Global distribution patterns of mycorrhizal associations: abundance, environmental drivers and ecological impacts
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CHAPTER 6

General discussion
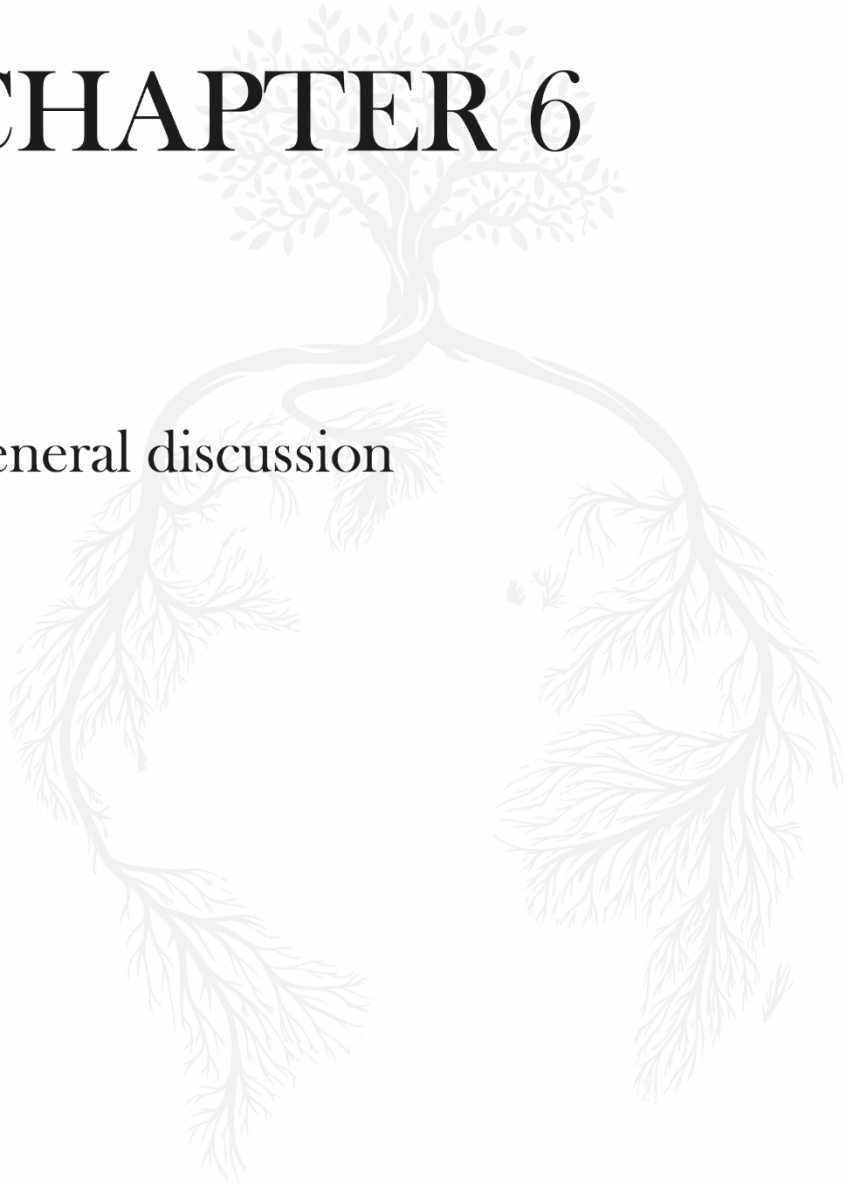
Our understanding of mycorrhizal interactions has increased substantially in the last decades (Smith and Read 2008, van der Heijden et al. 2015). The development of advanced molecular and soil analytical techniques, complex biogeochemical models and the multiplication of biological databases greatly advanced our knowledge of mycorrhizal physiology (Koltai and Kapulnik 2010), biodiversity (Kivlin et al. 2011, Tedersoo et al. 2012), evolution and ecology (van der Heijden et al. 2015). These recent findings increased our awareness of the importance of mycorrhizal symbiosis on the functioning of terrestrial ecosystems and their role as mediators of climate change impacts. However, mycorrhizas are complex interactions that involve radically different organisms (i.e. plants and fungi) and influence and connect several compartments of the earth system (i.e. lithosphere, atmosphere, biosphere) through intricate mechanisms. To add even more complexity, different mycorrhizal types differ in their ecophysiological dynamics with differential implications at ecosystem level (Tedersoo and Bahram 2019). Therefore, a complete understanding of the functioning and ecosystem implications of mycorrhizal associations remains a major challenge.

