

Pine wilt disease as a holobiont disorder: microbiome assembly as a potential management target

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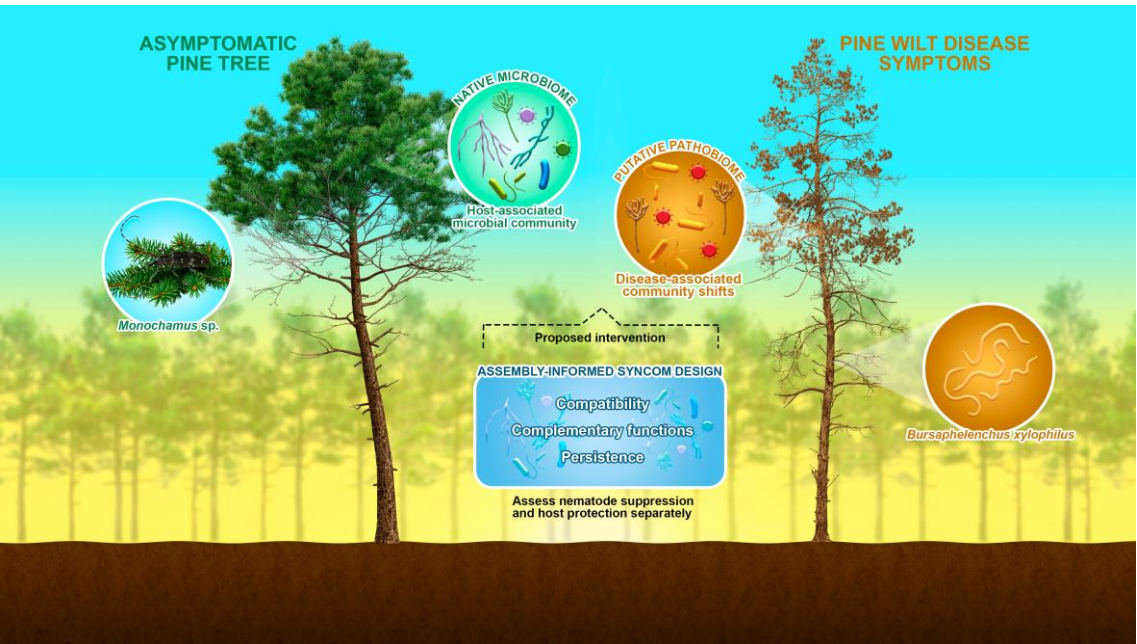
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Abstract

Pine wilt disease (PWD), caused by the pinewood nematode (PWN) *Bursaphelenchus xylophilus*, continues to expand globally despite decades of pathogen-focused management. Here, we propose that disruption of microbiome assembly within the pine holobiont may contribute to disease progression and help explain variation in disease outcomes and responses to microbial interventions. Drawing on recent advances in microbial ecology, multi-omics, and synthetic biology, we synthesize evidence of PWD-associated changes in host-associated microbial communities and examine how altered functional redundancy and pathobiome formation may contribute to disease progression. We propose that microbiome assembly, shaped by host traits, environmental filters, and biotic interactions, is a plausible and underexplored determinant of disease outcome. Building on this framework, we outline a shift from single-agent biocontrol toward assembly-informed microbiome engineering. To support reproducible design and future validation, we outline criteria for selecting candidate consortium members and assessing synthetic microbial community (SynCom) establishment, persistence and

functional stability. We further propose experimentally testable strategies to compare assembly-informed versus conventional interventions and identify measurable indicators of microbiome stability and disease suppression. By reframing PWD as a problem of microbiome assembly and stability, this work provides a testable framework for developing durable, holobiont-level management strategies. Integrating ecological theory with applied microbiome engineering offers a path forward to move beyond inconsistent control outcomes and toward resilient forest health under ongoing environmental change.

Graphical abstract



Keywords Biological control; *Bursaphelenchus xylophilus*; Community assembly; Microbiome engineering; Pathobiome; Pinewood nematode; Synthetic microbial communities.

1. Introduction

The continued spread of pine wilt disease (PWD) raises questions about how management can move beyond containment to improve host protection. Caused by the pinewood nematode (PWN, *Bursaphelenchus xylophilus*), the disease affects pine forests across East Asia and the Iberian Peninsula (Back et al., 2024), threatening ecosystems that make a substantial contribution to global carbon storage (Zhou et al., 2024). Its

economic, environmental and social consequences underpin the nematode's designation as an A2 quarantine organism by the European Plant Protection Organization and its ranking among the European Union's ten priority forestry pests (Schneider et al., 2025). International trade creates opportunities for new introductions (Robinet et al., 2009; Eyre et al., 2018), while climate change alters climatic suitability for the nematode, its pine hosts and *Monochamus* vectors, increasing the potential for further spread (Zhou et al., 2024). Reports from Portugal (Mota et al., 1999), Spain (Abelleira et al., 2011), Armenia (Arbuzova et al., 2025) and France (Folcher et al., 2026) illustrate the continuing need for surveillance and preparedness beyond established outbreak areas. Within affected regions, however, preventing further spread remains difficult. Clear-cutting has shown limitations as a containment measure (Robinet et al., 2020, 2024), and proposed improvements such as selective felling address the cost-effectiveness of tree removal (Sun et al., 2026), leaving scope for complementary approaches that target disease development within the host. Understanding the processes that influence susceptibility and disease progression, including interactions within the host-associated microbiome, may help identify such opportunities. PWD arises from a complex tripartite interaction among a susceptible host, the nematode, and its insect vector (Futai, 2013). However, the classical disease triangle is being reframed to incorporate a fourth dimension, the microbiome, which exerts a considerable influence on host–pathogen–vector dynamics (Leveau, 2024). Each component of the PWD pathosystem harbors distinct microbial communities that likely interact and collectively modulate disease development and progression. Furthermore, plant-associated microbiomes are dynamic rather than static, varying across species, spatial scales, and temporal contexts (Angulo et al., 2022; Thingujam et al., 2024). Accumulating evidence indicates that PWD is consistently associated with destabilization of these microbial communities, suggesting that disease progression reflects not only pathogen invasion but also disruption of microbiome structure and function. Such disease-associated disruption is commonly termed dysbiosis, a concept best treated as a descriptive label for community disruption associated with impaired host outcomes rather than a defined mechanistic state, given the difficulty of separating cause from consequence in observational studies (Thomas et al., 2026). The concept of the holobiont frames such systems as integrated biological units comprising a host and its associated microbiota (Margulis, 1991). This framework has expanded to encompass pathobiomes, in which certain microbial members (pathobionts) contribute to disease under specific environmental or genetic conditions (Bordenstein & Theis, 2015;

