentaient une forte diversité des trois groupes d'organismes, à des niveaux similaires à ce qui était observé dans les parcs urbains. Puis, peu de variation dans la structure des communautés était expliquée par les variables mesurées, supposant l'importance de la stochasticité. La méthode de séquençage utilisée dans cette étude qui utilise des séquences courtes pourrait aussi expliquer une partie de la grande variance inexpliquée. Cette dernière pourrait par contre aussi provenir de variables non mesurées, dont le substrat exact utilisé à la construction. Cependant, alors que pour les bactéries et les champignons, la physicochimie du sol semblait jouer un certain rôle pour la structure des communautés, pour les CMA seulement la structure des communautés de plantes jouait un rôle. De plus, les analyses phylogénétiques de ces derniers organismes nous ont permis d'apporter des hypothèses plus précises sur les mécanismes derrière l'assemblage des communautés, dont l'importance des processus neutres et des interactions biotiques.

Comme démontré dans ces deux études, cela reste très difficile de prédire la structure des communautés microbiennes des toits verts via les variables environnementales. Comme présenté par plusieurs, des variables spécifiques à l'écologie urbaine devront être intégrées aux futures études pour bien incorporer les spécificités de ces habitats comparativement aux milieux naturels. Yan et al., (2016) proposaient en autre de considérer la densité de la population et le produit intérieur brut par kilomètre carré (PIB km^{-2}), des variables spécifiques aux systèmes anthropiques. Même le pH qui est reconnu comme étant un prédicteur universel des communautés microbiennes

ne ressortait pas fortement dans certains de nos résultats. Cette absence d'effet de ce prédicteur commun des communautés microbiennes pourrait être causée par la faible différence de conditions de pH d'un toit à l'autre (entre 6,08 et 7,32). En effet, en conditions similaires, il peut être difficile de prédire les communautés microbiennes par les variables environnementales (Powell et Bennet, 2016).

Une des conclusions majeures de cette étude est que contrairement à ce qu'on aurait pu croire, les toits verts ont des communautés microbiennes très diversifiées et abondantes, autant pour les bactéries, les champignons et les CMA. Il y aurait ainsi probablement peu de besoins d'inoculation exogène pour combler les fonctions écosystémiques rendues par ces communautés. Toutefois, comme observées, les communautés des toits verts peuvent être distinctes de celles retrouvées dans d'autres environnements urbains. Ainsi, une question importante se pose, quelles sont les conséquences de cette unicité dans la capacité des toits verts à supporter les fonctions écosystémiques qui leur sont reconnus ?

On doit ainsi aborder les questions liées à la fonctionnalité des microbiomes des toits verts et comment les espèces présentes affectent les cycles biogéochimiques ou la performance des plantes par exemple. Gill et al., (2020) ont commencé cette réflexion alors qu'ils ont étudié la diversité microbienne fonctionnelle dans des infrastructures vertes, dont les toits verts. L'avancée dans l'association des taxons microbiens avec des processus métaboliques spécifiques permettra éventuellement d'avoir une meilleure compréhension des fonctions soutenues par les communautés sélectionnées sur les toits verts et éventuellement de faire des liens avec certaines fonctions clés des toits verts, dont l'isolation thermique et la rétention hydrique. Dans le même ordre d'idée, les avancées de la métagénomique pourraient éventuellement permettre aux microbiologistes urbains de prédire les conséquences sur les écosystèmes urbains et anthropiques des filtres uniques dans

ces milieux. De cette façon, il sera possible de vérifier la pertinence d'inoculations exogènes. Par exemple, est-ce que la faible abondance de Thaumarchaeota affecte le cyclage de l'azote et la productivité des écosystèmes? Si certaines fonctions semblent ne pas être soutenues pleinement par les communautés microbiennes en place, des inoculations exogènes spécifiques pourraient être envisagées. D'autre part, une telle approche fonctionnelle nous permettrait de savoir si les CMA généralistes observés en grande abondance sur les toits verts fournissent efficacement les différentes fonctions souhaitées des toits verts. Une préinoculation des plants des toits verts pourrait par exemple permettre à des taxons bénéfiques de coloniser aussi ces environnements.

Une autre avenue de recherche serait de s'attarder sur l'effet de différents taxons et compositions microbiennes sur les fonctions écosystémiques des toits verts et ainsi vérifier si les espèces présentes naturellement dans ces milieux sont les plus efficaces. Une expérience en mésocosme de toits verts en contrôlant les communautés microbiennes directement et en mesurant certaines fonctions, dont l'isolation thermique, la croissance des plantes et la rétention hydrique nous aiderait à répondre à cette question. Des expériences similaires sur l'effet différentiels d'espèces de plantes ont déjà eu lieu (Cook-Patton and Bauerle, 2012; Dunnett et al., 2008; Franzaring et al., 2016; Johnson et al., 2016; Lundholm et al., 2010; Lundholm et al., 2015; Xie et al., 2018.). En raison du grand conservatisme phylogénétique au niveau de la famille de CMA, il y aurait particulièrement un grand intérêt à manipuler ces communautés (Powell et al., 2009; Hart et Reader, 2002). Ce conservatisme permettrait d'associer des traits spécifiques de certaines familles à la performance de certaines fonctions (Yang et al., 2017). Dans le même ordre d'idée, alors que l'effet de l'âge sur la diversité et la structure des communautés microbiennes semblerait relativement faible, il serait pertinent de faire un suivi temporel dès la construction des toits verts pour vérifier combien de temps il est nécessaire pour que ces communautés atteignent une certaine stabilité. Ainsi, alors que

les toits les plus jeunes échantillonnés avaient déjà 2 ans, en échantillonnant dans les premiers jours ou semaines, nous pourrions voir une progression dans la structure des communautés ainsi que la diversité des espèces présentes. Est-ce que ces communautés microbiennes du sol initiales et précoces ont un impact sur celles qu'on observe quelques années plus tard ? Les effets historiques pourraient potentiellement avoir un plus grand impact sur l'assemblage des communautés que les filtres environnementaux mesurés dans les chapitres 2 et 3. Similairement, de telles informations nous permettront de vérifier la pertinence d'inoculations exogènes et si ces espèces sont capables de persister dans le temps. Si l'inoculation affecte significativement les communautés observées dans les sols des toits verts, une autre avenue de recherche serait d'évaluer si le moment d'inoculation, soit au moment de la construction ou quelques mois et mêmes années plus tard, affecte différemment comment les communautés s'assemblent. Ainsi, il pourrait potentiellement être inutile de procéder à de l'inoculation exogène quelques années après la construction du toit.

Références bibliographiques

Abarenkov, K., R. H. Nilsson, K. H. Larsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, T. Pennanen, R. Sen, A. F. S. Taylor, L. Tedersoo, B. M. Ursing, T. Vrålstad, K. Liimatainen, U. Peintner and U. Kõljalg. 2010. The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186:281–285.

Adams, R. I., M. Miletto, J. W. Taylor and T. D. Bruns. 2013. Dispersal in microbes: Fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. ISME Journal 7:1262–1273.

Aguilar-Trigueros, C. A., S. Hempel, J. R. Powell, W. K. Cornwell and M. C. Rillig. 2019. Bridging reproductive and microbial ecology: a case study in arbuscular mycorrhizal fungi. ISME Journal 13:873–884.

Akbari, H. and L. S. Rose. 2008. Urban Surfaces and Heat Island Mitigation Potentials. Journal of the Human-Environment System 11:85–101.

Al Horr, Y., M. Arif, A. Kaushik, A. Mazroei, M. Katafygiotou and E. Elsarrag. 2016. Occupant productivity and office indoor environment quality: A review of the literature. Building and Environment 105:369–389.

Alexandri, E. and P. Jones. 2008. Temperature decreases in an urban canyon due to green walls and green roofs in diverse climates. Building and Environment 43:480–493.

Allen, E. B., M. F. Allem, D. J. Helm, J. M. Trappe, R. Molina and E. Rincon. 1992. Patterns and regulation of arbuscular and ectomycrrhizal plant and fungal diversity: a hypothesis. Plant and Soil 170:47–62.

Allen, M. F. 2007. Mycorrhizal Fungi: Highways for Water and Nutrients in Arid Soils. Vadose Zone Journal 6:291.

Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42.

Augé, R. M. 2004. Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84:373–381.

Baas Becking L.G. M. 1934. Geobiologie of inleiding tot de milieukunde. W.P. Van Stockum & Zoon: The Hague, the Netherlands.

Bainard, L. D., J. N. Klironomos and A. M. Gordon. 2011. The mycorrhizal status and colonization of 26 tree species growing in urban and rural environments. Mycorrhiza 21:91–96.

Bardgett, R. D. and W. H. Van Der Putten. 2014. Belowground biodiversity and ecosystem functioning. Nature 515:505–511.

Barnard, R. L., C. A. Osborne and M. K. Firestone. 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME Journal 7:2229–2241.

Barrios, E. 2007. Soil biota, ecosystem services and land productivity. Ecological Economics 64:269–285.

Baryła, A., T. Gnatowski, A. Karczmarczyk and J. Szatyłowicz. 2019. Changes in temperature and moisture content of an extensive-type green roof. Sustainability, 11: 2498.

Bastida, F., I. F. Torres, M. Andrés-Abellán, P. Baldrian, R. López-Mondéjar, T. Větrovský, H. H. Richnow, R. Starke, S. Ondoño, C. García, F. R. López-Serrano and N. Jehmlich. 2017. Differential sensitivity of total and active soil microbial communities to drought and forest management. Global Change Biology 23:4185–4203.

Battistuzzi, F. U. and S. B. Hedges. 2009. A major clade of prokaryotes with ancient adaptations to life on land. Molecular Biology and Evolution 26:335–343.

Bell, M., D. L. Davis and T. Fletcher. 2004. A retrospective assessment of mortality from the London smog episode of 1952: The role of influenza and pollution. Environmental Health Perspectives 112:6–8.

Bellemain, E., T. Carlsen, C. Brochmann, E. Coissac, P. Taberlet. and H. Kauserud. 2010. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC microbiology 10:1-9.

Bengtsson, L., L. Grahn and J. Olsson. 2005. Hydrological function of a thin extensive green roof in southern Sweden. Nordic Hydrology 36:259–268.

Bennett, A. E. and J. D. Bever. 2009. Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in Plantago lanceolata. Oecologia 160:807–816.

Berardi, U., A. GhaffarianHoseini and A. GhaffarianHoseini. 2014. State-of-the-art analysis of the environmental benefits of green roofs. Applied Energy 115:411–428.

Berg, G. and K. Smalla. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68:1–13.

Berretta, C., S. Poë and V. Stovin. 2014. Moisture content behaviour in extensive green roofs during dry periods: The influence of vegetation and substrate characteristics. Journal of Hydrology 511:374–386.

Besir, A. B. and E. Cuce. 2018. Green roofs and facades: A comprehensive review. Renewable and Sustainable Energy Reviews 82:915–939.

Bever, J. D. 2015. Preferential allocation, physio-evolutionary feedbacks and the stability and environmental patterns of mutualism between plants and their root symbionts. New Phytologist 205:1503–1514.

Bianchini, F. and K. Hewage. 2012. Probabilistic social cost-benefit analysis for green roofs: A lifecycle approach. Building and Environment 58:152–162.

Borcard, D. and P. Legendre. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecological modelling 153:51-68.

Bouffaud, M. L., C. Bragalini, A. Berruti, M. Peyret-Guzzon, S. Voyron, H. Stockinger, D. van Tuinen, E. Lumini, D. Wipf, P. Plassart, P. Lemanceau, V. Bianciotto, D. Redecker and M. Girlanda. 2017. Arbuscular mycorrhizal fungal community differences among European long-term observatories. Mycorrhiza 27:331–343.

Brown, J. H. 1984. On the Relationship between Abundance and Distribution of Species. The American Naturalist 124:255–279.

Brown, J. S. and N. B. Pavlovic. 1992. Evolution in heterogeneous environments: Effects of migration on habitat specialization. Evolutionary Ecology 6:360–382.

Bruns, T. D. and J. W. Taylor. 2016. Comment on “Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism.” Science 351:826.

Bryant, J. A., C. Lamanna, H. Morlon, A. J. Kerkhoff, B. J. Enquist and J. L. Green. 2009. Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. In the Light of Evolution 2:127–148.

Cadenasso, M. L., S. T. A. Pickett and K. Schwarz. 2007. Spatial heterogeneity in urban ecosystems: Reconceptualizing land cover and a framework for classification. Frontiers in Ecology and the Environment 5:80–88.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson and S. P. Holmes. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature methods 13:581-583.

Cano, C. and A. Bago. 2005. Competition and substrate colonization strategies of three polyxenically grown arbuscular mycorrhizal fungi. Mycologia 97:1201–1214.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences of the United States of America 108:4516–4522.

Carter, M. R. and E. G. Gregorich. 2007. Soil sampling and methods of analysis. CRC press : Boca Raton, FL, USA.

Carter, T. L. and T. C. Rasmussen. 2006. Hydrologic behavior of vegetated roofs. Journal of the American Water Resources Association 42:1261–1274.

Chagnon, P. L. and J. Brisson. 2017. The role of mycorrhizal symbioses in phytotechnology. Botany, 95: 971-982.