The progress of mycorrhizal research has been seriously hindered by the lack of quantitative data on the abundance of different types of mycorrhizal associations (Soudzilovskaia et al. 2017). Quantifying their abundance in ecosystems at both plant and fungal sides of the interaction is key to disentangling the role of mycorrhizas on ecosystem functioning and biogeochemical cycles (Soudzilovskaia et al. 2015). Furthermore, better knowledge about mycorrhizal distribution patterns will lead to a better evaluation of changes in mycorrhizal-mediated processes under future climate scenarios (Rillig et al. 2002).

This thesis deepens our understanding of the characteristics of large-scale distribution patterns of Arbuscular mycorrhizas (AM), Ectomycorrhizas (EcM) and Ericoid mycorrhizas (ErM) and their relationship with environmental variables. The chapters included in the thesis address this topic from an aboveground (*Chapters 2 and 3*) and belowground (*Chapters 4 and 5*) perspectives, where both plant and fungal abundances are taken into account. In *Chapter 2* I related climatic and soil factors to the global distribution of plants featuring AM, EcM and ErM associations. Here I

showed that, at the global scale, climatic factors were the primary drivers of mycorrhizal plant distribution. These findings contradict the supposed casual connection between C-related soil properties and the distribution of different mycorrhizal types at the global scale (Read 1991, Read and Perez-Moreno 2003). The results of this chapter also highlight the uncertainties that still exist in tropical areas, which relates to the lack of data on mycorrhizal distribution and information about mycorrhizal impacts on biogeochemical cycles. Concerning this major knowledge gap, I explore the relationship between climatic, edaphic and microbial community parameters and the gradient of AM to EcM-dominated tropical forests in *Chapter 3*. Contrary to previously published data on patterns in the temperate zone, the results showed that in the tropical biome, an increase of EcM trees is not related to higher topsoil C accumulation. This analysis revealed that the mycorrhizal-associated biogeochemical syndromes found in temperate and boreal zones cannot be directly extrapolated to tropical forests. In the following two chapters, I shifted the focus to the belowground part of mycorrhizal associations. In *Chapter 4* I took up the challenge of creating the first high-resolution maps of fine root biomass associated with AM and EcM fungi. These maps were assembled by creating a hierarchical workflow that relates multiple publicly available biological datasets. This chapter serves as an important foundation for future mycorrhizal research. Finally, in *Chapter 5* I explicitly assess the existence of a relationship between the biomass of AM fungi inside the plant host roots and the extraradical mycelium biomass at plant community scale. The results showed that in a natural dune plant community the abundance of AM fungi in the soil increases linearly with higher AM fungal abundance in roots. These results open the possibility of using the abundance of mycorrhizal fungi in roots as a proxy for abundance in soils.

In the following sections of this chapter, I will explore in more detail the main findings of the previous chapters and how they advance our understanding of mycorrhizal ecology and more generally, the functioning of the terrestrial ecosystem. I will also examine future research directions to be taken to gain a comprehensive and complete knowledge of mycorrhizal systems.

6.1. Drivers and impacts of mycorrhizal host plant distribution.

In recent years, our understanding of the biogeography of plants featuring different mycorrhizal types has increased significantly due to the release of high-resolution maps of the global distribution of aboveground biomass fractions of different types of mycorrhizal associations (Soudzilovskaia et al. 2019, Steidinger et al. 2019). However, the driving forces of such distribution patterns at different geographical scales and their relationship with soil biogeochemical cycles remain unsolved.

