# Afforesting with microbes: disentangling the effects of soil biotic and abiotic characteristics on trees using soil inoculation

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# CHAPTER 7

# General Discussion

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Afforestation—the process of establishing forests on previously non-forested land (FAO, 2018)—fundamentally reshapes soil ecosystems by altering both abiotic (Ritter et al., 2003) and biotic properties (Yu et al., 2024). These changes, in turn, influence root-associated microbial communities, tree performance, and key ecosystem functions such as foliar herbivory (Georgopoulos et al., 2024) and greenhouse gas emissions (Gundersen et al., 2012; Georgopoulos et al., 2025). As afforestation gains prominence in restoration ecology, and techniques like soil inoculation are increasingly used to mitigate human impacts on natural systems, understanding how these evolving soil properties affect trees, their microbial symbionts, and their cascading effects on aboveground herbivores and ecosystem processes becomes ever more crucial. Although past studies have investigated how soils change over the course of afforestation, the effects of the abiotic and biotic characteristics of the soils have largely been observed together in field conditions (Wu et al., 2022; Guo et al., 2024). Therefore, my objective in this thesis was to disentangle the effects of changing abiotic and biotic soil characteristics and to further our understanding of how they contribute to tree performance, root associated microbes and symbionts and ecosystem functions, through the use of soil inoculation under controlled conditions in climate rooms.

In this thesis, I began by examining how abiotic and biotic soil characteristics change with stand age across an afforestation chronosequence (Chapter 2). I also investigated how these shifts in soil conditions influence soil fauna, bacterial and fungal functional groups (Chapter 2). Building on this fieldwork, I transitioned to controlled climate rooms and laboratory settings to focus on the early stages of afforestation. There, I explored how biotic and abiotic properties of forest soils affect *A. glutinosa* performance and foliar herbivory by a generalist insect herbivore (Chapter 3). Continuing this line of research, I focused more specifically on the biotic component of the soil. I conducted an experiment to test how the removal of soil biota—based on organism size—from young and mature forests impacts *A. glutinosa* performance, root-associated microbial communities, and GHG fluxes from mesocosms (Chapter 4). Motivated by nodulation-related findings in Chapters 3 and 4, I then designed a series of experiments to investigate how microbial communities from the same young and mature forests and also nitrogen and phosphorus availability, affect *F. alni* nodulation and *A. glutinosa* performance. This helped to clarify some of the soil characteristics that are influencing nodulation dynamics (Chapter 5). Finally, I explored the potential of using NIR spectroscopy as a novel method to distinguish between symbiotically fixed and soil-derived nitrogen in *A. glutinosa* (Chapter 6).

The upcoming sections outline key findings of my research and their significance. I then explore potential future applications of soil inoculation and share my perspective on incorporating *A. glutinosa* in afforestation efforts, along with the broader societal importance of this research.

## 7.1 Effects of abiotic and biotic soil characteristics on *A. glutinosa*

The central aim of this thesis was to disentangle the abiotic and biotic effects of forest soils of varying ages on tree performance, using *A. glutinosa* as a model species. Previous studies have similarly sought to separate these effects by sterilizing bulk soil to eliminate microbial life and through the use of various inoculation strategies—such as whole-soil inocula, microbial size fractions, or cultured taxa of interest (Wagg et al., 2014; Wang et al., 2019; Li et al., 2020; Oudor et al., 2022; Wang et al., 2022). My work contributes to this growing body of research by not only assessing abiotic influences through sterilization (Chapter 3), but also by evaluating biotic effects via three distinct inoculation approaches (Chapters 3, 4, and 5).

In Chapter 3, we observed that biomass production and stem width increased with forest age when soils were sterilized (Chapter 3, Fig. 1A, B), and that overall, trees grown in sterilized soils outperformed those in live soil. This pattern likely reflects the removal of soil pathogens and reduced microbial competition (Kuzyakov and Xu, 2013), as well as enhanced nutrient availability following sterilization. Notably, the increase in available N with forest age aligns with the chronosequence results in Chapter 2, which showed increasing $NO_3^-$ concentrations across older stands (Chapter 2, Fig. 2). These findings underscore the significant role of abiotic factors—particularly nutrient dynamics—as direct drivers of tree performance, with forest age acting as a key contextual variable.

