CellPress

Opinion

Sowing success: ecological insights into seedling microbial colonisation for robust plant microbiota engineering

Oscar Joubert [1,2,*], Gontran Arnault ![ID] [2], Matthieu Barret ![ID] [2], and Marie Simonin ![ID] [2,*]

Manipulating the seedling microbiota through seed or soil inoculations has the potential to improve plant health. Mixed in-field results have been attributed to a lack of consideration for ecological processes taking place during seedling microbiota assembly. In this opinion article, we (i) assess the contribution of ecological processes at play during seedling microbiota assembly (e.g., propagule pressure and priority effects); (ii) investigate how life history theory can help us identify microbial traits involved in successful seedling colonisation; and (iii) suggest how different plant microbiota engineering methods could benefit from a greater understanding of seedling microbiota assembly processes. Finally, we propose several research hypotheses and identify outstanding questions for the plant microbiota engineering community.

Manipulating the seedling microbiota to promote plant health

The successes of the Green Revolution in terms of widespread reduction of starvation and poverty were made possible by various inputs, including synthetic inorganic fertilisers and pesticides [1]. However, the associated environmental costs in terms of leaching of excess nitrogen and phosphorus fertilisers in groundwater, as well as pest resistance calls for the development of more sustainable agricultural practices. Plant hosts interact with a wide diversity of microorganisms that constitute the plant **microbiota** (see Glossary). The composition of the plant microbiota can affect **seedling** emergence [2,3] and plant growth [4–6] as well as diminish the effect of plant pathogens [5,7]. Using specific plant-beneficial microorganisms as an alternative to synthetic fertilisers and chemical pesticides would represent a significant achievement in the direction of a more sustainable agriculture [5,8,9].

Seeds are involved in the transmission of microorganisms within the plant life cycle [10–12]. Seeds contain **endophytic** and **epiphytic** microorganisms acquired from the parents, the atmosphere, and pollinators, of which a fraction may be transmitted to the growing seedling [12] (Figure 1A). The assembly of the seedling microbiota is the result of interactions between seed and soil microbiota in the **spermosphere** [13] (Figure 1B,C). The spermosphere is defined as a 'short-lived, rapidly changing and microbiologically dynamic zone of soil surrounding a germinating seed' [14], which is characterised by a temporary burst of **seed exudates**, including **primary metabolites** (e.g., nutrients) and **specialised metabolites**, some of which possess antimicrobial activities [5,13,15].

Early stages of the plant life cycle are crucial for crop establishment and production. Massive yield losses can occur during the first stages of crop production (germination and emergence) because of diverse abiotic and biotic stressors [16]. Among these, seed- and soil-borne pathogens pose a

Highlights

The plant microbiota is assembled dynamically from various sources that host a reservoir of beneficial taxa and functions for plant health.

Seed exudates can directly affect the interactions between seed- and soil-borne microbial communities that will constitute the primary inocula of seedling microbiota.

Common applications of microbiota engineering rely on either seed or soil inoculations of single strains or synthetic communities to modulate the composition of the plant microbiota.

Microbial community ecology, dispersal theory, and life history strategies can give us a framework for the study of seedling microbiota assembly and help develop robust microbiota engineering.

The taxonomic and functional composition of coalescent seed and soil communities may modulate colonisation success and competition intensity in the spermosphere and during seedling emergence.

[1]Master de Biologie, École Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Université de Lyon, 69342 Cedex 07 Lyon, France
[2]Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

*Correspondence:
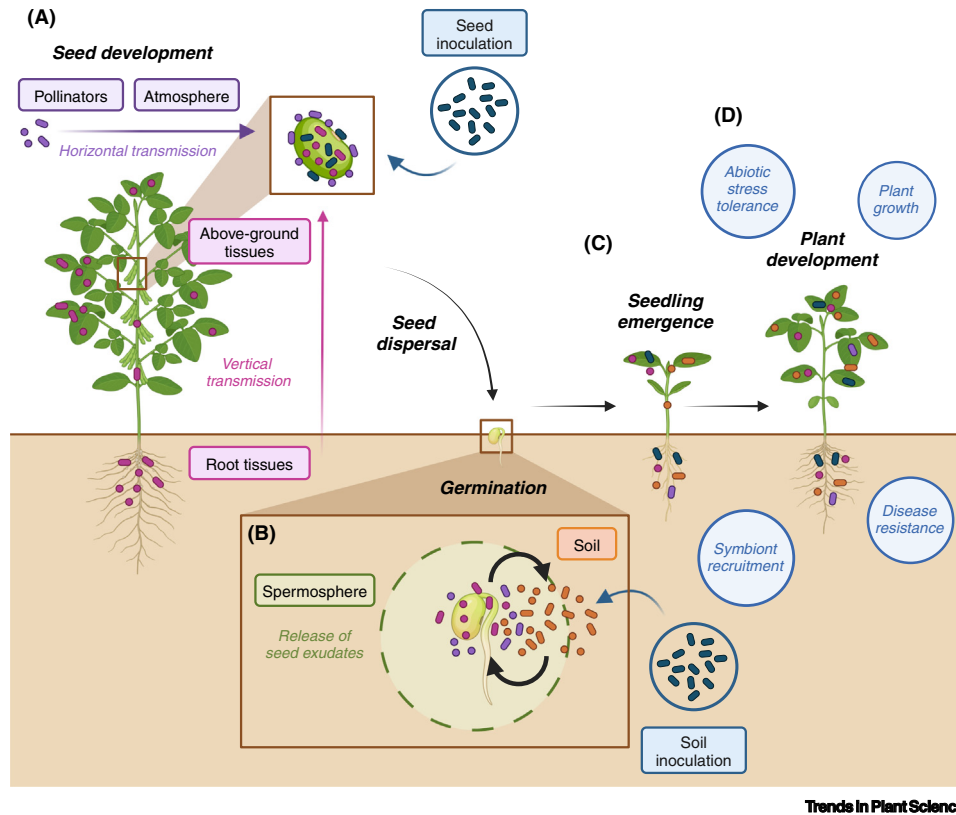oscar.joubert@ens-lyon.fr (O. Joubert) and marie.simonin@inrae.fr (M. Simonin).

Figure 1. Manipulating seedling microbiota assembly to harness microbe-driven plant-beneficial effects. (A) Seeds acquire microorganisms through vertical (from the mother plant root and the above-ground tissues) and horizontal transmission (from pollinators, the atmosphere, and during dispersal and storage). Seed and soil inoculations may influence the assembly of the seed and seedling microbiota. (B) Upon sowing and germination, seeds release a variety of compounds and seed-borne microorganisms interact with the soil microbiota in the spermosphere. (C) The resulting spermosphere microbiota colonises the above-ground tissues and roots of the emerging seedling. (D) The seedling microbiota can affect plant development and may be transmitted across generations. Created with BioRender.com.

major threat, leading to extensive use of chemical pesticides to enhance crop establishment. Manipulating the seedling microbiota through seed or soil inoculations has the potential to provide a more sustainable alternative to chemical seed treatments and resistance to global change drivers [17,18]. Phytostimulation (growth-promotion and abiotic stress-tolerance) as well as efforts to increase the nutritional value of plant-based food for human consumption could also benefit greatly from seedling **microbiota engineering** [2,19].

The study of the plant microbiota has highlighted the importance of the interplay between microbe-driven plant phenotypes and colonisation of the plant internal tissues [20]. Also, it has been suggested that the mixed in-field results observed for current inoculation approaches may be due to our incomplete knowledge of the drivers of successful colonisation of the seedling by microorganisms [21–23]. In this opinion article, we (i) assess the contribution of ecological processes at play during seedling microbiota assembly, (ii) investigate how life history theory can help us identify microbial traits involved in successful seedling colonisation, and (iii) suggest how targeted manipulations of the seedling microbiota could benefit from a greater understanding of seedling microbiota assembly.

