

Microbiome Engineering in Human Health and Agriculture: Rationale, Challenges, and Translational Pathways

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Abstract

Microbiome engineering represents a unified strategy to improve human health and promote agricultural sustainability. This approach is applicable across human medicine, crop production, and livestock systems by prioritizing the functional attributes of microbial communities over mere taxonomic composition. In medicine, the interplay between disrupted microbial metabolism and compromised barrier function has catalyzed the development of defined consortia and engineered live biotherapeutics, which are delivered using advanced materials and evaluated through activity-based outcomes. Multi-strain consortia consistently outperform single strains in dynamic field environments by enhancing nutrient utilization, stress resilience, and pest management. Co-culture methodologies further augment antifungal activity and crop development. Methane mitigation in ruminant agriculture is increasingly conceptualized as the regulation of electron flow within the rumen hydrogen/formate cycle, with functional activity metrics proving more predictive than abundance-based measures. Progress in any domain requires proactive strategies for microbial establishment, employing a triage framework that integrates Propagule Pressure, Environmental Filtering, and Biotic Interactions. Optimizing formulation, dosage, and timing is essential to maximize engraftment. Reliability and safety can be achieved through model-informed minimal consortia, standardized functional trait assessments, and One Health stewardship addressing antimicrobial resistance and ecosystem impacts. Future directions include scalable in situ manipulation of non-model commensals, multi-kingdom consortia, and the development of safety-by-design, regulatory-compliant products. Collectively, these principles will enable the translation of ecological insights into robust, scalable, and safe microbiome-based interventions for medicine and agriculture.

Keywords: Microbiome engineering; human health; agriculture; engineered live biotherapeutic products; defined consortia; materials-enabled delivery; genome engineering; in situ editing; persistence; Propagule Pressure-Environmental Filtering-Biotic Interactions; functional endpoint; rumen methane; One Health.

Introduction

Microbiome engineering has emerged as an integrated strategy to advance human health and agricultural sustainability by deliberately shaping both the composition and functional capacity of host-associated and environmental microbial communities. Microorganisms have co-evolved with plants, animals, and humans, playing pivotal roles in regulating metabolism, immunity, and growth. In soils and the rumen, microbial communities also drive biogeochemical cycles, influencing yield and climate at the systems level [1,7,25,23]. This review posits that transitioning from single-strain additions to community- and function-oriented design, supported by robust frameworks for delivery, establishment, and measurement, is essential for translating laboratory findings into effective field and clinical applications.

Dysbiosis is associated with inflammatory, metabolic, infectious, and oncogenic conditions via metabolite deficiencies (e.g., short-chain fatty acids, bile-acid derivatives), barrier dysfunction, and an inappropriate immune response [12,20]. Diet, probiotics, and fecal/defined microbiota transplants are broad tools that can restore colonization resistance or change metabolite pools, but their effectiveness varies and their mechanisms are often not clear [12,18]. Two technology arcs are making that change. First, genome and metabolic engineering of commensals allow for precise functions, such as delivering anti-inflammatory cytokines, rewiring the bile-acid pathway, breaking down ammonia or phenylalanine, and creating sensor-actuator circuits for "on-demand" therapy [15,11,17]. Second, materials science is making these functions

possible. For example, encapsulation and smart, region-specific release protect live microbes as they move through the stomach; organoids and gut-on-chip platforms let us model complex host–microbe interactions outside of the body; and ingestible and wearable biosensors add spatiotemporal readouts beyond taxonomy [14]. These capabilities emerge within a swiftly evolving clinical domain for live biotherapeutics—encompassing both defined consortia and engineered strains—characterized by successes (e.g., donor-derived spore products for recurrent *C. difficile*) counterbalanced by insights from initial trials where persistence or efficacy proved inadequate, highlighting the necessity for chassis selection, pharmacology, and manufacturing that adhere to drug-grade standards [18,17].

