DÉTERMINISME ET STOCHASTICITÉ DANS L'ASSEMBLAGE DES COMMUNAUTÉS MYCORHIZIENNES

par

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Sommaire

La vaste majorité des plantes terrestres sont impliquées dans des interactions symbiotiques avec des champignons du sol. Ces interactions, appelées mycorhizes, jouent un rôle clé dans l'écologie des plantes en influençant plusieurs facettes de leur croissance ou de leur reproduction (e.g., nutrition, protection contre les pathogènes, activation du système immunitaire). Toutefois, nous connaissons encore très peu de choses sur l'assemblage des communautés mycorhiziennes en milieu naturel : existe-t-il de la spécificité entre certaines espèces de plantes et de champignons, ou ces associations sont-elles le fruit du hasard et des conditions locales seulement? Cette question pose un défi tant sur le plan fondamental, où nous cherchons à comprendre comment les mutualismes persistent évolutivement, que sur la plan appliqué, où nous aimerions connaître comment les écosystèmes naturels s'assemblent pour guider nos pratiques de restauration écologique. Ainsi, mon doctorat a gravité autour de cette question : quels sont les mécanismes responsables de l'assemblage des communautés mycorhiziennes? En d'autres termes, qu'est-ce qui détermine qu'une plante s'associera avec certains champignons, et ne s'associera pas avec d'autres, en milieu naturel.

En premier lieu, j'ai approché cette question sur le plan théorique en utilisant la théorie des réseaux comme outil pour détecter les associations préférentielles entre plantes et champignons. J'ai aussi développé, pour prédire ces associations préférentielles, un cadre théorique basé sur les traits fonctionnels des organismes, en adaptant le triangle CSR de J.P. Grime. Finalement, j'ai pu tester mes hypothèses par des observations en milieu naturel et des expériences en milieu contrôlé. L'ensemble de mes travaux ont contribué à mettre en lumière deux éléments clés de l'assemblage des communautés mycorhiziennes. Premièrement, l'assemblage semble se faire de manière hiérarchique, où d'abord des contraintes neutres comme l'abondance et la distribution spatiale déterminent quelles espèces auront l'opportunité d'interagir entre elles et ensuite, une sélection déterministe des partenaires s'opère, où les

plantes ayant des traits fonctionnels similaires tendent à interagir avec un pool similaire de champignons mycorhiziens. Deuxièmement, bien qu'il semble y avoir de la sélection déterministe de partenaires, tant en milieu naturel qu'en milieu contrôlé, ce choix de partenaires demeure extrêmement flexible et dépend probablement des conditions locales et de phénomènes stochastiques (e.g., conditions du sol, luminosité, effets de priorité par les plantes voisines, etc.).

Ces résultats permettent de mieux comprendre la spécificité dans la symbiose mycorhizienne. Ils suggèrent aussi que ces communautés symbiotiques seront fortement résilientes aux perturbations (e.g., extinction locale d'une espèce), car la spécificité dans le choix de partenaires que l'on observe sur le terrain ne semble pas résulter d'évènements de coévolution réciproque et de spécialisation.

Mots clés : Mycorhizes, Réseaux, Écologie des communautés, Symbioses, Sélection de partenaires, Résilience, Nestedness, Modularité.

Remerciements

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Liste des abréviations

AM	Arbuscular mycorrhizal
AMF	Arbuscular mycorrhizal fungi
PCR	Polymerase chain reaction
NODF	Nestedness based on overlap and decreasing fill
CMA	Champignons mycorhiziens à arbuscules

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Chapitre 1

Introduction GÉNÉRALE

La symbiose entre les plantes terrestres et les champignons mycorhiziens à arbuscules (CMA) est sans doute la plus vieille et la plus répandue des symbioses végétales (Parniske 2008). Cette association influence à peu près tous les aspects de la croissance et de la reproduction végétales, tels la nutrition (Smith and Read, 2008), la production de phytohormones (Allen *et al.*, 1980), la compétition interspécifique (van der heijden *et al.*, 2003) et intraspécifique (Moora and Zobel, 1996), la fréquence des visites par les pollinisateurs (Wolfe *et al.*, 2005), etc. Cette symbiose est ainsi à la base de nombreux services écosystémiques tels la production de biomasse végétale, la réduction du lessivage des nutriments, la protection des cultures agronomiques contre les pathogènes, etc. Toutefois, en dépit de l'importance de cette symbiose dans les écosystèmes terrestres, nous savons encore très peu de choses sur l'assemblage des communautés mycorhiziennes naturelles. Ceci pose problème, car c'est en connaissant mieux l'assemblage actuel de ces communautés symbiotiques que nous serons en mesure d'établir des prédictions sur la façon dont ces communautés répondront aux changements environnementaux et aux perturbations d'origine anthropique. Tout au long de ce projet de doctorat, je me suis donc attardé à cette question centrale : quels sont les mécanismes via lesquels les communautés mycorhiziennes s'assemblent en milieu naturel? En d'autres termes, qu'est-ce qui détermine qu'une plante établisse des interactions avec certains CMA et n'en établisse pas avec d'autres? Cette question constitue en fait un volet substantiel de la recherche théorique sur la stabilité évolutive des mutualismes, où il est assumé que la sélection de partenaires est un des éléments clés permettant aux hôtes et aux symbiotes d'éviter les associations non bénéfiques (e.g. Bull and Rice, 1990). Avant d'aller plus loin dans la présentation du projet, il est nécessaire d'effectuer un recul historique pour mieux situer les connaissances que nous avions avant mon projet de doctorat, par rapport à la spécificité dans la symbiose entre plantes et CMA.

1.1 Spécificité au sein de la symbiose mycorhizienne à arbuscules, et implication pour sa stabilité évolutive

Les mutualismes sont omniprésents au sein des écosystèmes. Ceci peut sembler contre-intuitif d'un point de vue de sélection naturelle, où cette dernière devrait favoriser chez une espèce l'évolution de traits qui favorisent son propre succès, et non de traits qui bénéficient à une autre espèce. Ainsi, au sein de mutualismes, la sélection naturelle devrait constamment favoriser l'émergence de stratégies où une espèce tente de maximiser les bénéfices retirés du mutualisme en payant le moins de coûts possibles (Sachs et al. 2004). Les termes « tricheurs » ou « passagers clandestins » (de l'anglais « free riders ») ont souvent été employés pour désigner les espèces évoluant une telle stratégie. L'augmentation de l'abondance relative de tricheurs dans une communauté devrait, ultimement, mener à la rupture évolutive du mutualisme, car il deviendrait trop risqué pour les espèces de s'associer avec des tricheurs, et il serait donc plus avantageux de ne pas initier de mutualisme (Herre et al. 1999). Toutefois, certains mutualismes, comme les mycorhizes à arbuscules, montrent une stabilité évolutive surprenante : les mycorhizes à arbuscules sont formées depuis plus de 450 millions d'années dans les écosystèmes terrestres (Redecker et al. 2000). Différents mécanismes ont été proposés pour expliquer la stabilité évolutive des mutualismes en milieu naturel : la transmission verticale (i.e., parents-enfants) des mutualismes (REF), les limites à la dispersion qui empêcherait les tricheurs de se propager dans une communauté (Doebeli and Knowlton 1998) et la sélection de partenaires (soit a priori, ou par le biais de récompenses préférentielles envers les bons partenaires et/ou les sanctions envers les tricheurs) (e.g., Bull and Rice 1990; Bever et al. 2009; Kiers et al. 2011). Selon ce dernier mécanisme (la sélection de partenaires), on devrait voir des patrons d'associations préférentielles entre les différentes espèces impliquées dans le mutualisme en question. Cette spécificité dans les interactions entre les plantes et les CMA demeure encore très peu comprise, et ce fut l'objet de mon doctorat de mieux comprendre son ampleur et les mécanismes qui pourraient en être les causes.

Historiquement, il a été assumé que la symbiose mycorhizienne à arbuscules était non spécifique, et que les associations naturelles s'établissaient plus ou moins par le fruit du hasard (e.g., Allen *et al.*, 1995; Hoeksema, 1999). Cette assomption vient en partie du fait que jusqu'à relativement récemment, on ne distinguait que seulement ~ 200 espèces de CMA dans le monde, alors que des dizaines de milliers d'espèces végétales était connues pour former des associations avec ces champignons : on a donc assumé que ces ~ 200 CMA étaient des généralistes. Toutefois, avec l'avènement d'outils moléculaires pour définir les espèces de CMA en se basant sur leur ADN ribosomal, de nombreuses nouvelles espèces opérationnelles ont été définies (e.g., Öpik *et al.*, 2010). Ces mêmes outils ont aussi permis d'identifier directement les CMA colonisant les racines de différentes espèces de plantes coexistant en milieu naturel. Dans la vaste majorité des cas, on a trouvé que différentes espèces de plantes ne s'associaient pas avec les mêmes espèces de CMA (e.g., Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2002; Öpik *et al.*, 2009; Torrecillas *et al.*, 2012). Toutefois, ces études ont généralement utilisé des méthodes très différentes pour démontrer la présence d'associations préférentielles (e.g., arbres de parsimonie, regroupement hiérarchique, modèles de log de vraisemblance) et ont fourni une réponse qualitative (oui/non) à la question de la présence de spécificité dans la symbiose mycorhizienne. Il devenait donc avantageux de définir des indices numériques pouvant quantifier numériquement le phénomène d'associations préférentielles à l'échelle de la communauté. C'est dans cette optique que j'ai commencé à m'intéresser de plus près à la théorie des réseaux.

1.2 Mycorhizes, métacommunautés et réseaux

Les jeux de données sur les plantes-CMA sont essentiellement de nature matricielle, où on regroupe des vecteurs d'abondance (ou de présence/absence) d'espèces de CMA pour différentes espèces végétales : ces vecteurs deviennent les rangées d'une matrice d'interactions. Ces jeux de données sont en tous points semblables à ceux que l'on regroupe lorsqu'on analyse une métacommunauté, i.e. une matrice de vecteurs d'abondances d'espèces

dans différents sites. La seule distinction est donc ici que l'on substitue les sites par des espèces de plante hôtes, ce qui en soit est logique puisqu'on peut considérer la plante hôte comme un habitat pour le CMA qui colonise ses racines. Une telle analogie a aussi été remarquée par Mihaljevic (2012), qui suggérait que l'ensemble des outils théoriques issus de la littérature sur les métacommunautés (e.g., Leibold *et al.*, 2004; Cottenie *et al.*, 2005) étaient aussi applicables aux communautés symbiotiques. Parmi ces outils, Leibold and Mikkelson (2002) suggéraient trois patrons clés quantifiables mathématiquement pour caractériser la structure d'ensemble d'une métacommunauté. Ces patrons sont :

(1) la Cohérence (i.e. la continuité dans la distribution d'une espèce le long d'un gradient, où par exemple si une plante est présente dans un sol à pH 4 et dans un autre à pH 7, elle devrait aussi être présente dans un sol à pH 5);

(2) le Remplacement d'espèces (où la β-diversité entre les sites provient du fait que ces derniers ont différentes espèces, et non pas un nombre différents d'espèces);

(3) la Compartimentalisation (où on peut définir des groupes bien délimités d'espèces qui se distribuent de façon semblable dans l'environnement, rappelant la théorie de Clements (1916) sur les communautés en tant que superorganismes).

En parallèle, d'autres auteurs ont emprunté des outils mathématiques de la théorie des réseaux (exploitée dans l'étude des systèmes complexes et des réseaux sociaux, par exemple) pour analyser les communautés symbiotiques. Deux de ces outils ont connu une forte popularité dans la dernière décennie : (1) le « nestedness » (déjà présent en écologie dans la littérature sur la biogéographie insulaire, e.g. Atmar and Paterson, 1993) et (2) la modularité (Guimera and Amaral, 2005). Le nestedness est en fait un concept antithétique au remplacement d'espèces défini plus haut (e.g., Podani and Schmera, 2011; Carvalho *et al.*, 2013), et la modularité réfère au même concept que la compartimentalisation aussi définie plus haut, mais elle est mesurée d'une manière différente. Ainsi, la théorie des réseaux et la théorie autour des métacommunautés fournissent un certain lot d'outils mathématiques redondants. Toutefois, la

théorie des métacommunautés a ce désavantage que ses indices mathématiques sont fortement influencés par l'ordre dans lequel les rangées et les colonnes de la matrice de données sont ordonnées. Pour offrir une approche standard, Leibold and Mikkelson (2002) ont suggéré d'utiliser les scores d'une analyse de correspondance pour ordonner la matrice, de manière à faire ressortir de façon optimale les gradients écologiques « cachés » dans les jeux de données, et à maximiser la cohérence mesurée de ces matrices. Toutefois, cette méthode demeure plutôt arbitraire et il existe souvent des façons d'ordonner la matrice de données qui maximisent davantage sa cohérence (Chagnon, données non publiées). À l'inverse, les outils issus de la théorie des réseaux sont largement indépendants de l'ordre des rangées et des matrices dans le jeu de données, ce qui pourrait en faire des solutions préférables. Un nombre croissant d'auteurs utilisent en effet ces outils pour étudier l'assemblage de communautés symbiotiques ou trophiques (e.g., Vazquez *et al.*, 2009; Stang *et al.*, 2009).

Dans le prochain chapitre, je présente donc un article d'opinion visant à promouvoir l'utilisation des outils issus de la théorie des réseaux afin de mieux comprendre l'assemblage des communautés mycorhiziennes. Cet article est basé sur la ré-analyse d'un jeu de données publié et suggère que certains patrons dans les réseaux d'interactions plantes-CMA pourraient élucider l'importance relative de différents mécanismes dans l'assemblage de ces communautés symbiotiques.

Chapitre 2

USING ECOLOGICAL NETWORK THEORY TO EVALUATE THE CAUSES AND CONSEQUENCES OF ARBUSCULAR MYCORRHIZAL COMMUNITY STRUCTURE

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2.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are widespread and their symbiotic interactions involve a majority of terrestrial plant species (Wang and Qiu, 2006). These obligate biotrophs generally improve the nutrition and vigor of the host, thereby affecting individual plant traits (van der Heijden *et al.*, 1998) as well as the composition and functioning of entire plant communities (Moora and Zobel, 1996; Hartnett and Wilson, 1999; Bever, 2002). Studies on individual plant traits are useful in determining fitness benefits to the plant (e.g., increased growth, resistance to pathogens, etc.), whereas studies on community-level interactions can potentially explain constraints on host-symbiont web architecture (e.g. Blüthgen *et al.*, 2007). Community-level studies have been limited, however, to small subsets of natural plant communities, because processing and identifying AMF species associated to numerous plant root systems have proven costly and painstaking. Recent advances in next generation sequencing technologies (Margulies *et al.*, 2005) have removed this hurdle and improved the detection of rare AMF species (Öpik *et al.*, 2009). This increased capacity in describing whole plant-AMF networks provides an opportunity to identify the causes, and assess the functional consequences, of symbiotic network architectures (i.e., topology).

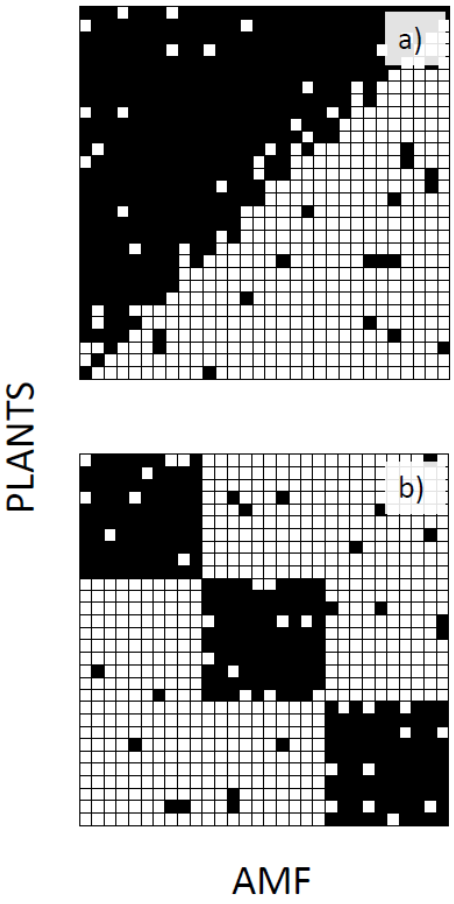


Figure 1. Hypothetical interaction matrices sorted so as to depict (a) the maximal nested state, or (b) the maximal modular state, of a plant-AMF network. Filled cells represent an interaction between a given plants and AMF species.

Network theory, originally developed to describe the flow of information within computational and social networks (e.g., Emerson, 1972), has more recently been applied to ecological studies of various mutualistic systems (e.g. Jordano *et al.*, 2003; Olesen *et al.*, 2007; Joppa *et al.*, 2010). The major advantage of an ecological network approach is that topological metrics can be quantified for any given network involving two or more groups of interacting organisms (e.g., plants and pollinators, food webs, etc.). For example, ecological networks may be described in terms of their "nestedness". High nestedness occurs when specialist species interact with a subset of partners with which generalist species also interact. For example, a specialist pollinator would tend to specialize on a generalist plant, and vice-versa (Fig. 1a). This absence of reciprocal specialization was shown to be a pervasive feature of pollination networks (Bascompte *et al.*, 2003; Joppa *et al.*, 2009; Joppa *et al.*, 2010) that potentially favors diversity and stability of ecological communities (Memmott *et al.*, 2004; Burgos *et al.*, 2007; Bastolla *et al.*, 2009; Thébault and Fontaine, 2010). Ecological networks can also be described according to their "modularity", that is the tendency of species to be grouped into modules in which interactions are more frequent than with the rest of the community (Fig. 1b). Thompson (2005) suggested that communities may assemble into distinct modules based on the functional complementarity of their traits, and this may offer some insight into coevolutionary dynamics between symbiotic species (Guimarães *et al.*, 2007).

In this *Letter*, we argue that an ecological network approach could provide a framework by which to characterize and compare plant-AMF communities from different environments or at different successional stages. This, in turn, could improve our understanding of mechanisms structuring mycorrhizal communities and bring mycorrhizal science to a more predictive level (Johnson *et al.*, 2006). In a recent study, Öpik *et al.* (2009) used pyrosequencing to describe AMF communities associated with 10 plant species in a forest understory community. Here, we have used their published data set to demonstrate the applicability of ecological network theory to characterize plant-AMF communities. Our exercise revealed that this particular plant-AMF network was both highly nested and modular. We discuss possible reasons and implications for such topological features, and stress the potential for ecological network theory to direct future research on plant-AMF communities. In concluding, we argue that there may be a reciprocal advantage for advancing ecological network theory using plant-AMF communities as a model experimental system.

2.2 Data set

Öpik *et al.* (2009) sampled individual root systems from 10 plant species in a 100 m^2 plot established in a hemiboreal forest in Estonia. A total of 458 root systems were sampled, from which DNA was individually extracted. DNA extracts were pooled by plant species and PCR amplified using the AMF specific primer AM1 and the general eukaryotic primer NS31. The exact conditions for PCR are described in Öpik *et al.* (2009). Amplicons were pyrosequenced, yielding the number of sequence reads of each AMF taxon associated with each plant species.

2.3 Plant-AMF network topology

An interaction matrix was drawn using the data published by Öpik *et al.* (2009) (Fig. 2). The matrix nestedness was calculated using the *bipartite* package of R statistical software (R Development Core Team, 2007). This metric varies from 0 to 100 (perfectly nested matrix). To assess the statistical significance of this nested structure, random matrices were generated

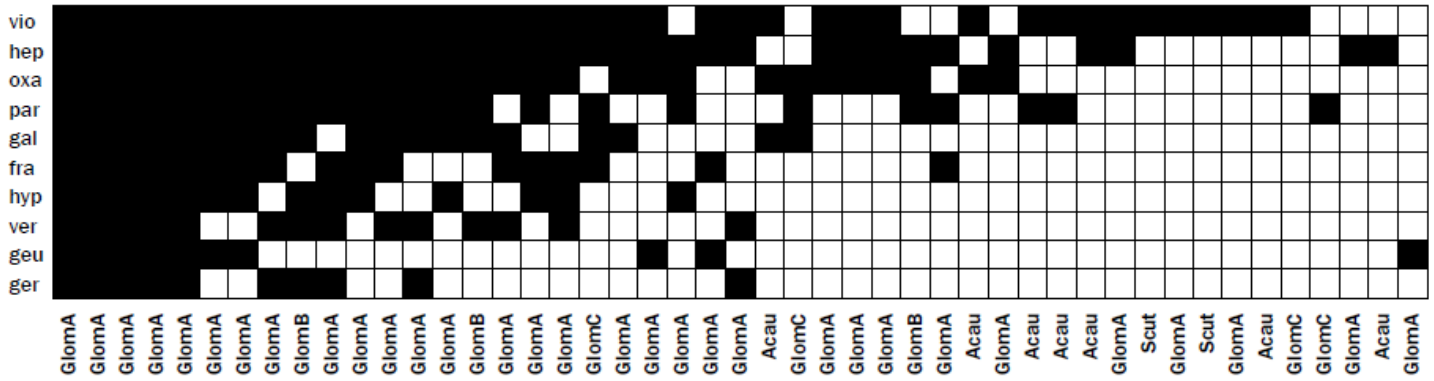


Figure 2. An interaction matrix in its maximal nested state, drawn from the published data set of Öpik *et al.* (2009). Rows and columns respectively represent plant and AMF species sampled in a 100 m^2 forest plot. Abbreviations for plant species : oxa – *Oxalis acetosella*, gal – *Galeobdolon luteum*, vio – *Viola mirabilis*, par – *Paris quadrifolia*, hep – *Hepatica nobilis*, fra – *Fragaria vesca*, hyp – *Hypericum maculatum*, geu – *Geumrivale*, ver – *Veronica chamaedrys*, ger – *Geranium pratense*. Abbreviations for AMF taxa : Acau – genus *Acaulospora*, Scut – genus *Scutellospora*, GlomA – genus *Glomus* group A, GlomB, genus *Glomus* group B, GlomC – genus *Glomus* group C.

using three different null models. These models use constrained randomizations of the original interaction matrix and, according to the level of constraints, can be prone to either type I or type II errors. The first model, originally developed by Atmar and Patterson (1993), is a full randomization of the filled cells across the matrix. Here, the probability (ρ_{ij}) of each cell (ij) to be filled in the random matrices is equal to $1/N$, where N is the total number of filled cells in the original matrix. This model has been criticized (e.g. Ulrich *et al.*, 2009) for overestimating the statistical significance of nestedness (i.e., type I error). The second model, proposed by Bascompte *et al.* (2003), partially controls for row and column totals, so that the probability (ρ_{ij}) of cell (ij) to be filled is equal to $(\rho_i + \rho_j)/2$, where ρ_i and ρ_j are respectively the proportion of filled cells in row i and column j. This second null model is more conservative than the first in estimating the statistical significance of nestedness. The third null model fully controls for row and column totals, so that the probability (ρ_{ij}) of cell (ij) to be filled is equal to ($\rho_i\rho_j$). This third model is the most conservative of the three (i.e., most prone to Type II error), as the total number of filled cells for each row and each column in each random matrix is equal to the corresponding total in the original data matrix from Öpik *et al.* (2009). The third model thus controls for the effects of a species' abundance on its level of generalism in partner choice (Vazquez, 2005). For each null model, 100 randomizations were performed and

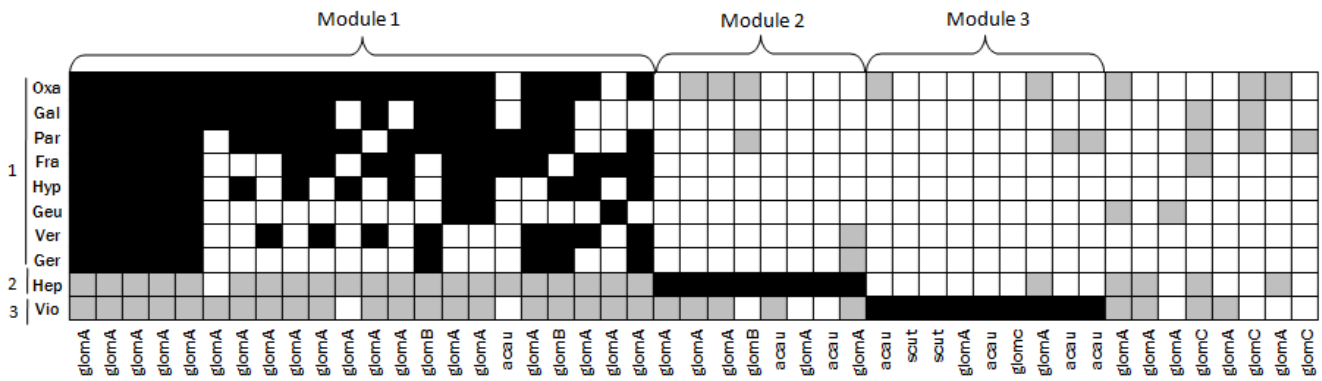
the nestedness of each outcome was calculated as above. We considered nestedness to be significant if 95% or more of the random matrices of a given null model were less nested than the original data matrix.

To analyze the modularity of the mycorrhizal network described by Öpik *et al.* (2009), we implemented an algorithm in R software that was developed by Guimerà and Amaral (2005). The algorithm uses a simulated annealing procedure to distribute the species of the community in different modules in order to reach maximal modularity (M_{max}). For more details about the algorithm, see Guimerà and Amaral (2005). After determining $M_{\max}$ for the original interaction matrix, we assessed its statistical significance by performing 100 randomizations while controlling for row and column totals (i.e., using the third null model described above), and recalculating $M_{\max}$. We considered modularity to be significant if 95% or more of the random matrices were less modular than the original data matrix. We then performed a Chi-squared test to assess the non-randomness of AMF taxa among the modules identified by the algorithm.

2.4 Results

The interaction matrix drawn from the mycorrhizal community described by Öpik *et al.* (2009) demonstrated significantly higher nestedness (N) than the randomly generated matrices under the first two null models (Original matrix, $N = 82.6$; Null model I, $N = 40.5 \pm 3.4$ (1 SD) ; Null model II, $N = 55.2 \pm 4.3$). Under the third null model, six out of 100 random matrices were more nested than the original data matrix, and 25 had a nestedness value above 80 ($N = 76.9 \pm 4.6$).

The original mycorrhizal network was also found to be significantly modular ($P < 0.01$), as all of the randomized matrices had lower $M_{\max}$ values (0.204 ± 0.02 SD) than the original data matrix (0.264). Figure 3 shows the original network divided into distinct modules according to the modularity algorithm. AMF taxa were not randomly distributed across these modules ($\chi^2 = 66.6$, df = 36, $P < 0.01$). More specifically, members of the genera *Acaulospora* and



Scutellospora were mostly confined to a single module associated with *Viola mirabilis*, a

Figure 3. An interaction matrix in its maximal modular state, drawn from the published data set of Öpik *et al.* (2009). Black cells represent recorded interactions found within one of the three modules identified by the modularity algorithm. Gray cells are interactions not included into any module, and white cells indicate that no interactions were observed between the corresponding species. Module affiliation is shown for AMF (above the matrix) and for plants (numbers in the left).

"forest specialist plant" (*sensu* Öpik *et al.*, 2009). On the other hand, members of the *Glomus* group A clade were the most generalist in their partner choice and mainly found in the module comprising the most plant species.

2.5 Plant-AMF network structure

Historically, all AMF species were considered broad generalists (Smith and Read, 2008), as laboratory assays demonstrated nearly complete compatibility between a range of host plants and cultured AMF species (Klironomos, 2000). This belief may have arisen from experimental artifact, as compatibility assessments can only be conducted with cultured fungi that are likely to exclude specialist and unculturable species (Sýkorová *et al.*, 2007). Hence, both plants and AMF seemed to have a broad fundamental niche regarding their partner choice. It was later observed, however, that neighboring plants under field conditions can differ widely in their root-borne AMF communities (e.g., Vandenkoornhuyse *et al.*, 2003; Alguacil *et al.*, 2009). Here, we suggest that ecological network analysis can provide a valuable platform to evaluate

the relative contribution of niche-based *vs.* neutral mechanisms involved in plant-AMF community assembly.

A highly nested structure, as we found in the interaction matrix drawn from data by Öpik *et al.* (2009), suggests that some AMF taxa specialize for only a few plant species. We thus argue that niche-based processes, driven by specific functional traits, may play a key role in the assembly of plant-AMF communities. For example, recent studies have suggested that the development of distinct AMF communities in the rhizosphere of different plant species (e.g. Johnson *et al.*, 1992; Bever *et al.*, 1996) may be driven by preferential allocation of plant carbon to the most beneficial fungal partner (Bever *et al.*, 2009; Kiers *et al.*, 2011). Also, both plants and AMF species have distinct seasonal peaks in their activities (Daniell *et al.*, 2001; Pringle and Bever, 2002; Oehl *et al.*, 2009), implying that phenological compatibility may be another niche-based mechanism driving partner choice. Likewise, our modularity analysis revealed a phylogenetic trend in the distribution of AMF taxa into different network modules. That important functional traits are conserved across major AMF lineages (Powell *et al.*, 2009) lends more support to the notion that plant-AMF communities are constructed so as to maximize functional matching among partners (Thompson 2005).

Ecological network analysis may also evoke neutral mechanisms for plant-AMF community assembly, based on the abundance and spatial distribution of each species. For example, the nestedness of the interaction matrix drawn from data by Öpik *et al.* (2009) was significant only when compared to null models that did not control for the observed abundance of each species. Given the correlation that should exist between the abundance of a species and its degree of generalism in partner choice, our results suggest that the plant-AMF network studied by Öpik *et al.* (2009) relied at least partly on neutral assembly processes. One such process was proposed by Dumbrell *et al.* (2010), who found that a single fungal species displayed strong dominance in many AMF communities, with a disproportionately high number of subordinate AMF species. They suggested that fungal dominance was likely the result of a positive feedback occurring during the build-up of the plant-AMF community. A "founder AMF" species colonizing plant roots earlier during ecological succession would benefit from

more plant-derived carbon than "latecomers", which would favor its growth and spread through the soil, and increase its probability of colonizing newly formed roots. This positive feedback, termed "preferential attachment" in the network theory literature (Barabasi and Albert, 1999), has been found to cause nestedness in other types of mutualistic networks (Medan *et al.*, 2007).

Our network analysis thus allowed us to conjure the existence of both niche-based and neutral mechanisms involved in structuring plant-AMF communities. From these, we may hypothesize a general assembly process based on successive filters (*sensu* Diamond, 1975), the first one being neutral and determined by overlapping spatial patterns, the second one being niche-based and determined by functional traits. Hence, during community build-up, AMF communities randomly associated to different plant species may gradually differentiate, subdividing the network into distinct functional modules. This is corroborated by data from Davison *et al.* (2011), who found that AMF communities associated to different plant species were more differentiated later in the growing season. To further verify this hypothesis, we suggest that more work be done to characterize the functional traits of AMF species belonging to same modules. This could be done by establishing pure cultures of AMF collected from a given site, growing them in standardized conditions and measuring ecologically relevant traits such as mycelial structure, hyphal life span, nutrient uptake and C acquisition (van der Heijden and Scheublin, 2007).

2.6 Functional consequences of plant-AMF network topology

Besides providing insights on the mechanisms that may be responsible for plant-AMF community assembly, a network approach could also help us understand the functional consequences of community structure. For example, high nestedness should limit interspecific competition among plants for AMF symbionts, thus favoring a higher diversity of co-existing species (Bastolla *et al.*, 2009). Nested networks have also been shown to be more resistant to species extinction than randomly assembled communities (Thébault and Fontaine, 2010), thus conferring a greater stability to disturbance. However, those results arose from modeling

studies that assumed only niche-based processes. In other words, two non-interacting species were assumed to be fundamentally incompatible. The recent demonstration that neutral mechanisms can also produce nested structures as those observed in nature (e.g. Krishna *et al.*, 2008) calls for more work incorporating neutral mechanisms and their functional consequences.

Even though less work has been conducted to explore the ecological consequences of modularity, this topological metric may be important from an evolutionary viewpoint. Coevolution between plant and AMF species has naturally been studied on a pairwise basis, where a plant is inoculated with a "home" or "away" mycorrhizal community (e.g. Johnson *et al.*, 2010; Callaway *et al.*, 2011). Modularity analysis may allow us to refine our understanding by predicting that species belonging to the same modules should be better co-adapted to each other (Guimarães *et al.*, 2007). Yet another consideration in modularity analysis is the turnover of species within and across modules. As AMF community structure may vary over time (Dumbrell *et al.*, 2011), it is likely that some species change modules and perhaps even alter between being a specialist or generalist species, suggesting that reciprocal selective pressures exerted between plants and fungi may be themselves fluctuating over time. Such temporal variability in the generalism of a species has been reported in other mutualistic networks (e.g., Diaz-Castelazo *et al.*, 2010; Lazaro *et al.*, 2010) and should be investigated in plant-AMF communities.

2.7 Advancing ecological network theory using plant-AMF communities

As we've discussed, ecological network theory is a promising approach to test the relative importance of niche-based *vs.* neutral mechanisms involved in structuring plant-AMF communities, as well as to provide insights on the functional consequences of these structures. To face these challenges, there needs to be an empirical platform for testing various hypotheses. Most ecological networks that have been studied do not easily lend themselves, however, to experimental manipulation of community interaction patterns. For example, most data on mutualistic networks come from studies on pollination systems, because this

mutualism is widespread and data are readily available. Those systems are, however, rather unsuitable for manipulative experiments, as it is hard to control which organisms will interact. For this reason, recent advances in ecological network theory have relied on modeling work to depict the causes and consequences of divergent network topologies (Dunne *et al.*, 2002; Thébault and Fontaine, 2010). Inevitably, these models simplify the interactive complexity of real communities. For example, community simulations have mainly used fixed interaction matrices depicting constant species interactions through time, a scenario that is unlikely to occur in nature (e.g., Petanidou *et al.*, 2008; Diaz-Castelazo *et al.*, 2010; Lazaro *et al.*, 2010). There is thus a need to design manipulative experiments that will test predictions made by these models.

The plant-AMF symbiosis may comprise a model experimental system for understanding the causes and consequences of different network topologies. It is possible to inoculate individual plants with specific AMF species and to grow these in a common garden. In other words, it is possible to build specific plant-AMF communities knowing the identity and initial abundance of each species, and the structure of the network. Such a model system could be used, for example, to test the importance of neutral mechanisms in structuring mutualistic communities, by testing the relationship between a species' initial relative abundance and its level of generalism following the build-up of the community. Conversely, experimental manipulations of plant-AMF networks could be used to test the importance of niche-based mechanisms. For example, it was shown that phylogenetically distant AMF species have more distinct and complementary niches (Maherali and Klironomos, 2007; Powell *et al.*, 2009) than closely related AMF species. This provides the opportunity to test whether roots colonized by phylogenetically overdispersed AMF assemblages are more apt to limit *de novo* root colonization (i.e., invasion) than those with phylogenetically clustered assemblages, as it is assumed that fewer empty niches would be left available in the formers (Elton, 1958). Finally, artificially constructed plant-AMF communities could be used to evaluate the functional consequences (in terms of species persistence, plant diversity, productivity, etc.) of different network structures. For example, it would be possible to test various hypotheses regarding the correlation between network nestedness and the rate of species extinction (Thébault and

Fontaine, 2010), using experimental protocols designed to test soil microbial stability following stress and disturbance (e.g., Lacombe *et al.*, 2008; Royer-Tardif *et al.*, 2010).

2.8 Limitations of our analysis

In other mutualistic systems, it is generally accepted that interaction frequency is a good proxy for the functional impact of one species on its partner (Vazquez *et al.*, 2005). On the other hand, we lack evidence that the number of AMF sequence reads in plant roots is indicative of the functional impact of the fungus on its host, especially when considering the wide variation in biomass allocation inside *vs.* outside the roots (Powell *et al.*, 2009). For this reason, we restricted our present analysis to two topological metrics that are quantified from binary (i.e. presence/absence) data matrices. Future work should strive, however, to find appropriate quantitative measures of interaction strengths in plant-AMF systems. For example, the number of independent interactions recorded in replicated data sets could be one way of corroborating results like those presented by Öpik *et al.* (2009).

The fact that Öpik *et al.* (2009) may not have sampled all potential host plants in their plot could bias our estimate of nestedness. Nevertheless, Nielsen and Bascompte (2007) showed that estimates of nestedness were generally robust against incomplete sampling designs. Moreover, Bascompte *et al.* (2003) showed that larger networks were consistently more nested than smaller ones, which implies that our estimation of nestedness was probably conservative.

2.9 Conclusions

New molecular tools, such as pyrosequencing technology, have increased our capacity to thoroughly describe plant-AMF communities in natural settings. Ecological network theory provides quantitative tools to study such data sets and to generate hypotheses related to selective partnering and community-level functional attributes. Conversely, the plant-AMF symbiosis, or perhaps mycorrhizal symbioses in general, comprise a model experimental system for advancing ecological network theory, such as testing hypotheses related to neutral

vs. niche-based mechanisms controlling community structure, and to the functional consequences of different network topologies.

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Dans ce dernier article, la théorie des réseaux est présentée comme un outil prometteur pour étudier l'assemblage des communautés plantes-CMA. Toutefois, elle porte aussi son lot de problèmes potentiels. En effet, ces nouveaux outils mathématiques en écologie des communautés ont aussi introduit un nouveau vocabulaire. Par exemple, très vite, les « nœuds » des réseaux d'interactions écologiques avec peu de liens (d'interactions) ont été identifiés comme des spécialistes. Toutefois, tel que noté par Poisot *et al.* (2011a), la spécialisation réalisée sur le terrain peut être le fruit de différents facteurs qui n'ont rien à voir avec un phénomène réel et évolutif de spécialisation : elle peut être le fruit d'une faible abondance relative ou d'effets historiques stochastiques, par exemple. Dans le cadre de la théorie des réseaux, une grande diversité d'indices ont été développés pour quantifier la spécialisation des espèces quant à leur choix de partenaires (e.g., Blüthgen *et al.*, 2006; Albrecht *et al.*, 2010; Poisot *et al.*, 2011b). Toutefois, l'interprétation de ces indices a souvent omis la nuance mentionnée ci-dessus quant aux causes d'une spécialisation apparente sur le terrain. L'article publié par Toju *et al.* (2013) en est un bon exemple. Les auteurs utilisent l'indice *d'* développé par Blüthgen *et al.* (2006) pour quantifier la « spécialisation » des espèces de plantes et de champignons endophytes en milieu naturel. Toutefois, l'interprétation qu'ils font de ces indices est purement basée sur des phénomènes évolutifs, alors que leur système d'étude implique des organismes sessiles, et donc limités par la dispersion. Ainsi, plusieurs espèces pourraient être perçus comme étant spécialistes parce qu'ils ont une faible abondance relative ou une distribution spatiale très agrégée (e.g. Blüthgen *et al.*, 2008). Dans l'article qui suit, je démontre, par la ré-analyse de leurs données, que les inférences que l'on peut faire en calculant simplement des indices de spécialisation, sans données externes pour élucider les causes d'une telle spécialisation apparente, sont faibles (*sensu* Platt, 1964). Ceci démontre le besoin de complémenter des jeux de données sur les interactions entre les espèces avec d'autres données écologiques pertinentes (e.g., traits fonctionnels, abondance et distribution spatiale, phénologie).

Chapitre 3

PLANT-FUNGAL SYMBIOSES AS ECOLOGICAL NETWORKS: THE NEED TO CHARACTERIZE MORE THAN JUST INTERACTION PATTERNS

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3.1 Abstract

Next-generation sequencing technologies are providing us with new opportunities to characterize plant-fungal communities in more depth and with better replication than ever before. The application of network concepts and numerical tools to analyze those extensive data sets is also rapidly increasing. Here we show, however, that network-based tools will further advance our understanding of the ecology of plant-fungal symbioses if (1) researchers characterize both the interaction patterns among species, and investigate the likely biotic and abiotic drivers of such interactions (e.g. species' abundance, functional traits, environmental conditions) and (2) researchers make sure that the assumptions made by their network-based numerical tools are met by their data sets.

3.2 Results and Discussion

The increasing accessibility of next-generation sequencing technologies has sparked a new wave of studies that have characterized interaction patterns naturally occurring between plants and their fungal symbionts (e.g. Montesino-Navarro *et al.*, 2012; Martos *et al.*, 2012). One way to analyze such data sets, is to describe community structure using novel indices/metrics

derived from ecological network theory (Bascompte, 2009; Bahram *et al.*, 2014). The advantage of this approach is that it is possible to detect community-level patterns and to evaluate, through the use of null models, their statistical significance and their ecological correlates (Chagnon *et al.*, 2012). Thus, this approach has the potential to shed new light on the processes underpinning the ecological and co-evolutionary dynamics of symbiotic communities (Bascompte, 2009; Ulrich and Gotelli, 2013). However, field sampling schemes and numerical analyses need to be carefully designed in order to maximize the inference that can be extracted from data sets (Heleno *et al.*, 2014). To emphasize this point, we have re-analyzed data describing the interactions found between plants and root-colonizing fungi in an oak-dominated temperate forest in Japan (Toju *et al.*, 2013). This recently published data set provides useful insights on the quantitative nature of plant-fungal interactions in a natural forest setting. Our re-analysis of their data suggests, however, that by strictly characterizing interaction patterns among plant and fungal taxa, the study provides little information about the relative importance of neutral versus niche-based processes that determine the assembly of plant–fungal communities. Given the growing importance of next-generation sequencing studies in belowground community ecology (Poisot *et al.*, 2013), future network studies may have to invest less effort in characterizing interaction patterns among species to be able to invest more in investigating the biotic and abiotic drivers of community-level patterns.

Toju *et al.* (2013) sampled a 59 m x 15 m grid comprising 960 soil sampling points. At each point, they collected one root fragment from which DNA was extracted. From these extracts, they identified plant species by amplifying and sequencing chloroplastic DNA. They also used the same DNA extracts to amplify and sequence fungal DNA (using general fungal ITS primers) to determine the fungal taxa composition inside of roots. After thorough bioinformatic filtering of the data set (see Toju *et al.*, 2013), the authors identified a network of 10 plant species interacting with 49 fungal taxa. The aim of the study was to determine the degree of specialization in plant-fungal interactions in a natural forest setting. They calculated the specificity of associations between plants and fungi by computing the *d'* index, which is an information-derived index (like Shannon diversity, see Blüthgen *et al.*, 2006). This index is bounded between 0 and 1: high values indicate a low diversity of partners (i.e. specialist

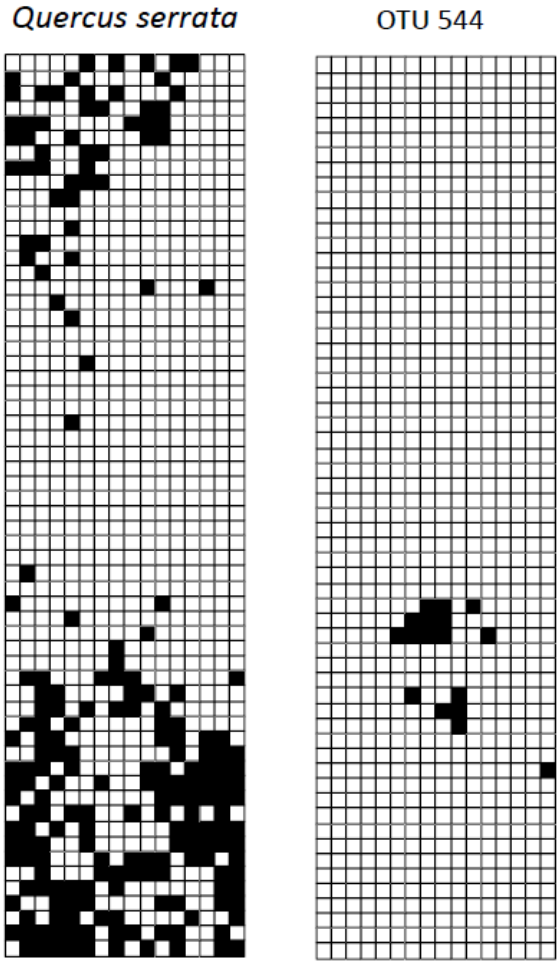

Figure 4. Examples of species that had clearly and significantly aggregated spatial distributions (i.e. the plant species *Quercus serrata*, and the fungal OTU 544). The matrices represent species' occurrences across each spatial sampling units (i.e. cells) in a binary way (occurrences = filled cells).

species). This index was explicitly stated by its developers to be useful in studies focusing on spatial scales that are small enough to avoid situations where the absence of an interaction between two species could be simply ascribed to the absence of overlap in their spatial distribution (Blüthgen *et al.*, 2006). In other words, at large spatial scales, the *d'* index does not strictly address the issue of partner selection and association specificity, but it is also biased by the neutral effects of species abundances and spatial distributions. Thus, in the study by Toju *et al.* (2013), to assume that the *d'* index actually characterizes association specificity, it is necessary to demonstrate that species are homogeneously distributed across the sampling points. To verify this assumption, we plotted the spatial distribution of plant and fungal taxa across the spatial grid sampled by Toju *et al.* (2013). For many species, there were obvious visual patterns of aggregation (see examples in Fig. 4). To test for the significance of this aggregation, we calculated Besag's L function (Besag, 1977), an improved version of Ripley's K function, which calculates and compares the frequency with which events occur at small pairwise distances with those predicted from Monte Carlo random simulations. For spatial scales between 1–10 m, we found that most plant species and about half of the fungal taxa were significantly aggregated (fig. 5). This confirms that species cannot be assumed to be homogeneously distributed across the landscape, and that the *d'* index cannot be interpreted here to strictly infer preferential partner selection. For example, partners

that were found to interact more often than predicted by chance may simply have had overlapping spatial distributions arising from stochastic dispersion processes or from their similar responses to environmental gradients (e.g. soil properties).