Glossary

Community coalescence: interactions and interchanges between two or more mixing communities (source communities) in a given environment. The resulting community displays different properties and functions from its source communities.

Life history strategies: ecological theory that describes how organisms differentially invest in growth, reproduction, and survivorship. Depending on the environment, life history strategies may affect fitness.

Mass effects: the way high dispersal rates from suitable environments enable the establishment of organisms where their populations cannot be self-sustained, thus preventing environmental filters from operating.

Metabolite: term used to describe small molecules produced by the metabolism of an organism.

Microbial invasion: establishment of a microorganism in a microbial community where it was not present before.

Microbiota: community of living microorganisms (bacteria, archaea, fungi, protists, algae) present in a given environment.

Microbiota engineering: manipulation of the composition or function of complex microbial communities through the inoculation of microbial strains or consortia, genetic engineering approaches, prebiotics, drug administration, or environmental modification.

Niche: the set of environmental conditions in which a species can live and reproduce (fundamental environmental niche).

Primary metabolites: metabolites that are directly involved in growth, development, and reproduction. They include carbohydrates (alcohol, saccharides) and certain amino acids.

Priority effect: the effect of the timing of arrival of a species in a given environment on community dynamics.

Propagule pressure: the effect of the number of individuals per dispersal event on community dynamics.

Realised environmental niche: a restriction of the fundamental environmental niche taking into account biological interactions.

Rhizosphere: microbial habitat associated with the zone of soil surrounding and influenced by the root.

Seed endophytic microbiota: microbial species residing in internal

Ecological processes shaping microbial community coalescence during seedling emergence

From invasion to community coalescence

A major step forward in understanding microbiota assembly is the translation of the concept of biological invasion to microbial communities and the definition of **microbial invasion** as 'the entrance of a foreign microorganism into a resident community of microbes' [24]. An invasion is successful when the newly introduced invader overcomes abiotic and biotic barriers and grows and spreads through the acquisition of resources in the environment [24,25]. In nature, invasion processes often involve whole microbial communities and their environments merging together. These observations have led to the conceptualisation of community-driven invasion or microbial **community coalescence** [26]. Microbial community coalescence describes the interactions and interchanges between two or more mixing communities and their environments called 'source communities', as well as the properties of the resulting 'coalescent community' [27].

In the context of seedling microbiota assembly, microbial community coalescence occurs when the seed microbiota and soil microbial communities mix during sowing and germination. Applying ecological theory to microbial community coalescence can give us the framework within which we may study seedling microbiota assembly. Walter *et al.* theorise that successful engraftment of the human gut by microbial communities depends on the interplay between host-mediated effects, community-level processes, and intermicrobial interactions [25]. Here, we investigate how this framework may apply to the study of seedling microbiota assembly.

Host-mediated effects on community coalescence during seedling emergence

Niche theory describes how an organism or population responds to the distribution of resources and competitors. It postulates that microbial communities can be described by the distribution and performance of the microbial species that compose it along an environmental gradient [28] (Figure 2A). Upon microbial community coalescence, niche occupation patterns may overlap, leading members of source communities to compete for nutrients or space [23] (Figure 2B). Seed germination and seedling emergence are crucial steps for plant development characterised by important and rapid changes in physiology [5]. The host-related effects driven by these changes may exert significant selective pressure on seed and soil microbial communities and contribute to seedling microbiota assembly. Seed imbibition and the release of exudates in the spermosphere can directly affect the interactions between resident soil communities and members of the seed microbiota, driving expansion or reduction of **realised environmental niches** [14,29] (Figure 2C).

The plant immune system is known to tightly regulate microbiota composition [23]. However, the study of vertical transmission of seed-borne plant pathogens evidenced the absence of defence response induction in seedlings, allowing early efficient multiplication of seed-borne pathogens and seedling colonisation [30,31]. Still, the shift from immune permittivity to a mature immune system in later stages of seedling development (i.e., microbe-associated molecular pattern recognition) may play a significant role in seedling microbiota assembly through inhibition or facilitation of certain microbial species [5] (Figure 2D). Additionally, the passive release of antimicrobial compounds [e.g., glucosinolates, reactive oxygen species (ROS)] in the spermosphere and inside plant tissues may also exert selective pressure on microbial communities [15]. The transient release of ROS during germination known as the 'oxidative burst' has been shown to select seed-borne microorganisms displaying high ROS-processing catalase activity [32].

seed tissues. They often come from the endosphere and are vertically transmitted to progeny seedlings.
Seed epiphytic microbiota: microbial species that colonise seed surfaces. They often come from the environment or the plant surface and are both vertically and horizontally transmitted to progeny seedlings.
**Seed exudates:** cellular and vacuolar constituents of the seed released in the environment following seed imbibition.
Seedling: young plant emerging from the seed.
Specialised metabolites: metabolites derived from primary metabolites that have an important ecological function (i.e., a relational function) and are often restricted to a given taxon. They include alkaloids, antibiotics, terpenoids, and peptides.
Spermosphere: microbial habitat associated with the zone of soil surrounding a germinating seed and under the influence of seed exudates.
Synthetic community: microbial community designed by assembling several cultured microorganisms and used for the study of host–microbe and microbe–microbe interactions or for performing microbiota engineering.
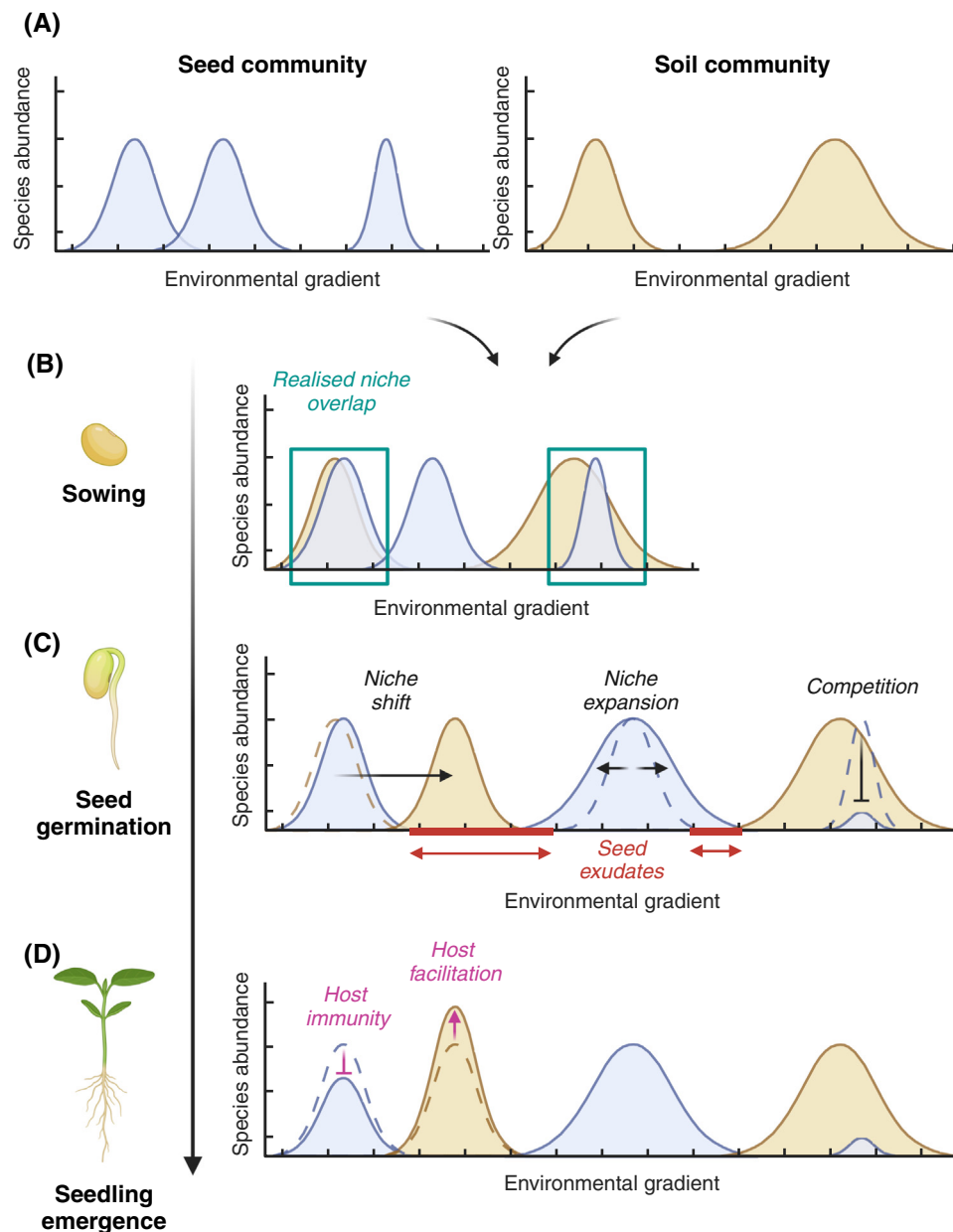
Figure 2. Niche availability, interactions between microbial communities, and host-mediated effects shape the seedling microbiota. (A) Microbial communities can be described along an environmental gradient by the occupancy of realised niches. (B) Coalescence events upon sowing may cause some realised niches to overlap. (C) During germination, overlap between niches may lead to competition between strains and changes in species abundances in the spermosphere. Seed exudates expand the environmental gradient by creating new niches. This may lower competition pressure, with certain species shifting their realised niches and niche expansion of others. (D) The emerging seedling may control microbial abundances through its maturing inducible immune system and specialised metabolite secretion. Created with BioRender.com.