Chagnon, P. L., C. Brown, G. C. Stotz and J. F. Cahill. 2018. Soil biotic quality lacks spatial structure and is positively associated with fertility in a northern grassland. Journal of Ecology 106:195–206.

Chagnon, P. L., R. L. Bradley and J. N. Klironomos. 2012. Using ecological network theory to evaluate the causes and consequences of arbuscular mycorrhizal community structure. New Phytologist 194:307–312.

Chagnon, P. L., R. L. Bradley and J. N. Klironomos. 2015. Trait-based partner selection drives mycorrhizal network assembly. Oikos 124:1609–1616.

Chagnon, P. L., R. L. Bradley, H. Maherali and J. N. Klironomos. 2013. A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18:484–491.

Chapin, F. S. 1991. Integrated Responses of Plants to Stress. BioScience 41:29–36.

Chase, J. M. 2007. Drought mediates the importance of stochastic community assembly. Proceedings of the National Academy of Sciences of the United States of America 104:17430–17434.

Chase, J. M. and J. A. Myers. 2011. Disentangling the importance of ecological niches from stochastic processes across scales. Philosophical Transactions of the Royal Society B: Biological Sciences 366:2351–2363.

Chaudhary, V. B., E. L. Sandall and M. V. Lazarski. 2018. Urban mycorrhizas: predicting arbuscular mycorrhizal abundance in green roofs. Fungal Ecology:1–8.

Chaudhary, V. B., M. K. Lau and N. C. Johnson. 2008. Macroecology of microbes - Biogeography of the glomeromycota. Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics (Third Edition):529–563.

Chaudhary, V. B., S. Nolimal, M. A. Sosa-Hernández, C. Egan and J. Kastens. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238–252.

Chen, H., X. Zhao, Q. Lin, G. Li and W. Kong. 2019. Using a combination of PLFA and DNA-based sequencing analyses to detect shifts in the soil microbial community composition after a simulated spring precipitation in a semi-arid grassland in China. Science of the Total Environment 657:1237–1245.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–66.

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R. D., Wardle, D. A. & Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science, 339(6127), 1615-1618.

Cline, L. C. and D. R. Zak. 2014. Dispersal limitation structures fungal community assembly in a long-term glacial chronosequence. Environmental microbiology 16:1538–1548.

Compant, S., M. G. A. Van Der Heijden and A. Sessitsch. 2010. Climate change effects on beneficial plant-microorganism interactions. FEMS Microbiology Ecology 73:197–214.

Cook-Patton, S. C. and T. L. Bauerle. 2012. Potential benefits of plant diversity on vegetated roofs: A literature review. Journal of Environmental Management 106:85–92.

Correia, M., R. Heleno, L. P. da Silva, J. M. Costa and S. Rodríguez-Echeverría. 2019. First evidence for the joint dispersal of mycorrhizal fungi and plant diaspores by birds. New Phytologist 222:1054–1060.

Cotrufo, M. F., M. D. Wallenstein, C. M. Boot, K. Denef and E. Paul. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: Do labile plant inputs form stable soil organic matter? Global Change Biology 19:988–995.

Czemiel Berndtsson, J. 2010. Green roof performance towards management of runoff water quantity and quality: A review. Ecological Engineering 36:351–360.

Dalpé, Y. and S. M. Séguin. 2013. Microwave-assisted technology for the clearing and staining of arbuscular mycorrhizal fungi in roots. Mycorrhiza 23:333–340.

Davis, K. E. R., P. Sangwan and P. H. Janssen. 2011. Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony-forming soil bacteria. Environmental Microbiology 13:798–805.

Davison, J., L. Ainsaar, S. Burla, A. G. Diedhiou, I. Hiiesalu, T. Jairus, N. C. Johnson, A. Kane, K. Koorem, M. Kochar, C. Ndiaye, R. Singh, M. Vasar and M. Zobel. 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science, 349: 970-973.

Davison, J., M. Moora, M. Öpik, L. Ainsaar, M. Ducousso, I. Hiiesalu, T. Jairus, N. Johnson, P. Jourand, R. Kalamees, K. Koorem, J. Y. Meyer, K. Püssa, Ü. Reier, M. Pärtel, M. Semchenko, A. Traveset, M. Vasar and M. Zobel. 2018. Microbial island biogeography: isolation shapes the life history characteristics but not diversity of root-symbiotic fungal communities. ISME Journal 12:2211–2224.

Davison, J., M. Moora, M. Semchenko, S. B. Adenan, T. Ahmed, A. A. Akhmetzhanova, J. M. Alatalo, S. Al-Quraishy, E. Andriyanova, S. Anslan, M. Bahram, A. Batbaatar, C. Brown, C. G. Bueno, J. Cahill, J. J. Cantero, B. B. Casper, M. Cherosov, S. Chideh, A. P. Coelho, M. Coghill, G. Decocq, S. Dudov, E. C. Fabiano, V. E. Fedosov, L. Fraser, S. I. Glassman, A. Helm, H. A. L. Henry, B. Hérault, I. Hiiesalu, I. Hiiesalu, W. N. Hozzein, P. Kohout, U. Kõljalg, K. Koorem, L. Laanisto, Ü. Mander, L. Mucina, J. P. Munyampundu, L. Neuenkamp, Ü. Niinemets, C. Nyamukondiwa, J. Oja, V. Onipchenko, M. Pärtel, C. Phosri, S. Põlme, K. Püssa, A. Ronk, A. Saitta, O. Semboli, S. K. Sepp, A. Seregin, S. Sudheer, C. P. Peña-Venegas, C. Paz, T. Vahter, M. Vasar, A. J. Veraart, L. Tedersoo, M. Zobel and M. Öpik. 2021. Temperature and pH define the realised niche space of arbuscular mycorrhizal fungi. New Phytologist.

Davison, J., M. Öpik, M. Zobel, M. Vasar, M. Metsis and M. Moora. 2012. Communities of arbuscular mycorrhizal fungi detected in forest soil are spatially heterogeneous but do not vary throughout the growing season. PLoS ONE 7.

Davison, J., M. Moora, M. Öpik, A. Adholeya, L. Ainsaar, A. Bâ, S. Burla, A. G. Diedhiou, I. Hiiesalu, T. Jairus, N. C. Johnson, A. Kane, K. Koorem, M. Kochar, C. Ndiaye, M. Pärtel, Ü. Reier, Ü. Saks, R. Singh, M. Vasar and M. Zobel. 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349: 970– 973

de Bello, F., S. Lavorel, S. Díaz, R. Harrington, J. H. C. Cornelissen, R. D. Bardgett, M. P. Berg, P. Cipriotti, C. K. Feld, D. Hering, P. M. da Silva, S. G. Potts, L. Sandin, J. P. Sousa, J. Storkey, D. A. Wardle and P. A. Harrison. 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodiversity and Conservation 19:2873–2893.

De Cáceres, M., F. Jansen and N. Dell. 2020. Package 'indicspecies'. Relationship between species and groups of sites. R package version, 1(7).

De Kimpe, C. R. and J. L. Morel. 2000. Urban soil management: a growing concern. Soil science, 165 : 31-40.

De La Providencia, I. E., F. A. De Souza, F. Fernández, N. S. Delmas and S. Declerck. 2005. Arbuscular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. New Phytologist 165:261–271.

de Vries, F. T., A. Williams, F. Stringer, R. Willcocks, R. McEwing, H. Langridge and A. L. Straathof. 2019. Changes in root-exudate-induced respiration reveal a novel mechanism through which drought affects ecosystem carbon cycling. New Phytologist 224:132–145.

de Vries, F. T., R. I. Griffiths, M. Bailey, H. Craig, M. Girlanda, H. S. Gweon, S. Hallin, A. Kaisermann, A. M. Keith, M. Kretzschmar, P. Lemanceau, E. Lumini, K. E. Mason, A. Oliver, N. Ostle, J. I. Prosser, C. Thion, B. Thomson and R. D. Bardgett. 2018. Soil bacterial networks are less stable under drought than fungal networks. Nature Communications 9.

de Vries, F. T., R. I. Griffiths, M. Bailey, H. Craig, M. Girlanda, H. S. Gweon, S. Hallin, A. Kaisermann, A. M. Keith, M. Kretzschmar, P. Lemanceau, E. Lumini, K. E. Mason, A. Oliver, N. Ostle, J. I. Prosser, C. Thion, B. Thomson and R. D. Bardgett. 2018. Soil bacterial networks are less stable under drought than fungal networks. Nature Communications 9.

Dechezleprêtre, A., N. Rivers and B. Stadler. 2020. The economic cost of air pollution: Evidence from Europe. OECD Economics Department Working Paper 1584.

Deeb, M., P. M. Groffman, J. L. Joyner, G. Lozefski, A. Paltseva, B. Lin, K. Mania, D. L. Cao, J. McLaughlin, T. Muth, B. Prithiviraj, J. Kerwin and Z. Cheng. 2018. Soil and microbial properties of green infrastructure stormwater management systems. Ecological Engineering 125:68–75.

Delavaux, C. S., L. M. Smith-Ramesh and S. E. Kuebbing. 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. Ecology 98:2111–2119.

Delgado-Baquerizo, M., F. T. Maestre, P. B. Reich, T. C. Jeffries, J. J. Gaitan, D. Encinar, M. Berdugo, C. D. Campbell and B. K. Singh. 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7:1–8.

Delgado-Baquerizo, M., P. B. Reich, A. N. Khachane, C. D. Campbell, N. Thomas, T. E. Freitag, W. Abu Al-Soud, S. Sørensen, R. D. Bardgett and B. K. Singh. 2017. It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environmental Microbiology 19:1176–1188.

Diamond, J. M. 1969. Avifaunal equilibria and species turnover rates on the Channel Islands of California. Proceedings of the National Academy of Sciences 64:57-63.

Díaz, S., S. Lavorel, F. De Bello, F. Quétier, K. Grigulis and T. M. Robson. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences of the United States of America 104:20684–20689.

Dickie, I. A., T. Fukami, J. P. Wilkie, R. B. Allen and P. K. Buchanan. 2012. Do assembly history effects attenuate from species to ecosystem properties? A field test with wood-inhabiting fungi. Ecology Letters 15:133–141.

Dodd, J. C., T. A. Dougall, J. P. Clapp and P. Jeffries. 2002. The role of arbuscular mycorrhizal fungi in plant community establishment at Samphire Hoe, Kent, UK - The reclamation platform created during the building of the Channel tunnel between France and the UK. Biodiversity and Conservation 11:39–58.

Dotzler, N., C. Walker, M. Krings, H. Hass, H. Kerp, T. N. Taylor and R. Agerer. 2009. Acaulosporoid glomeromycotan spores with a germination shield from the 400-million-year-old Rhynie chert. Mycological Progress 8:9–18.

Dumbrell, A. J., M. Nelson, T. Helgason, C. Dytham and A. H. Fitter. 2010. Idiosyncrasy and overdominance in the structure of natural communities of arbuscular mycorrhizal fungi: Is there a role for stochastic processes? Journal of Ecology 98:419–428.

Dumbrell, A. J., P. D. Ashton, N. Aziz, G. Feng, M. Nelson, C. Dytham, A. H. Fitter and T. Helgason. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190:794–804.

Dunnett, N., A. Nagase, R. Booth and P. Grime. 2008. Influence of vegetation composition on runoff in two simulated green roof experiments. Urban Ecosystems 11:385–398.

Dvorak, B. and A. Volder. 2010. Green roof vegetation for North American ecoregions: A literature review. Landscape and Urban Planning 96:197–213.

e Silva, M. C. P., A. C. F. Dias, J. D. van Elsas and J. F. Salles. 2012. Spatial and Temporal Variation of Archaeal, Bacterial and Fungal Communities in Agricultural Soils. PLoS ONE 7.

Egan, C., D. W. Li and J. Klironomos. 2014. Detection of arbuscular mycorrhizal fungal spores in the air across different biomes and ecoregions. Fungal Ecology 12:26–31.

Engelmoer, D. J. P., J. E. Behm and E. Toby Kiers. 2014. Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance. Molecular Ecology 23:1584–1593.

Evans, S. E. and M. D. Wallenstein. 2014. Climate change alters ecological strategies of soil bacteria. Ecology Letters 17:155–164.

Evans, S., J. B. H. Martiny and S. D. Allison. 2017. Effects of dispersal and selection on stochastic assembly in microbial communities. ISME Journal 11:176–185.

Farrell, C., X. Q. Ang and J. P. Rayner. 2013. Water-retention additives increase plant available water in green roof substrates. Ecological Engineering 52:112–118.

Feijen, F. A. A., R. A. Vos, J. Nuytinck and V. S. F. T. Merckx. 2018. Evolutionary dynamics of mycorrhizal symbiosis in land plant diversification. Scientific Reports 8:1–7.

Ferenci, T. 2016. Trade-off Mechanisms Shaping the Diversity of Bacteria. Trends in Microbiology 24:209–223.

Fierer, N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nature Reviews Microbiology 15:579–590.

Fierer, N., C. L. Lauber, K. S. Ramirez, J. Zaneveld, M. A. Bradford and R. Knight. 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME Journal 6:1007–1017.