In agriculture, decades of research demonstrate that the soil–root–endosphere microbiome supports nutrient utilization efficiency, stress resilience, disease suppression, and product quality [1,4]. Microbes that help plants grow move nitrogen, phosphorus, and micronutrients; make phytohormones and siderophores; and cause systemic resistance, which means that plants don't need as many synthetic inputs [7,24,21]. Field evidence is nuanced: in protected, high-input environments, elite single strains can perform as well as consortia, but under low-P, alkaline, sandy, or arid conditions, microbial consortia restore rhizosphere diversity, unlock P, and raise yields—highlighting context dependence and the advantage of complementary division-of-labor designs [5]. Community assembly within roots is not entirely deterministic; colonization frequently depends on particular traits (e.g., chemotaxis, cell-wall–degrading enzymes) and stochastic processes, with mixed inocula preventing the undesirable over-dominance observed with certain single strains [3]. Co-culture strategies can also turn on dormant biosynthetic gene clusters, which can help plants grow and make antifungal treatments more effective. However, the dose and spectrum must be adjusted to avoid unwanted side effects [13]. More generally, population growth, climate stress, and the known costs of using a lot of agrochemicals (loss of biodiversity, soil function, and off-target harm) make the case for plant microbiome engineering as a way to make things more sustainable [6,8,9].

Pastoral systems introduce a climatic aspect: the rumen microbiome regulates the majority of enteric methane (CH_4), a potent greenhouse gas and a direct energy deficit for the animal. Comparative studies demonstrate that the overall abundance of methanogens is a poor predictor of emissions. Instead, the composition of methanogenic clades, the availability of reductants (H_2 , formate, methyl compounds), and, crucially, their activity (metatranscriptomic signatures of methanogenesis) elucidate the distinctions between high and low-emitter phenotypes [25]. This changes mitigation from counting taxa to controlling the flow of hydrogen and the activity of methanogenesis, along with diet and management. Rumen-focused "function-over-composition" thinking is similar to the main idea of this review: to get reliable results, we need to measure and change what microbes are doing, not just who is there.

Establishment is a common problem in both areas. Introduced organisms, even if they are "right" in terms of how they work, often don't last long in complicated communities of native organisms. Based on invasion and restoration ecology, establishment relies on Propagule Pressure (dose, frequency, delivery), Environmental Filtering (niches, timing, disturbances), and Biotic Interactions (competition, antagonism, predation, facilitation). Historically, most microbiome programs have put too much emphasis on the strain and not enough on these axes. Recent frameworks have focused on PP–EF–BI triage and decision trees to fill this gap [2,10]. Field inconsistency is also caused by abiotic factors (pH, moisture, temperature, nutrients), host genotype and exudates, and the structure of the background community. Inoculants that work for everyone don't always work for all soils, seasons, and patients [1,4,6,5,12]. There is still a translation gap on the human side for tooling (editing non-model commensals, in situ delivery vectors), safety (biocontainment, horizontal gene transfer, immunogenicity), and manufacturing/regulation (GMP, stability, pharmacology, and acceptance), even though genome engineering pipelines and species-specific parts libraries are growing [11,15,18].

Lastly, a One Health perspective connects the effects on people, animals, and the environment: microbial communities work with hosts to break down carbon and nitrogen, change how people respond to cancer treatments and radiation, and move antibiotic resistance genes between different groups and species [23,21]. This systems perspective advocates for intersectoral surveillance and coordinated, microbiome-informed policy to mitigate zoonotic spillover and antimicrobial resistance while harnessing advantageous functions.

Size and shape. This review builds on these ideas by putting together (i) the reasons for microbiome engineering in agriculture and human health; (ii) the common problems that happen during translation (establishment failures, context dependence, tooling and regulatory gaps); and (iii) solution playbooks based on persistence-aware design (dose/timing/formulation), consortia engineering, materials-enabled delivery and measurement, genome/metabolic engineering of commensals, and field-aware validation. Case snapshots—from engineered live biotherapeutics and defined consortia to co-cultured biocontrols and rumen methane mitigation—illustrate how a function-first, context-tuned approach can convert ecological principles into reliable interventions [16,17,24,22]. The papers reviewed here show both how important it is to make microbiome engineering a reliable part of medicine and agriculture and how to do it.