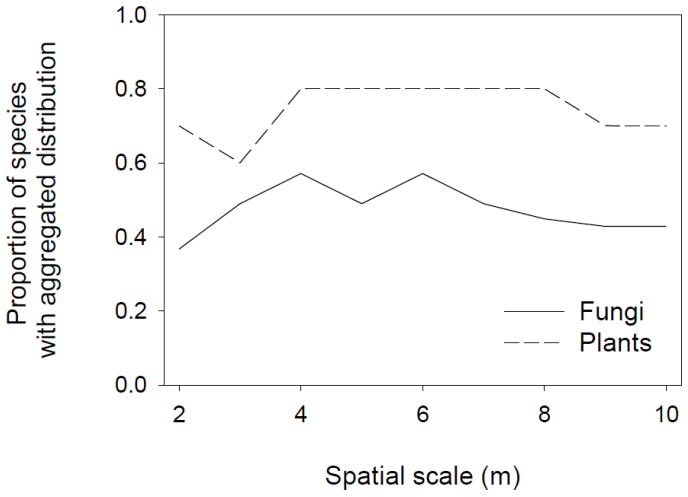

Figure 5. Proportion of plant and fungal species in the data set having a significantly aggregated spatial distribution (test using Besag's *L* function and Monte-Carlo randomizations) at spatial scales ranging from 1 to 10 meters.

To test whether spatial co-occurrence patterns could predict the interactions observed between plants and fungi, we constructed a null model that allocated interactions in the network based on the co-occurrence patterns of plant and fungal taxa. First, we calculated a pairwise co-occurrence index under the form of a *z*-score. Briefly, for each plant-fungal pair, we compared the total number of co-occurrences observed in the field to a null distribution that was obtained by shuffling the spatial distribution of the fungus. We thus ended up calculating $z_c = \frac{C_{obs} - \overline{C_{null}}}{SD_{C_{null}}}$, where z_c is the co-occurrence index, C_{obs} is the total number of co-occurrences between the plant and fungal taxa in the field, $\overline{C_{null}}$ is the mean number of co-occurrences from 1000 simulations, and $SD_{C_{null}}$ is the standard deviation around $\overline{C_{null}}$. We then built 1000 random networks, allocating interactions using the *z*-scores calculated above as probabilities (i.e. high *z*-score = higher probability of interacting in simulated networks). It should be noted that the *z*-scores can be negative if there are less co-occurrences than expected by chance between two species. To allow using them as probabilities in our simulations, we transformed the values by bounding them between 0 and 1, using the function *decostand* as implemented in the R package *vegan* (Oksanen *et al.*, 2012). While assembling our random networks, we constrained interaction probabilities according to two important network attributes: (1) the

total number of interactions (i.e. connectance) in the network, and (2) the total number of interactions per fungal taxa. Controlling for connectance is a routine procedure when simulating random interaction networks, because connectance is highly correlated to many network metrics (e.g. Almeida-Neto *et al.*, 2008; Blüthgen *et al.*, 2008). Controlling for the total number of interactions per fungal taxa was also important because the large number of taxa with very few interactions in the original data set would have artificially inflated the number of empty columns in the simulated random networks. Interactions that were found in more than half (i.e. >500) of our null network simulations were then assumed to be predictable by spatial co-occurrence patterns. As a result, we found that 257 of the 274 interactions present in the original data set (i.e. 94%) could be predicted by spatial co-occurrence patterns.

The close relationship between spatial distributions and the observed interaction patterns was not surprising, given the nature of the data set: at each sampling point, fungal DNA was sequenced from roots of a single plant species. Thus, if a fungus co-occurred at a given sampling point with a given plant species, it was necessarily because it was found interacting with that plant (i.e. sequenced from its roots). In other words, co-occurrence and interaction were not independent, and should have been disentangled by independently characterizing the spatial distribution of plants and fungi. One way of doing this could have been to collect, at each sampling point: (1) roots and/or soil to characterize fungal community composition, (2) a compound sample of roots to characterize plant community composition, and (3) a single root fragment to characterize plant-fungal interactions (only the last point was done in the original study). Such a design would have increased sampling effort at each sampling point, but this could have been compensated by visiting less sampling points overall. At least, with such a design, the study would have provided valuable information on the relative importance of spatial overlap in driving plant-fungal interactions. Otherwise, it remains unsure whether the preferential interactions found by Toju *et al.* (2013) arose from co-evolutionary specialization, or simply from both partners responding similarly to environmental gradients (Lewinsohn *et al.*, 2006). Alternatively, frequent pairs might result from overlapping spatial distributions that had been generated by stochastic processes, or even simply be an artefact of the sampling design. For example, it is known that spatial autocorrelation is high for ectomycorrhizal

communities that are less than 3m apart (Lilleskov *et al.*, 2004). Thus frequent interactions in the data set may simply reflect the repeated sampling of a single interaction within a small neighborhood. Without any supplementary data from this system, we cannot interpret preferential associations in relation to ecological (neutral vs. niche-based) or evolutionary processes.

There is no doubt that Toju *et al.* (2013) presented a valuable data set, which provides us with valuable true estimates of plant-fungal interaction frequencies in a natural forest setting. Their sequencing effort is the first to characterize plant-fungal interaction patterns so intensively and thus represents a major contribution. Our comment does not specifically seek to criticize their work, as many other plant-fungal interaction studies have used a network approach but are limited in their abilities to infer processes from patterns (e.g. Jacquemyn *et al.*, 2010, 2011; Chagnon *et al.*, 2012; Montesino-Navarro *et al.*, 2012). Instead, here, we wish to emphasize the value of designing "network studies" that not only characterize interaction patterns, but also explore the likely biotic and abiotic drivers of these interaction patterns. For example, future studies should focus on collecting additional data on plant and fungal functional traits (e.g. Stang *et al.*, 2009; Chagnon *et al.*, 2013), on spatial variation in soil properties (Dumbrell *et al.*, 2010) and on plant and fungal phenology (Olesen *et al.*, 2011). By integrating such information, network analyses may shift from being a simple descriptive tool to a powerful approach for advancing our ecological and evolutionary understanding of community-level symbiotic interaction patterns.

3.3 Ackowledgements

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Dans l'article qui précède, je souligne l'importance d'utiliser les outils de la théorie des réseaux non pas seulement comme outil exploratoire pour tester la présence ou l'absence de différents patrons reliés au nestedness, à la modularité ou à la spécialisation. Je préconise plutôt l'utilisation des ces nouveaux outils mathématiques pour tester de véritables hypothèses écologiques reliées à l'assemblage des communautés symbiotiques. Ainsi, il apparaît clair que l'analyse de données basées uniquement sur les patrons d'interactions entre les espèces est insuffisante : il faut aussi amasser des données sur les mécanismes potentiels qui déterminent quelles espèces interagiront ensemble.

Dans le cadre de la sélection de partenaires dans la symbiose mycorhizienne, il existait très peu de théorie permettant de prédire quelles espèces de plantes devraient préférer quelles espèces de CMA. Certaines études très fragmentaires avaient suggéré que les plantes plus susceptibles aux pathogènes du sol devraient sélectionner de façon préférentielle les CMA qui fournissent une meilleure protection face aux pathogènes (e.g. Sikes *et al.*, 2009). D'autres ont montré que certains CMA tendent à demeurer plus près de la racine lorsqu'ils colonisent le sol, suggérant que leur rôle dans la nutrition serait redondant avec celui des longs poils racinaires de la plante (e.g., Koide, 2000; Smith *et al.*, 2000), suggérant ainsi que les plantes produisant beaucoup de poils racinaires auraient peu d'avantage à interagir avec de tels CMA. Toutefois, ces données demeurent largement insuffisantes pour prédire les associations préférentielles en milieu naturel, car dans la vaste majorité des cas, nous ignorons complètement l'aptitude des CMA à protéger les plantes contre les pathogènes où à pousser près de la racine dans le sol. Nous ignorons mêmes souvent la susceptibilité des plantes aux pathogènes du sol et l'abondance ou la longueur moyenne de leur poils racinaires. Ainsi, il y a un urgent besoin de développer des cadres conceptuel basés sur les traits fonctionnels des espèces impliquées (plantes et CMA), de manière à déterminer quels traits fonctionnels doivent être mesurés.

C'est précisément ce que je propose de faire dans le prochain article. Je développe un cadre théorique déjà largement accepté en écologie végétale : le triangle CSR de J.P. Grime (e.g., Grime, 1977). Ce cadre théorique semble utile pour plusieurs raisons :

- Il permet de caractériser non pas seulement des traits fonctionnels uniques, mais aussi comment ces traits sont intégrés à des stratégies d'histoire de vie plus large, qui ont permis de résoudre des défis écologiques donnés comme la survie en milieu aride ou en milieu fortement perturbé (i.e. Stearns 1976);
- En ayant un cadre théorique commun pour les plantes et les CMA, il est plus facile de faire des prédictions sur les associations préférentielles. Par exemple, une plante tolérante au stress, avec une stratégie axée sur la conservation des ressources (avec une biomasse coûteuse mais longévive), n'aurait probablement pas avantage à s'associer avec un champignon qui a une stratégie rudérale axée sur l'acquisition de ressource (où la plante devrait constamment fournir du carbone au champignon pour remplacer sa biomasse peu longévive);
- Puisque très peu d'études ont jusqu'à maintenant mesuré de façon systématique les traits des CMA, un cadre théorique précis permettrait de focaliser les efforts et de suggérer certains traits clés à mesurer dans un futur proche. Par exemple, le triangle CSR mettant l'emphase sur les perturbations et le stress, on pourrait vouloir mesurer d'abord chez les CMA des traits reliés à leur taux de croissance ou à leur réponse à un stress nutritionnel (e.g., faible investissement en carbone de la part d'une plante à l'ombre). Ceci permettrait donc de faire converger les efforts déployés par différents groupes de recherche.

Toutefois, bien que le prochain article soit centré autour du triangle CSR, je mets aussi l'emphase sur le fait que d'autres cadres théoriques pourraient être aussi valables et que l'étude ne constitue pas en effet le début d'un effort formel pour calibrer mathématiquement un triangle CSR à partir des traits des champignons. L'effort ici est davantage conceptuel, et vise plutôt à initier une recherche sur les traits fonctionnels des CMA qui soit ciblée et efficace, et surtout qui puisse contribuer à faire la lumière sur les associations préférentielles entre plantes et CMA.

Chapitre 4

A TRAIT-BASED FRAMEWORK TO UNDERSTAND LIFE HISTORY OF MYCORRHIZAL FUNGI

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4.1 Abstract

Despite the growing appreciation for the functional diversity of arbuscular mycorrhizal (AM) fungi, our understanding of the causes and consequences of this diversity is still poor. In this Opinion article, we review published data on AM fungal functional traits and attempt to identify major axes of life history variation. We propose that a life history classification system based on the grouping of functional traits, such as Grime's C-S-R (competitor, stress tolerator, ruderal) framework, can help to explain life history diversification in AM fungi, successional dynamics and the spatial structure of AM fungal assemblages. Using a common life-history classification framework for both plants and AM fungi could also help predict likely species associations in natural communities and increase our fundamental understanding of the interaction between land plants and AM fungi.

4.2 Functional diversity in arbuscular mycorrhizal fungi: the need for a conceptual framework

The symbiosis between plants and arbuscular mycorrhizal (AM) fungi (phylum Glomeromycota, see Glossary) originated some 450 million years ago (Redecker *et al.*, 2000), and is thought to have facilitated the transition of plants from water to land. This symbiosis

occurs in a majority of species in the plant kingdom and may be a major driver of the assembly, dynamics and productivity of plant communities (e.g. van der heijden *et al.*, 1998; Klironomos *et al.*, 2000). There is a need, therefore, to understand the mechanisms through which AM fungi influence a wide range of plant responses in different environmental contexts.

The historical notion that AM fungi are a functionally homogeneous group specialized in the provision of phosphorus (P) to their host plants (Gerdemann, 1975) has been expanded to consider other types of functions. It has been known for some time that AM fungi can confer plant pathogen protection as well as improve plant tolerance to drought and heavy metal contaminants (e.g. Schenck, 1981; Harris *et al.*, 1985; Griffioen and Ernst, 1989). More recently, it has been demonstrated that AM fungi may alter plant hormone dynamics (Hause *et al.*, 2007) as well as stabilize soil aggregates, which could have physical and resource benefits for the plant (Rillig *et al.*, 2002). There is also interspecific variation for these functions and their attendant traits, suggesting the existence of functional trade-offs among AM fungal species (Daft, 1983). For instance, different AM fungal species can vary in their carbon demand from host plants (Pearson and Jakobsen, 1993), P translocation to roots (Ravnskov and Jakobsen, 1995), carbon storage (van Aarle and Olsson, 2003), and relative investment into extra-radical versus intra-radical biomass (Hart and Reader, 2002). To understand the origin of this variation and to predict its ecological consequences, it is necessary to develop a conceptual framework that organizes AM fungal species according to functional groups.

Several advantages arise from classifying AM fungal species according to broad functional groups. Identifying sets of correlated functional traits within each group could help us to define major life-history strategies. Those strategies, in turn, could be used to predict biodiversity patterns and successional trajectories in a tractable way. For example, ecologists have been using r and K selection strategies (MacArthur and Wilson, 1967) to describe the early establishment of populations with a short generation time, rapid growth and low resource use efficiency (i.e. r strategy), and their eventual replacement by populations with delayed reproduction, high parental care and a few large off-spring (i.e. K strategy) (Reznick *et al.*,

2002). In the case of AM fungi, as obligate plant biotrophs, an additional challenge would be to develop a common framework that categorizes both plant and AM fungal life-history strategies, since the level of matching between the life histories of interacting plant and fungal symbionts may predict the relative benefit that each partner will derive from the interaction.

Frameworks that group species into functional groups along a few trait axes have helped to summarize biological variation, and has led to the development of hypotheses to explain the origins of functional diversity (MacArthur and Wilson, 1967), the distribution and abundance of species (Winemiller, 2005), and the consequences of functional traits for ecosystem functioning (Westoby and Wright, 2006). Of the many frameworks that have been proposed, the r-K selection model (MacArthur and Wilson, 1967) has likely been the most influential. Nevertheless, this framework has been criticized for its oversimplification of life history strategies along a single axis that combines both disturbance and resource availability (Winemiller, 2005). Other models that integrate additional axes have thus been proposed to more completely characterize diversity while remaining simple and tractable. One example in aquatic science is the Winemiller-Rose triangular model (Winemiller and Rose, 1992), which integrates both disturbance frequency and predictability, thus defining three main strategies: opportunistic (highly disturbed systems), seasonal (periodically disturbed systems) and equilibrium (undisturbed systems). One limitation of the triangular model, however, is that even though it provides a clearer role for two different qualitative aspects of disturbance in selecting for distinct life histories, it does not account for additional and potentially major aspects of life history, such as resource availability and abiotic stressors.

In plant science, Grime's C-S-R (competitor, stress tolerator, ruderal) framework overcomes some limitations of other models by classifying plant life history strategies according to the functional traits associated with responses to two major environmental filters, namely stress and disturbance (Grime, 1979). Stress refers to persistent adverse environmental conditions (e.g. low soil fertility and limited light availability) whereas disturbance refers to events leading to significant loss of functional biomass (e.g. fire and windthrow). The C-S-R

framework identifies three main life-history strategies. 'Competitors' thrive in low-stress and low-disturbance environments, where they gain a competitive advantage by delaying reproduction so as to invest in structures that optimize the acquisition of resources (Hodgson *et al.*, 1999). 'Stress tolerators' endure sub-optimal environments owing to resource conservation strategies, such as the production of long-lived biomass, which increases resource use efficiency in the long term (Chapin, 1980; Cornellissen *et al.*, 2001). 'Ruderals' cope with frequent disturbance by relying on high colonization ability, rapid production of low-cost biomass, and short reproductive cycles (Grime, 1979; Hodgson *et al.*, 1999). According to the framework, no species can withstand both high levels of stress and disturbance, thus preventing the existence of a fourth life history strategy. As a whole, the C-S-R framework has been useful for understanding the assembly of plant communities undergoing land-use change (Hodgson, 1991), and for predicting successional trajectories of plant communities after disturbance events (Cacciagana *et al.*, 2006; Navas *et al.*, 2010). In addition to plant studies, the C-S-R framework has been used to study functional variations in coral reef communities (Darling *et al.*, 2012), and it has also been proposed as a means of studying life-history strategies of phyllosphere microorganisms (Nix-Stohr *et al.*, 2008), thus supporting its generalizability to various systems. In this Opinion article, we employ the C-S-R framework as an example of how trait-based classification approaches can advance our knowledge of the relationship between AM fungal life history traits, plant life history traits and environmental abiotic filters.

4.3 Applying the C-S-R framework to AM fungi

To better understand the biology and life history of AM fungi requires a mycocentric perspective, that is, an appreciation of AM fungi not only as plant symbionts but as organisms that have developed traits that maximize their own fitness in different environments (Fitter *et al.*, 2000; Alberton *et al.*, 2005). We must recognize, therefore, that what benefits the plant is not necessarily what benefits the AM fungus, and vice versa. For example, high soil P availability may promote the growth of the plant, but will in turn reduce the amount of carbon transferred to the AM fungal symbiont (Mosse and Hayman, 1973). Thus, when applying a C-

S-R framework to AM fungi, we must consider which environmental conditions cause stress or disturbance to AM fungi, and then explore which functional traits improve the AM fungal response to those environmental filters (Figure 1).

4.3.1 Competitive AM fungi

The competitive ability of an individual derives from its capacity to acquire growth-limiting resources. Considering previous work on AM fungal foraging strategies, the main growth-limiting resource for AM fungi appears to be plant-derived carbon (Olsson *et al.*, 2002). Consequently, competitive AM fungi should be those with functional traits that improve carbon acquisition from the host plant. It is generally recognized that soil P deficiency increases the flow of plant carbon to AM fungi (Ratnayake *et al.*, 1978). Furthermore, it has also been shown that the flow of plant carbon to the fungus is proportional to the amount of P that the fungus returns to its host (Kiers *et al.*, 2011), thus supporting models of metabolic coupling between carbon and P transfer (Bücking and Shachar-Hill, 2005; Fitter, 2006). A high rate of P transfer to the host is related to extra-radical hyphal production (Jansa *et al.*, 2005; Avio *et al.*, 2006) rather than to the intensity of root colonization (Ravnskov and Jakobsen, 1995). Hence, competitive AM fungi are likely to be those that allocate large quantities of carbon to growing mycelial biomass for soil exploration and soil P solubilization. In this situation, the trade-off traits are likely to be a lower investment in root-borne carbon storage structures (e.g. vesicles) and a delay in the reproductive effort.

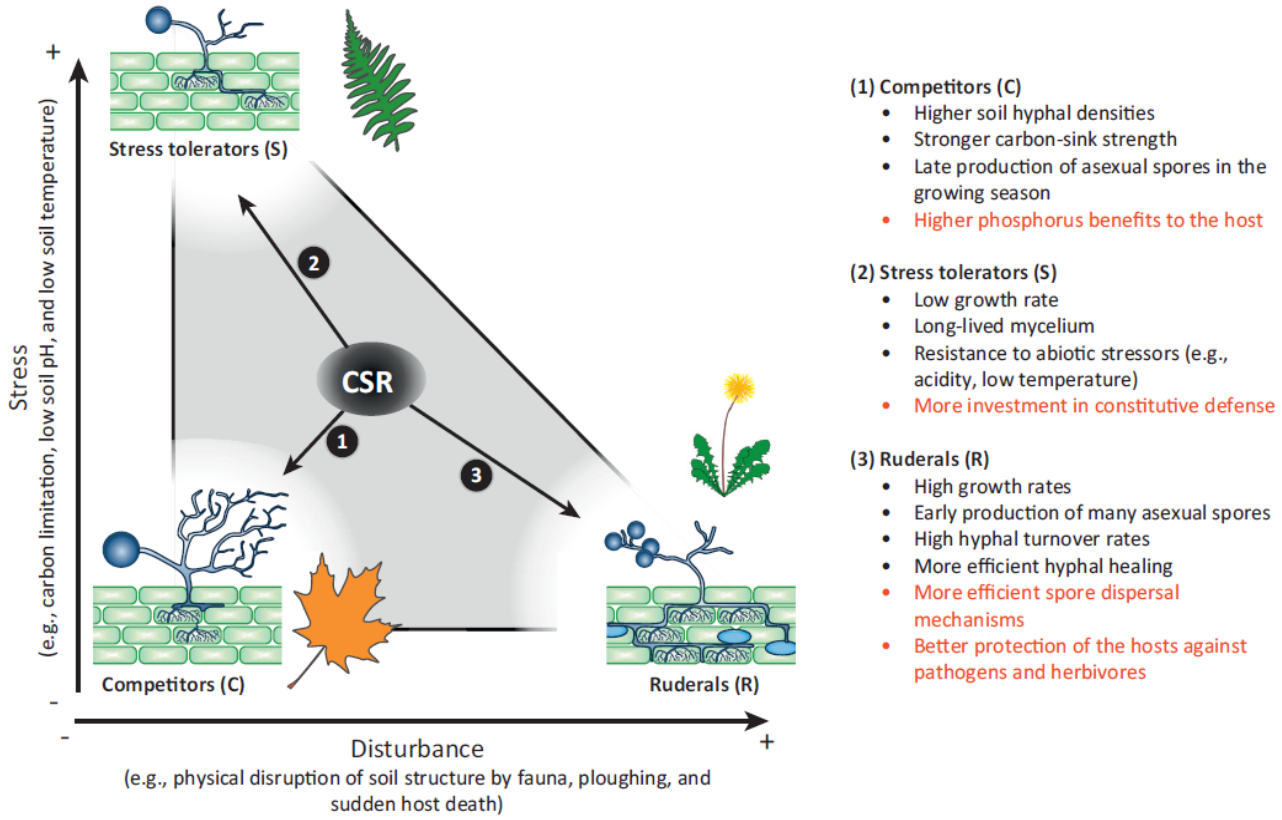

Figure 6. A C-S-R triangle identifying stress and disturbance factors as well as phenotypic traits of AM fungi classified as competitors, stress tolerators or ruderals. Empirical evidence is lacking for the suggested traits highlighted in red. On the triangle, we also illustrate the corresponding plant life-history strategy that would match each fungal strategy given that preferential associations are likely between plants and fungi with similar C, S or R strategies (see text).

There is evidence that AM fungi in the Gigasporaceae family show traits associated with a competitive life-history strategy. For example, members of the Gigasporaceae invest more biomass in extra-radical hyphae than in root-borne structures, compared with other phylogenetic groups (Hart and Reader, 2002; Maherali and Klironomos, 2007). Moreover, members of the Gigasporaceae increased dramatically in abundance in nitrogen (N)-fertilized plant communities where P availability in the soil was limited (Johnson *et al.*, 2003). In this case, added N increases the carbon-fixing potential of plant hosts, which exacerbates P limitation and consequently prompts them to provide more carbon to their fungal symbionts. Several isotope tracer studies have also provided direct evidence of a 'competitive' strategy among the Gigasporaceae, revealing that these fungi are stronger carbon sinks for plant carbon than other lineages (Pearson and Jakobsen, 1993; Lerat *et al.*, 2003). Finally, the Gigasporaceae in temperate ecosystems sporulate later in the growing season than AM fungi

from other taxa (Klironomos and Hart, 2002; Oehl *et al.*, 2009), which is consistent with a competitive life-history. Taken collectively, these traits indicate that AM fungal communities in low P, or high N-to-P, environments should favor members of the Gigasporaceae family owing to their shared traits related to high carbon acquisition from their host plants.

Plant species that would benefit most from competitive AM fungi are likely to be those with high soil P requirements and high carbon-fixing potential. This is likely to exclude ruderal plants, owing to their short life cycle and lack of nutrient limitation on disturbed, early-successional soils. Likewise, stress-tolerant plants would not fully benefit from competitive AM fungi because of their low growth rate and high resource use efficiency. Preferential associations between competitive AM fungi and competitive host plants are, therefore, likely, particularly under low soil P supply. Besides their matched nutritional benefits, their matched delay in reproduction effort would allow both organisms to invest in vegetative growth so as to derive reciprocal nutritional benefits for an extended period of the growing season. According to functional equilibrium models (Johnson, 2010), this matching of functional traits should create a positive feedback favoring dominance and stability of both organisms in their respective communities, and delay ecological succession.

4.3.2 Stress-tolerant AM fungi

AM fungi are stressed, for example, when the carbon supply from their host is consistently low. Under such conditions, successful AM fungi may be those that use carbon most efficiently, through the slow production of high cost, long-lived biomass. Reduced turnover rates should then reduce carbon costs in the long term (Chapin, 1980). To date, hyphal turnover rates in the order of a week have been measured for a few AM fungal strains belonging to the Glomeraceae (Staddon *et al.*, 2003). Measuring turnover rates across a broader phylogenetic spectrum may reveal that some taxa use plant carbon more efficiently than the Glomeraceae and, thus, correspond to a 'stress-tolerant' strategy. Efficiency in the use of host carbon could also be expressed by the ability of the fungus to complete its life cycle

with low biomass production, because this would reduce metabolic maintenance costs. Producing little extra-radical biomass would also reduce exposure to abiotic stress agents such as soil acidity or heavy metals.

There is some evidence that stress-tolerant strategies do exist among AM fungal species. For example, shading experiments have shown reduced root colonization by whole AM fungal assemblages (Tester *et al.*, 1985), suggesting a competitive advantage for carbon-efficient strains. This is corroborated by data from (Heinemeyer *et al.*, 2004) who reported a shift in AM fungal community structure in response to shading. Likewise, abiotic stress such as high soil acidity has frequently been shown to drive AM fungal community structure (Porter *et al.*, 1987; Johnson *et al.*, 1991; Oehl *et al.*, 2010). Specifically, AM fungi belonging to the Acaulosporaceae family are commonly reported in lower pH environments (e.g. Porter *et al.*, 1987; Oehl *et al.*, 2010; Morton, 1986). Also, high elevation sites with harsher climatic conditions frequently show a higher proportion of species belonging to the Acaulosporaceae family than is commonly seen in grasslands. Moreover, some Acaulosporaceae species are found exclusively in alpine environments (Oehl *et al.*, 2011). Consistent with the expectation of stress tolerance, members of this family produce less biomass (both extra-radical hyphae and internal root structures) than members of the Glomeraceae and Gigasporaceae (Hart and Reader, 2002; Maherali and Klironomos, 2007).

As with competitive AM fungi, we propose that there are likely to be preferential associations between stress-tolerant fungi and specific plant functional groups. For example, shade-tolerant plants will sparingly invest carbon in AM fungal symbionts because of their low rates of photosynthesis (Heinemeyer *et al.*, 2004). Indeed, plants growing under any adverse condition that limits carbon fixation are likely to limit the amount of carbon supplied to the AM fungal symbiont. Given that stress-tolerant AM fungi may be slow to provide nutritional and other benefits to their hosts, the initial cost of a fungal symbiont to their host may be high, although these could be offset by their long term benefits. Thus, the plants that are likely to benefit the most from stress-tolerant AM fungi are those with slow growth rates, long life spans and resource conservation strategies: in other words, stress-tolerant plants. It is important to note

that the predicted matching between stress tolerating plants and AM fungi strictly relates to the life histories of the partners. Although AM fungi can improve plant tolerance to various stresses such as drought or heavy metals (Schenck, 1981; Harris *et al.*, 1985; Griffioen and Ernst, 1989), the potential ability of AM fungi to alleviate host stress is not the basis for our prediction. Instead, we suggest that similarity in resource allocation to various components of the life history (i.e. growth and reproduction) may lead preferential associations between stress tolerant plants and AM fungi.

4.3.3 Ruderal AM fungi

From a mycocentric perspective, disturbance occurs when hyphal networks are broken, either by physical disruption of the soil structure or by faunal grazing. Disturbance could be an ecological filter selecting for ruderal traits that enable the rapid re-establishment of functional hyphal networks and symbiotic interactions with a plant host. A ruderal life history could be achieved through high growth rates and efficient hyphal fusion mechanisms by which fragmented hyphae can be reconnected to form functional mycelia (Avio *et al.*, 2006). Another way for ruderal AM fungi to reestablish a symbiosis following disturbance is by maximizing *de novo* colonization of roots by propagules. Thus, a short life-cycle leading to an early and constitutive investment in asexual spores could be a strategy by which ruderal AM fungi cope with disturbance. Likewise, efficient healing mechanisms that prolong the viability of colonized roots and soil hyphae that have been severed (e.g. Klironomos and Hart, 2002; De la Providencia *et al.*, 2005) would be consistent with an AM ruderal strategy.

Frequently tilled agricultural soils are likely to select for ruderal AM fungal strategies. Studies have shown that these soils tend to have low AM fungal diversity, and are dominated by species belonging to the Glomeraceae, more specifically to the *Glomus* group (Gr.) A clade (e.g. Helgason *et al.*, 1998; Jansa *et al.*, 2002; Maherali and Klironomos, 2012). Compared with other AM fungal families, *Glomus* Gr. A species: (i) grow faster (Powell *et al.*, 2009), (ii) fuse hyphae more readily (De la Providencia *et al.*, 2005), (iii) invest earlier and more

abundantly in spore formation (Oehl *et al.*, 2009), and (iv) form cross-walls that enable infected root pieces and severed hyphal fragments to heal and recolonize host roots (Klironomos and Hart, 2002; De la Providencia *et al.*, 2005). All of these traits are consistent with a ruderal life-history strategy. Also, the ratio of intra-radical relative to extra-radical hyphal abundance appears to be higher in the Glomeraceae than in other AM fungal families (Hart and Reader, 2002), which may comprise a disturbance-avoidance strategy.

Ruderal AM fungi with high growth rates and short life cycles should produce low-cost, albeit non-enduring, biomass. The cost of having to replace this short-lived biomass represents, therefore, a disadvantage to long-lived plants. Hence, ruderal plants with a similar short-term investment in low-cost biomass should preferentially interact with ruderal AM fungi. Given that ruderal plants colonize early-successional habitats where soil nutrients are rarely limiting (Navas *et al.*, 2010), the primary benefit they derive from AM fungi may not be P uptake, but rather an increased protection against phytopathogens (Newsham *et al.*, 1995). This is supported by the finding that early-successional ruderal plant species may be more prone to pathogen attacks than other plant functional groups (e.g. Kulmatiski *et al.*, 2008). Accordingly, *Glomus* Gr. A strains are more efficient at providing protection to plant hosts than other AM fungal lineages (Maherali and Klironomos, 2007). It has been suggested that this protection relies partly on a jasmonate-based plant hormonal pathway that also activates a number of anti-herbivore mechanisms (Pozo and Azcon-Aguilar, 2007). Hence, it is possible that ruderal AM fungi are involved in priming plant responses against herbivores as well (Kempel *et al.*, 2010).

Despite our use of the C-S-R framework to organize functional variation in AM fungi, we emphasize that the aim of this opinion paper is not to simplistically allocate species or even families to C, S or R strategy, nor to promote the C-S-R framework as the best way to make sense of functional diversity in the AM fungi. Rather, our aim is to identify the traits that are likely to be the most important components of AM fungal life histories. Likewise, preferential associations between plants and fungi may not follow the idealized cases where C, S and R plants would interact with C, S and R AM fungi, respectively. Associations in nature will

likely be much more complex because (i) plants and AM fungi involved will rarely be at any of the three extremes of the C-S-R triangle, but most of the time will rather have an intermediate life history and (ii) many factors, other than preferential partner selection, will influence the assembly of fungal assemblages in plant roots (e.g. plant neighborhood, spatial constraints on fungal species' availability, stochastic events). Still, the C-S-R framework offers a basis from which to develop a trait-based approach for AM fungi and advance our understanding of their life history strategies. In the following section, we identify five research areas where such a better understanding of AM fungal life history strategies would be particularly useful.

4.4 Potential advances in AM fungal ecology using a trait-based approach

4.4.1 Preferential association patterns with host communities

Some plants and AM fungi are known to interact preferentially in natural communities (e.g. (Vandenkoornuyse *et al.*, 2003); however, it is as yet unknown whether those over-represented interactions in communities are between symbionts that share compatible life-history strategies. If so, this would suggest a strong influence of niche-based (i.e. deterministic) processes underlying the assembly of plant–AM fungal communities. Such determinism could arise either from the matching of functional traits that optimize mutual benefits, or from both partners being similarly filtered along environmental gradients. Evidence for such determinism has been found (Chagnon *et al.*, 2012) in a previously described plant–AM fungal community (Öpik *et al.*, 2009): AM fungi from different families interacted preferentially with different plant species. Given the apparent phylogenetic conservatism of AM fungal traits at the family level (Powell *et al.*, 2009), these results would suggest a strong influence of deterministic (i.e. niche-based) mechanisms driving plant–AM fungal community assembly. Nevertheless, the pattern described in Chagnon *et al.* (2012) was mainly the result of one plant species that interacted with distinct fungal species compared to the rest of the community. Hence, more field surveys are needed to test this hypothesis. One fruitful avenue

would be to couple data on interaction patterns at given sites with a characterization of plant and fungal traits from those sites, to test for correlations between the two.

4.4.2 Succession patterns in AM fungal communities

A major debate in plant ecology over the past century has been the theoretical basis for ecological succession (e.g. Clements, 1916; Gleason, 1926; Odum, 1969; Tilman, 1990). Although the C-S-R framework was mainly focused on describing plant history traits in contrasting environments, it implicitly drew linkages between plant traits and autogenic succession, particularly when reconciled with a resource-based theory of competition and succession (Tilman, 1985; Grace, 1990). From these two frameworks, the paradigm of secondary succession that has evolved is one whereby short-lived ruderal plants colonize newly disturbed environments, to be replaced by competitive plants that optimize resource-use over the longer term, which are themselves eventually replaced by stress-tolerant plants once the demand for resources exceeds supply. By extension, a C-S-R approach could provide a trait-based explanation of temporal patterns that have been reported in AM fungal communities. For example, in a microcosm succession experiment, the early-stage communities were dominated by *Glomus mosseae* (Oehl *et al.*, 2009), which is often found dominating in agricultural fields (e.g. Helgason *et al.*, 1998). Similarly, later-successional AM fungal inocula produced relatively more soil hyphae than early successional ones (Sikes *et al.*, 2012), which is consistent with a switch from ruderal towards competitive life-history traits. Finally, late-successional fungi tended to form either larger spores or sporocarps (Allen *et al.*, 2003; Oehl *et al.*, 2011). More studies that link AM fungal traits and succession would help us to understand the potential interplay between plant and fungal succession, and its implications for ecosystem function.

4.4.3 Specificity of responses in plant–AM symbioses

A paradox of AM fungal ecology is that, although the specificity of association between different plant and AM fungal species is low (Smith and Read, 2008), the specificity of the

response to such associations is relatively high. Thus, the fitness consequences for both partners are highly dependent on the identity of the species involved (e.g. Sanders and Fitter, 1992; Klironomos 2003). This is likely to be related to the compatibility of measurable traits in each partner. For example, plants with coarse root systems may be more apt to derive a P benefit, whereas those with ramified root systems may rather derive pathogen protection from their AM fungal symbionts (Newsham *et al.*, 1995; Sikes *et al.*, 2009). This is only one example of how trait-matching may promote mutualistic benefit in the symbiosis, and many possibilities can still be explored (Fitter, 2006). By integrating several functional traits into discrete life-history strategies, a C-S-R framework would provide a more predictive approach for studying the specificity of response of various associations. Such predictive power would be valuable for agriculture or horticulture where best matches between various plant and AM fungal genotypes would enhance production.

4.4.4 Linkages between plant and AM fungal diversity

A trait-based approach could also provide insights to link plant and AM fungal diversity at fine spatial scales (i.e. within-site β-diversity). It is known that AM fungal community structure is highly heterogeneous at a one meter scale (e.g. Wolfe *et al.*, 2007). Given the specificity of the response of plants towards different AM fungal species, such a spatial structure in AM fungal communities may influence plant recruitment (van der Heijden, 2004) and contribute to the fine-grain spatial structure in plant communities. If there is preferential matching between AM fungi and plant hosts with analogous life histories, then it is likely that the spatial distribution of plants and fungi are tightly linked. There is thus an opportunity to test for such linkages in the spatial distribution patterns of plants and AM fungi that share similar life history strategies.

4.4.5 Phylogeny as a proxy for life-history traits in AM fungi

We suggested above that life-history traits of AM fungi may drive their biogeography and interaction patterns with host plant species (Fitter, 2005). To study the importance of this

Table 1. Examples of comparative studies with AM fungal isolates[a]

Trait measured	AM fungal taxa	Trait value	C-S-R	Explanation	Refs
Healing ability	*Glomus* Gr. A	Efficient healing, rapid re-growth	R	Re-establish functional mycelium after disturbance	[1]
	Gigasporaceae	Efficient healing, moderate regrowth	–		
Growth rate	*Glomus intraradices*	High	R	Replace biomass lost after disturbance	[2]
	Glomus etunicatum	Intermediate	–		
	Gigaspora gigantea	Low	C/S		
Hyphal turnover rate	*Glomus* spp.	High	R[b]	Low resource use efficiency (i.e. high tissue turnover rates)	[3]
Carbon sink strength	*Gigaspora rosea*	Strong	C	Relates to the ability of AM fungi to compete for plant carbon	[4]
	Glomus mosseae	Weak	S/R		
	Glomus intraradices	Weak	S/R		
Hyphal fusion	*Glomus* Gr. A	Frequent	R	Re-establish functional mycelium after disturbance	[1]
	Gigasporaceae	Infrequent	C/S		
Timing to sporulation	Glomeraceae	Early and constitutively	R	Short generation time	[5,6,7]
	Gigasporaceae	Fall in temperate systems	C	Delayed reproduction to favor resource acquisition	
	Acaulosporaceae	Spring in temperate systems	–		
Biomass allocation	Glomeraceae	Low in soil, high in roots	R	Reduced exposure to soil disturbance	[8,9]
	Gigasporaceae	High in soil, low in roots	C	High P acquisition and transfer to host	
	Acaulosporaceae	Low in both soil and roots	S	Low metabolic costs and exposure to soil stressing agents	

[a]We present functional trait values and their associated life-history strategy. No C-S-R strategy is assigned to a trait value when it does not constitute an explicit prediction of the C-S-R framework.

[b]Measured turnover rates are thought to be high, but comparisons with other taxa are needed. Ruderals are likely to have the highest turnover rates.

[c]references : [1] De la Providencia *et al.,* (2005), [2] Hart & Reader (2005), [3] Staddon *et al.,* (2003), [4] Lerat *et al.,* (2003), [5] Oehl *et al.,* 2009, [6] Klironomos *et al.,* 2001, [7] Pringle & Bever 2002, [8] Hart & Reader (2002), [9] Maherali & Klironomos 2007.

phenomenon, we must characterize the life-history strategies of AM fungal species based on their functional traits. The obvious way to achieve this is by collecting AM fungal strains from

a wide range of environments, cultivating these strains in pure cultures, and measuring a standardized set of traits. Given the enormity of this task, and considering that many AM fungal species are difficult to cultivate, it may be preferable to validate an established classification scheme that might correlate with AM fungal life histories. As we have alluded to in previous sections, many functional traits of AM fungi appear to be similar among close relatives within broad phylogenetic groupings (examples given in Table 1), particularly at the family level (Powell *et al.*, 2009). Such phylogenetic conservatism indicates that phylogeny would be a viable proxy for predicting the life-history strategy of AM fungal species and their relative performance in the field. For example, phylogenetic data were recently used to show that environmental filtering and dispersal limitations are important drivers of AM fungal community assembly (Kivlin *et al.*, 2011; Maherali and Klironomos, 2012). However, at this stage, AM fungal phylogeny is still undergoing major revisions (Redecker and Raab, 2006; Oehl *et al.*, 2011b). Moreover, the Glomeraceae family is a very heterogeneous one, with some *Glomus* species found dominating mature stands (Öpik *et al.*, 2009) or late stages of AM fungal succession (Oehl *et al.*, 2009, 2011a). Furthermore, considerable functional variability has been found among isolates of the same species in the genus *Glomus* (Hart and Reader, 2002; Munkvold *et al.*, 2004). We thus acknowledge that an eventual mapping of life histories onto AM fungal phylogeny will yield a portrait much more complex than what is outlined here. Future work should capitalize on the development of high-throughput sequencing to define a reliable phylogeny for AM fungi (e.g. Krüger *et al.*, 2012), and meanwhile, more effort should be placed to characterize life history traits of AM fungi with known phylogenetic affiliation.

4.5 Moving forward

The need for a trait-based approach in AM fungal ecology is not a novel idea in the literature (van der Heijden and Scheublin, 2007; Parrent *et al.*, 2010; Powell *et al.*, 2013). In this

opinion paper, however, we argue that grounding such a trait-based approach into an established life history classification scheme such as the C-S-R framework can provide more mechanistic insights about the relationship between AM fungal traits, plants traits and abiotic environment filters. In addition to its potential for summarizing the ecological niche of AM fungi based on functional traits, a C-S-R (or other similar) framework may help us to predict preferential associations between plant and AM fungal species in the field, as well as the specificity of response to these associations. Moreover, a trait-based functional grouping may improve our understanding of plant–AM fungal successional dynamics as well as biodiversity patterns in natural communities. However, moving our understanding forward will require that progress be made in at least two research areas.

First, it may be tenuous to compare trait values and life-history strategies of AM fungal species based on data from disparate studies because trait variation may be biased by differences in experimental design. We need to develop, therefore, a standard trait database for AM fungi with standardized protocols for plant growth conditions, host choice, stages in ontogenic development, and other factors that influence fungal trait states. Second, we need to refine our understanding of the basic biology of AM fungi to link morphology to functions that are targeted by agents of natural selection such as plant hosts, other biota and the abiotic environment. For example, members of the Gigasporaceae tend to produce thicker-walled hyphae than members of other AM fungal families (e.g. Thonar *et al.*, 2011), but it remains unknown whether this trait affects hyphal lifespan, resistance to fungivores, and the efficiency of nutrient translocation to hosts.

In plant science, the trait-based functional grouping is one of the conceptual advances that spurred the rapid expansion of databases that classify plants on the basis of their traits, the climatic and soil resource conditions under which they grow, and the interactions between plants and other biota (Westoby and Wright, 2006; Katge *et al.*, 2011). Such databases have facilitated comparative studies that correlate plant functions to their evolutionary history and their ecological consequences (Reich *et al.*, 1997; Wright *et al.*, 2004), leading to many insights about the mechanisms that govern the distribution and abundance of plants (McGill *et*

al., 2006). We suggest that an analogous database for AM fungi offers similar opportunities for understanding the causes of AM fungal distribution and abundance, and may eventually have important ramifications for applied fields such as agriculture and ecological restoration, where a judicious manipulation of the symbiosis could increase crop yields and the stability of introduced plant communities, respectively.

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Après l'ensemble des articles présentés ci-dessus, un objectif clair émergeait : j'avais besoin d'un échantillonnage intensif sur un site donné pour collecter à la fois des données sur :

1. les interactions entre plantes et CMA, i.e. le résultat de l'assemblage de la communauté symbiotique;
2. les abondances relatives ou fréquences relatives des espèces de plantes et CMA interagissant sur ce site : il est facilement concevable que les interactions puissent être simplement le résultat de l'abondance, où les espèces rares ont tendance à peu interagir entre elles, puisqu'elles ne se rencontrent pas fréquemment dans l'environnement (e.g., Stang *et al.*, 2007, Krishna *et al.*, 2008, Chamberlain *et al.*, 2010);
3. des données sur la distribution spatiale des plantes et des CMA : une plante et un CMA auront plus d'opportunités pour interagir s'ils sont distribués de façon similaire dans l'espace, un point crucial considérant que nos organismes à l'étude sont sessiles (e.g., Lewinsohn *et al.*, 2006);
4. des données sur les propriétés abiotiques du sol : une plante et un CMA pourraient être distribués de façon similaire dans l'environnement parce qu'ils répondent de façon similaire aux paramètres du sol (e.g., Dumbrell *et al.*, 2010);
5. des données sur les traits des plantes, afin de déterminer si les plantes qui ont des traits semblables interagissent avec des CMA semblables, tel que prédit par l'article qui précède.

Ainsi, l'article qui suit présente une étude observationnelle où j'ai collecté collecté l'ensemble de ces données en milieu naturel afin de déterminer l'importance relative de différents mécanismes neutres (e.g., abondance, distribution spatiale) ou basés sur la niche des espèces (e.g., sélection de partenaires basée sur les traits) dans l'assemblage d'une communauté mycorhizienne naturelle.

Chapitre 5

TRAIT-BASED PARTNER SELECTION DRIVES MYCORRHIZAL NETWORK ASSEMBLY

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5.1 Abstract

Plants and their microbial symbionts are often found to interact non-randomly in nature, but we have yet to understand the mechanisms responsible for such preferential species associations. Theory predicts that host plants should select symbiotic partners bearing traits complementary to their own, as this should favor cooperation and evolutionary stability of mutualisms. Here, we present the first field-based empirical test for this hypothesis using arbuscular mycorrhizas (AM), the oldest and most widespread plant symbiosis. Preferential associations occurring within a local plant-AM fungal community could not be predicted by the spatial distributions of interacting partners, nor by gradients in soil properties. Rather, plants with similar traits preferentially hosted similar AM fungi and, likewise, phylogenetically related AM fungi (assumed to have similar functional traits) interacted with similar plants. Our results suggest that trait-based partner selection may have been a strong force in maintaining plant-AM fungal symbioses since the evolution of land plants.

5.2 Introduction

Communities containing species having mutualistic relationships are often assembled in a non-random way, and a current challenge facing community ecologists is to identify the mechanisms driving such patterns (Vazquez *et al.*, 2009). Preferential partner selection is thought to be one important assembly mechanism for symbiotic communities, as it may be a way to avoid interacting with compatible, yet non-cooperative, symbionts (Sachs *et al.*, 2004). This is corroborated by laboratory studies suggesting that host organisms may reward cooperative symbionts (Kiers *et al.*, 2011), impose sanctions against cheating symbionts (Kiers *et al.*, 2003), or selectively screen for partners displaying specific traits (Nyholm and McFall-Ngai, 2004). However, the relative importance of preferential partner selection in the assembly of natural symbiotic communities remains an open question.