Fierer, N., M. A. Bradford and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria. Ecology 88:1354-1364.

Fierer, N., M. S. Strickland, D. Liptzin, M. A. Bradford and C. C. Cleveland. 2009. Global patterns in belowground communities. Ecology Letters 12:1238–1249.

Finlay, B. J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063.

Finlay, B. J., G. F. Esteban, K. J. Clarke and J. L. Olmo. 2001. Biodiversity of terrestrial protozoa appears homoqeneous across local and global spatial scales. Protist 152:355–366.

Fitter, A. H. 2005. Darkness visible: reflections on underground ecology. Journal of Ecology, 93:231-243.

Fitzsimons, M. S., R. M. Miller and J. D. Jastrow. 2008. Scale-dependent niche axes of arbuscular mycorrhizal fungi. Oecologia 158:117–127.

Flynn, D. F. B., N. Mirotchnick, M. Jain, M. I. Palmer and S. Naeem. 2011. Functional and phylogenetic diversity as predictors of biodiversity- Ecosystem-function relationships. Ecology 92:1573–1581.

Franzaring, J., L. Steffan, W. Ansel, R. Walker and A. Fangmeier. 2016. Water retention, wash-out, substrate and surface temperatures of extensive green roof mesocosms—Results from a two year study in SW-Germany. Ecological Engineering 94:503–515.

Friesen, M. L. 2013. Microbially Mediated Plant Functional Traits. Molecular Microbial Ecology of the Rhizosphere 1:87–102.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan and R. B. Allen. 2010. Assembly history dictates ecosystem functioning: Evidence from wood decomposer communities. Ecology Letters 13:675–684.

Fulthorpe, R., J. S. MacIvor, P. Jia and S.-L. E. Yasui. 2018. The Green Roof Microbiome: Improving Plant Survival for Ecosystem Service Delivery. Frontiers in Ecology and Evolution 6:1–10.

Gai, J. P., P. Christie, X. B. Cai, J. Q. Fan, J. L. Zhang, G. Feng and X. L. Li. 2009. Occurrence and distribution of arbuscular mycorrhizal fungal species in three types of grassland community of the Tibetan Plateau. Ecological Research 24:1345–1350.

Garcia, M. O., P. H. Templer, P. O. Sorensen, R. Sanders-DeMott, P. M. Groffman and J. M. Bhatnagar. 2020. Soil Microbes Trade-Off Biogeochemical Cycling for Stress Tolerance Traits in Response to Year-Round Climate Change. Frontiers in Microbiology 11:1–18.

Gardes, M. and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118.

Gaston, K. J. and J. H. Lawton. 1990. Effects of Scale and Habitat on the Relationship between Regional Distribution and Local Abundance. Oikos 58:329–335.

Gaston, K. J., T. M. Blackburn and J. H. Lawton. 1997. Interspecific Abundance-Range Size Relationships: An Appraisal of Mechanisms. The Journal of Animal Ecology 66:579.

Gause, G. F. 1934. Experimental analysis of Vito Volterra's mathematical theory of the struggle for existence. Science, 79:16-17.

Gerhold, P., J. F. Cahill, M. Winter, I. V. Bartish and A. Prinzing. 2015. Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). Functional Ecology 29:600–614.

Getter, K. L., D. B. Rowe, J. A. Andresen and I. S. Wichman. 2011. Seasonal heat flux properties of an extensive green roof in a Midwestern U.S. climate. Energy and Buildings 43:3548–3557.

Gill, A. S., K. Purnell, M. I. Palmer, J. Stein and K. L. McGuire. 2020. Microbial Composition and Functional Diversity Differ Across Urban Green Infrastructure Types. Frontiers in Microbiology 11.

Gill, S. E., J. F. Handley, A. R. Ennos and S. Pauleit. 2018. Adapting cities for climate change: The role of the green infrastructure.Built environment 33:115-133.

Gregg, J. W., C. G. Jones and T. E. Dawson. 2003. Urbanization effects on tree growth in the vicinity of New York City. Nature 424:183–187.

Griffiths, B. S. and L. Philippot. 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 37:112–129.

Grime, J. P. 1973. Competitive exclusion in herbaceous vegetation. Nature 242:344-347.

Grime, J. P. 1977. Evidence for the Existence of Three Primary Strategies in Plants and Its Relevance to Ecological and Evolutionary Theory. The American Naturalist 111:1169–1194.

Grimm, N. B., S. H. Faeth, N. E. Golubiewski, C. L. Redman, J. Wu, X. Bai and J. M. Briggs. 2008. Global change and the ecology of cities. Science 319:756–760.

Guilland, C., P. A. Maron, O. Damas and L. Ranjard. 2018. Biodiversity of urban soils for sustainable cities. Environmental Chemistry Letters 16:1267–1282.

Guzman, L. M., R. M. Germain, C. Forbes, S. Straus, M. I. O'Connor, D. Gravel, D. S. Srivastava and P. L. Thompson. 2019. Towards a multi-trophic extension of metacommunity ecology. Ecology Letters 22:19–33.

Hanada, S. and Y. Sekiguchi. 2014. The phylum Gemmatimonadetes. In: The Prokaryotes: Other Major Lineages of Bacteria and the Archaea (eds Rosenberg, E., E. F. DeLong., S. Lory., E. Stackebrandt. and F. Thompson), pp. 677–681. Springer Berlin Heidelberg, Berlin, Heidelberg.

Hanson, C. A., J. A. Fuhrman, M. C. Horner-Devine and J. B. H. Martiny. 2012. Beyond biogeographic patterns: Processes shaping the microbial landscape. Nature Reviews Microbiology 10:497–506.

Hardy, O.J. 2008. Testing the spatial phylogenetic structure of local communities: statistical performances of different null models and test statistics on a locally neutral community. Journal of Ecology 96: 914– 926.

Hart, M. M. and R. J. Reader. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153:335–344.

Hart, M. M., R. J. Reader and J. N. Klironomos. 2001. Life-history strategies of arbuscular mycorrhizal fungi in relation to their successional dynamics. Mycologia, 93:1186-1194.

Hartmann, M., I. Brunner, F. Hagedorn, R. D. Bardgett, B. Stierli, C. Herzog, X. Chen, A. Zingg, E. Graf-Pannatier, A. Rigling and B. Frey. 2017. A decade of irrigation transforms the soil microbiome of a semi-arid pine forest. Molecular Ecology 26:1190–1206.

Hausmann, N. T. and C. V. Hawkes. 2010. Order of plant host establishment alters the composition of arbuscular mycorrhizal communities. Ecology 91:2333–2343.

Hazard, C., P. Gosling, C. J. Van Der Gast, D. T. Mitchell, F. M. Doohan and G. D. Bending. 2013. The role of local environment and geographical distance in determining community composition of arbuscular mycorrhizal fungi at the landscape scale. ISME Journal 7:498–508.

Heim, A. and J. Lundholm. 2014. Species interactions in green roof vegetation suggest complementary planting mixtures. Landscape and Urban Planning 130:125–133.

Helgason, T. and A. H. Fitter. 2009. Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany 60:2465–2480.

Helgason, T., J. W. Merryweather, J. Denison, P. Wilson, J. P. W. Young and A. H. Fitter. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. Journal of Ecology 90:371–384.

Helgason, T., J. W. Merryweather, J. P. W. Young and A. H. Fitter. 2007. Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community. Journal of Ecology 95:623–630.

Hildebrandt, U., M. Regvar and H. Bothe. 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146.

Ho, A., D. P. Di Lonardo and P. L. E. Bodelier. 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS microbiology ecology 93:1–14.

Hobbs, R. J., S. Arico, J. Aronson, J. S. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vilà, R. Zamora and M. Zobel. 2006. Novel ecosystems: Theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography 15:1–7.

Hoch, J. M. K., M. E. Rhodes, K. L. Shek, D. Dinwiddie, T. C. Hiebert, A. S. Gill, A. E. S. Estrada, K. L. Griffin, M. I. Palmer and K. L. McGuire. 2019. Soil microbial assemblages are linked to plant community composition and contribute to ecosystem services on urban green roofs. Frontiers in Ecology and Evolution 7.

Hood-Nowotny, R., N. Hinko-Najera Umana, E. Inselbacher, P. Oswald-Lachouani and W. Wanek. 2010. Alternative methods for measuring inorganic, organic and total dissolved nitrogen in soil. Soil Science Society of America Journal 74:1018–1027.

Hoogsteen, M. J. J., E. A. Lantinga, E. J. Bakker, J. C. J. Groot and P. A. Tittonell. 2015. Estimating soil organic carbon through loss on ignition: Effects of ignition conditions and structural water loss. European Journal of Soil Science 66:320–328.

Hooper, D. U., E. C. Adair, B. J. Cardinale, J. E. K. Byrnes, B. A. Hungate, K. L. Matulich, A. Gonzalez, J. E. Duffy, L. Gamfeldt and M. I. Connor. 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105–108.

Hui, N., A. Jumpponen, G. Francini, D. J. Kotze, X. Liu, M. Romantschuk, R. Strömmer and H. Setälä. 2017. Soil microbial communities are shaped by vegetation type and park age in cities under cold climate. Environmental Microbiology 19:1281–1295.

Husband, R., E. A. Herre, S. L. Turner, R. Gallery and J. P. W. Young. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Molecular ecology 11:2669–78.

Janos, D. P., C. T. Sahley and L. H. Emmons. (1995). Rodent dispersal of vesicular-arbuscular mycorrhizal fungi in Amazonian Peru. Ecology, 76:1852-1858.

Janusz, G., A. Pawlik, J. Sulej, U. Świderska-Burek, A. Jarosz-Wilkolazka and A. Paszczyński. 2017. Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiology Reviews 41:941–962.

Jayne, B. and M. Quigley. 2014. Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: a meta-analysis. Mycorrhiza 24:109–119.

John, J., G. Kernaghan and J. Lundholm. 2017. The potential for mycorrhizae to improve green roof function. Urban ecosystems 20:113-127.

John, J., J. Lundholm and G. Kernaghan. 2014. Colonization of green roof plants by mycorrhizal and root endophytic fungi. Ecological Engineering 71:651–659.

Johnson, C., S. Schweinhart and I. Buffam. 2016. Plant species richness enhances nitrogen retention in green roof plots. Ecological Applications 26:2130–2144.

Johnson, N. C., D. Tilman and D. Wedin. 1992. Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–2042.

Jongmans, A. G., N. Van Breemen, U. Lundström, P. A. W. Van Hees, R. D. Finlay, M. Srinivasan, T. Unestam, R. Giesler, P. -A. Melkerud and M. Olsson. 1997. Rock-eating fungi. Nature, 389:682-683.

Jongmans, A. G., van B. N, L. U, van H. P. A. W, R. Giesler, M. Olsson and P.-A. Melkerud. 1997. Rock-eating fungi. Nature 389:682–683.

Joyner, J. L., J. Kerwin, M. Deeb, G. Lozefski, B. Prithiviraj, A. Paltseva, J. McLaughlin, P. Groffman, Z. Cheng and T. R. Muth. 2019. Green infrastructure design influences communities of urban soil bacteria. Frontiers in Microbiology 10.

Kaiser, K., B. Wemheuer, V. Korolkow, F. Wemheuer, H. Nacke, I. Schöning, M. Schrumpf and R. Daniel. 2016. Driving forces of soil bacterial community structure, diversity and function in temperate grasslands and forests. Scientific Reports 6:1–12.

Kaisermann, A., P. A. Maron, L. Beaumelle and J. C. Lata. 2015. Fungal communities are more sensitive indicators to non-extreme soil moisture variations than bacterial communities. Applied Soil Ecology 86:158–164.

Kavamura, V. N. and E. Esposito. 2010. Biotechnological strategies applied to the decontamination of soils polluted with heavy metals. Biotechnology Advances 28:61–69.

Kawahara, A., G. H. An, S. Miyakawa, J. Sonoda and T. Ezawa. 2016. Nestedness in arbuscular mycorrhizal fungal communities along soil pH gradients in early primary succession: Acid-tolerant fungi are pH generalists. PLoS ONE 11:1–20.

Kaye, J. P., P. M. Groffman, N. B. Grimm, L. A. Baker and R. V. Pouyat. 2006. A distinct urban biogeochemistry? Trends in Ecology and Evolution 21:192–199.

Kembel, S.W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg and C. O. Webb. (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics, 26, 1463–1464.

Kennedy, A. C., T. L. Stubbs, J. Hall and W. State. 2006. Soil Microbial Communities 45:287–308.

Kibblewhite, M. G., K. Ritz and M. J. Swift. 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B: Biological Sciences 363:685–701.

Kivlin, S. N., C. V. Hawkes and K. K. Treseder. 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. Soil Biology and Biochemistry 43:2294–2303.

Kivlin, S. N., G. C. Winston, M. L. Goulden and K. K. Treseder. 2014. Environmental filtering affects soil fungal community composition more than dispersal limitation at regional scales. Fungal Ecology 12:14–25.

Klironomos, J. N. and M. M. Hart. 2002. Colonization of roots by arbuscular mycorrhizal fungi using different sources of inoculum. Mycorrhiza 12:181–184.

