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OPINION

Soil Microbiome Inoculation for Resilient and Multifunctional New Forests in Post-Agricultural Landscapes

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ABSTRACT

Afforestation is increasingly recognized as a critical strategy to restore ecosystems and enhance biodiversity on post-agricultural landscapes. However, agricultural legacies, such as altered soil structure, nutrient imbalances, and depleted microbial diversity, can slow down forest establishment or cause ecosystems to deviate from expected successional trajectories. In this opinion paper, we explore the potential of soil inoculations as a tool to overcome these challenges by introducing beneficial microbial communities that can accelerate ecosystem recovery and forest development. Restoring soil biodiversity is a crucial aspect of this process that drives broader ecosystem functionality and resilience. We highlight the need to carefully consider the type and timing of inoculations and to ensure compatibility between the inoculum and recipient site characteristics to optimize the establishment of introduced species. While tree productivity is often a central focus of afforestation efforts, the restoration of soil biodiversity, which will also contribute to increased ecosystem-level functions, should also be a priority for long-term forest resilience. Agricultural legacies add complexities to the restoration process, creating unique challenges that need to be addressed in restoration planning. Thus, successful inoculation strategies require a thorough understanding of both donor and recipient site characteristics, also in relation to potential mismatches related to soil physiochemical properties to avoid unintended consequences such as the non-establishment of introduced species. Additionally, we call for the re-evaluation of afforestation targets and the development of standardized monitoring protocols that track the success of inoculation efforts, particularly regarding soil health, microbial community establishment, and biodiversity recovery. By integrating inoculation practices within a broader restoration framework, we can enhance the resilience, biodiversity, and ecosystem functionality of newly afforested landscapes. Ultimately, this approach may play a critical role in ensuring the success of large-scale afforestation projects.

1 | Forest Restoration and Climate Mitigation in Post-Agricultural Land

Tree-planting holds high potential to simultaneously mitigate the climate and biodiversity crises if done appropriately (Fleischman et al. 2020), being a top societal and economic priority globally (Bastin et al. 2019). The European Union (EU) biodiversity strategy for 2030 tackles the protection and restoration of nature under the European Green Deal (European Commission 2021a). One of the commitments is to plant at least three billion additional trees in the EU by 2030 (European Commission 2021b). Yet, efficient strategies to plant resilient forests that promote stable functions while supporting biodiversity in our changing world are still lacking (Cantarello et al. 2024). Most global ecosystem restoration efforts have underperformed, failed, or have been reduced due to high costs required for upscaling (Silliman et al. 2024). Planting three billion additional trees in the EU requires 2–3 million hectares of land (European Commission 2021b), which may only be taken from abandoned agricultural land (Castillo et al. 2021). This poses significant decision-making challenges, such as determining optimal locations and choosing the most efficient tools to facilitate the conversion of abandoned agricultural land into forests. Planted forests are especially vulnerable in the initial decades after establishment, particularly in conditions that require land use transformation (Banin et al. 2022) due to the radical changes in both abiotic and biotic features associated with agricultural practices towards those typical for forest ecosystems.

It is well established that competitive herbaceous vegetation and unfavorable soil conditions can hinder the successful establishment of new forest ecosystems (Thrippleton et al. 2017; Kepfer-Rojas et al. 2015). Previous land use can result in soil legacies that strongly influence soil properties, subsequently affecting tree establishment and growth, which potentially delays or reduces the development of biodiversity-rich and functional ecosystems (Foster et al. 2003). These legacy effects can impact diverse biotic and abiotic elements across various spatial and temporal scales. They not only influence the trajectory of ecosystem development (Hermy and Verheyen 2007) but also act as biotic filters, shaping the succession of the belowground microbial community toward a forest microbiome (Osburn, Aylward, and Barrett 2021). To overcome these negative legacy impacts, here we discuss the potential of inoculations, including the introduction of soil microbiomes, to speed up ecosystem recovery. We propose windows of opportunity throughout the development of a forest during which the chances of success may increase. Our focus extends beyond tree productivity and includes the restoration of soil biodiversity and ecosystem-level functions within afforestation practices, i.e., planting trees on sites that were cleared of original forest cover for agricultural use. A key question in this context is whether the target ecosystem at the location in focus of restoration is a forest or instead a natural grassland. While there is currently a lot of emphasis worldwide on tree planting, aiming for the wrong target ecosystem may lead to improper afforestation and to poor biodiversity outcomes (Veldman et al. 2015). We address the multifaceted challenges posed by agricultural legacies when planting trees and establishing forests on former agricultural land. First, we summarize how land-use legacies specifically challenge the establishment, development, and restoration of forest ecosystems in post-agricultural landscapes. Secondly, we present our view on the rationale for using soil inoculation to accelerate and steer the development of forest ecosystems in the presence of land-use legacies. We also argue for the necessity of a whole-ecosystem approach and suggest that optimizing methods for soil inoculation can improve the outcomes of forest restoration. Finally, we discuss factors that can hinder the success of soil inoculations, potential implications, and suggest avenues for future research.