In *chapter 2*, I related the biomass fraction of AM, EcM and ErM plants from the mycorrhizal vegetation global maps published by Soudzilovskaia et. al. (2019) with a set of 39 different environmental variables, including temperature, precipitation, seasonality and soil physicochemical properties. Surprisingly, the global distributions of the three main predominant mycorrhizal types were mainly explained by temperature-related factors, while soil properties had only a marginal role. In a similar analysis but focusing only on forest ecosystems, Steidinger et. al. (2019) found also a strong climatic signal explaining the global distribution patterns of AM and EcM forests. The lack of edaphic control on mycorrhizal distribution contrasts with the current paradigm of mycorrhizal plants being globally distributed according to the capabilities of the fungal partners to exploit soil organic matter (SOM) (Read 1991, Read and Perez-Moreno 2003). While strong biogeochemical differences have been reported between AM- and EcM-dominated ecosystems at the biome level (Phillips et al. 2013, Cheeke et al. 2017, Craig et al. 2018), these are not reflected at a global scale. The results of *Chapter 2* suggest that the physiological constraints of different mycorrhizal fungi to perform at different temperature ranges might be a significant driver at global scale. In this way, AM associations would avoid low temperatures zones where AM fungal intraradical colonization (Hetrick and Bloom 1984, Gavito and Azcón–Aguilar 2012), extraradical mycelium growth (Rillig et al. 2002, Gavito et al. 2003, Heinemeyer and Fitter 2004) and fungal responsiveness (Veresoglou et al. 2019) are reduced. In contrast, EcM and ErM fungal adaptations to colder temperatures would favour the predominance of these types of mycorrhizal associations at higher latitudes. Alternatively, Steiniger et al. (2019) proposed that climatic factors controlling

mycorrhizal distributions are the same as those that control decomposition rates at global scale. Therefore, they hypothesize that distinct mycorrhizal associations are distributed globally according to differences in soil nutrient release rates. Independently on the mechanisms behind the climatic control of mycorrhizal distribution, it is clear that there are no strong mycorrhizal-associated biogeochemical syndromes at global scale.

Although the temperature-driven distribution model proposed in C*hapter 2* can explain main global trends, biome level particularities are not well captured. This is especially relevant in tropical areas. While AM is the predominant type of association across the tropical biome, EcM trees are also frequent (Brearley 2012, Nouhra et al. 2019, Steidinger et al. 2019), reaching monodominance in certain locations (Corrales et al. 2018). Therefore, even in theoretically unfavourable climatic conditions, specific local properties may enhance the relative abundance of tropical EcM symbiosis. Field measurements of mycorrhizal abundance and soil properties are scarce in tropical areas, limiting our understanding of EcM trees' ecological niche and their impact on ecosystem functioning. In C*hapter 3* I deepen the understanding of this topic by exploring the relationship between plot-level environmental variables and the relative abundance of EcM trees (used as a proxy of the AM-EcM transition). I found that an increase in EcM tree abundances was associated with lower nutrients and C content in the topsoil and a reduction in biomass and simplification of saprotrophic microbial communities. These results reinforce the view of EcM symbiosis as being an adaptation to low nutrient soils (Read and Perez-Moreno 2003). Moreover, the differences in edaphic properties suggest that distinct biogeochemical syndromes between AM and EcM systems are also present in tropical forests. However, the mechanisms that trigger these distinct biogeochemical syndromes between AM and EcM-dominated tropical forests remain unknown. Lower litter quality or litter inputs enhanced SOM decomposition or lower N-fixing symbionts in EcM- relatively to AM-dominated forests are plausible explanations for the patterns found. Further mycorrhizal research in tropical areas is needed to explore the mechanisms underpinning the patterns found in this study and to provide further insight into the mycorrhizal impacts on biogeochemical cycling.