The diversity–invasion relationship

The impact of the biodiversity of a microbial community (in terms of species richness and species evenness) on its resistance to invasion is known as the diversity–invasion relationship [24].

Observations that diverse microbiota are more resistant to colonisation can be attributed to an increase in overlap in the nutrient-utilisation profile between resident microbial communities and invaders [33]. In the context of community coalescence between seed and soil communities, the low richness observed on seed compared with soil microbiota might explain why soil microorganisms often dominate seedling communities in terms of relative abundance [34,35]. This hypothesis is supported by a study by Walsh *et al.* showing that inoculation of seeds with highly diverse soil slurries decreases the contributions of seed-associated bacteria to the seedling microbiota [36].

Competition-relatedness theory

Competition-relatedness theory postulates that competition should be more intense between closely related species that may share similar niches (e.g., similar substrate affinities) [37]. Community coalescence experiments in soil demonstrated that suppressed resident communities were phylogenetically closer to invaders compared with those that survived [38]. However, this observation seems to depend on the taxonomic level considered, with a study showing that the level of antagonism and phylogenetic distance are positively correlated within the *Bacillus* genus [39]. As of today, competition-relatedness theory in the context of seedling microbiota assembly has not yet been investigated.

Propagule pressure and priority effects shape the outcome of dispersal events

Dispersal, defined as the movement of species across space and time, is at the basis of every microbial community coalescence event [40]. **Propagule pressure** describes both the frequency and size of dispersal events during microbial coalescence [40,41]. Intuitively, in the absence of obstacles to dispersal, high propagule pressure would lead to higher colonisation (Figure 3A). The number of individuals that compose bacterial communities on seeds is often lower compared with those in other plant compartments or soil [36], which may explain why soil microorganisms often outcompete seed communities [35]. The importance of propagule pressure for colonisation success is supported by external inoculations of seeds with **synthetic communities** (SynComs) that show that SynCom colonisation of seedlings is positively correlated to its concentration inoculated on seeds [42].

The timing of arrival of microbial species in a community may also influence their colonisation success and their short and long term effects on the environment. Species that arrive earlier may gain a numerical advantage over late colonisers and therefore outcompete the latter (Figure 3B). This phenomenon, known as **priority effect**, has been extensively reviewed for several microbiota [43]. In particular, competition experiments between *Pantoea agglomerans* strains in wheat reveal an advantage for early incomers for seedling colonisation [44].

Towards a trait-based prediction of seedling colonisation success upon microbial community coalescence

Under given environmental conditions, it is often suggested that the outcome of community coalescence would be primarily driven by resource competition and other microbe–microbe interactions [23,38,45,46]. Competition and mutualism result from trade-offs in traits displayed by microbial populations [23,25]. The concept of **life history strategies** describes the way specific environments select certain trait combinations (or strategies) that maximise the fitness of an organism within a given context [47,48] (Box 1). Identifying which strategies are selected in the spermosphere and seedlings could help predict which microbial taxa would successfully colonise these environments upon community coalescence.
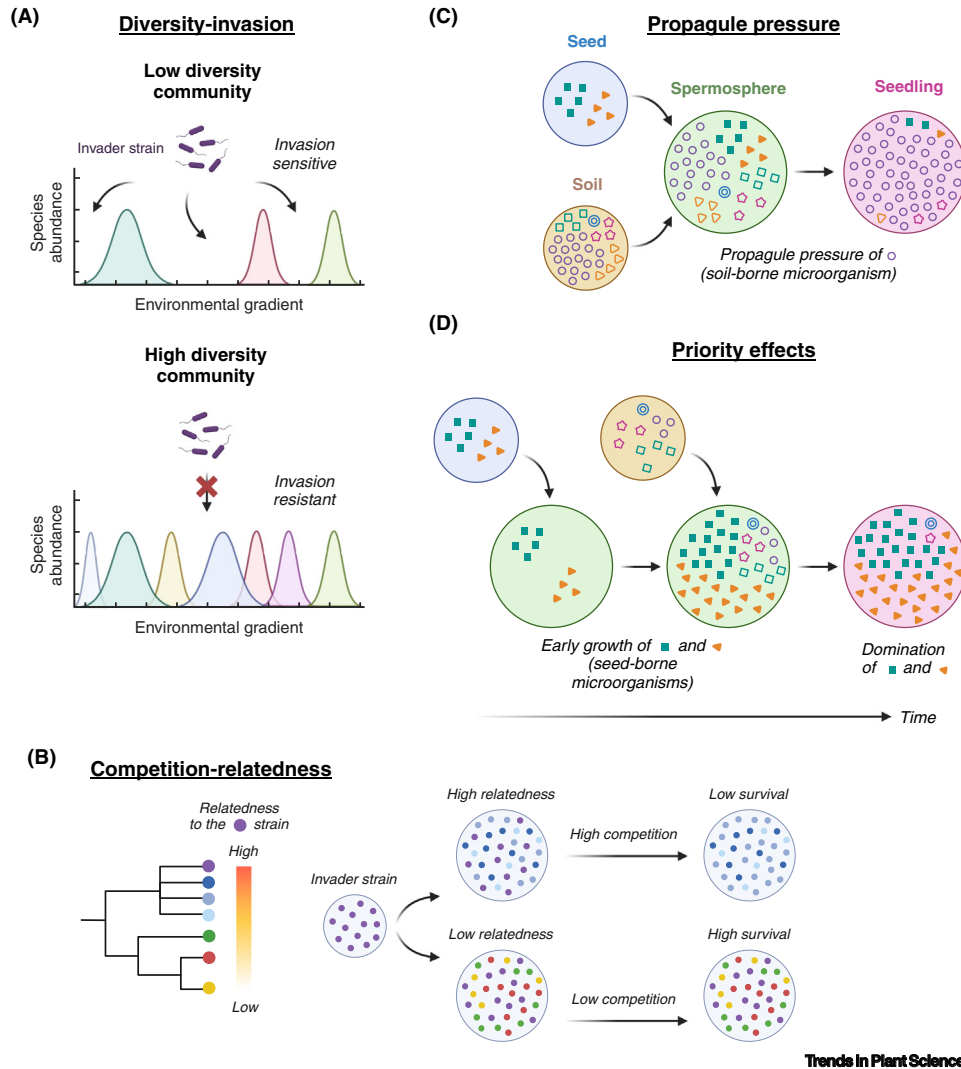
Figure 3. Composition of resident communities as well as the size and timing of dispersal influence the composition of coalescent communities in the spermosphere. Diversity of the resident community (A) and relatedness with invaders (B) affect the outcome of community coalescence. (C) The size of microbial communities contributes to their colonisation success through propagule pressure. (D) Priority effects theory posits that early incomers are advantaged compared with late incomers. Created with BioRender.com.