Koziol, L. and J. D. Bever. 2016. AMF, phylogeny and succession: Specificity of response to mycorrhizal fungi increases for late-successional plants. Ecosphere 7:1–11.

Kraft, N. J. B., W. K. Cornwell, C. O. Webb and D. D. Ackerly. 2007. Trait evolution, community assembly and the phylogenetic structure of ecological communities. American Naturalist 170:271–283.

Krause, S., X. Le Roux, P. A. Niklaus, P. M. van Bodegom, J. T. Lennon T., S. Bertilsson, H. P. Grossart, L. Philippot and P. L. E. Bodelier. 2014. Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. Frontiers in Microbiology 5:1–10.

Laforest-Lapointe, I., C. Messier and S. W. Kembel. 2017. Tree leaf bacterial community structure and diversity differ along a gradient of urban intensity. MSystems 2:1–16.

Laliberté, E. 2017. Below-ground frontiers in trait-based plant ecology. New Phytologist 213:1597–1603.

Lau, J. A. and J. T. Lennon. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences of the United States of America 109:14058–14062.

Lauber, C. L., M. Hamady, R. Knight and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Lauber, C. L., M. Hamady, R. Knight and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111–5120.

Lauber, C. L., M. S. Strickland, M. A. Bradford and N. Fierer. 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40:2407–2415.

Law, R. and D. H. Lewis. 1983. Biotic environments and the maintenance of sex–some evidence from mutualistic symbioses. Biological Journal of the Linnean Society 20:249–276.

Lee, J., S. Lee and J. P. W. Young. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65:339–349.

Legendre, P. and L. Legendre. 2012. Numerical ecology. Elsevier.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau and A. Gonzalez. 2004. The metacommunity concept: A framework for multi-scale community ecology. Ecology Letters 7:601–613.

Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau and A. Gonzalez. 2004. The metacommunity concept: A framework for multi-scale community ecology. Ecology Letters 7:601–613.

Lekberg, Y. and L. P. Waller. 2016. What drives differences in arbuscular mycorrhizal fungal communities among plant species? Fungal Ecology 24:135–138.

Lekberg, Y., J. Meadow, J. R. Rohr, D. Redecker and C. A. Zabinski. 2011. Importance of dispersal and thermal environment for mycorrhizal communities: lessons from Yellowstone National Park. Ecology, 92:1292-1302.

Lessard, J. P., M. K. Borregaard, J. A. Fordyce, C. Rahbek, M. D. Weiser, R. R. Dunn and N. J. Sanders. 2012. Strong influence of regional species pools on continent-wide structuring of local communities. Proceedings of the Royal Society B: Biological Sciences 279:266–274.

Li, H., Y. Chen, G. Yu, F. Rossi, R. De, X. Cheng, W. Wang, R. Li and N. Sciences. 2019. Multifaceted diversity traits of crucial microbial groups in biological soil crusts promote soil multifunctionality:1–36.

Li, S. peng, P. Wang, Y. Chen, M. C. Wilson, X. Yang, C. Ma, J. Lu, X. yong Chen, J. Wu, W. sheng Shu and L. Jiang. 2020. Island biogeography of soil bacteria and fungi: similar patterns, but different mechanisms. ISME Journal 14:1886–1896.

Lin, L., Y. Chen, G. Xu, Y. Zhang, S. Zhang and K. Ma. 2021. Impacts of Urbanization Undermine Nestedness of the Plant–Arbuscular Mycorrhizal Fungal Network. Frontiers in Microbiology 12:1–13.

Lovett, G. M., M. M. Traynor, R. V. Pouyat, M. M. Carreiro, W. X. Zhu and J. W. Baxter. 2000. Atmospheric deposition to oak forests along an urban - Rural gradient. Environmental Science and Technology 34:4294–4300.

Luck, G. W., R. Harrington, P. A. Harrison, C. Kremen, P. M. Berry, R. Bugter, T. R. Dawson, F. De Bello, S. Diaz, C. K. Feld, J. R. Haslett, D. Hering, A. Kontogianni, S. Lavorel, M. Rounsevell, M. J. Samways, L. Sandin, J. Settele, M. T. Sykes, S. Van Den Hove, M. Vandewalle and M. Zobel. 2009. Quantifying the contribution of organisms to the provision of ecosystem services. BioScience 59:223–235.

Lundholm, J. T. 2015. Green roof plant species diversity improves ecosystem multifunctionality. Journal of Applied Ecology 52:726–734.

Lundholm, J., J. S. MacIvor, Z. MacDougall and M. Ranalli. 2010. Plant species and functional group combinations affect green roof ecosystem functions. PLoS ONE 5.

Lundholm, J., S. Tran and L. Gebert. 2015. Plant functional traits predict green roof ecosystem services. Environmental Science and Technology 49:2366–2374.

MacArthur, R. H. and E. O. Wilson. (2001). The theory of island biogeography (Vol. 1). Princeton university press.

Maestre, F. T., J. L. Quero, N. J. Gotelli, A. Escudero, M. Delgado-baquerizo, M. García-gómez, M. A. Bowker, S. Soliveres, C. Escolar, P. García-palacios, M. Berdugo, B. Gozalo, A. Gallardo, L. Aguilera, T. Arredondo, B. Boeken, D. Bran, A. A. Conceição and O. Cabrera. 2013. Europe PMC Funders Group Plant species richness and ecosystem multifunctionality in global drylands 335:214–218.

Maestre, F. T., M. Delgado-Baquerizo, T. C. Jeffries, D. J. Eldridge, V. Ochoa, B. Gozalo, J. L. Quero, M. García-Gómez, A. Gallardo, W. Ulrich, M. A. Bowker, T. Arredondo, C. Barraza-Zepeda, D. Bran, A. Florentino, J. Gaitán, J. R. Gutiérrez, E. Huber-Sannwald, M. Jankju, R. L. Mau, M. Miriti, K. Naseri, A. Ospina, I. Stavi, D. Wang, N. N. Woods, X. Yuan, E. Zaady and B. K. Singh. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Sciences of the United States of America 112:15684–15689.

Maherali, H. and J. N. Klironomos. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748.

Maherali, H. and J. N. Klironomos. 2012. Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. PLoS ONE 7.

Mangan, S. A. and G. H. Adler. 2002. Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131:587–597.

Manzoni, S., J. P. Schimel and A. Porporato. 2012. Responses of soil microbial communities to water stress: Results from a meta-analysis. Ecology 93:930–938.

Marin, J. A., T. Hernandez and C. Garcia. 2005. Bioremediation of oil refinery sludge by landfarming in semiarid conditions: Influence on soil microbial activity. Environmental Research 98:185–195.

Matthews, T. J., H. E. W. Cottee-Jones and R. J. Whittaker. 2015. Quantifying and interpreting nestedness in habitat islands: A synthetic analysis of multiple datasets. Diversity and Distributions 21:392–404.

Mayer, H. 1999. Mayer: Cities. Atmospheric Environment 33:4029–4037.

McDonnell, M., S. Pickett, P. Groffman, P. Bohlen, R. Pouyat, W. Zipperer, R. Parmelee, M. Carreiro and K. Medley. 1997. Ecosystem processes along an urban-to-rural gradient. Urban Ecosystems 1:21–36.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New phytologist, 115: 495-501.

McGuire, K. L., S. G. Payne, G. Orazi and M. I. Palmer. 2015. Bacteria and fungi in green roof ecosystems. In Green Roof Ecosystems (pp. 175-191). Springer, Cham.

McGuire, K. L., S. G. Payne, M. I. Palmer, C. M. Gillikin, D. Keefe, S. J. Kim, S. M. Gedallovich, J. Discenza, R. Rangamannar, J. A. Koshner, A. L. Massmann, G. Orazi, A. Essene, J. W. Leff and N. Fierer. 2013. Digging the New York City Skyline: Soil Fungal Communities in Green Roofs and City Parks. PLoS ONE 8.

Meisner, A., S. Jacquiod, B. L. Snoek, F. C. Ten Hooven and W. H. van der Putten. 2018. Drought legacy effects on the composition of soil fungal and prokaryote communities. Frontiers in Microbiology 9:1–12.

Menge, B. A. and J. P. Sutherland. 1976. Species diversity gradients: synthesis of the roles of predation, competition and temporal heterogeneity. The American Naturalist, 110:351-369.

Miller, E. T., D. R. Farine and C. H. Trisos. 2017. Phylogenetic community structure metrics and null models: a review with new methods and software. Ecography 40:461–477.

Molineux, C. J., A. C. Gange and D. J. Newport. 2017. Using soil microbial inoculations to enhance substrate performance on extensive green roofs. Science of the Total Environment 580:846–856.

Molineux, C. J., A. C. Gange, S. P. Connop and D. J. Newport. 2015. Are microbial communities in green roof substrates comparable to those in post-industrial sites?—a preliminary study. Urban Ecosystems 18:1245–1260.

Molineux, C. J., S. P. Connop and A. C. Gange. 2014. Manipulating soil microbial communities in extensive green roof substrates. Science of the Total Environment 493:632–638.

Montesinos-Navarro, A., J. G. Segarra-Moragues, A. Valiente-Banuet and M. Verdú. 2012. The network structure of plant-arbuscular mycorrhizal fungi. New Phytologist 194:536–547.

Moora, M., M. Öpik, M. Metsis, T. J. Daniell and M. Zobel. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytologist 184:424–437.

Morgan, B. S. T. and L. M. Egerton-Warburton. 2017. Barcoded NS31/AML2 Primers for Sequencing of Arbuscular Mycorrhizal Communities in Environmental Samples. Applications in Plant Sciences 5:1700017.

Moyersoen, B., R. E. Beever and F. Martin. 2003. Genetic diversity of Pisolithus in New Zealand indicates multiple long-distance dispersal from Australia. New Phytologist 160:569–579.

Mummey, D. L., P. M. Antunes and M. C. Rillig. 2009. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. Soil Biology and Biochemistry 41:1173–1179.

Mummey, D. L., P. M. Antunes and M. C. Rillig. 2009. Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. Soil Biology and Biochemistry 41:1173–1179.

Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chimica acta 27:31-36.

Nemergut, D. R., S. K. Schmidt, T. Fukami, S. P. O'Neill, T. M. Bilinski, L. F. Stanish, J. E. Knelman, J. L. Darcy, R. C. Lynch, P. Wickey and S. Ferrenberg. 2013. Patterns and Processes of Microbial Community Assembly. Microbiology and Molecular Biology Reviews 77:342–356.

Newsham, K. K., Fitter, A. H., & Watkinson, A. R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. Journal of ecology, 991-1000.

Niemelä J, Breuste J, Guntenspergen G, McIntyre N, Elmqvist T. (2011). Urban Ecology: Patterns, Processes and Applications. Oxford University Press: New York,USA.

Oberndorfer, E., J. Lundholm, B. Bass, R. R. Coffman, H. Doshi, N. Dunnett, S. Gaffin, M. Köhler, K. K. Y. Liu and B. Rowe. 2007. Green Roofs as Urban Ecosystems: Ecological Structures, Functions and Services. BioScience 57:823–833.

Oehl, F., E. Sieverding, K. Ineichen, P. Mäder, A. Wiemken and T. Boller. 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different

agroecosystems in long-term microcosms. Agriculture, Ecosystems and Environment 134:257–268.

Oehl, F., E. Sieverding, K. Ineichen, P. Mäder, T. Boller and A. Wiemken. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. Applied and Environmental Microbiology 69:2816–2824.

Oke, T. R. 1982. The energetic basis of the urban heat island. Quarterly Journal of the Royal Meteorological Society, 108: 1-24.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. Mcglinn and M. H. H. Steven. 2020. Package—vegan: Community ecology package. R package version 2.5-6.

Ortiz, N., E. Armada, E. Duque, A. Roldán and R. Azcón. 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. Journal of Plant Physiology 174:87–96.

Partel, M., M. Zobel, K. Zobel and E. Van Der Maarel. 1996. The Species Pool and Its Relation to Species Richness : Evidence from Estonian Plant Communities. Oikos 75:111–117.

Paterson, E., T. Gebbing, C. Abel, A. Sim and G. Telfer. 2007. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytologist 173:600–610.

Peay, K. G., M. G. Schubert, N. H. Nguyen and T. D. Bruns. 2012. Measuring ectomycorrhizal fungal dispersal: Macroecological patterns driven by microscopic propagules. Molecular Ecology 21:4122–4136.

Peay, K. G., M. Garbelotto and T. D. Bruns. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. Ecology 91:3631–3640.

Peay, K. G., M. Garbelotto and T. D. Bruns. 2010. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. Ecology 91:3631–3640.

Peay, K. G., T. D. Bruns, P. G. Kennedy, S. E. Bergemann and M. Garbelotto. 2007. A strong species-area relationship for eukaryotic soil microbes: Island size matters for ectomycorrhizal fungi. Ecology Letters 10:470–480.

Pester, M., C. Schleper and M. Wagner. 2011. The Thaumarchaeota: An emerging view of their phylogeny and ecophysiology. Current Opinion in Microbiology 14:300–306.

Philippot, L., J. M. Raaijmakers, P. Lemanceau and W. H. Van Der Putten. 2013. Going back to the roots: The microbial ecology of the rhizosphere. Nature Reviews Microbiology 11:789–799.