While sterilization provides insight into abiotic effects, it complicates efforts to fully isolate the influence of microbial communities, as sterilization itself alters the soil environment (Manea et al., 2024). However, by integrating results from our inoculation experiments, we gain a clearer picture of how biotic and abiotic factors interact to influence the performance of *A. glutinosa*. In Chapter 2, we showed that as forests mature, changes in soil abiotic properties—particularly nutrient levels, pH, and SOM—drive shifts in microbial community composition (Chapter 2, Fig. 3). In the later chapters we show that these microbial communities, in turn, exert significant effects on *A. glutinosa*. Across chapters, we identified microbial taxa such as *Streptomyces* and *Rokubacteriales* (Chapter 3 and 5) that were positively associated with aboveground biomass, suggesting that *A. glutinosa* selectively benefits from specific root-associated microbes. These patterns are further supported in Chapter 4, where inoculation with soils from different forest developmental stages resulted in distinct root-associated community compositions. Surprisingly, inoculation with mature forest soil, particularly with the largest size fraction (assumed to have the most complex microbial consortia), consistently reduced tree growth. This suggests a possible shift from mutualistic to antagonistic or competitive interactions in more developed microbial communities, again potentially due to increased microbial competition for nutrients (Kuzyakov and Xu, 2013). This finding was unexpected, given that Chapter 2 highlighted the dominance of ectomycorrhizal fungi in mature forests, which are generally considered beneficial for both *A. glutinosa* and its primary symbiont, *F. alni* (Orfanoudakis et al., 2010). Chapter 5 provides further resolution, showing that fungal communities from mature soils suppressed the growth-promoting effects of *F. alni*, even though nodule biomass remained unchanged. In contrast to past studies (Wagg, et al., 2014), these results indicate that microbial diversity does not always translate to functional benefit or better tree performance as some taxa may directly disrupt symbiotic efficiency. This will be discussed further in section 7.4.

## 7.2 Integrating across scales and stages

Taken together, these findings depict a nuanced interaction between abiotic and biotic soil properties in determining tree performance. While microbial communities can facilitate growth and enhance symbiosis, their effects are highly context-dependent, influenced by nutrient availability, forest age, and soil physiochemical conditions. At the same time, abiotic factors not only directly impact tree performance but also shape the structure and function of microbial communities.

The chronosequence analysis in Chapter 2 reinforces the importance of abiotic drivers—particularly pH, soil nutrients and SOM—in microbial succession and functional turnover. These abiotic characteristics influence the composition of soil biota that interact with *A. glutinosa*, offering critical ecological context for interpreting the outcomes of controlled inoculation experiments.

## 7.3 Effects of abiotic and biotic soil characteristics on *F. alni*

In this thesis I demonstrate that both abiotic soil conditions and microbial community composition influence the establishment and effectiveness of the symbiosis between *F. alni* and *A. glutinosa*. Across Chapters 3, 4, and 5, it becomes evident that nutrient availability and the presence of specific microbial groups can either support or suppress nodulation and symbiotic N fixation.

In Chapter 5, we showed that *F. alni* nodulation is particularly sensitive to N and P availability. Elevated soil N significantly reduced nodule biomass (Chapter 5, Fig. 4A), supporting earlier findings that trees reduce investment in this energy-intensive symbiosis when mineral N is readily available (Bélanger et al., 2011; Ballhorn et al., 2017). Conversely, P availability was positively correlated with nodule biomass (Chapter 5, Fig. 4A), in line with studies indicating that P not only promotes nodulation (Wall et al., 2000; Gentili and Huss-Danell, 2002) but is also essential for its initiation (Huss-Danell, 1997). These findings provide insight into patterns observed in Chapter 3, where we found that nodulation declined with increasing forest stand age and was nearly absent in 25-year-old live forest soils (Chapter 3, Fig. 1H). This coincided with a trend of rising $NO_3^-$ concentrations across older soils (Chapter 3, Fig. S5), which is also evident in the field when examining the forest chronosequence data from Chapter 2 (Chapter 2, Fig. 2). These results suggest that as N availability increases in aging forests, *A. glutinosa* reduces its reliance on symbiotic N fixation. Further support comes from the sterile soil pilot experiment in Chapter 5, where varying the concentration of *F. alni* inoculum alone did not affect nodulation, indicating that nodulation success is shaped by the surrounding abiotic and biotic soil context rather than the concentration of *F. alni* in the soil (Chapter 5, Methods S2).