2 | Challenges due to Land Use Legacies

Agricultural practices such as tillage, pesticide use, fertilization, and liming make cropland soils very different from soils in forests with long continuity, i.e., ancient forests (Wirth et al. 2009), in terms of both abiotic and biotic properties (Figure 1a, Table S1). For example, the homogenized plough layer of cropland soils has higher pH, higher nitrogen (N) and phosphorus (P) contents, and lower C/N ratio compared to the heterogeneous topsoil of ancient forests of the same origin (Guo and Gifford 2002; McLauchlan 2006; Murty et al. 2002), and the soil microbiome is usually bacterial-dominated in croplands but fungal dominated in forests (Siles et al. 2023). These characteristics form a substantial legacy that deters the establishment of forest-associated biota, such as soil microbes, including mycorrhizal fungi, flora, and fauna, which are often sensitive to soil nutrient levels. As a result, forests planted on agricultural land may often exhibit species compositions and functions divergent from ancient forests within the same landscape, potentially leading to the development of ecosystems that differ from those typically considered as reference ancient forests (De Frenne et al. 2011).

Upon afforestation, trees establish, and after a decade or two, the canopy closes, creating a forest microclimate, and gradually, a more diverse forest structure develops (Figure 1a). Despite the cessation of agricultural practices, cropland soil properties persist as agricultural legacies that may hinder the establishment of forest-adapted species. Over time and with forest succession, soil properties slowly change at variable rates (see details for each soil property in Table S1). Available nitrogen, particularly NO_3, declines rapidly within the initial 5–10 years (Hansen et al. 2007) as fertilization stops and due to increased nitrogen demand by young trees and ground vegetation. Topsoil pH, however, declines gradually over several decades (Brasseur et al. 2018; Wei et al. 2012). Tree planting increases aboveground carbon stocks and carbon storage in the topsoil, which also benefit from the production of recalcitrant litter. Simultaneously, the ceased ploughing reduces disturbance, increasing carbon stabilization concurrently with enhanced nitrogen retention through tree and understory growth. High phosphorus levels in former agricultural soils are often considered an important long-term legacy that strongly influences the composition of flora, fauna, and soil microbes in post-agricultural forests, and it is only slowly removed through plant uptake (Turley et al. 2020) while available P decreases within the first decades (B. Wang 2024). The development of a complex vertical and horizontal forest