Conceptual Foundations

Microbiome engineering is based on the idea that microbial communities are not separate organisms, but rather dynamic ecosystems. Each microbiome, whether in soil, plant roots, rumen, or the human gut, consists of thousands of interacting species whose collective metabolism dictates system behavior. The transition from single-strain inoculants to community-level, function-oriented interventions signifies a paradigm shift observed in both agricultural and biomedical domains [1,4,7,9,12,16].

2.1 From the idea of a "Single Microbe" to the idea of a "Microbiome"

In the past, microbial applications focused on single strains that were chosen to help plants grow or fight off disease. However, single-strain inoculants often do not last or provide consistent benefits in real-world situations because the outcome depends on the ecosystem. Empirical evidence from agricultural microbiome studies demonstrates that interactions among bacteria, fungi, archaea, and protists produce emergent properties, including nutrient cycling, pathogen suppression, and stress tolerance, which cannot be replicated by a single species [1,5,6,24]. Likewise, in human systems, health and disease are contingent not on a singular pathogen or commensal, but on the equilibrium and intercommunication among microbial guilds that govern immune and metabolic pathways [12,17,20].

2.2 Ecological Principles That Guide Engineering

Microbiomes adhere to traditional ecological principles of niche occupation, colonization resistance, and succession. New strains must find the right niches, the right times to disturb them, and positive interactions with other species in order to get past these barriers. Theoretical and experimental frameworks characterize persistence as a function of Propagule Pressure (PP), Environmental Filtering (EF), and Biotic Interactions (BI) [2,10]. Microbiome engineering necessitates the design around these axes—choosing suitable delivery methods, aligning ecological niches, and considering competition and cooperation within resident communities.

2.3 Function over composition

A major agreement among studies is that "who's there" is less important than "what they do." Functional redundancy and metabolic cross-feeding enable different taxa to fulfill similar functions. Metatranscriptomic analyses of low- and high-methane ruminants indicate that variations in microbial activity levels, rather than taxonomic composition, account for emission differences [22,25]. In soils and roots, metabolic traits—like phosphorus solubilization, siderophore production, or ACC deaminase activity—are better indicators of how plants will grow than their genus-level identity [4,5,7]. In human health, metabolites like short-chain fatty acids, bile acids, and tryptophan derivatives regulate the immune system and metabolism, regardless of the specific taxa that produce them [12,14,15,20].

2.4 Integration of Systems and Synthetic Biology

Modern microbiome engineering combines knowledge of ecological systems with synthetic biology. Genome-scale metabolic models (GEMs) and high-throughput ‘omics now allow the design and simulation of microbial communities based on metabolic fluxes, rather than guesswork [11,15,17]. In both human and agricultural contexts, synthetic consortia—strategically organized groups with complementary roles—are supplanting empirical mixtures. These advancements allow researchers to forecast community outcomes, reduce ecological disruption, and cultivate stability through labor division and metabolic interdependence.

The conceptual foundation of microbiome engineering integrates principles from ecology, evolution, and molecular design. Recognizing microbes as components of complex, adaptive networks—rather than isolated tools—enables the development of interventions in health, food, and environmental systems that are predictable, scalable, and durable.

Human Health Applications

The human microbiome operates as a decentralised organ that influences immunity, metabolism, and neuroendocrine communication. Dysbiosis, characterised by altered microbial composition or function, is associated with inflammatory bowel disease (IBD), obesity, type 2 diabetes or T2D, non-alcoholic fatty liver disease or NAFLD, neurodegeneration, autoimmunity, infection, and cancer [12,15,17,19,20]. The therapeutic aim of microbiome engineering is to restore or reprogram community function to attain quantifiable clinical advantage.