Philippot, L., S. G. E. Andersson, T. J. Battin, J. I. Prosser, J. P. Schimel, W. B. Whitman and S. Hallin. 2010. The ecological coherence of high bacterial taxonomic ranks. Nature Reviews Microbiology 8:523–529.

Pianka, E. R. 1970. On r-and K-selection. The american naturalist, 104:592-597.

Pierce, S., A. Vianelli and B. Cerabolini. 2005. From ancient genes to modern communities: The cellular stress response and the evolution of plant strategies. Functional Ecology 19:763–776.

Pirozynki, K. and Y. Dalpé. 1989. Geological history of the Glomaceae with particular reference to mycorrhizal symbiosis. Symbiosis 7:1–36.

Podani, J. and D. Schmera. 2011. A new conceptual and methodological framework for exploring and explaining pattern in presence–absence data. Oikos, 120:1625-1638.

Pope, C. A., M. J. Thun, M. M. Namboodiri, D. W. Dockery, J. S. Evans, F. E. Speizer and C. W. Heath. 1995. Particulate air pollution as a predictor of mortality in a prospective study of U.S. Adults. American Journal of Respiratory and Critical Care Medicine 151:669–674.

Powell, J. R. and A. E. Bennett. 2016. Unpredictable assembly of arbuscular mycorrhizal fungal communities. Pedobiologia 59:11–15.

Powell, J. R., J. L. Parrent, M. M. Hart, J. N. Klironomos, M. C. Rillig and H. Maherali. 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proceedings of the Royal Society B: Biological Sciences 276:4237–4245.

Powell, J. R., J. L. Parrent, M. M. Hart, J. N. Klironomos, M. C. Rillig and H. Maherali..

Powell, J. R., S. Karunaratne, C. D. Campbell, H. Yao, L. Robinson and B. K. Singh. 2015. Deterministic processes vary during community assembly for ecologically dissimilar taxa. Nature Communications 6.

Powell, J. R., S. Karunaratne, C. D. Campbell, H. Yao, L. Robinson and B. K. Singh. 2015. Deterministic processes vary during community assembly for ecologically dissimilar taxa. Nature Communications 6:1-10.

Prober, S. M., J. W. Leff, S. T. Bates, E. T. Borer, J. Firn, W. S. Harpole, E. M. Lind, E. W. Seabloom, P. B. Adler, J. D. Bakker, E. E. Cleland, N. M. Decrappeo, E. Delorenze, N.

Hagenah, Y. Hautier, K. S. Hofmockel, K. P. Kirkman, J. M. H. Knops, K. J. La Pierre, A. S. Macdougall, R. L. Mcculley, C. E. Mitchell, A. C. Risch, M. Schuetz, C. J. Stevens, R. J. Williams and N. Fierer. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. Ecology Letters 18:85–95.

Pruesse, E, J. Peplies and F. O. Glöckner. 2012. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics 28: 1823-1829

Quah, E. and T. L. Boon. 2003. The economic cost of particulate air pollution on health in Singapore. Journal of Asian Economics 14:73–90.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research 41:590–596.

Ramirez, K. S., J. M. Craine and N. Fierer. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology 18:1918–1927.

Ranalli, M. and J. Lundholm. 2008. Biodiversity and ecosystem function in constructed ecosystems. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 3.

Reese, A. T., A. Savage, E. Youngsteadt, K. L. Mcguire, A. Koling, O. Watkins, S. D. Frank and R. R. Dunn. 2016. Urban stress is associated with variation in microbial species composition - but not richness - in Manhattan. ISME Journal 10:751–760.

Reich, P. B. 2014. The world-wide "fast-slow" plant economics spectrum: A traits manifesto. Journal of Ecology 102:275–301.

Rillig, M. C. and D. L. Mummey. 2006. Mycorrhizas and soil structure. New Phytologist 171:41–53.

Ripley, B.V., D. M. Bates, K. Hornik, A. Gebhardt, D. Firth, M. B. Ripley. 2013. Package 'MASS'.

Rizwan, A. M., Dennis, L. Y., & Chunho, L. I. U. (2008). A review on the generation, determination and mitigation of Urban Heat Island. Journal of environmental sciences, 20:120-128.

Rolfe, S. A., J. Griffiths and J. Ton. 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current Opinion in Microbiology 49:73–82.

Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (2014) The Prokaryotes: Actinobacteria. Springer Berlin Heidelberg, Berlin, Heidelberg.

Rowe, D. B. 2011. Green roofs as a means of pollution abatement. Environmental Pollution 159:2100–2110.

Ruiz-Lozano, J. M., R. Azcon and M. Gomez. 1995. Effects of arbuscular-mycorrhizal Glomus species on drought tolerance: Physiological and nutritional plant responses. Applied and Environmental Microbiology 61:456–460.

Ruiz-Lozano, J. M., R. Porcel, G. Bárzana, R. Azcón and R. Aroca. 2012. Contribution of arbuscular mycorrhizal symbiosis to plant drought tolerance: state of the art. In Plant responses to drought stress (pp. 335-362). Springer, Berlin, Heidelberg.

Rumble, H. and A. C. Gange. 2009. Soil microarthropod community dynamics in extensive green roofs 44:1–40.

Rumble, H., P. Finch and A. C. Gange. 2018. Green roof soil organisms: Anthropogenic assemblages or natural communities? Applied Soil Ecology 126:11–20.

Ruth, B., M. Khalvati and U. Schmidhalter. 2011. Quantification of Mycorrhizal water uptake via high-resolution on-line water content sensors. Plant and Soil 342:459–468.

Saadatian, O., K. Sopian, E. Salleh, C. H. Lim, S. Riffat, E. Saadatian, A. Toudeshki and M. Y. Sulaiman. 2013. A review of energy aspects of green roofs. Renewable and Sustainable Energy Reviews 23:155–168.

Sailor, D. J. 2008. A green roof model for building energy simulation programs. Energy and Buildings 40:1466–1478.

Saks, Ü., J. Davison, M. Öpik, M. Vasar, M. Moora and M. Zobel. 2014. Root-colonizing and soil-borne communities of arbuscular mycorrhizal fungi in a temperate forest understorey. Botany 92:277–285.

Schellenbaum, L., J. Müller, T. Boller, A. Wiemken and H. Schüepp. 1998. Effects of drought on non-mycorrhizal and mycorrhizal maize: Changes in the pools of non-structural carbohydrates, in the activities of invertase and trehalase and in the pools of amino acids and imino acids. New Phytologist 138:59–66.

Schlaeppi, K., S. F. Bender, F. Mascher, G. Russo, A. Patrignani, T. Camenzind, S. Hempel, M. C. Rillig and M. G. A. van der Heijden. 2016. High-resolution community profiling of arbuscular mycorrhizal fungi. New Phytologist 212:780–791.

Schlatter, D., L. Kinkel, L. Thomashow, D. Weller and T. Paulitz. 2017. Disease suppressive soils: New insights from the soil microbiome. Phytopathology 107:1284–1297.

Schloss, P. D., M. L. Jenior, C. C. Koumpouras, S. L. Westcott and S. K. Highlander. 2016. Sequencing 16S rRNA gene fragments using the PacBio SMRT DNA sequencing system. PeerJ 2016:1–16.

Schröder, R., M. Mohri and K. Kiehl. 2019. AMF inoculation of green roof substrate improves plant performance but reduces drought resistance of native dry grassland species. Ecological Engineering 139:105583.

Schueler, T. (1994) The importance of imperviousness. Watershed Protect. Technol. 1, 100–111

Sepp, S. K., J. Davison, T. Jairus, M. Vasar, M. Moora, M. Zobel and M. Öpik. 2019. Non-random association patterns in a plant–mycorrhizal fungal network reveal host–symbiont specificity. Molecular Ecology 28:365–378.

Setälä, H. and M. A. McLean. 2004. Decomposition rate of organic substrates in relation to the species diversity of soil saprophytic fungi. Oecologia 139:98–107.

Shafique, M., R. Kim and M. Rafiq. 2018. Green roof benefits, opportunities and challenges – A review. Renewable and Sustainable Energy Reviews 90:757–773.

Sharma, A., P. Conry, H. J. S. Fernando, A. F. Hamlet, J. J. Hellmann and F. Chen. 2016. Green and cool roofs to mitigate urban heat island effects in the Chicago metropolitan area: Evaluation with a regional climate model. Environmental Research Letters 11.

Shochat, E., P. S. Warren, S. H. Faeth, N. E. McIntyre and D. Hope. 2006. From patterns to emerging processes in mechanistic urban ecology. Trends in Ecology and Evolution 21:186–191.

Sikes, B. A., K. Cottenie and J. N. Klironomos. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97:1274–1280.

Six, J., C. Feller, K. Denef, S. Ogle, J. C. de Moraes Sa and A. Albrecht. 2002. Soil organic matter, biota and aggregation in temperate and tropical soils-Effects of no-tillage. Agronomie, 22: 755-775.

Six, J., S. D. Frey, R. K. Thiet and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70:555–569.

Šmilauer, P., J. Košnar, M. Kotilínek and M. Šmilauerová. 2020. Contrasting effects of host identity, plant community and local species pool on the composition and colonization levels of arbuscular mycorrhizal fungal community in a temperate grassland. New Phytologist 225:461–473.

Smith, S. E., & Read, D. J. (2008). Mycorrhizal symbiosis. Academic press.

Speak, A. F., J. J. Rothwell, S. J. Lindley and C. L. Smith. 2013. Reduction of the urban cooling effects of an intensive green roof due to vegetation damage. Urban Climate 3:40–55.

Staddon, P. L., R. Gregersen and I. Jakobsen. 2004. The response of two Glomus mycorrhizal fungi and a fine endophyte to elevated atmospheric CO2, soil warming and drought. Global Change Biology 10:1909–1921.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30: 1312-1313

Stovin, V., S. Poë and C. Berretta. 2013. A modelling study of long term green roof retention performance. Journal of Environmental Management 131:206–215.

Susca, T., S. R. Gaffin and G. R. Dell'Osso. 2011. Positive effects of vegetation: Urban heat island and green roofs. Environmental Pollution 159:2119–2126.

Sutton, R. K. 2008. Media Modifications For Native Plant Assemblages On Green Roofs. Sixth Annual Greening Rooftops for Sustainable Communities Conference, Awards and Trade Show:1–12.

Talbot, J. M., T. D. Bruns, J. W. Taylor, D. P. Smith, S. Branco, S. I. Glassman, S. Erlandson, R. Vilgalys, H. L. Liao, M. E. Smith and K. G. Peay. 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences of the United States of America 111:6341–6346.

Tedersoo, L. and B. Lindahl. 2016. Fungal identification biases in microbiome projects. Environmental Microbiology Reports 8:774–779.

Telford, R. J., V. Vandvik and H. J. B. Birks. 2006. Dispersal limitations matter for microbial morphospecies. Science 312:1015.

Tilman, D., S. S. Kilham and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. Annual review of ecology and systematics. 13:349–372.

Torrecillas, E., P. Torres, M. M. Alguacil, J. I. Querejeta and A. Roldán. 2013. Influence of habitat and climate variables on arbuscular mycorrhizal fungus community distribution, as revealed by a case study of facultative plant epiphytism under semiarid conditions. Applied and Environmental Microbiology 79:7203–7209.

Vályi, K., U. Mardhiah, M. C. Rillig and S. Hempel. 2016. Community assembly and coexistence in communities of arbuscular mycorrhizal fungi. ISME Journal 10:2341–2351.

van der Heijden, M. G. A., J. Klironomos, N. John, M. Ursic, P. Moutoglis, R. Streitwolf-engel, T. Boller, I. R. Sanders and J. Klironomos. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 74:69–72.

van der Heijden, M. G. A., R. D. Bardgett and N. M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Van Der Heijden, M. G. A., R. D. Bardgett and N. M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Van der Heijden, M. G. A., T. Boller, A. Wiemken and I. R. Sanders. 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091.

Vannier, N., A. K. Bittebiere, C. Mony and P. Vandenkoornhuyse. 2020. Root endophytic fungi impact host plant biomass and respond to plant composition at varying spatio-temporal scales. Fungal Ecology 44:100907.

Vanuytrecht, E., C. Van Mechelen, K. Van Meerbeek, P. Willems, M. Hermy and D. Raes. 2014. Runoff and vegetation stress of green roofs under different climate change scenarios. Landscape and Urban Planning 122:68–77.

VanWoert, N. D., D. B. Rowe, J. A. Andresen, C. L. Rugh, R. T. Fernandez and L. Xiao. 2005. Green Roof Stormwater Retention. Journal of Environmental Quality 34:1036–1044.

Vellend, M. 2010. Conceptual synthesis in community ecology. Quarterly Review of Biology 85:183–206.

Ventura, M., C. Canchaya, A. Tauch, G. Chandra, G. F. Fitzgerald, K. F. Chater and D. van Sinderen. 2007. Genomics of Actinobacteria: Tracing the Evolutionary History of an Ancient Phylum. Microbiology and Molecular Biology Reviews 71:495–548.