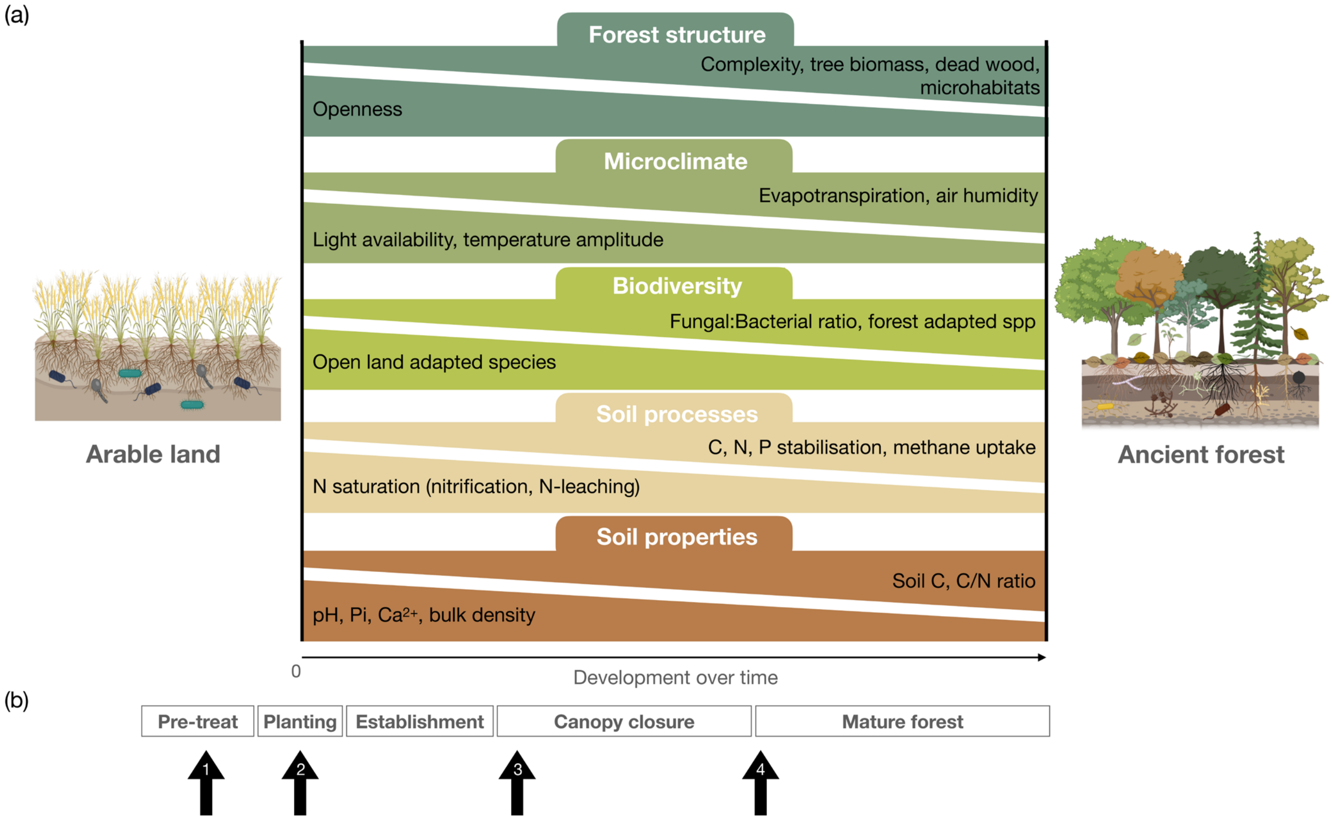

FIGURE 1 | (a) Development of new forests on arable land, including changes in forest structure, microclimate, biodiversity, soil processes, and soil properties (see details on processes and thresholds on Table S1). (b) Multiple forest stages are depicted in the boxes at the bottom following the development over time. Black arrows indicate specific time points proposed for inoculations to speed up forest development.

structure and characteristic habitats is slow, resulting in low structural variation of the tree layer in the early stages. As canopy closure progresses, forest-associated species replace open habitat specialists, and the negative impact of agricultural legacies diminishes. However, the influence of the persistent agricultural legacies during this phase on the development of soil biota is highly understudied. The forest structural variation and soil conditions continue to reflect the agricultural legacy, resulting in forests that poorly resemble ancient forests (Bergès and Dupouey 2021; Brubaker and Cosentino 2021).

After planting tree saplings, smaller seeds that are typical of open habitats germinate quickly, giving rise to ruderal or opportunistic species. Importantly, forest-adapted species are often absent from the post-agricultural seed bank, emphasizing a key challenge in vegetation transition from agriculture to forest during early phases (Bergès and Dupouey 2021). During the initial stages of forest development, bacterial dominance persists, as is typical in agricultural soils (Siles et al. 2023), but within one or two decades, we observed a fast shift in the fungal microbiome to forest-associated species (Barsotti 2024), whereas the establishment of other organisms, such as soil fauna or larger animals, may encounter difficulties due to varying sensitivities to nutrient levels, lack of favorable habitats, or dispersal limitation (Malica et al. 2024; Osburn, Aylward, and Barrett 2021). Consequently, we argue that the soil development during the transition from agricultural land to a forest ecosystem is a process often characterized by low biodiversity and limited ecosystem functions.

3 | Soil Inoculations Can Enhance and Steer Forest Ecosystem Development

Soil biodiversity is key to promoting terrestrial life, supporting, among others, soil health, water purification, and carbon sequestration (Bardgett and van der Putten 2014; Mercado-Blanco et al. 2018). The approach to use inoculations, i.e., the introduction of target organisms or communities to overcome natural migration barriers, is gaining traction in forestry, agriculture, and restoration ecology (Robinson et al. 2023). Inoculation intends to make use of intraspecific facilitation theory (Silliman et al. 2024) through the introduction of beneficial soil microorganisms, such as plant growth-promoting bacteria and (mycorrhizal) fungi, either by a targeted culture-based approach (i.e., inoculating specific microbial strains) or directly inoculating living soil (i.e., soil inoculations from a donor site) from a target ecosystem (Robinson et al. 2023). The introduction of microbial symbionts enhances the establishment of higher trophic level species, promoting facilitation cascades, and consequently boosting ecosystem functions (Li et al. 2024; Requena et al. 2001). While such inoculation methods can have positive results in both sterile laboratory settings and experimental field conditions (Hoeksema et al. 2010), the success of commercial inoculants in natural conditions may be hampered by low compatibility with specialized host trees and local edaphic or climatic conditions (O'Callaghan, Ballard, and Wright 2022; Salomon et al. 2022). Using soil from a tree's native habitat as an inoculant is perceived as generally enhancing tree growth (Averill et al. 2022). However, both positive and negative outcomes have been reported, and the effects appear to depend on whether trees associate with arbuscular or ectomycorrhizal fungi (Bennett et al. 2017; Delavaux et al. 2023; Teste et al. 2014). The plant–soil feedback theory advertises that certain plant species exhibit reduced growth in their own soil due to the build-up of specific pathogens (van der Putten et al. 2013), as has been commonly shown for oaks, despite these associating with ectomycorrhizal fungi (Kwaśna and Szewczyk 2016). Understanding species-specific effects of microbial symbiont inoculation and its interaction with the local microbial assemblage, particularly at a broader scale, is still an open area of research.