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The most intriguing result from C*hapter 3* is the lack of topsoil C accumulation in EcM tropical plots compared to AM plots, which contrasts with the patterns found in temperate forests (Phillips et al. 2013, Averill and Hawkes 2016). I hypothesized that the discrepancies between mycorrhizal-associated temperate vs tropical topsoil C accumulation patterns might relate to a more important role of microbially stabilized SOM in the topsoil C stocks of tropical forests. In AM tropical systems, higher microbial biomass production favours an increase of C accumulation in the upper soil layers. Microbial growth might be even more stimulated in AM tropical forests compared to AM temperate forests due to the higher proportion of N-fixing trees in the tropics. Additionally, AM associations in tropical areas might be favoured in soils where SOM is stabilized and nutrient losses by leaching are minimized.

Unfortunately, the nature of the dataset used in this chapter did not allow us to explore these potential mechanisms that explain the patterns found here. Future mycorrhizal research should specifically measure SOM transformation processes rates and nutrient and C stocks in different depths to disentangle the mechanisms that lead to changes in biogeochemical properties in the AM to EcM continuum in tropical forests and the differences with temperate patterns.

6.2. Patterns of mycorrhizal distributions belowground

In the last decades, the study of mycorrhizal distribution has been primarily focused on aboveground patterns. Recent research has greatly advanced our knowledge in this field, and now the biogeography of different types of mycorrhizal vegetation (Soudzilovskaia et al. 2019, Steidinger et al. 2019), as well as their environmental drivers (Steidinger *et al.,* 2019; *Chapter 2)*, ecosystem impacts (e.g. Phillips *et al.,* 2013; Averill *et al.,* 2014; *Chapter 3)* and responses to global change (Talbot et al. 2008, Mohan et al. 2014) are better understood. However, when we switch our focus to belowground patterns, the picture becomes fuzzy. The evaluation of mycorrhizal fungi community composition and diversity is relatively common due to the development of molecular techniques (Tedersoo et al. 2010, Kivlin et al. 2011), in contrast, the quantification of mycorrhizal biomass in both soil and root compartments is still rare.

This lack of quantitative data makes the actual belowground abundance of mycorrhizas in ecosystems unknown.

Determining the total abundance of mycorrhizal association inside the plant root systems is methodologically challenging, as it does not only require assessing the level of intimacy between plant and fungi (i.e. root intensity of colonization) but also quantifying the standing root stocks. Only the combination of these two parameters will provide a useful metric to complete our understanding of the effect of mycorrhizas on ecosystem nutrients and C fluxes and pools (Soudzilovskaia et al. 2015). In *Chapter 4* I assembled for the first time a high-resolution global map of the abundance of mycorrhizal fungi in plant roots, expressed as the biomass of fine roots that are colonized by AM and EcM fungi. The maps are structured over 881 different spatial units that result from the combination of global ecoregions, and land cover types across six continents. The root stocks colonized by AM and EcM were calculated for each of these ecoregions, enabling a high level of spatial resolution. To assemble the maps I created a workflow that combines different publicly available datasets proving information on root biomass C (Spawn et al., 2020), the proportion of AM and EcM trees biomass (Soudzilovskaia et al., 2019), plot-level relative abundance of plant species (Sabatini et al., 2021) and intensity of AM and EcM root colonization (Soudzilovskaia et al., 2020).

It was not the goal of this chapter to provide an analysis of the ecological implications of the belowground distribution patterns revealed by these maps. Instead, I focused on creating the most accurate and high-quality product that serves as a basis for further research where estimates of root mycorrhizal stocks are needed. Including quantitative data on mycorrhizal-associated root stocks on global analysis of mycorrhizal impacts on ecosystem functioning, assessments of the mycorrhizal-mediate response to global change or global C cycling models have the potential to greatly improve our current understanding of the mycorrhizal influence on soil processes.

While C*hapter 4* provides the first steps toward a better knowledge of mycorrhizal abundance belowground, it only covers the fungal biomass that develops inside the roots of the host plants. The biomass patterns of the fungal part that extends through

the soil matrix (i.e extraradical mycelium) remains unexplored due to the lack of quantitative data. The mycorrhizal extraradical mycelium has direct implications for soil ecosystem functioning and it is an important precursor of stable SOM (Wallander et al. 2011, Clemmensen et al. 2013). Despite its importance, the quantification of mycorrhizal fungal mycelium biomass in soils is labour- and time-consuming (Leake et al. 2004), constraining its use in mycorrhizal research. A shortcut to overcoming the lack of extraradical mycelium biomass data is to relate these parameters to intraradical abundance estimates.