Yet, biotic factors cannot be overlooked. While earlier studies have focused primarily on the interactions between *Frankia* and AM or EcM fungi (Pozzi et al., 2017; Zhou et al., 2017; Chen et al., 2020)—which are generally shown to facilitate nodulation (Orfanoudakis et al., 2004)—this thesis expands the lens to include entire soil microbial communities. The results reveal that while nutrient availability directly drives nodulation outcomes, biotic interactions can indirectly suppress the beneficial effects of *F. alni*. Fungal communities from mature forests, in particular, appear to play a pivotal role in this suppression.

## 7.4 Interactions between bacterial symbionts and soil communities

A key theme across this thesis is that the relationship between *F. alni* and soil microbial communities plays a central role in shaping the *F. alni–A. glutinosa* symbiosis. Findings from Chapters 3, 4, and 5 reveal that these interactions can be facilitative, neutral, or antagonistic, depending on the composition of the microbial community and the developmental stage of the forest.

In Chapter 3, as discussed, nodulation declined with forest age in live soils and was almost absent in soils from 25-year-old stands. While this pattern aligns with increasing $NO_3^-$ levels, a similar, though non-significant, trend was observed in the second experiment involving inoculation of sterile soils (Chapter 3, Fig. 2H). This indicates that biotic factors also contribute to reduced nodulation, even when abiotic conditions are controlled. This was further supported in Chapter 4, where a decline in nodule density with forest age was again observed (Chapter 4, Fig. 4C). Existing literature suggests that as forests mature, soil microbial communities shift from bacterial to fungal dominance (Kang et al., 2018), increasing the influence of fungi in older soils. In my research I show that this influence is not always facilitative but sometimes also suppressing, The most compelling evidence of fungal-mediated suppression was found in Chapter 5, where co-inoculation with fungal communities from mature forests consistently reduced *F. alni*-mediated growth promotion, despite no significant change in nodule biomass (Chapter 5, Fig. 2). Notably, this suppression effect was absent when fungal communities from younger forests were used, suggesting that it emerges with changes in fungal community structure and dominance over time. Although results from Chapter 2 showed an increase in EcM fungi with forest age (Chapter 2, Fig. 4)—which are generally associated with enhanced nodulation (Chen et al., 2020)—results from Chapter 5's microbial culture experiments revealed that some fungal and bacterial isolates directly inhibited *F. alni*-driven growth promotion (Chapter 5, Fig. 3). This provides direct evidence of microbial antagonism within the soil community.

Together, these findings suggest that interactions between fungal and bacterial members of the soil microbiome can significantly influence the functionality of N-fixing symbioses, with broader implications for tree nutrient status, growth, and resilience under nutrient-limited conditions. Whether these interactions are beneficial or detrimental depends on the microbial community composition, nutrient availability, and forest successional stage.

These insights have important implications for afforestation and restoration efforts. While inoculation with *Frankia* may suffice in early successional or low-competition environments, the presence of complex fungal communities in mature forest soils may require more nuanced management. Where maximizing biological N fixation is a key goal, it may be necessary to account for and potentially mitigate microbial antagonism within the soil.