The introduction of soil from target ecosystems like grasslands or heathlands has shown lasting effects on vegetation, soil microbes, nematodes, and arthropods (Wubs et al. 2016). Yet, the outcomes in relation to target ecosystems can be unpredictable, and only about half of the experiments have yielded persistent positive effects (Cornell et al. 2021). In forests, understanding how soil inoculations impact ecosystem development remains in its early stages, and transfer of knowledge from grassland ecosystems to forests should be done cautiously, due to system-related differences in the effects of soil legacies and in establishment processes. Forests produce high aboveground and belowground biomass and relatively recalcitrant lignin-rich litter, making these ecosystems particularly dependent on soil microbes with high enzymatic capacities. Therefore, forest ecosystems may benefit in particular from inoculation of soil microbial communities that promote specific ecosystem functions such as organic matter decomposition (Benetková et al. 2022; Han et al. 2022; Moradi et al. 2018; Wubs et al. 2016).

A recent meta-analysis provided inconclusive evidence for the hypothesis that the introduction of species is a sustainable way to conserve and restore ecosystems, but this could be related to the small number of studies available for the analysis (Langhammer et al. 2024). This highlights that knowledge on the effects of species introductions on ecosystem development and functioning is critically limited. In grassland or heathland systems, soil inoculation interventions have proven to be effective (Han et al. 2022; van der Bij et al. 2018; Wubs et al. 2016). As the same principle and mechanisms may also work in other ecosystems, soil inoculation could be a potential tool to aid in the transition towards ecosystems exhibiting characteristics reminiscent of ancient forests. Integrating soil inoculations in tree planting schemes is already being implemented in some initiatives (e.g., *The Carbon Community* in the UK, www.carboncommunity.org), however many questions remain open and this may prohibit maximizing its benefits and suitability (see *Key considerations for implementing soil inoculations* Section below). Inoculation with donor soil from nearby target ecosystems can be beneficial in aiding the germination of native species in grasslands (Duell, Hickman, and Wilson 2022) and facilitating the establishment of rare or late successional plant species (Koziol and Bever 2017; Soteras, Renison, and Becerra 2014). Thus, we foresee that science-informed soil inoculations can become a widespread, compelling tool for enhancing efforts to restore forest ecosystems.

4 | Implementing Soil Inoculations for Forest Restoration

The development of soil from abandoned cropland to mature forests occurs through a combination of physiochemical processes mediated by biological activity and species interactions (Robinson et al. 2024). In afforestation, soil biogeochemical processes typically evolve gradually towards, but do not necessarily reach, the characteristics of ancient forest ecosystems (Figure 2; non-inoculation pathway). This is influenced by long-term forest development and feedback mechanisms, including changes in canopy structure, soil hydrology, physical environment, C/N dynamics, microbial communities, and tree species composition (Christiansen and Gundersen 2011; Gatica-Saavedra et al. 2023). While previous studies have primarily focused on the direct enhancement of plant growth through inoculation, the broader impact involves the establishment of mycorrhizal and saprotrophic hyphal networks, along with the introduction of bacteria and archaea crucial for soil nutrient cycling (Crowther et al. 2019). Collectively, these microbial communities enhance soil carbon sequestration, mitigate nutrient leaching, and reduce greenhouse gas emissions, in particular N_2O and CH_4 (Bakken and Frostegård 2020; Praeg et al. 2020; Scholz et al. 2020).

The success of inoculation depends on the degree to which the inoculated species or communities establish and on the composition and ecological functions of the establishing communities. However, this success can be hindered by abiotic conditions and biotic interactions, as highlighted by Albright et al. (2022). As the strength of these filters changes throughout forest development, optimizing the timing and the method of inoculation can increase its success and efficiency while reducing efforts (Figure 1b). In the early stages of afforestation—the most vulnerable phase—efforts are considerably more intensive and carry a higher risk of failure, as this is the critical period for jumpstarting forest growth. This requires a carefully designed strategy to rapidly mitigate the persistent effects of agricultural legacies. A key component of this approach is optimizing the compatibility between donor and recipient soils in terms of physicochemical properties and species competitiveness. Once the canopy closes, priorities may shift toward guiding forest development to achieve specific objectives, making the choice of these targets particularly important in order to stimulate the establishment of mature forest species or desired services. Evidence is still lacking on whether and how fast soil inoculations will mitigate the agricultural legacy effects by accelerating the development towards a forest ecosystem. This could occur either by creating conditions that converge with those of mature or donor forests or by establishing novel forests with microbial communities that combine legacy and donor diversity and functions, shaped through species interactions and niche filling (Figure 2; inoculation pathway). In parallel to that, management practices should be revisited and adapted during the process of forest development. In the next paragraphs, we propose mechanisms and simultaneous measures that can be taken to optimize the success of soil inoculations at different stages of forest development.