A positive relationship between the intra- and extraradical AM mycelium biomass has been detected in single fungal isolates (Hart and Reader 2002), but it has never been proven at ecosystem scale. In natural plant communities, where the coexistence of different mycorrhizal species with different root vs soil C allocation strategies is expected, the extrapolation of the results found in the lab becomes less straightforward. In *Chapter 5* I found that, despite differences in community composition between soil and root compartments, the AM root and soil biomass correlation found in the lab also exists in a natural dune plant community. These results suggest that, at plant community level, different AM colonization strategies are structured in a way that the differences in the extraradical and intraradical fungal mycelium biomass get compensated. The coexistence of different AM colonization strategies may ultimately lead to maximising plant hosts' fitness by delivering different benefits to the plant. *Chapter 5* provides the first empirical proof of a correlation between root and soil AM biomass in natural ecosystems. This suggests that the maps developed in *Chapter 4* could be potentially used to infer the biomass of mycorrhizas in soil, which can have large implications for soil functioning studies. However, similar analyses in other ecosystems are needed to generate general equations that can be used in larger-scale applications.

6.3. Implications: are mycorrhizal-associated biogeochemical syndromes context-dependent?

The association between plants and mycorrhizal fungi has profoundly shaped terrestrial ecosystems, being key regulators of soil biogeochemical cycling from local

to global scale. Understating how different mycorrhizal types distribute at different geographical scales and how they affect ecosystem functioning will not only improve our knowledge of mycorrhizal ecology but will also lead to better spatial predictions of ecosystem responses to future climate scenarios. Through the chapters of this thesis, I explored the validity of theoretical frameworks dealing with the biogeography of vegetation featuring different mycorrhizal types and their ecosystem implications as well as shedding some light on the question of how much mycorrhizal fungal biomass exists belowground.