### 7.5 General implications for nodulation

Looking at the broader implications, a picture emerges where nodulation is a finely tuned outcome of plant-microbe interactions modulated by the soil environment. Favorable abiotic conditions set the stage for nodule formation, while biotic factors (the presence of effective symbionts, absence of strong competitors or pathogens) largely determine the success and efficiency of the symbiosis. Recent research into nodulating symbionts has reinforced these concepts with specific case studies: for instance, identifying moisture and native microbiota as key limits in agricultural fields (Kasper et al., 2019), uncovering how mycorrhizal networks assist rhizobia in legumes (He et al., 2024) and showing that both legumes and actinorhizal plants benefit from fungal co-symbiosis (Tedersoo et al., 2009). Across legumes and actinorhizal plants, the mechanisms show convergence – both types of plants evolved strategies to optimize nodulation under favorable conditions and curtail it under stress or surplus of N. Therefore, managing soil nutrients and biological inputs can significantly influence nodulation outcomes in both agricultural and natural ecosystems, regardless of the specific plant–microbe partnership involved.

### 7.6 The “picky” nature of *A. glutinosa*

In Chapter 2 we show that changes in the abiotic characteristics of the soils with increasing forest age also affect the composition of the soil biotic community (Chapter 2, Fig. 3). A key finding from this study is that across multiple experiments in this thesis, *A. glutinosa* consistently demonstrated a remarkable ability to select and assemble its root-associated microbial community, despite being exposed to highly variable soil biotic environments and inocula. Whether grown in whole forest soils (Chapter 3), community fractions from different forest developmental stages (Chapter 4), or cultured microbial inocula (Chapter 5), *A. glutinosa* maintained a high degree of host-driven filtering, often overriding differences in inoculum composition. This “picky” nature of *A. glutinosa* suggests a tightly regulated selection process, likely shaped by root exudation chemistry, immune signaling and symbiotic compatibility mechanisms (Ruiz Palomino et al., 2005). In Chapter 3, despite clear differences in bulk soil communities across 10-, 15-, and 25-year-old forests (Chapter 3, Fig. S9), the root-associated microbial communities remained largely unchanged (Chapter 3, Fig. 3,4), indicating strong host filtering. Chapter 4 extended this by showing that when the contrast between soil communities was increased—comparing 10- and 100-year-old forests—*A. glutinosa* root associated communities did reflect these broader differences (Chapter 4, Fig. 3). However, simplifying the microbial inoculum within each age group (via size fractionation) did not alter the root microbiome, again highlighting the tree’s ability to selectively recruit from a limited pool (Chapter 4, Fig. 3). Similarly, in Chapter 5, even when exposed to cultured bacterial and fungal communities with distinct compositions from the same young and mature forests (Chapter 5, Fig. 1), *A. glutinosa* still assembled root microbiomes that were compositionally consistent across treatments (Chapter 5, Fig. S4). Together, these three chapters paint a consistent picture: *A. glutinosa* is not just a passive recipient of soil microbiota, but rather a selective gatekeeper—capable of assembling tailored microbial communities under a wide range of biotic and abiotic contexts. The “picky” nature of *A. glutinosa* appears to be a core ecological strategy that allows it to thrive across heterogeneous environments, optimize beneficial symbioses, and potentially buffer against suboptimal microbial or nutrient inputs during afforestation.

Despite this, the selective association of trees with root microbiota is not unique to *A. glutinosa*, but rather reflects a broader pattern observed across diverse tree species.. It has been shown that different plant species harbor distinct root-associated microbial communities even when grown in the same soil (Berendsen et al., 2012). In other words, the host tree selectively recruits or filters certain microbes from the surrounding soil. For example, a controlled field experiment comparing monoculture stands of European beech (*Fagus sylvatica*, a deciduous broadleaf) and Norway spruce (*Picea abies*, a conifer) demonstrated clear species-specific effects on the composition of soil and rhizosphere microbiomes. Despite identical soil conditions, each tree species fostered a unique microbial community structure and abundance profile, highlighting the strong influence of host identity on microbiome assembly (Uroz et al., 2016) supporting the view that selective recruitment of root microbiota is a widespread and ecologically significant trait among trees

## 7.7 Cascading effects on herbivory and GHG emissions

Beyond direct effects on *A. glutinosa* growth and nodulation, soil abiotic and biotic characteristics also generate cascading above- and belowground consequences. Two important downstream responses—leaf herbivory and GHG emissions—were explored in Chapters 3 and 4, respectively. These outcomes reflect not just plant condition but the broader ecosystem functions regulated by soil–plant–microbe interactions.