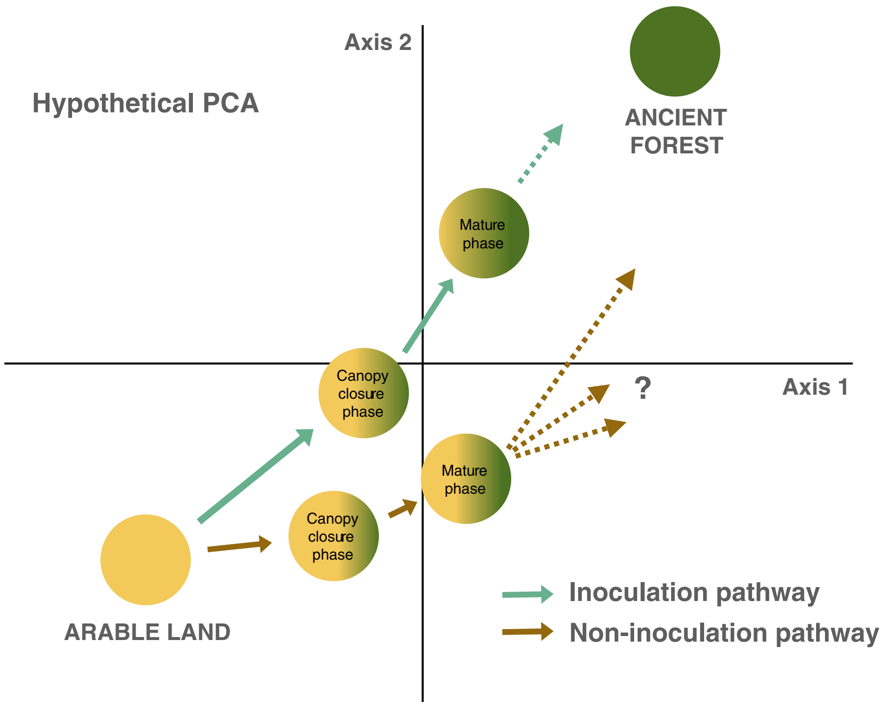

FIGURE 2 | Conceptual hypothesis of forest development with and without microbial inoculations in relation to agricultural legacy. Axis 1 represents the successional stages and species composition changes; Axis 2 relates to soil functions, e.g., C sequestration, methane oxidation, and nutrient immobilization (see Figure 1 for a complete view of functions and direction of change). Colors in the circles represent the presence of arable land legacy (yellow) and resemblance to target ancient forest (green) in the canopy closure and mature phases during forest development when following the inoculation (green arrows) or non-inoculation (brown arrows) pathways. Concept for forest development inspired by results from soil inoculations in grasslands (Wubs et al. 2016).

4.1 | Nursery Stage

Inoculation can start before tree planting (Figure 1b, arrow 1). At the nursery stage, seeds or early-developing seedlings can directly be sown or planted in inoculated soil, and the saplings can then later be transplanted to abandoned agricultural fields. Trees establish symbiotic relationships with soil microbes—importantly, but not exclusively, with mycorrhizal fungi—which contribute to their uptake of nutrients and water, helping them tolerate stressful abiotic conditions (Allsup and Lankau 2019) and play a pivotal role for soil aggregation. Theory predicts that plants select their core microbiome very early after seedling emergence and that early colonizers generally determine the final community (Costello et al. 2012; Edwards et al. 2015; Paredes and Lebeis 2016). The advantage of inoculating at this stage is that the success of colonization by the inoculated communities has been shown to be higher for seeds and seedlings when they have not been in contact with resident communities (Aleklett et al. 2022; Pera and Parlade 2005). Hence, the nursery stage is an ideal instance for inoculating the trees (Repáč 2011). Moreover, at this stage, the confined boundaries of the nursery enable that the amount of material or soil that is needed for inoculation is rather limited, and this also reduces the burden on the donor ecosystem where the soil is collected from.

4.2 | Tree Planting

By the time of planting trees in agricultural soils (Figure 1b, arrow 2), biotic and abiotic agricultural legacies are the strongest. The high nutrient and light levels, low organic carbon, and biotic communities associated with a productive and light-open environment result in a harsh and stressful environment that can limit the colonization and establishment of inoculated forest-associated species (Lopes, Dias-Filho, and Gurgel 2021). Inoculating trees at the planting stage can significantly enhance their chances of acquiring beneficial symbionts, which in turn promotes survival and growth. The aim of early inoculation is to boost tree establishment and reduce mortality and accelerate the positive soil feedbacks that result from successful tree growth. (Requena et al. 2001; Robinson et al. 2023). While the establishment of the soil microbiome can benefit from interacting with planted host tree species (Habiyaremye et al. 2021), the amelioration of the agricultural legacy entailed by the trees (nutrient depletion, litter input, and organic layer build-up) can facilitate the later establishment of understory plants and meso- and macrofauna assemblages (Ganault et al. 2021). Nevertheless, inoculation at this stage is more time-consuming and extensive than at the nursery stage, as it requires a higher inoculum quantity added in the rooting zone, and the introduced microorganisms must be compatible with the biotic and abiotic conditions of the site to ensure the successful colonization of trees.