Pitlik & Koren, 2017; Mesny et al., 2023; Alfaro-García et al., 2025). Understanding PWD, therefore, requires examining how microbial communities merge and reorganize, a process known as community coalescence, where distinct communities integrate into a new, inseparable entity (Rillig et al., 2015). These coalesced networks often exhibit emergent properties, as cooperative or competitive interactions among microbes drive collective functions beyond the capabilities of individual taxa (Brenner et al., 2008; Xie & Shou, 2021). Within this framework, we propose that disruption of microbiome assembly within the pine holobiont may contribute to PWD progression through changes in community composition, functional redundancy (i.e., overlap in key ecological functions among taxa), and the emergence of pathobiome states. This claim concerns the determinants of disease progression and outcome, and not the capacity of the nematode to initiate infection: axenic *B. xylophilus* induces wilt in the absence of its associated bacteria, a long-contested claim now confirmed across a graded series of host systems (Zhu et al., 2012; Faria et al., 2015; Zhu et al., 2026; reviewed by Nascimento et al., 2015). Because plants actively recruit and reshape their microbiomes throughout their life cycle (Berg et al., 2017; Santoyo, 2022; Spooren et al., 2024), microbiome assembly, shaped by host traits, environmental filters, and biotic interactions, emerges as a plausible, yet underexplored, determinant of disease trajectories. Moreover, proof-of-concept studies with engineered endophytes demonstrate the feasibility of endophyte-based interventions against PWD (Li et al., 2023, 2026a, 2026b). Preemptive inoculation with *Esteya* spp. reduced nematode reproduction in *P. pinaster* seedlings without consistent improvements in disease outcomes (Pires et al., 2026), motivating tests of whether assembly-informed interventions can improve the translation of nematode suppression into host protection. Future controlled comparisons will be essential to determine whether assembly-informed designs outperform existing management approaches, a hypothesis that warrants systematic evaluation. Such insights are especially relevant in tolerant pine species and can potentially provide substantial benefits for future research on targeted biopesticides employing synthetic microbial communities (SynCom), defined as deliberately assembled consortia of functionally complementary microorganisms.

Here, we synthesize evidence of PWD-associated microbiome changes, focusing on pine-associated communities and their interactions with the nematode, insect vectors, and environmental conditions. We use this evidence to develop a testable framework linking microbiome assembly to variation in disease progression and microbial

intervention outcomes. To support reproducible design and future validation, we outline criteria for selecting candidate consortium members and assessing SynCom establishment, persistence and functional stability. Finally, we outline research directions aimed at supporting assembly-informed, microbiome-based strategies capable of delivering durable and resilient management outcomes.

2. Advances in environmental microbiome profiling

Testing whether microbiome assembly contributes to PWD progression requires approaches that distinguish community composition from the processes governing microbial establishment, interaction, and function within the holobiont. The study of environmental microbial communities has undergone a rapid evolution in recent years, largely driven by advances in molecular and computational technologies. Classical culture-dependent methods capture only a fraction of this diversity. In contrast, modern tools such as high-throughput sequencing platforms enable comprehensive surveys to unravel the complexity of microbial communities across diverse biological systems, including trees, insect vectors, and soils (White et al., 2016; Bronzato Badial et al., 2018). Metagenomics, metatranscriptomics, and metaproteomics allow for the simultaneous detection and functional assessment of microbial taxa, revealing not only who is present but also the roles they may play in disease dynamics (Aguiar-Pulido et al., 2016; Hassa et al., 2018; Sereika et al., 2025; Xu et al., 2026). Critically, these approaches provide a foundation for linking shifts in community composition to functional changes associated with microbiome assembly destabilization and pathobiome emergence. For example, these technologies have been applied to identify shifts in bacterial and fungal communities in pine tissues colonized by *B. xylophilus* or its insect vectors, providing insights into potential synergistic or antagonistic interactions within the pathosystem of PWD (Jones et al., 2008; Rodrigues et al., 2021; Zheng et al., 2022; Ge et al., 2023; Koski et al., 2024). The use of amplicon sequencing of ribosomal markers (16S, ITS) has become a standard tool for comparing microbial diversity between healthy and infected environments, uncovering microbial indicators of disease progression (Fukuda et al., 2016). Likewise, interactions among SynCom candidates can be evaluated in co-inoculation experiments by tracking their relative abundance using amplicon sequencing (Carlström et al., 2019). Nevertheless, RNA-based amplicon sequencing has limitations and can be biased by primer choice, rRNA operon copy number, RNA integrity, and

rRNA abundance may not reflect activity (Straub et al., 2020; Shi et al., 2021; Wang et al., 2023). These constraints highlight the need for integrative approaches capable of disentangling presence, activity, and ecological function. Multi-omics datasets are increasingly integrated with advanced bioinformatics and machine learning, providing cross-validation for community inference and helping identify keystone taxa and ecological networks relevant to disease dynamics. Such integrative analyses are particularly important for identifying assembly rules, interaction networks, and potential tipping points associated with microbiome instability. Furthermore, a holo-omics framework that jointly tracks host, nematode, vector, and microbiome will be required to resolve timing and directionality in this system and guide targeted interventions (Nyholm et al., 2020; Xu et al., 2021; Berruto & Demirer, 2024). By capturing these cross-kingdom interactions, holo-omics approaches provide a pathway toward predictive models of microbiome assembly and disease progression. Beyond sequencing, imaging techniques combined with molecular probes allow visualization of microbial colonization within host tissues (Wang et al., 2020; Li et al., 2023), and metabolomics or stable isotope probing shed light on functional roles and host defense (Uhlik et al., 2013; Aguiar-Pulido et al., 2016; Adejor et al., 2025). These complementary methods enable spatial and functional resolution of microbial processes, which is essential for understanding how assembly dynamics unfold within specific host compartments.