A substantial body of research has explored how interactions occurring belowground can shape aboveground trophic dynamics in plants (Koes et al., 2015; Heinen et al., 2018; Huberty et al., 2020). Findings indicate that soil biota—such as bacteria, fungi, and broader microbial communities—can significantly alter the levels of primary and secondary metabolites produced by plants (Bezemer et al., 2005), potentially increasing their vulnerability to herbivores. Additionally, *F. alni* have been shown to enhance foliar nutritional quality, thereby increasing the attractiveness of plants to herbivorous organisms (Ballhorn et al., 2017). Interestingly, in Chapter 3, trees grown in 10-year-old live soils experienced the highest levels of herbivore damage (Chapter 3, Fig. 5), coinciding with the highest observed root nodulation rates (Chapter 3, Fig. 1H). In contrast, trees in 25-year-old soils, where nodulation was virtually absent, showed lower herbivory, despite similar leaf nitrogen levels. One plausible explanation is that in older soils, higher background nitrogen availability—as also noted in Chapter 2 with increasing $NO_3^-$ concentrations across the chronosequence—may compensate for the absence of symbiotic nitrogen fixation, thereby maintaining comparable foliar N content. However, Chapter 6 provides critical insight into this pattern: although fertilized and *F. alni*-inoculated trees exhibited similar chlorophyll levels and overall leaf N percentages, NIR spectroscopy was able to differentiate between the two treatments, detecting a unique spectral signature associated with *Frankia*-mediated nitrogen fixation (Chapter 6, Fig. 2B). This suggests that the leaf N content may still differ between symbiotic and inorganic N sources, even when total N appears equal. It is therefore likely that in Chapter 3, *M. brassicae* herbivores were responding to such subtle qualitative differences in nitrogen content—perhaps perceiving *Frankia*-derived N as more accessible or nutritious—leading to greater leaf consumption in the 10-year-old live soils. These results imply that microbial-mediated N acquisition can influence plant-herbivore interactions beyond what is captured by standard foliar nutrient metrics, and highlight the value of physiological tools like NIR spectroscopy in detecting such subtle effects.

In contrast to herbivory, GHG emissions appeared largely uncoupled from microbial complexity and developmental stage. Chapter 4 tested the effects of soil microbial fractionation and forest developmental stage on net $CO_2$, $CH_4$, and $N_2O$ fluxes. Surprisingly, neither the size of the microbial inoculum nor its origin (young vs. mature forest) had significant effects on any of the measured gas fluxes (Chapter 4, Fig. 5). This was despite clear differences in root-associated microbial communities and tree performance across treatments (Chapter 4, Fig. 3 and 4). While Chapter 4 discusses the potential role of functional redundancy (Yang et al., 2018) or the disruption of key microbial guilds—such as methanotrophs—through soil handling and inoculation (Kumaresan et al., 2011), additional insights emerge from the forest chronosequence presented in Chapter 2. There, we observed a general scarcity of methanotrophs, methane oxidizers, and bacterial taxa involved in denitrification and nitrate respiration across all stages (Chapter 2, Fig. 4). This consistent absence or low abundance of functional groups central to GHG cycling suggests that their limited presence may underlie the muted gas flux responses observed in our experiments. In other words, if the microbial communities lacked sufficient representation of key functional taxa to begin with, then variations in microbial composition or complexity—whether through fractionation or forest age—would be unlikely to yield measurable differences in GHG emissions.

## 7.8 Future perspectives and societal implications

### 7.8.1 The role of black alder and *Frankia alni* in afforestation

In recent years, afforestation has gained momentum as a promising strategy for land restoration, offering potential benefits for addressing both the climate and biodiversity crises (Doelman et al., 2020; Fleischman et al., 2020). However, it faces significant challenges (Kaine et al., 2023), as land degradation, nutrient-poor and eroded soils, and the impacts of climate change make it difficult for trees to establish in unfavorable soil conditions (Gomes et al., 2025). In this context, actinorhizal trees and more specifically *A. glutinosa* may serve as a valuable tool for "kick-starting" and supporting the success of afforestation efforts (Diagne et al., 2013). Several findings presented in this thesis offer insights into how this species could be effectively utilized in such projects.