Planting trees with the addition of soil inoculum into the field can have further benefits by introducing other soil organisms with specialized functions. Not only the agricultural legacy but also the lack of specialized resources will limit the success of the establishment of forest-specific soil organisms in this stage. Considering that decomposers are key for nutrient cycling, stimulating forest-specific decomposers at early stages of forest plantation, e.g., by adding litter (Contos et al. 2021), decomposing logs, or mulch (Parkhurst et al. 2022), may accelerate the introduction of key taxa that will speed up nutrient cycling and other ecosystem functions. Additionally, with the addition of forest soil at the tree planting stage, seeds and root pieces from forest habitats can be introduced to overcome dispersal limitation (i.e., soil transplantation as a tool for assisted dispersal), and the addition of a plant litter layer can further increase seedling survival (Douterlungne et al. 2018).

4.3 | Canopy Closure

Canopy closure is a decisive stage of forest development. The development of the canopy stabilizes the microclimatic conditions, decreases light availability, and alters nutrient inputs on the forest floor (Figure 1b, arrow 3) (Verheyen et al. 2024). At this stage, soil inoculations may be less targeted at the introduction of tree microbial symbionts but can be used to promote the establishment of larger soil organisms augmenting the restoration potential, in particular for those that need specific abiotic conditions different from the harsh environment of bare cropland soil. Furthermore, the potential of the inoculated communities to further steer the development of the forest communities into a distinct trajectory is lower at this stage and onwards since the established trees and other organisms already had time to shape the direction of ecosystem trajectory and reduce the initial agricultural legacies. Still, interventions at this stage may greatly contribute to increasing biodiversity in planted forests by bringing in target species when abiotic conditions are sufficiently ameliorated to support their establishment.

4.4 | Mature Phase

Soil inoculation or translocation of species at the mature stage serves the purpose of restoring forest-associated species or species of conservation concern associated with mature forest conditions when specialized resources and environmental conditions are finally made available locally through forest maturation (Figure 1b, arrow 4). This can be particularly important for species with unusually strong roles in influencing ecosystem functions (i.e., metacommunity hubs, *censu* Moreno-Mateos et al. (2020)) found in mature forests or higher-order organisms like understory plants and invertebrates (Grimbacher and Catterall 2007). Colonization and establishment of organisms from mature forests through direct soil inoculation is expected to have an increased chance of survival due to more suitable abiotic forest conditions at this stage (Benetková et al. 2022). Early successional plant species generate strong negative feedbacks due to their weak defense against pathogens and unresponsiveness to microbial mutualists, while late-successional plant species are generally more dependent on microbial mutualists and better defended against pathogens (Kardol, Martijn Bezemer, and van der Putten 2006). As a result, soil pathogens and soil mutualists may both inhibit early successional species and facilitate late successional species (Bauer, Mack, and Bever 2015; Jing, Bezemer, and van der Putten 2015; Wubs et al. 2019).

5 | Key Considerations for Implementing Soil Inoculations

Soil or tree inoculations with forest-associated microbiomes and invertebrates are not a one-time self-sufficient solution. Several factors need to be considered for successful implementation, for example, the recipient site characteristics (e.g., soil type and strength of agricultural legacy) and the inoculum source and quantity. These are especially important when considering that the microorganisms provided with the inoculum may come from a well-developed system (ancient forest as donor soil), and their survival and establishment may be hampered by the abiotic and biotic legacies of agricultural systems at the recipient sites. In this section, we address key considerations to overcome these adverse initial conditions with the aim of facilitating the inoculant establishment and promoting a beneficial effect for the recipient afforested ecosystem. Finally, we reflect on how to evaluate the success of the inoculation by redefining the concept of "reference site."

5.1 | Recipient Site Characteristics

Recipient site characteristics profoundly impact the success of introduced communities through inoculation, potentially undermining the effectiveness of such interventions (Contos et al. 2021; Gerrits et al. 2023). Unsuitable soil properties of the recipient site can hinder microbial community establishment and functionality, with soil texture and water content being key predictors of success. Gerrits et al. (2023) found that loamy soils exhibit the highest restoration success, although additional soil amendments may be necessary to mitigate challenges. Microtopography is also a critical aspect that influences the spreading and establishment of the inoculated microbiome or mesofauna in the recipient site, as demonstrated in post-mining site restoration (Moradi et al. 2018). The degree of heterogeneity of topsoil microtopography depends on land use, former management, and developmental stage of the established ecosystem, requiring careful consideration in the timing of microbiome inoculations for afforestation efforts (Figure 1b).