Seedling emergence is expected to favour copiotrophs and opportunists

Seeds are often considered a resource-poor and stressful environment that would be dominated by oligotrophs and stress-tolerant microorganisms [12]. Comparatively, soils are spatially and temporarily variable environments that would favour opportunists and oligotrophs [49]. However, because abiotic variables (e.g., soil pH, nutrient concentrations, organic carbon pools) may differ between soil types, selected strategies may differ accordingly [36]. Seedling emergence is associated with high nutrient availability following seed imbibition, and seed exudates may provide a wide diversity of potential substrates for microbial growth, such as sugars and amino acids [5,50]. We can thus hypothesise that the contribution of soil and seed communities to the seedling microbiota would be mainly composed of copiotrophic, opportunistic, and competitor microorganisms.

Investigating trait combinations required for seedling microbiota assembly

In order to test the hypotheses derived from life history theory for seedling microbiota assembly, we need to identify trait combinations that are specifically selected during seedling colonisation. The functional potential of a microbial community can be inferred from metagenome-assembled genomes reconstructed from shotgun-sequencing data [51]. Acquiring causal mechanistic understanding of interaction between microorganisms during seedling microbiota assembly can be achieved through gene mutation experiments. Work by van Dijk and Nelson evidenced that knocking-out genes involved in fatty acid metabolism modified competition and biocontrol activity of *Enterobacter cloacae* against *Pythium ultimum* [52]. Genome-wide transposon mutagenesis approaches relying on randomly barcoded transposon sequencing (RB-TnSeq) will also identify microbial genes participating in seedling colonisation [53,54]. Studying the changes in the metatranscriptome of microbial communities during seedling microbiota assembly may also reveal gene expression patterns during seedling emergence [55].

Most studies investigating seedling microbiota assembly have focused on the differences in taxonomic composition between source communities (soil and seed) and the seedling community. Although no clear phylogenetic pattern seems to emerge, bacteria from the *Pseudomonas* and *Pantoea* genera are often among the dominant seedling taxa. Interestingly, these taxa have also been shown to be part of a core seed microbiota shared by some 20 plant species [56]. Studies comparing the functional potential of the seed, soil, and seedling microbiota are scarce. Torres-Cortés *et al*. found that seedling emergence selected microbial genes associated with carbohydrate metabolism, amino acid metabolism, and transcription [51]. They correlated this with the release of saccharides and amino acids during seed germination, suggesting that successful taxa are those able to use these substrates. Furthermore, they found that the genome of successful seedlings colonisers had higher *rrn* copy numbers. These observations support the hypothesis of an enrichment in fast-growing copiotrophic microorganisms during seedling emergence. More studies linking taxonomic composition and functional potential are needed to confirm these results.

Addressing inconsistencies in inoculation outcome and improving colonisation success

Research on the plant microbiota and its applications to agriculture, pollution, and climate change mitigation has been booming in the last decade thanks to major advances in meta-
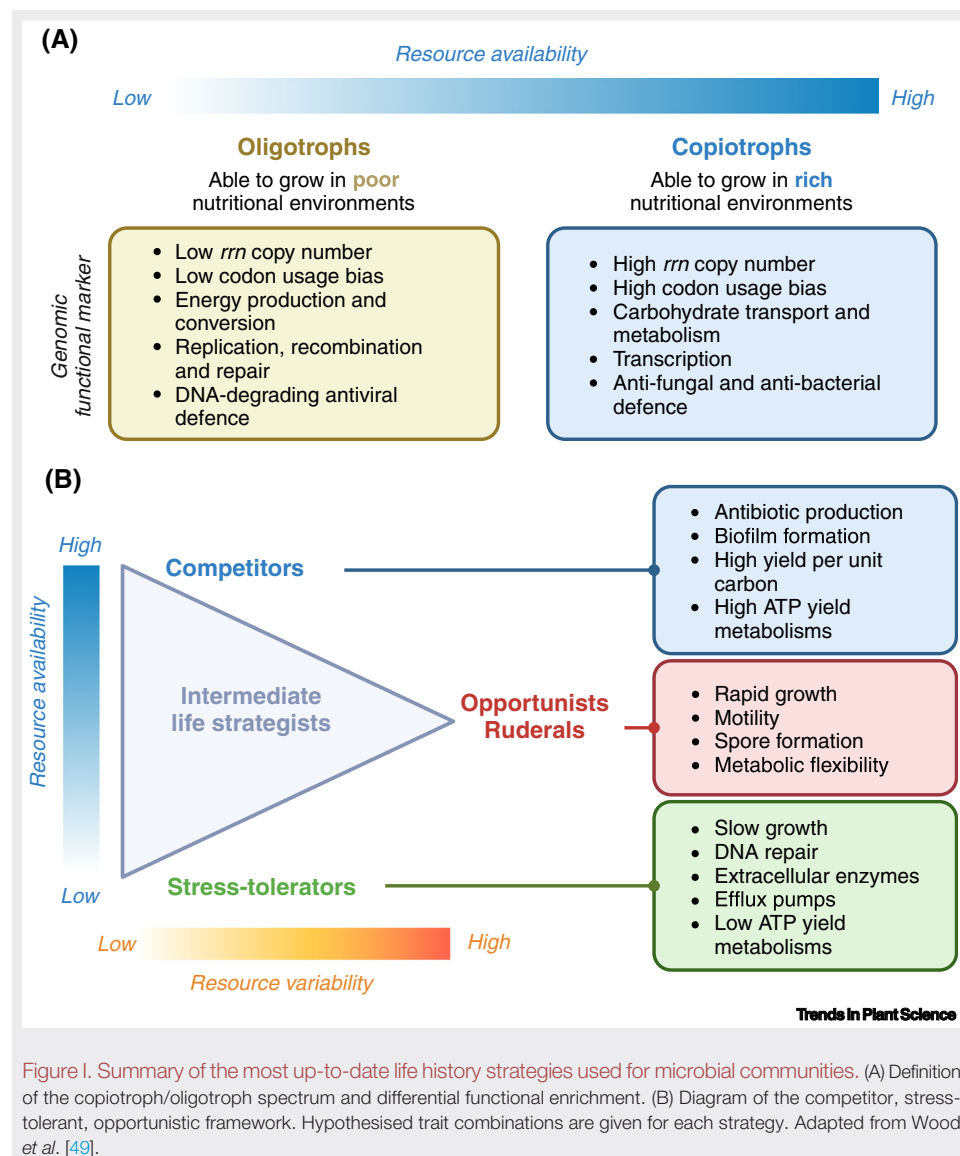

Box 1. Adapting life history theory to the study of microorganisms

r/K and copiotrophs/oligotrophs continuums

A fundamental classification derived from macro-ecology is the *r*- to *K*-selection continuum [47], which has since been adapted to the microbial world as the copiotroph/oligotroph continuum [81]. Copiotrophs (and *r*-strategists) usually thrive when resources are abundant, whereas oligotrophs (and *K*-strategists) often outcompete copiotrophs when resources are scarce [82]. Copiotroph-specific traits included gene families involved in the transport and metabolism of carbohydrates, as well as transcription. Oligotroph-specific traits included gene families involved in energy production, as well as replication-related genes [83,84] (Figure IA).