The arbuscular mycorrhizal (AM) association between plants and AM fungi is one of the most ancient and widespread terrestrial symbioses (Parniske, 2008). AM fungi are thought to have facilitated the colonization of terrestrial habitats by plants more than 450 million years ago, and it has been suggested that preferential partner selection has contributed to the surprising evolutionary stability of this symbiosis (Bever *et al.*, 2009; Kiers *et al.*, 2011). In natural communities, plants and AM fungi are generally found to interact non-randomly (e.g. Öpik *et al.*, 2009, Wehner *et al.*, 2014). Even though it has been argued that preferential partner selection may be important in structuring plant-AM fungal communities, any reported interaction patterns may also result from other mechanisms such as the ecological filtering of both partners along the same environmental gradients (Oehl *et al.*, 2010), or from neutral mechanisms affecting the spatial distribution of each partner group (e.g. Dumbrell *et al.*, 2010). Thus, the relative importance of preferential partner selection in driving the assembly of plant–AM fungal communities so far has remained elusive. Recently, it was suggested that functional traits and life history strategies may lead to preferential partner selection in the plant-AM fungal symbiosis (Chagnon *et al.*, 2013). More specifically, this framework predicts that preferential interactions observed in natural plant-AM fungal communities should involve hosts and symbionts sharing similar resource management strategies. For example, a stress-tolerant plant, whose strategy is predicated on carbon-use efficiency, may preferentially associate with AM fungi that are able to complete their life cycle with low biomass production

and turnover (Chagnon *et al.*, 2013). Given the evidence that AM fungi with similar functional traits appear to have close phylogenetic relatedness (Powell *et al.*, 2009), we predict that plants with similar functional traits should interact with groups of AM fungi that are phylogenetically clustered.

Here, we provide the first field-based empirical test for this hypothesis. We sampled a small natural plant–AM fungal community from which we calculated its modularity (Thébault 2013). This index measures the tendency of a community to be subdivided into subgroups of species that interact more frequently together than with the rest of the community (i.e. preferential associations). Such "modules" may arise from either neutral or niche-based mechanisms. For that reason, we then used various statistical and numerical methods to evaluate how the observed modular pattern was related to plant and AM fungal spatial distributions, soil chemical properties, plant functional traits and AM fungal phylogeny. Under trait-based partner selection, our prediction was that there would be significant modules, and that species found in the same modules would share similar traits (plants) or phylogenetic affiliation (AM fungi).

5.3 Materials and Methods

5.3.1 Field sampling

We sampled an old-field meadow near Sherbrooke, Canada (45° 24' N, 71° 54' W) that had not been cultivated for more than 40 years due to its low fertility. At the time of sampling, it was dominated by *Populus tremuloides Michx.*, with a herbaceous understory. We randomly established a 5 m x 5 m plot in which we identified a total of 9 plant species that could potentially host AM fungi (verified by root staining and microscopic observations) and that had at least 10 individuals (shoots) in the plot. Within the plot, we established a square, 16 point, sampling grid and noted the presence/absence of each plant species in a 30 cm radius around each point. *P. tremuloides Michx.* was excluded from the sampling because its root system was deemed too large relative to the size of the grid, thereby precluding an unbiased

estimation of its belowground spatial distribution. At each point, we also took a 15 cm diameter soil core (10-15 cm deep) in order to characterize the local AM fungal community (to monitor the spatial distribution of AM fungi in our grid) as well as soil chemical properties. These cores were placed on ice packs in a cooler and transported to the laboratory, where they were kept at 4 °C until the time of processing (within 8 h after sampling).

Ten individuals of each plant species were randomly selected and destructively sampled to collect fine root material, which eventually would be used to characterize plant-AM fungal interactions. These samples thus served to build our bipartite network from which modularity was characterized (see below). Plant roots were stored in sealed plastic bags at -20 °C until DNA extraction (explained below). We collected fresh leaves and roots from an additional seven individuals per species to measure three traits: (1) specific leaf area (leaf area per dry mass), (2) leaf dry mass content (dry weight to fresh weight ratio) and (3) specific root length (root length per dry mass). These traits were chosen because they provide complementary information on plant life history strategy and leaf economics (Roumet *et al.*, 2006; Pierce *et al.*, 2013).

5.3.2 Soil physico-chemical properties

For each soil core, we measured the following: (1) % organic matter (% mass loss after ignition in a muffle furnace at 400°C for 16 h), (2) total C:N ratio (as measured by an Elementar Vario Macro analyzer), (3) % humidity (% mass loss after oven heating at 105°C for 36 hours), (4) Mehlich-III extractable phosphorus (P) (extraction of P with Melich-III solution and quantification by spectophotometry at 882 nm), and (5) pH in water and in KCl (using a Accumet AB-15 pH meter). More details about the methods can be found in Carter and Gregorich (2007).

5.3.3 Characterizing AM fungal assemblages

The root systems of the sampled plants were washed thoroughly under tap water, and fine roots (≤1 mm diameter) were isolated and collected with forceps and scissors. We ensured that AM fungal interactions were associated to the correct plant species by only keeping fine root material still attached to the source plant after root washing. Approximately 300 mg (fresh wt) of each fine root sample were transferred to 1.5 mL tubes, and DNA was extracted with MoBio Ultra Clean Plant DNA isolation kits following the manufacturer's instructions. To characterize AM fungal communities in each soil core, we extracted DNA from 5 soil subsamples (ca. 1-2 g) as well as from 5 fine root subsamples taken from each core. For the soil subsamples, we used MoBio Power Soil DNA isolation kits. By sampling DNA in soil as well as in roots from each core, we minimized the bias that could arise from some AM fungal species producing less biomass than others, or some AM fungal species investing different amounts of biomass in roots than in soil (e.g. Hart and Reader 2002).

AM fungal DNA was amplified using a nested PCR approach, given that preliminary attempts at amplifying AM fungal DNA directly from the DNA extracts were unsuccessful. In the first round, total fungal DNA from each DNA extract was amplified using 2 µL of DNA extract solution, 10 µL of HotStart Taq Master Mix kit solution (QIAGEN), 0.125 µL of T4Gene32 protein solution (New England Biolabs), 4 µL of 0.5 µM NS1-SR5 fungal-specific primer solution (White *et al.*, 1990, RytasVilgalys' lab, http://biology.duke.edu/fungi/mycolab/primers .htm) and 3.875 µL of ultra-pure water. In the second round of PCR, amplicons from round 1 were used as templates, and the primer set was the AM fungal specific AM1-NS31 couple (Helgason *et al.*, 1998). Because PCR products were meant to be sequenced by 454 sequencing, additional nucleotides were attached to those primers, following instructions from the sequencing facility (Génome Québec, Montreal). All PCR products that belonged to the same plant species or soil core were tagged with a similar molecular identifier (MID – Roche) integrated in their primer to allow pooling of the different amplicons for sequencing. Thus, plant-AM fungal interactions were not monitored at the plant individual level, but rather at the species level. DNA was purified using Agencourt AMPure beads (Beckman Coulter) to isolate long, double-stranded DNA from single DNA strands,

remaining primers and impurities. DNA concentration in each sample was then quantified with replicated spectrophotometry (Nanodrop) lectures and an equimolar amount of each amplicon was added to the final pool, which was sent to be sequenced at Genome Québec facilities (Montréal, QC).

For an unknown reason, no amplicon was sequenced for 1 of our 9 plant species. For the remaining samples, a sequence-based phylogenetic tree was built using the open source QIIME software package (Caporaso *et al.*, 2010). First, the MIDs were split into separate sequence libraries for plant roots and soil cores. Sequences were excluded from the dataset if (1) their primer and/or barcode sequence was missing or erroneous, (2) their length after trimming out the primer was less than 200 (i.e. poor sequence) or more than 500 (i.e. too high for the 454 technology at that time), or (3) they included homopolymers longer than 5 base pairs. An equal number of sequence reads was randomly drawn from each library and AM fungal operational taxonomic units (OTUs) were based on a 97% sequence similarity threshold, using the USEARCH clustering algorithm (Edgar, 2010). Before clustering, sequences were sorted by length to increase the probability that longer sequences would develop into centroids of OTU clusters. This clustering procedure was performed once again, this time by identifying clusters based on comparisons with the MaarjAM database for Glomeromycota (Öpik *et al.*, 2010). A subsequent Mantel test revealed a highly significant correlation between the two outputs ($r = 0.784$, $P < 0.0001$). Below, we present results from the first clustering output. Singletons as well as rare OTUs (with 5 or less occurrences), which may represent artifacts or transient species (Tedersoo *et al.*, 2010) were removed from the dataset. We also excluded OTUs that exclusively were found in soil DNA extracts, as these were not relevant to the study, where we looked at preferential associations with host plants.

After filtering the dataset, the remaining OTU sequences were blasted against the MaarjAM online database (Öpik *et al.*, 2010). Sequences that did not match any entry in MaarjAM were blasted in GenBank and most of these were found to be either plant or non-AM fungal DNA. After eliminating non-AM fungal sequences, the remaining sequences were aligned and a phylogenetic tree was built using the MUSCLE algorithm (Edgar 2004).

5.3.4 Modularity analysis

We computed modularity from our bipartite network, which consisted of plant-fungal interactions pooled at the plant species level. Modularity was calculated following Barber's index (Barber 2007), which provides more consistent results than alternative indices (Thébault 2013). Briefly, the algorithm uses a simulated annealing procedure to find the maximal modular configuration of the network. Simulated annealing is a heuristic optimization technique that allows exploring suboptimal configurations in the initial stages of the iteration process, which avoids getting "trapped" in local maxima (of modularity, in our case) and ensures converging on the global maximum. More details are given in Cěrny (1985). Statistical significance of the observed modularity pattern was tested using a null model that randomized the interactions in the matrix while preserving the total number of interactions as well as the number of interactions for each plant and AM fungal species (i.e. row and columns totals). This is argued to be the optimal null model to avoid type I errors (e.g. Ulrich and Gotelli 2013).

5.3.5 Testing various factors that could explain plant-AM fungal interaction patterns

We first tested for a relationship between the frequency of occurrence of plants or AM fungal OTUs (i.e. around or inside our soil cores, respectively) and their number of interactions, using Pearson's correlation coefficient. We then tested whether the observed plant-AM fungal interaction pattern could be predicted by the spatial distributions of hosts and symbionts. For this, we randomized our bipartite network using a second null model that kept the same constraints as the first (described above), but allocated interactions between pants and AM fungi based on the relative overlap of their spatial distribution (monitored around and in our soil cores, respectively). In other words, two species that co-occurred frequently in our plot had a higher probability of interacting together in our simulations. To compute these probabilities, we compared the observed co-occurrence frequency of each species pairs to 1000 randomized scenarios where the spatial distribution of plants and AM fungi had been

shuffled in our sampling grid. We calculated co-occurrence indices as *z*-scores ($z = \frac{observed - mean_{random}}{sd_{random}}$), which we then scaled to values between 0 and 1 representing a probability for each species pair to interact. Thus, a plant and an AM fungus that co-occur frequently in our sampling grid would get a high *z*-score, and consequently a probability of interacting in our null matrices close to 1. We then used those interaction probabilities for each species pair to draw from a binomial distribution, in order to build our null matrices. We repeated the draws until each null matrix was filled according to our constraints (i.e. row and column totals identical to our real network).

We investigated a possible link between plant and AM fungal spatial distribution and soil properties using canonical correspondence analysis (CCA), which correlated presence/absence of plants and AM fungi around or inside each soil core, respectively, to the measured soil physico-chemical properties of that core. This was done to ensure that preferentially interacting plants and AM fungi were not doing so simply because they were similarly filtered by the abiotic environment. CCA was also used to explore a possible link between plant-AM fungal interaction patterns and plant functional traits. Based on evidence that some AM fungal traits are phylogenetically conserved (Powell *et al.*, 2009), we used AM fungal phylogenetic distance as a proxy for functional distance among AM fungal OTUs. To determine whether fungal phylogeny was linked to plant-AM fungal interaction patterns, we used (1) a Mantel test correlating AM fungal phylogenetic distances to Bray-Curtis distances that measured the similarity in plant host choice, and (2) a permutation-based test of the phylogenetic relatedness of AM fungi found within modules. This latter test calculated a ratio $R = \frac{\sum_{k=1}^{n} \overline{x_k}}{nX}$, where *k* identifies the network modules, *n* is the total number of modules, x_k is the mean phylogenetic distance between AM fungal taxa comprised in module *k*, and *X* is the mean phylogenetic distance between all AM fungal taxa in the network. We compared this ratio to 1000 random values obtained by shuffling the tips of the phylogenetic tree and recalculating the ratio *R*, using a one-tailed *z*-test.

All statistical analyses were coded in R. CCAs were run using the *vegan* package (Oksanen *et al.*, 2013), whereas phylogenetic analyses and tree plotting were respectively performed using the packages *picante* (Kembel *et al.*, 2010) and *ape* (Paradis *et al.*, 2004). Modularity analysis used the C++ executable program MODULAR (Marquitti *et al.*, 2014).

5.4 Results

Our plant–AM fungal network was significantly modular under both null models, and revealed 3 subgroups in which interactions between specific plant species and AM fungal taxa were significantly over-represented (fig. 7). This high modularity occurred despite a strong positive relationship between the spatial frequency of soil-borne AM fungal taxa and their number of host plants (fig. 8): such neutral assembly of networks based on abundance or frequency could be expected to generate a strongly nested pattern with low modularity (e.g. Krishna *et al.*, 2008). Given that our second null model controlled for the spatial overlap of plant and AM fungal OTUs, our results

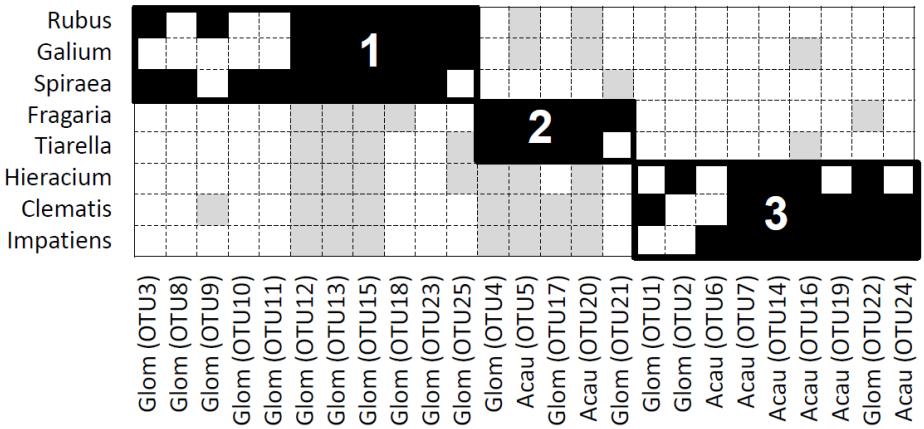

Figure 7. The observed plant-AM fungal interaction matrix (with plants as rows and AM fungi as columns) sorted into its maximally modular state. The calculated modularity index was 0.245, and was significant (P < 0.01) with both null models. Black cells represent interactions belonging to one of 3 modules (modules are boxed and numbered), grey cells are interactions that did not belong to any module, whereas white cells depict the absence of an interaction. Plant species: *Rubus pubescens*; *Galium sp.*; *Spiraea alba* var. *latifolia*; *Fragaria virginiana*; *Tiarella cordifolia*; *Hieracium aurantiacum*; *Clematis virginiana*; *Impatiens capensis*. AM fungal taxa: Glom = Glomeraceae family; Acau = Acaulosporaceae family.

confirm that the modular pattern was not generated by spatial distributions. More precisely, most of the interactions that were not predicted by spatial overlap occurred within network modules, while the majority of interactions that could be predicted by spatial patterns occurred outside network modules (i.e. grey cells in fig. 7), thus contributing to blur the modularity pattern (χ^2 = 17.8, P < 0.0001). Furthermore, CCAs revealed no significant relationships between soil chemical properties and plant or soil-borne AM fungal spatial distributions (P = 0.32 and P = 0.12, respectively). Instead, CCA revealed a significant relationship between plant traits and the structure of the root-borne AM fungal community (fig. 9). This relationship was marginally significant (P = 0.059) when all three measured plant traits were considered in the analysis, but was highly significant (P < 0.01) when considering only leaf dry mass content. Furthermore, mean phylogenetic distance among AM fungal taxa, considered to be a proxy for functional distance (Powell *et al.*, 2009), was significantly lower within than among modules (z = -4.48, P < 0.0001, fig. 10). Thus, module affiliation was not distributed randomly across AM fungal phylogeny. Likewise, the similarity of plant host choice among fungal taxa, as measured by Bray-Curtis distances, was significantly related to fungal phylogenetic distance (Mantel's r = 0.27, P = 0.006). It should be noted that Mantel tests are expected to display higher rates of type II errors as compared to alternative methods such as phylogenetic eigenvectors, so our correlation estimates should be considered conservative (Tedersoo *et al.*, 2013).

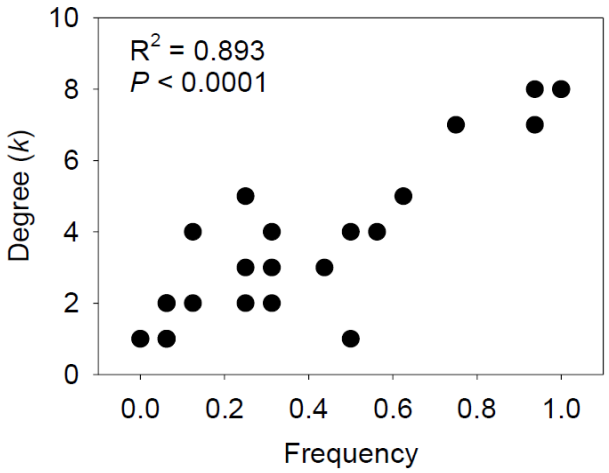

Figure 8. Positive relationship between the number of interactions (i.e. degree, *k*) of AM fungal taxa and their frequency of occurrence. Each circle represents an AM fungal taxon.

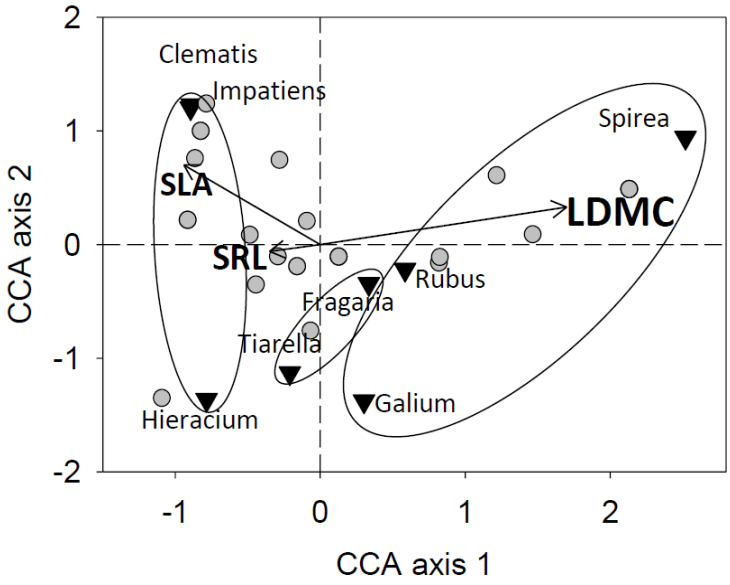

Figure 9. CCA biplot showing the relationship between plant traits and AM fungal assemblages in their roots. Plant species = black triangles and AM fungal taxa = grey circles. Displayed plant morphological traits are leaf dry mass content, specific leaf area, and specific root length. Ellipses delineate plant-AM community modules.

5.5 Discussion

The present results show clear evidence for preferential partner selection in the plant-AM symbiosis. Our modularity analysis defined three subsets (i.e. modules) of preferentially interacting plant and AM fungal species. These modules were not comprised of species that responded similarly to gradients in soil properties, nor species that showed similar spatial distributions. Instead, network modules were comprised of plants with similar traits and AM fungi that were phylogenetically clustered. Taken collectively, these results provide strong support for our initial hypothesis that trait-based partner selection is an important mechanism driving plant-AM fungal interaction patterns and community structure (Chagnon *et al.*, 2013). Our results thus provide a functional framework for understanding the non-random interaction patterns between plants and mycorrhizal symbionts that have previously been reported (e.g. Öpik *et al.*, 2009; Tedersoo *et al.*, 2013; Wehner *et al.*, 2014).

Sikes *et al.* (2009) showed that the morphology of the root system was a major determinant of the type of benefit provided by a given AM fungal taxon (i.e., P acquisition vs. protection against pathogens). A highly branched root system (i.e. high specific root length) may increase a plant's ability to forage for P, but it could at the same time increase its exposure to pathogens. Thus, specific root length is a root trait considered by some to be a major driver of plant-AM fungal interactions (Hetrick *et al.*, 1992; Newsham *et al.*, 1995). It was, therefore,

counter-intuitive that specific root length turned out to be the poorest predictor of the plant-AM fungal interaction pattern in our study. Instead, the plant trait that best predicted the structure of this below-ground symbiotic community was leaf dry mass content, an above-ground plant trait. As leaf dry mass content is related to a plant's resource conservation strategy (Pierce *et al.*, 2013), this result indicates that plants preferentially associate with AM fungal partners based on a broad set of traits related to life history strategies (Chagnon *et al.*, 2013), thus suggesting that plant-AM fungal cooperation may go beyond simple resource trading (Werner *et al.*, 2014). In other words, partner selection in this system may not only come from selective reward and resource allocation: we may as well see hosts as habitat patches displaying specific traits that cause AM fungal species sorting (e.g. Mihaljevic, 2012; Chagnon *et al.*, 2013).

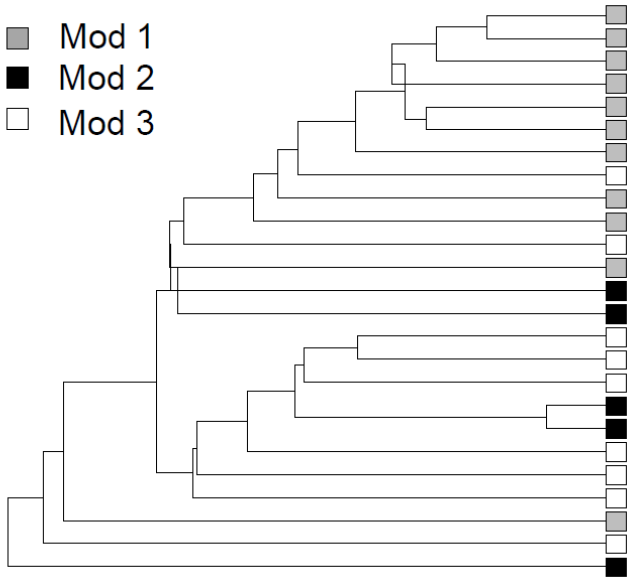

Figure 10. Phylogenetic tree showing the module affiliation for AM fungal taxa.The tips of the tree are colored following the module affiliation of each AM fungal taxon.

Partner selection, as suggested by our results, may be the basis for the evolution of cooperation in interspecific symbioses, by ensuring optimal fitness alignments between hosts and symbionts (e.g. Sachs *et al.*, 2004). This theory is discordant, however, with some laboratory-based evidence that plants may accumulate detrimental AM fungal species in their rhizosphere, thus generating negative plant-soil feedbacks (e.g. Bever 2002). We can think of three reasons for this apparent contradiction: (1) laboratory-based plant-soil feedback experiments involve AM fungi that grow easily in pots, namely ruderal species that are not representative of natural AM fungal communities in the field (Sykorova *et al.*, 2007; Hodge and Fitter, 2013), (2) mechanisms controlling resource trading and, by implication, partner

selection are not perfect, and may even promote cheaters under some circumstances (e.g. Walder *et al.*, 2012), and (3) partner selection is not driven by preferential carbon allocation by the host, but by immutable host traits that only benefit the fungal symbiont. To argue that partner selection stabilizes the AM fungal symbiosis at evolutionary timescales, it is necessary to demonstrate that the preferentially selected partners are those that maximize host fitness, which we cannot achieve using only observational data as we have.

We chose here to sample a small plot, specifically to limit heterogeneity in the abiotic environment (i.e. light and water availability, soil physico-chemistry). It should be of no surprise, then, that we found no effect of environmental filtering in generating the modular patterns. It seems very plausible, thus, that at larger spatial scales, modular patterns may results from the joint consequence of partner selection and spatial segregation due to environmental filtering. Also, given our small plot size, the plant niche gradient along which AM fungi could have been sorted in our study was relatively short as compared to the wide diversity of AM fungal hosts found in nature. More specifically, our sampled plants were mainly perennials displaying an intermediate tolerance to shade, whereas the regional pool of potential AM fungal hosts include ruderals (e.g. *Plantago major*) as well as long-lived competitive species (e.g. *Acer saccharum*). Thus, over a wider range of plant functional types, it is likely that trait-based partner selection may be much more apparent than what we have shown here.

Our approach also provides insights on the hierarchical organization of symbiotic networks. First, we revealed a strong relationship between the spatial frequency of AM fungal taxa and their number of host plant species. This indicates a role for stochastic neutral processes as a first filter driving the interaction patterns of plants and AM fungi (i.e. determining co-occurrence patterns). Then we provided strong evidence that plants are able to locally select for specific AM fungal partners (and/or vice versa) according to trait-based and phylogenetic features. This indicates a role for niche-based processes as a second filter driving the interaction patterns of co-occurring plants and AM fungi. Thus, overall, our approach highlights the value of complementary data sets (e.g. species spatial distributions, soil or other

environmental gradients, functional traits, phylogenetic relatedness, etc.) that allows us to characterize interaction patterns among species, and also to disentangle the likely drivers of such interactions (Chagnon *et al.*, 2014).

In conclusion, this research raises several research areas that should be explored to better understand the assembly of plant-AM fungal networks. For example, future work should:

- *Characterize plant-AM fungal network structure along seasons:* we know that network structure may change over the growing season (Bennett *et al.*, 2013), but we do not yet understand the biological mechanisms underlying such changes (e.g. facilitating interactions among AM fungi, progressive AM fungal species sorting in plant roots, demographic stochasticity, accumulation of AM fungal DNA through deposition in storage propagules such as vesicles, etc.);
- *Characterize "active" plant-AM fungal networks:* when using crude root DNA, there is a high probability of including transient or inactive species. One way to circumvent that problem is to use stable isotope probing methods to only capture DNA synthesized from recently transferred C to AM fungi by the hosts (e.g. Vandenkoornhuyse *et al.*, 2008);
- *Repeatedly sample the same plant-AM fungal networks over several years:* Modularity may have important consequences for species' coevolution, as it may be expected that species that preferentially interact (i.e. belonging to the same module) will progressively become more tightly adapted to one another (Guimaraes *et al.*, 2011). However, the underlying condition for this is that module affiliation is stable over generations (Chagnon *et al.*, 2012);
- *Quantifying interaction strength in nature:* Here we only report interactions in a binary way (presence/absence). Some species may depend more strongly on a small subset of their potential partners, and this would have important consequences on the resistance and resilience of communities facing perturbations (e.g. McCann *et al.*, 1998). One way to assess interaction strength would be to sample replicated networks in a given study system: this would allow us to tease apart the species pairs that (a) never interact in spite

of frequent co-occurrence in a small plot (avoidance), (b) interact and co-occur frequently (potentially neutral/opportunistic interactions) and (c) species that are rare but always found co-occurring and interacting together (potentially tracking each other in the environment, i.e. very strong interaction);

- *Combine field surveys with laboratory studies:* To provide direct evidence that plants tend to preferentially associate with AM fungi that best improve their own fitness, we need to combine natural observations of their interaction patterns with laboratory assays with pure AM fungal cultures. It may be that a plant's traits make it a good habitat for specific AM fungi that do not necessarily improve host fitness.
- *Investigate for trait-based partner selection in early community assembly and over short intervals:* There is evidence that priority effects exist in the assembly of AM fungal communities in plant roots (e.g. Mummey and Rillig 2009, Hausman and Hawkes 2010). Thus, the build-up of preferential interactions in the rhizosphere may be spread out over multiple generations. Here, we sampled a relatively mature system with long-lived perennials. It would be interesting to investigate community-level patterns in early successional communities, or short pot experiments. Regarding the latter, there is evidence that plants may build up specific AM fungal communities over a single generation (e.g. Bever *et al.*, 1996; Eom *et al.*, 2000; Bever, 2002).

Furthermore, our results suggest that, more broadly, a trait-based framework could be developed to better understand the feedback dynamics between plants and their non-symbiotic rhizosphere or phyllosphere microbial communities. There is growing evidence that such interactions may dramatically influence plant community dynamics (e.g. Kardol *et al.*, 2006), but our understanding of the underlying processes so far has remained limited. By incorporating information on plant functional traits and microbial phylogeny in plant-microbial feedback studies, we may find, for example, that the accumulation of beneficial/detrimental microbes may also be linked to hosts' life-history strategies and reciprocal reward/sanction systems (e.g. Mitchell *et al.*, 2010; Kobe and Vriesendorp, 2011; Chagnon *et al.*, 2013). Plant-microbial network structures could, therefore, become more predictable, and their ecological and evolutionary significance better understood.

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L'article qui précède démontre clairement la présence d'associations préférentielles entre plantes et CMA en milieu naturel, et l'implication potentielle des traits des plantes dans la sélection de partenaires. Toutefois, les patrons observés représentent ce qu'on pourrait qualifier de niche réalisée des espèces en ce qui a trait aux interactions mycorhiziennes. En d'autres termes, nous ignorons, avec cet échantillonnage, l'ensemble des interactions *possibles*, mais non réalisées, entre les plantes et les CMA échantillonnés. Les espèces apparaissant comme spécialistes sur le terrain le sont-elles à cause d'une réelle spécialisation ou simplement parce qu'elles sont localement rares ou ont une distribution spatiale agrégée? Poisot *et al.* (2011a) font à cet égard la distinction entre spécialisation potentielle et spécialisation réalisée : une espèce pourrait avoir peu d'interaction sur le terrain mais avoir la capacité d'interagir avec un bien plus grand nombre d'espèces (i.e. forte spécialisation réalisée, mais faible spécialisation potentielle).

Afin d'adresser la question de la spécialisation dans la symbiose mycorhizienne, dans le prochain article je présente une étude empirique où j'ai fait pousser des plantes soit en communauté ou individuellement. Ainsi, dans un cas (plantes seules) la sélection de partenaires peut se faire sans aucune contrainte (hormis la disponibilité des différentes espèces de CMA dans le sol), alors que dans l'autre (plantes en communauté), la sélection peut être contrainte par les interactions compétitives avec les plantes voisines ou les effets de priorité dus à celles-ci. En effet, des études ont démontré que l'identité des plantes voisines peut influencer les communautés de CMA dans les racines d'une plante donnée (e.g., Mummey and Rillig, 2006; Hawkes *et al.*, 2006; Hausmann and Hawkes, 2009), et Hausmann and Hawkes (2010) ont aussi trouvé que l'ordre d'établissement des plantes peut jouer un rôle dans l'établissement des interactions entre plantes et CMA. Ainsi, dans le cas où une plante aurait une forte spécialisation potentielle (i.e. réellement spécialisée pour interagir avec certains CMA seulement), on devrait trouver peu de variation entre ses interactions lorsqu'elle pousse seule ou en présence de plantes voisines. Cette étude constituait aussi une opportunité pour tester l'hypothèse selon laquelle la sélection de partenaires mycorhiziens par les plantes basée sur leurs traits peut s'effectuer à l'intérieur d'une seule saison de croissance. En effet, puisque l'étude présentée dans l'article précédent impliquait des plantes pérennes, les associations

préférentielles auraient pu résulter de l'effet additif de plusieurs années de sélection de partenaires, où des communautés différenciées de CMA se seraient bâties d'une année à l'autre dans la rhizosphère de chaque espèce de plante.

Chapitre 6

MYCORRHIZAL NETWORKS HAVE A DETERMINISTIC, YET FLEXIBLE, ARCHITECTURE

Chagnon, P.L., Bradley, R.L. and Klironomos, J.N.

(In preparation, to be submitted to Ecology Letters)

6.1 Abstract

Most studies looking at the resilience and/or resistance of ecological networks to local disturbances such as species removal have only considered current interactions and neglected the ability of networks to rewire. Such rewiring, which may take place through the establishment of novel interactions or through shifts in interaction strengths, is likely to buffer the effects of disturbances on ecological networks. We thus need to better take it into account in future studies. Here, we assessed the flexibility in mycorrhizal networks' structure, and thus its potential for rewiring under different circumstances. We show that although deterministically organized through trait-based partner selection, mycorrhizal networks have flexible structure. This is because most species can interact with a wide range of partners in different contexts. Thus, coextinction cascades are more likely to be buffered by flexible interaction patterns than by static network properties such as nestedness. This goes against received wisdom about the implication of network structure on community dynamics, and call for an assessment of interaction flexibility in other mutualistic systems.

6.2 Introduction

One of the current major challenges for ecologists is to predict how species will evolve (Gienapp *et al.*, 2014) or shift their distributional ranges (Savage and Vellend, 2014) in response to current global changes. But given that species are also involved in a myriad of interactions with other species (e.g., mutualisms, parasitisms, trophic interactions) an additional challenge is to predict how global change might influence such interactions. For example, many land plants rely on animal pollinators for reproduction, and it remains an open question whether the independent response of both guilds to global warming, for example through shifts in their phenology, is likely or not to decouple these pollination interactions and have consequences on community dynamics (e.g., Memott *et al.*, 2007). On one hand, if an interaction becomes impossible between a plant and a pollinator, it may decrease pollen transfer for the plant, and even lead to its population collapse. On the other hand, the plant may simply experience more pollen transfer by its other current pollinators or establish novel interactions with new partners. Some species removal experiments have indeed showed that networks of interacting species can rewire after a disturbance such as species removal, thus giving rise to novel interactions (Borvall *et al.*, 2000). However, for most ecological networks, we only have short-term data on interactions that occur at a given place and time, and we know little about the whole range of interactions that are possible between groups of species such as plants and pollinators. In other words, we may say that we only have data on realized interactions niches of species, that is, the interactions that are actually realized and observed in the field. However, we rarely have data on fundamental interaction niches, i.e. the whole range of possible interactions between species. Yet, the latter type of data is needed to evaluate the resistance and resilience of ecological networks to disturbances such as local species extinction. Indeed, broad fundamental interaction niches may contribute to buffer the effect of local extinctions and to avoid cascades of secondary extinctions (e.g., Loeuille 2010, Blüthgen 2010).

Mycorrhizal networks, that is, networks involving most land plants and soil symbiotic fungi, are no exception to the rule in that we still know very little about species' fundamental and

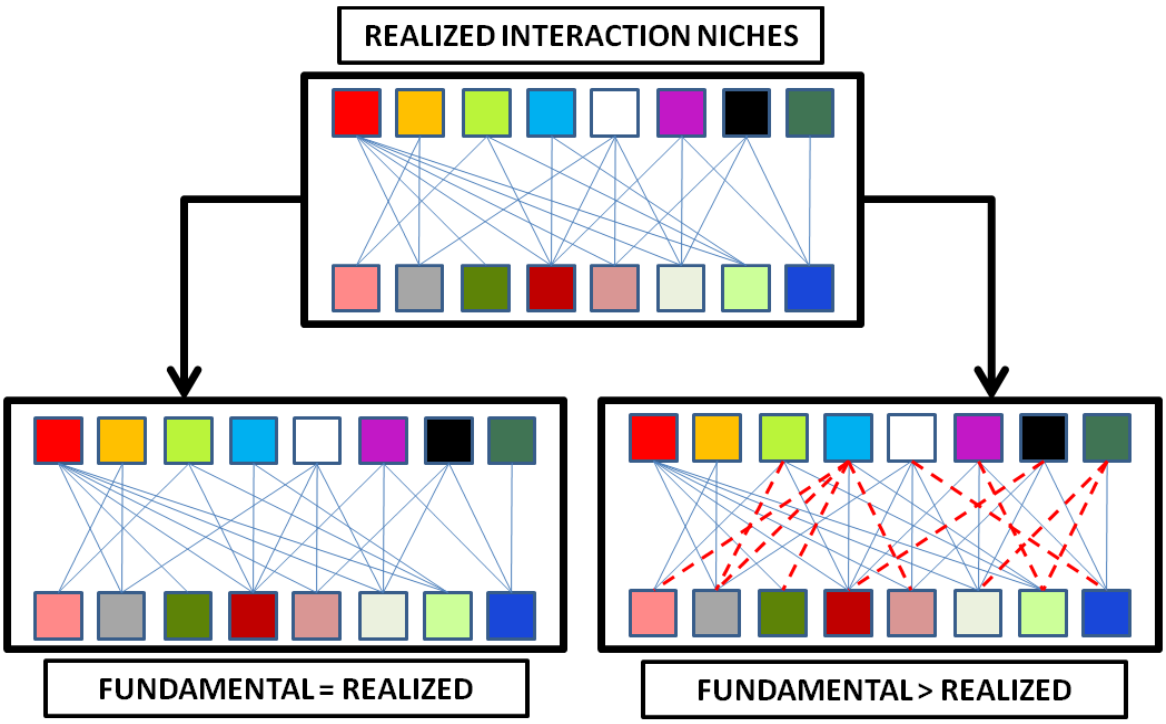

Figure 11. Two potential scenarios of interaction niches. In the rigth one, we see that on top of interactions that are realized in the field (blue lines in the top panel), many more interactions are possible but not realized in the field (dashed red lines in the right panel), thus part of the fundamental interaction niche. Conversely, in the left panel, no additional interactions are possible, indicating that fundamental interaction niche is no broader than the realized interaction niche. In this case, the system may be prone to coextinction cascades.

realized interaction niches. New sequencing technologies have allowed an unprecedented effort in characterizing realized interactions in the field. Yet, we are still mostly ignorant of species' fundamental interaction niches. As a result, we know virtually nothing about the resilience and resistance of these networks when facing local disturbance such as stochastic species extinction. If the fundamental interaction niches are very broad (i.e. much broader than what is observed in a single temporal/spatial snapshot in the field), then there is a high probability that the network will be very resilient to disturbances because it will allow extensive rewiring of the interactions following the disturbance, thus buffering its impact (fig 11). On the other hand, if the interactions recorded in the field represent most of the possible interactions between plants and fungi (i.e. fundamental interaction niche no broader than realized ones), then very little rewiring of interactions is allowed after a disturbance, thus increasing the likelihood of coextinction cascades. In order to characterize those fundamental

interactions niches, it is necessary to evaluate the relative importance of various deterministic and stochastic mechanisms in driving mycorrhizal interactions. For example, absences of interactions may be determined by species' local relative abundances, where rare species rarely encounter each other in the landscape, or by priority effects whereby an AM fungus that colonized a root system earlier prevents other fungi to colonize it. Alternatively, interactions may be driven by patterns of specialization and deterministic partner selection. The former scenario would suggest that fundamental niches are broad, while the latter would suggest the reverse.

To address this issue, we studied the assembly of mycorrhizal networks between 20 plant species and a natural community of arbuscular mycorrhizal (AM) fungi in 2 contrasted scenarios: when plants were growing individually in pots, vs. when plants were growing in a community context. In pots, the plants were thus selecting AM fungal partners in the soil with no constraint other than the availability of the different fungal species in the soil. In communities, other neutral or stochastic factors could also play a role in network assembly. For example, it has been shown that a plant's root-associated fungal community can be influenced by the identity of its plant neighbors (e.g., Mummey and Rillig, 2006, Hausmann and Hawkes, 2009). Alternatively, assembly history may also influence interactions between a given plant species and AM fungi through priority effects (e.g., Hausmann and Hawkes, 2010). Thus, our expectations were that in communities, there would be a larger role for stochastic mechanisms in driving network assembly, while in pots the assembly would be mostly deterministic and determined by preferential partner selection. Our results show evidence for stochastic network assembly in communities, although deterministic signals remain detectable. We also show that fundamental interaction niches are likely to be broad, which may confer stability to mycorrhizal networks.

6.3 Materials and Methods

6.3.1 Experimental design

We grew 20 plant species in natural soil collected in an old-field near Sherbrooke (Canada, 45°24' N 71°53' W) that was abandoned more than 40 years ago. Plants were either grown alone in 650 mL cone tainers (Stuewe and Sons, USA) filled with the natural soil or in 20L wooden boxes containing 3 individuals from 10 randomly drawn species. Plants were placed in a growth chamber at 16 h daylight and 22 °C – 20 °C (day – night). Given logistical constraints, we were limited to cultivating 4 different plant communities, and 3 replicates per individual species (N=180 individuals). We also kept samples of the soil inoculum for eventual DNA extractions to identify the fungal taxa that were originally present in our inoculum, and their frequency, in order to see if frequent/abundant fungi in the original inoculum were those that became generalists during the build-up of the community. Because AM fungi can colonize roots from hyphae, roots and spores, we identified AM fungi from the soil inoculum using various types of samples: (1) 10 whole soil samples (~ 0.5 mL), containing hyphae, spores and roots, (2) 5 samples of spores extracted from 500 g of soil using a standard centrifugation-flotation protocol (Chagnon and Bradley, 2011) and (3) 5 samples of extraradical hyphae, isolated using the same protocol, but where hyphae were collected after having let spores decant in the bottom of the tubes. Those latter extractions allowed us to characterize the fungal material for a much larger soil volume than typical crude DNA extraction from soil directly.

6.3.2 Harvest

Plants were harvested after 130 days of growth. Fresh leaves were taken from each individual to measure the following traits: leaf dry mass content (dry mass / fresh mass), specific leaf area (area/dry mass), leaf [C] and [N] as measured by an Elemental analyzer, and mean leaf area. Remaining shoot material was dried to determine total shoot weight. Roots were separated from surrounding soil, thoroughly washed under tap water and rinsed with distilled water. Then, the root system was scanned, and cut in ~ 1 cm fragments to take a random subsample (~ 0.5 mL). This subsample was transferred to a 1.5 ml tube and stored at -20 °C until DNA extraction (to identify AM fungal community within the roots). The rest of the root system was dried and weighed.

6.3.3 Characterizing AM fungal communities

To identify AM fungal taxa within roots, we first extracted total root DNA using MoBio UltraClean Plant DNA isolation kits following manufacturer's instructions. We also extracted DNA from the soil subsamples (mentioned above) using MoBio PowerSoil DNA isolation kits. AM fungal DNA was amplified using a nested PCR approach. In the first round, total fungal DNA from each DNA extract was amplified using 2 µL of DNA extract solution, 10 µL of HotStart Taq Master Mix kit solution (QIAGEN), 0.125 µL of T4Gene32 protein solution (New England Biolabs), 4 µL of 0.5 µM NS1-SR5 fungal-specific primer solution (White *et al.*, 1990, RytasVilgalys' lab, http://biology.duke.edu/fungi/mycolab/ primers.htm) and 3.875 µL of ultra-pure water. In the second round of PCR, amplicons from round 1 were used as templates, and the primer set was the AM fungal specific AML2-NS31 couple (Lee *et al.*, 2008). Because PCR products were meant to be sequenced by 454 sequencing, additional nucleotides were attached to those primers, following instructions from the sequencing facility (Génome Québec, Montreal). PCR amplicons were purified using Agencourt AMPure beads (Beckman Coulter) to isolate long, double-stranded DNA from single DNA strands, remaining primers and impurities. DNA concentration in each sample was then quantified with replicated spectrophotometry (Nanodrop) lectures and an equimolar amount of each amplicon was added to the final pool, which was sent to be sequenced at Genome Québec facilities (Montréal, QC).

The resulting sequences were analyzed using the QIIME pipeline (Caporaso *et al.*, 2010). We excluded from the dataset sequences that did not match our quality criteria (see chapter 5 for more details). We identified operational species of AM fungi using the published MaarjAM database (Öpik *et al.*, 2010).

6.3.4 Plant-AM fungal network structure

Plant-AM fungal networks were characterized using 3 structural metrics: nestedness, C-score and modularity. Nestedness (here characterized using the NODF metric, Almeida-Neto *et al.*,

2008) refers to a pattern where specialist species consistently interact with a subset of the partners with which more generalist species also interact. In other words, specialists tend to interact with generalists, while generalists interact with both generalists and specialists. The C-score is used to measure the frequency of co-occurrences between pairs of species in a metacommunity or an interaction network (e.g., Stone and Roberts, 1990; Gotelli and Rohde, 2002). A high C-score has been suggested to indicate strong interspecific competition, where some species co-occur less than by chance. Finally modularity refers to the presence of well-defined groups of species (i.e. modules) that interact preferentially among themselves rather than with the rest of the community. This metric has been suggested to be useful in detecting preferential associations in ecological networks (e.g., Olesen *et al.*, 2007; Chagnon *et al.*, 2012). To evaluate the statistical significance of these network patterns, we compared the observed values to 1000 random values, calculated from null matrices. Those matrices were generated using a conservative randomization algorithm that conserves the total number of interactions per row and columns in the matrix. Such null model is thus not prone to type I errors. Network indices (NODF, C-score and modularity) were thus expressed as z-scores (observed – mean(null) / sd(null)) and statistical significance was assessed by a *Z*-test. NODF and C-score were calculated using the R packages *vegan* and *bipartite*, respectively (Oksanen *et al.*, 2012; Dormann *et al.*, 2009). Modularity was calculated through simulated annealing using the C++ executable MODULAR (Marquitti *et al.*, 2014).

6.3.5 Trait-based and phylogenetic analyses

In order to evaluate whether different plant species associated with different AM fungal partners, we compared communities of AM fungi associated with different plant individuals by calculating their pairwise bray-curtis distances. We then compared those distances within vs. among species. We also performed a canonical correspondence analysis (CCA) to see how plant traits could predict their interactions with AM fungi. Those analyses were performed using, respectively, the *adonis* and *cca* functions of the R package *vegan* (Oksanen *et al.*, 2012). We characterized the phylogenetic structure of AM fungal assemblages within each root system using the mean nearest taxon distance (MNTD) metric. The observed metric was

compared to 1000 random values generated by shuffling the tips of the AM fungal phylogenetic tree (Kembel *et al.*, 2010).