The results presented in *Chapter 2 and Chapter 3* suggest that the distribution of AM and EcM plants relates to a different set of environmental properties at biome and global scale (Figure 6.1). At the global scale, the distribution of different mycorrhizal types responds mainly to temperature gradients (*Chapter 2*). In contrast, within biomes where the temperature becomes more stable, the transition from AM to EcM domination is related to changes in soil biogeochemical proxies (Phillips *et al.*, 2013, *Chapter 3*). But, the magnitude and direction of these relationships are not constant across biomes (Figure 6.1), suggesting that taking the relative abundance of a given type of mycorrhizal vegetation in an ecosystem as a proxy of specific biogeochemical syndromes may be misleading without considering its specific environmental context. In temperate forests, a transition from AM to EcM tropical forests generally results in the accumulation of topsoil C, while in tropical forests the pattern is reversed, being the AM systems that are the major accumulators of topsoil C. Moreover, even within the same biomes, ecosystem impacts related to the abundance of a given type of mycorrhizal plant have been proven to be context-dependent (Kyaschenko et al. 2017, Fernandez et al. 2020). These findings imply that for more spatially explicit predictions on mycorrhizal impacts on nutrient and C cycling new theoretical frameworks need to be developed than include the interaction between specific plant and fungal traits and their soil conditions.
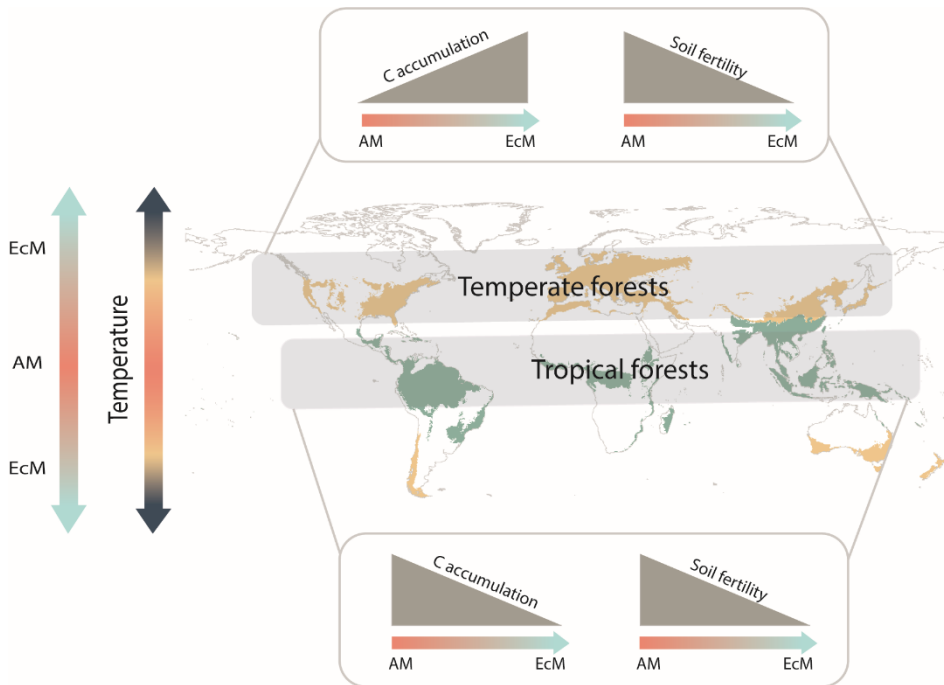
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Figure 6.1: *Schematic representation of the main correlations between environmental factors and EcM and AM dominance at global and biome scales. At global scale, the AM to EcM transition is driven mainly by temperature differences. While AM plants have preferences for warmer climates, EcM vegetation dominates in colder zones. At biome scales, the AM to EcM transition relates mainly to C accumulation and soil fertility. In temperate ecosystems, EcM plants dominate in low fertility soils with high topsoil C stocks. In tropical forests, the abundance of EcM vegetation is also related to low soil fertility, but low topsoil C accumulation.*

It is also important to highlight that assessing mycorrhizal-mediated ecosystem processes based only on the abundance of mycorrhizal vegetation might be misrepresentative as the biomass dynamics of plant and fungal partners are not necessarily correlated. In this way, an ecosystem with a high relative abundance of a given type of mycorrhizal vegetation but low root fungal colonization might not show the same mycorrhizal impacts on ecosystem functioning as a similar ecosystem with higher levels of root colonization. Moreover, different levels of mycorrhizal root colonization can also result in different quantities of extraradical mycelium in the soil, with subsequent differential effects on ecosystem functioning processes. This issue has

been seriously undervalued in mycorrhizal research and the actual abundance of mycorrhizal fungi in roots and soils is rarely taken into consideration when assessing mycorrhizal impacts on ecosystem functioning. Improving our understanding of mycorrhizal abundance in roots is now possible thanks to the great accumulation of biological data that has been recently collected into public datasets (*Chapter 4*), allowing us to overcome methodological limitations of assessing this parameter in the field. Finally, accounting for extraradical mycorrhizal biomass is still a major challenge. Therefore, data on mycorrhizal extraradical mycelium is currently absent at global scale. Although being methodologically possible, obtaining such data will require setting up a global network that applied standardized protocols that allow within-site comparisons and global analysis. Alternatively, a correlation between soil and root biomass can be found at the species level (Hart and Reader 2002) and plant community level (*Chapter 5*), opening the possibility of inferring mycorrhizal extraradical abundances from intraradical estimates (*Chapter 4*) which avoids methodological biases of field measurements.

Taking all the chapters of this thesis together, I conclude that the current paradigms on drivers and impacts of mycorrhizal types distribution should be revised. Despite different mycorrhizal types having distinctive ecophysiological characteristics (Tedersoo and Bahram 2019), their environmental preferences and impacts on ecosystem functioning depend not only on the abundances of AM, EcM or ErM plants but are also influenced by a broader set of factors such as climatic conditions, the abundances of the fungal partners, the microbial community composition or species-specific plant and fungal traits. Acknowledging this is critical for advancing our perspectives on mycorrhizal ecology and the mechanisms behind mycorrhizal involvement in C and nutrient fluxes of terrestrial ecosystems.