The competitor, stress-tolerator, opportunistic framework for microbial communities

Grime's competitor, stress-tolerator, ruderal (CSR) theory is a more complete framework which takes into account external factors limiting growth such as stress (conditions that restrict biomass production) and disturbance (partial or total destruction of biomass) [48]. First developed in plants, CSR theory was adapted to microbial communities by Malik *et al*. as the high yield (Y), resource acquisition (A) and stress tolerance (S) framework (YAS), then by Wood *et al*. as a competitor (C), stress-tolerator (S), opportunistic (O) strategy trade-off (CSO), with a focus on resources [49,85]. Here, disturbance is considered in terms of variable resource availability. Competitors thrive in highly productive environments, stress-tolerators show higher survival than other strategies in underproductive environments, and opportunists harbour traits adapted to environments where resource availability is temporally variable [49] (Figure IB).

Figure I. Summary of the most up-to-date life history strategies used for microbial communities. (A) Definition of the copiotroph/oligotroph spectrum and differential functional enrichment. (B) Diagram of the competitor, stress-tolerant, opportunistic framework. Hypothesised trait combinations are given for each strategy. Adapted from Wood *et al.* [49].

omics [8,57]. However, the translation of this research into commercially available products has been lagging behind [23]. As of 2021, only 20 fungal and bacterial strains were approved as biocontrol active substances in the European Union and the composition of biocontrol products is largely dominated by bacteria from the *Bacillus* genus [58,59]. Kaminsky *et al.* suggest that the mixed results observed in field settings might be due to trade-offs between plant beneficial traits and colonisation traits, with the latter being vastly overlooked in the microbial inoculant design process [22]. Microbiota research in recent years has been marked by the shift from single-strain approaches to SynCom inoculation. A recent meta-analysis by Liu *et al.* has evidenced improved biofertilisation and bioremediation when using SynComs compared with single-strain inoculations [60]. Both single-strain and SynCom approaches could benefit from a greater understanding of seedling microbiota assembly processes.

Improving our understanding of niche dynamics during seedling emergence

Acquiring knowledge on host-mediated effects during germination and seedling emergence will be pivotal to understand why certain microorganisms are able to survive and thrive in these transient and dynamic niches. Published studies characterising these niche dynamics in the spermosphere are scarce. We recommend that plant species-specific characterisation of seed exudates be performed. The capacity of seed and soil microorganisms to use the diversity of carbon sources released during seed imbibition as well as the impact of antimicrobial compounds should also be investigated with *in vitro* growth assays and microcosm experiments [61,62].

Applying diversity–invasion and competition-relatedness theory to SynCom design

For biocontrol purposes, seedling microbiota manipulation through inoculation aims at preventing pathogens from interfering with germination and seedling health. In this context, diversity–invasion theory would suggest that this could be achieved by increasing the overlap in nutrient-utilisation profile between pathogens and inoculated strains [63]. Increasing the functional diversity in SynCom inoculations may serve this purpose. Potential microbial interactions within SynComs should also be taken into account as they might make inoculation formulas more or less effective for colonisation. Machado *et al*. revealed the existence of a polarisation between cooperative and competitive natural microbial communities. While competition decreases with the addition of new species for cooperative communities, the opposite effect is observed for competitive communities [64]. Therefore, without complete knowledge of interactions between strains that compose SynComs, it is difficult to predict whether increasing the diversity may inadvertently increase competition within the SynComs or not. Combining the metagenomic data derived from colonisation studies with microorganism **metabolite** utilisation patterns can also be used to build metabolic models to simulate interactions within microbial communities [61,65]. Such models can then be used to predict the intensity of competition (direct antagonism or competition for nutrients) as well as facilitative interactions such as cross-feeding [66].

With regards to competition-relatedness theory, we can hypothesise that inoculated single strains or SynComs will have higher colonisation success if they have a low phylogenetic similarity with resident communities (i.e., native soil communities) [38]. Human gut inoculation experiments showed that the persistence of an inoculated *Bifidobacterium longum* strain was negatively correlated to the abundance of resident *B. longum* bacteria in the gut microbiota [67]. However, the taxonomic level at which strains should be dissimilar from resident communities is likely to depend on the identity of the strains themselves. Competition may be stronger at the species, genera, or family level, depending on the level of functional diversity and redundancy within a given taxon. Trying to design inoculants that are dissimilar from resident soil or seed communities may also contradict the need for bacterial traits that promote seedling colonisation, as such traits may be found in soil or seed communities. Finally, too few studies have investigated competition-relatedness for seedling microbiota assembly, calling for caution. Immense microbial diversity surveys for soil bacterial communities have shown that only a few taxa are ubiquitous worldwide and a recent meta-analysis on seed bacterial and fungal communities revealed that seed microbiota are extremely variable between plant species [56,68]. Competition-relatedness might explain different outcomes for inoculants, depending on soil and seed community composition, and should be taken into account in the interpretation of such observations.

Making the most out of priority effects and propagule pressure through seed inoculation

When it comes to designing microbial inoculation strategies for field applications, both theoretical and technical considerations suggest that seed inoculations should be preferred to leaf or soil inoculations for manipulation of seedling microbiota assembly [8]. Although leaf inoculations are a

relevant approach for phyllosphere and plant microbiota engineering, they arrive too late to affect seed vigour, seedling growth, and the colonisation of soil-borne pathogens [69]. Soil inoculations currently rely on propagule pressure to increase colonisation efficiency; however, this strategy requires high volumes of inoculation solutions which hinders upscaling [70]. Large-scale spreading of inoculation might also have detrimental environmental effects [8,71]. The volume of inoculation solutions required to treat an entire field would be lesser in the case of seed inoculations compared with soil inoculations, making seed inoculation approaches more easily upscalable. From the perspective of priority effects, seed inoculations may provide inoculants with a higher proximity to the spermosphere and growing seedlings compared with local soil communities. Seed inoculation strategies may also benefit from technical expertise acquired by the seed industry, with the translation of seed coating methods developed for pesticide application to microbial inoculation [72].

Overcoming trade-offs and improving trait selection for efficient seedling colonisation

Difficulties faced when designing efficient inoculation strategies may be explained by trade-offs predicted by life history theory between plant-beneficial, survival, and colonisation traits [22,23]. Improving inoculation strategies may be achieved by screening strains for multiple traits simultaneously guided by colonisation and genetic modification experiments. The environment from which we isolate candidates for inoculation will be important in this endeavour. Most microbial strains currently found in commercially available products have been isolated from soil or roots. Seeds play a prominent role in the transgenerational transmission of microorganisms. Therefore, isolating strains from seeds may yield candidates that can simultaneously survive abiotic stress during seed development and germination and drive plant-beneficial effects through colonisation of the seedling. However, discovering a microorganism that would maximise all elements of its fitness simultaneously in the wild may prove difficult. Using SynCom approaches to engineer seedling microbiota might help to overcome the trade-offs between survival and colonisation, with complementary functional mechanisms arising from synergistic relationships between SynCom members. Compared with single-strain approaches, microbial SynComs might be more versatile and less dependent on environmental factors that may favour or be a hindrance to the growth of certain SynCom members, but not to others. SynComs may also lead to complementarity and redundancy in the event of unsuccessful colonisation by a SynCom member [23].