6.3.6 Beta-diversity partitioning methods

Beta-diversity partitioning methods are increasingly used in community ecology to refine our understanding of species distributional patterns in metacommunities (e.g., Carvalho *et al.*, 2013). One recent way of partitioning beta-diversity among sites was proposed by Podani and Schmera (2011). This method, called the SDR simplex, partitions beta-diversity in two additive components: nestedness and species turnover. Indeed, two sites can be different (i.e. show a non null beta-diversity) because they have a different number of species (nestedness), or because they have species of different identity (species turnover). For example, consider a pair of sites A and B that contain, or not, the species 1 to 6. In the first scenario, let's assume that site A contains species 1, 2, 3, 4, 5, and 6, while site B only contains species 1 and 2. Here, this is a case of extreme nestedness, because the only difference between the two sites relates to the number of species they contain, but not to their identity: the species poor site does not have unique species. On the other hand, if site A contains species 1, 2 and 3, while site B contains species 4, 5 and 6, then the only difference is in the identity of species (i.e., species turnover), their number being equal (i.e., 3). Such partitioning of beta-diversity has proved to offer new insights in understanding the structure of metacommunities (e.g., Carvalho *et al.*, 2013). Because the structure of the data is similar for metacommunities (sites x species matrix) and ecological networks (e.g., plants x AM fungi matrix), those analytical tool may also be relevant to study patterns of species interactions between plants and CMA. Here, we partitioned beta-diversity within each networks using the SDR simplex (Podani and Schmera, 2011) as a way to validate our nestedness analysis. Indeed, a major component of the SDR simplex is the concept of nestedness, yet the pattern is characterized using a different method.

We also partitioned the dissimilarity of our different networks into 2 additive components according to the framework developed by Poisot *et al.* (2012). The rationale of this framework

is that two mycorrhizal networks can be different because (1) they don't involve the same plant and fungal species (i.e. species turnover) or (2) they involve the same species, but those species don't interact similarly in the two networks (i.e. interaction turnover). This partitioning allowed us to investigate the level of flexibility in mycorrhizal interactions in our system (i.e. the important of interaction turnover).

6.4 Results

6.4.1 Network structure

Interactions were significantly nested for both plants and AM fungi when plants were growing in pots, but not in communities (fig 12a-b). This was mainly mediated by the presence of highly generalist AM fungal taxa: the removal of those generalists from our dataset resulted in a loss of significance of this nested interaction pattern, while the removal of specialists or a random removal of taxa had no effect (fig 13). Thus, it does not seem that interactions are nested because specialists interact preferentially with generalists. The lower nestedness in the communities vs. in pots was present in spite of a higher connectance (i.e. proportion of filled cells in an interaction matrix) in the former treatment, while nestedness is well known to correlate positively with connectance in binary matrices (e.g. Olesen and Jordano, 2002). Further examination of the data revealed that nestedness was lower in communities because of a lower variance in the number of interactions per species: in pots, such variance was high, with some very generalist and some very specialist species. In communities, however, there was a consistent trend for plant species which were more specialist in pots to be more generalist (data not shown). This may explain the discrepancy between nestedness in communities vs. its corresponding counterpart in pots.

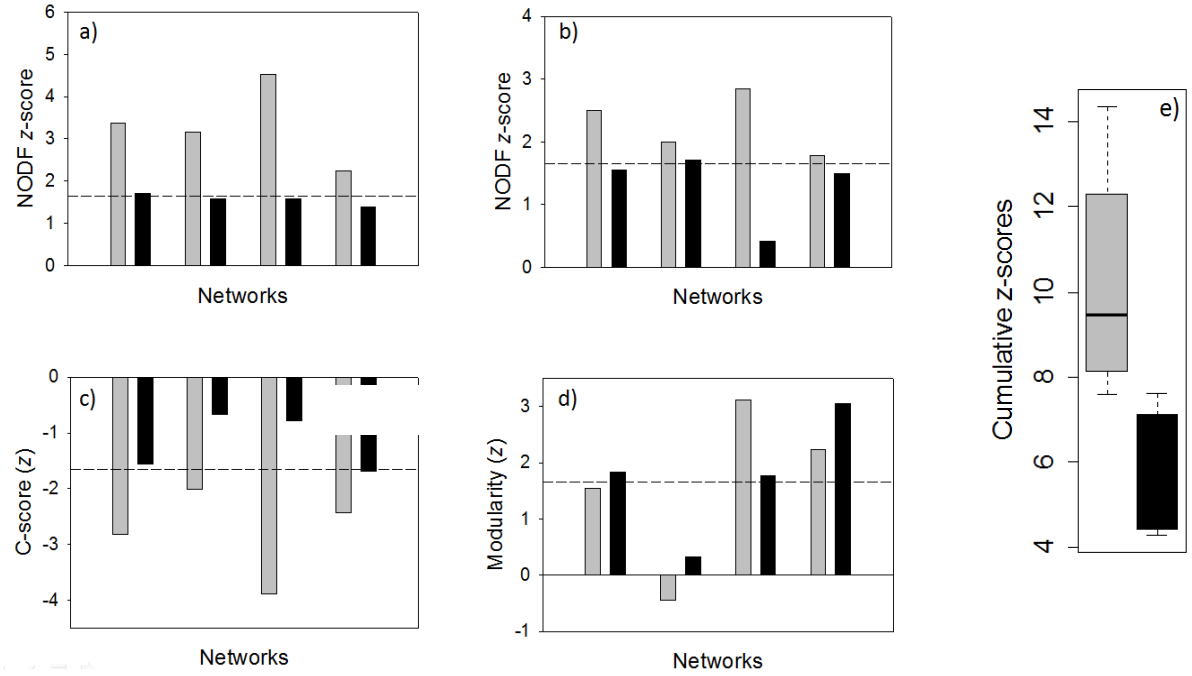

Figure 12. Network structure in pots (grey bars) vs in communities (black bars). Nestedness (NODF) was calculated for both plants, a), and AM fungi, b). The dashed lines indicate the level above which (or below which, for C-score) the metric is significantly different from 0. In e) we plot cumulative z-scores (in absolute values) for pots vs. communities.

C-scores were always found to be lower than what would be expected by chance, yet the pattern was significant only for pot-based data, and not for communities (fig 12c). However, because C-score correlated strongly and negatively with interaction nestedness of both plants and AM fungi (r = -0.88 and r = -0.84, respectively), it remains unsure whether such C-score pattern arose from a true biological effect or as a negative correlate to nestedness (or vice versa).

There were no clear patterns regarding modularity data (fig 12d). Although in most cases observed modularity was higher than what was realized in our null matrices (z-score higher than 0), this was not significant. Moreover, there were no clear trends for modularity to be higher or lower in pots. However, if we cumulate all z-scores (in absolute values) related to network architecture (i.e. nestedness, C-score and modularity), we see that the values were significantly further away from 0 in pots

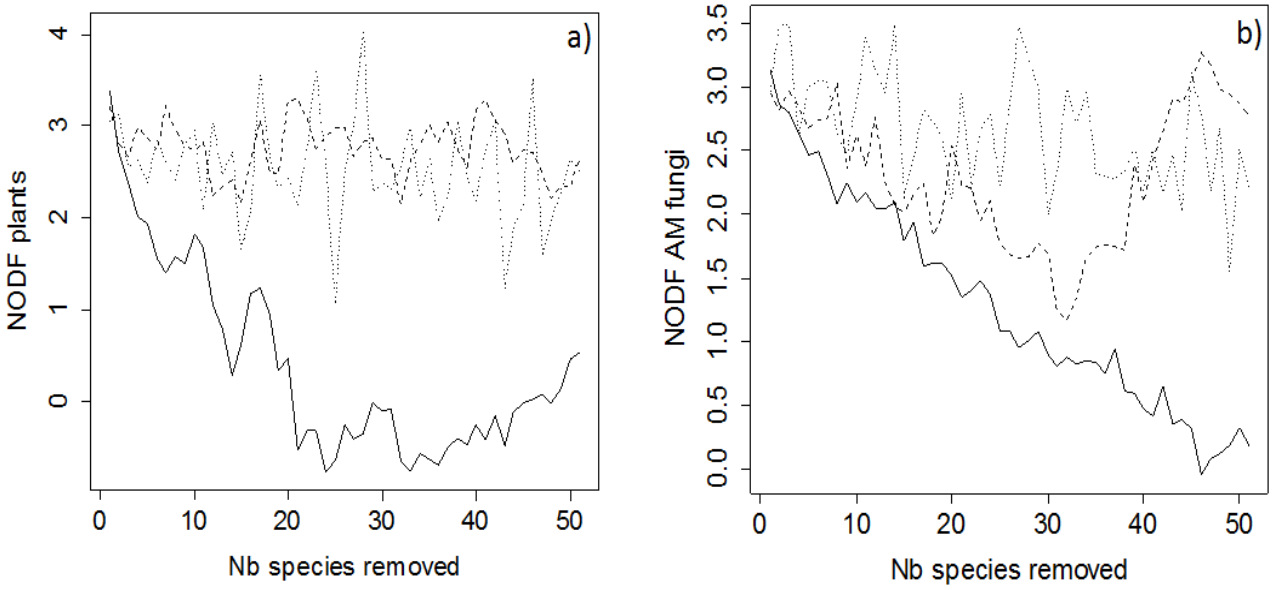

Figure 13. Role for generalist and specialist AM fungi in driving the nested network pattern. We plot the NODF z-scores for both plants in a), and AM fungi in b), as a function of the number of AM fungal taxa removed from the dataset. We either removed generalist AM fungal taxa first (solid lines), specialists first (dashed lines) or randomly (dotted lines). In both cases, when generalists are removed first, z-scores rapidly get close to 0 (nestedness not statistically significant).

vs. communities. This suggests that assembly was further away from randomness in pots. (fig 12e)

Plant species that were generalist with respect to their fungal partners when grown in mixtures were not necessarily more likely to be so in monocultures, while there was a strong tendency for AM fungi that were generalist with respect to their plant hosts in multispecies communities

to be also generalist when growing in plant monocultures (Plants: Mantel's r = 0.15, *P* = 0.198 ; AM fungi: Mantel's r = 0.89, *P* = 0.001) (Table 2).

Table 2. Correlations between the number of interactions of plants and AM fungi in given communities vs. corresponding data in pots.

	Plants		AM fungi	
Community	**pearson's r**	**p-val**	**pearson's r**	**p-val**
A	-0.061	0.87	0.83	<0.0001
B	0.39	0.27	0.87	<0.0001
C	0.41	0.24	0.78	<0.0001
D	0.24	0.5	0.84	<0.0001

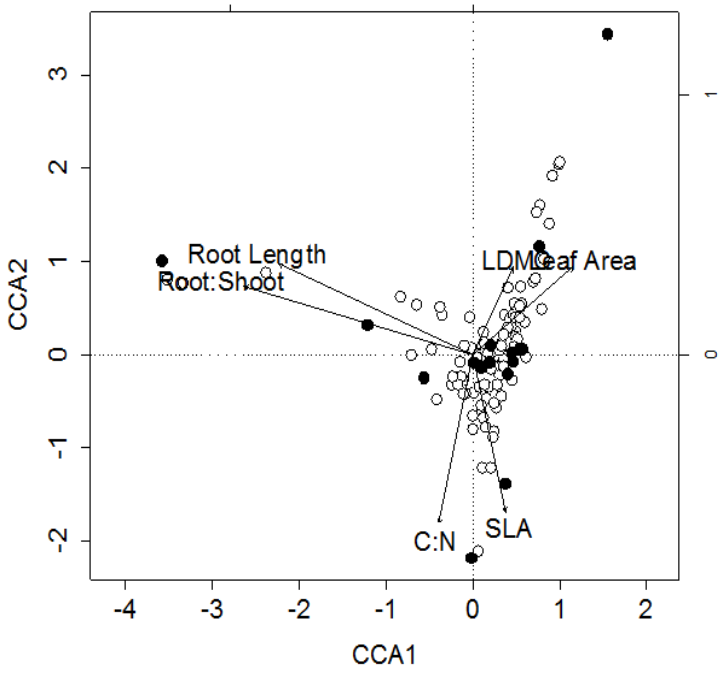

Figure 14. CCA biplot showing the association between plant traits and interactions with AM fungi. Plants and AM fungi are respectively closed and open circles. LDMC = leaf dry mass content, SLA = specific leaf area, C:N = leaf [C] / leaf [N], Root:Shoot = root dry mass / shoot dry mass.

6.4.2 Trait-based and phylogenetic analyses

Different plant species associated with different AM fungal partners, but the signal was stronger for pots vs. communities (*paired-t* = 2.67, *P* = 0.03). When pooling all data for pot-based plant-fungal interactions, we see that plant traits can significantly predict their interactions with AM fungi (*P* = 0.027) with two dominant axes: the most important around root production and allometry, and the second one around leaf traits (fig 14).

Phylogenetic structure of AM fungal assemblages in single root systems showed a consistent trend towards clustering:

MNTD z-scores were negative for 95% of the communities, and it was significant for about 73% of them. Regarding the role for plant phylogeny in driving their interactions with AM fungi, a Mantel test revealed a weak and marginally significant correlation between plant phylogenetic distance and bray-curtis distance (in terms of fungal partners) (Mantel's $r = 0.18$, $P = 0.09$). Interestingly, 6 AM fungal OTUs were only interacting with some clusters of the plant phylogeny (i.e. significantly negative MNTD z-scores). Three of those OTUs seemed to show a preference for Asteraceae species (e.g., *Hieracium spp.*, *Leontodon autumnalis*) and another showed a preference for Rosaceae (e.g., *Fragaria vesca*, *Potentilla recta*).

6.4.3 Beta-diversity partitioning within and among networks

Within networks, partitioning of beta-diversity according to the SDR simplex (Podani and Schmera, 2011) confirmed results from nestedness analyses: in pots, plants tend to be different more because they have a different number of partners than because they have different ones, while the reverse is true for communities (fig 15).

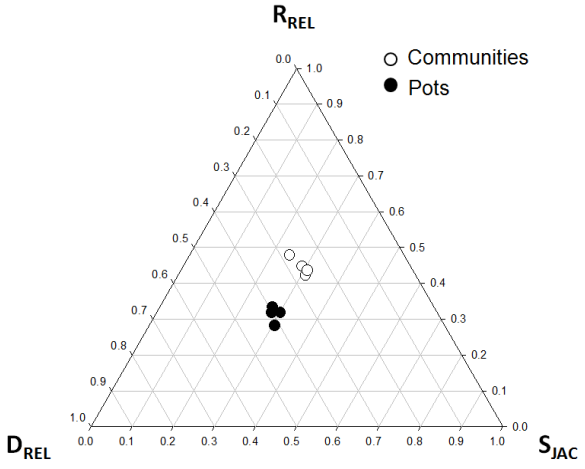

Figure 15. Beta-diversity partitioning within networks according to the SDR simplex (Podani & Schmera 2011). S_{JAC} = jaccard similarity, D_{REL} = relativised richness difference (i.e. nestedness), R_{REL} = relativised species replacement/turnover. Each point represents the centroïd of all pair-wise comparisons within a community (open circles) and corresponding data in pots (closed circles).

When partitioning the dissimilarity of different networks in its interaction vs. species turnover components according to Poisot *et al.* (2012), we found that much of the variation across plant communities could be explained by flexibility in interactions (fig 16). Indeed, around 65-70% of the dissimilarity among pairs of networks was explained by the fact that species present in both networks had

different interactions, and not by the fact that the two networks were simply formed of different species. Also, this high proportion of pairwise network dissimilarity explained by variation in interactions cannot be solely explained by a corresponding low variation in species composition. All pairwise networks were designed to have a dissimilarity in plant species composition of around 70%, and were calculated to have a dissimilarity in AM fungal OTUs composition of around 20%. Thus, combined, those dissimilarities in community composition would have been expected to explain a higher proportion of network-level dissimilarity if interactions were not flexible.

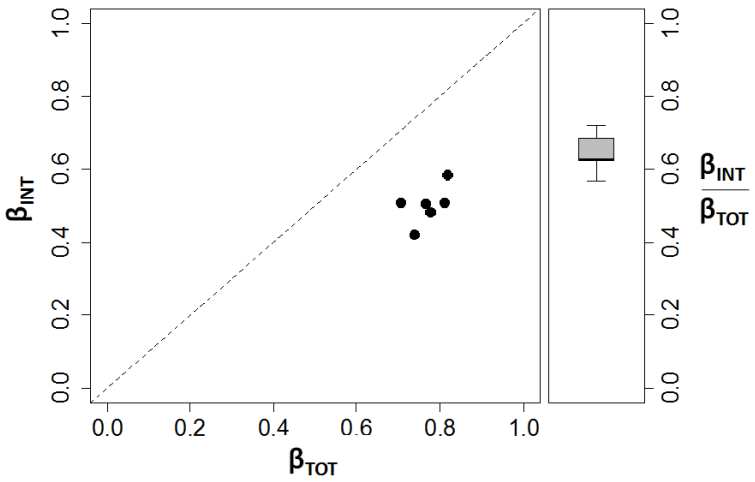

Figure 16. Beta-diversity partitioning among networks according to Poisot *et al.*, (2012). β_{TOT} = total variation between two networks, β_{INT} = variation due the fact that species present in both networks interact with different partners (i.e. flexibility in interactions across networks). The dashed line is the 1:1 relationship, i.e. the theoretical maximum for β_{INT}.

6.5 Discussion

In this study, we were interested in evaluating the relative importance of deterministic vs. stochastic mechanisms as drivers of mycorrhizal network assembly. We showed clear evidence for determinism, for example with the consistent phylogenetic clustering of AM fungal assemblages within single root systems. Also, in accordance with previous theoretical work (Chagnon *et al.*, 2013), we found that plant leaf economics were related to their interaction patterns. This may suggest that plants are able to select AM fungal partners whose resource economics strategy is aligned with their own. For example, it seems a priori unlikely that a fast-growing ruderal plant species would benefit from associating with a slow-growing fungus that produces costly but persistent biomass in the soil. We also found root-shoot

allometry (as measured by total root length and root:shoot ratio) to be related to plant interactions with AM fungi. This may have arisen because plants with various amounts of roots rely on AM fungi for different reasons. For example, a plant with limited root surface in the soil may rely on AM fungi for nutrition, while plants with massive amounts of roots in the soil may benefit more from AM fungi through their ability to protect them from soil-borne diseases. This old hypothesis (Newsham *et al.*, 1995) has indeed received some empirical support (Sikes *et al.*, 2009). Since different phylogenetic groups of AM fungi provide pathogen protection to various extents (e.g., Maherali and Klironomos, 2007), this could explain why plants with various amounts of roots associate with different AM fungi. However, most assumptions around this hypothesis remain to be further investigated. For example, it is largely unknown whether there is a clear trade-offs in AM fungi between the two functions (providing nutrients vs. protecting against pathogens). It even remains unclear what are the optimal root traits to evaluate a plant's reliance on AM fungi for nutrition (e.g., Maherali 2014) or susceptibility to soil-borne pathogens (Newsham *et al.*, 1995). An alternative explanation for the role of root-shoot allometry in driving plant-fungal interactions would be that plants forming massive amounts of roots will provide much more colonizing opportunities for AM fungi, thus favoring those with better colonizing abilities. Conversely, plants with fewer roots may rather favor AM fungal species with better competitive abilities (assuming a trade-offs between colonizing and competitive abilities (e.g., Cadotte *et al.*, 2006) among AM fungi). Overall, phylogenetic and trait-based analyses suggested a clear role for determinism in driving mycorrhizal interactions.

In spite of such determinism, we also found strong evidence for flexibility in interactions. This is exemplified by the structure of networks in communities vs. in pots: communities appeared to have network structure closer to randomness. For example, interaction nestedness was consistently significant in pots, while it was not in communities. Conversely, C-scores showed the exact opposite pattern. Indeed, C-scores were consistently lower than expected by chance in pots. Given that high C-scores are generally assumed to represent the signature of interspecific competition in communities (e.g., Stone and Roberts, 1990), some authors have interpreted low C-scores as indicative of facilitation, an antithetic concept to competition

(Gorzelak *et al.*, 2012; Pickles *et al.*, 2012). Thus, from our results, it could appear that AM fungal communities within root systems assemble through facilitation rather than competition. However, it is in fact an oversimplification to expect facilitation to result in low C-score. Indeed, if subgroups of AM fungal species facilitate each other, this would result in interaction modules at the network scale. Such modular network configuration is in fact associated with a high C-score, which is progressively lost as we randomize the network (Chagnon, unpublished data). In our opinion, here, the low C-scores are not indicative of a specific ecological mechanism, but rather a by-product of the high nestedness (the two metrics are strongly and negatively correlated). We have shown that such nestedness was strongly mediated by the presence of generalist AM fungi, which were the most abundant species in the initial inoculum. This is the first empirical evidence showing that the generalism level of AM fungi can arise as a consequence of high local abundance, while up to now only correlative evidence was available (e.g., Chapter 5, fig 8 of this thesis). However, more direct tests for this hypothesis are needed to draw stronger inferences. Indeed, it is not impossible that fungi that were abundant in our inoculum achieved such high abundance in the field (where we took the inoculum) because they were host generalists. Future studies should build artificial plant-AM fungal networks from pure cultures as fungal inocula, where fungal abundance can be manipulated directly. Regarding the number of interactions for plants, it was largely unstable from a network to another, indicating that no single plant species had a clear propensity to be a generalist or a specialist.

An even clearer indication of flexibility in mycorrhizal interactions in our system was demonstrated by our partitioning of the beta-diversity among our networks as suggested by Poisot *et al.* (2012). Indeed, we found that for most plant species, their set of fungal partners was likely to vary considerably from one community (plant species mixtures) to another, and vice versa for mycorrhizal fungal species. Thus, it seems clear that mycorrhizal interactions are very flexible and context dependent. This potentially illustrates the role for stochastic and historical processes in driving mycorrhizal network assembly (e.g., Hausmann and Hawkes 2010). Future studies should explore the importance of such stochasticity along ecological gradients. For example, Chase (2007) has shown that as environmental filtering becomes more

important, community structure becomes, in turn, increasingly deterministic. It might be that in drier or colder climates, plants rely on more specific subsets of AM fungi. Alternatively, we should explore longer gradients of plant life history strategies. In our system, all potential hosts were herbaceous angiosperms, while a much broader range exists for AM fungi in nature (e.g., ferns, horsetails, shade-tolerant trees, gymnosperms). It might be that plants with highly contrasted life history strategies will consistently share very little AM fungal partners and display a deterministic network structure at the community level.

Overall, our results suggest that mycorrhizal interactions can show some level of determinism. While numerous observational studies had reported similar patterns (e.g., Öpik *et al.*, 2009; Torrecillas *et al.*, 2012; Montesinos-Navarro *et al.*, 2012), it remained unclear whether field-based patterns were due to a progressive community assembly where perennial plants progressively build-up specific AM fungal communities in their rhizosphere year after year. Here we show that deterministic patterns can arise within 4 to 5 months of growth in pots, which mirrors results of other studies investigating sporulation dynamics in the rhizosphere of single hosts (e.g., Bever *et al.*, 1996; Eom *et al.*, 2000). It also suggests that even if pot-based studies may select for a biased subset of AM fungi, potentially more host-generalist and ruderal (e.g., Sykorova *et al.*, 2007), they remain relevant to investigate mycorrhizal interactions: in our case, we found clear patterns of specificity of association among various plant species and a broad range of AM fungal taxa (not only ruderal *Glomus* taxa, for example). Our results also show that even if determinism can arise at a local scale, mycorrhizal interactions are very flexible. This suggests that mycorrhizal communities should be very resilient to local disturbances causing the local extinction of some potential partners. Indeed, such flexibility in interactions would allow networks to rewire and novel interactions would buffer the effect of local extinctions. This might explain why Urcelay *et al.* (2009) found that AM fungal communities were very resilient to the removal of plant functional groups, for example. As a whole, our results show that specificity in mycorrhizal interactions is at the realized niche level, rather than at the fundamental niche level (e.g., Aldrich-Wolfe, 2007). In other words, preferential interactions recorded in the field are not likely to have arisen from fundamental specialization of the partners.

This points to an urgent research need in the literature surrounding ecological networks and their stability in nature. Most focus has been placed around how specific patterns promoted or not stability of trophic or symbiotic communities using either static (e.g., Memott *et al.*, 2004; Burgos *et al.*, 2007) or dynamic (e.g., May, 1973; Thébault and Fontaine, 2010; Gravel *et al.*, 2011) simulations. Those simulations typically make the assumption that interactions are inflexible through time (i.e. realized interaction niche = fundamental interaction niche), which in itself stands in contradiction with field observations showing that species can switch partners across years (e.g., Petanidou *et al.*, 2008; Lazarro *et al.*, 2010). In line with this, our results suggest that the opportunity for a network to rewire after a disturbance (through broad fundamental interaction niches of species) may be central to the resilience of natural communities. Community ecologists now have to (1) find creative ways to incorporate such process in their simulation studies and (2) gather much more data about fundamental interaction niche through long-term and/or manipulative studies.

6.6 Acknowledgements

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L'article précédent montre clairement la flexibilité dans les interactions plantes-CMA. Bien que des signaux déterministes dans l'assemblage des communautés soient perceptibles, ils ne résultent nullement de spécialisation stricte des plantes envers certains groupes de CMA. Toutefois, cette étude impliquait des organismes qui n'ont aucune histoire de coévolution, puisque les graines de plantes ne provenaient pas du même site où l'inoculum fongique a été prélevé. Ainsi, pour valider ces résultats, une étude observationnelle a été conduite. Plutôt que de manipuler ou non la présence et l'identité de plantes voisines, j'ai caractérisé les interactions plantes-CMA de façon répliquée dans l'espace. Ainsi, si certaines plantes étaient spécialisées sur certains groupes de CMA, leurs patrons d'interactions devraient être les mêmes à travers les différentes communautés locales.

Ce projet de recherche s'inscrit aussi dans un cadre plus large de recherche sur les réseaux d'interactions écologiques. En effet, il a été suggéré que certaines espèces ont un rôle plus important à jouer dans de telles communautés (typiquement les espèces généralistes) (e.g., Bascompte *et al.*, 2003). Certains auteurs sont même allé jusqu'à les considérer comme des « keystone species » (*sensu* Paine, 1969) et qu'elles devraient être les cibles principales des efforts de conservation (e.g., Tylianakis *et al.*, 2010). Toutefois, ce genre d'argumentation fait la supposition suivante : le généralisme d'une espèce est une propriété intrinsèque de cette espèce, et non pas le fruit de contingences locales. Le corollaire est donc que les espèces spécialistes sur le terrain le sont à cause de propriétés intrinsèques (e.g., faible compatibilité phénotypiques avec d'autres espèces), et non pas simplement parce qu'elles sont rares localement. Ainsi, le projet de recherche présenté dans le chapitre qui suit visait aussi à déterminer si le nombre d'interactions d'une espèce (ainsi que différents indices de centralité dans les réseaux écologiques) variait beaucoup d'une communauté locale à une autre.

Chapitre 7

MYCORRHIZAL NETWORKS ARE NOT BUILT AROUND KEYSTONE SPECIES OR INFLEXIBLE INTERACTION MODULES

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(In preparation, to be submitted to Ecology Letters)

7.1 Abstract

In most ecological networks investigated to date, a few species tend to have disproportionately more interactions than the rest of the species. Some have argued that we should consider these generalists as keystone species, because static or dynamic simulation models suggested that their removal would have catastrophic consequences on the whole community. Likewise, mutualistic communities are often found to be built around modules of preferentially interacting species, that are thought to reflect coevolutionary history among subsets of species. Here, we show that 18 mycorrhizal networks show similar features, with the occurrence of both highly generalist species and preferential interactions. However, we also show that the level of generalism of a given species was highly fluctuating across space, and so were preferential interactions. Those results thus suggest that (1) being a generalist locally is an insufficient criterion to be considered a keystone species in an ecological network, and (2) preferential interactions can arise from local and contemporary partner selection without involving coevolutionary events. We argue that such flexibility in interaction patterns (either the number of interactions or the identity of the partners) urgently needs to be characterized in other systems to guide modeling studies on ecological networks. We also discuss the relevance of seeing the assembly of ecological networks as an optimization process.

7.2 Introduction

Ecosystem services or functions are performed largely through species interactions, rather than by species alone. For example, nearly 100% of a plant's phosphorus may be derived from its mycorrhizal fungal partner in the soil (Smith *et al.*, 2004). Thus, ecosystem functioning relies on networks of interacting species, and a challenge facing community ecologists is to understand and predict how such networks will be resistant and resilient to natural or anthropogenic perturbations. Other scientific disciplines have used network-based tools to evaluate the potential fragility of networks when confronted to various forms of perturbations. For example, Watts and Strogatz (1998) have shown that various types of complex networks (e.g., neural, social, computational) were built around a few elements or nodes that were involved in many more link than the average nodes. Those central nodes have been typically referred to as hubs. Albert *et al.* (2000) have shown that the existence of such hubs increased the general efficiency of the network in performing its corresponding function (e.g., signal transmission when talking about the internet, or passenger flow when looking at networks of connected airports), yet it also made it more vulnerable to disturbances if they involved the loss of these hubs. Similar reasoning was transposed to species interaction networks, where it was argued that hubs in ecological communities should be considered as keystone species whose role is disproportionately important as compared to the rest of species comprised in the network (e.g., Sole *et al.*, 2003). It was even argued that, in this view, the study of species interaction networks should become a central tool in conservation to provide guidance in settling management priorities (Tylianakis *et al.*, 2010). However, a nuance should be made regarding the actual role of hubs in ecological networks. Indeed, while we often see natural networks organized around hub species, we don't know how replaceable they are: it may well be that a disturbance eliminating a hub would simply lead to its rapid replacement by another hub species. Such rewiring was missing in the original simulations of network dynamics by Albert *et al.* (2000) and must be considered in ecological networks, where observed interaction patterns only reflect the realized "interaction niche" of a species, while its fundamental counterpart may be much broader (e.g., Blüthgen 2010).

The challenge when tackling the question of the replaceability of hubs in ecological networks is that extensive datasets are required to evaluate whether the identity of hubs vary in space and time. Indeed, we need to know whether hub species are so because, for example, they are locally abundant and so very prone to be interacting with many species, or because it is an intrinsic property of this species (for example, because it has a phenotype that makes it morphologically compatible with many other species). In other words, we need to separate between local contingency vs. deterministic species attribute. However, very few datasets have traced ecological network assembly through time over multiple years. When it has been done, some have found that the number of interactions of a species was highly likely to change drastically from one year to another (e.g., Petanidou *et al.*, 2008; Lazzaro *et al.*, 2010), and many have found that generalists were simply the most abundant species (e.g., Ollerton *et al.*, 2003; Vazquez *et al.*, 2005). Others that have studied interaction networks in spatial designs have found that some species tend to be regionally abundant and to always appear as hubs, potentially by being superior competitors (e.g., Dattilo *et al.*, 2014). However, in the latter case, if hubs are so because they are superior competitors, it might be that if they are removed, subordinate species may quickly take advantage of the situation and become hubs themselves. Hence, it remains unclear whether the existence of hubs in ecological networks has strong implications for their stability and functioning, because we still have very limited knowledge about the replaceability of these hubs, and more generally about flexibility and rewiring in species interaction networks. Recent theoretical work has provided tools to study such variation across networks (e.g., Poisot *et al.*, 2012), but much data has yet to be collected to address this important issue.

Mycorrhizal networks are widespread worldwide, with a vast majority of land plants forming these symbiotic associations (Wang and Qiu, 2006). Those networks provide important ecosystem functions such as improved plant nutrition and reduced nutrient runoff (e.g., van der Heijden, 2011), protection of plants against pathogens (e.g., Sikes *et al.*, 2009), increased soil aggregation and physical protection of organic matter (e.g., Wilson *et al.*, 2009) and increased plant C fixation by acting as carbon sinks (e.g., Wright *et al.*, 1998; Miller *et al.*, 2002). There is still, though, limited data available at the whole community level to

characterize the structure of these ecological networks. This is because (1) mycorrhizal interactions have typically been characterized for a small proportion of plant species within communities (i.e. 2-3 plant species at most), and (2) until the development of high-throughput sequencing techniques, it was quite laborious to identify most mycorrhizal fungal partners associating with a given plant species. However, there has been recently a burst in community-level studies characterizing the structure of mycorrhizal networks (e.g., Chagnon *et al.*, 2012; Montesino-Navarro *et al.*, 2012; Torrecillas *et al.*, 2014). Yet, those studies, like most other studies of ecological networks, have been performed as snapshots of species interaction patterns, in one given place and at one given time. Thus, while it has been found that those mycorrhizal networks are also organized around interaction hubs (e.g., Chagnon *et al.*, 2012), we still have no insight about their potential replaceability. Mycorrhizal networks have also been shown in many instances to be subdivided into modules of preferentially interacting species (e.g., Chagnon *et al.*, 2012; Montesino-Navarro *et al.*, 2012; Martos *et al.*, 2012; Bahram *et al.*, 2014). The existence of such modules in species interaction networks has been suggested to result from coevolutionary processes among species to favor trait matching (e.g., Olesen *et al.*, 2007) but as noted by Chagnon *et al.* (2012), it must first be demonstrated that the species composition of such interaction modules is stable across space and time to argue in favor of coevolutionary processes.

To address these issues, we studied the spatial variation in mycorrhizal network structure for 18 local networks, in three different sites with contrasted successional status. We used high-throughput sequencing to identify the mycorrhizal fungal partners present in the roots of every host plant species present in each local network. We then characterized species centrality in the network to reveal the potential hubs in each local network, and to assess whether a hub species in one network was more likely to be so in another network (i.e. if the hub "quality" was an intrinsic species property repeatable through space). We also subdivided our local networks into interaction modules to evaluate whether two species in the same module in one network were more likely to be so in another network. Our results show that although we could detect deterministic partner selection within local networks, interaction patterns were flexible through space (i.e. from one local network to another), which implies that hubs were

replaceable across sites. Also, module composition was flexible through space, implying that there was no evidence for tight reciprocal coevolution going on in these mycorrhizal communities, involving inflexible subsets of species.

7.3 Materials and Methods

7.3.1 Sampling

We sampled 3 different sites in or near the city of Sherbrooke (Canada, 45° 24' N 71° 53' W): (1) a grassland disturbed the previous year, (2) an ancient agricultural field uncultivated since >40 years currently being colonized by *Salix spp.* and *Populus spp.* and (3) an old growth forest with a relatively closed canopy of ectomycorrhizal hosts. At each site, we established a 30 m transect along which we delimited six 1 m^2 quadrats (i.e. our local networks). Within each quadrat, we carefully excavated all potential host plants associated with arbuscular mycorrhizal (AM) fungi, and separated the root systems by species. Only the part of the root system still attached to the plant was conserved to guarantee host plant identity. Fresh root systems were placed in plastic bags and transported on ice to the laboratory, where they were thoroughly washed to take off attached soil and contaminating roots from other plants. The washed root systems were pooled by plant species separately for each local network, and kept at -20°C until processing within a week (see section 7.3.2). We also sampled supplementary leaf and root tissues from replicated individuals of each of the plant species to characterize the following traits: average root diameter, specific root length (root length per unit dry mass), root dry mass content (root dry mass per unit fresh mass), specific leaf area (leaf area per unit dry mass), leaf dry mass content (leaf dry mass per unit fresh mass) and leaf area. Those traits were selected for their relevance in mycorrhizal interactions: root coarseness is thought to be related to a plant's dependency on AM fungi and susceptibility to pathogens (e.g., Hetrick *et al.*, 1992; Newsham *et al.*, 1995; Sikes *et al.*, 2009; but see Maherali, 2014) and leaf traits collected provide information about plant resource acquisition and conservation strategy (e.g., Pierce *et al.*, 2013). Such resource management strategies have been argued to be a key trait driving partner selection in the AM symbiosis (Chagnon *et al.*, 2013). Thus, with these

additional data on plant traits, we could test for deterministic, trait-based partner selection in our mycorrhizal networks. However, some plants were rare in each site, so we were unable to sample additional individuals to measure functional traits, so in some cases data on plant traits was unavailable. Also, we did not measure trait data for the first site, which comprised mostly ruderal species, because at the time of sampling (end of July), some species had already flowered and leaves were beginning to die out.

7.3.2 Characterizing AM fungal communities

For each plant species of each local network, sampled root systems were cut in small pieces (~1 cm long) and a random subsample was selected and transferred to a 1.5 mL tube for DNA extraction, which was performed using MoBio UltraClean Plant DNA isolation kits following manufacturer's instructions. AM fungal DNA was amplified using a nested PCR approach, given that preliminary attempts at amplifying AM fungal DNA directly from the DNA extracts were unsuccessful. In the first round, total fungal DNA from each DNA extract was amplified using 2 µL of DNA extract solution, 10 µL of HotStart Taq Master Mix kit solution (QIAGEN), 0.125 µL of T4Gene32 protein solution (New England Biolabs), 4 µL of 0.5 µM NS1-SR5 fungal-specific primer solution (White *et al.*, 1990, RytasVilgalys' lab, http://biology.duke.edu/fungi/mycolab/ primers.htm) and 3.875 µL of ultra-pure water. In the second round of PCR, amplicons from round 1 were used as templates, and the primer set was the AM fungal specific AML2-NS31 couple (Lee *et al.*, 2008). Because PCR products were meant to be sequenced by 454 sequencing, additional nucleotides were attached to those primers, following instructions from the sequencing facility (Génome Québec, Montreal). PCR amplicons were purified using Agencourt AMPure beads (Beckman Coulter) to isolate long, double-stranded DNA from single DNA strands, remaining primers and impurities. DNA concentration in each sample was then quantified with replicated spectrophotometry (Nanodrop) lectures and an equimolar amount of each amplicon was added to the final pool, which was sent to be sequenced at Genome Québec facilities (Montréal, QC).

The resulting sequences were analyzed using the QIIME pipeline (Caporaso *et al.*, 2010). We excluded from the dataset sequences that did not match our quality criteria (see chapter 5 for more details). We identified operational species of AM fungi using previously the published MaarjAM database (Öpik *et al.*, 2010).

7.3.3 Variation in species' centrality

We characterized plant and AM fungal species centrality in all local networks, in order to identify local hubs. Many indices exist to characterize such a species-level property. We chose a simple and potentially more intuitive method that simply compares the number of interactions of a focal species to the rest of the species under the form of a z-score (i.e. $z_i = \frac{nb_i - mean(nb)}{sd(nb)}$), where nb_i is the number of interactions involving the focal species, and *mean(nb)* and *sd(nb)* are respectively the mean and standard deviation in the number of interactions among species in the network. This approach has been used by various authors to discriminate central vs. peripheral species in ecological networks, using the arbitrary criterion that species with z > 1 are central/core species (e.g., Diaz-castelazo *et al.*, 2010; Dattilo *et al.*, 2014). In our case, we used the crude z-scores as continuous input variables rather than to label each species as core or peripheral. Other centrality metrics have been argued to contain more information by also considering indirect paths between all species in a network. However, such metrics can become irrelevant when there are some species in the network linked to all other species (extreme generalists). In such case, indirect paths between species don't exist, because all species are interlinked together by these extreme generalists. In such cases, indices like Freeman's betweenness centrality (Freeman, 1977) take a value of 0 for all species, and thus provide no relevant information. Therefore, we chose to focus on a simple and relatively intuitive measure of centrality in our networks.

7.3.4 Variation in module affiliation

Every local network was subdivided into modules of preferentially interacting species using a simulated annealing optimization procedure that maximizes Barber's modularity (Barber, 2007). This routine is implemented in the C++ executable MODULAR (Marquitti *et al.*, 2014). For each of our 18 networks, we tested the statistical significance of network modularity by comparing the observed value to 1000 randomized matrices. Those randomized matrices were generated using a null model that constrains for species number of interactions (and thus indirectly for connectance). Such null model is conservative and thus not prone to type I errors (Ulrich and Gotelli, 2013).

7.3.5 Deterministic partner selection

To evaluate whether mycorrhizal associations were deterministically driven by either plant or fungal characteristics, we evaluated (1) the relationship between plant traits and their interactions with AM fungi using a canonical correspondence analysis (CCA), and (2) the phylogenetic structure of fungal communities associated with a given host. For the latter, we used the mean nearest taxon distance (MNTD) index to look at phylogenetic clustering of fungal communities, and we compared the observed values to 1000 random scenarios where the tips of the phylogenetic tree were shuffled. These analyses were run in R using the packages *vegan* (Oksanen *et al.*, 2012) and *picante* (Kembel *et al.*, 2010), respectively. We also partitioned network beta-diversity, which we refer to as variation in interaction patterns between local networks. Poisot *et al.* (2012) have suggested to partition this measure in two additive components: variation in species composition in each network, and variation in interactions between the species shared by two networks (the latter being due to flexibility in interactions).

7.4 Results

7.4.1 Interaction modules and deterministic partner selection

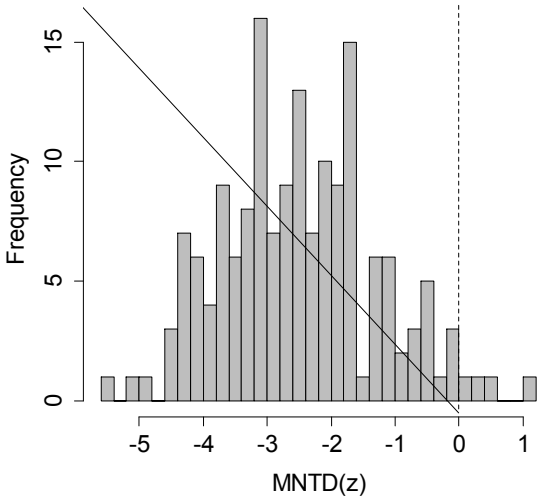

Figure 17. AM fungal phylogenetic community structure within roots, as measured by MNTD *z*-scores. Negative *z*-scores indicate phylogenetically clustered communities.

Of the 163 compound root samples that were analysed, 160 presented a phylogenetically clustered AM fungal community, and the pattern was significant for 132 of them (82.5%) (fig. 17). In all but two networks we could build a CCA model that significantly ($\alpha = 0.05$) predicted plant-fungal interactions based on plant traits. It should be noted that for one of these two networks, we only had trait data for three plant species in the network, which resulted in very few degrees of freedom in CCA models and thus low statistical power to detect a link between plant traits and interactions. By looking at the correlation between the number of times a plant and a fungal species co-occurred, and the number of times they interacted, we noted that among the species that co-occur often (i.e. more than 6 times), only 12 interacted more than 6 times, while 30 interacted only once and 38 did not interact at all. Thus, this provides little evidence for tightly specialized species that track each other in the environment. Conversely, some species pairs may apparently avoid each other or co-occur in a given m^2 while not co-occurring at finer spatial scales where

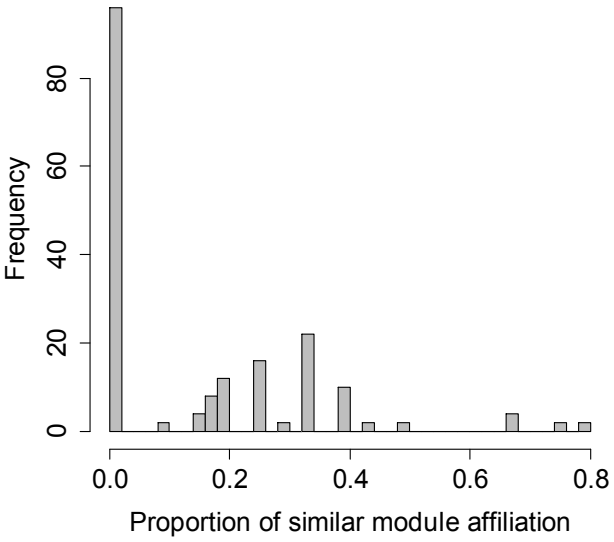

Figure 18. Among the species pairs that co-occurred more than three times, how many times were those species pairs found to be in the same module?

interactions take place. Likewise, network modularity was significantly higher than expected by chance in only 3 networks out of 18. When comparing module affiliations for each pair of plant species, we see that there is no clear tendency for pairs of plant species to be consistently affiliated to the same module in different plot. Notable exceptions are *Galium sp.*, *Tiarella cordifolia* and *Oxalis montana* which all tended to be in the same module 70-80% of the time (figure 18).

By partitioning the network beta-diversity as suggested by Poisot *et al.* (2012), we show that most of the variation among networks is due to flexibility in interactions, which consistently explains ~80% of the total network beta-diversity (fig. 19). It should be noted that the high proportion of the total variation between interaction networks explained by interaction flexibility could not arise simply because there was a low species turnover among the networks (i.e. the other additive component of total network dissimilarity). Indeed, bray-curtis distance in terms of fungal and plant species composition among networks was consistently around 0.40 and sometimes up to 0.70. Thus, species turnover in itself could have been expected to account for a larger proportion of total network dissimilarity.

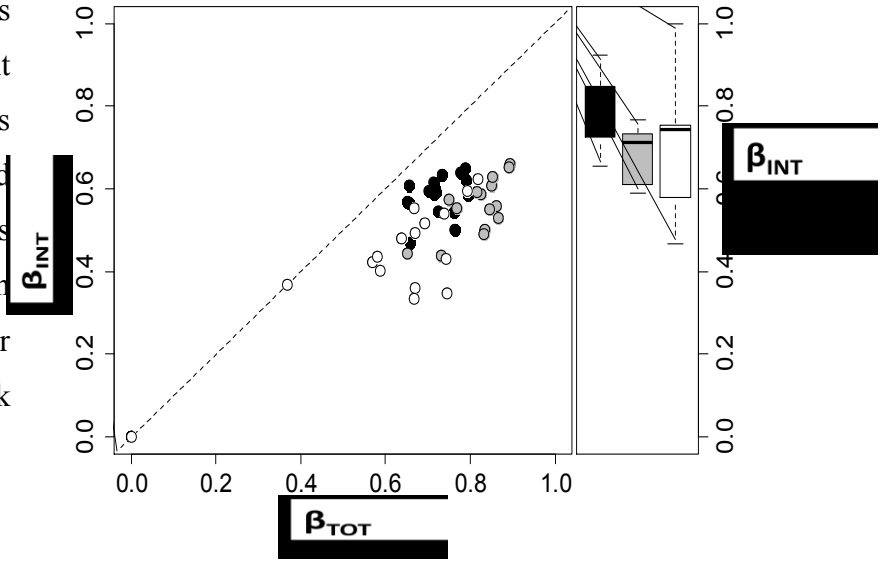

Figure 19. As for figure 16 (chapter 6), we plot the variation among networks that was due to variation in interaction patterns among shared species. The boxplots in the right panel show these values as proportion. Refer to figure 16 for more details.