3.1 Dysbiosis, the integrity of barriers, and immune–metabolic regulation

Disruptions in gut communities frequently result in deficiencies of short-chain fatty acids or SCFAs and other metabolites that maintain epithelial tight junctions and immune tolerance, accompanied by heightened permeability ("leaky gut") and the translocation of inflammatory ligands [12,20]. Microbial modifications of bile acids and aromatic amino acids generate ligands for FXR, TGR5, and AhR, which govern glucose and lipid homeostasis and inflammatory responses; dysregulation of these pathways is evident in metabolic disorders and NAFLD [12,15]. The goal of therapeutic restoration of these functions is to bring metabolism back to normal, strengthen barrier integrity, and bring immune balance back to normal.

3.2 Strategies for modulation

3.2.1 Diet, prebiotics, probiotics, and synbiotics

Prebiotics (substrates that favour beneficial taxa) and probiotics (defined live microbes) continue to be essential tools. Certain *Lactobacillus* and *Bifidobacterium* strains can ameliorate glycaemic control and lipid profiles in Type 2 Diabetes (T2D) and Non-Alcoholic Fatty Liver Disease (NAFLD), though results differ due to strain specificity and individual variability; synbiotics integrate both to enhance resilience and functionality [12,21].

3.2.2 Transplantation of microbiota and established consortia

Faecal microbiota transplantation (FMT) restores colonisation resistance and metabolic output, demonstrating high efficacy against recurrent *Clostridioides difficile* infection; defined consortia are being developed to enhance safety, dose regulation, and mechanism [16,18]. 11-strain immune-active consortia and propionate-producing mixes that fix dysbiosis caused by antibiotics are two examples [17,18].

3.2.3 Engineered live biotherapeutic products (eLBPs)

Engineered probiotics and commensals can detect disease signals and provide therapeutic functions in situ. Reported designs encompass *Lactococcus lactis* secreting IL-10, GLP-1, or GAD65+IL-10; *Escherichia coli* engineered to degrade phenylalanine (phenylketonuria) or reduce ammonia levels (hyperammonemia); and *Saccharomyces boulardii* expressing a tetra-specific antibody to neutralise *C. difficile* toxins [15,18]. These chassis serve as programmable, localised "pharmaceutical production facilities."

3.3 Toolkits for genome and metabolic engineering

CRISPR systems, integrases, and transposons have been modified for a growing array of gut commensals; however, effective DNA delivery continues to be a significant obstacle for numerous non-model species [11]. In situ editing utilising phage- or conjugation-based vectors is being developed to alter resident strains directly within the gut [11,18]. Genome-scale metabolic models (GEMs) and iterative Learn-Design-Build-Test cycles direct strain/consortium design and forecast interspecies fluxes [15,17]. There are still tooling gaps for anaerobic taxa that can't be genetically modified [11,15].

3.4 Delivery and measurement made possible by materials

Materials science deals with survival, targeting, and keeping an eye on things. Encapsulation, enteric coatings, and stimuli-responsive polymers safeguard cargos during gastric transit and facilitate region-specific release; organoids and gut-on-chip models emulate human tissue microenvironments for mechanistic studies and screening; ingestible and wearable biosensors offer spatiotemporal functional readouts that extend beyond taxonomy [14].

3.5 Clinical landscape, persistence, and safety

Over 5,400 microbiome-related trials encompass metabolic, inflammatory, infectious, and oncologic indications [12]. There are still some important translational problems to solve. For example, many eLBPs/probiotics have a short residency time (about 48 hours for engineered *E. coli* Nissle), which means they need to be re-dosed and carefully chosen; their effectiveness varies between groups; and drug-class requirements for cGMP manufacturing, biocontainment (kill switches, auxotrophy), horizontal gene transfer mitigation, and pharmacology/PK-PD characterisation [18,19,20]. Resolving these limitations is crucial for widespread clinical implementation.

3.6 Overview

Human-health microbiome engineering combines genetic and metabolic design with materials-enabled delivery and high-resolution measurement to change the functions that control metabolism, immunity, and barrier homeostasis. The field is evolving from empirical probiotics and faecal microbiota transplantation (FMT) to engineered, precision biotherapeutics and well-defined consortia, driven by mechanisms, modelling, and clinical pharmacology [11–20].