Past studies in South Africa and China have shown that other actinorhizal trees, such as *Casuarina* species, have been widely used in degraded land restoration projects to improve soil fertility and even increase crop yields in agroforestry systems (Ndiaye et al., 1993; Zhong et al., 2010). Similar to *Alnus* (Yamanaka et al., 2005; Quoreshi et al., 2007), *Casuarina* trees benefit from forming symbiotic relationships with *Frankia*, which enhances their growth and biomass production (Sayed, 2011) and increases their resistance to both abiotic and biotic stresses (Bargali, 2011). Despite these functional similarities, the use of *Alnus* in restoration projects remains comparatively underexplored (Quoreshi et al., 2007). In my research, I found that nodule biomass production was positively correlated with aboveground biomass in *A. glutinosa* (Chapters 3 and 4). Furthermore, when trees were inoculated with crushed *Frankia* nodules, they consistently produced more aboveground biomass and had higher leaf nitrogen and chlorophyll content compared to uninoculated controls (Chapters 5 and 6). Since the soils used in Chapters 4 and 5 were gamma-sterilized sandy soils—more similar to poor conditions typical of abandoned or degraded land—these results highlight not only *A. glutinosa*'s capacity to perform well in unfavorable environments, but also the essential role of *F. alni* in supporting its success. This suggests that *A. glutinosa* could be employed similarly to *Casuarina* as a pioneer species in afforestation projects, promoting initial forest establishment and improving soil quality (Lefrançois et al., 2010). However, it is important to note that most degraded soils are not naturally infected with *Frankia* (Benson et al., 2004). Therefore, for reforestation efforts to succeed, it is essential to introduce stress-tolerant alder trees along with compatible and effective *Frankia* strains.

In Chapters 3 and 4, we observed that inoculating with soil communities from older forests led to reduced root nodulation and tree performance—a trend that, as shown in Chapter 5, can be linked to specific fungi and bacteria such as *Trichoderma harzianum*, *Pseudomonas* sp., and *T. koningii*. Additionally, Chapter 5 demonstrates that elevated levels of available N in the soil result in a near-complete absence of root nodules in *A. glutinosa*. For future applications, it is therefore vital to consider the compatibility among *Frankia*, the host plant, and the local environment, as black alder may exhibit varied nodulation responses under different environmental conditions (Sayed, 2011). It is also worth noting that even under N-rich conditions where nodulation was suppressed, *A. glutinosa* absorbed all available nitrogen during its growth. By the end of the experiments in Chapter 5, nearly no $NO_3^-$ or $NH_4^+$ remained in the soil. Given that previous research has shown actinorhizal plants can enrich nutrient-poor soils with nitrogen (Diagne et al., 2013), these findings suggest that during its early growth stages, *A. glutinosa* can instead uptake soil N and, in the absence of *F. alni*, may still form beneficial interactions with other soil microbes, such as those that positively correlated with aboveground biomass production in Chapters 3 and 4.

### 7.8.2 Kick-Starting Forests: Can soil inoculation make a difference?

Soil inoculation has been proposed as a tool to accelerate ecosystem restoration (Robinson et al., 2023), with multiple studies demonstrating its potential to establish target soil communities that resemble those of donor ecosystems (Contos et al., 2012; Waterhouse et al., 2014; Wubs et al., 2016). However, its effectiveness in promoting tree establishment and growth during afforestation is less clear. In this thesis, I explored the potential of different inoculation approaches, each with varying effects on the growth and establishment of *Alnus glutinosa*. Specifically, Chapter 3 focused on whole forest soil transplantation, while Chapters 4 and 5 investigated size-fractionated forest soil communities and cultured bacterial and fungal inocula, respectively.