7.4.2 Centrality of plant and fungal species

To our surprise, plant centrality did not correlate with the number of individuals

of that plant species in its network (R^2 = 0.015, P = 0.15). However, we found a weak link between some plant traits and centrality. For example, leaf area and dry mass content contributed to increase plant centrality (P = 0.035 and P = 0.024, respectively). Yet, those results should be interpreted with caution, because a visual inspection of data revealed that this effect was mainly due to 3 particular plant species: *Fragaria virginiana*, *Rubus pubescens* which had high leaf dry mass content and *Onoclea sensibilis* which had much larger leaves than other species. Those species were consistently dominant in terms of root biomass in their local network, which may have contributed to their high number of interactions, and thus high centrality in the network.

Regarding AM fungi, most species tended to have only few interactions, with a small minority of species were highly generalist. Within sites, the centrality of the different AM fungal species were weakly correlated from one plot to another (i.e., when making pairwise comparisons among the 6 plots found in a single site). However, in some comparisons the correlation was very close to 0 or even negative. We also compared centrality of AM fungal species among our three sites (for those AM fungal species present in more than one site). Again, correlation was extremely weak and never significant (fig 20). At the regional level (across sites), there were only 2 AM fungal taxa that tended to always be central. Indeed those taxa had centrality z-scores above 1 in all three sites, which corresponds to an arbitrary threshold sometimes used to delimit core vs. peripheral species in ecological networks (e.g. Diaz-Castelazo *et al.*, 2010). Those taxa were not clustered in the AM fungal phylogeny, one being an *Acaulosporaceae* and the other a *Claroideoglomeraceae*. Given that in each site, there were respectively 26, 22 and 18 AM fungal taxa with an interaction z-score above 1, this shows how variable was the centrality of AM fungi.

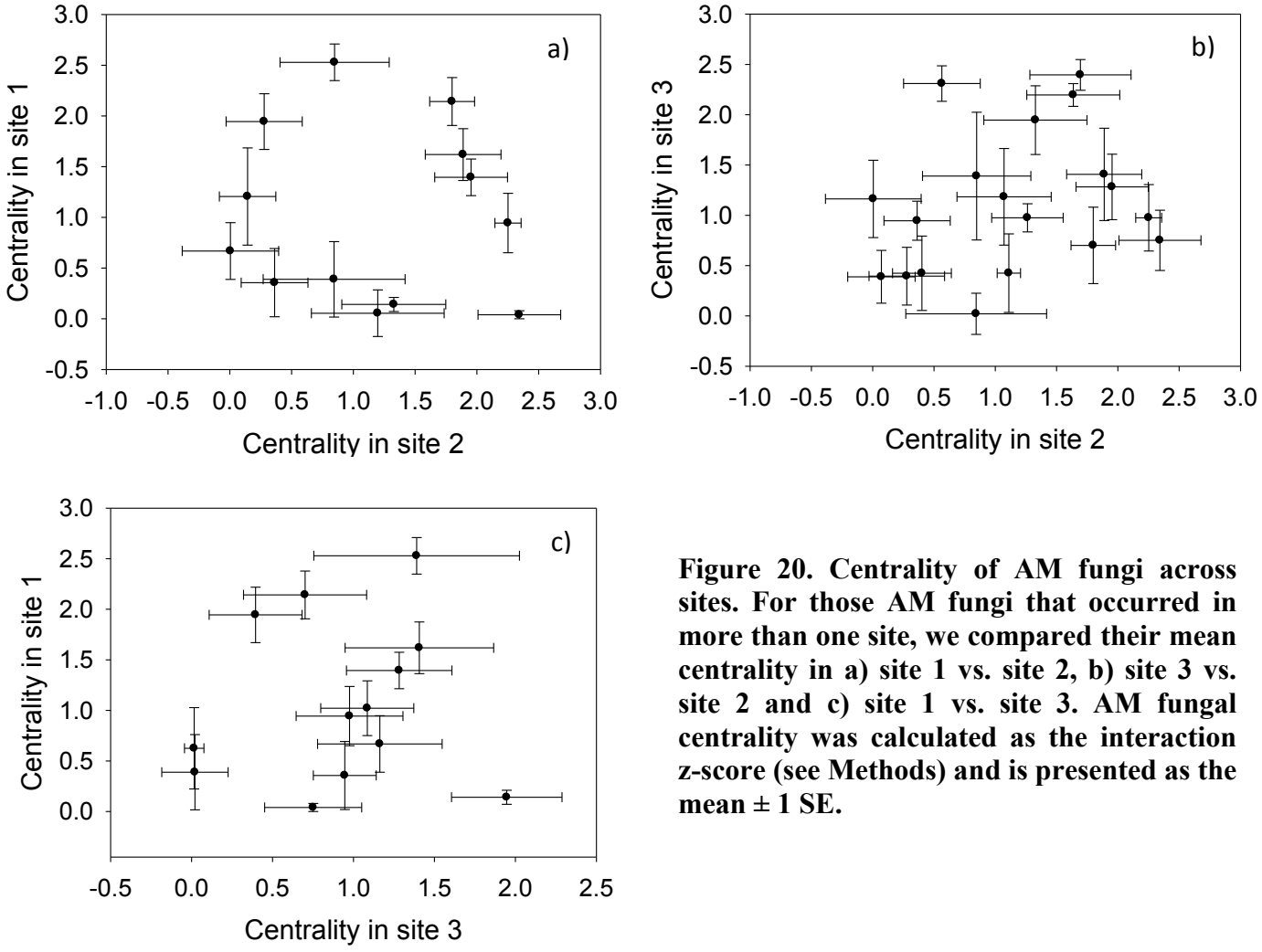

Figure 20. Centrality of AM fungi across sites. For those AM fungi that occurred in more than one site, we compared their mean centrality in a) site 1 vs. site 2, b) site 3 vs. site 2 and c) site 1 vs. site 3. AM fungal centrality was calculated as the interaction z-score (see Methods) and is presented as the mean ± 1 SE.

7.5 Discussion

While we did not find any evidence for strong modularity at the community level, CCA analyses revealed in most cases a significant role for plant traits in driving interaction patterns with AM fungi. Also, we found some pairs of species that tended to be consistently affiliated to the same interaction module (according to our modularity optimization by simulated annealing) across networks. It should be noted that those species (*Galium sp.*, *Tiarella*

cordifolia and *Oxalis montana*) all have very shallow roots. This might reflect a vertical structuring of mycorrhizal fungal niches in our system, as reported elsewhere for ectomycorrhizal fungi (Pickles and Pither, 2014) or AM fungi in agricultural systems (Oehl *et al.*, 2005). Indeed, in our two sites where these plant species occur, the soil profile changes drastically with soil depth, and roots of these species are predominantly found in the upper organic layer. Future studies should further investigate a vertical niche differentiation between organic and mineral soil among AM fungal species in late-successional systems. Such differentiation might reveal new insights about AM fungal life history strategies, with AM fungi dominating organic horizons potentially being ruderal with fast growth rates that would allow to scavenge for nutrients recently mineralized by saprotrophs. Alternatively, these fungi may be exerting a priming effect on the saptrotrophic community through exudation of plant-derived carbon.

The lack of stable interaction modules repeated across local mycorrhizal networks suggests that there is no tight and reciprocal coevolution in those systems at the site level, mediated by repeated interactions with the same species in the network (e.g., Olesen *et al.*, 2007). We rather found high flexibility in those interactions, as evidenced by the β-diversity partitioning of these interaction networks (fig. 19). It is indeed remarkable that as much as 90% of the variation between two networks could be explained by the fact that the species shared by these networks interacted with different partners. However, at the site level, there were consistently a few AM fungal species that tended to be generalists in all local networks. This stable core of generalists may be more important in driving coevolution (Dattilo *et al.*, 2014), especially if they are generalist because they are more abundant in the soil. Indeed, by being more abundant, they necessarily would exert a stronger selection pressure on the plant community. In this view, recently published evidence of local coevolution between plants and AM fungal assemblages (e.g., Johnson *et al.*, 2010; Callaway *et al.*, 2011) might reveal plant adaptation to the most abundant fungal partners in the soil. Future work using pure cultures should investigate this hypothesis.

When comparing AM fungal interaction centrality across site, we show a total absence of correlation, suggesting that centrality is not an inherent property of AM fungal species. It rather seems that the centrality of a fungus will be affected by local site contingencies such as its relative abundance or the identity of the hosts available. Only 2 AM fungi tended to be generalist in all three sites. It is yet to be determined if any key functional trait makes AM fungi more or less susceptible to become generalists in interaction networks. Helgason *et al.* (2007) have shown that fungi that recovered quickly after a disturbance tended to be those which had been seen to interact with many host species in previous studies globally. From this, it may be hypothesized that ruderal fungi are more likely to be generalists in interaction networks. Likewise, a meta-analysis using the maarjAM database (Öpik *et al.*, 2010) revealed that a few AM fungal taxa tended to be widespread globally and also recorded in many different host species. Those fungi, such as *Funneliformis mosseae*, are well known to display a ruderal behavior with high growth rates within roots and early colonization (Jansa *et al.*, 2008; Oehl *et al.*, 2009; Chagnon *et al.*, 2013). One reason that might make ruderal fungi potentially good generalists is the nature by which plant-fungal communities assemble. Indeed, the new young roots that are made available to fungi may be first colonized by AM fungi with better colonizing abilities. And because necessarily, every plant species (although at various degrees) has to turnover a part of its root system and produce new roots every year, every plant species should provide a window of opportunity for ruderal fungi to colonize. If there is any role for a competition-colonization trade-offs to structure AM fungal communities within roots, then those ruderal species should eventually be replaced by better colonizers as the root ages. Future work should thus compare AM fungal communities between parts of a root system of different ages, to see whether younger roots are consistently dominated by a typical subset of the fungal community, i.e. potentially the ruderal species.

Regarding plant interaction centrality, we could not predict it consistently with any species-level property, thus suggesting that as for AM fungal centrality, plant centrality in mycorrhizal networks is the result of local contingencies rather than an inherent species property. The only trends that we could detect were (1) a higher centrality for species with high leaf dry mass content, which was mediated almost solely by two of our ~50 plant species investigated that

were very abundant in two of our three sites (i.e. *Fragaria virginiana* and *Rubus pubescens*), and (2) a positive effect of leaf area on plant centrality, which was solely mediated by a fern species (*Onoclea sensibilis*) that produced massive amounts of root biomass per individual when present in a local network. Thus, plant centrality rather appears to be very flexible for a given species from one site to the other, and it remains unclear what are the factors that control it. Although here we found no effect of a plant species' abundance on its centrality, it should be reminded that even though we collected roots from all individuals of a given species, we did not include more root biomass in our root DNA extractions when a plant was abundant. Therefore, it is very likely that the more abundant a plant was in a local network, the more we underestimated the number of fungal partners with which it associated (because its root-associated AM fungi were not sampled with an effort corresponding to its abundance). It thus remains possible that abundance could explain plant centrality in our networks. Such positive correlation between local abundance and number of interactions is in fact apparent in other mycorrhizal studies (e.g., Öpik *et al.*, 2009; Montesino-Navarro *et al.*, 2012).

7.6 Conclusion

Overall, our results highlight an important feature of mycorrhizal networks: although we can detect deterministic community assembly (e.g., plant interaction patterns predicted by their traits), mycorrhizal interactions remain highly flexible across space. Also, interaction modules rarely appear to be stable across space, indicating that there is no strong evidence for reciprocal coevolution at the site level between small subgroups of species: it may rather be the most abundant and generalist species that drive coevolution in those networks (e.g., Thompson 2005). Finally, the variation in plant and fungal centrality in the networks from one local patch to another, or among sites, indicate that mycorrhizal networks are not, in our system, built around a few keystone species. It rather appears that hubs are highly replaceable, which should contribute to the resilience of mycorrhizal networks when facing perturbations such as the stochastic extinction of a species locally. And if a species local abundance determines its propensity to become a hub in a mycorrhizal network, as suggested by other studies (e.g., Öpik *et al.*, 2009; Montesino-Navarro *et al.*, 2012; chapter 5 of this thesis), then

those species are the least likely to experience demographic stochasticity and local extinction, so the disappearance of a hub would be less likely.

Those findings contrast with patterns evidenced in other types of networks. For example, airport networks tend to have few very big and connected airports that are crucial to the service provided by that network: passenger flow (e.g., Barrat *et al.*, 2004). Likewise, electrical power grids have a tendency for similar network architecture (e.g., Watts and Strogatz, 1998). Such centralized network structure had been related in other fields to its efficiency or its stability when facing perturbation (e.g., Albert *et al.*, 2000). It was thus appealing for ecologists a few years ago to find that ecological communities also display a trend towards network centralization around a few important generalists (e.g., Jordano *et al.*, 2003; Olesen *et al.*, 2006), Yet, ecological networks remain different in one key aspect that has seemed to be rarely considered up to now. In other kinds of networks such as airports or power grids, there is an agency (private or governmental) that mediates the network assembly guided by some specific interests. It thus makes sense to see network assembly as an optimization procedure, where we want to maximize the service provided by the whole network. However, no such agency controls the assembly of ecological networks to maximize some community-level property. It has been suggested that communities naturally evolve towards higher stability, as the less stable communities may disassemble and leave room for the assembly of more stable ones (e.g., Fontaine *et al.*, 2011). They based their reasoning on the fact that some simulation models predicted the emergence of a given structure of interaction networks, which was in fact frequent in nature. For example, most mutualistic networks show significant nestedness in nature, and Thébault and Fontaine (2010) have found that nested mutualistic networks were more stable in their simulations. However, such models rely on questionable assumptions. For example, they assume that (1) species interactions do not vary through time and/or space, while empirical studies (e.g., Petanidou *et al.*, 2008; Lazarro *et al.*, 2010; present study) have shown the opposite, (2) the only drivers of species dynamics are local interactions (i.e. no source-sink / mass effects of dispersal from nearby populations), which would only be typical of strictly isolated insular habitats, (3) no dispersal constraint in meeting the potential interaction partners, which is clearly unrealistic when

dealing with organisms with limited dispersal abilities (mycorrhizal networks are an extreme example involving two guilds of sessile organisms), etc. Hence, current modeling studies may have limited generality and it might have been an excess of enthusiasm to have concluded from them that ecological networks evolve towards more optimal structures (here optimizing stability in simulation models), as it is seen in other kinds of networks. Our results suggest that we may have pushed the analogy between abiotic networks and ecological communities too far. Maybe ecological networks do not evolve in a way that optimizes a community-level property such as stability or functioning: maybe ecological networks are rather built around individual-based interests, one of which would be for species to remain flexible in their interaction patterns, in order to buffer the potential loss of some partners. Such individual-based interests would better fit an optimization scenario, where natural selection can select for optimal strategies in partner selection.

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Les chapitres 1 à 7 forment le cœur de mon projet doctoral. Toutefois, plusieurs éléments périphériques ont été produits pendant ce doctorat, qui ont un lien parfois assez indirects avec mon projet mais témoignent tout de même de ma productivité scientifique. Je souhaitais donc les ajouter ici en tant que chapitres supplémentaires. Le Chapitre 8 formera une suite au chapitre 4, visant à définir un cadre théorique pour mieux comprendre les stratégies d'histoire de vie des CMA. Dans le chapitre 8, je me concentre sur un trait clé pour ces champignons, soit leur capacité à fusionner les hyphes pour échanger du matériel génétique ou pour reformer un réseau d'hyphes intact après une perturbation. Je revois la littérature sur le sujet, et discute de la façon dont ce trait peut s'insérer dans la stratégie d'histoire de vie d'un CMA. Le chapitre 9, quant à lui, porte sur les interactions entre CMA et autres champignons de la rhizosphère, par le biais indirect de la plante hôte. En effet, si le présent projet doctoral a mis l'emphase sur les interactions plantes-CMA, ces dernières ne sont qu'une forme des nombreuses interactions que la plante entretient avec des microorganismes du sol. Dans le même ordre d'idées, le chapitre 10 utilise un système d'étude très utile en phytopathologie (i.e. des souches de pathogènes de la tomate) pour déterminer l'importance du système hormonal de la plante dans la médiation des interactions CMA-champignons pathogènes. Dans le chapitre 11, je m'intéresse plutôt aux rétroactions plantes-sol, avec un système d'étude très intéressant impliquant diverses espèces d'asclépiades, où il est possible de séparer l'effet de la phylogénie des plantes de l'effet de leur stratégie de réponse aux ennemis.

Finalement, je présente dans les chapitres 12 à 15 différentes analyses sur les aspects méthodologiques de mon doctorat. Dans le chapitre 12, j'évalue l'influence de divers protocoles d'extraction de spores de CMA sur leur viabilité. Dans le chapitre 13, je réalise des simulations afin d'estimer le biais potentiel dans les études sur les CMA qui emploient les techniques de biologie moléculaire, du à un sous-échantillonnage des communautés naturelles. Les chapitres 14 et 15 traitent plutôt des analyses numériques réalisées sur les matrices de données d'interactions plantes-CMA. Le chapitre 14 s'intéresse à l'influence de la connectance d'une matrice (nombre d'interactions réalisées sur le nombre total d'interactions possibles) sur notre aptitude à détecter des patrons comme les interactions nichées (i.e. « nestedness ») ou la modularité. Finalement, le chapitre 15 évaluent la performance de deux

méthodes alternatives pour calculer et tester la significativité statistique de la modularité dans des matrices binaires.

En somme, même si ces chapitres (8 à 15) n'ont pas nécessairement contribué de façon directe à tester les hypothèses primaires ayant trait à mon projet de doctorat (concentré sur la présence de spécialisation dans la symbiose plantes-CMA), ils témoignent tout de même de ma productivité scientifique soutenue pendant ce projet doctoral. De plus, certains chapitres (i.e. 12-13-14-15) ont contribué à éclairer mes choix quand à mes décisions méthodologiques pour répondre à mes questions d'ordre biologique.

Chapitre 8

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF HYPHAL FUSION IN ARBUSCULAR MYCORRHIZAL FUNGI

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8.1 Abstract

Arbuscular mycorrhizal (AM) fungi are important plant symbionts widespread worldwide. Like other fungi, they have the ability to perform hyphal anastomosis, that is, the fusion of encountering vegetative hyphae. Research in other fungal phyla has evidenced numerous potential functional and evolutionary consequences of anastomosis. Yet, in AM fungal research, anastomosis has almost strictly been discussed in the context of fungal response to disturbance and inter-individual genetic exchange. Here, I review more broadly the implications of anastomosis for AM fungal ecology and evolution. I also identify major knowledge gaps, and research prospects to better ground hyphal anastomosis strategies of AM fungi in their general life history strategies.

8.2 Introduction

Arbuscular mycorrhizal (AM) fungi (*Glomeromycota*) are widespread plant symbionts representing a significant portion of soil microbial biomass (Leake *et al.,* 2004). As with other filamentous fungi, they have the capacity – unusual in the tree of life - to perform inter-individual fusion of vegetative cells, a process termed hyphal anastomosis (or hyphal fusion). This finely tuned process has been relatively well studied in *Ascomycota* and *Basidiomycota* (e.g. Read *et al.,* 2009), and has been argued to have multiple, far-reaching implications for ecology and evolution of fungi (Pontecorvo, 1946; Rayner, 1991). However, even though anastomosis has long been known to occur in AM fungi (e.g. Mosse, 1959), only recent research has started unearthing its potential functional consequences (e.g. Avio *et al.,* 2006; Croll *et al.,* 2009), and anastomosis has mostly been discussed as a mechanism promoting response to disturbance and speeding up asexual evolution (e.g. Young, 2009; Sanders and Croll, 2010). Here consequences of anastomosis on fungal ecology and evolution are broadly reviewed (see fig.21 for a visual overview) and potential selection pressures mediating fusion rates in nature, as well as research needs for the AM fungal system more specifically, are discussed.

8.3 Functional consequences of anastomosis

8.3.1 Homeostasis maintenance

Most fungal species on earth are modular organisms formed by septate hyphae in which only small amounts of cytoplasmic material flows through pores in the septa. Some hyphal networks in soil can extend over large spatial scales (e.g. Smith *et al.,* 1992 ; Beiler *et al.,* 2010), which poses a challenge for the maintenance of homeostasis. Intra-individual cross-connections by hyphal fusion may help maintain significant levels of cytoplasmic flow through the network (Glass *et al.,* 2000; Fu *et al.,* 2011), and contribute significantly to maintenance of homeostasis (Read *et al.,* 2009). Indeed, microscopic real-time observation of hyphal fusion events in *Neurospora crassa* revealed dramatic shifts in cytoplasm streaming after the fusion (Hickey *et al.,* 2002; Leeder *et al.,* 2011). Some fungi, however, are

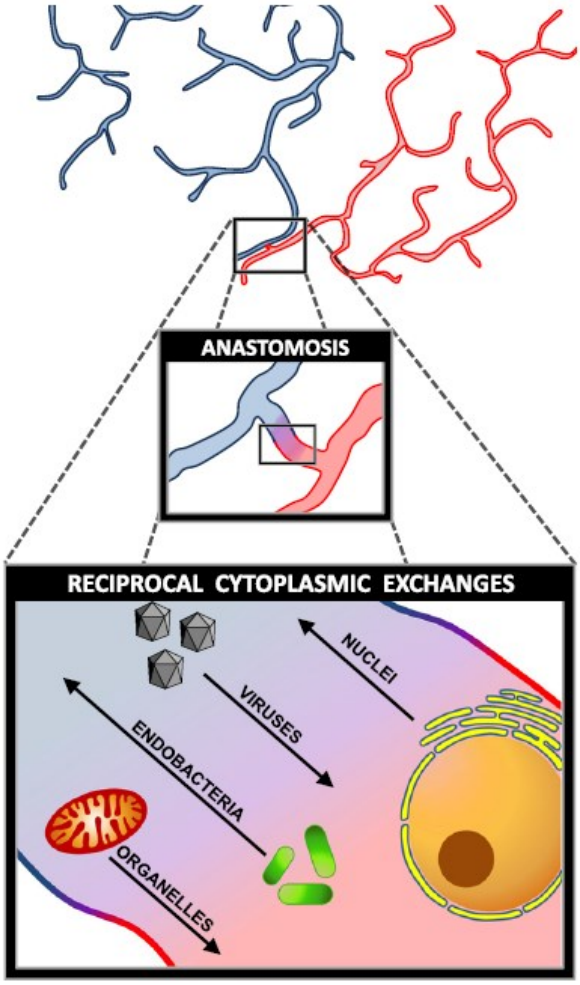

Figure 21. Inter-individual exchanges during anastomosis events. Two individuals of a given AM fungal species (here represented by blue and red hyphae, respectively), grow towards each other (potentially involving positive chemotropism, e.g. Sbrana & Giovannetti, 2005). Their hyphae anastomose, which allows a bidirectional flow of cytoplasmic material between individuals. Material transferred involves nuclei, bearing alleles that may be beneficial in the given environment where they grow, or conversely coding for aggressive replication, and not for a function likely to increase organism fitness. Mycoviruses may also be transferred, with potentially positive or negative effect on fungal fitness. Likewise, parasitic or mutualistic endobacteria may be exchanged, as well as debilitated organelles (organelles that show a decreased function, while keeping replicating and thus draining energetic resource).

coenocytic (i.e. lack septa in their hyphae), which allows cytoplasm to stream rather freely and thus rapidly across the hyphal network (Jany and Pawlowska, 2010; Purin and Morton, 2011). This is the case for *Zygomycota* and *Glomeromycota* (AM fungi). For those fungi, the homeostasis balance is potentially easier to maintain, which may explain lower incidence of fusion events found in those fungal phyla (e.g. Gregory *et al.,* 1984; Purin and Morton, 2011). Nevertheless, as no study has yet compared the frequency of fusion events in different fungal phyla in the same experiment, it is still too soon to speculate whether the differences observed among phyla result from experimental set-ups or from a true divergence of functional strategies.

8.3.2 Genetic exchange and diversification

Giovannetti *et al.* (1999) visually observed that nuclei could be transferred during fusion events between encountering AM fungal hyphae. By promoting nuclei exchange, anastomosis is likely to have great evolutionary consequences for AM fungi. Although heterokaryosis in AM fungi remains a debated issue (e.g. Pawlowska and Taylor, 2004), there is increasing empirical support to it (e.g. Croll *et al.*, 2009). Thus, the nuclei acquired through anastomosis may bring new alleles (Glass *et al.,* 2000). Croll *et al.* (2009) corroborated this by showing that following

anastomosis between two genetically distinct AM fungal isolates, specific molecular markers from both parents were passed to progeny. This shows that anastomosis in AM fungi may have consequences for the genetic structure of populations by allowing genotypic mixing across different isolates, and it was noticeable from their study that even isolates pairs that anastomosed at very low frequencies exchanged detectable amounts of genetic material (Croll *et al.,* 2009). Not all AM fungal isolates of a given species readily anastomose, though. For example, while Croll *et al.* (2009) found broad compatibility between isolates of a given AM fungal species (*Rhizophagus irregularis*) from a single site, Giovannetti *et al.* (2003) didn't observe any anastomosis between geographically distant isolates of *Funneliformis mosseae*. The genes mediating the compatibility system in AM fungi are yet unkown, but in *Ascomycota*, *het*-genes have been identified as regulators of vegetative hyphal compatibility, through the additive effect of multiple *het* alleles (Pearson *et al.,* 2009). Divergences in too many *het*-genes alleles between two encountering hyphae cause the heterokaryon generated by anastomosis to be unstable, by triggering a programmed cell death reaction (Glass and Kaneko, 2003). Nevertheless, even incompatible strains can exchange some genetic material through leakages during the incompatible interaction (Papazova-Anakieva *et al.,* 2008), but to what extent this process may affect population genetic structure is still uncertain. It has been shown that strains compatibility in nature is negatively correlated to phylogenetic and geographic distances (Park *et al.,* 2006; Roper *et al.,* 2011; Mehrabi *et al.,* 2011). This is consistent with the observation that variability at *het* loci appears to be maintained evolutionarily at noticeably high levels (Saupe *et al.,* 2000), which suggests that without genetic homogeneization of fungal populations through anastomosis, between-isolates genetic differences at *het* loci will rapidly accumulate to eventually hinder any fusion event. More work at various spatial scales with AM fungi will clarify the process of natural genetic divergence between AM fungal populations.

Genetic material exchange may significantly affect the ability of AM fungi to rapidly evolve in a changing environment not only by providing new alleles on which selection can act, but

also by potentially allowing recombination between nuclei of distinct origins, following karyogamy (Pawlowska, 2005). Empirical evidence is still lacking for AM fungi, but such post-fusion recombination is thought to be a major mechanism generating genetic diversity in pathogenic fungi, thus promoting virulence in their arms race with hosts (Wang and McCallum, 2009). Indeed, as fusion events may happen more frequently than sexual cycles in fungi, genetic exchange and recombination may represent an important way to quickly adapt to novel environmental conditions (Mehrabi *et al.,* 2011). Such horizontal gene transfer has long been recognized in bacteria to promote genome plasticity, and thus adaptation (Juhas *et al.,* 2009).

Along with nuclei exchange, nuclei segregation (i.e. the transfer of only a random fraction of total nuclei from a parent to its progeny, thus generating a genetic bottleneck effect), has more recently been identified as a potentially important mechanism generating genetic variability in AM fungal populations (Angelard *et al.,* 2010). It has been found that segregated lines of a given AM fungal isolate (i.e. sub-cultures of this isolate initiated from single spores of a parent culture) varied widely in their phenotypic characteristics (Angelard and Sanders, 2011), as well as on their effect on host plants (Angelard *et al.,* 2010). It may be thought that inter-individual anastomosis events and nuclei segregation act in concert to contribute to AM fungal evolvability through fission-fusion dynamics (see fig. 22). On one hand, segregation reduces allele population size in a newly formed individual, thus favoring drift in the relative abundance of different alleles and promoting a novel phenotype (Angelard *et al.,* 2010), and on the other hand, anastomosis may help reshuffling alleles in the population, promoting heterokaryosis maintenance (Bever and Wang, 2005). Combined, those two mechanisms may provide explanations to two major issues originally thought to hinder long term maintenance of an asexual strategy, namely (1) the Red Queen Hypothesis (asexual organisms should adapt more slowly because of absence of sex-based alleles recombination; Bell, 1982) and (2) the Mueller's Ratchet (asexual organisms will accumulate deleterious mutations over generations because of absence of allele segregation through meiosis; Müller, 1932). Segregation and

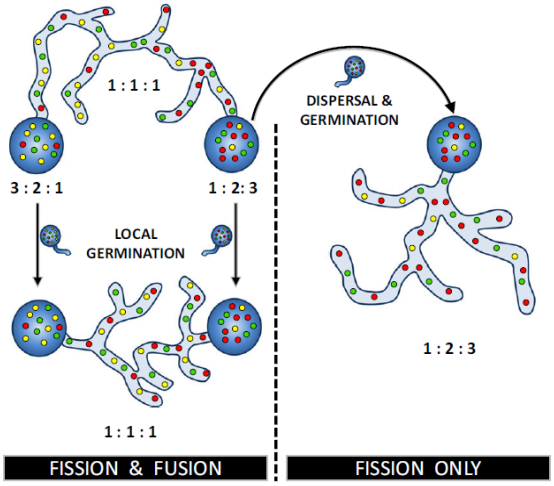

Figure 22. Evolutionary fission-and-fusion events (*sensu* Grant & Grant, 2008) in AM fungi. A growing mycelia (top-left corner) may harbor a balanced set of three nuclei (represented by red, green and yellow circles, respectively, and having a relative abundance ratio of 1:1:1). During spore formation, a subset of those nuclei reach the spore and through drift, the relative abundance ratios among nuclei type may change (e.g. here to 3:2:1 and 1:2:3, respectively. This fission event is known as segregation. If those spores germinate locally (i.e. nearby their formation sites) or if they co-disperse, a potential hyphal anastomosis may reshuffle nuclei among the fused mycelia and potentially re-balance the relative abundance of nuclei types (or not, if the abundance ratios among nuclei was not as symmetrical as shown here). Conversely, if spores disperse at different locations and germinate without possibility for anastomosis (right panel of the figure), the new abundance ratios among nuclei is likely to be preserved and is predicted to display a contrasted phenotype, as compared to the parent individual. Thus, like Darwin's finches populations in Galapagos, populations of some AM fungal species are likely to undergo evolutionary fission (i.e. segregation) and fusion (anastomosis), which may contribute to fast, asexual evolution.

genetic exchange both generate novel phenotypes on which natural selection can act (Red Queen), and segregation can limit the transfer of deleterious mutations to progeny by genetic drift (Mueller's Ratchet). Those mechanisms may thus, at least partly, explain the apparent maintenance of asexuality in AM fungi, which is presumed to have lasted for the last 450 million years. The upcoming challenge will be to investigate how frequent are fusion events in natural AM fungal populations, and how widespread they are across different AM fungal lineages. Estimates for anastomosis frequency are also lacking in an ecologically relevant context, although significant progress has been made in designing novel study systems more relevant to natural conditions (e.g. Avio *et al.,* 2006; Purin and Morton, 2011). Another future prospect for research is to investigate whether evidences from recombination in AM fungi are partly due to cryptic sexual events, as multiple meiosis genes and putative sex pheromones have been identified in *Glomus* spp. (Halary *et al.,* 2011, 2013).

8.3.3 Deleterious cytoplasmic elements (DCEs) transfer

Cytoplasmic continuity between fungal individuals established through anastomosis results in bidirectional transfer of cytoplasmic elements (fig.

21). Among those elements, some may be deleterious. For example, transposons could be transferred via anastomosis, and even if they could facilitate recombination and thus adaptation (Mehrabi *et al.,* 2011), they could also induce undesirable deleterious mutations, which are thought to be especially threatening for asexual fungi like AM fungi (Pawlowska, 2005). Aggressive nuclei are also considered as DCEs (Glass *et al.,* 2000). By aggressive nuclei, one means nuclei that contribute little to organism fitness, while coding for enhanced rates of anastomosis frequency to promote their own transfer (i.e. selfish nuclei) (Saupe *et al.,* 2000). Debilitated organelles, that can replicate but show decreased functional activities, can also be transferred during anastomosis and decrease fitness of the recipient individual by draining metabolic resource without providing any functional benefit in return (Caten, 1972; Milgroom *et al.,* 1999). Currently, we have no information regarding (1) the frequency of transfer of such DCEs in AM fungi, and (2) their persistence in the recipient hyphae. Finally, mycoviruses can also take advantage of hyphal fusion events to spread in fungal populations (Ihrmark *et al.,* 2002). They generally consist of double-stranded (ds) RNA molecules encapsidated (or not) in a peptide (Ghabrial *et al.,* 1998). As they do not have any extracellular vector to spread between hosts, they must be transferred either vertically to progeny, or horizontally through anastomosis (Ghabrial *et al.,* 1998). While most dsRNA have been shown to have limited effect on host phenotype, some viruses can be quite beneficial (Pearson *et al.,* 2009) or detrimental (Dalzoto *et al.,* 2006; Wu *et al.,* 2007) to their host. In fact, reduced virulence of plant fungal pathogens caused by mycoviruses (i.e. hypovirulence) has raised much attention for its potential as a biocontrol strategy to protect economically important crops. In AM fungi, the only study reporting and manipulating mycoviruses was done using a single fungal isolate (Ikeda *et al.,* 2012). The authors have found various dsRNA genomes in the AM fungal isolate, and they were able to raise an AM fungal line free of one particular dsRNA genome. They observed that this virus-free line produced twofold greater spore number and better promoted host plant growth than the infected isolate (Ikede *et al.,* 2012), which clearly indicates the potential top-down pressure that mycoviruses may exert on natural AM fungal populations. In natural populations of other fungal phyla, when surveys have been done, it has generally been observed that dsRNA are frequent in fungal hyphae in nature, thus suggesting that spread efficiency of mycoviruses in fungal populations offsets the

effect of selection against infected hosts (e.g. van Diepeningen *et al.,* 1998). The vegetative incompatibility system can act as an effective transmission barrier in ascomycetes (e.g. Park *et al.,* 2006), and theoretical work has suggested that DCEs such as viruses can even select for higher levels of vegetative incompatibility (Brusini *et al.,* 2011). Nevertheless, for dsRNA to effectively act as a selective pressure restricting vegetative compatibility in fungal populations, they must have a negative (even if small), impact on fungal fitness (Brusini *et al.,* 2011). More work needs to be done to determine fitness costs of dsRNA (e.g. Ikede *et al.*, 2012; Dalzoto *et al.*, 2006) and to characterize their prevalence in natural AM fungal populations. Only with such data we can determine whether top-down regulation of AM fungal populations by parasitic dsRNA genomes is important (Purin and Rillig, 2008).

8.3.4 Response to disturbance

Fungal mycelia can experience various forms of disturbance (i.e. loss of functional biomass due to physical rupture of the mycelium), either caused by soil fauna, tillage, soil freezing or wetting-drying cycles. As obligate biotroph, AM fungi are dependent upon a functional hyphal network to colonize roots for carbon uptake and soil for mineral nutrition. Anastomosis of disrupted hyphae to form back a connected network after disturbance events has been suggested as a crucial mechanism allowing persistence of AM fungi in frequently disturbed environments (de la Providencia *et al.,* 2005; Avio *et al.,* 2006). Some even suggested that disturbance may be the strongest selection pressure maintaining high anastmosis rates in nature (Young, 2009). Even before post-disturbance hyphal fusion, healing mechanisms of disrupted hyphae are crucial to prevent cytoplasm leaking from hyphal breakpoints (de la Providencia *et al.,* 2005). This is especially true for fungi with coenocytic hyphae (i.e. *Zygomycota* and AM fungi), which lack septa to limit cytoplasm leaking to the local injured section of the mycelium. In fact, it has been observed that in ecosystem recently invaded by earthworms, which cause physical disturbance in soils, there was a loss of *Zygomycota* species from the fungal community, probably due to their incapacity to cope with chronic injuries and cytoplasm leak (McClean *et al.,* 2006). Other fungal species are known to have specific

organelles, the Woronin bodies, which rapidly plug the pores of the septa surrounding the injury to prevent excessive loss of cytoplasm after disturbance (e.g. Jedd and Chua, 2000). In AM fungi, little is known about hyphal healing. De la Providencia *et al.* (2005) observed distinct healing strategies in different AM fungal families, namely the *Glomeraceae* and the *Gigasporaceae*. In *Glomeraceae*, after hyphal disruption, a septal plug was formed at both injured tips, and multiple new hyphal branches were formed at each tip, which presumably reveals a strategy evolved to quickly reconnect the disrupted hyphae into a cohesive network after disturbance (de la Providencia *et al.,* 2005). In *Gigasporaceae*, after disruption, a septal plug was assembled 50-300 μM away from the disrupted hyphal tips, resulting in considerable cytoplasm leakage, and four to six hours later, one or two hyphal branches were produced behind those septa. The authors argued that this strategy could reflect more a strategy to survive to adverse conditions, rather than a strategy to reconnect the hyphal network. Also, the growth of hyphal branches was directed towards each other if they were at short distances (40-100 μM), but not if they were further away, indicating that repaired hyphae were not seeking to anastomose. Taken together, those results indicate that some AM fungal species should benefit from hyphal fusion to restore cohesive hyphal networks after disturbance, while other species may be more adapted to environments displaying less frequent disturbance. This is corroborated by the observation that *Glomeraceae* typically dominate conventionally tilled arable sites (Daniell *et al.,* 2001; Oehl *et al.,* 2003) while *Gigasporaceae* are at disproportionately low abundances in those sites compared to surrounding natural environments (e.g. Jansa *et al.,* 2002, 2003).

8.4 Synthesis and future work

As shown above, anastomosis should be regulated evolutionarily following a balance between negative and positive functional consequences. If positive aspects (e.g. increasing genotypic

diversity and plasticity, favoring response to disturbance, etc.) offset negative ones (e.g. virus transmission, aggressive nuclei transfer, etc.), anastomosis should be maintained at high rates in natural AM fungal populations. Estimates of anastomosis rates in laboratory studies have been shown to vary according to the species taxonomic affiliation (e.g. Avio *et al.,* 2006) and to the cultivation system used (Giovannetti *et al.,* 2004; Voets *et al.,* 2006; Purin and Morton, 2011), thus preventing reliable predictions as to how frequent hyphal fusion events should be in natural AM fungal populations. Nevertheless, it was found by inspecting the genomes of other fungal phyla that high levels of allele polymorphism were conserved in *het*-genes, mainly through conservation of ancestral variability and unusually high substitution rates (Saupe *et al.,* 2000). This suggests that the rate of anastomosis in natural populations is under tight evolutionary control. Fusion rate must be selected in fungal population in a way to maintain positive effects of inter-individual cytoplasmic exchange while limiting the costs through DCEs transfer (Brusini *et al.,* 2011). As it has been shown that even short and infrequent fusion events can lead to substantial material transfer between fungi (Papazova-Anakieva *et al.,* 2008; Croll *et al.,* 2009), it may be expected that fusion rates in nature are maintained at low levels through rapid evolution of fungal vegetative incompatibility.

From an applied viewpoint, by bringing together most selection pressures acting on hyphal fusion rates in natural AM fungal populations, this short review may help envisaging how agricultural practices may affect AM fungal phenotypes. Indeed, as frequent ploughing may select for higher hyphal fusion rates in order for AM fungi to maintain a functional hyphal network (e.g. Avio *et al.,* 2006), this may impact on AM fungal biology by promoting virus transmission for example, which may influence AM fungal symbiotic function either positively or negatively (Ikeda *et al.,* 2012). Hence, a more comprehensive understanding of functional and evolutionary consequences of hyphal fusion in AM fungal populations will help addressing applied issues such as consequences of agricultural practices on symbiotic performance of AM fungi.

Future research should especially strive to link hyphal fusion strategies to overall species' life history strategies. Here I list 3 potentially fruitful research avenues to explore:

(1) We know that some AM fungal species can anastomose very frequently, while for other species we never observe any fusion event. Is this related to a live-fast die-young strategy (Promislow and Harvey; 1990), where the most ruderal species, adapted to disturbed environments, will grow quickly and readily fuse hyphae, while accepting the cost of accumulating deleterious organelles, nuclei and mycoviruses?

(2) Can we link spore size to hyphal fusion strategy? Large-spored Gigasporaceae species may be less prone to experience nuclei segregation during spore formation (because nuclei populations may be larger). Thus, hyphal fusion could be avoided in order to limit the spread of deleterious mutations because nuclei segregation is not likely to purge such mutations out. Conversely, as small spores may be likely to disperse over larger distances and thus encounter very different environmental conditions, there could be a selection pressure for small-spored species to rapidly adapt to novel environments, which could be achieved through both nuclei segregation and hyphal fusion with native individuals at their "invasion" site, allowing allele exchange.

(3) Hyphal fusion strategy vs. fungal response to hyphal grazing. It is not unreasonable to expect that AM fungi display a trade-offs between tolerance and resistance to fungivory (e.g. van der Meijden *et al.,* 1988). Tolerant species may rely on high anastomosis frequency and high growth rate to quickly rebuild a functional hyphal network after fungivory (which is a form of disturbance), while resistant species may have traits that limit fungivory (e.g. thick and tough walls, repellent compounds, etc.).

Hence, overall, to better understand the ecological and evolutionary bases of hyphal anastomosis, this particular function has to be considered in the broader context of organisms' life history strategies. This may help to unearth trait syndromes (i.e. strategies) that may be evolutionarily conserved across the AM fungal phylum (e.g. Chagnon *et al.,* 2013). Future

work on AM fungi will thus have to also include a wider range of species and genera of AM fungi, as past research has mainly focused on cosmopolitain, generalist taxa such as *Rhizophagus irregularis* or *Funneliformis mosseae*, which are known to display a fast-growing and massive reproduction strategy, typical of a ruderal life history strategy (Chagnon *et al.*, 2013).

8.5 Concluding remark

This short review is an example of how insights may be gained in AM fungal ecology by considering the knowledge gained with other fungal phyla. It is essential to capitalize on this latter literature which benefits from the availability of gene-deletion libraries with few model fungal species (e.g. *Neurospora crassa*, *Aspergillus nidulans*) to explore the functional roles of single genes on fungal phenotypic expression (Fu *et al.,* 2011). This highlights the need to consider AM fungi first as fungi, not strictly as plant symbionts (Fitter 2000, 2005). Such mycocentric viewpoint will better enable mycorrhizal ecologists to broaden their view of AM fungi and to recognize the similarities those symbionts share with the rest of the fungal Kingdom.

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Chapitre 9

EVIDENCE THAT SOIL NUTRIENT STOICHIOMETRY CONTROLS THE COMPETITIVE ABILITIES OF ARBUSCULAR MYCORRHIZAL *VS.* ROOT-BORNE NON-MYCORRHIZAL FUNGI

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9.1 Abstract

A majority of plant species have roots that are colonized by both arbuscular mycorrhizal (AM) and non-mycorrhizal (NM) fungi. The latter group may include plant mutualists, commensals, parasites and pathogens. The co-occurrence of these two broad groups may translate into competition for root volume as well as for plant-derived carbon (C). Here we provide evidence that the relative availability of soil nitrogen (N) and phosphorus (P) (i.e., soil nutrient stoichiometry) controls the competitive balance between these two fungal guilds. A decrease in the soil available N:Pratio resulted in a lower abundance of AM fungi and a corresponding increase in NM fungi. However, when the same fertilization treatments were applied in a soil in which AM fungi were absent, lowering the soil available N:P ratio did not affect NM fungal abundance. Taken collectively, our results suggest that the increase in NM fungal abundance was not a direct response to soil nutrient stoichiometry, but rather a competitive release from AM fungi responding negatively to higher soil P. We briefly discuss the mechanisms that may be responsible for this competitive release.

9.2 Introduction

Fungal competition has mainly been studied within various functional groups such as saprotrophs (Song *et al.,* 2012), plant pathogens (Leonard *et al.,* 1999) and ectomycorrhizal fungi (Kennedy *et al.,* 2007). On the other hand, relatively few studies have examined inter-guild fungal competition (Kennedy, 2010). As a result, little is known about the drivers of competitive interactions between broad groups of fungi occupying the same territory or volume. Given the wide diversity of fungal groups that may naturally colonize the interior of plant roots (Vandenkoornhuyse *et al.*, 2002), the "endorhizosphere" may be a suitable arena for studying these interactions.

Both arbuscularmycorrhizal (AM) and non-mycorrhizal (NM) fungi colonize the roots of a majority of terrestrial plants (Wang and Qiu, 2006). Members of the former group are obligate symbionts constituting the phylum Glomeromycota, whereas the latter group may include plant mutualists, commensals, parasites and pathogens.Both of these broad fungal groups may affect plant growth and population dynamics (Klironomos, 2002), and both depend on plant-derived carbon(C) for energy (Olsson *et al.,* 2002; Singh *et al.,* 2000; Jeger *et al.,* 2008). Hence, relative plant C allocation to these different groups of fungi may influence their competitive abilities within the root. Soil nutrient stoichiometry, notably the available N:P ratio, is considered a major driver of plant C transfer to AM fungi (Johnson, 2010). This is because the symbiosis is strongly related to the reciprocal exchange of C and P between plants and AM fungi (Hammer *et al.,* 2011; Kiers *et al.,* 2011; Smith and Smith, 2012). The more soil available P there is relative to soil available N, the less benefit a plant may derive from its AM symbiont (Johnson *et al.,*1997). Conversely, a high soil available N:Pratiomay stimulate plant C investment to AM fungi (Johnson *et al.,* 2003). For their part, root-colonizing NM fungi do not acquire plant C through such intimate coupling mechanisms of nutrient transfer. Thus, soil nutrient stoichiometry may not be as important in determining their competitive ability within roots.

Here, we report on a study demonstrating how soil nutrient stoichiometry, more specifically the soil available N:P ratio, may drive competitive interactions between AM and NM fungal groups. Our results were obtained serendipitously from a plant-soil feedback experiment that was originally designed to test hypotheses regarding positive or negative plant-soil feedbacks in the absence or presence of AM fungi, and at low and high soil available P. Although results did not supportour initial hypotheses, they provided someunexpected insightsas to the role that soil nutrient stoichiometry may play in driving competitive interactions among different groups of root fungal endophytes.