Agricultural Applications

The agricultural microbiome, which includes communities in the soil, rhizosphere, phyllosphere, and endophytic, is very important for crop productivity, nutrient cycling, disease resistance, and resistance to abiotic stresses. Microbiome engineering in agriculture seeks to utilise, restore, and stabilise microbial functions to enhance sustainable yields while decreasing reliance on synthetic fertilisers and pesticides [1,4,6,7,8,9,24].

4.1 The soil-plant microbiome is the basis for sustainability

There are billions of microbial cells at the soil-plant interface that help with nitrogen fixation, phosphorus solubilisation, siderophore production, and phytohormone synthesis. Microbes that live on plants work together to help plants take in nutrients, fight off pathogens, and send signals to each other [1,4,24]. Rhizosphere communities, especially plant growth-promoting rhizobacteria (PGPR) like *Pseudomonas*, *Azospirillum*, *Bacillus*, and *Rhizobium*, help roots grow, make photosynthesis more efficient, and make plants more resistant to different kinds of stress [7,21]. These natural alliances make the microbiome a living part of the "plant holobiont," which is important for keeping the ecosystem in balance and keeping the soil fertile for a long time.

4.2 Microbial inoculants: single strains vs. groups

Conventional inoculants concentrated on individual strains with specific roles—such as nitrogen fixation or phosphate solubilization—yet field outcomes frequently exhibited variability in diverse soils and climates. Multi-strain consortia get around these problems by combining functions that work well together and making it easier for bacteria to colonise. Bradáčová et al. (2019) showed that using multiple strains of microbes in tomato production improved nutrient uptake and yield more consistently than using just one strain, especially when there weren't many nutrients available [5]. Zhang et al. (2022) demonstrated that mixed endophytic bacterial inocula exhibited superior colonisation and enhanced disease protection compared to individual isolates [3]. These results confirm that microbial consortia replicate the collaborative ecology of natural microbiomes and exhibit superior performance across various environments.

4.3 Dependence on context and differences in the field

Field inconsistency continues to be a significant impediment to the large-scale application of beneficial microbes. Abiotic and biotic factors, including soil type, pH, moisture, temperature, plant genotype, and the background microbial community, significantly affect inoculant survival and functionality [1,6,8]. Research indicates that outcomes from controlled environments seldom directly apply to open-field systems where multiple stressors operate concurrently. For instance, Afridi et al. (2022) stressed that soil type and time accounted for as much as 15% of the variation in the structure of the root microbiome, and that climate factors like temperature and rainfall had a big effect on bacterial communities [8]. Consequently, the design of microbiome interventions necessitates site-specific customisation and a long-term ecological perspective.

4.4 Mechanistic insights: nutrient cycling, stress tolerance, and biocontrol

Microbiome engineering aids various agronomic functions:

Getting nutrients: Rhizobia fix nitrogen from the air, and phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi move around phosphates and micronutrients that are not soluble [4,24].

Phytohormone production: PGPR secrete auxins, cytokinins, gibberellins, and ACC deaminase, which change the shape of roots and lower ethylene stress [7,21].

Biocontrol: Some species of *Bacillus*, *Pseudomonas*, and *Streptomyces* make antibiotics, lipopeptides, and siderophores that stop bacteria and fungi from growing [5,13].

Activation of secondary metabolism through co-culture: Co-culturing in the rhizosphere of *Streptomyces* strains activates dormant biosynthetic gene clusters, boosting antifungal activity and stimulating plant growth by increasing phytohormone production [13].

Abiotic stress tolerance: Microbes enhance water-use efficiency, salinity tolerance, and drought resilience by modifying plant hormone signalling and osmolyte equilibrium [6,7,8].