Together, these approaches can link community profiles to microbial location, activity, and host responses. Combined with longitudinal sampling and controlled perturbations, they can help distinguish disease-associated changes from microbial processes that contribute to disease progression. The following section synthesizes the available patterns and considers their implications for microbiome assembly.

3. Diversity and structure of microbial communities associated with PWD

Studies of PWD report changes in microbial composition, diversity, and inferred function across locations, host compartments, and disease stages. Recurrent observations include reduced representation of ectomycorrhizal fungi (EMF), enrichment of opportunistic or saprotrophic taxa, and changes in inferred microbial networks. These patterns are consistent with altered community assembly, but do not by themselves establish the underlying processes or whether microbial changes contribute to disease

progression. Variation among studies further indicates that disease-associated responses depend on host identity, environmental conditions, and the compartment examined.

3.1. Geographical location

Geographic variation provides a natural lens through which to examine how environmental filtering shapes microbiome assembly and contributes to disease-associated destabilization. The assembly of the pine microbiome appears to be influenced by environmental filters, but current evidence is primarily correlational across sites. Microbial communities associated with PWD display distinct biogeographic patterns, indicating that assembly trajectories diverge across locations rather than converging toward a single disease-associated configuration (Figure 1). Although the bacterial assemblages carried by the PWN appear broadly consistent across regions, reflecting flexible yet recurrent associations rather than strict specificity, the endophytic microbiomes inhabiting PWD-infected pine wood show pronounced spatial differentiation. This contrast suggests that, while the nematode may introduce a relatively conserved microbial consortium, local environmental conditions and host context exert stronger control over *in planta* assembly processes. Differences in community structure between forest sites, together with the increase in bacterial diversity observed with disease severity, likely reflect a relaxation of host-mediated filtering rather than enhanced functional resilience, and suggest that local environmental factors and host physiological status strongly shape the composition of wood-associated bacterial communities (Proença et al., 2010, 2014, 2017). The increase in diversity with disease severity is consistent with the view that dysbiosis often manifests as increased stochasticity and among-host dispersion, and that the direction of diversity change is context-dependent rather than a reliable indicator of host health (Thomas et al., 2026). However, because current insights are drawn from heterogeneous and largely observational studies, quantitative variance partitioning and manipulative experiments are needed to establish causality and estimate effect sizes. Disentangling the relative contributions of environmental filtering, host condition, and stochastic colonization will be essential to determine whether geographic variation reflects alternative stable states or transient trajectories of assembly disruption.

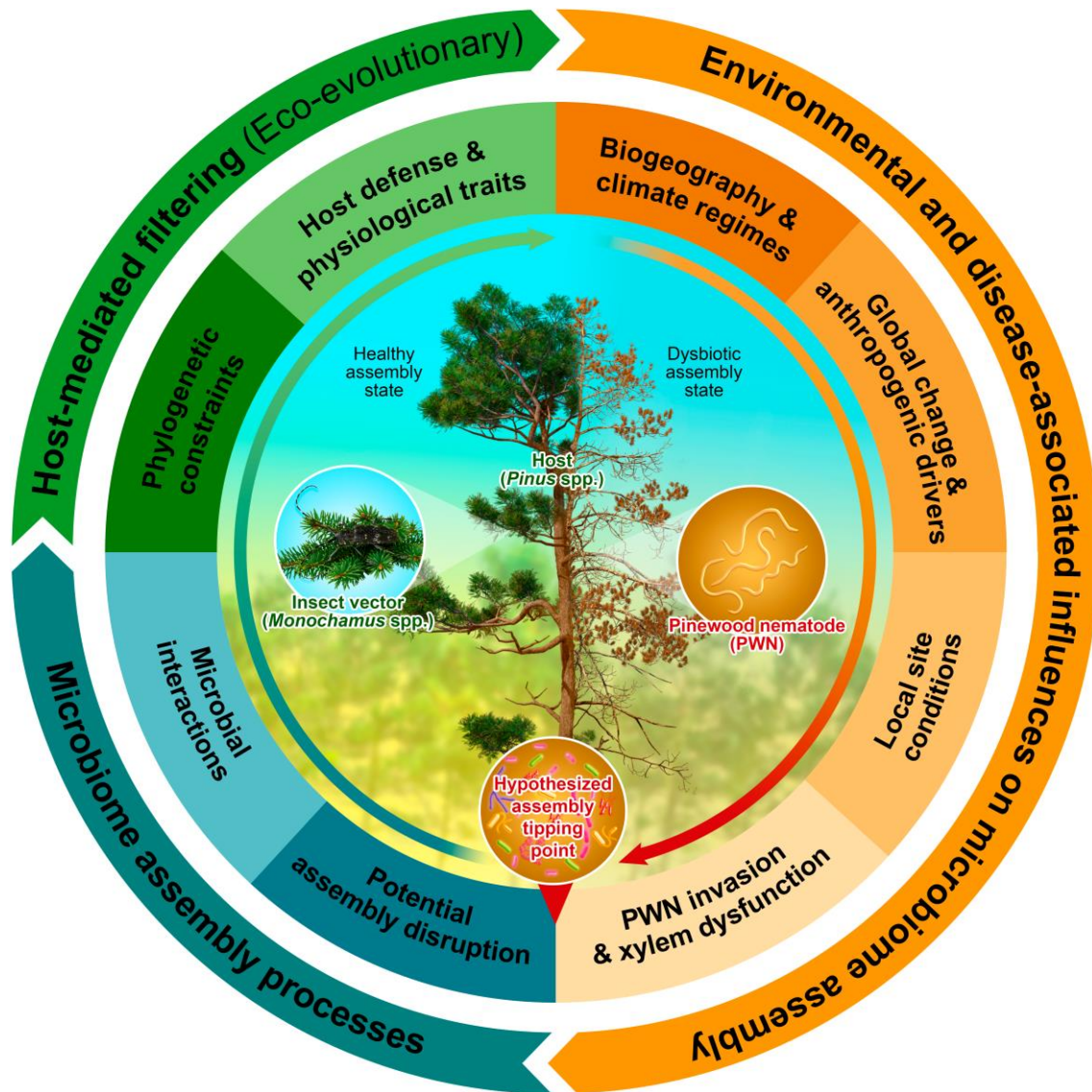


Figure 1. Conceptual framework linking host-mediated filtering, environmental and disease-associated influences, and microbiome assembly processes in pine wilt disease. Outer arrows indicate the direction of the conceptual sequence, while inner arrows represent hypothesized feedbacks among host condition, microbiome assembly and disease progression. The central illustration depicts the proposed transition from healthy to dysbiotic assembly, including a hypothesized tipping point. Colors distinguish host-mediated filtering (green), microbiome assembly processes (teal), and environmental and disease-associated influences (orange).