Vierheilig, H., A. P. Coughlan, U. Wyss and Y. Piché,. 1998. Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. Appl. Environ. Microbiol. 64, 5004–5007

von Rein, I., A. Gessler, K. Premke, C. Keitel, A. Ulrich and Z. E. Kayler. 2016. Forest understory plant and soil microbial response to an experimentally induced drought and heat-pulse event: the importance of maintaining the continuum. Global change biology 22:2861–2874.

Wagg, C., J. Jansa, M. Stadler, B. Schmid and M. G. A. Van Der Heijden. 2011. Mycorrhizal fungal identity and diversity relaxes plant-plant competition. Ecology 92:1303–1313.

Wagg, C., S. F. Bender, F. Widmer and M. G. A. Van Der Heijden. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 111:5266–5270.

Wall, D. H., R. D. Bardgett and E. Kelly. 2010. Biodiversity in the dark. Nature Geoscience 3:297–298.

Wall, D. H., V. Behan-Pelletier, K. Ritz, T. H. Jones, J. Six, D. T. Strong and W. H. van der Putten, W. H. 2012. Soil ecology and ecosystem services. Oxford University Press.

Wang, P., S. P. Li, X. Yang, J. Zhou, W. Shu and L. Jiang. 2020. Mechanisms of soil bacterial and fungal community assembly differ among and within islands. Environmental Microbiology 22:1559–1571.

Wang, Y., U. Naumann, S. T. Wright and D. I. Warton. 2012. Mvabund- an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3:471–474.

Ward, N. L., J. F. Challacombe, P. H. Janssen, B. Henrissat, P. M. Coutinho, M. Wu, G. Xie, D. H. Haft, M. Sait, J. Badger, R. D. Barabote, B. Bradley, T. S. Brettin, L. M. Brinkac, D. Bruce, T. Creasy, S. C. Daugherty, T. M. Davidsen, R. T. DeBoy, J. C. Detter, R. J. Dodson, A. S. Durkin, A. Ganapathy, M. Gwinn-Giglio, C. S. Han, H. Khouri, H. Kiss, S. P. Kothari, R. Madupu, K. E. Nelson, W. C. Nelson, I. Paulsen, K. Penn, Q. Ren, M. J. Rosovitz, J. D. Selengut, S. Shrivastava, S. A. Sullivan, R. Tapia, S. Thompson, K. L. Watkins, Q. Yang, C. Yu, N. Zafar, L. Zhou and C. R. Kuske. 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Applied and Environmental Microbiology 75:2046–2056.

Warner, R. R. and P. L. Chesson. 1985. Coexistence mediated by recruitment fluctuations: a field guide to the storage effect. The American Naturalist 125: 769-787.

Warton, D. I., S. T. Wright and Y. Wang. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods in Ecology and Evolution 3:89–101.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: An example for rain forest trees. American Naturalist 156:145–155.

Weemstra, M., L. Mommer, E. J. W. Visser, J. van Ruijven, T. W. Kuyper, G. M. J. Mohren and F. J. Sterck. 2016. Towards a multidimensional root trait framework: a tree root review. The New phytologist 211:1159–1169.

Williams, A. and F. T. de Vries. 2020. Plant root exudation under drought: implications for ecosystem functioning. New Phytologist 225:1899–1905.

Williams, N. S. G., J. Lundholm and J. Scott Macivor. 2014. Do green roofs help urban biodiversity conservation? Journal of Applied Ecology 51:1643–1649.

Wilson, G. W. T., C. W. Rice, M. C. Rillig, A. Springer and D. C. Hartnett. 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecology Letters 12:452–461.

Wilson, J. M. 1984. Competition for infection between vesicular-arbuscular mycorrhizal fungi. New Phytologist, 97: 427-435.

Wong, N. H., Y. Chen, C. L. Ong and A. Sia. 2003. Investigation of thermal benefits of rooftop garden in the tropical environment. Building and Environment 38:261–270.

Wright, I. J., P.B. Reich., M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J Midgley, M. -L. Navas, Ü. Niimets, J. Oleksyn, N. Osaka, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.

Xie, G., J. T. Lundholm and J. Scott MacIvor. 2018. Phylogenetic diversity and plant trait composition predict multiple ecosystem functions in green roofs. Science of the Total Environment 628:1017–1026.

Xu, H. J., S. Li, J. Q. Su, S. Nie, V. Gibson, H. Li and Y. G. Zhu. 2014. Does urbanization shape bacterial community composition in urban park soils? A case study in 16 representative Chinese cities based on the pyrosequencing method. FEMS Microbiology Ecology 87:182–192.

Yan, B., J. Li, N. Xiao, Y. Qi, G. Fu, G. Liu and M. Qiao. 2016. Urban-development-induced Changes in the Diversity and Composition of the Soil Bacterial Community in Beijing. Scientific Reports 6:2–10.

Yang, C., C. Hamel, M. P. Schellenberg, J. C. Perez and R. L. Berbara. 2010. Diversity and functionality of arbuscular mycorrhizal fungi in three plant communities in semiarid Grasslands National Park, Canada. Microbial Ecology 59:724–733.

Yang, J., Q. Yu and P. Gong. 2008. Quantifying air pollution removal by green roofs in Chicago. Atmospheric Environment 42:7266–7273.

Young, T., D. D. Cameron and G. K. Phoenix. 2015. Using AMF inoculum to improve the nutritional status of Prunella vulgaris plants in green roof substrate during establishment. Urban Forestry and Urban Greening 14:959–967.

Zak, D. R., K. S. Pregitzer, A. J. Burton, I. P. Edwards and H. Kellner. 2011. Microbial responses to a changing environment: Implications for the future functioning of terrestrial ecosystems. Fungal Ecology 4:386–395.

Zanne, A. E., K. Abarenkov, M. E. Afkhami, C. A. Aguilar-Trigueros, S. Bates, J. M. Bhatnagar, P. E. Busby, N. Christian, W. K. Cornwell, T. W. Crowther, H. Flores-Moreno, D. Floudas, R. Gazis, D. Hibbett, P. Kennedy, D. L. Lindner, D. S. Maynard, A. M. Milo, R. H. Nilsson, J. Powell, M. Schildhauer, J. Schilling and K. K. Treseder. 2020. Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. Biological Reviews 95:409–433.

Zhang, J., F. Wang, R. Che, P. Wang, H. Liu, B. Ji and X. Cui. 2016. Precipitation shapes communities of arbuscular mycorrhizal fungi in Tibetan alpine steppe. Scientific Reports 6:1–10.

Zhou, J. 2017. Stochastic Community Assembly : Does It. Microbiology and Molecular Biology Reviews 81:1–32.

Zhou, J. and J. J. Fong. 2021. Strong agricultural management effects on soil microbial community in a non-experimental agroecosystem. Applied Soil Ecology 165:103970.

Zobel, M. and M. Öpik. 2014. Plant and arbuscular mycorrhizal fungal (AMF) communities - which drives which? Journal of Vegetation Science 25:1133–1140.

Annexes

Annexe I : Figure S2.1

Fig S2.1: Map of the sampled sites across the island of Montreal (45.4-45.6 N and 73.5-73.8 W), urban parks are in green and green roofs are in blue

Annexe II: Figure S2.2

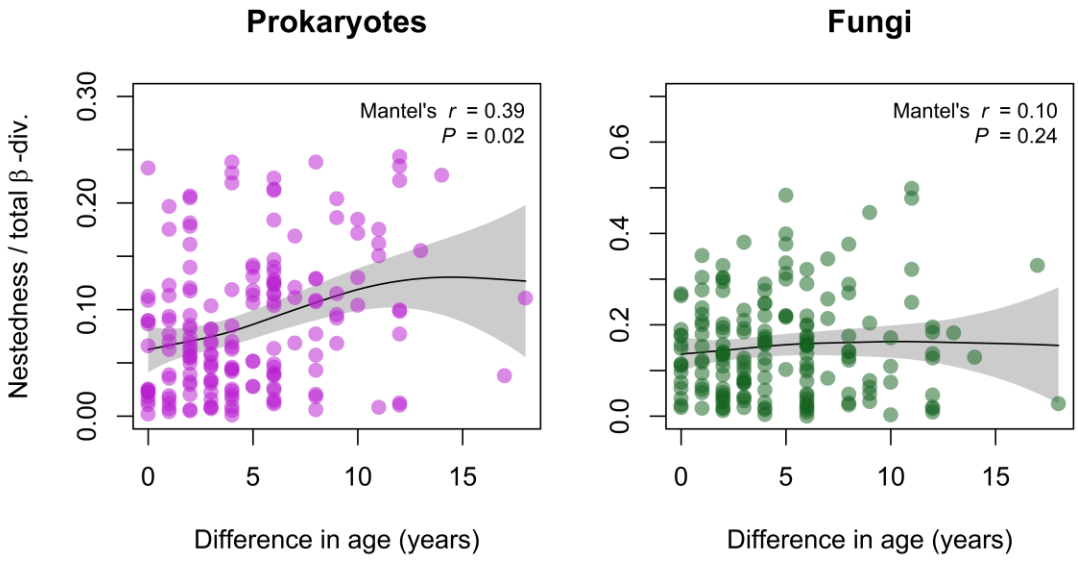

Fig S2.2: Nestedness component of β-diversity between green roofs depending of the difference in age

Annexe III: Figure S2.3

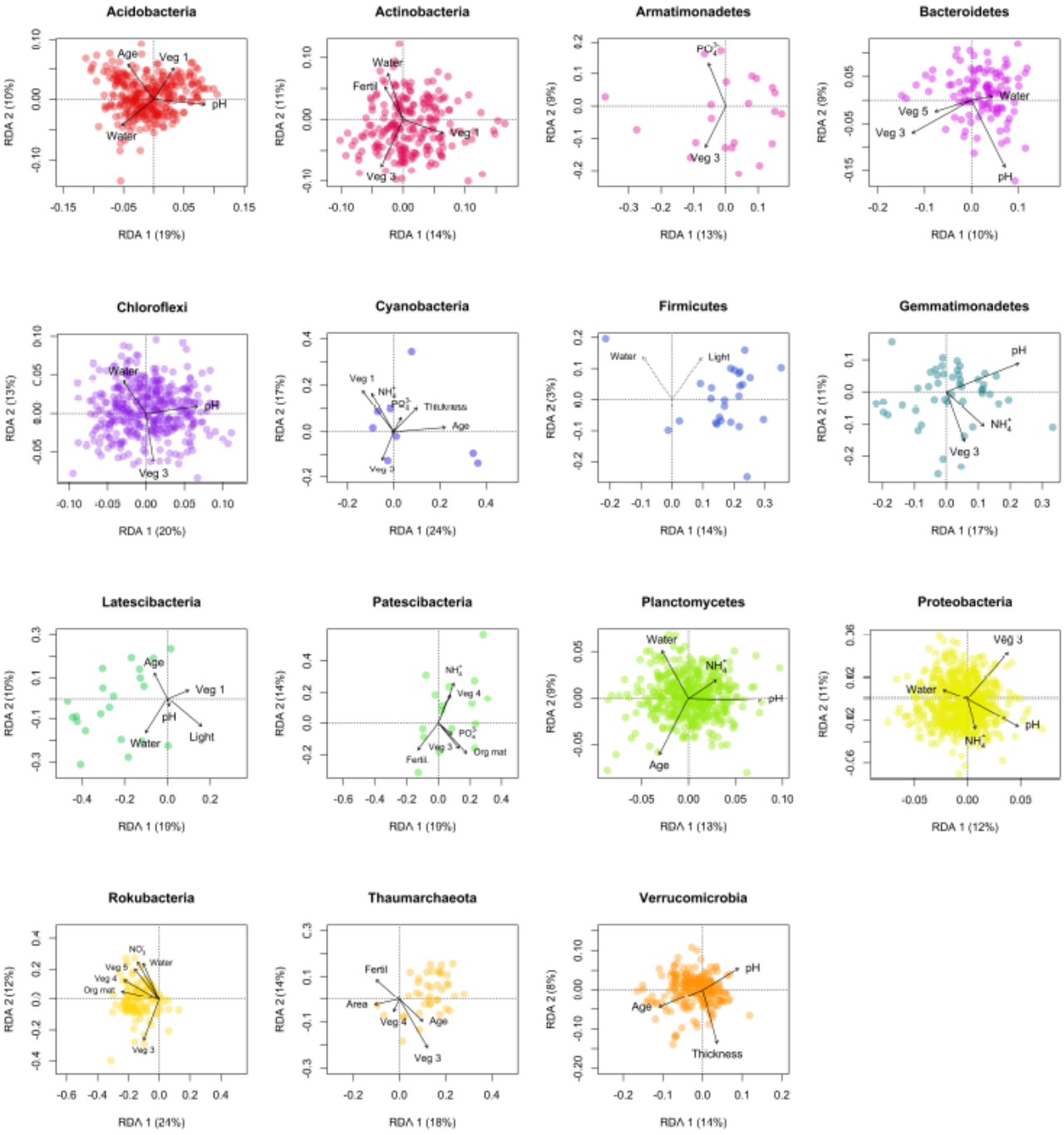

Figure S2.3a. Individual RDAs on every prokaryotic Phylum separately. Predictors were selected using an automated stepwise algorithm as implemented in the R package vegan (Oksanen et al., 2015). The retained predictors have their vectors shown on the graphs and each symbol represents an individual ASV. The numbers in parentheses represent the percent variance constrained by the canonical ordination.