Trait selection for seed inoculation should also be guided and tailored based on the choice of inoculation method. Seed coating is the surface application of a microbial inoculant for plant health-promoting purposes using a binder compound [70]. Due to low microbial survival and viability in the coating material inoculated, microorganisms used for seed coating applications may be selected for stress-tolerance traits such as spore formation, desiccation tolerance, or 'persister cell' behaviour. However, co-selected trade-offs might include low microbial growth upon the transition from a dry to a hydrated environment during seed imbibition. This would make 'seed-coating tolerant' species poor competitors not suited for spermosphere and seedling colonisation. Seed coating is often opposed to seed biopriming methods that rely on soaking seeds into a 'fresh' microbial suspension rather than using binder compounds for dry coating. Although shelf life may be reduced due to high potential for contamination, survival and stress-tolerance traits might not be required, allowing inoculant formulation efforts to focus on colonisation traits. Another approach is to compensate for stressful conditions induced by the coating process by adding growth- or survival-promoting compounds (i.e., prebiotics [73]). Either way, the development of seed microbial inoculants should include survival experiments to assess the compatibility of coating or priming methods with the viability of the microbial inoculant. Finally, field inoculation

strategies aiming at modulating seed microbiota assembly preharvest have been gaining traction for their ability to pre-emptively suppress seed-borne pathogens [74]. This may be achieved by introducing microorganisms during seed development, by treating the parental plant at multiple timepoints, from flowering to ripening [75,76]. **Mass effects** predict that repeated dispersal events allowing the constant influx of a species from a source community could lead to the maintenance of that species in areas where it cannot establish self-sustaining populations by overwhelming environmental and biotic filters [77]. Repeated inoculations of the parental plant during flowering and seed ripening could allow microorganisms that are unadapted to the seed environment to still establish themselves on seeds until sowing and germination. As a notable example, Mitter *et al*. managed to successfully introduce the plant growth-promoting bacteria *Paraburkholderia phytofirmans* PsJN in seeds of various plant species through floral inoculation [76].

Legacy effects of microbial inoculants

Achieving successful colonisation of the spermosphere and seedling environment by inoculated plant-beneficial microorganisms would be a major step forward towards efficient plant microbiota engineering. However, the potential negative impacts of the introduction of alien species in the environment should not be underestimated [8]. Recent studies have raised awareness on the legacy of microbial inoculants on soil indigenous microbial communities and the impact of community coalescence on keystone taxa [71,78]. In a comprehensive literature-search, Mawarda *et al*. investigated the resilience (return to the initial state) of soil communities following deliberate inoculation and found that in 86% of cases, the inoculation modified the structure of soil communities. In many cases, this change in community structure was correlated with changes in enzymatic activity, showing that inoculation has the potential to alter nutrient cycling, denitrification, organic matter decomposition, and other important soil ecosystem functions [79]. Finally, coalescence experiments in soil microcosms also evidenced that survival of the invader is not required to induce significant community shift [38]. Recent seedling microbiota engineering attempts using SynCom seed inoculations support this hypothesis. Seed inoculations led to significant changes in **rhizosphere** microbial community structure and taxonomic composition, yet without colonisation of the rhizosphere by SynCom microbial strains [42]. Furthermore, inoculation could affect microbial soil functions without modifying or with weak effects on dominant microbial microbial taxa. Papin *et al*. observed a significant increase in soil nitrate concentration after recurrent inoculation with a strain of *Pseudomonas fluorescens*, despite weak effects on dominant resident soil microbial taxa [80]. They suggested that rare taxa might be responsible for the observed nitrate accumulation, highlighting that considering only dominant taxa is not sufficient to assess the impact of microbial inoculants on the environment. There is currently a gap in studies investigating the long-term persistence and impact of microbial inoculants in plants and soils that should be filled. Additionally, the potential for long-term persistence, dispersal, or impact of inoculated microbial strains on the environment should be investigated before making bioinoculants commercially available.

Concluding remarks and future directions

Several studies show that engineering the seedling microbiota holds the potential to improve crop establishment and plant health (Figure 1). Microbial community ecology theory allows us to better understand the drivers of seedling microbiota assembly upon community coalescence between soil and seed-borne microorganisms (Figures 2 and 3). Finally, identifying microbial life history strategies and traits involved in the successful colonisation of the seedling will help us select microorganisms for inoculant design and solve current limitations faced during field trials (Box 1). Ultimately, the limiting factor to our understanding of seedling microbiota assembly is the lack of experimental data. In order to validate the main hypotheses developed in this opinion article, we recommend that more seedling colonisation experiments, focusing on both taxonomic and functional

Outstanding questions

Do diversity–invasion and competition-relatedness theory apply to seedling microbiota assembly?

What is the relative proportion of trait- and frequency-based processes during seedling microbiota assembly?

How do the primary and specialised metabolites released in the spermosphere shape microbe–microbe interactions during germination?

Are high concentrations of inoculated microorganisms correlated with high propagule pressure and seedling colonisation success?

Is the seed microbiota subjected to priority effects during germination and seedling emergence thanks to its proximity with the emerging seedling?

What is the magnitude and persistence of potential negative legacy effects of seed inoculations on the seedling, seedling rhizosphere, and surrounding soil microbiota?

Can we identify specific microbial traits linked with successful colonisation of seedlings?

How can the lab–industry–farmer transition facilitate the deployment of microbiota engineering strategies?

Key figure

Synthesis of the key considerations and perspectives for robust seedling microbiota engineering using soil or seed microbial inoculation
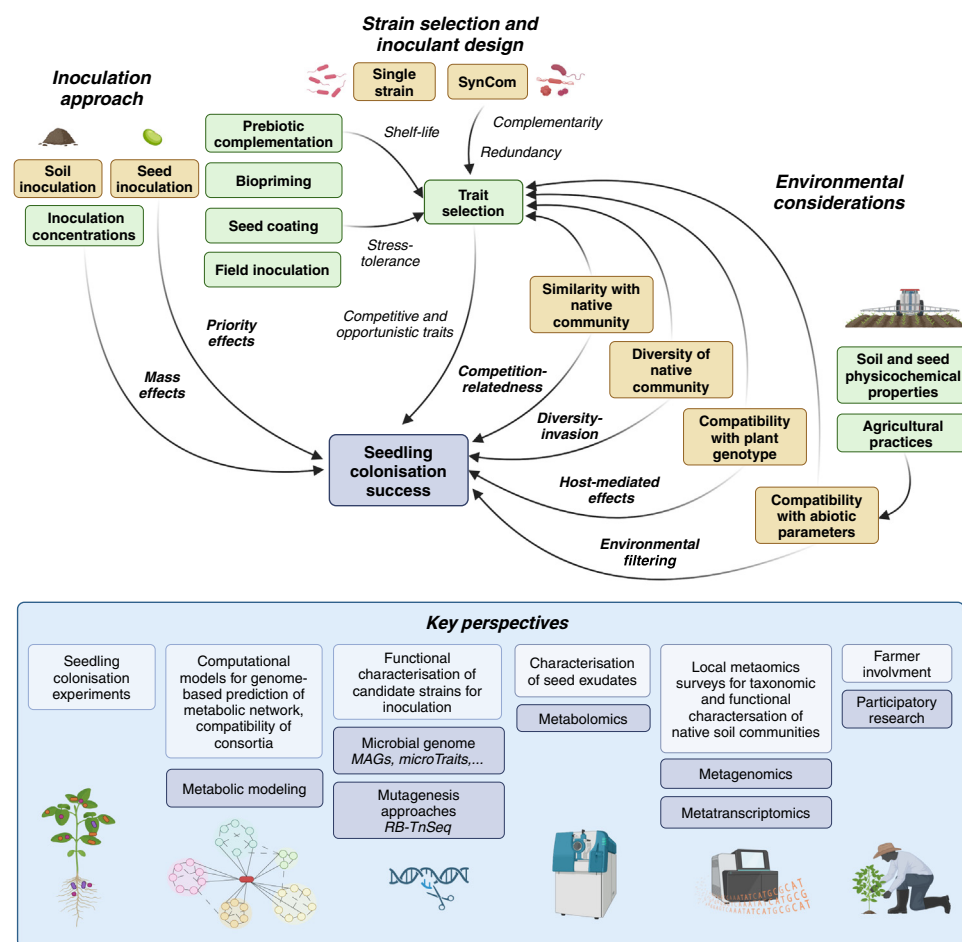
Figure 4. Abbreviations: MAGs, metagenome-assembled genomes; RB-TnSeq, randomly barcoded transposon sequencing; SynCom, synthetic community. Created with BioRender.com.