A key finding across all experiments was that the age of the forest stand from which the inoculum was sourced had a marked influence on the performance of *A. glutinosa*. In most cases, regardless of the inoculation method, trees inoculated with communities from older forest stands exhibited thinner stems (Chapter 3, Fig. 2B) and produced less aboveground biomass (Chapter 4, Fig. 4A) compared to those grown in sterilized control soils. This is consistent with previous studies showing that soil sterilization can sometimes enhance plant growth (Tian et al., 2009; Qin et al., 2014), likely due to increased nutrient availability, reduced competition with microbes and the removal of soil pathogens (Li et al., 2019). These findings might lead one to question the overall value of soil inoculation, potentially viewing it as an ineffective and labor-intensive approach. However, is this conclusion justified? In contrast to our results and those of similar studies, other research has shown positive effects of soil inoculation on tree growth and establishment (Brown and Bedford, 1997; Rowe et al., 2009; Middleton and Bever, 2012; Averill et al., 2022). While soil inoculation is often grounded in intraspecific facilitation theory—introducing beneficial soil organisms to support plant growth (Silliman et al., 2024)—*A. glutinosa* may respond differently to mature forest inocula compared to other trees, due to potential interactions between the introduced microbial assemblages and its primary symbiont, *F. alni*.

Notably, in Chapters 3 and 4, I observed that inocula from older forests negatively affected root nodulation. In Chapter 3, the higher available N in soils from the 25-year-old forest stand, aligning with the direct inhibitory effects of high N on nodulation observed in Chapter 5 made it more challenging to conclude that this was an effect of the soil community. In contrast, Chapter 4 involved soils with similar abiotic characteristics across treatments, suggesting that the observed effects stemmed from biotic factors—specifically, the presence of antagonistic microbes in the inoculum—a hypothesis further supported by findings in Chapter 5. Despite these negative effects, there were also several specific microbial taxa that were identified in Chapters 3, 4, and 5 that positively influenced both tree performance and *Frankia* symbiosis. This highlights the potential for a more targeted inoculation approach, focusing on introducing select beneficial microbes rather than entire soil communities. It is also important to consider that in all experiments, inocula were applied at the seedling stage—when trees are most vulnerable and potentially less competitive against microbial communities adapted to mature forest environments. This suggests that younger forest inocula may be more appropriate for early-stage inoculation efforts.

Taken together, these findings indicate that the effects of soil inoculation are highly context-dependent. Future research should therefore test inoculation strategies across different tree species and at multiple developmental stages to determine the optimal timing and conditions for successful application.

## 7.9 Closing remarks

This thesis explored how changes in soil biotic and abiotic properties across influence tree performance, N-fixing root symbiosis, and broader ecosystem functions. My findings show that both soil abiotic characteristics and microbial communities critically shape tree growth and symbiotic success—often in complex and context-dependent ways.

While certain microbial taxa and soil inocula enhanced nodulation and biomass, others—especially from mature forests—suppressed these benefits, challenging the assumption that higher microbial diversity is always advantageous. These outcomes highlight the need for targeted, rather than generalized, microbial inoculation in restoration efforts.

Even though I did not observe strong effects on foliar herbivory and greenhouse gas, the results suggest that belowground interactions—particularly those related to nitrogen fixation—can subtly influence aboveground herbivory. This highlights the context-dependent nature of soil–plant–microbe dynamics and their potential to shape broader ecosystem processes.

Altogether, this research supports the use of actinorhizal trees like *A. glutinosa* in afforestation but calls for precision in inoculating with soil biota. As afforestation becomes central to climate and biodiversity strategies, integrating microbial ecology into practice will be key to building resilient, multifunctional ecosystems.

To build on these findings and inform future restoration efforts, I propose that further research should focus on:

- Conducting long-term field trials to assess the field applicability of inoculation effects, testing whether outcomes observed in controlled environments persist under natural conditions.
- Comparing inoculation effects across different stages of afforestation, different growth stages of the tree and in varied biogeographic regions.
- Expanding research to other tree species to determine whether the observed soil–microbe–plant interactions and inoculation effects can be generalized across different actinorhizal and non-actinorhizal species.