9.3 Methods

The hypothesis that initially motivated our study was that AM fungal mediated plant-soil feedbacks, defined as the difference in crop yield over 2 successive generations (Kardol *et al.,* 2006), would be positive at high soil N:P ratio and negative at low soil N:P ratio. Thus, we used 4 treatments comprising a factorial array of 2 soil N:P ratios × 2 soil fungal communities. *Panicummiliaceum L.* seedlings,grown from surface sterilized seeds (soaked in 70% ethanol for 1 min), were transplanted in 0.7 L pots filledwith 400g (dry wt. equiv.) soil that had been collected from an abandoned field(pH in H_2O = 5.69; pH in KCl = 5.18;Mehlich-III extractable P = 0.41 ppm; organic matter = 13.4% (w/w); total N = 0.6 %(w/w)). The seedlings were grown for 10 weeks in a growth chamber at 20 °C with a 16 hour diurnal daylight (600 μmol photons m^{-2} sec^{-1}) period. Twice every week, half of the pots received a +N solution (5.65 mg NH_4NO_3 in 20 mL H_2O) in order to alleviate any possible N deficiency to plants. The remaining pots were fertilized with a +N+P solution (N solution + 1 mg KH_2PO_4 in 20 mL H_2O), which ensured no N deficiency but lowered the soil available N:P ratio.

Additional NM “control” treatments were included in order to investigate the isolated effect of the soil N:P ratio on NM fungal root colonization. These were prepared by autoclaving the

same soil, and re-inoculating each pot with 50 mL of AM-free soil solution. This AM-free solution was prepared by blending 1 kg of non-autoclaved soil in 6 L of water, and filtering the suspension through a 30 μm nylon mesh. This filtering procedure excluded the larger AM fungal spores, but not the smaller NM fungal spores (e.g. Koide and Li 1989). Ideally, our experimental design would also have included AM "control" pots, that is, pots with plant roots colonized exclusively by AM fungi and grown at each soil N:P ratio. We were not able to produce these 2 treatments because preliminary tests showed that a too large number of AM fungal spores was required to achieve satisfactory root colonization, and that it was equally impossible to obtain a NM free sample by centrifugation. All 4 treatments (+N, +N+P, +N control, +N+P control) were replicated 20 times.

Plants from the first generation were destructively sampled in order to measure AM and NM fungal root colonization. First, roots were cleared for 1 wk in a KOH aqueous solution (10% w/v) at room temperature and then stained in a blue ink-vinegar solution (5% v/v) (Vierheilig *et al.,* 1998). Morphological discrimination of AM and NM hyphae was based on the criteria described by Rillig *et al.* (1998). Data were analyzed using t-tests, or non-parametric Mann-Whitney rank sum tests when homoscedasticity between groups was not satisfied.

9.4 Results and Discussion

AM fungal colonization was significantly higher in the +N than in the +N+P treatment ($U=0$, $P<0.01$), while the opposite was true ($t=-3.04$, $P<0.01$) for NM fungi (Fig. 23). This resulted in a significant difference ($U=0$, $P<0.01$) in the AM:NM fungal colonization ratio between the two treatments (+N=2.2 *vs*. +N+P=0.2). In theNM control treatments, no AM fungal structures were found, and the soil N:P ratio had no effect ($t=-1.417$, $P=0.16$) on percent root colonization by NM fungi (Fig. 23). Thus, the positive effect of higher P on NM fungal biomass, within roots bearing both fungal guilds, ostensibly came about as a result of reduced competition from AM fungi, as opposed to a direct positive response to P fertilization.

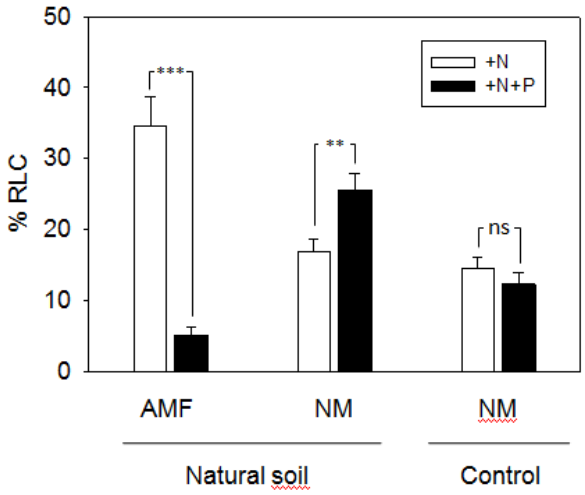

Figure 23. Effect of soil N fertilization, with or without P, on percent root colonization (%RLC) of arbuscular mycorrhizal (AM) and non-mycorrhizal (NM) fungi in *Panicum miliaceum L.* The control treatment refers to pots containing AM-free plant roots. Means are shown ± 1SE. * *P*< 0.001; ** *P*< 0.01; ns = non significant.**

We note that NM fungal root colonization was lower in the control soil than in the natural soil, which might suggest that AM fungi actually facilitate the development NM fungi. A difference in the absolute amounts of NM fungal colonization in each soil type does not, however, corroborate such a conclusion. In the natural soil, NM fungal propagules were likely to be present throughout the soil, such that newly forming roots had a high probability of encountering NM fungal propagules. The control soil, on the other hand, had been sterilized and re-inoculated, such that NM fungal propagules were likely to be more patchy and in lower densities. Thus, it is understandable that NM fungal root colonization in the control soil would take longer to develop than in the natural soil. This circumstance precludes us comparing the absolute amounts of NM colonization between soil types. The only valid comparisons are, in fact, between fertilizer treatments within each soil type, which allows us to verify whether differences in soil N:P ratio has a similar relative effect on NM colonization in the presence or absence of AM fungi. Thus, the only plausible interpretation of our data is that a high soil N:P ratio improves the competitive ability of AM fungi relative to root-borne NM fungi.

Given the generally accepted relationship between soil N:P stoichiometry and plant C allocation to AM fungi (Johnson *et al.,* 2003; Johnson 2010), we hypothesize that competitive interactions between AM and NM fungi were mediated, at least in part, by preferential plant C allocation to AM fungi when both guilds are present. Other factors that may have affected these competitive interactions are the production of antibiotics by different fungal guilds (St-

Arnaud *et al.,* 1997; Cairney and Meharg, 2002), which may reduce metabolic activity of competitors (Kjøller and Rosendahl, 1997), or the priming of plant defenses and bacterial strains, which may suppress the growth of fungal pathogens and other NM fungi (Pozo and Azcon-Aguilar, 2007; Bharadwaj *et al.,* 2012). It is not clear, however, how these alternative mechanisms could be linked to soil N:P stoichiometry. Furthermore, as only 15–35% of total root length was colonized by fungi across all treatments, we surmise that competition for limited root volume was an unlikely cause for our results.

Our findings surfaced serendipitously from an experiment designed to test an entirely different research question than the topic of this short communication. Our interpretations are based, therefore, on a limited data set and need to be substantiated with future studies addressing specific data gaps. For example, it is still not clear how the competitive interactions that we reported would develop in unfertilized or N-limited soil. Secondly, autoclaving and re-inoculating soil, in order to study the isolated effects of soil N:P stoichiometry on NM fungi, generates obvious experimental artifact such as altering soil nutrient concentrations and eliminating soil fauna. Thirdly, we only reported percent root colonization instead of total AM and NM fungal biomass estimates.

In spite of its shortcomings, the small data set that we are presenting should motivate us to seek a better understanding of how different fungal guilds interact between themselves within competitive environments, and how these interactions are shaped by environmental factors such as soil nutrient stoichiometry. Previous work describing interactions between AM and root-borne NM fungi was mainly conducted with pure cultures (e.g. Larsen and Bødker, 2001). Among the few trials that used native fungal assemblages, Klironomos *et al.* (1996) observed a relative increase in NM abundance following soil fertilization. Their study used a single fertilizer N:P ratio and did not control for a possible direct response of NM fungi to fertilization, thus precluding any inference of competitive interactions. More recently, Wehner*et al.* (2011) observed a decrease in NM fungal abundance when roots were co-inoculated with AM fungi. While these results did suggest competitive interactions between the two guilds of fungi, they did not reveal the drivers of this competition. Our results are,

therefore, incremental to those of Wehner *et al.* (2011), as they point to soil N:P stoichiometry as a possible factor driving the competitive balance between AM and NM fungi.

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Chapitre 10

THE RELATIVE IMPORTANCE OF HOST VIGOR AND HORMONAL RESPONSE TO PATHOGENS IN CONTROLLING THE DEVELOPMENT OF ARBUSCULAR MYCORRHIZAL FUNGI

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10.1 Abstract

Plants are routinely colonized by both beneficial and detrimental microorganisms. These two microbial guilds may indirectly interact with each other via their host, either by modifying its vigor, or by altering its hormonal/defense status. Here, we studied indirect interactions between arbuscular mycorrhizal (AM) fungi and three plant pathogens. We show that AM fungal sporulation was only triggered by the least aggressive fungal pathogen, which is known to induce a jasmonate-based hormonal response by the host without affecting its vegetative growth and vigor. Conversely, the most aggressive fungal pathogen considerably reduced host vigor but did not alter AM fungal growth and sporulation. Our results thus suggest that the plant hormonal system is an important component of indirect interactions between AM fungi and plant pathogens.

10.2 Results and discussion

Most herbaceous plants are simultaneously colonized by pathogens and by arbuscular mycorrhizal (AM) fungi, both of which affectthe host plant's competitive ability (van der

Heijden *et al.,* 2003; Mordecai, 2011) and its fitness (Mills and Bever, 1998; Philip *et al.,* 2001). Although pathogens produce disease whereas AM fungi tend to provide benefits to the host plant, both depend on plant derived carbon (C) to grow and to reproduce. It is expected, therefore, that these two broad endophytic microbial groups may develop competitive or mutualistic strategies with respect to each other within the host plant. Moreoever, there is evidence that indirect interactions between the two groups can be mediated by the host's physiological status (e.g. Tester *et al.,* 1985; Chagnon and Bradley, 2013) or by its hormonal response to infection (e.g. Pozo and Azcon-Aguilar, 2007). Here, we present a small data set from which we can infer the relative importance of these two mechanisms in driving indirect interactions between plant pathogens and AM fungi.

We used two fungal strains of *Botrytis cinerea* differing in their virulence. This pathogen has a necrotrophic lifestyle that promotes host cell death in order to acquire C and nutrients. The main component of plant defense against necrotrophic pathogens is the elicitation of the jasmonic acid-based (JA) pathway, which results in the production of JA and a subsequent increase in the expression of defense effector genes. This response is also elicited by other forms of stresses such as wounding or insect herbivory (Glazebrook, 2005). The strains of *B. cinerea* used in our experiment both have a necrotrophic lifestyle, but they differ in their interactions with the host's hormonal defense system. The more aggressive strain has evolved a mechanism to down-regulate the host plant's jasmonic acid-based (JA) hormonal response, but has a strong negative effect on host vigor (El'Oirdi *et al.,* 2011). Conversely, the weaker strain elicits the JA pathway and more broadly antifungal defense compounds such as systemin (El'Oirdi *et al.,* 2011), but it has a much lower effect on the physiological status of the host. Hence, both *B. cinerea* strains can lead, via different mechanisms, to a decrease in host quality for AM fungi. Either way, deteriorating conditions for AM fungi should lead to a decrease in mycelial biomass production and/or an increase in energy storage structures such as spores and vesicles (Douds *et al.,* 2005). Our study system thus allowed us to measure the relative importance of host vigor vs. hormonal response in controlling the indirect effect of *B. cinerea* on AM fungi. Our study system also included a host infection treatment using *Pseudomonas syringae*, a biotrophic bacterial pathogen. Host infection by biotrophic

pathogens should trigger the anti-biotrophic salicylic acid-based (SA) hormonal pathway (Bari and Jones, 2009), without causing cell death to the host. As obligate biotrophs, AM fungi rely on a low expression of the anti-biotrophic pathway to maintain root colonization (Klopholz *et al.,* 2011). We thus predicted that host infection by *P. syringae* would decrease somatic growth and/or increase energy storage structures by AM fungi.

Tomato (*Solanum lycopersicum* cv. Moneymaker) plants were grown in a soil collected from an abandoned field left uncultivated for >40 years. Before potting, the soil was coarse-sieved (1 cm mesh) to remove large roots and other fragments, stored at 4 °C for 2 wk and then potted in 0.5 L pots. Three surface-sterilized (10% bleach) tomato seeds were planted in the middle of each pot. Pots were then transferred to a growth chamber set to a 16 h daylight period and temperatures of 20 °C day / 18 °C night. Within 2 wk of emergence, the two least vigorous seedlings were removed. Seedlings that germinated from the soil's seed bank over the course of the experiment were also plucked and discarded. Symptoms of nutrient deficiencies appeared after 5 wk growth, therefore each pot was amended with 60 mg of N (NH_4NO_3) and 50 mg of P (KH_2PO_4) suspended in water. This amount of fertilizer has been shown to not significantly reduce root colonization by AM fungi in nutrient-poor soils (e.g. Collins and Foster, 2009).

Following 8 wk growth, plants were inoculated with one of the three pathogen types according to the methods outlined in Yangui *et al.,* (2010), with seven replicates per treatment. For the two *B. cinerea* strains, a spore suspension (10^6 per mL in H_2O) was sprayed on two leaves. For *P. syringae*, we soaked the two leaves for 30 s in a cell suspension of this pathogen (10^6 per mL in 10 mM $MgCl_2$) (Chakravarthy *et al.,* 2003). Inoculated plants were placed in a polyethylene bag to retain moisture, and placed in a dark growth chamber for 24 h, after which the bags were removed and the normal light cycle was restored. Along with the pathogen infection treatments, the experiment included two control treatments: (1) two leaves sprayed with distilled water, and (2) two leaves soaked in $MgCl_2$ solution (because the bacterial cells were suspended in $MgCl_2$ in the biotroph inoculation treatment).

Plants were harvested 2 wk after inoculation, and total fresh shoot and leaf mass were recorded for each pot. Whole root systems from each pot were washed and digitized using a desktop optical scanner. Total root length and average root diameter were calculated using GIAROOTS image analysis software (Galkovkyi *et al.,* 2012). Roots were then dried at 55 °C and weighed. To assess AM fungal colonization, roots were rehydrated and cut into ~1 cm fragments, cleared for 1 wk in a 10% p/v KOH solution, dipped 15 min in 2% v/v acetic acid, and then soaked for 1 d in an ink-vinegar (5% v/v) staining solution. Root fragments were then mounted onto glass slides and percent colonization of AM arbuscules, hyphae and vesicles were recorded using the gridline intersect method (McGonigle *et al.,* 1990). A soil subsample from each pot was used to determine gravimetric moisture content, while the remaining soil was divided into two subsamples. The first was used to count spores by means of wet-sieving and centrifugation (Chagnon and Bradley, 2011), whereas the second was used to measure soil hyphal length (Johnson *et al.,* 2003). Mycorrhizal hyphae were distinguished from non mycorrhizal ones using established criteria, such as absence of clamp connections or regular septation (e.g., Rillig *et al.,* 1998). The densities of soil-borne spores and hyphae were calculated on a dry soil mass basis. Normality and homoscedasticity of the data were verified respectively using Shapiro-Wilk's and Bartlett's tests. The effects of treatments on plant biomass and fungal structures were tested using ANOVA, and the separation of means was performed using Tukey's HSD test. All statistical analyses were coded in R statistical package (R Core Team, 2013).

Pathogenic infection was deemed 100% successful, based on visual symptoms. The more aggressive *Botrytis* strain caused the loss of the infected leaves, and the leaves formed after inoculation were pale and small and accounted for a significantly smaller proportion of total aboveground biomass ($F = 49.66$, $P < 0.0001$) than in the other treatments (fig. 24). Also in this treatment, plants had significantly fewer roots ($F = 3.25$, $P < 0.05$), which were of higher diameter ($F = 2.71$, $P < 0.05$) than all other treatments. The weaker *B. cinerea* strain only caused the yellowing of the two inoculated leaves. Infection with *P. syringae* resulted in yellow spots on the inoculated leaves only. Treatments had no effect on the density of

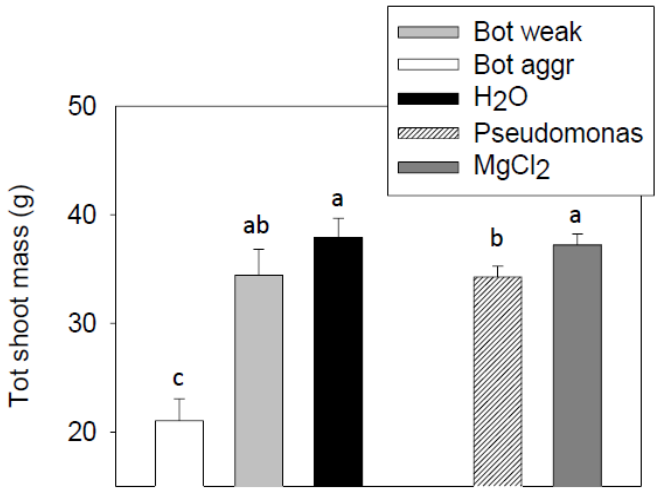

Figure 24. Plant aboveground biomass in response to the treatments. Bars = mean ± SE. Treatments were: Bot weak and Bot aggr = leaves sprayed with weak and aggressive strains of *Botrytis cinerea*, respectively; H_2O = leaves sprayed with water ; Pseudomonas = leaves dipped in a *Pseudomonas syringae* suspension; $MgCl_2$ = leaves dipped in a $MgCl_2$ control. Letters above bars are results from Tukey's HSD test.

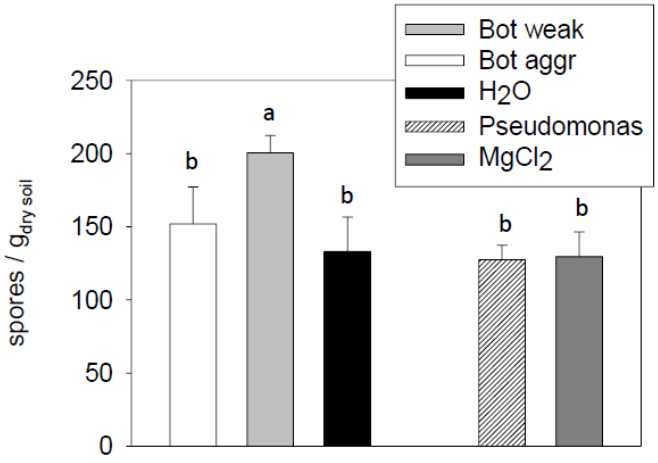

Figure 25. AM fungal sporulation in response to the treatments. Bars = mean ± SE. Treatments were: Bot weak and Bot aggr = leaves sprayed with weak and aggressive strains of *Botrytis cinerea*, respectively; H_2O = leaves sprayed with water ; Pseudomonas = leaves dipped in a *Pseudomonas syringae* suspension; $MgCl_2$ = leaves dipped in a $MgCl_2$ control. Letters above bars are results from Tukey's HSD test.

arbuscules or vesicles occurring within roots, nor on soil hyphal length produced in the soil (not shown). However, soil-borne spores were significantly higher (F = 19.03, P < 0.001, fig. 25) with the weak *B. cinerea* strain.

Plants infected by the aggressive strain of *B. cinerea* were likely to transfer less C to their AM fungal symbionts due to a greater loss of photosynthetic tissues. However, this loss of host vigor did not trigger a sporulation response by AM fungi. Conversely, the weak *B. cinerea* strain did not substantially decrease host vigor, but AM fungal sporulation increased by nearly 60% in this treatment (fig. 25). Given that this weaker strain is known to normally elicit an anti-fungal JA-based hormonal response (El'Oirdi *et al.,* 2011), our results suggest that such a response to pathogen infection has a stronger effect, than a loss of host vigor, on AM fungal development. This has important implications for agriculture, as seemingly minor aboveground symptoms of pathogen infection may mask consequential impacts on the

belowground development of symbiotic AM fungi. By triggering a sporulation response, the weaker strain of *B. cinerea* appeared to cause a shift towards a C storage strategy by AM fungi. This may, in turn, reduce nutrient uptake for the plant and impact its fitness, especially for high P-demanding crops such as tomato. It may seem counter-intuitive that the weaker strain of *B. cinerea* did not increase vesicle production, as these structures are involved in storing plant-derived C (e.g., Denison and Kiers, 2011). One may speculate that when the quality of a host deteriorates due to local pathogen accumulation in the surrounding environment, AM fungi may better store C in soil propagules that are more likely to disperse (i.e. spores) instead of in root-borne propagules more likely to survive locally. Alternatively, it may be that vesicles are not optimal indicators of C storage by AM fungi (e.g., Lekberg *et al.,* 2013). Finally, contrary to our hypothesis, the biotrophic pathogen did not elicit any response in AM fungal development. This may be due to *Pseudomonas* infection being too low to trigger a significant hormonal response, as corroborated by the slighter visual infection symptoms in this treatment. Alternatively, the biotrophic pathogen may exert both positive and negative effects on AM fungi, which cancel each other out. For example, the elicitation of anti-biotrophic defense genes may harm AM fungi, but a successful infection by the pathogen may also indicate the suppression of other plant defense genes, which would benefit AM fungi (e.g., Abramovitch and Martin, 2004).

Overall, our results suggest that a plant hormonal response to pathogens may have a strong impact on beneficial symbioses such as associations with AM fungi. This supports broader theories invoking potential trade-offs between defense and mutualisms in plants (e.g., Agrawal, 2011; Adler *et al.,* 2012). Still more could be learned about plant-mediated interactions between pathogens and AM fungi. For example, what happens when a plant is infected early in the growing season, when AM fungal root colonization is not yet fully established? Does this initiate a plant screening process for AM fungi that are better at providing bioprotection against such pathogens (e.g., Newsham *et al.,* 1995)? Also, what are the underlying physiological and hormonal causes for variation in AM fungal growth patterns following pathogen infection? Experimenting with mutant plants bearing defense-related knockout genes may be one way to shed light on these questions.

10.3 Acknowledgements

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CHAPITRE 11

CAN WE LINK A PLANT'S LIFE HISTORY STRATEGY TO ITS FEEDBACK DYNAMICS WITH SOIL BIOTA?

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(Manuscript in preparation, to be submitted to Journal of Ecology)

11.1 Abstract

Plant-soil feedback dynamics have rarely been studied in the context of plant life history strategies. Here, we build a framework linking plant resource management, life history, dispersal and interactions with enemies and mutualists. Central to our framework is the well-known trade-offs in plants between tolerance and resistance to herbivores. We hypothesized that resistant plant species should accumulate fewer enemies in their rhizosphere (by being better defended), and have more neutral to positive feedbacks. The reverse should be true for tolerant, poorly-defended plants. We tested this hypothesis using two pairs of milkweed species. Each pair consisted of two phylogenetically close species that have contrasting enemy resistance-tolerance strategies. We found support for our hypothesis in one of the two species pairs, where the resistant plant species accumulated both more beneficial arbuscular mycorrhizal fungi and more beneficial (or less detrimental) non-mycorrhizal microorganisms. The lack of support for our hypothesis with the other species pair was driven by the accumulation of detrimental non-mycorrhizal microorganisms in the rhizosphere of the more resistant species. This may be explained by the fact that this species had finer roots than the rest of species, thus being more exposed to belowground enemies. Overall, this study suggests

that plant interactions with belowground biota may be integrated into broader evolutionary trade-offs related to defense or symbiotic strategies, and that root characteristics should be taken into account when investigating plant-soil feedback dynamics. Further studies are needed to test the generality of those findings.

11.2 Introduction

Microorganisms are major components of terrestrial ecosystems (van der Heijden *et al.*, 2008), and they have the potential to drive plant community structure (e.g., O'connor *et al.*, 2002; Schnitzer *et al.*, 2010; Maron *et al.*, 2011; Mordecai 2011). Plants are well known to exert bottom-up control on belowground microbial communities through qualitative and quantitative carbon allocation (reviewed in Wardle 2002; Ayres *et al.*, 2009) and, in turn the microbial communities developed in the rhizosphere may influence positively or negatively plant performance through so-called plant-soil feedback effects (Bever, 1999; Kulmatiski *et al.*, 2008; Harrison and Bardgett, 2010). Such feedback dynamics are likely to be important drivers of major plant community-level processes such as coexistence (Bever, 1999; Hart *et al.*, 2003), invasion (Callaway *et al.*, 2004; Agrawal *et al.*, 2005), and succession (Kardol *et al.*, 2006; Carbajo *et al.*, 2011).

Plant enemies are well known to induce feedback dynamics and, for example, there is much empirical evidence that plants can accumulate detrimental pathogens in their rhizosphere (e.g., Mills and Bever, 1998; Klironomos, 2002). Similar patterns have been found at the aboveground level (e.g., Bagchi *et al.*, 2010). Likewise, soil mutualistic microorganisms may also produce feedback dynamics. Arbuscular mycorrhizal (AM) fungi are among the most widespread plant mutualists in terrestrial ecosystems, and again there is much empirical evidence that they can induce either positive plant-soil feedbacks (e.g., Klironomos, 2002; Mangan *et al.*, 2010; Zhang *et al.*, 2010) or negative ones (Bever, 2002). Yet, we still know very little about the relative importance of these two microbial guilds in driving plant-soil feedbacks in natural communities (Hodge and Fitter, 2013). In fact, quantifying such relative

importance of pathogens, mutualists and plant competitive interactions in driving plant community structure has been identified elsewhere as one of the most pressing issue in plant community ecology (Agrawal *et al.*, 2007; Klironomos *et al.*, 2011). Several bodies of research suggest that pathogens – or more broadly enemies - and AM fungi may not simply be additive components of natural plant-soil feedbacks. For example, AM fungi may protect plants from enemies through a variety of mechanisms (reviewed in Borowicz, 2001; Koricheva *et al.*, 2009). Thus it is likely that in presence of AM fungi, ennemy-mediated plant-soil feedbacks would be reduced. Conversely, various plant enemies are known to modify AM fungal community structure, thus having a potential impact on AM fungal-mediated plant-soil feedbacks (e.g., Kowalchuk *et al.*, 2002; Alguacil *et al.*, 2011). Also, it is increasingly acknowledged that most organisms interacting with plants (herbivores, pathogens, commensal endophytes, mutualists) elicit and/or are influenced by common and multifaceted components of the plant defense system, of which the most important are salicylic and jasmonic acids (e.g., Glazebrook, 2005; Hause and Schaarschmidt, 2009; Bezemer *et al.*, 2005). It should not be surprising, then, to find that those organisms simultaneously interacting with plants have reciprocal influence on each other (e.g., Goverde *et al.*, 2000; De Roman *et al.*, 2011). It then becomes very difficult to predict the outcome of those multipartite interactions even in simplified laboratory systems. There is thus a need to develop a coherent theoretical framework integrating all plant interactions with those various guilds of organisms.

Several theories have already been independently developed to link some key plant interactions together. For example, at a coarse level, it has been suggested that plant defense against enemies could be traded-offs against interactions with beneficial mutualists (Agrawal, 2011). Accordingly, Adler *et al.* (2012) found that plants producing more defense compounds also relied less on pollinators for reproduction, potentially because those compounds acted as deterrent for the mutualists. Others have suggested that defense should be traded-offs against growth (e.g., Coley *et al.*, 1985). Accordingly, it has been found that (1) predator preferentially feed on palatable (i.e. less defended) species that also grow and recruit at faster rates (Loh *et al.*, 2014), (2) species suffering more from herbivory (less defended) are also

those that grow fast and do not tolerate low nutrient concentrations in soil (Lind *et al.*, 2013) and (3) species that grow slowly and tolerate low nutrient concentrations accumulate less pathogens (Mitchell *et al.*, 2010). Another premise at the core of the evolutionary theory of plant defense is the trade-offs between plant tolerance and resistance to enemies (Hay *et al.*, 2011). Following this trade-offs, plants should be either good at quickly re-growing after enemy attack, or good at resisting against enemy attack through defense. Mooney *et al.* (2010) found evidence for such a trade-offs in plant response to herbivory: plants that grew faster and responded positively to high nutrient concentrations were less resistant to herbivores. Finally, another body of research that links the interactions of plants with other biota to their life history strategy is the work on succession. There is increasing evidence, indeed, that successional replacement of species can be understood partly through plant interactions with soil biota. For example, Kardol *et al.* (2006) showed that early-successional plant species tended to consistently develop negative plant-soil feedbacks in microcosms, while late-successional species developed positive ones. This suggests that early-successional species may be prone to accumulate soil-borne enemies, as suggested by the work of van der Putten *et al.* (1993) on sand dune succession. Those results could easily be reconciled with a growth-defense trade-offs, where faster-growing, early-successional species tend to be less well defended against enemies, and thus are eventually replaced, along the successional trajectory of the community, by species that are better defended but grow more slowly. Hence, it seems clear from the work cited here that plant growth rate and responses to enemies and mutualists should be viewed as key components of their life history strategy. Here, we try to merge these aspects into a coherent framework and to include mycorrhizal fungi into it (which has yet to be done, in spite of the fact that most terrestrial plant species are mycorrhizal). We build upon the above-mentioned theories by predicting a continuum of strategies with the two following extremes: (1) plants that tolerate attack instead of resisting it, by having fast growth rates. Those species are likely to be short-lived and to disperse their seeds far away from their parents to avoid locally accumulated enemies. They would prefer early-successional, richer habitats. Such habitats are likely to favor a low dependency on AM fungi, because nutrients are readily available to plants (Chapin, 1980). Also, it has also been shown that short-lived annuals depended less on AM fungi than perennials (Boerner *et al.*, 1992; Collier *et al.*, 2003).

Such plants would have a resource management strategy that maximizes acquisition instead of conservation, with high specific leaf area, low leaf dry mass content and thin roots (e.g., Wright *et al.*, 2004; Roumet *et al.*, 2006; Pierce *et al.*, 2013). This may overall be considered akin to a live-fast die-young trade-offs (Pearl, 1928), where a plant would maximize fast acquisition of resource and short term fitness gains instead of surviving and spanning its fitness over multiple generations. At the other end of the extreme, we would have (2) plants that invest more into defense, thus resisting to enemies. Those plants would have slower growth rates, would tolerate poorer environments and depend more on AM fungi for nutrient acquisition. They would be more efficient at conserving resource and would live longer. Overall, one might see our framework simply as an extension of the tolerance-resistance trade-offs cited above, which would now include broader expectations regarding successional dynamics, plant resource economics and relationships with AM fungi. Regarding plant-soil feedbacks, our prediction would be that tolerant plants would predominantly develop negative plant-soil feedbacks, mostly driven by the accumulation of enemies. On the other hand, resistant plants would show neutral enemy-mediated feedbacks, and would rather develop positive feedbacks with AM fungi. The positive nature of AM fungal-mediated feedbacks is based on the empirical evidence that plants and AM fungi can control resource allocation to pay more when their partners also pays more (Bever *et al.*, 2009; Hammer *et al.*, 2010; Kiers *et al.*, 2011; Fellbaum *et al.*, 2012). Thus, at the community level, one would expect that plants progressively select optimal fungal partners (and vice versa), which should be beneficial to the plant in the next generation.

Here, we tested this hypothesis using two pairs of milkweed species (*Asclepias spp.*). Each pair consisted of phylogenetically related species that showed contrasting tolerance-resistance strategies to herbivores (Mooney *et al.*, 2010). This allowed us to test for the role of the tolerance-resistance trade-offs in driving plant-soil feedbacks, while controlling for confounding phylogenetic effects. We found mixed support to our framework, suggesting that it may be oversimplified and need further refinements at finer scales.

11.3 Materials and Methods

11.3.1 First generation

We germinated seeds of four milkweed species: *Asclepias syriaca, Asclepias tuberosa, Asclepias subverticillata* and *Asclepias currassavica*. The two former species formed the first pair (tolerant and resistant species, respectively) and the two latter, the other pair (tolerant and resistant species, respectively). To help germination, we nicked the seeds with a clean, disinfected razor blade, and cold-stratified them between moist filter papers at 4°C for three weeks. Then, we transferred them to a 28°C environment for germination. We then transplanted the germinated seedlings into 1 L pots filled with a bottom layer of 300 mL of turface®-natural soil 1:1 mixture, 600 mL of natural soil and 100 mL of turface®. Turface® was added to improve drainage. The natural soil was collected in an old-field near Sherbrooke, Canada (45° 24' N, 71° 54' W) that had not been cultivated for more than 40 years. Plants were grown for four months (March to July 2012) in a growth chamber with 16 hours of light per day, at 22°C during day and 20°C during night. After four months, plant shoots and roots were separately harvested to measure leaf economics traits (specific leaf area, leaf dry mass content and leaf thickness) and root traits (specific root length, mean root diameter). Soil was thoroughly mixed to prepare microbial inocula for the second generation. AM fungal inocula was prepared by extracting spores from 20 g equivalend dry mass of soil using a standard wet-sieving and centrifugation technique (Chagnon and Bradley, 2011). Spores were collected on a 30 μm nylon mesh and rinced three times with 10% bleach to surface sterilize them, in order to exclude as much non mycorrhizal (NM) microorganisms as possible. A second, NM microbial fraction was prepared by suspending 20 g equivalent dry mass of soil in 0.5 L of distilled water, and sieving through a 30 μm nylon mesh. The < 30 μm fraction, comprising small NM fungal spores, and bacterial cells and spores was collected.

11.3.2 Second generation

For the second generation, plants were grown in 600 mL cone-tainers filled with 200 mL of sand-turface®-perlite 1:1:1 mix, 200 mL of sterilized natural soil, 150 mL of sand-turface®-perlite 1:1:1 mix to which the microbial inocula were added and 50 mL turface ®. The microbial inocula consisted of either AM fungal spores, NM microbial filtrate, or both, and each plant species was inoculated with either its "home" microorganisms or the microorganisms developed in the rhizosphere of the other species of the pair (i.e. "away" microorganisms). Each treatment was replicated ten times, for a total of 240 experimental units. To ensure that AM fungal spore density was comparable to NM microbial densities as compared to what was found originally in generation one, we took care to inoculate each pot in generation two precisely with the microorganisms that were found in 15 g equivalent dry mass of soil from generation one. This was crucial to our design, where we want to assess the relative importance of the two microbial guilds in mixed inoculations. Seedlings were germinated and transplanted as explained for generation one. To avoid runoff of AM fungal spores or NM propagules during the first week of the seedlings establishment, we watered the plants frequently but with very small amounts of water using a transfer pipette. Plants were grown for four months, after which they were harvested as in generation one. Plant-soil feedback was calculated as (Performance home) – (performance away) / (performance away), which is alike to calculating a response ratio, considering the presence of . Incertitude around this feedback measure was calculated using a bootstrap approach, as in Carvalho *et al.* (2010). Also, in addition to the measurements made for generation one, we also measured in generation the proportion of root surface area that represented the pivot area. We did so using scanned images of the root systems, and the software WinRhizo. The rationale behind this is that pivot production may be an important component of a plant's next generation performance, owing to its resource storage function. By considering pivot production in a second generation, we might thus get information on how a plant may perform in a third generation.

11.4 Results

We found support to our hypothesis in one of the two species pairs. Indeed, the herbivore-tolerant species *A. subverticillata* developed negative plant-soil feedbacks, while the herbivore-resistant *A. currassavica* developed positive ones (fig. 26). However, the negative feedbacks of *A. subverticillata* were driven mostly by AM fungi, not by NM microorganisms. On the other hand, for the other species pair, both species developed negative plant-soil feedbacks. It should be noted, for this species pair, that the resistant species, *A. tuberose*, had significantly finer roots than all other species.

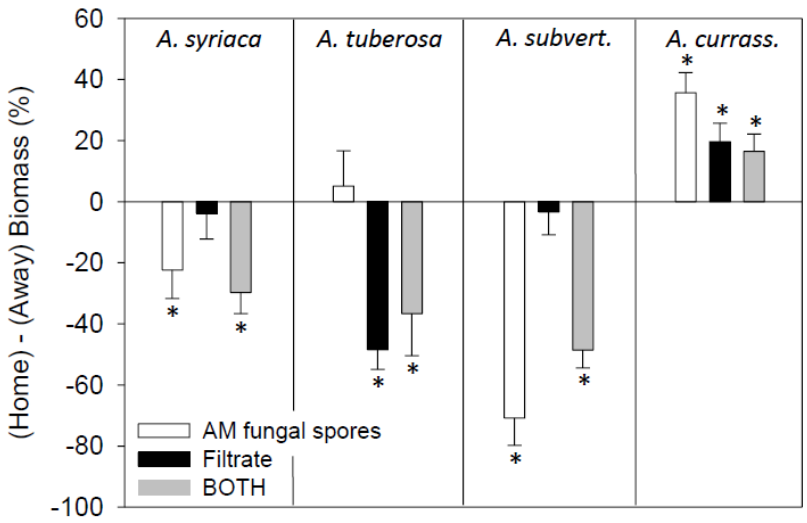

Figure 26. Plant performance in home vs. away soil fractions. The values are reported as response to home inoculum (i.e. (home – away) / away. In this figure plant performance was monitored as shoot dry mass, but similar trends were revealed when using root dry mass or total dry mass. Asterisks indicate performance indices significantly deviating from 0.

We also found that the effects of AM fungi vs NM microorganisms tended to be largely additive in the two species of the first pair, and clearly not additive for the species of the second pair. This is visually obvious from fig 26, and it was confirmed numerically using a bootstrapping approach (data not shown).

Here we measured plant-soil feedbacks from various biomass compartments: shoot mass, root mass, and

pivot area. We found that using shoot vs. root biomass, or both, to estimate plant-soil feedback had very minor influence on the results, as the three were all highly correlated to each other (fig 27a-c). Conversely, when comparing feedback values in terms of root length production vs. pivot area, we found that feedbacks based on root length were consistently of larger magnitude than those based on pivot production (fig 27d). In other words, when looking at root length, one would consistently overestimate positive feedbacks and underestimated negative ones, as compared to pivot production.

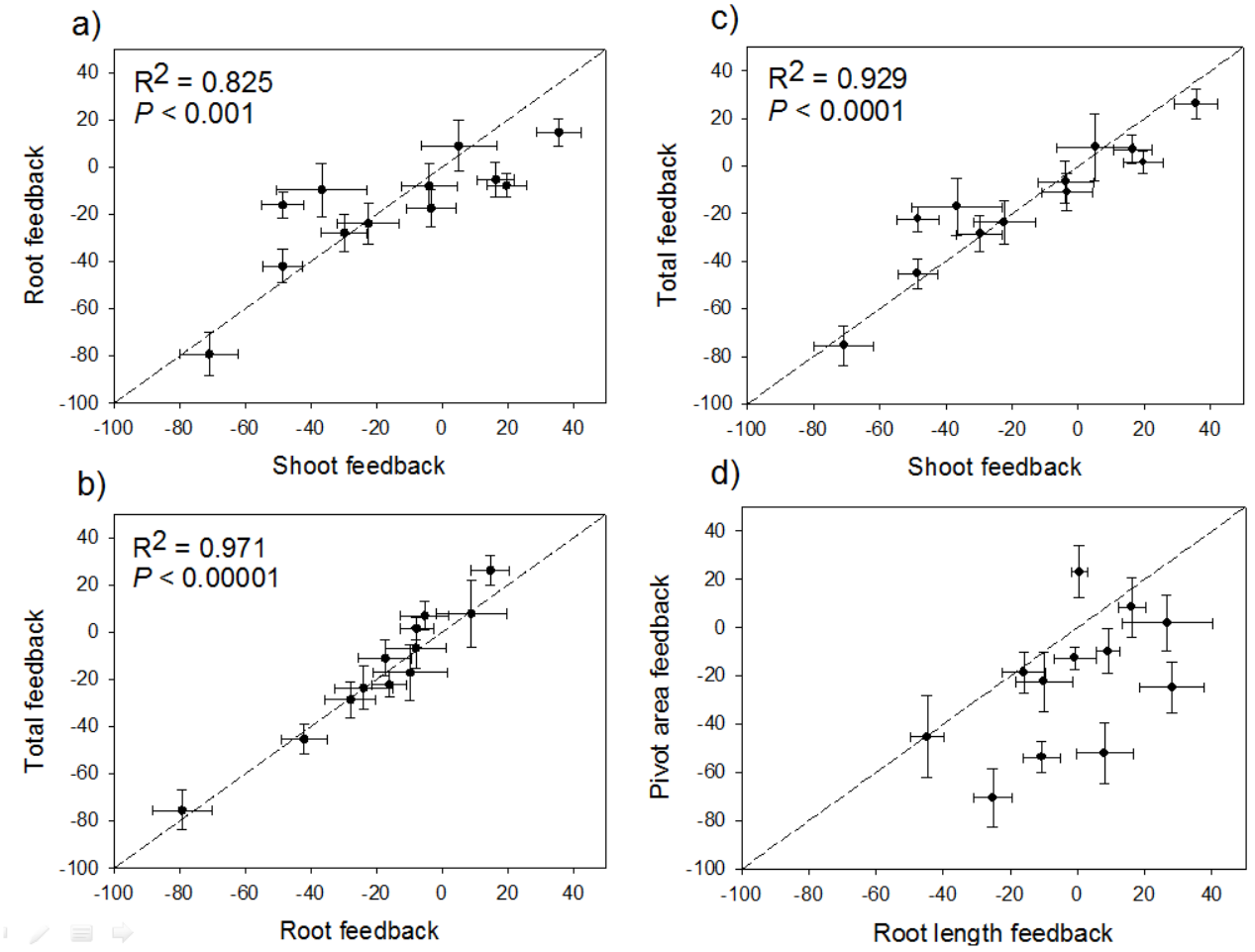

Figure 27. Comparisons of feedback values when using root dry mass vs. shoot dry mass (a), shoot dry mass vs. total dry mass (b), or root dry mass vs. total dry mass. In (d), we plot the feedback values for root length vs. for the area of the pivot (a positive feedback thus indicating that the plant formed a larger pivot in its own soil, for example).

11.5 Discussion

Here, we tried to develop a coherent framework for predicting feedback dynamics between plants and their associated enemies and mutualists, with special emphasis on soil microorganisms. Plant interactions with such biota may be considered as traits integrated into broader life history strategies or tactics (Stearns, 1976). Traditionally, theoretical work on plant life history strategies has mostly focused on nutrient foraging and response to stress and disturbance (Grime, 1974), while neglecting interactions with other biotas. However, empirical evidence is accumulating regarding potential linkages between resource management strategies and interactions with enemies or mutualists. For example, shade tolerant species tend to suffer less from negative plant-soil feedbacks mediated by enemies (McCarthy-Neumann and Kobe, 2008; Kobe and Vriesendorp, 2011), and stress tolerating species tend to accumulate less pathogens in general (Mitchell *et al.*, 2010). Conversely, fast-growing early successional species tend to experience negative plant-soil feedbacks (Kardol *et al.*, 2006), and growth rate has been associated to reduced investment in defense (Lind *et al.*, 2013; Loh *et al.*, 2014). Central to our framework was a trade-offs between tolerance and resistance to enemies (e.g., Agrawal *et al.*, 2004). We predicted that tolerant species would develop more negative feedbacks due to accumulation of enemies. This was expected because tolerant species usually have faster growth rates, which is generally associated to increased carbon transfer belowground (Bardgett *et al.*, 2005; De Deyn *et al.*, 2008) that can be used by opportunistic soil enemies. We also hypothesized that the effect of AM fungi and NM microorganisms on plant performance would be non additive, owing to the repeated evidence in the literature that those microbial guilds interact in the rhizosphere (e.g., Newsham *et al.*, 1995a; Sikes *et al.*, 2009; Chagnon and Bradley, 2013). Our results lent mixed support to both our hypotheses.

11.5.1 Tolerance vs. resistance to enemies

In our first milkweed species pair (*A. syriaca – A. tuberose*), both species experienced mostly negative feedbacks with both microbial guilds. However, the tolerant species did not

accumulate detrimental NM enemies, but rather suffered more from its "own" AM fungi (fig 26). Such negative AM-mediated feedbacks have been found elsewhere, although with a different methodological approach (inoculation with pure AM fungal cultures, Bever, 2002). This may indicate a poor ability for the plant to down-regulate investment into a non beneficial association. This would be consistent with recent empirical evidence showing that shaded plants that did not benefit from AM fungi were unable to stop carbon investment into the symbiosis (Olsson *et al.*, 2010; Grman, 2012). Hart *et al.* (2013) also found that cheaters could accumulate in a diverse AM fungal community. Negative AM mediated feedback could also arise through a trade-offs for AM fungi between competitive ability and benefits provided to hosts (Bennett and Bever, 2009). It is possible that some AM fungi have costly strategies that make them good competitors (e.g., fast growth rates, allelopathic interference with other fungi), and in turn cannot afford to also provide extensive nutritional benefits to the host. Conversely, *A. tuberosa,* which is more resistant to herbivores (Mooney *et al.*, 2010) and was expected to develop neutral to positive feedbacks, actually experienced negative ones with NM microorganisms. This may have been explained by the fact that this species had significantly finer roots than all the rest of our species (data not shown), which increased its exposure to soil pathogens (Newsham *et al.*, 1995a, Sikes *et al.*, 2009). This highlights the need to include root characteristics when trying to predict plant interactions with soil belowground microorganisms.

In our second species pair (*A. subverticillata – A. currassavica*), the tolerant species, *A. subverticillata*, showed the same pattern as the tolerant species in the first pair (*A. syriaca*); it suffered from negative feedbakcs mediated solely by AM fungi. Regarding the resistant species of the pair, *A. currassavica*, as expected it experienced neutral feedback with NM microorganisms, and positive ones with AM fungal mutualists. This does not support a defense-mutualism trade-offs, as observed for other mutualisms (Adler *et al.*, 2012): it does not seem that being well defended compromises the ability of a plant to interact with beneficial symbionts in general. It should also be noted that while NM microorganisms did not trigger a feedback response when considering biomass overall, they did modify the relative biomass allocation of *A. currassavica*. Indeed, in the presence of "home" NM

microorganisms, *A. currassavica* invested proportionally more biomass to shoots and less to roots. This could be a response of pathogens avoidance, and points to a caveat in our framework: while we consider the ability of plants to tolerate or to resist to enemies, we do not integrate avoidance in our range of strategies to cope with enemies. Yet, all three strategies are known to play important roles in plant response to other abiotic stressors such as drought (Gurevitch *et al.*, 2006).