diversity, in different plant species and environments be performed (see Outstanding questions and Figure 4, Key figure).

Declaration of interests

The authors declare no competing interests.

References

1. Pingali, P.L. (2012) Green Revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U. S. A.* 109, 12302–12308
2. Fiodor, A. *et al.* (2023) Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. *Front. Microbiol.* 14, 1142966
3. Simonin, M. *et al.* (2023) Transmission of synthetic seed bacterial communities to radish seedlings: impact on microbiota assembly and plant phenotype. *Peer Commun. J.* 3, e95
4. Leite, H.A.C. *et al.* (2013) *Bacillus subtilis* and *Enterobacter cloacae* endophytes from healthy *Theobroma cacao* L. trees can systemically colonize seedlings and promote growth. *Appl. Microbiol. Biotechnol.* 97, 2639–2651
5. Schiltz, S. *et al.* (2015) A review: what is the spermosphere and how can it be studied? *J. Appl. Microbiol.* 119, 1467–1481
6. Verma, S.K. *et al.* (2018) Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant Soil* 422, 223–238
7. Matsumoto, H. *et al.* (2021) Bacterial seed endophyte shapes disease resistance in rice. *Nat. Plants* 7, 60–72
8. O'Callaghan, M. *et al.* (2022) Soil microbial inoculants for sustainable agriculture: limitations and opportunities. *Soil Use Manag.* 38, 1340–1369
9. Rétif, F. *et al.* (2023) Seed fungal endophytes as biostimulants and biocontrol agents to improve seed performance. *Front. Plant Sci.* 14, 1260292
10. Shade, A. *et al.* (2017) Ecological patterns of seed microbiome diversity, transmission, and assembly. *Curr. Opin. Microbiol.* 37, 15–22
11. Newcombe, G. *et al.* (2018) A hypothetical bottleneck in the plant microbiome. *Front. Microbiol.* 9, 1645
12. Abdelfattah, A. *et al.* (2023) From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. *Trends Microbiol.* 31, 346–355
13. Nelson, E.B. (2018) The seed microbiome: origins, interactions, and impacts. *Plant Soil* 422, 7–34
14. Nelson, E.B. (2004) Microbial dynamics and interactions in the spermosphere. *Annu. Rev. Phytopathol.* 42, 271–309
15. Rosa, S. *et al.* (2022) Antifungal activity and biochemical profiling of exudates from germinating maize Nostrano di Storo local variety. *Plants (Basel)* 11, 2435
16. Lamichhane, J.R. *et al.* (2018) Abiotic and biotic factors affecting crop seed germination and seedling emergence: a conceptual framework. *Plant Soil* 432, 1–28
17. Lamichhane, J.R. *et al.* (2020) Revisiting sustainability of fungicide seed treatments for field crops. *Plant Dis.* 104, 610–623
18. Olofintila, O.E. and Noel, Z.A. (2023) Soybean and cotton spermosphere soil microbiome shows dominance of soilborne copiotrophs. *Microbiol. Spectr.* 11, e0037723
19. Chávez García, S.N. *et al.* (2023) Sprouts as probiotic carriers: a new trend to improve consumer nutrition. *Food Chem. (Oxf.)* 7, 100185
20. Liu, H. *et al.* (2017) Inner plant values: diversity, colonization and benefits from endophytic bacteria. *Front. Microbiol.* 8, 2552
21. Murphy, B.R. *et al.* (2018) From concept to commerce: developing a successful fungal endophyte inoculant for agricultural crops. *J. Fungi* 4, 24
22. Kaminsky, L.M. *et al.* (2019) The inherent conflicts in developing soil microbial inoculants. *Trends Biotechnol.* 37, 140–151
23. Russ, D. *et al.* (2023) Deep discovery informs difficult deployment in plant microbiome science. *Cell* 186, 4496–4513
24. Mallon, C.A. *et al.* (2015) Microbial invasions: the process, patterns, and mechanisms. *Trends Microbiol.* 23, 719–729
25. Walter, J. *et al.* (2018) To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. *Curr. Opin. Biotechnol.* 49, 129–139
26. Rillig, M.C. *et al.* (2015) Interchange of entire communities: microbial community coalescence. *Trends Ecol. Evol.* 30, 470–476
27. Custer, G.F. *et al.* (2023) Toward an integrative framework for microbial community coalescence. *Trends Microbiol.* 32, 241–251
28. Malard, L.A. and Guisan, A. (2023) Into the microbial niche. *Trends Ecol. Evol.* 38, 936–945
29. Qiao, H. *et al.* (2024) Ecological niche conservatism spurs diversification in response to climate change. *Nat. Ecol. Evol.* 8, 729–738
30. Darrasse, A. *et al.* (2010) Transmission of plant-pathogenic bacteria by nonhost seeds without induction of an associated defense reaction at emergence. *Appl. Environ. Microbiol.* 76, 6787–6796
31. Ortega-Cuadros, M. *et al.* (2022) Seed transmission of pathogens: non-canonical immune response in *Arabidopsis* germinating seeds compared to early seedlings against the necrotrophic fungus *Alternaria brassicicola*. *Plants (Basel)* 11, 1708
32. Gerna, D. *et al.* (2020) Hydrogen peroxide metabolism in interkingdom interaction between bacteria and wheat seeds and seedlings. *Mol. Plant Microbe Interact.* 33, 336–348
33. Spragge, F. *et al.* (2023) Microbiome diversity protects against pathogens by nutrient blocking. *Science* 382, eadj3502
34. Guo, J. *et al.* (2021) Seed-borne, endospheric and rhizospheric core microbiota as predictors of plant functional traits across rice cultivars are dominated by deterministic processes. *New Phytol.* 230, 2047–2060
35. Rochefort, A. *et al.* (2021) Transmission of seed and soil microbiota to seedling. *mSystems* 6, e0044621
36. Walsh, C.M. *et al.* (2021) Variable influences of soil and seed-associated bacterial communities on the assembly of seedling microbiomes. *ISME J.* 15, 2748–2762
37. Russel, J. *et al.* (2017) Antagonism correlates with metabolic similarity in diverse bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 114, 10684–10688
38. Liu, X. and Salles, J.F. (2024) Lose-lose consequences of bacterial community-driven invasions in soil. *Microbiome* 12, 57
39. Xia, L *et al.* (2022) Biosynthetic gene cluster profiling predicts the positive association between antagonism and phylogeny in *Bacillus*. *Nat. Commun.* 13, 1023
40. Custer, G.F. *et al.* (2022) Ecological and evolutionary implications of microbial dispersal. *Front. Microbiol.* 13, 855859
41. Colautti, R.I. *et al.* (2006) Propagule pressure: a null model for biological invasions. *Biol. Invasions* 8, 1023–1037
42. Arnault, G. *et al.* (2024) Seedling microbiota engineering using bacterial synthetic community inoculation on seeds. *FEMS Microbiol. Ecol.* 100, fiae027
43. Debray, R. *et al.* (2022) Priority effects in microbiome assembly. *Nat. Rev. Microbiol.* 20, 109–121
44. Soluch, R. *et al.* (2021) Colonization dynamics of *Pantoea agglomerans* in the wheat root habitat. *Environ. Microbiol.* 23, 2260–2273
45. Leff, J.W. *et al.* (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10967–10972
46. Wang, Y. *et al.* (2022) Substrate utilization and competitive interactions among soil bacteria vary with life-history strategies. *Front. Microbiol.* 13, 914472
47. MacArthur, R.H. and Wilson, E.O. (2001) *The Theory of Island Biogeography*, Princeton University Press
48. Grime, J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111, 1169–1194
49. Wood, J.L. *et al.* (2023) Rethinking CSR theory to incorporate microbial metabolic diversity and foraging traits. *ISME J.* 17, 1793–1797
50. Roberts, D.P. *et al.* (1996) Amino acids as reduced carbon sources for *Enterobacter cloacae* during colonization of the spermospheres of crop plants. *Soil Biol. Biochem.* 28, 1015–1020
51. Torres-Cortés, G. *et al.* (2018) Functional microbial features driving community assembly during seed germination and emergence. *Front. Plant Sci.* 9, 902
52. van Dijk, K. and Nelson, E.B. (2000) Fatty acid competition as a mechanism by which *Enterobacter cloacae* suppresses *Pythium ultimum* sporangium germination and damping-off. *Appl. Environ. Microbiol.* 66, 5340–5347
53. Lohrke, S.M. *et al.* (2002) Mutation of rpiA in *Enterobacter cloacae* decreases seed and root colonization and biocontrol of damping-off caused by *Pythium ultimum* on cucumber. *Mol. Plant Microbe Interact.* 15, 817–825
54. Cole, B.J. *et al.* (2017) Genome-wide identification of bacterial plant colonization genes. *PLoS Biol.* 15, e2002860
55. Vannier, N. *et al.* (2023) Genome-resolved metatranscriptomics reveals conserved root colonization determinants in a synthetic microbiota. *Nat. Commun.* 14, 8274