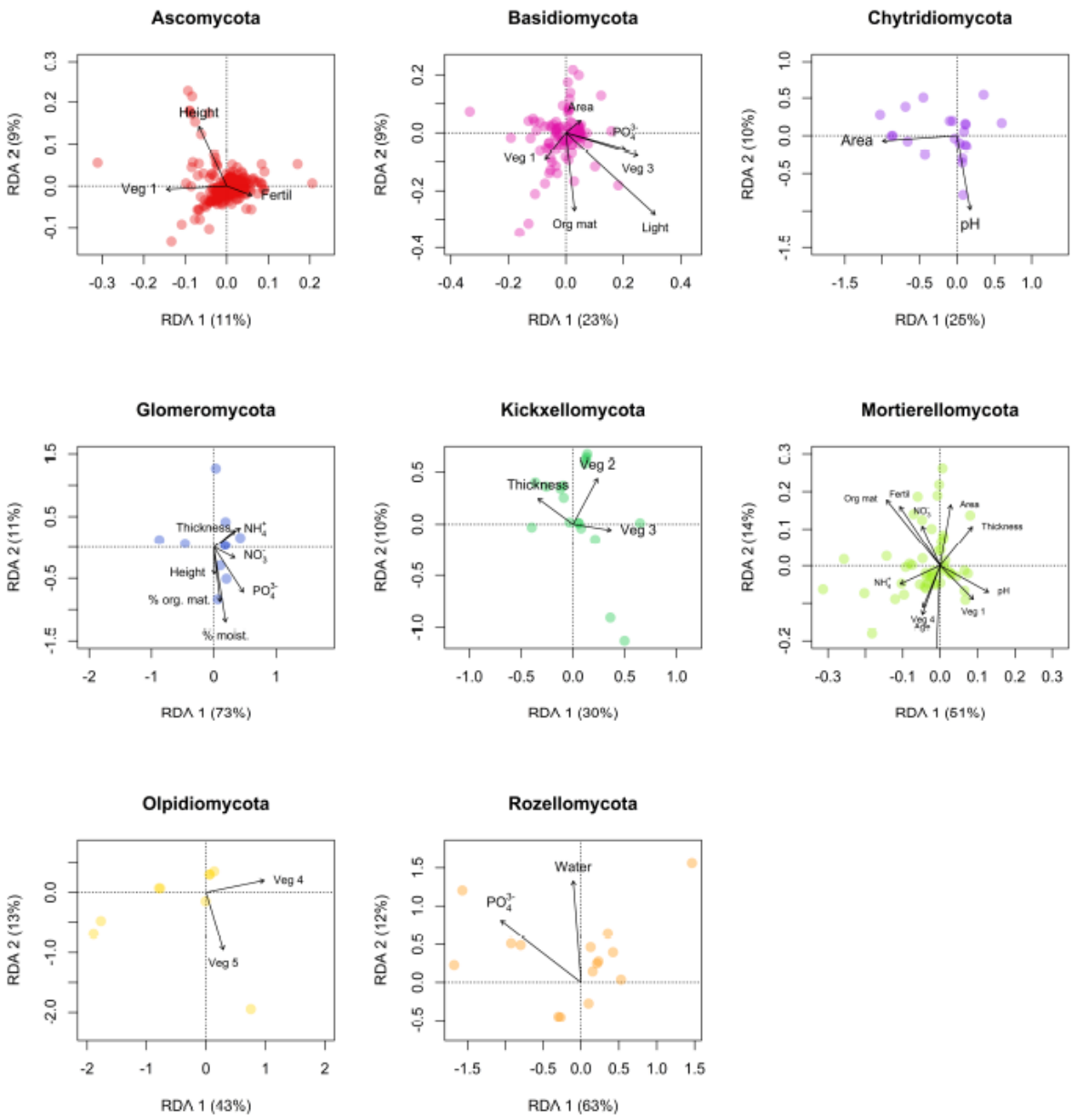

Figure S2.3b. Individual RDAs on every fungal Phylum separately. Predictors were selected using an automated stepwise algorithm as implemented in the R package vegan (Oksanen et al., 2015). The retained predictors have their vectors shown on the graphs and each symbol represents an individual ASV. The numbers in parentheses represent the percent variance constrained by the canonical ordination.

Annexe IV: Figure S2.4

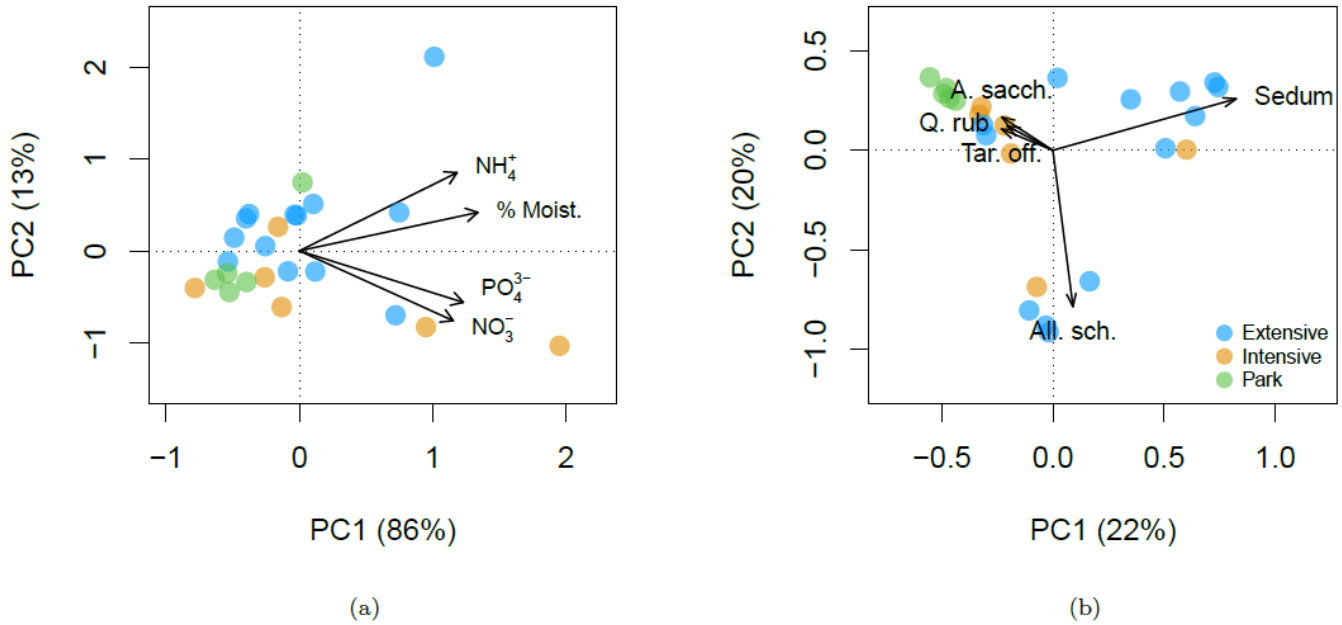

Figure S2.4 : Principal component analyses on (a) soil abiotic properties and (b) vegetation structure (Hellinger- transformed plant relative abundances). Percentages in parentheses represent percent explained variances by the ordination axes.

Annexe V: Figure S2.5

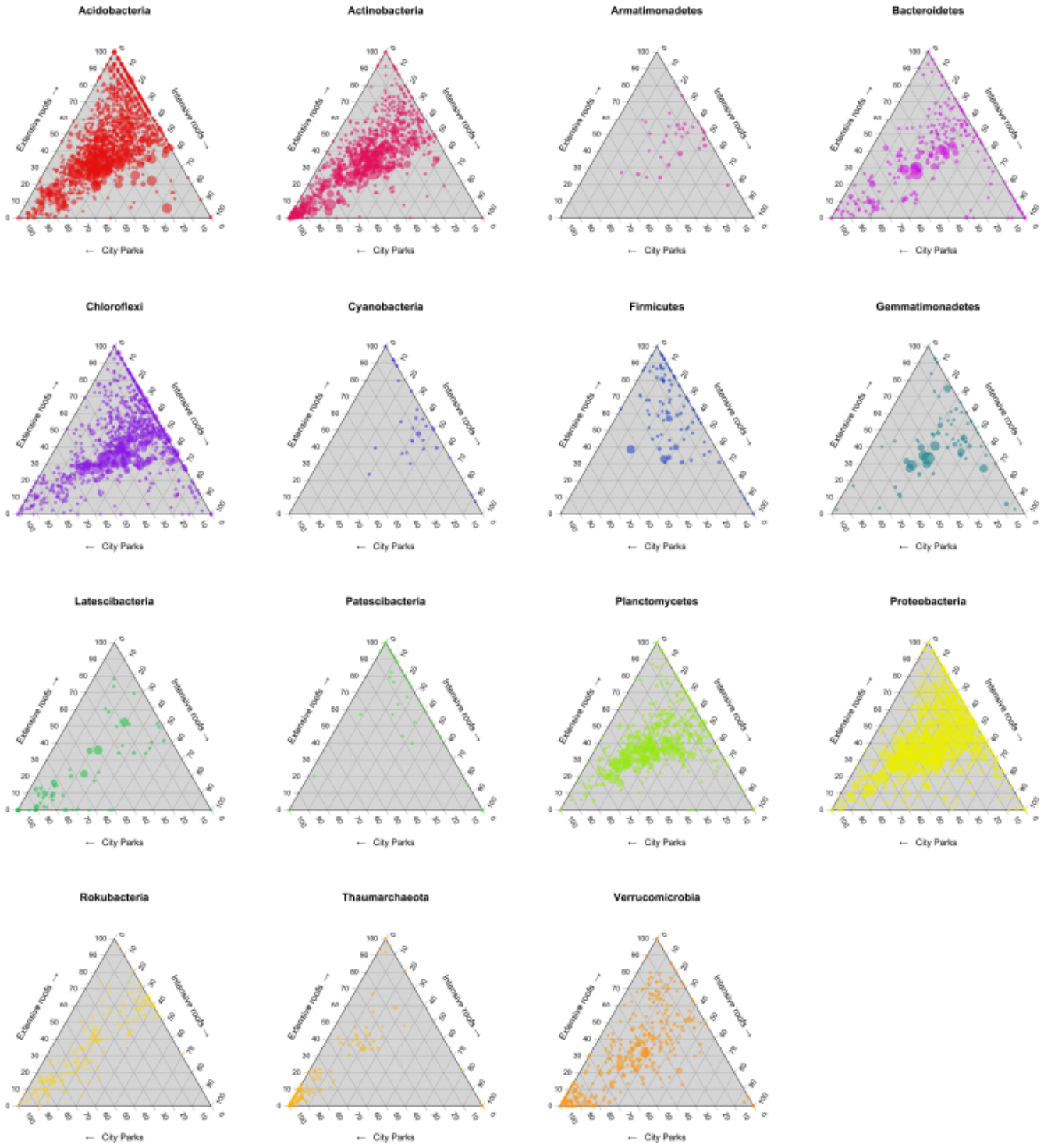

Figure S2.5a: Ternary plots showing the relative distribution of prokaryotic ASV reads in the three habitat classesstudied. Every symbol represents a given ASV and for each ASV, coordinates in the ternary plot sum up to 100%.