11.5.2 Additivity in the effects of AM fungi and NM microorganisms

In our first species pair, the two microbial guilds had very additive influence on plant performance, while in the second pair there were clear evidences of interactions in their effects (fig 26). Evidence for the additivity of various endophytes' impact on plant performance is mixed in the literature (e.g., Newsham *et al.*, 1995b, Larimer *et al.*, 2012). One clear prediction that we had regarding interactions between AM fungi and NM microorganisms was that the former would counter-act any negative feedback response driven by the latter, because AM fungi are expected to provide bioprotection against NM pathogens (Bever, 2003; Wehner *et al.*, 2011). Here, we found no evidence for such mechanism, potentially because plant protection from pathogens by AM fungi has mostly been observed when the AM fungus was inoculated to the plant well before the pathogen (e.g., Declerc *et al.*, 2002). In our case, both microbial guilds were inoculated simultaneously. It can then be questioned whether pathogen protection by AM fungi is relevant to natural conditions, where pre-inoculation by AM fungi may not be the norm.

11.5.3 Feedback and pivot production

One striking result in our experiment was that feedbacks in terms of pivot production were always of lower magnitude than feedbacks in overall root production. This may be highly relevant for a perennial plant which would have to face an eventual third generation with soil microorganisms. In our system, it suggests that the negative feedbacks we found are in fact underestimation of microbial influence on plant performance: in those cases, not only the plant

produced less root biomass in the second generation with its "own" microorganisms, but it also stored less resource in the pivot for the next generation. This is likely to compromise even more plant performance on generation three. Conversely, regarding our positive feedback estimates, they may be overestimations of the positive microbial influence on plant performance. Thus, our results highlight the value of investigating other indicators of performance than simple shoot or root biomass in feedback studies, and use measures more directly related to a plant's vegetative and sexual reproduction (e.g. genet spread, seeds), and thus fitness.

11.5.4 Routes to refine our framework

Our mixed results call for a refinement of several aspects of our framework. First, the definition of our microbial guilds here was very coarse, especially when considering NM microorganisms as a homogeneous group. Such microbial fraction, although convenient to isolate and inoculate, combines various fungi and bacteria, which may be either pathogens, parasites, commensals or mutualists. In other words, we could not directly test for a pathogen-mediated or enemy-mediated feedback. This may explain the lack of negative NM-mediated feedbacks with our tolerant species: the balanced effect of enemies and mutualists comprised in the NM fraction. In fact, it could even prove to be a large oversimplification to consider all enemies as a homogeneous group: a plant may display tolerance to herbivores and resistance to soil fungal pathogens, for example. In fact, Ali and Agrawal (2014) have recently shown, using the same *A. syriaca – A. tuberosa* species pair that two specialist enemies responded very differently to the presence of the other on the plant. Much mechanistic understanding of plant defense in face of enemies is thus needed to guide future framework developments (Agrawal, 2011). Also, here, our framework considered AM fungi as strict mutualists, while it is well known that their impact on plant performance ranges along a continuum from parasitism to mutualism (Johnson *et al.*, 1997).

Future plant-soil feedback studies should also include additional effort to track microbial community structure (identity and abundances of microorganisms accumulated in the

rhizosphere). For example, typical plant-soil feedback studies are done in controlled conditions, in pots, and with shorter generations (i.e. 2-4 months) than what is usually achieved in a real-field situation. Such conditions may select for generalist, fast-growing ruderal AM fungi (e.g., Sykorova *et al.*, 2007; Hodge and Fitter, 2013), which may be clustered in the AM fungal phylogeny (Chagnon *et al.*, 2013). Indeed, Oehl *et al.* (2009) have shown that successional replacement of AM fungi occurs over 36 months in microcosms, with fast-growing fungi dominating early stages, and some other fungal taxa being restricted to later stages. Sikes *et al.* (2012) found that late-successional AM fungi tended to invest more biomass to extraradical hyphae, which may suggest a higher carbon cost to plants. Accordingly, Allen *et al.* (2003) found that late-successional AM fungal inocula were more costly to the plant and induced growth depressions, while early-successional inocula were beneficial. Thus, by working only with fast-growing ruderal AM fungi in short-term, greenhouse plant-soil feedbacks experiments, we may miss an important component of natural plant-soil feedbacks. Another reason for tracking AM fungal community structure in plant-soil feedbacks is to test whether the plants growing in their own soil preferentially screen for AM fungal partners that are better able to provide protection against "home" pathogens. Such AM fungi may also be clustered in the AM fungal phylogeny (Maherali and Klironomos, 2007; Chagnon *et al.*, 2013). Also, monitoring changes in the NM community composition would allow to estimate the proportion of pathogens that are actually shared by the species involved. Given the repeated evidence that phylogenetically close species tend to share enemies (e.g., Ness *et al.*, 2011; Yguel *et al.*, 2011; Locke *et al.*, 2013; Paine *et al.*, 2012; Callaway *et al.*, 2013), it may be that our milkweed species accumulated similar NM enemy communities in their rhizosphere.

It may also be useful to work with longer plant strategy gradients in future work. Here, we wanted to draw a link between plant tolerance-resistance and its feedback dynamics with soil microorganisms. However, while we identified extreme strategies in our framework as (1) fast-growing ruderal species tolerant to enemies and (2) more conservative and resistant species, here we worked on a short gradient of plant life history strategies. All our species were perennials with no clear distinction in leaf economic traits (non metric multidimensional

scaling shown in fig S1), and they were all similarly and positively responsive to AM fungi in a previous trial (not shown). A longer gradient of species may be needed to find any effect. For example, on theoretical grounds, Bennett *et al.* (2006) found that short-lived annuals should invest resource gains derived from AM fungi to growth rather than to defense. Such annuals may also display a fast growth and long distance dispersal strategy to evade enemies accumulated in the rhizosphere of the maternal plant (van der Putten *et al.*, 2001). Thus, including such kind of species to test our framework would be relevant.

Finally, as stated above, working in artificial, microcosms systems may not capture adequately the complexity of natural plant-soil feedbacks. It thus follows that our results should not be over-extrapolated, but rather validated by field plant-soil feedback assays. However, some clear advantages of microcosm systems should be exploited in future work on plant-soil feedbacks. For example, there is a great opportunity to follow root behavioral responses to soil biota in microcosms. Indeed, while greenhouse plant-soil feedback experiments typically force a plant to grow in "home" or "away" soils, in nature a plant invests its root production in a heterogeneous mixture of "home" and "away" soils. It should be tested whether plant can preferentially allocate root production to "away" soil in order to avoid enemies accumulated in its own rhizosphere ("own" soil). There is much evidence that plant respond to heterogeneity in soil abiotic components (Cahill and McNickle, 2011), so it is not unreasonable that plants also adopt complex root behaviors to respond to soil biota. It may be then possible to link root behavior with soil biota to their structural and functional traits (e.g., Hetrick *et al.*, 1992; Hummel *et al.*, 2007).

11.6 Conclusion

Overall, we presented here an attempt to include plant interactions with soil biota into a broader life-history strategies framework. Our results offered mixed support to our hypotheses, calling for much future work to refine this framework. We still think, however,

that it represents a valuable starting point to make plant-soil feedbacks more predictable. This will enable us to better integrate such feedbacks to applied issues such as restoration of degraded sites, conservation of endangered species and biodiversity management.

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Chapitre 12

ON THE USE OF SODIUM HEXAMETAPHOSPHATE TO EXTRACT SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI FROM SOIL

Chagnon, P.L., and Bradley, R.L.

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12.1 Abstract

Extraction of arbuscular mycorrhizal fungal (AMF) spores from soil is widely used to assess AMF community structure and abundance. The most widely used protocol relies on a water-sucrose gradient flotation technique. Na-hexametaphosphate has also been used to deflocculate soil aggregates prior to spore extraction in order to optimize recovery, but its effect on spore viability remains unknown. Here, we report that Na-hexametaphosphate increases average spore yield in a high clay soil by about 15%, but decreases average spore viability by about 20%. Na-hexametaphosphate should therefore be used cautiously where the extracted spores are destined to be used as inoculum for subsequent studies.

12.2 Results and Discussion

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are a prevalent group of soil microbes, comprising up to 30 % of total microbial biomass in some soils (Leake *et al.*, 2004). These organisms have co-evolved with plants as these moved from water to land about 400 million years ago (Redecker *et al.*, 2000). AMF live as obligate biotrophs, colonizing roots

and scavenging for soil nutrients in exchange for plant photosynthates (Jakobsen, 1986; Pfeffer *et al.,* 2004). Part of the carbon transferred from the host plant to the fungus is stored as asexual spores associated with extramatrical hyphae. These spores serve as propagules to initiate de novo colonization of young roots growing in their vicinity. Because AMF spores are large and easily discernible compared to those of other fungi, they are commonly extracted to quantify AMF abundance and fitness in natural and experimental systems (e.g. Pearson *et al.,* 1994, Bever, 2002). AMF spore extraction and morphotyping have been used to determine which factors mediate species distribution across environmental or successional gradients (e.g. Johnson *et al.,* 1991,1992; Fitzsimons *et al.,* 2008). AMF spores have also been extracted in order to isolate, propagate and study the functional aspects of specific AMF species (e.g. Siqueira *et al.,* 1994; Brundrett and Juniper; 1995).

The most common AMF spore extraction protocol relies on a flotation technique using a water-sucrose gradient whereby AMF spores float over sucrose (60% p/v) but not over water (e.g. Brundrett *et al.,* 1996). Spores can thus be harvested at the interface between water and sucrose after centrifugation. If the researcher's objective is to quantify AMF spores from a given amount of soil, his extraction technique must strive to optimize spore recovery regardless of spore viability. If his objective is, however, to use the extracted spores as inocula for subsequent growth experiments, spore viability then becomes an important criterion guiding his choice of techniques. Hence, spore extraction protocols must be adapted to the specific objectives of the experiment.

Na-hexametaphosphate is a dispersing agent often used in soil analyses to deflocculate soil aggregates. Consequently, treating soils with this agent may help release AMF spores embedded within soil aggregates (Moutoglis *et al.,* 1995) and consequently optimize spore recovery. Studies have shown, however, that Na-hexametaphosphate may be an effective antimicrobial agent, as it damages the cell membranes of bacteria (Fukao *et al.*, 2000). It is uncertain whether Na-hexametaphosphate would have a similar negative impact on AMF spore viability, given that spore walls are complex multilayered structures that contain polysaccharides, protein, lipids, chitin and melanin (Sward, 1981; Purin and Rillig, 2008).

In order to test the effects of adding Na-hexametaphosphate on AMF spore recovery and viability, we used soil from an agricultural field in southern Québec, Canada (45°47' N, 73°21' W). The soil is classified as a clay loam (Soil Classification Working Group, 1998), with a pH of 7.15 (1:2 = soil:water), 0.48% organic matter content, and 66,0% clay. A subsample of the 0-20 cm surface layer was collected, sieved (2 mm mesh), hand-mixed into a homogeneous substrate and separated into 24 x 5 g subsamples. Twelve subsamples were soaked (10 h at 4 °C) in 25 mL of Na-hexametaphosphate (3.9 % p/v) while the other twelve subsamples were soaked in distilled water (i.e., control treatment). Each subsample was wet sieved at 710 µm to remove coarse material, and at 53 µm to retain AMF spores. The content of the finer sieve was rinsed under water to remove most clay and silt particles, transferred in a 50 mL centrifuge tube containing 20 mL of sucrose 60% (p/v) overlain with 20 mL of distilled water, and centrifuged at 1900 g (5 min at 10°C). For half the tubes (n=6), the spore fraction at the interface between water and sucrose was spread on 30 µm nylon mesh and hand counted under a 50x stereomicroscope. For the other tubes (n=6), spores were dark-incubated (48 h at 20°C) in 0.1% iodonitrotetrazolium (INT) salt. Following the incubation, total and viable (i.e., red coloration) spores were counted, as described above (Callaway *et al.,* 2008).Treatment means were compared with student t-tests using the R statistical package (R Development Core Team, 2007).

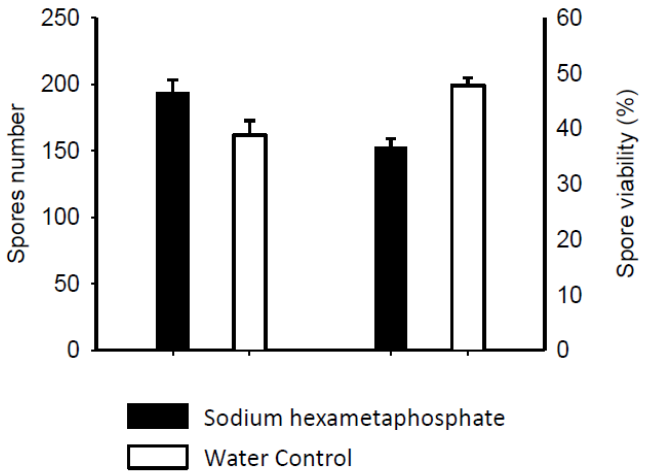

Figure 28. Effect of pre-treating soil samples with Na-hexametaphosphate on spore recovery and viability. Error bars = 1 SEM (n=6).

A higher (t=2.27, P=0.04) number of spores were recovered from soils soaked in Na-hexametaphosphate prior to extraction (fig. 28). Conversely, spore viability was lower (t=5.39, P<0.01) in Na-hexametaphosphate treated soil. In both cases, the effect size was approximately

15-20%. Since Na-hexametaphosphate optimizes spore recovery but compromises spore viability by approximately the same proportion, we conclude that there is no net gain in using Na-hexametaphosphate to produce inocula for subsequent growth experiments. Given the increasing awareness of the wide functional diversity among AMF species (e.g. Klironomos 2000; Cavagnaro *et al.,* 2005; Smith *et al.,* 2000) and of the disproportionate contribution of rare species to community function (e.g. Lyons and Schwartz, 2001), there is a need to develop and test alternative methods (e.g., low energy sonication) that will optimize both spore recovery and viability.

12.3 Acknowledgements

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Chapitre 13

IS ROOT DNA A RELIABLE PROXY TO ASSESS ARBUSCULAR MYCORRHIZAL COMMUNITY STRUCTURE?

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13.1 Abstract

Arbuscular mycorrhizal (AM) fungi are widespread plant symbionts that extensively colonize both soil and roots. Given their influence on ecosystem processes such as plant growth, soil carbon storage and nutrient cycling, there is great interest in understanding the drivers of their community structure. AM fungal communities are increasingly characterized by selectively amplifying their DNA from plant roots, thus assuming that AM fungal community structure within roots provides a reliable portrait of the total (i.e. soil + roots) community. Below, through numerical simulations, we test this assumption using published data. We show that community structure and diversity is well preserved when analyzing only a subset of the community biomass (i.e. roots or soil), provided that the community shows a typical skewed abundances distribution, with few very dominant species and a high prevalence of rare species. Given that this community structure has been shown to be common in natural AM fungal communities, the present work would suggest that characterizing AM fungal communities using only roots or soil can provide a reliable portrait of the overall community. However, we show through additional analyses that the proportion of sample biomass used for

molecular methods must be over a minimal threshold to properly characterize the community. Using published molecular datasets, we validate those results which suggest that typical molecular protocols using low amounts of biomass may strongly influence AM fungal community characterization. Finally, we also discuss other assumptions implied by the molecular analysis of AM fungal communities, and point urgent knowledge gaps.

13.2 Results and Discussion

Arbuscular mycorrhizal (AM) community ecology, that is, the study of the mechanisms governing AM fungal community assembly and dynamics, is a very active area (e.g. Mangan *et al.,* 2004; Maherali and Klironomos 2007; Fitzsimmons *et al.,* 2008; Verbruggen *et al.,* 2010; Dumbrell *et al.,* 2010a). Many researchers have been interested in quantifying AM fungal biodiversity and relate it to land use (Oehl *et al.,* 2003), abiotic stress (Alguacil *et al.,* 2011), host plant identity (Alguacil *et al.,* 2009) or ecosystem function (van der Heijden *et al.,* 1998). For such purposes, selective amplification of AM fungal DNA from environmental or experimental samples has become the dominant approach (Öpik *et al.,* 2014), because it is fast and requires little expertise in taxonomy, as compared to morphological identification of AM fungal spores. To date, most molecular studies characterizing AM fungal communities have used root DNA extracts, as it is thought to better reflect AM fungi actively colonizing roots (e.g. Krüger *et al.,* 2009). However, root AM fungal biomass only represents a fraction of the community, as a considerable amount of energy and biomass is rather allocated to the soil compartment (Helgason and Fitter, 2009). Moreover, there is substantial interspecific variation in the relative investment of biomass inside vs. outside roots among AM fungal taxa (e.g. Hart and Reader, 2002). Accordingly, it has been found that, for a given soil core, characterizing the AM fungal community from soil or from roots yielded very different results (e.g. Hempel *et al.,* 2007). Hence, it remains unclear whether root-derived AM fungal communities provide a reliable portrait of the total (i.e. roots + soil) community. Below, we explore this assumption in more details by simulating artificial AM fungal communities using published data. More specifically, we verify that (1) root AM fungal biodiversity is correlated to total (i.e. roots +

soil) biodiversity, and (2) the pairwise distances among communities are preserved if we use root community data as a proxy for the total community.

We used data from Hart and Reader (2002), which is the most comprehensive effort to date that has quantified the relative biomass investment of AM fungal species inside vs. outside roots. From this data, we defined a pool of 30 species that allocated biomass according to the data from figure 6 of Hart and Reader (2002). We then built artificial AM fungal communities by picking up 20 AM fungal species from the pool, and assigning an abundance to each of them (i.e. a number of biomass units). Abundances were sampled from a lognormal distribution, which commonly fits natural AM fungal communities (Dumbrell *et al.,* 2010b). In all communities, each AM fungal species allocated its biomass units in roots vs. soil according to data from Hart and Reader (2002). We then compared the structure of the total community (i.e. all biomass units) with the structure of the root community (i.e. only root biomass units), using two common biodiversity indices: Shannon's and Simpson's diversities. Shannon's diversity was calculated as $\exp\left(-\sum_{i=1}^{R}\ln(p_i)p_i\right)$, where R is the total number of species in the community, and p_i is the relative abundance of species i in the community. Simpson's diversity was calculated as $\left(\sum_{i=1}^{R}{p_i}^2\right)^{-1}$ (Jost 2007). Root and total community diversities were compared using Pearson's correlation coefficient.

Figure 29 shows that total and root AM fungal Shannon's diversity tends to be highly correlated. This pattern was robust to the structure of the community (i.e. the mean and the standard deviation of the lognormal distribution used to draw species' abundances). The only exception to this is in cases where the standard deviation of the lognormal community tends to become small (i.e. when the community becomes increasingly normal, with a lower proportion of very rare species, data not shown). In such cases, root diversity is very poorly correlated with total diversity (even sometimes negatively correlated with it), and it overestimates total community diversity. However, a recent meta-analysis has shown that natural AM fungal

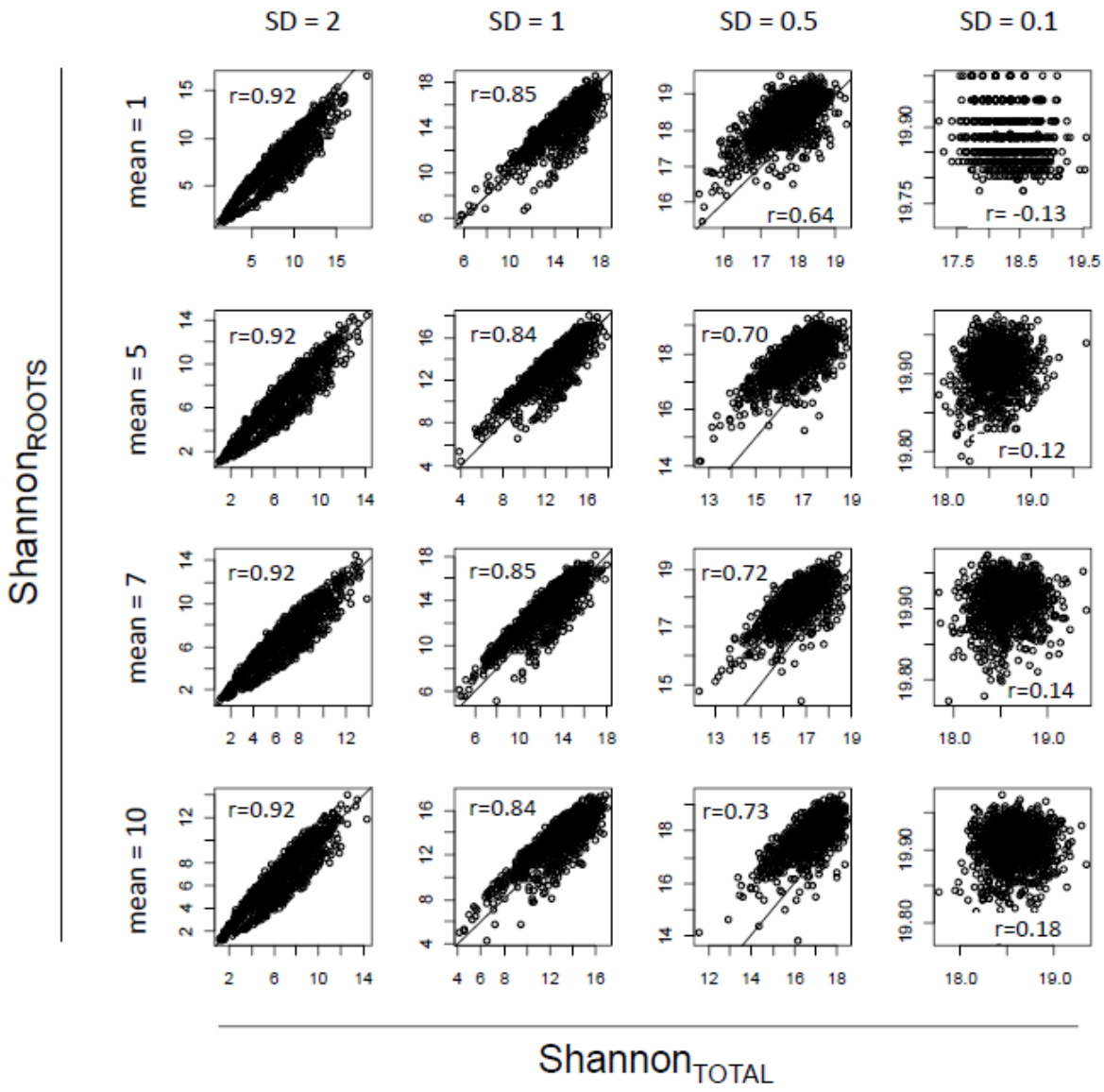

Figure 29. Correlation between root (*x* axis) and total community (*y* axis) diversities for simulated AM fungal communities. Each graph shows results for communities drawn from a lognormal distribution with a given mean and a given standard deviation (SD). On each graph, each point corresponds to a single community (*N* = 1000 per graph). Diversity was calculated here as Shannon's diversity (results for Simpson's diversity are shown in fig. S1). On each graph, the solid line indicates the 1:1 relationship (at SD=0.1, 1:1 relationship is not part of the graph, because root diversity largely underestimates total diversity).

communities tend to be highly dominated by a single taxon, and display a very high number of very rare species (Dumbrell *et al.,* 2010b). Thus, it seems unlikely, based on existing literature that the conditions that make root diversity a poor predictor of total community diversity are frequently, if ever, met in nature.

Another assumption that is made when using root AM fungal community as a proxy for total AM fungal community is that it preserves the ecological distance among communities/samples. In other words, two samples that show dissimilar overall (i.e. roots + soil) AM fungal communities should also display dissimilar root AM fungal communities, and vice versa. To verify this assumption, we built metacommunities each comprising 20 local communities (all simulated as described in the previous paragraph). we then calculated pairwise bray-curtis distances between local communities, using either total biomass or only root biomass data. This generated, for each metacommunity, two distance matrices: one for total biomass data, and one for root biomass data. We compared those distance matrices using a Mantel test, and repeated the overall procedure 999 times to evaluate the frequency distribution of the Mantel's correlation coefficient (r) between the two matrices. We used the R package ecodist to compute bray-curtis distances (R Core team 2013; Goslee and Urban 2007).

Figure 30 shows that in metacommunities, pairwise community distances are very similar, whether we use root data or total biomass data. This trend was robust to variation in initial community structure (fig. 30). In fact, Mantel's r were hardly ever below 0.95, and always

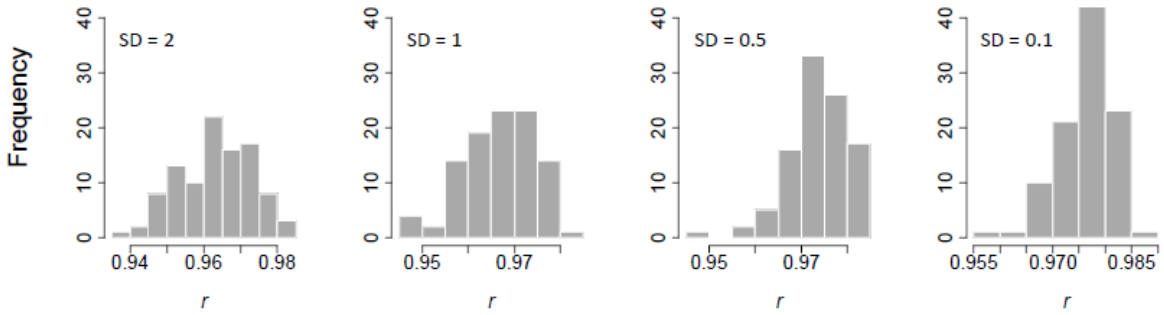

Figure 30. Frequency distributions of Mantel's *r*, when correlating bray-curtis pairwise distances derived from root communities vs. total communities. As for fig. 29, different SD values have been tested.

highly significant ($P<0.001$).

Overall, data from those simulations suggest that the interspecific variation in biomass investment within vs. outside roots in AM fungi does not compromise the use of root DNA to characterize AM fungal communities. The level of diversity within roots was highly correlated with the total community diversity when the community abundances distribution was right skewed (i.e. high prevalence of very rare species), a pattern found to be common in natural AM fungal communities (Dumbrell *et al.,* 2010b). Also, community pairwise distances were well preserved when using root biomass as a proxy for total biomass. This would tend to suggest that root biomass data may provide a reliable proxy for detecting spatio-temporal variation of AM fungal communities (e.g. Dumbrell *et al.,* 2010a). However, those results are conflicting with empirical evidences that soil-borne AM fungal communities are frequently very different from root-borne or spore-based communities (e.g. Clapp *et al.,* 2002 and references therein; Hempel *et al.,* 2007; Saks *et al.,* 2014). In fact, when using different sampling approaches, often some AM fungal taxa are even missing from some compartments (e.g. taxa found only as spores, or only colonizing roots). This suggests that other mechanisms, different from simple interspecific variation in biomass allocation to roots vs. soil, bias our samplings of natural AM fungal communities. One possibility is that our sampling imposes a strong bottleneck effect, as we only process small fractions of the total AM fungal biomass present in a sample. Indeed, typically AM fungal communities are only characterized from a very small proportion of the colonized roots or the soil available in the original environmental sample. For example, widely used commcercial DNA isolation kits allow around 250 mg of root or soil biomass while the whole AM fungal biomass in a small soil core may be orders of magnitude larger. To explore this potential bias numerically, we re-conducted the analyses above while including a bottleneck in the sampling of biomass units. In other words, we constructed the communities as described above, but we subsampled only given fractions of all the biomass units present in the community (i.e. from 0.1 to 10%). Figure 31 shows the percentage of species that are recovered under different percentages of biomass subsampling. We can clearly see that there is a sharp decrease when the sampling effort is below 2% of the biomass available in the sample. This also translates into a decrease in

correlation regarding the species composition (fig. 32) and the biodiversity indices (fig. 33). This may explain at least in part the discrepancies that are found when comparing AM fungal communities derived from different compartments of the same samples. Data presented here suggest that there is a threshold of minimal biomass to use in order to get a reliable picture of the AM fungal community: this needs to be substantiated by direct empirical tests.

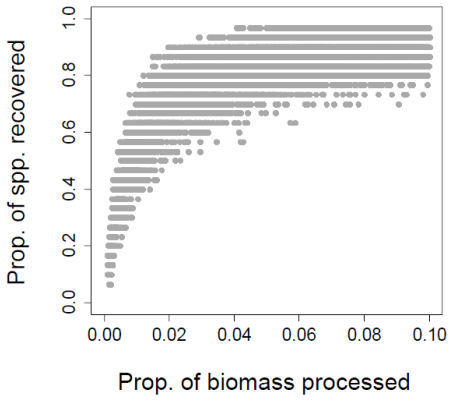

Figure 31. Proportion of species recovery in surveys with increasing biomass sampling effort.

Because all the simulations presented above are derived from a single dataset (Hart and Reader, 2002) that is based on morphological (not DNA-based) characterization of AM fungi, we wanted to validate our results using published molecular datasets, as our inferences are meant to be done at the molecular level. Moreover, Hart and Reader (2002) collected biomass data in controlled greenhouse conditions, which does not take into account behavioral shifts in biomass allocation that AM fungi may display in a variable, natural environment (e.g. Lekberg *et al.,* 2010). However, no published molecular dataset can be argued to properly characterize the total AM fungal community; instead, published data are based on DNA extracted from a small subset of total

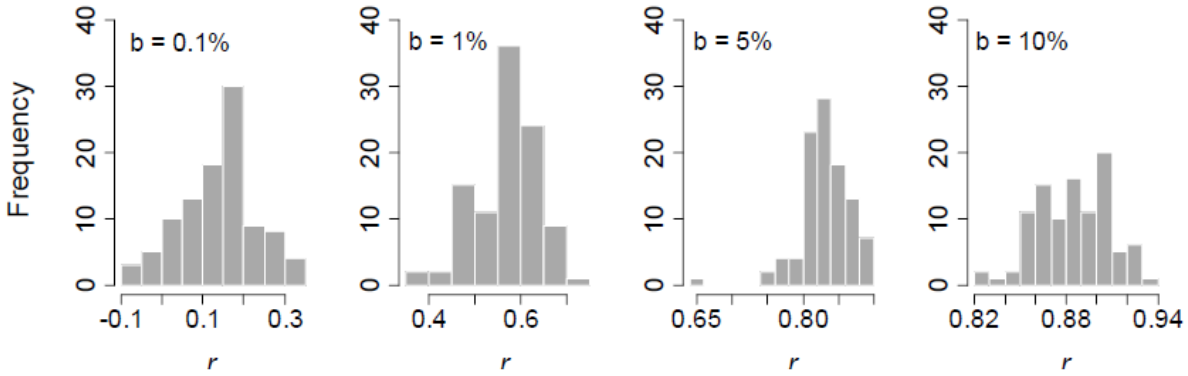

Figure 32. As for fig. 30, a frequency distribution of Mantel's *r*, but with variation in the bottleneck effect of biomass sampling (from 0.1% to 10% of total biomass sampled). Here mean and SD of the lognormal species abundance distribution have been set to 5 and 1, respectively.

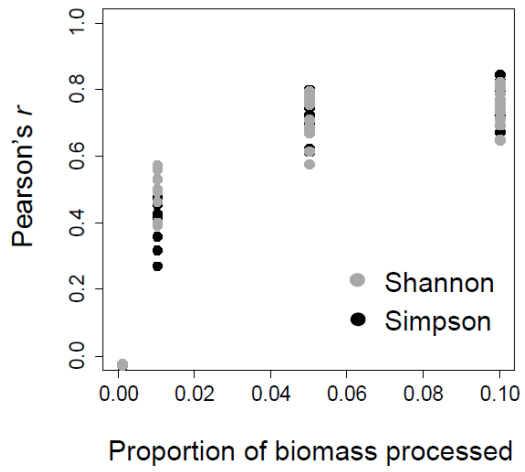

Figure 33. Pearson's correlation coefficient between total community diversity vs. sampled root community, as a function of the proportion of biomass units sampled. Results from both Shannon's and Simpson's diversity indices are reported.

biomass available in the original communities characterized. Thus, for validation purposes, we did not correlate properties of total vs. root AM fungal communities (as done in our previous simulations): we rather compared root vs. soil-borne communities. Figure 34 presents results from analyses similar to those shown in fig. 32, but where the Mantel test was correlating root AM fungal species composition to soil AM fungal species composition. To see how published data map on this figure, we performed a similar Mantel test (again using the bray-curtis index to compute pairwise distances among root and soil samples) on two datasets. The first (Bainard *et al.,* 2014) is derived from 454-sequencing of root and soil DNA in a Canadian prairie agroecosystem, while the second (Bainard *et al.,* 2011) comes from T-RFLP analyses of root and soil DNA sampled in a tree-based intercropping system. In both datasets, Mantel's r was very low (r = 0.15, P = 0.003 ; r = 0.046, P = 0.14, respectively).

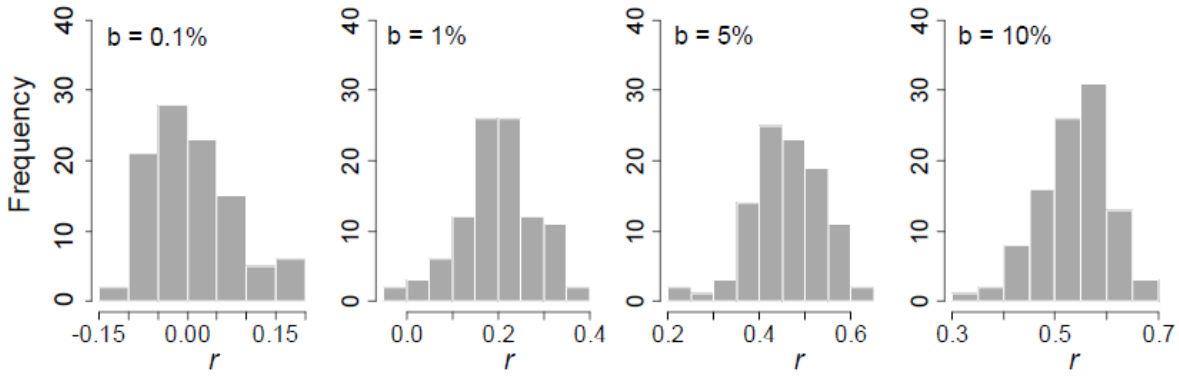

Figure 34. Results similar to those presented in fig. 4. Here, Mantel's *r* is correlating root community structure to soil community structure (*vs.* correlating root to total community structure for fig. 4). As for fig.4, b indicates the proportion of biomass in the sample that is processed for community characterization (e.g. DNA extraction and sequencing).

According to our simulations, this would correspond to a sampling of c.f. 0.1 to 1% of total biomass available (see fig. 34). Interestingly, this seems to make sense with the sampling protocols used in our original studies (Bainard *et al.,* 2011, 2014). Indeed, according to the original sampling design (i.e. volume of soil cores and soil bulk density, approximate amount of roots from which the subsample was drawn to perform DNA extraction, etc.), the biomass sampling effort should be, for these studies, around 0.05% to 0.1%. However, if we consider that only a fraction of the DNA extract is generally used as a template for PCR amplification, then those values should be even lower.

Overall, our results suggest that interspecific variation in biomass allocation within vs. outside roots seems to have most dramatic impacts on AM fungal community characterization, only when a very low amount of sample biomass is used for DNA extraction (which is the case for our current molecular protocols). This bias was verified here to correlate well with patterns found in published molecular data. It should be noted, though, that several additional biases are inherent to characterizing root AM fungal communities by selective DNA amplification: (1) the amount of DNA per nuclei, or the number of copies of the operon sequenced for community characterization (most of the time, ribosomal DNA) could exhibit interspecific variation (Corradi *et al.,* 2007), (2) the number of nuclei per unit of functional biomass may also exhibit interspecific variation, (3) the primer set used to amplify AM fungal DNA may not have a balanced affinity towards all AM fungi present in the community (Krüger *et al.,* 2009), (4) PCR biases may induce changes in relative abundances distribution of different AM fungal sequences (Kanagawa, 2003), and (5) even the choice of the DNA extraction method/commercial kit is likely to influence the outcome of the results (Vishnivetskaya *et al.,* 2014). Next-generation sequencing also introduces new challenges, as there are a wide variety of approaches to deal with sequencing errors (frequent with such technology, see Tedersoo *et al.,* (2010)), and to cluster sequence reads into OTUs. However, for most of those issues, data is either absent, fragmentary or does not even come from the AM fungal system. There is thus an urgent need for further research to evaluate how those numerous potential biases affect the viability of root DNA as a proxy for AM fungal community characterization. Until comprehensive data is generated to assess the importance of such biases, researchers may try

multiple approaches to deal with their datasets a posteriori (and report results as supplementary material) or at least provide raw data for others to do so.

13.3 Acknowledgements

PLC acknowledges the financial support of NSERC through a doctoral Vanier scholarship. PLC designed and performed the study. LB provided molecular datasets. PLC wrote a first draft and both authors contributed to revisions.

13.4 References

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Chapitre 14

CHARACTERIZING TOPOLOGY OF ECOLOGICAL NETWORKS ALONG GRADIENTS: THE LIMITS OF METRICS' STANDARDIZATION

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14.1 Abstract

Species interact in nature to form complex ecological networks. There has been a rising interest in recent years to characterize the topology of such networks along various gradients (e.g. successional, climatic, elevational) to better understand how they assemble in space and time. However, to compare structure of networks that vary in size, shape and connectance, topological metrics need to be standardized (as most metrics covary with such network attributes). Traditionally, this has been done by transforming network metrics into *z*-scores prior comparisons. Here, I show that such standardized metrics are not independent of basic network properties such as connectance. Instead, I found that there was a consistent tendency for *z*-scores to approach 0 when connectance progressively decreased and approached its minimal value. This is probably due to the reduced null space available for null models to randomize interactions at such low connectance. I discuss ways to circumvent the problem in future studies.

14.2 Introduction

In the last decade, there has been a growing interest in characterizing the network structure of bipartite communities (i.e. two guilds of species interacting in a community context). Such

network-based approach to community ecology has been argued to allow uncovering universal constraints to the assembly of ecological communities (e.g. Bascompte *et al.*, 2003; Solé *et al.*, 2003), or at least to identify drivers for local community assembly (e.g. Vazquez *et al.*, 2009a, 2009b ; Stang *et al.*, 2009). More specifically, it has been suggested that characterizing network structure along gradients (e.g. disturbance, successional, seasonal, climatic, elevational,…) was the key to understanding how ecological interactions respond to a changing environment, and thus how climate change is likely to impact on ecological communities (e.g. Memmott *et al.*, 2007; Benadi *et al.*, 2014). However, it is well known that most metrics used to characterized network structure are influenced by basic network/matrix properties such as size, fill/connectance and shape) (e.g. Blüthgen *et al.*, 2008; Almeida-Neto *et al.*, 2008; Ulrich *et al.*, 2009). Thus, when comparing structure of different networks along a gradient, one risks to compare apples with oranges if those matrix properties differ among networks. To circumvent this problem, it has been argued that network metrics should be standardized prior comparisons by transforming them into *z*-scores (Ulrich *et al.*, 2009). This is done by comparing the actual metric value for the observed network to random values (i.e. calculated from randomized networks, generated following a given null model). Such standardized network metrics are expected to be independent from network size, connectance and shape, because null matrices display identical values for those latter attributes. Here, I re-evaluate this assumption by computing *z*-score values for two well known network properties (i.e. nestedness and modularity) along a connectance gradient.

14.3 Nestedness analyses

I start with a 20 spp. x 20 spp. interaction matrix, half-filled (connectance = 0.5) and perfectly nested. (see fig 35). Then, interactions are progressively removed, while maintaining perfect nestedness of interactions for both rows and columns, up to the minimal connectance conformation (fig 35). This generated a gradient of 18 matrices, with connectance values ranging from 0.5 to 0.0975. For each matrix, 150 corresponding null matrices were generated using a null model that preserves rows and columns marginal totals. This number of null matrices to compute *z*-scores was chosen based on preliminary trials, which indicated that a higher number of null matrices did not increase significantly the precision of the *z*-scores (fig

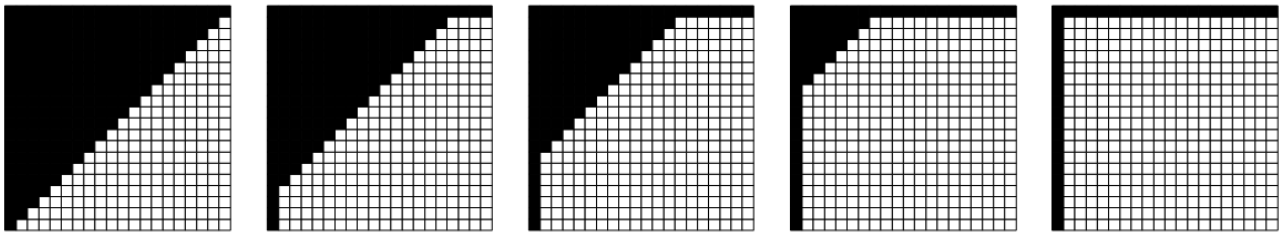

Figure 35. Connectance gradient for perfectly nested matrices. Interactions are progressively removed, while conserving the optimal nestedness.

36). Nestedness was calculated for the initial matrices and for the null matrices, using the NODF index (Almeida-Neto *et al.*, 2008). This allowed to compute *z*-scores, as: $z = (obs - mean_{null}) / SD_{null}$, where *obs* is the NODF value for the initial matrix, $mean_{null}$ and SD_{null} are respectively the mean and the standard deviation of NODF values for null matrices. NODF calculations were computed using the R package *vegan* (Oksanen *et al.*, 2013).

14.4 Modularity analyses

Likewise, a gradient of perfectly modular matrices with decreasing connectance was generated. This was done by starting with a 2-modules matrix (connectance = 0.5), and then progressively increasing the number of modules and, accordingly, decreasing module size (connectance values ranging from 0.5 to 0.05, see fig 37). Modularity was calculated using a simulated annealing procedure that maximizes Barber's modularity index, using the C++ executable MODULAR (Marquitti *et al.*, 2014). The *z*-scores were computed using the same procedure as for nestedness.

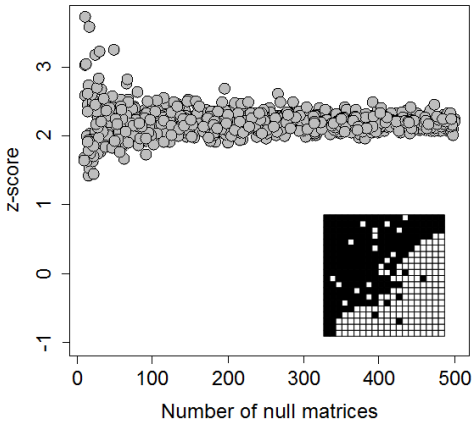

Figure 36. How many null matrices are required to get a reliable z-score? For the matrix shown here, we see that the z-score converges with as little as ~150-200 null matrices.

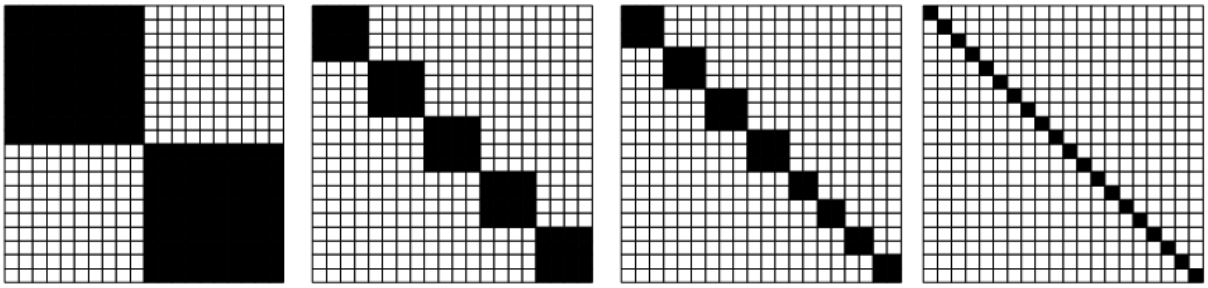

Figure 37. Connectance gradient for perfectly modular matrices. Interactions are progressively removed, while conserving the optimal modularity.