Key bacterial genera such as *Pseudomonas*, *Rhodococcus*, and *Acinetobacter* are consistently detected across PWD systems, yet their relative abundance and community composition vary across regions and hosts, reflecting distinct biogeographic and host-associated patterns (Proença et al., 2014; Cao et al., 2024). Likewise, fungal communities

also show site-specific differences, with diversity and dominant taxa varying between locations (Vicente et al., 2022). Some studies report richer or more complex microbial communities in asymptomatic trees, although the direction of diversity changes varies among microbial groups, host compartments, and study systems. For instance, asymptomatic *P. pinaster* hosts exhibit richer fungal assemblages, with balanced representation across major fungal phyla such as Ascomycota and Basidiomycota (Vicente et al., 2022, 2025). This diverse mycobiome likely contributes to host resilience by maintaining key functional guilds, including EMF that support nutrient acquisition and stress tolerance. Beyond host-specific effects, the composition of microbial communities in pine forests also varies geographically. Site-specific environmental conditions shape fungal species richness, diversity, and community structure across different regions (Brodde et al., 2025; Vicente et al., 2025). These spatial patterns underscore the ecological importance of local microbial assemblages in sustaining host health under changing abiotic and biotic pressures. Understanding these baseline dynamics is essential for i) assessing how the pine holobiont responds to biotic disturbances such as PWN infection, and ii) elucidating how the holobiont can self-organize under stress.

3.2. Host tree

Within the pine host, microbial communities occupy compartments that differ in resource availability, host regulation, and exposure to environmental inputs. Comparing these compartments can reveal where disease-associated changes occur and generate hypotheses about the assembly processes involved.

3.2.1. Rhizosphere (roots)

The rhizosphere represents a primary interface between the host and soil microbiome, where environmental filtering and host-mediated selection jointly shape community assembly. PWD significantly reshapes the rhizosphere microbiome. Infected trees exhibit pronounced shifts in fungal community structure, typically characterized by a decline in EMF and an increase in saprotrophic guilds, while overall fungal diversity may rise or fall depending on host species and site conditions. Bacterial assemblages likewise change, with decreases in beneficial groups such as *Actinobacteria* and increases in taxa associated with stress and disease progression. These shifts suggest an early

relaxation of host filtering and reconfiguration of community composition under biotic stress, with responses modulated by host identity, management practices, and local soil properties (Guo et al., 2023; Jiao et al., 2023; Ahmed et al., 2025; Hou et al., 2025; Mannaa et al., 2025). Recent research further shows that soil bacteria colonize roots in spatially distinct hotspots associated with developmental zones and disruptions in Casparian strip integrity (Tsai et al., 2025). Glutamine released from vascular tissues, including at emerging lateral roots, acts as a major attractant and is also metabolically exploited by recruited bacteria. These findings demonstrate that root microbiome assembly depends on localized host-derived cues that may be altered under disease, potentially affecting fine-scale community composition and functional partitioning (Tsai et al., 2025). Among four *Pinus* species (*P. densiflora*, *P. koraiensis*, *P. rigida*, and *P. thunbergii*) sampled across various locations in Korea, roots exhibited the highest relative abundance and richness of putative bacterial endophytes (Liu et al., 2019). Roots and stems also shared more bacterial isolates than stems and needles, while only *P. densiflora* and *P. thunbergii* contained endophytes common to all three tissues (Liu et al., 2019). Whether PWD alters these spatially structured recruitment processes, and whether such changes precede or follow host decline, remains to be tested. Building on this evidence, recent studies suggest that pine trees actively modulate their root-associated microbiota in response to stress. In *P. pinaster*, for instance, Lasa et al. (2025) reported a stress-induced enrichment of plant growth-promoting microorganisms (PGPM) such as *Bacillus*, *Neobacillus*, *Peribacillus*, and *Streptomyces* in both the rhizosphere and root endosphere. This enrichment is consistent with a compensatory “cry for help” response, although its effects on community function and host condition remain to be established. These findings highlight the capacity of pine roots to selectively recruit beneficial microbes under biotic and abiotic stress (Santoyo, 2022; Park et al., 2023; Lasa et al., 2024, 2025).

3.2.2. Endosphere (stem)

In the stem, microbiome assembly is more tightly constrained by host internal conditions. PWD nevertheless causes substantial changes in endophytic communities of stems and wood. In some cases, bacterial diversity increases with disease severity, potentially reflecting weakened plant defenses and subsequent colonization by soil

microbes, while fungal and bacterial communities show increased representation of pathogenic and saprotrophic taxa and reduced beneficial endophytes. Together, these changes suggest a shift from a host-regulated microbiome toward a more permissive and opportunistic assembly regime. Studies across different *Pinus* species and geographic regions consistently show that PWD profoundly disrupts the native endophytic microbiome. In *P. pinaster*, the invasion of *B. xylophilus* caused strong shifts in bacterial composition between sites, with *Proteobacteria* and *Firmicutes* dominating, yet the overall effect of infection was comparable, with microbial diversity peaking in severely affected trees. Similarly, in *P. koraiensis*, nematode infection led to pronounced structural changes in endophytic communities, with fungal diversity and abundance increasing while bacterial diversity declined, accompanied by compositional and functional reorganization of dominant taxa such as *Graphilbum* and *Pseudoalteromonas*. In *P. massoniana*, infected trees exhibited increased abundance of pathogenic and saprotrophic fungi, including *Saitozyma*, *Graphilbum*, and *Ophiostoma*, and changes in metabolic potential consistent with weakened resin defenses and altered nutrient cycling. Collectively, these patterns are consistent with altered host-mediated filtering and microbial resource use, but their contribution to subsequent disease progression remains unresolved (Proença et al., 2017; Hao et al., 2022; Li et al., 2024). Across four *Pinus* species, stems harbored 30% of all bacterial isolates, second only to roots, and showed the highest diversity and evenness of putative bacterial endophytes among the three tissues examined (Liu et al., 2019).