56. Simonin, M. *et al.* (2022) Seed microbiota revealed by a large-scale meta-analysis including 50 plant species. *New Phytol.* 234, 1448–1463

57. Jansson, J.K. *et al.* (2023) Soil microbiome engineering for sustainability in a changing environment. *Nat. Biotechnol.* 41, 1716–1728

58. Sellitto, V.M. *et al.* (2021) Microbial biocontrol as an alternative to synthetic fungicides: boundaries between pre- and postharvest applications on vegetables and fruits. *Fermentation* 7, 60

59. Meyer, M.C. (2022) *Bioinsumos na Cultura da Soja*, Embrapa

60. Liu, X. *et al.* (2023) Inoculated microbial consortia perform better than single strains in living soil: a meta-analysis. *Appl. Soil Ecol.* 190, 105011

61. Schäfer, M. *et al.* (2023) Metabolic interaction models recapitulate leaf microbiota ecology. *Science* 381, eadf5121

62. Hubert, B. *et al.* (2024) A method to determine antifungal activity in seed exudates by nephelometry. *Plant Methods* 20, 1

63. Wei, Z. *et al.* (2015) Trophic network architecture of root-associated bacterial communities determines pathogen invasion and plant health. *Nat. Commun.* 6, 8413

64. Machado, D. *et al.* (2021) Polarization of microbial communities between competitive and cooperative metabolism. *Nat. Ecol. Evol.* 5, 195–203

65. Cerk, K. *et al.* (2024) Community-scale models of microbiomes: articulating metabolic modelling and metagenome sequencing. *Microb. Biotechnol.* 17, e14396

66. Gonçalves, O.S. *et al.* (2023) Designing a synthetic microbial community through genome metabolic modeling to enhance plant–microbe interaction. *Environ. Microbiome* 18, 81

67. Maldonado-Gómez, M.X. *et al.* (2016) Stable engraftment of *Bifidobacterium longum* AH1206 in the human gut depends on individualized features of the resident microbiome. *Cell Host Microbe* 20, 515–526

68. Delgado-Baquerizo, M. *et al.* (2018) A global atlas of the dominant bacteria found in soil. *Science* 359, 320–325

69. Zhan, C. *et al.* (2022) Pathways to engineering the phyllosphere microbiome for sustainable crop production. *Nat. Food* 3, 997–1004

70. Rocha, I. *et al.* (2019) Seed coating: a tool for delivering beneficial microbes to agricultural crops. *Front. Plant Sci.* 10, 1357

71. Mallon, C.A. *et al.* (2018) The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader's niche. *ISME J.* 12, 728–741

72. Ehsanfar, S. and Modarres-Sanavy, S.A. (2005) Crop protection by seed coating. *Commun. Agric. Appl. Biol. Sci.* 70, 225–229

73. Wen, T. *et al.* (2023) Tapping the rhizosphere metabolites for the prebiotic control of soil-borne bacterial wilt disease. *Nat. Commun.* 14, 4497

74. Kashyap, N. *et al.* (2023) Biocontrol screening of endophytes: applications and limitations. *Plants (Basel)* 12, 2480

75. Medison, R.G. *et al.* (2022) Use of beneficial bacterial endophytes: a practical strategy to achieve sustainable agriculture. *AIMS Microbiol.* 8, 624–643

76. Mitter, B. *et al.* (2017) A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Front. Microbiol.* 8, 11

77. Shmida, A. and Wilson, M.V. (1985) Biological determinants of species diversity. *J. Biogeogr.* 12, 1–20

78. Liu, X. *et al.* (2022) The legacy of microbial inoculants in agroecosystems and potential for tackling climate change challenges. *iScience* 25, 103821

79. Mawarda, P.C. *et al.* (2020) Deliberate introduction of invisible invaders: a critical appraisal of the impact of microbial inoculants on soil microbial communities. *Soil Biol. Biochem.* 148, 107874

80. Papin, M. *et al.* (2024) Survival of a microbial inoculant in soil after recurrent inoculations. *Sci. Rep.* 14, 4177

81. Koch, A.L. (2001) Oligotrophs versus copiotrophs. *BioEssays* 23, 657–661

82. Fierer, N. *et al.* (2007) Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364

83. Weissman, J.L. *et al.* (2021) Estimating maximal microbial growth rates from cultures, metagenomes, and single cells via codon usage patterns. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2016810118

84. Finn, D.R. *et al.* (2021) Functional trait relationships demonstrate life strategies in terrestrial prokaryotes. *FEMS Microbiol. Ecol.* 97, fiab068

85. Malik, A.A. *et al.* (2020) Defining trait-based microbial strategies with consequences for soil carbon cycling under climate change. *ISME J.* 14, 1–9