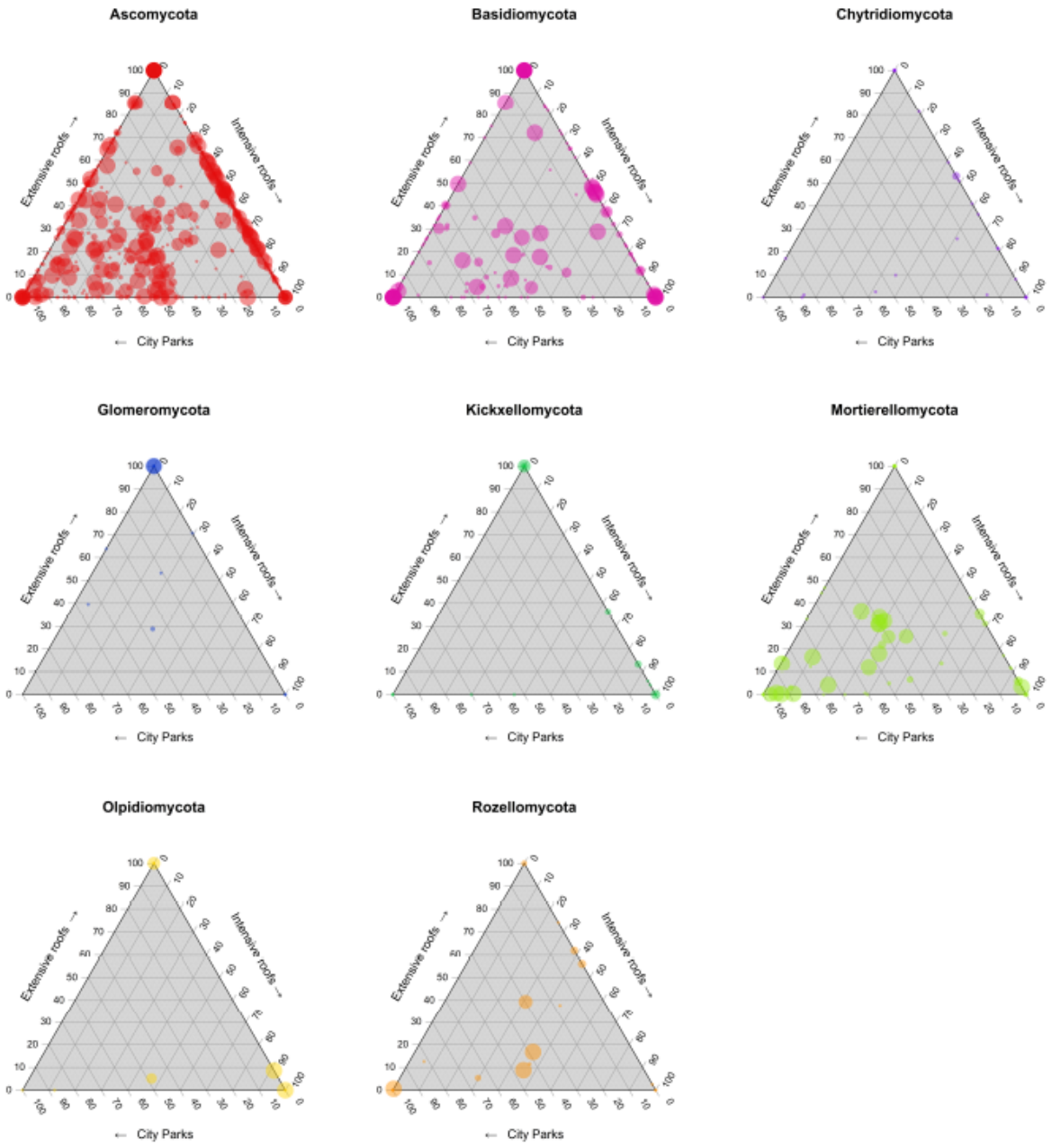

Figure S2.5b: Ternary plots showing the relative distribution of fungal ASV reads in the three habitat classes studied. Every symbol represents a given ASV and for each ASV, coordinates in the ternary plot sum up to 100%.

Annexe VI: Figure S3.1

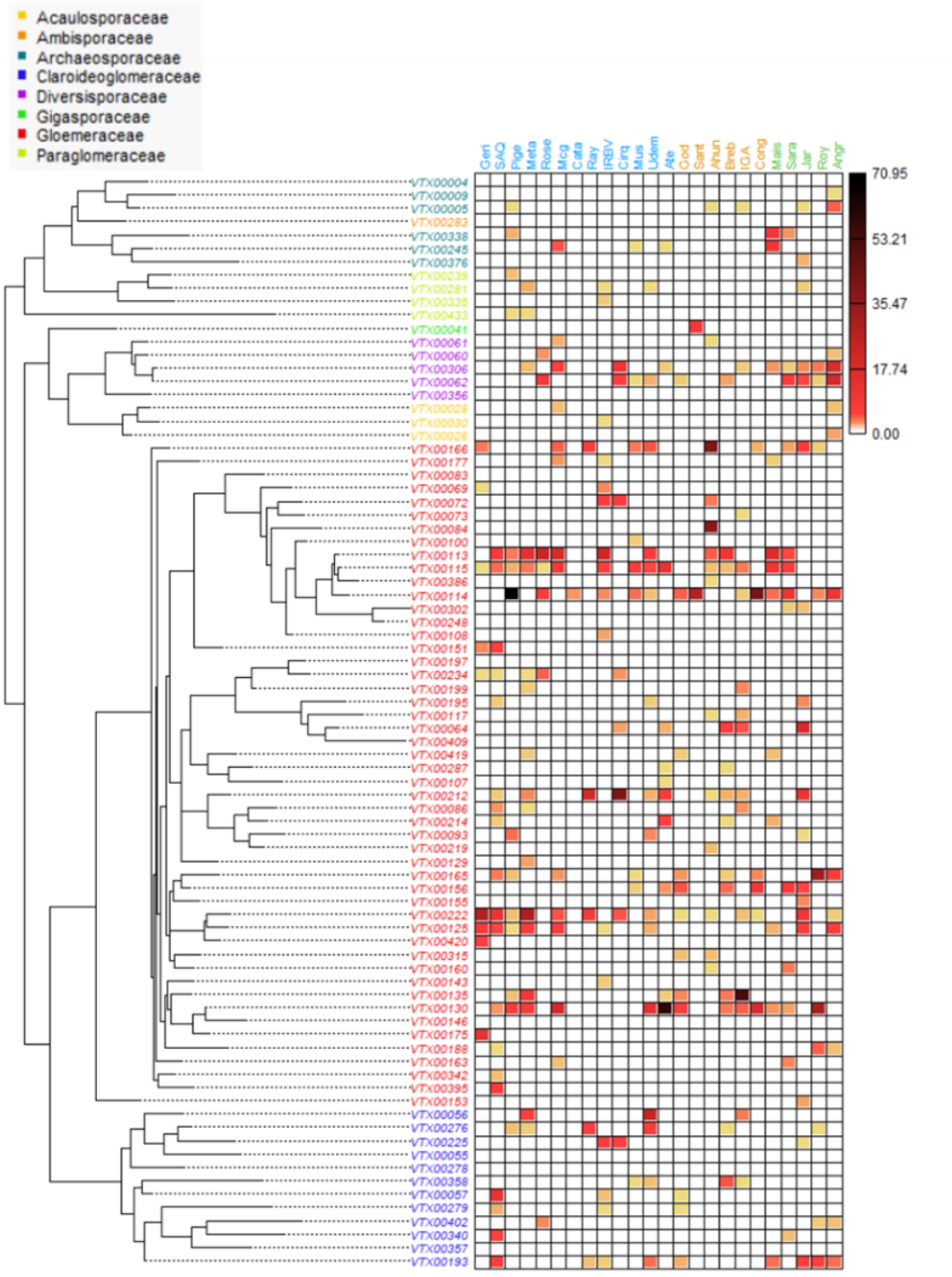

Figure S3.1: Maximum likelihood phylogeny of the VTX identified in green roofs and urban parks with their relative abundance in each site.

Annexe VII: Figure S3.2

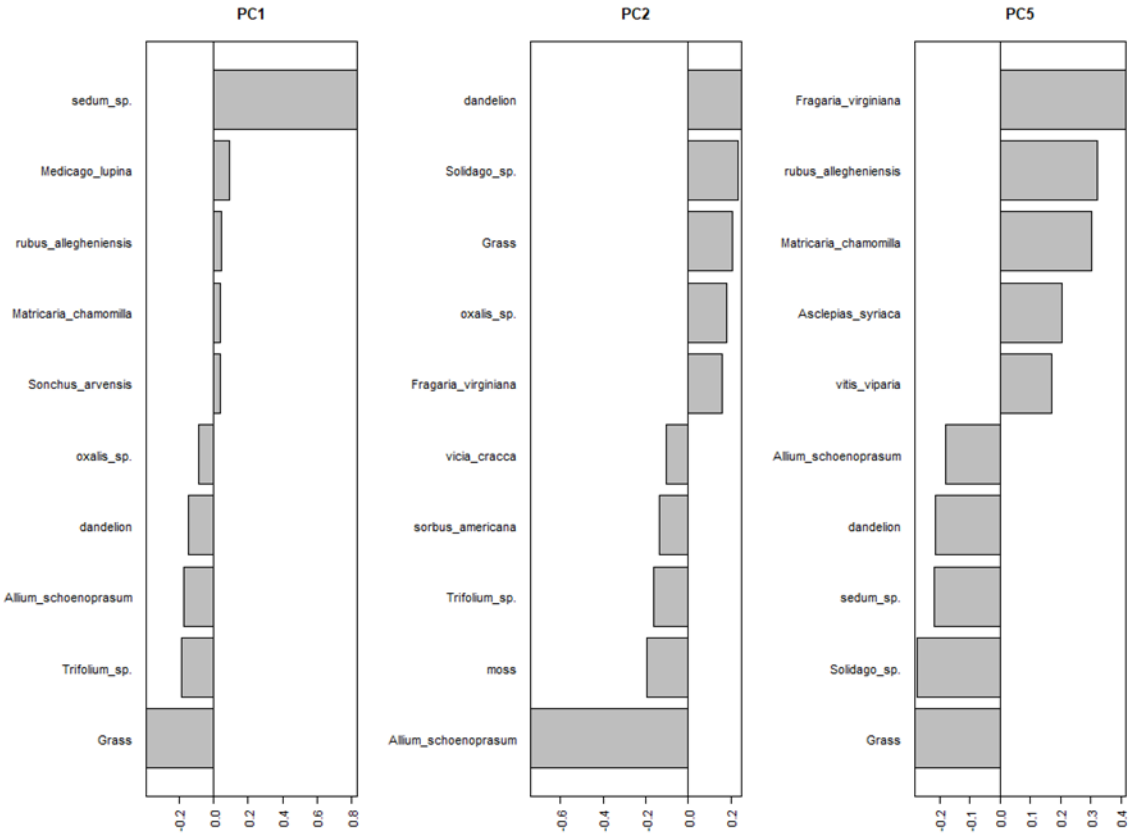

Figure S3.2: Plants species most positively and negatively associated with the first, second and fifth axis of the principal component analysis (PCA) on Hellinger-transformed plant community data.

Annexe VIII: Figure S3.3

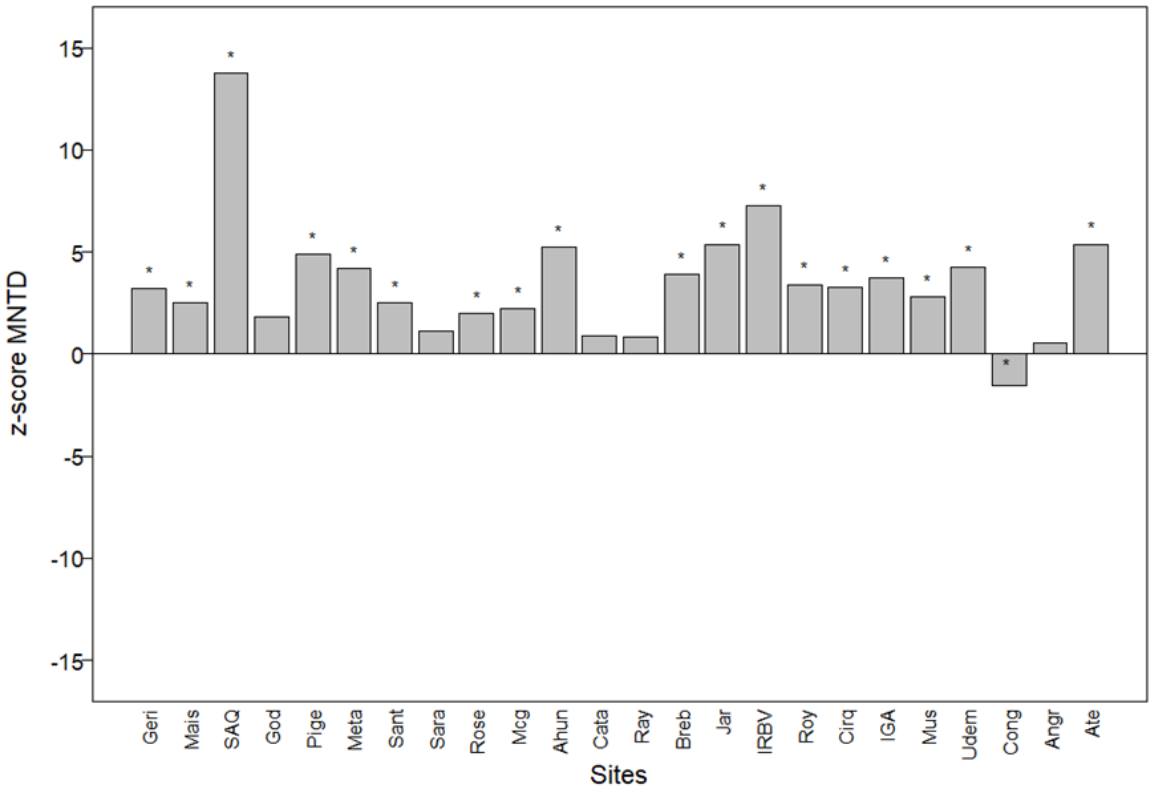

Figure S3.3: Z-score of the mean nearest taxon distance (MNTD) of the sampled sites compared to 1000 random local communities drawn from the regional pool (Lessard et al., 2012; Miller et al., 2017). "*" mean a significative value.

Annexe IX: Table S3.1

Table S3.1: Identified generalist AM fungi virtual taxon in our study sites as the ones occurring in more than 10 sites and with more than 1% of the reads per sire on average.

Family	VTX
Glomeraceae	VTX000113
	VTX000114
	VTX000130
	VTX000212
	VTX000165
	VTX000135
	VTX000064
	VTX000166
	VTX000222
	VTX000125
	VTX000156
Diversisporaceae	VTX000062
	VTX000306
Claroideoglomeraceae	VTX000193

Annexe X: Base de données

https://osf.io/9t6kc/