3.2.3. Phyllosphere (needles)

The phyllosphere is the most environmentally exposed host compartment and may therefore be particularly sensitive to PWD-associated microbiome disruption. Infected trees generally show reduced fungal richness, diversity, and evenness in needles, accompanied by a shift from symbiotrophic to saprotrophic lifestyles, with diseased needles dominated by saprotrophic taxa and healthy needles retaining a higher proportion of symbiotrophs, consistent with reduced representation of mutualistic associations under disease (Liu et al., 2021). In *P. thunbergii*, nematode infection significantly altered the fungal community and functional structure of needles, whereas the microbiota of roots and surrounding soil remained largely unchanged. Bacterial responses appear subtler: in *P. massoniana*, compositional differences were detected mainly in roots, whereas functional reorganization was more pronounced in diseased needles (Ma et al., 2020; Liu

et al., 2021). These compartment-specific responses highlight that microbiome assembly destabilization is not uniform but follows distinct trajectories depending on tissue-specific assembly constraints (Ma et al., 2020; Liu et al., 2021). The phyllosphere contains relatively few putative bacterial endophytes and exhibits lower bacterial richness and diversity than other pine tissues (Liu et al., 2019). Although not directly linked to PWD, studies of pine phyllosphere communities further illustrate strong environmental and spatial filtering. In *P. radiata*, bacterial and fungal communities varied with canopy position, with adjacent sections sharing more taxa than non-adjacent sections (Addison et al., 2023). Similarly, across the geographic range of *P. flexilis* and co-occurring pine species, site-specific factors were stronger determinants of bacterial community assembly than host species identity (Carper et al., 2024).

3.3. Biotic stressors

Biotic stressors act as direct drivers of microbiome assembly disruption by altering host physiology, modifying microbial interactions, and reshaping ecological selection within the holobiont. Upon infection, the PWN alters its host physiology and immune response by secreting effector proteins into pine tissues (Espada et al., 2025; Li et al., 2025; Song et al., 2025). Recent work provides direct mechanistic evidence that these effectors can actively modulate microbiome assembly: a PWN-secreted antifungal effector reshapes host-associated fungal communities, suppressing specific taxa while promoting others in ways that enhance nematode fitness (Li et al., 2026c). Together, these findings demonstrate that microbiome shifts observed during PWD are not solely indirect consequences of host decline but can result from targeted pathogen-driven manipulation of community composition. This provides a mechanistic example of pathogen-mediated community modification, while leaving open how broadly this mechanism explains the community shifts reported across PWD systems. These changes reshape selective pressures and promote reassembly toward disease-associated configurations across the holobiont (Liu et al., 2021; Vicente et al., 2022, 2025; Li et al., 2026c). For instance, PWN-infected *P. pinaster* trees exhibit simplified fungal communities compared to the complex mycobiome of asymptomatic trees (Vicente et al., 2022, 2025). Such simplification is indicative of reduced ecological complexity and loss of stabilizing interactions within the microbiome. These communities are particularly enriched in Ophiostomatales species, which likely reflects their ecological association with the PWN, as they serve as food sources during its mycophagous phase (Vicente et al., 2022, 2025).

This enrichment suggests a shift toward functionally aligned, but less diverse, microbial consortia that reinforce pathogen success. Similar microbial shifts have been observed in *P. koraiensis*, where PWD has been shown to increase fungal α -diversity in the rhizosphere, while microbial diversity in the root endosphere and bulk soil remains largely unaffected (Hou et al., 2025). A recent study has reported higher fungal (e.g., *Leptographium*) and bacterial (e.g., *Burkholderia-Caballeronia-Paraburkholderia*) diversity in diseased pines, suggestive of active microbiome modulation during disease progression (Yang et al., 2026). As observed in other compartments, increases in diversity under disease conditions likely reflect relaxation of host filtering and opportunistic colonization rather than enhanced functional resilience. Nevertheless, the taxonomic composition of both fungal and bacterial communities in the rhizosphere and bulk soil is significantly altered. Specifically, PWN infection reduces the relative abundance of EMF and favors saprotrophic taxa both in the rhizosphere and bulk soil, further reshaping soil microbial functions and nutrient dynamics (Hou et al., 2025). These compositional shifts are consistent with a transition from mutualistic to opportunistic functional states, reinforcing microbiome assembly destabilization. Microbial community simplification in response to biotic stress is not exclusive to the PWN. A recent study has found that the feeding activity of longhorn beetles such as the PWN vector *M. saltuarius* significantly disrupts the native fungal community in *P. koraiensis*, favoring plant pathogens and diminishing the abundance of lignocellulosic depolymerizing fungi (Zhu et al., 2025). Likewise, in declining *P. pinaster* trees attacked by *Matsucoccus feytaudi*, simplified root microbial networks were also reported compared to asymptomatic counterparts (Lasa et al., 2025), suggesting that biotic stress broadly disrupts the root microbiome and potentially hinders microbial functions. These examples suggest that different biotic stressors can produce partly overlapping community responses, although changes in diversity or inferred network structure do not alone demonstrate reduced functional stability. Nevertheless, an enrichment in PGPM was observed in the rhizosphere and root endosphere of symptomatic *P. pinaster* trees, pointing to a selective recruitment of beneficial microbes by plants under biotic stress (Lasa et al., 2025). Indeed, plants under biotic stress are known to release root exudates as a “cry for help” to attract natural enemies (Dicke, 2009; Rolfe et al., 2019). This compensatory recruitment represents an active host attempt to re-stabilize microbiome assembly under stress. On the other hand, pathogens have been shown to secrete effectors that inhibit this recruitment to successfully invade plant hosts (Kraege et al., 2025). The interplay between host-driven

recruitment and pathogen-mediated suppression highlights a dynamic feedback loop that ultimately shapes microbiome assembly outcomes.