14.5 Results and Discussion

Our analyses clearly show that *z*-scores covaried with connectance (fig 38). Decreasing matrix connectance made *z*-score values converge towards 0, both for nestedness and modularity. Accordingly, matrix connectance and *z*-score values were strongly correlated for both

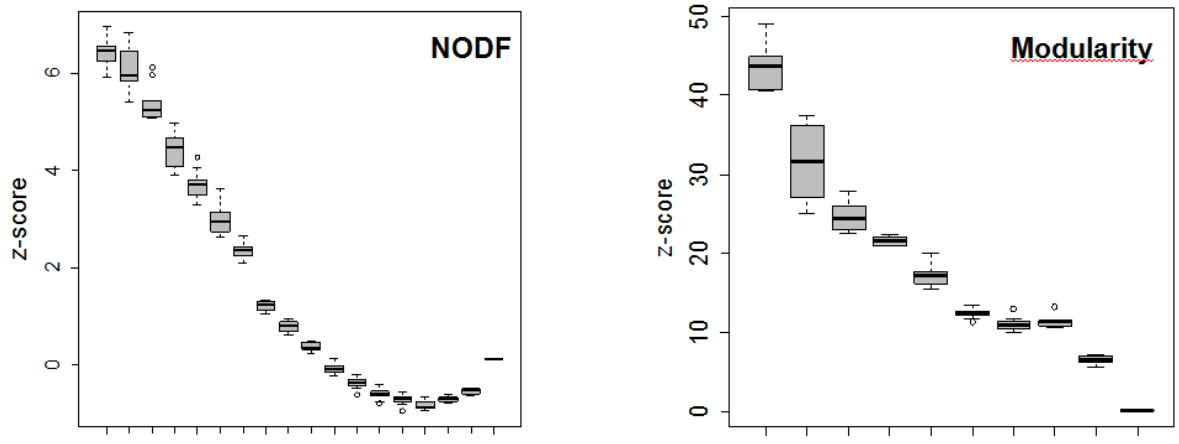

Figure 38. Nestedness (left panel) and modularity (right panel) z-scores covary with matrix connectance. Each boxplot represents replicates for a given initial perfectly nested or modular matrix along the connectance gradient. The boxplots are ordered in both cases from the most connected (left) to the least connected (right).

nestedness (pearson's $r = 0.99$, $P < 0.0001$) and modularity (pearson's $r = 0.95$, $P < 0.0001$). Of course, those analyses were based on matrices that displayed either a perfectly nested or a perfectly modular pattern, which may not reflect the usual "noisier" configuration of natural ecological networks. To ensure that our results could be generalized to real-world networks, similar analyses were conducted on "noisier" networks. Briefly, for nestedness analyses, noisy nested matrices were generated using a similar approach to Krishna *et al.* (2008): row and column species were given abundances drawn from a heterogeneous distribution (i.e. lognormal) and the probability of each species pair to interact was computed as the product of their relative abundances. Thus, if two species were very abundant, they had a probability of interacting close to 1, and vice versa for rare species. Given that the interactions were determined from binomial draws, using those probabilities explained above, the level of noise and the connectance of the matrices could both be manipulated by modifying those

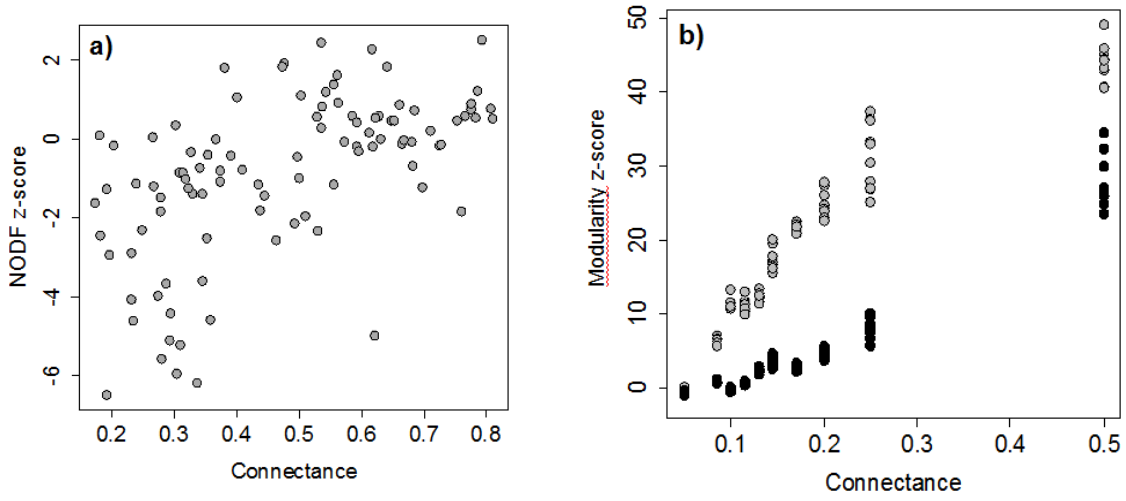

Figure 39. Nestedness and modularity both covary with connectance in noisy matrices. In b), black symbols represent the noisy matrices, and grey symbols represent the original, perfectly modular matrices.

probabilities. For example, to build a low-connectance matrix, the probability for abundant species to interact could be set at only 0.5 instead of 0.95. 100 nested matrices were generated using this general procedure. For modularity analyses, the procedure was even simpler: I directly used the original, perfectly modular interaction matrices (fig 37), and switched a given proportion of 0's to 1's and vice versa (i.e. 10%). Note that this simpler procedure could not

be used to generate noisy nested matrices, because random switching from 0's to 1's and vice versa would have reduced heterogeneity in degree distributions (i.e. made specialists less specialist and generalists less generalist). Heterogeneous degree distribution is a prerequisite to a nested pattern (because it is based on specialists interacting preferentially with generalists), so using such an approach would have lead to loss in nestedness in the noisy matrices, and thus *z*-scores would have converged towards 0, regardless of connectance, thus hampering any test of our hypothesis. Conversely, random switching from 0's to 1's and vice versa is not a problem for modular matrices, because degree distributions are already highly homogeneous. The noisy matrices showed the same trend as the perfectly nested or modular matrices. Both NODF and modularity *z*-scores were positively correlated to connectance (NODF: $r = 0.57$, $P < 0.0001$, Modularity: $r = 0.97$, $P < 0.0001$) (fig 39).

Hence, our results clearly show that z-scores are not independent from network basic properties such as connectance, as traditionally assumed (e.g. Almeida-Neto *et al.*, 2008; Dattilo *et al.*, 2014). Instead, a progressive decrease towards minimal matrix connectance causes a consequent reduction in *z*-score values for topological metrics such as nestedness or modularity. This may be due to the reduced null space available for null models to randomize the matrix at such low connectance. Indeed, as connectance is reduced, there is a progressive increase in matrix information and a corresponding decrease in entropy (Shannon, 1948; Atmar and Patterson, 1993): there are less 1's to place, and important constraints as to how they must be placed in the matrix (i.e. very heterogeneous degree distributions for nested pattern and very even ones for modular pattern). Thus, null matrices tend to resemble more the original matrix when a constrained null model is used (that controls for row and/or column marginal totals). Overall, our results suggest that when comparing network structure along ecological gradients, the use of metrics standardized as *z*-scores should be used with caution: if there is a steep connectance gradient correlated to the ecological gradient of interest (e.g. Ramos-Jiliberto *et al.*, 2010), this may bias the *z*-scores and introduce artefactual (i.e. statistical, not ecological) differences among the networks. In such situations, the use of other metrics that do not covary with connectance may be preferable. As an example, Baselga (2010) proposed a method to partition β-diversity into its nestedness and Simpson turnover

additive components, which allows to disentangle between species turnover and nestedness. This partitioning is unaffected by matrix connectance in perfectly nested matrices (data not shown) and thus, is not dependent upon matrix randomizations and calculation of *z*-scores. However, as for NODF used here, it covaries with connectance in noisy nested matrices. An alternative approach would be to regress network metrics directly with connectance (or other basic network properties that covary with the ecological gradient of interest) and use the regression residuals as topological properties of networks (e.g. Olesen and Jordano, 2002). In conclusion, I believe that characterizing network structure along gradients (or following response of network topology to experimental treatments [e.g. Yodzis, 1988; Woodward and Hildrew, 2002; Zhou *et al.*, 2011; Chagnon *et al.*, 2012]) is an important challenge that community ecologists need to tackle, but caution needs to be taken in order to compare apples with apples. As clearly stated by Ulrich (2009), the biggest upcoming challenge will be to "disentangle statistical from ecological processes" in driving ecological network topology.

14.6 Acknowledgements

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Chapitre 15

BARBER'S MODULARITY OUTPERFORMS BOUNDARY CLUMPING TO DETECT COMPARTMENTS IN BINARY MATRICES

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(In preparation, to be submitted to Oikos)

15.1 Abstract

Modularity is a central concept in biogeography and community ecology. Here, two distinct methods to evaluate the presence of compartments in a binary matrix are compared. I evaluate more specifically their propensity to type I and type II errors. The boundary clumping method seems to perform more poorly, especially when statistical significance is tested using a chi-square test. I thus recommend the use of Barber's modularity optimization with simulated annealing in future studies aiming at detecting modules in binary matrices.

15.2 Introduction

Understanding the factors determining species distribution in metacommunities is a central issue in community ecology. This dates back to the debate between Clements and Gleason: the former suggested that species within local communities are tightly interlinked and coevolved units, while the latter argued that every species is distributed independently in the environment, owing to local abiotic conditions and to chance historical events. Thus, according to Clements, species distributional patterns across sites in a metacommunity should be clustered, in such a way that we can define clear-cut groups or compartments comprising

species that are distributed similarly. On the other hand, a Gleasonian view would not predict the existence of such well-defined groups or compartments. More recently, similar questions have been asked in the study of interaction networks, to determine whether some groups or compartments of species interact more together than with the rest of species in a community. A Clementsian view of ecological networks would predict that some species interact preferentially because they are more tightly coevolved to each other, thus forming interaction compartments in the whole community, while a Gleasonian view would predict a fairly random distribution of interactions, or at least the absence of any well-defined compartments of preferentially interacting species. Thus, testing for the existence of compartments of species is a key component of the study of both species' distributional and interaction patterns in natural communities.

As for many other areas of community ecology, different tools have been developed in parallel to answer the same question. Leibold and Mikkelson (2002) suggested that the Morisita's I index (Morisita, 1971) should be used to quantify the relative dispersion in species' range boundaries: a Clementsian community pattern would predict those boundaries to be aggregated. Such analyses of boundary clumping have also been applied to interaction networks (e.g., Dallas *et al.*, 2014). However, modularity analyses (e.g. Guimera and Amaral, 2005) have become more popular in the study of interaction networks to detect compartments or modules of preferentially interacting species (e.g., Olesen *et al.*, 2007; Donnatti *et al.*, 2011; Chagnon *et al.*, 2012). While the statistical performance of other metrics widely used in metacommunity analysis (e.g., nestedness indices, c-score) has been repeatedly evaluated to select the optimal metrics (e.g., Ulrich *et al.*, 2009; Podani and Schmera, 2012), very few studies have looked at the performance of group-detecting methods such as the Morisita's I index or modularity. Some studies have compared the performance of different modularity indices (e.g., Martin-Gonzales *et al.*, 2012; Thébault, 2013), but to my knowledge no study has yet formally compared Morisita's I index to the modularity optimization approach. Here, I present such analysis, where I evaluate how prone those two approaches are to type I and type II errors.

Two critical statistical properties are expected from an ideal metric when trying to characterize given pattern in a data matrix: (1) ability to detect the pattern when it is present (i.e. avoiding type II errors) and (2) ability to detect the absence of a pattern when it is absent (i.e. avoiding type I errors). To evaluate the former, I constructed artificial data matrices, that can be viewed either as species x sites (metacommunity) or species x species (interaction network) matrices. I started by constructing a perfectly modular or compartmented matrix. I then progressively deconstructed the modular pattern in the matrix by sequentially switching one filled and one empty cell in the matrix (thus preserving the total number of filled cells). This switching procedure was repeated 1000 times, to eventually obtain a matrix with fully randomized filled cells (fig 40). For ten matrices along this gradient, I calculated both the Morisita's I index, using the R package metacom (Dallas, 2013), and Barber's modularity (recently shown by Thébault (2013) to perform better than other modularity indices) using the C++ executable MODULAR (Marquitti *et al.*, 2014). To evaluate whether the pattern was detected as significant using those two indices, I computed 150 null matrices for every of those 10 original matrices, using a conservative null model that preserves the number of filled cells for both

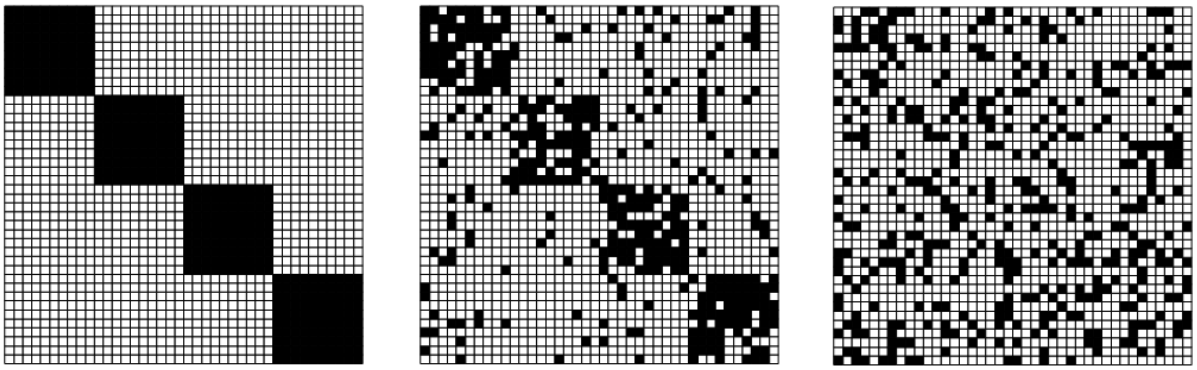

Figure 40. Gradient of matrices from perfect to absent modularity. Those matrices were generated to perform what has been termed a noise test (e.g. Gotelli 2000), in order to see how rapidly a metric loses the ability to detect a pattern in a progressively noisier matrix. If it loses this ability too soon, we are prone to make type II errors, while if it loses it too late or not at all, we are prone to make type I errors.

rows and columns, as frequently suggested in the literature (e.g., Ulrich and Gotelli, 2013). Morisita's I index and modularity were thus calculated as z-scores, by comparing the observed index value to the range of values calculated for the corresponding null matrices (z=observed-mean(null)/sd(null)). Statistical significance was thus assessed using a z-test. For the Morisita's I index, Hoagland and Collins (1997) had proposed an alternative way to test for statistical significance (based on a randomization of the range boundaries and a chi-square test) that is also implemented in the R package metacom. For the sake of comparisons, I also recorded those P-values provided by the package.

Figure 41 shows how z-score and P-values change along the gradient of matrix randomization. Of course, the path of an ideal index would start at very low P-values, and then progressively increase above our set alpha type I error rate (most often 0.05). However, the P-value should not increase too fast and early during the randomization process, so that we can detect existing

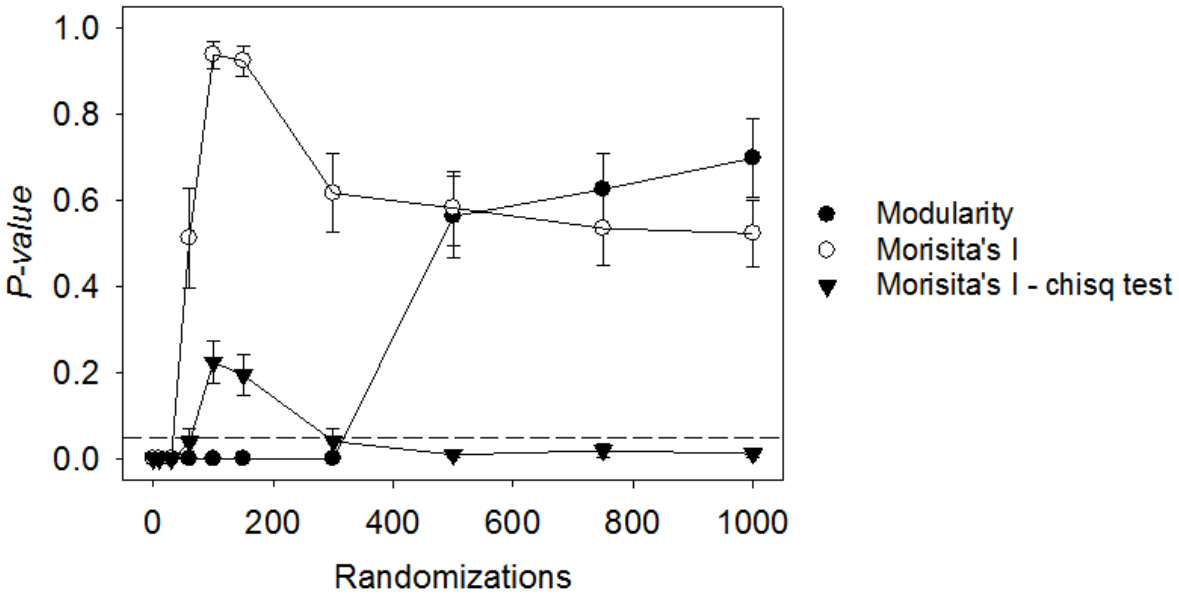

Figure 41. Statistical significance of the modularity/compartmentalization pattern along the gradient of matrix randomization. The circles represent the cases where the statistical significance was tested using null matrices and z-test, while the triangle represent the case where statistical significance of the Morisita's I index was tested using the chi-square based test developed by Hoagland & Collins (1997).

but imperfect patterns. Figure 41 clearly shows that (1) the modularity index follows an expected path, (2) the Morisita's I index is very prone to type II errors, as the pattern soon becomes insignificant after only 60 randomizations (while the matrix still shows a clear compartmentalization: as an example, the middle panel of figure 40 is taken after 150 randomizations) and (3) the chisquare-based significance test is very prone to type I errors, as even the most random matrices remain significantly compartmentalized.

Because matrix fill, or connectance, is well-known to influence its architectural patterns such as nestedness or modularity (e.g., Ulrich *et al.*, 2009), I conducted additional analyses to evaluate how type I error rates would covary with connectance. I thus constructed random matrices with a connectance ranging from 10% to 90% fill. I then evaluated the statistical significance of both Morisita's I index and modularity as described above. It is striking to see how prone high-connectance matrices are to type I errors when using Morisita's I and the chisquare-based significance test (figure 42a). Virtually all random matrices with connectance above 30% were detected as significantly compartmentalized. On the other hand, when using a null model and z-score approach, neither Morisita's I nor modularity were more sensitive to

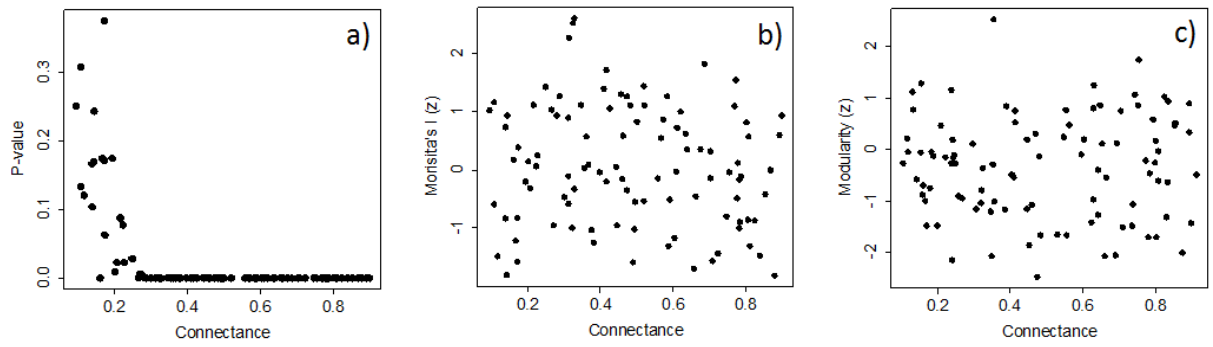

Figure 42. Statistical significance of the modularity/compartmentalization in random matrices (in order to assess type I error rates). In a), we show the P-value of Morisita's I index as assessed by the chi-square test. In b) and c), we assess the statistical significance of the Morisita's I and Modularity indices respectively, using null models. As expected in b) and c), z-scores tend to converge around 0, regardless of the connectance level.

type I errors at high connectance. (Fig 42b-c).

Overall, the above analyses suggest that modularity outperforms Morisita's I index in detecting compartments in binary matrices. The former is better able to detect existing but imperfect patterns. The chi-square based statistical significance test is clearly to be avoided in future research, as it is very prone to type I errors. Ever since Clements and Gleason, researchers have debated around the existence of compartments in metacommunities, and more recently in interaction networks. The work by Whittaker (1956) had appeared to better support the Gleasonian view, especially when observations were conducted along continuous environmental gradients (rather than abrupt ecotones). However, a re-analysis of these data by Leibold and Mikkelson (2002) had found a much higher proportion of significant compartments in metacommunities, thus arguing back in favor of Clements. Yet, their analyses were based on Morisita's I index which was tested for significance using a chi-square based statistical test. The present work, however, clearly shows that such approach is very prone to type I error, which may shift the balance back in favor of Gleason. Hence, this study shows how having the right statistical tools is a crucial technical challenge when drawing inferences in community ecology. From this work it seems that the modularity index and its null-model based significance assessment should be kept as a useful part of the community ecologist's toolbox.

15.3 Acknowledgements

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Chapitre 16

Discussion générale et conclusion

16.1. Le débat autour de la spécialisation dans la symbiose mycorhizienne à arbuscules

Avant le développement des outils moléculaires pour identifier directement les interactions entre plantes et CMA, il était traditionnellement assumé que ces interactions étaient non-spécifiques, et que l'ensemble des plantes et des CMA étaient des généralistes (e.g. Allen *et al.*, 1995; Smith and Read, 2008). En effet, les essais de colonisation en milieux contrôlés, avec des cultures pures de CMA révélaient que l'ensemble des champignons cultivables pouvaient coloniser une vaste gamme de plantes hôtes, et donc que la spécificité d'association due à des incompatibilités phénotypiques était peu probable en nature (e.g. Smith and Read, 2008). Toutefois, certaines études ont montré qu'il existait de grandes variations dans la réponse de différentes plantes à différents CMA (e.g., Klironomos, 2003), et vice versa pour la réponse des CMA à différentes plantes hôtes (e.g., Bever *et al.*, 1996;Eom *et al.*, 2000; Bever, 2002). Ainsi, il semblait exister une spécificité dans la réponse des hôtes et des CMA, reliée à l'identité de leur partenaire. Plus tard, avec le développement des outils moléculaires, on a trouvé que des associations préférentielles semblaient exister en milieu naturel entre certaines plantes et certains CMA (e.g., Husband *et al.*, 2002; Vandenkoornhuyse *et al.*, 2003). Ceci a remis en question l'existence de spécialisation chez certains CMA, qui ne se retrouvaient que sur certaines plantes hôtes (e.g., Fitter, 2005). Il a été suggéré que les outils moléculaires ouvraient la voie à une meilleure définition du concept opérationnel d'espèce chez les CMA et que l'existence de CMA fondamentalement spécialistes était plausible. Toutefois, à cette époque les évidences manquaient toujours pour soutenir une telle affirmation. Pour clarifier cette question, une étude empirique a été conduite par Aldrich-Wolfe (2007) au Panama. L'auteure a caractérisé les CMA dans les racines d'une espèce d'arbre dans deux contextes

contrastés : sur des plantules poussant naturellement en forêt vs. sur des plantules transplantées dans une prairie voisine. Elle a trouvé que les CMA associés à l'arbre en prairie n'étaient ni ceux retrouvés en forêt, ni ceux retrouvés dans les racines des plantes de prairie voisines, mais plutôt une communauté tout à fait distincte de CMA. Ces résultats montrent que les CMA présents dans les racines de cet arbre ne sont pas le fruit d'une spécialisation fondamentale où l'arbre dépend de certaines espèces pour croître et se reproduire. Ils montrent aussi que les CMA présents dans les racines ne sont pas non plus simplement le fruit de l'environnement local (i.e. quels partenaires sont disponibles via les plantes voisines). En somme, ces résultats raffinent notre conception de la spécialisation dans cette symbiose : il ne semble pas y avoir de spécialisation fondamentale où certaines espèces dépendent d'un petit nombre de partenaires, mais il y a tout de même une sélection de partenaires sur le terrain, c'est-à-dire que les interactions ne sont pas aléatoires et qu'il existe des préférences entre plantes et CMA. Toutefois, ces données étaient pour le moins fragmentaires, car l'étude n'a étudié qu'une seule espèce de plante. Ici, nos résultats complémentent ces données en montrant que (1) des préférences existent entre plantes et CMA, (2) ces préférences sont liées aux traits des plantes et à la phylogénie des CMA, et (3) bien que des préférences existent, les interactions plantes-CMA demeurent flexibles localement et sont sans doute modulées par d'autres facteurs comme l'environnement abiotique ou des effets stochastiques et historiques. Nos données montrent aussi que l'ensemble des interactions possibles entre plantes et CMA semble très vaste (les courbes de raréfaction d'interactions sont très loin de saturer avec notre effort d'échantillonnage), corroborant la faible spécificité d'association suggérée par certains auteurs (e.g., Smith and Read, 2008). Un tel cadre théorique pour la spécialisation des plantes et des CMA est cohérent avec ce à quoi on s'attendrait considérant la biologie de ces organismes. En effet, les CMA sont totalement dépendants des plantes pour acquérir le carbone nécessaire à leur métabolisme (Smith and Read, 2008). Puisqu'ils sont sessiles et que des études montrent leur faible potentiel de dispersion (e.g., Dumbrell *et al.*, 2010; Egan and Klironomos, 2014), la spécialisation d'un CMA sur certaines plantes hôtes seulement semble peu probable, car une absence de ces plantes hôtes localement entrainerait inévitablement son extinction locale (e.g. Hoeksema, 1999). De même, puisque les plantes rencontrent en milieu naturel des populations très agrégées de CMA (e.g., Whitcomb and Stutz, 2007), une espèce

de plante spécialisée sur un petit nombre de CMA aurait de fortes probabilités de se disperser sur des parcelles locales d'environnement où les CMA dont elle dépend sont absents.

Avoir une stratégie de généraliste pourrait aussi être avantageux pour la plante du point de vue des bénéfices qu'elle retire de la symbiose. En effet, puisque différents CMA ont différents traits et contribuent à fournir différentes fonctions (e.g., Smith *et al.*, 2000; Burleigh *et al.*, 2002; Sikes *et al.*, 2009), une communauté diverse de CMA dans ses racines pourrait générer des effets de complémentarité quant aux bénéfices que les CMA lui fournissent (e.g., Koide, 2000; Maherali and Klironomos, 2007). Ceci est analogue à la vaste littérature développée autour de la relation biodiversité-fonctionnement des écosystèmes (e.g., Loreau *et al.*, 2001; Hector *et al.*, 2006). De plus, il pourrait même être favorable pour une plante de maintenir des CMA qui fournissent des bénéfices semblables (i.e. qui sont redondants fonctionnellement) en tant qu'assurance si l'environnement devenait mauvais pour un CMA donné par exemple (le niveau de bénéfices retirés par la plantes des CMA serait donc très résilient aux perturbations locales) (e.g., Yachi and Loreau, 1999).

Par ailleurs, ces résultats montrent aussi que les plantes peuvent constituer une force déterminante dans l'assemblage des communautés locales de CMA. Depuis les années 1990, un débat persiste à savoir si les CMA répondent davantage à l'environnement abiotique qu'ils rencontrent dans le sol, ou aux plantes hôtes qui sont disponibles. Certains auteurs ont trouvé que les CMA étaient différents dans la rhizosphère ou dans les racines de différentes plantes (e.g., Johnson *et al.*, 1992; Bever *et al.*, 1996; Öpik *et al.*, 2009), alors que d'autres ont trouvé que les CMA suivaient davantage les gradients abiotiques, particulièrement le pH du sol (e.g., Dumbrell *et al.*, 2010). Certains ont même avancé que puisqu'une portion majeure de la biomasse des CMA (jusqu'à 90% selon Olsson *et al.*, 1999) est placée dans le sol et que les plantes offrent un environnement relativement stable et homéostatique, les CMA sont avant tout sélectionnés pour performer mieux dans le sol, sans égard à la plante hôte qu'ils colonisent (Helgason and Fitter, 2009). Toutefois, avec un éventail aussi large de plantes hôtes pour les CMA, il semblerait douteux de considérer des racines de plantain et d'onoclée comme étant des habitats similaires pour un CMA. En effet, les différentes plantes hôtes disponibles

pour les CMA varient énormément pour une grande variété de traits (e.g., phénologie, présence de canaux longitudinaux d'air dans les racines, finesse et taux de production des racines, etc.) qui forcément font de ces plantes des habitats contrastés pour les CMA (e.g., Brundrett and Kendrick, 1988, 1990; Newsham *et al.*, 1995; Chagnon *et al.*, 2013). Nos résultats vont dans ce sens. J'ai trouvé une relation claire entre les traits des plantes et les CMA qui colonisent leurs racines. Nos résultats ne ferment pas le débat autour de l'importance relative des plantes vs. du sol sur les communautés de CMA. En effet, il existe probablement un continuum d'importance relative entre les deux, qui sera déterminé par l'échelle spatiale, l'étendue des traits des plantes hôtes et l'étendue des conditions du sol. Par exemple, les études qui ont montré un rôle prépondérant du sol dans l'assemblage des communautés de CMA ont été réalisées généralement le long de gradients de pH du sol très prononcés (e.g., Porter *et al.*, 1987; Dumbrell *et al.*, 2010; Torrecillas *et al.*, 2014).

16.2 Structure des réseaux d'interactions plantes-CMA

Un nombre croissant d'études utilisent de nouveaux indices dérivés de la théorie des réseaux pour caractériser la structure des communautés bipartites, où deux types d'organismes (ici plantes et CMA) interagissent (e.g., Bascompte *et al.*, 2003; Jordano *et al.*, 2003; Olesen *et al.*, 2007). Plus récemment, cette nouvelle tendance en écologie des communautés a aussi rejoint le domaine des symbioses mycorhiziennes (e.g., Martos *et al.*, 2012; Chagnon *et al.*, 2012; Bahram *et al.*, 2014). Toutefois, la plupart des études se sont contentés de révéler des patrons significatifs en spéculant vaguement sur leurs causes potentielles. Par exemple, Montesinos-Navarro *et al.* (2012) ont trouvé que leur réseau d'interaction était fortement niché, c'est-à-dire que les espèces de plantes et de CMA spécialistes interagissaient davantage avec des partenaires généralistes. De plus, ils ont trouvé que cette tendance des spécialistes à avoir des interactions nichées dans celles des généralistes était plus forte pour les plantes que pour les CMA. Ils ont interprété ce patron, de façon assez élusive, comme une évidence que les plantes compétitionnent moins pour les CMA que les CMA compétitionnent pour les plantes. Toutefois, plusieurs études ont montré une aptitude des CMA à transférer plus de nutriments aux plantes qui leur donnaient plus de carbone en échange (e.g., Hammer *et al.*, 2010; Kiers *et*

al., 2011). Ainsi, lorsque deux plantes s'associent avec le même CMA, elles entrent véritablement en compétition et on devrait s'attendre à ce que la plante qui fournissent plus de carbone retire plus de nutriments de ce CMA commun. Ceci n'est qu'un exemple du genre de dérapage qui peut se produire lorsqu'on se contente de décrire des patrons d'interactions et de seulement spéculer sur leurs causes potentielles plutôt que de véritablement récolter des données biologiques pour déterminer directement ces causes. Ce dernier problème était en fait l'objet du troisième chapitre de cette thèse.

Un autre aspect évalué dans le présent projet de recherche était la présence de modules d'interaction où certaines espèces interagissent préférentiellement entre elles. Dans l'ensemble de nos travaux, j'ai délibérément évalué ce patron à faible échelle spatiale, pour la raison suivante : des modules d'interactions peuvent être formés parce que certaines espèces sélectionnent des partenaires de façon non aléatoires (tel qu'observé dans nos études) ou parce que ces espèces ont des distributions spatiales avec beaucoup de recoupement. Torrecillas *et al.*, (2014) ont en effet remarqué que certaines interactions préférentielles, dans un réseau mycorhizien à l'échelle régionale, étaient dues au fait que certains CMA et certaines plantes ne se retrouvaient que dans les microsites acides et d'autres dans les microsites alcalins. Ainsi, si dans nos projets j'ai volontairement évalué la modularité à faible échelle spatiale pour limiter la variation environnementale, il importe de souligner que la modularité sera fort probablement un patron fortement dépendant de l'échelle spatiale et du niveau de variation dans l'environnement abiotique (e.g., Lewinsohn *et al.*, 2006). La présence de modularité, sans autre données disponible, ne devrait donc pas être considérée comme suffisante pour détecter de la sélection de partenaires à proprement parler.

Finalement, un autre patron clé qui émerge de nos données est l'absence frappante d'espèces clés (i.e. keystone species) dans nos réseaux mycorhiziens. En effet, l'étude des réseaux d'interaction a souvent montré que la majorité des interactions impliquaient une poignée d'espèces fortement généralistes, et des simulations mathématiques ont montré que retirer des généralistes de la communauté pouvait engendrer une cascade de coextinction (e.g., Memott *et al.*, 2004; Burgos *et al.*, 2007). Toutefois, nos données montrent que le niveau de généralisme

d'une espèce ne semble pas du tout être une propriété intrinsèque des plantes ou des CMA, et qu'au contraire il y a de fortes variations dans ce paramètre d'une communauté locale à une autre. Ceci contredit certains arguments en faveur de l'utilisation de la théorie des réseaux pour guider les pratiques de conservation (de manière à conserver les « keystone » généralistes) : il semble en effet que, à tout le moins pour les réseaux mycorhiziens, ces généralistes soient facilement remplaçables par d'autres espèces d'une communauté à une autre.

En somme, la structure des réseaux mycorhiziens que j'ai trouvée au fil de nos études semble entrer en contradiction avec les études de modélisation sur les réseaux entre mutualistes (e.g., Thébault and Fontaine, 2010; Okuyama *et al.*, 2008). En effet, ces études ont suggéré que les interactions nichées (i.e. nestedness) tendent à favoriser la stabilité des mutualismes (Okuyama *et al.*, 2008) et surtout que la modularité tend à réduire cette stabilité, en favorisant l'extinction locale et les cascades de coextinction. À l'inverse, ici j'ai trouvé que les interactions nichées semblent simplement être le fruit de la présence de CMA généralistes, et non pas une propriété générale de l'ensemble des interactions mycorhiziennes. De plus, j'ai montré l'existence, dans certains cas, de modules d'interactions basés sur la sélection déterministes de partenaires. Ainsi, nos données empiriques ne corroborent pas du tout les prédictions faites autour de la stabilité des mutualismes (e.g., Thébault and Fontaine, 2010). Dans la prochaine section, j'explique comment cette divergence pourrait être due au fait que les modèles théoriques utilisés pour faire des prédictions sur la stabilité des mutualismes ne reflètent pas du tout le fonctionnement d'une communauté mycorhizienne naturelle.

16.3 Pourquoi les modèles théoriques ne reflètent pas le fonctionnement des communautés mycorhiziennes naturelles

Depuis les travaux pionniers de Robert May sur la relation entre diversité et stabilité des réseaux d'interactions (May, 1973), de nombreuses études ont tenté de peaufiner l'approche en manipulant, par exemple, la présence de compétition interspécifique à l'intérieur d'un guilde (e.g., Encinas-Viso *et al.*, 2012), la symétrie dans les interactions (Okuyama and Holland,

2008), la structure des communautés d'interactions (e.g., Thébault and Fontaine, 2010), le nombre d'interactions (connectance) (Dunne *et al.*, 2002), etc. Toutefois, la vaste majorité de ces études utilisent des extensions d'équations de type Lotka-Volterra, avec une croissance populationnelle densité-dépendante de chacune des espèces et des réponses fonctionnelles additives de chaque espèce à l'ensemble de ses partenaires mutualistes. Ainsi, en modélisant la dynamique des communautés de cette façon, ce genre d'études implique les assomptions suivantes (entre autres) :

- Absence de limite à la dispersion (modèles spatialement implicites): à chaque génération dans le modèle, chaque espèce de plante va interagir avec toute espèce de CMA avec laquelle elle est compatible et dont la population est non nulle. Toutefois, en milieu naturel la symbiose mycorhizienne implique des organismes sessiles qui ont des distributions spatiales agrégées (e.g., Boerner *et al.*, 1996; Whitcomb and Stutz, 2007; Dumbrell *et al.*, 2010; Maherali and Klironomos, 2012). De plus, l'assemblage des communautés plantes-CMA a ceci de particulier que de nouvelles « patch » sont constamment rendues disponibles pour les CMA (les racines nouvellement produites). Il devrait donc y avoir un rôle important des phénomènes de dispersion locale dans la colonisation de ces jeunes racines, et il serait même envisageable que l'assemblage des communautés de CMA dans ces racines jeunes soit en partie stochastique (e.g., Hausmann and Hawkes, 2010; Dumbrell *et al.*, 2010b; Chagnon *et al.*, 2012). Ceci ne cadrerait pas avec des interactions purement déterministes telles que simulées dans les modèles;
- Absence de variation temporelle dans les interactions : j'ai montré dans nos travaux que les partenaires mycorhiziens d'une plante donnée sont sujets à changer à la fois dans le temps et l'espace. Cette variation est importante, et n'est pas modélisée dans les simulations traditionnelles, où la matrice d'interaction initiale est la même pour des centaines de générations. De plus, les coefficients de réponses des plantes et des CMA à leurs partenaires symbiotiques (i.e. coefficient qui déterminent dans le modèle à quel degré une espèce bénéficie d'un partenaire donné en terme de croissance populationnelle) sont les mêmes durant la totalité des simulations, alors que nous savons très bien qu'un même CMA peut être parfois bénéfique et parfois parasite dans différents contextes

environnementaux (e.g., microsites avec des conditions abiotiques différentes, années avec différentes conditions en eau ou en lumière, etc.) (e.g., Johnson *et al.,* 1997);

- Le bénéfice retiré par une plante d'une association avec un CMA donné (et vice versa) est représenté comme une fonction saturante, en fonction de la densité du partenaire symbiotique. Ainsi, plus le partenaire est abondant, plus il est bénéfique pour l'hôte (et vice versa). Toutefois, des études ont montré que les bénéfices fournis ne suivent pas une fonction saturante (e.g., Vanette and Hunter, 2013).

Ainsi, il semble clair qu'en milieu naturel, la dynamique des communautés mycorhiziennes sera probablement tout autre que ce que l'on modélise dans les simulations classiques. Par conséquent, ce genre de simulations semblent peu utiles pour prédire les conséquences de patrons d'interaction donnés (e.g., les interactions nichées ou la modularité) sur la dynamique de communautés mycorhiziennes naturelles (Chagnon *et al.*, 2012). Toutefois, de telles interprétations basées sur des études de modélisation deviennent fréquentes dans la littérature sur les réseaux mycorhiziens (e.g., Martos et al, 2012; Haug *et al.*, 2013).

Un problème additionnel avec les études de modélisation est relié à l'interprétation qu'elles font de la modularité dans les réseaux écologiques. La plupart des auteurs discutent de la modularité comme d'une caractéristique d'un réseau qui limite les effets de contagion lorsqu'une perturbation survient. Cette notion, assez intuitive si on prend le cas de la contagion dans un réseau épidémiologique, a été étendue aux fluctuations démographiques dans les réseaux trophiques (e.g., Melian and Bascompte, 2002). Il a été argumenté que la présence de compartiments empêcherait des cascades de coextinction si certaines espèces venaient à s'éteindre localement. Toutefois, encore une fois, ce genre d'étude a négligé une caractéristique clé des réseaux naturels d'interaction : leur capacité à se réorganiser après une perturbation. En effet, de nombreuses études, dont la nôtre, montrent que les patrons d'interactions réalisés sur le terrain ne sont pas inflexibles, ce qui suggère que si une espèce d'un réseau vient à s'éteindre localement, de nouvelles interactions peuvent s'établir et venir tamponner l'effet négatif de cette extinction locale, prévenant ici des cascades de coextinction. À la lumière de nos travaux, la grande flexibilité dans les interactions mycorhiziennes suggère

que la réorganisation du réseau après une extinction locale est en fait potentiellement bien plus importante que la présence de compartiments ou de modules pour tamponner l'effet négatif des extinctions locales.

16.4 La question de l'optimisation dans l'assemblage des systèmes complexes

Les problèmes mentionnés dans la section précédente avec les études de modélisation de réseaux écologiques prend peut-être racine dans un problème philosophique de plus grande ampleur : l'obsession pour l'optimisation et le déterminisme dans l'assemblage des systèmes complexes. On peut remonter aussi loin que jusqu'à la théorie des Idées de Platon, qui voyait le développement des sociétés humaines comme un chemin directionnel vers la dégénérescence (Leroux, 2002). De même, en sciences naturelles, le déterminisme de Newton a eu des retombées incalculables dans toutes les grandes sciences, soit avec Malthus et ses travaux sur la croissance des populations humaines (reprenant le principe de l'inertie), ou encore avec Adam Smith, en économie, qui voyait l'assemblage des marchés comme étant un phénomène émergent (s'opérant par la « main invisible ») d'optimisation de l'alignement des intérêts des différents acteurs du marché. La théorie de l'évolution par sélection naturelle est aussi largement une extension des concepts Newtoniens de force et d'inertie. Plus récemment, vers la fin des années 1990, plusieurs études sur les systèmes complexes ont montré que ces derniers étaient articulés autour de quelques éléments clés, ce qui rendait ces systèmes plus résistants et résilients aux perturbations aléatoires (e.g., le bris d'un serveur dans un réseau internet). Ainsi, ces systèmes semblaient aussi avoir atteint une structure relativement optimale pour favoriser le fonctionnement et/ou la stabilité du système. Il a donc été fort attrayant pour des écologistes de trouver que les réseaux d'interactions semblaient montrer des structures semblables, suggérant ainsi que l'assemblage des communautés naturelles pourrait aussi être issue de l'optimisation. Toutefois, tel qu'argumenté dans le chapitre 7, il y a un problème logique à considérer l'assemblage des communautés comme un phénomène d'optimisation. En effet, aucun agent ne contrôle l'assemblage de ces communautés, comme c'est le cas dans les réseaux computationnels optimisés par l'homme, ou les réseaux neuronaux optimisés par la sélection naturelle agissant sur l'individu.

Néanmoins, au moins deux volets de la théorie autour des réseaux écologiques ont été développés en suivant une logique d'optimisation. Le premier est le concept d'ascendance, développé par Robert E. Ulanowicz (1986, 1997), qui prédit que l'information (*sensu* Shannon, 1948) dans les réseaux d'interactions écologiques devrait toujours augmenter au fil de la succession (i.e. de l'assemblage des communautés). Ce postulat de base avait été énoncé pour expliquer formellement des tendances empiriques observées précédemment dans l'étude des successions écologiques, comme le rétrécissement des niches écologiques et la tendance vers des organismes avec des stratégies conservatrices d'utilisation des ressources (e.g. Odum, 1969). Le deuxième volet théorique suivant une logique d'optimisation est celui voulant que les communautés aient certains états stables alternatifs (e.g., Fontaine *et al.*, 2011; Scheffer *et al.*, 2012), et qu'au fil du temps les fluctuations dans la communautés fassent converger celle-ci vers son état le plus stable. Ces deux volets théoriques, bien qu'offrant des solutions attrayantes pour expliquer l'existence de patrons observés dans les communautés naturelles (e.g., interactions nichées, modularité, distribution d'abondances biaisées vers une poignée de généralistes, etc.), ont ce problème commun qu'ils sont tous les deux dérivés de la thermodynamique. Ceci pose deux obstacles majeurs :

- En thermodynamique, l'état des molécules est déterminé par leur énergie libre. Ce dernier concept demeure élusif en écologie, et ce manque de définition claire contribue à empêcher l'articulation d'hypothèses falsifiables autour de celui-ci. En admettant que les communautés tendent vers la « minimisation de l'énergie libre », comment peut-on mesurer cette entité? Le manque d'hypothèse falsifiable contribue donc à faire de ce volet théorique un élément non scientifique, *sensu* Popper (1934, 1963).
- Le principe de base de la thermodynamique est de considérer seulement les phénomènes à larges échelles car les dynamiques à faible échelles sont imprévisibles ou inobservables. On peut donc considérer les atomes d'un récipient de gaz comme étant des entités équivalentes et soumises aux mêmes conditions, dont les dynamiques individuelles sont imprévisibles, mais dont la dynamique collective peut être prédite par des lois définies (e.g., relation entre température, pression et volume). Toutefois, les individus d'une communauté peuvent-ils réellement être considérés comme des atomes? Ces individus ne

sont certainement pas équivalents, et surtout, par le biais de la sélection naturelle, sont soumis à des pressions évolutives qui leur sont propres. Ainsi, peut-on vraiment s'attendre à pouvoir prédire la dynamique collective de ces individus comme nous prédisons l'état statistique moyen des molécules d'un gaz? Si en thermodynamique classique on se permet de négliger les phénomènes à l'échelle des molécules, en écologie de nombreuses évidences empiriques montrent que la stochasticité à petite échelle peut avoir de grandes répercussions sur les dynamiques à plus grande échelle. Par exemple, les dynamiques évolutives ont été montrées comme étant dépendantes de l'histoire d'assemblage des communautés bactériennes (e.g., Fukami *et al.*, 2007).

Ainsi, les cadres théoriques de l'assemblage des réseaux d'interaction basés sur l'optimisation et les analogies à la thermodynamique semblent être basés sur des assomptions non falsifiables, et pourraient donc constituer des avenues non productives de la recherche scientifique.

16.5 Conclusion et perspectives

À la lumière de ce projet de recherche, je suggère que l'avenue la plus productive pour faire avancer l'étude de l'assemblage des réseaux d'interactions consiste à récolter une grande quantité de données empiriques permettant d'élucider les mécanismes qui causent les patrons d'interactions observés en milieu naturel. Sans prétendre que l'écologie théorique n'a pas son rôle dans l'étude de tels réseaux, je suggère que l'accumulation d'études empiriques permettra de bâtir des modèles de simulations qui se rapprocheront davantage des dynamiques réelles des communautés écologiques. En effet, puisque la modélisation des dynamiques de communautés consiste à spécifier un nombre limité de règles d'assemblage simples sous forme mathématique, il semble naturel de guider de tels modèles par les réelles règles d'assemblage que l'on observe en milieu naturel.

Ce projet de recherche suggère aussi plusieurs pistes de recherche pertinentes pour étudier la symbiose mycorhizienne. Par exemple, il sera urgent de travailler avec des cultures pures de

CMA isolées du terrain pour savoir si (1) les CMA sélectionnés par une plante donnée en milieu naturel sont ceux qui lui fournissent le plus de bénéfices, tel que prédit par les théories évolutives autour des mutualismes (e.g., Bull and Rice, 1991; Sachs *et al.*, 2004; Bever *et al.*, 2009) et (2) le nombre d'interactions pour un CMA est une conséquence directe de son abondance dans le sol. De telles recherches permettront de mieux comprendre le rôle de la symbiose mycorhizienne dans la dynamique des communautés végétales, et la susceptibilité des communautés mycorhiziennes face aux perturbations comme l'extinction locale de partenaires. Puisque cette symbiose fournit des services écosystémiques primordiaux (e.g., van der Heijden, 2010; Bender *et al.*, 2015), de telles connaissances apparaissent comme cruciales pour mieux comprendre la dynamique des écosystèmes terrestres.

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