5.2 | Landscape Positioning

Habitat fragmentation and isolation of new forests from ancient ones also pose significant landscape-scale challenges by creating barriers for the dispersal of forest species (Brunet 2007). This has profound implications for afforestation outcomes as it reduces the natural potential species pool available for the establishment of forest-associated species in planted forests, both in terms of species richness (Brunet 2007; Brunet et al. 2021; Peterken and Game 1984) and composition (Dzwonko and Loster 1992; Flinn and Marks 2007; Matlack 1994). Forest-adapted plant species are generally rare in newly planted forests, underscoring the simultaneous need to promote the protection of existing native and ancient ecosystems from where these target species can be naturally sourced (Hua, Liu, and Wang 2024). Seed dispersal and recruitment are often recognized as bottlenecks in the recovery of specialized plant biodiversity in such forests (Flinn and Vellend 2005; Hermy and Verheyen 2007) due to their limited dispersal (Dzwonko and Loster 1992). With soil inoculations, seeds are also transferred. As both the seeds of target plant species and their associated microbial communities (Green and Bohannan 2006) are introduced with soil inoculation, this can be a promising strategy to steer plant communities towards the diversity found in the reference site (Gerrits et al. 2023; Wubs et al. 2016). The same idea extends to soil fauna (Gómez-Martínez et al. 2020; Malica et al. 2024; Seibold et al. 2019). These species with limited dispersal abilities across several taxonomic groups are affected by the distance to the forest and the quality of the surrounding landscape (Fahrig 2007; Peay, Garbelotto, and Bruns 2010). Considering landscape positioning can aid in determining the priority of sites for soil inoculation; e.g., sites next to ancient forests may not need inoculations when the target species have good dispersal capability. Therefore, it is essential to consider alternative restoration designs and adapt optimal designs that are context dependent on the landscape's positioning relative to the target abandoned land intended for afforestation (Hua, Liu, and Wang 2024; Pedersen, Schmidt, and Kepfer-Rojas 2023).

5.3 | Inoculum Source

The choice of donor site requires careful consideration as soil characteristics significantly influence plant germination, establishment, soil nutrient status, and pH at the recipient site (Carbajo et al. 2011; Gerrits et al. 2023). The origin of the donor soil plays a pivotal role in plant community responses, with implications for early and late successional species. Inoculum from late successional soils may promote beneficial symbionts and enhance the responsiveness of arbuscular mycorrhizal plants (Bauer, Mack, and Bever 2015; Kardol, Martijn Bezemer, and van der Putten 2006; Rowe, Brown, and Claassen 2007). Also, local inoculum usually outperforms arbuscular mycorrhizal commercial inoculations (Emam 2016). Rowe, Brown, and Claassen (2007) showed that all tested late-successional plant species responded positively to field inoculum, as opposed to early-successional plant species. However, mismatches between donor and receiver soil properties should be minimized, as the composition of fungal communities (e.g., ectomycorrhizal) changes with forest succession stages, which include factors like forest age, maturity, and tree species identity (Jones, Durall, and Cairney 2003). Soil conditions in mature forests are very contrasting compared to those in agricultural soils (Figure 1a); thus, donor soils from forests in different developmental stages should be considered as possibilities if that increases compatibility in terms of soil properties, in particular pH, of inoculum establishment. One possibility to consider is to use soils from forests in the succeeding developmental stage as inoculum to optimize compatibility. In addition, risks associated with non-native microorganisms should be evaluated, as they can become invasive and negatively impact soil biodiversity and functioning (Hart, Antunes, and Abbott 2017). For instance, the common forest root rot fungus *Armillaria* spp., prevalent in old forests, can colonize both old and young trees. Similarly, the tree decay fungus *Heterobasidion parviporum* causes white rot and is particularly problematic in spruce plantations in post-agricultural soil (Gunulf et al. 2013).

5.4 | Inoculum Quantity and Type

Finding a balance between the amount of soil to source from donor sites and the thickness of the soil layer to inoculate is of high importance since the quantity of inoculum positively influences its efficacy (Barsotti 2024; Han et al. 2022; McMahen et al. 2022). Simultaneously, sourcing substantial amounts of donor soil from mature forests for inoculating newly planted forests may not be sustainable at a larger scale in afforestation projects, highlighting the need to develop methodologies to maximize the inoculation success in order to minimize the negative impacts on donor ecosystems. Therefore, research on the minimum amounts of inoculum soil needed for successful establishment of the target microbiome will be important to reduce donor disturbance. Furthermore, viable biotechnological strategies may involve isolating and mass propagating essential microbes to avoid extensive disruption of natural donor ecosystems through the removal of large quantities of soil. Alternatively, the identification of beneficial microbes that will thrive in the afforested sites and pairing them with trees in the nursery stage can greatly reduce the need to rely on large quantities of soil from donor sites. A recent meta-analysis indicates that while bacterial diversity often increases after inoculation, the effects on fungal diversity in the field vary (Cornell et al. 2021). Notably, some studies have shown that desired effects were not sustained in recipient soil even after repeated inoculations (L. Wang et al. 2016). Cornell et al. (2021) also investigated the effects of inoculating single species or mixed inocula and found that mixed inocula more often increased bacterial diversity, although data on mixed inocula for fungi were lacking. Using bacterial consortia shows promise for enhancing plant growth, surpassing single-strain soil inoculations in forestry. However, this area remains underexplored and mainly focuses on bacteria, neglecting other soil microbiome components such as fungi and protists (Liu, Mei, and Salles 2023). Nonetheless, scaling up the most appropriate tools to provide sufficient inoculum for large-scale application still presents significant challenges.