Beyond community structure, stress conditions can trigger functional shifts in microbial behavior. For instance, *Diplodia sapinea*, a common endophyte and known causal agent of diplodia tip blight, can shift to a latent pathogenic state under abiotic or biotic stress in Scots pine (*P. sylvestris*) (Brodde et al., 2025). These transitions illustrate how microbiome members can switch functional roles under disrupted assembly conditions, thereby amplifying disease progression and exacerbating host decline.

3.4. Abiotic and dispersal-driven filters of microbiome assembly

In addition to biotic drivers, abiotic stressors act as external filters that reshape microbiome assembly by altering environmental conditions, resource availability, and host physiological status. Like biotic stressors, abiotic filters do not merely reshape community composition but can redirect microbiome assembly trajectories toward alternative, potentially less stable states. Abiotic factors such as soil pH, moisture, nutrient availability, and forest management practices (e.g., thinning, replanting) strongly influence microbial community structure and function. These factors modulate the strength and direction of environmental filtering, thereby influencing which taxa can establish, persist, or dominate within the microbiome. PWD alters microhabitat conditions, such as increased light and temperature resulting from the wilting canopy, which can further shift microbial diversity and composition. Such feedbacks between disease progression and abiotic conditions can reinforce microbiome assembly destabilization by creating increasingly permissive environments for opportunistic taxa. Depending on how they are implemented and on site-specific conditions, management interventions may either help stabilize microbial communities or unintentionally intensify these ecological changes (Qu et al., 2020; Guo et al., 2023; Zhang et al., 2025). Global change is also anticipated to induce profound and unprecedented shifts in microbial community composition. For instance, although prolonged drought does not significantly affect the total abundance or biomass of soil microorganisms, it alters community structure by favoring desiccation-tolerant taxa. Specifically, prokaryotic communities tend to shift from copiotrophic to oligotrophic lifestyles under sustained water stress in *P. sylvestris* mesocosms, potentially resulting in the loss of ecosystem functions mediated by the soil microbiome (Jaeger et al., 2024). Such shifts indicate that abiotic stress can

alter community composition through selection for stress-tolerant taxa; their consequences for functional redundancy and ecosystem resilience require direct assessment. Beyond local environmental conditions, microbiome assembly is also shaped by dispersal processes and early-life microbial inheritance, which define the initial inoculum available for host colonization. Recent evidence indicates that pine reproductive structures, including seeds and pollen, harbor distinct microbial communities that can act as reservoirs of vertically or horizontally transmitted symbionts (Deckert et al., 2019; Armstrong et al., 2024). These early-life microbial assemblages may pre-condition subsequent microbiome assembly trajectories, influencing which taxa are available to colonize emerging seedlings and how communities respond to later environmental or biotic perturbations. In pollen, for example, microbial communities have been shown to include metabolically active taxa with potential roles in host physiology and defense, suggesting that dispersal units can carry functionally relevant microbiota across spatial scales (Madmony et al., 2005; Armstrong et al., 2024). Such propagule-associated microbiomes introduce an additional layer of ecological filtering, linking dispersal processes with microbiome assembly outcomes in new hosts. Host genetic background further interacts with abiotic conditions to shape microbiome composition. In common garden experiments, pine species have been shown to structure their associated fungal communities despite shared environmental conditions, indicating that host identity imposes selective constraints on microbiome assembly (Romeralo et al., 2022). This interaction between host genotype and environmental filtering suggests that abiotic stress effects cannot be fully understood without considering host-mediated selection.

Collectively, these findings indicate that microbiome assembly is shaped by local environmental conditions, host-specific filtering, and the inherited and dispersal-associated microbial pools available for colonization. Under changing environmental conditions, these factors may alter assembly trajectories from the earliest stages of host development, with potential consequences for microbiome stability, disease susceptibility, and intervention outcomes. Microbiome-based interventions should therefore account for the ecological processes governing microbial establishment, interactions, and function within the pine holobiont.

4. Engineering the host microbiome to manage PWD

If microbiome assembly contributes to variation in PWD progression, manipulating microbial establishment and function could complement pathogen- and vector-directed management. An assembly-informed approach therefore tests whether introduced microorganisms establish within resident communities rather than remain transiently present, and whether their functional activity translates into host protection. Axenic *B. xylophilus* completes its life cycle and induces wilt across pine calli, axenic seedlings and potted trees up to eight years old, with disease severity comparable to non-axenic inoculations (Zhu et al., 2026). These findings establish that nematode-associated bacteria are not required for wilt under the tested conditions, but leave resident microbiome effects unresolved because host-associated communities were not experimentally controlled in the older potted trees. Interventions targeting nematode-borne bacteria (Kwon et al., 2010; Liu et al., 2017) therefore address a different question from those targeting the host microbiome.

Numerous biological control agents have demonstrated efficacy in suppressing the PWN (Paiva et al., 2013; Proença et al., 2019; Dou & Yan, 2022; Pires et al., 2022; Su et al., 2025). However, reliable field-level control remains limited, motivating closer examination of inoculant establishment and activity alongside delivery, host susceptibility and environmental conditions. Microbial engineering offers one approach to enhancing endophyte performance. For instance, *Pseudomonas koreensis* BM06, isolated from healthy stems of *P. massoniana*, efficiently colonizes its host and promotes seedling growth. An engineered derivative, BM06-P60, expressing a nematocidal peptidase, achieved approximately 70% control efficacy against the PWN in greenhouse trials. This example supports the feasibility of enhancing endophyte-mediated suppression under greenhouse conditions, while field performance, persistence, and ecological effects require further evaluation (Li et al., 2026a).