6.4. Future perspectives: stepping forward from considering the AM-EcM vegetation as a binary phenomenon

In the last decades, great advances had been made in the field of mycorrhizal ecology. However, we are still scratching the surface in discerning the complex dynamics that involve this symbiotic interaction. Previous knowledge in the field has been built up

based on the premise than AM and EcM associations differ in key physiological and morphological aspects. While this binary classification has been proven useful in certain ecosystems as a predictive tool of biogeochemical processes (Phillips et al. 2013, Averill et al. 2014) or ecosystem responses to global change (Terrer et al. 2016), the observed context-dependency of mycorrhizal effects on C and nutrient dynamic makes this approach insufficient to the future progress of our knowledge.

From my perspective, the future of mycorrhizal ecology research lies in building up new frameworks that expand beyond simplistic classifications of AM vs EcM vegetation. In this sense, understanding the functional diversity of mycorrhizal fungi is critical. Even within AM and EcM fungal types, distinct taxonomic groups differ in the expression of functional traits such as nutrient mobilization capabilities (Bödeker et al. 2014, Yang et al. 2017), extraradical mycelium biomass production (Hart and Reader 2002, Weigt et al. 2012) or protection against plant pathogens (Veresoglou and Rillig 2012, Mohan et al. 2015). The relative abundance in which these functional traits appear in an ecosystem can determine the magnitude and direction of the mycorrhizal-mediated impacts on ecosystem functioning (Clemmensen et al. 2015, Fernandez et al. 2020). Therefore, understanding the abundance patterns of key mycorrhizal functional traits might be of great relevance than focusing only on traditional mycorrhizal types. This will help to solve some of the contradictions found in the mycorrhizal–associated biogeochemical syndromes framework and to improve predictions of mycorrhizal impacts on ecosystem functioning. Going in this direction will require improving our knowledge in three strategic areas of research:

1. Information about the functional diversity of mycorrhizal fungi across different taxonomic groups should be accumulated in open-access databases. Despite the development of molecular techniques, knowledge about the functional properties of mycorrhizal fungi is scarce and comprehensive functional characterization of a wide range of mycorrhizal genotypes is needed. Important progress has been made through the creation of fungal trait databases (Nguyen et al. 2016, Põlme et al. 2020), but no specific database exists that focuses specifically on mycorrhizal fungi including key functional

traits such as hyphal growth and turnover rates, biomass allocation strategy or nutrient acquisition strategy.

2. A better understanding of how distinct mycorrhizal functional traits affect ecosystem functioning is also a promising area of research. Laboratory and field experiments aiming to link mycorrhizal fungal traits with ecosystem processes will provide an important step forward in mycorrhizal ecology.

3. Quantification of community mean functional traits at different spatial scales should be encouraged. This will allow performing spatial explicit analysis on the distribution of these functional traits and their relevance for ecosystem functioning.

Hopefully, in the next years, new methodological techniques will be developed to overcome the issues identified here, allowing us to disentangle the mechanism through which mycorrhizas shape terrestrial ecosystems.

6.5. Concluding remarks

Mycorrhizas are complex interactions that profoundly affect the functioning of terrestrial ecosystems. Understanding how different mycorrhizal types distribute across different spatial scales and relate to ecosystem properties has been a major research challenge. This thesis provides valuable knowledge on the abundance, drivers and ecological implications of mycorrhizal types distribution from both fungal and plant perspectives. Despite these great advances, the future of the field depends on recognizing the complexity of mycorrhizal associations and steeping forwards from old conceptual frameworks and answering ecological questions from a broader perspective. I am sure that the coming years will bring fascinating discoveries that advance our understanding of the mechanisms that drive the biogeochemical fluxes of terrestrial ecosystems.