5.5 | Criteria to Assess Inoculation Success

To assess the success of inoculations, standardized biodiversity monitoring methods are essential to verify ecosystem recovery and health improvements over time (Robinson et al. 2024). It is crucial to ensure that added species can thrive in the recipient environment and fulfill their expected functions, considering both abiotic mismatches and competition with resident species. Compatibility between recipient and donor characteristics should be assessed beforehand to predict and enhance the effectiveness of inoculation efforts in accelerating the process of creating new forests. Moreover, monitoring ecosystem recovery should align with expected changes linked to the specific donor inoculum characteristics. However, in many cases, afforested sites bear little resemblance to the native ancient forests often used as reference points, likely due to legacy effects (Brockerhoff et al. 2008), leading to unrealistic comparisons with native ancient forests. Current reference systems and newly afforested sites develop under distinct pressures (e.g., historic land use, climate change, and human influences). Therefore, we propose to redefine the concept of reference sites to account for realistic expectations of future biodiversity and ecosystem functions in these newly planted ecosystems. This concept should consider multiple ecosystem trajectory possibilities imposed by post-agricultural status, tree planting methods with or without the use of assisted dispersal and inoculations. In large-scale afforestation efforts, where topsoil removal to reduce legacy effects is generally impractical or not widely used as a common practice (but see (Wubs et al. 2016) for a successful example in the Netherlands), evaluating the success of ecosystem development with soil inoculations requires careful consideration. The interaction between donor inoculum—typically obtained from the target reference site—and the biotic and abiotic characteristics of the recipient site will shape ecosystem development trajectories (Figure 2). Accordingly, targets to assess forest establishment success should acknowledge divergent starting conditions and recognize that success does not always entail direct convergence towards the reference (i.e., typically donor) sites. Establishing science-informed definitions of target points as references for forest development assessment is crucial. This involves accepting that newly planted forests may not reach the standard set for current mature forests, and targets and success expectations should be adjusted accordingly.

6 | Outstanding Questions for Future Research Avenues

In exploring the potential of soil microbiome inoculation for establishing resilient and multifunctional new forests in post-agricultural landscapes, several critical questions remain unanswered. Addressing these outstanding questions through targeted research can provide valuable insights and practical guidance for improving the resilience and multifunctionality of new forests established on post-agricultural lands. We emphasize that the proposed strategy of multiple inoculation time points, focusing on key developmental stages for timely interventions, along with ensuring compatibility between donor and recipient sites, can significantly contribute to the restoration of other ecosystems.

- How should targets in afforestation be defined to optimize ecological and functional outcomes? *Clear and scientifically grounded targets* are essential for guiding afforestation projects, ensuring they meet ecological, social, and economic objectives. Defining the goals, whether they are carbon sequestration, biodiversity enhancement, or ecosystem service provision, will help in designing effective afforestation strategies.
- Should nutrient removal be accelerated in post-agricultural soils to create conditions more conducive to forest establishment and soil microbiome inoculation? Excess nutrients can favor fast-growing, competitive plant species and microbes that might not support long-term forest development. Investigating methods to *accelerate nutrient removal* or immobilization in soil and biomass to balance soil nutrient levels can be crucial for successful forest restoration.
- At what stages during the development of planted forests should inoculation and other management practices be reassessed? The *timing of interventions* such as soil microbiome inoculation, nutrient management, and other silvicultural practices is critical. Continuous monitoring and adaptive management throughout forest development are needed to optimize forest health and resilience.
- How can soil inoculations be done effectively on a large scale? The development of machinery to aid soil spreading adapted to run in between tree rows is needed for an efficient adoption of soil inoculations by foresters or other end users.
- What proportion of the living inoculum can successfully establish in the recipient site and impact soil processes? Understanding the *survival, establishment, and functional integration of inoculated microbes* into the native soil community is essential for assessing the effectiveness of inoculation strategies. This includes evaluating the persistence of inoculated species, their impact on soil health and forest development, and the minimum amount needed for inoculated communities to perform the desired functions.
- What is the *natural rate of inoculum spread* through the soil and forest ecosystem? Investigating the dispersal mechanisms and rates of inoculated microbes can inform the design of inoculation strategies, including the optimal placement and frequency of inoculations.
- What are the *long-term implications of soil microbiome inoculation* for forest development and ecosystem functioning? Longitudinal studies are needed to assess the sustained impact of inoculation on forest health, productivity, biodiversity, and ecosystem services over decades.
- To what extent can the *manipulation of soil microbial communities facilitate or follow vegetation changes* in restoration projects? Understanding the interplay between soil microbes and plant communities is crucial for designing interventions that support desired vegetation outcomes and ecosystem functions.

Author Contributions

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.