Alongside strain engineering, the ecological traits of microbial antagonists warrant consideration. Studies of *Esteya vermicola* and *E. floridanum*, two promising fungal PWN antagonists, demonstrate that both species can directly compete with key ophiostomatoid fungi associated with PWD (e.g., *Leptographium terebrantis* and *Ophiostoma ips*). The broader metabolic versatility of *E. vermicola* may favor its establishment in pine tissues, and these findings suggest that prophylactic deployment, rather than reactive application, is likely to maximize protective outcomes (Pires et al.,

2024). Tests of prophylactic inoculation have produced mixed results. In *P. densiflora*, *E. vermicola* reduced nematode density in inoculated logs and improved seedling survival when applied 10 days to one month before challenge, whereas application two days before challenge only delayed mortality (Wang et al., 2011, 2017). *Esteya floridanum* delayed wilt and death in *P. koraiensis* without preventing mortality (Li et al., 2021). In *P. pinaster*, despite a longer pre-challenge interval (5 weeks), preemptive inoculation reduced *B. xylophilus* reproduction by both *Esteya* species, yet symptom severity, disease incidence and disease progression did not differ significantly from the nematode-challenged control despite lower mean severity, and a survival advantage over that control was resolved only in the time-to-event analysis and only for *E. floridanum* (Pires et al., 2026). That trial also used a lower nematode inoculum than earlier studies (approximately 500 vs. 1,000–5,000 nematodes per plant). Across studies, outcomes varied with host, inoculum density, inoculation protocol and assessment, and reduced nematode reproduction was not consistently accompanied by improved host outcomes (Figure 2A). Observational evidence also highlights the need to distinguish nematode presence, density, and host condition: in a Portuguese production forest, *B. xylophilus* was recovered from the canopy of 58% of visually asymptomatic *P. pinaster*, and its detection did not differ significantly between healthy and declining trees; only nematode density did, increasing 21.9-fold among declining trees where the nematode was present. Nematode density also correlated with blue-stain fungi and bark beetles, while decline was concentrated on drier topographic positions (Pimentel et al., 2026). Thus, pathogen presence alone poorly discriminates host status, although endpoint sampling cannot establish whether elevated density drives decline or accumulates in already-compromised tissue. Individual inoculant performance must also be evaluated in relation to resident communities and environmental conditions. For many antagonists, that context dependence is inherent to their biology: nematophagous fungi alternate between saprotrophic and antagonistic lifestyles, as *Esteya* spp. do on maritime pine wood (Pires et al., 2024), and in trap-forming genera such as *Arthrobotrys* the shift to predation is a regulated response to nutrient limitation and prey-derived signals rather than a constitutive trait (Hsueh et al., 2013; Kuo et al., 2024; Tay et al., 2026). Antagonism is therefore a conditional phenotype whose activating conditions in pine tissues remain unidentified, so colonization cannot be equated with antagonistic activity. Community-level experiments should therefore test whether interactions with resident

microorganisms improve, limit or leave unchanged the protection achieved by individual strains.

To support reproducible and rational microbiome engineering, SynCom standards should consider three criteria: i) taxonomy, ii) functional traits, and iii) ecological compatibility of the microbial inoculants (Northen et al., 2024). Consortium members should establish in their intended compartments, remain ecologically compatible and provide complementary or synergistic functions under the relevant environmental and host conditions (Table 1; Figure 2B). A SynCom is considered stable when its members can successfully colonize pine tissues, integrate with or compete effectively against the native microbiome, maintain their intended functions, and persist over time. Transient presence following inoculation should therefore be distinguished from establishment and sustained functional activity over the period required for protection. Persistence and functional stability should be assessed through repeated measurements of member-specific absolute abundance and relevant activities over periods appropriate to the intended application, with relative abundance used to describe changes in community composition. Stability should not, however, be equated with rigidity: because plants remodel their microbiomes under stress, including through “cry for help” recruitment, consortia that remain stable under baseline conditions yet responsive to host stress signals may be preferable to rigidly engineered communities (Thomas et al., 2026).

Table 1. Candidate microbial taxa for assembly-informed SynCom design against the pinewood nematode, organized by functional role, mechanisms of antagonism, and predicted contribution to community assembly and stability. This non-exhaustive selection highlights taxa with well-documented antagonistic activity and ecological functions relevant to microbiome assembly and disease suppression.

Taxon	Function	Mechanism	SynCom role	References
<i>Bacillus subtilis</i>	Nematode antagonist; host defense inducer	Secondary metabolites; hydrolytic enzymes; ISR	Antagonist; defense priming	Xia et al., 2011; Manna et al., 2024, 2025
<i>B. thuringiensis</i>	Nematode antagonist	Secondary metabolites;	Antagonist; functional redundancy	Cheng et al., 2018; Huang et al., 2018;

		hydrolytic enzymes		En-jie et al., 2019; Park et al., 2020; Guo et al., 2022; Han et al., 2025
<i>Pseudomonas</i> spp.	Nematode antagonist; microbiome stabilizer	Secondary metabolites; ISR	Antagonist; competitive exclusion; defense priming	Li et al., 2026b
<i>Streptomyces</i> spp.	Nematode antagonist; microbiome stabilizer	Secondary metabolites	Antagonist; community stabilization	Feng et al., 2019; Kim et al., 2020; Kang et al., 2026
<i>Arthrobotrys</i> spp.	Nematode antagonist	Predation	Antagonist	Hao et al., 2023; Wu et al., 2023; Jia et al., 2024
<i>Beauveria</i> spp.	Nematode antagonist; insect-associated biocontrol agent	Secondary metabolites; hydrolytic enzymes; parasitism	Antagonist; vector suppression; functional redundancy	Wu et al., 2022; Sánchez-Gómez et al., 2023, 2025; Zhang et al., 2025
<i>Esteya floridanum</i>	Nematode antagonist	Parasitism	Antagonist; competitive interactions	Li et al., 2021; Pires et al., 2024, 2026
<i>E. vermicola</i>	Nematode antagonist	Parasitism	Antagonist; competitive interactions	Wang et al., 2011, 2017; Pires et al., 2024, 2026

<i>Trichoderma</i> spp.	Nematode antagonist; host defense inducer	Mycoparasitism ; hydrolytic enzymes; ISR	Antagonist; competitive interactions; defense priming	Chen et al., 2024, 2026
EMF (<i>Amanita vaginata</i> , <i>Suillus bovinus</i> , <i>Pisolithus orientalis</i>)	Microbiome stabilizer; host resilience	Nutrient exchange; stress tolerance	Community stabilization; host buffering	Akema & Futai, 2005; Chu et al., 2021; Chen et al., 2023

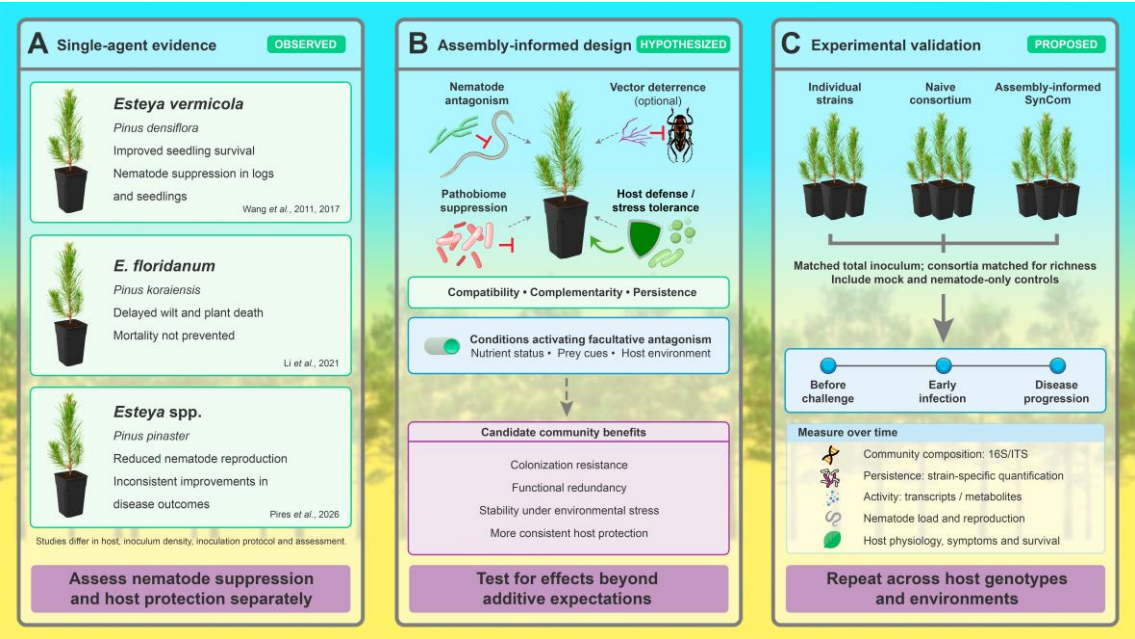


Figure 2. From nematode suppression to testable community design. **(A)** Observed outcomes of single-agent *Esteya* inoculation across hosts and conditions. **(B)** Hypothesized assembly-informed SynCom combining complementary functions, with the conditions activating facultative antagonism treated as design parameters (Table 1). **(C)** Proposed comparison of individual strains, naive consortia (assembled without compatibility or functional criteria) and assembly-informed SynComs over time. Dashed arrows = hypothesized effects; T-bars = inhibition.

Together, these findings support testing assembly-informed deployment of native or engineered microbial candidates. The central question is whether explicit consideration of ecological compatibility, functional expression, and resident communities improves the consistency of host protection relative to individual strains or consortia assembled

without these criteria. Validation across host species and environmental settings will be necessary to establish the scope and practical value of this approach.

5. Conclusions and future perspectives

This review proposes microbiome assembly as a framework for investigating variation in PWD progression and the performance of microbial interventions. Interactions between introduced microorganisms and the resident microbiome depend on environmental conditions, host genotype and ecological setting, constraining establishment and function (Causevic et al., 2025). Existing microbial antagonists provide candidate members for assembly-informed SynComs, but their combination should be justified by ecological compatibility and complementary functions rather than an assumption that consortia outperform individual strains. Disease-associated community shifts provide a rationale for investigating these relationships, while the causal contribution of assembly disruption to disease progression remains to be established. The distinction between infection, symptom expression, and transmission also matters for containment. Asymptomatic trees harboring nematodes may represent reservoirs of infection, although their contribution to subsequent transmission remains unresolved (Pimentel et al., 2026). Host protection and transmission risk should therefore be assessed separately when evaluating microbiome-based interventions. A central experimental priority is to determine when microbial establishment and antagonistic activity translate into host protection. Preemptive *Esteya* inoculation reduced nematode reproduction without consistent improvements across disease endpoints (Pires et al., 2026), illustrating why suppression and host outcomes require separate assessment. Longitudinal studies should jointly track microbial persistence and activity, nematode dynamics, host physiology and symptom development. For microorganisms whose antagonistic functions depend on nutrient conditions or prey-derived signals, colonization alone cannot establish that the intended activity is expressed. Identifying these conditions is necessary for designing interventions and interpreting their performance. Assembly-informed SynComs offer a testable approach to combining nematode antagonism with complementary functions supporting host performance. Controlled comparisons with individual strains and consortia assembled without explicit compatibility or functional criteria should account for inoculum density and consortium richness, and should test whether consortium effects exceed those expected from the additive contributions of

individual members. Community profiling should be complemented by measurements of absolute abundance and target activity, alongside nematode reproduction and host outcomes. Validation across host genotypes, resident microbiomes and environmental conditions will be essential to determine whether assembly-informed design improves the consistency of protection. Microbiome-based interventions should complement phytosanitary and vector-directed measures. This framework establishes research priorities for testing whether manipulation of microbiome assembly can contribute to reliable and ecologically grounded PWD management.

CRediT authorship contribution statement

David Pires: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Margarida Espada:** Writing – review & editing. **Diogo N. Proença:** Writing – review & editing. **Cláudia S. L. Vicente:** Conceptualization, Writing – review & editing.

Data availability

No new datasets were generated or analyzed for this review.

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Declaration of competing interests

The authors declare that they have no financial or non-financial interests to disclose.

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