4.5 Uses in pastoral and rumen settings

The rumen microbiome controls how well livestock converts feed into energy and how much methane (CH₄) it releases. Methanogenic archaea take hydrogen and carbon dioxide from other rumen microbes and convert them into methane, a greenhouse gas 28 times more likely to cause global warming than CO₂ [25]. Microbiome-targeted strategies—like changing the diet, adding probiotics, or directly stopping methanogenesis pathways—have two benefits: they lower CH₄ emissions and help animals use energy better. Transcriptomic analyses indicate that variations in methane yield are predominantly due to methanogen activity rather than abundance, highlighting the necessity for functional rather than compositional interventions [22,25].

4.6 Problems with translation and deployment

Even after decades of research, many microbial products still don't work well in the field. Some of the main problems are:

Inconsistent establishment and persistence: Introduced microbes frequently do not endure competition from indigenous communities [2,10].

Abiotic factors like soil chemistry, organic matter, and water availability affect how well microbes can live and interact with each other [6,8].

Regulatory challenges: Lengthy and complicated approval processes make it harder to sell products, especially in areas with strict rules about biopesticides [7].

Quality and formulation problems: Shelf life, storage stability, and compatibility with agrochemicals make it hard to use [9].

To get past these problems, we need to use ecological modelling, systems biology, and formulation science in the process of making new products.

4.7 Microbiome engineering for precision agriculture

Next-generation agricultural biotechnology combines omics, genome editing, and synthetic ecology to make microbiome systems that can handle stress. To predict how well an inoculant will survive and work in certain environmental conditions, scientists are using predictive models of community metabolism, ecological niche modelling, and genome-scale reconstructions [7,8,10]. Some new trends are the use of synthetic communities made for soils that are low in nutrients or high in salt, biofilm-based formulations that help colonisation, and the use of bioinformatics to help design consortia. These new ideas show a shift from biofertilizers based on experience to engineered microbiome solutions that use molecular precision and ecological principles to make crops and livestock more productive in the long term.

In summary, agricultural microbiome engineering recognizes that soil, plant, and rumen ecosystems function as dynamic engines of productivity. Integrating ecological knowledge with modern biotechnologies enables agriculture to move beyond chemical dependence, fostering sustainable, resilient, and climate-adaptive food systems [1–10,13,21,24,25].

Pastoral Systems & Rumen Methane

Ruminant livestock are very important for food production around the world because they turn fibrous plant material into protein-rich food. However, this process lets out a lot of methane (CH_4), which is a strong greenhouse gas and a waste of energy for the animal. Pastoral microbiome engineering aims to comprehend and modify the rumen microbial ecosystem—the principal fermentation site—to diminish methane emissions while enhancing feed efficiency and animal health [22,25].

5.1 The rumen microbiome and its role in making methane

The rumen is a complex, anaerobic microbial community made up of bacteria, archaea, protozoa, and anaerobic fungi. These microbes break down plant polymers into volatile fatty acids (VFAs) that the host can use. Methane is made when methanogenic archaea eat hydrogen (H_2) and carbon dioxide (CO_2) that are made by fermentative microbes [25]. The rumen produces more than 87% of all ruminant methane, while the hindgut produces the rest [25].

The overall abundance of methanogens exhibits a weak correlation with emission levels; however, community composition and functional activity—particularly within the *Methanobrevibacter* SGMT clade—are strongly associated with high-methane phenotypes. Conversely, *Methanosphaera* and specific methylotrophic lineages correlate with reduced emissions, indicating taxon-specific functional niches [25].

5.2 The diversity and function of microbes in methane phenotypes

Research employing metagenomics and metatranscriptomics indicates that disparities between high- and low-emitting animals are primarily influenced by gene expression and metabolic pathways rather than the identity of the individuals present [25]. High-emitters exhibit elevated levels of methanogenesis transcripts, especially those associated with hydrogenotrophic pathways. Low-emitters frequently display an increased relative abundance of propionate-producing bacteria, including *Quinella*, *Fibrobacter*, *Olsenella*, and *Prevotella*, which redirect H_2 from methanogens by generating alternative reductant sinks [25].

Protozoa and anaerobic fungi also have an effect on the amount of methane that is produced. During carbohydrate fermentation, protozoa produce a lot of H_2 , which methanogens use as a substrate. When defaunation is done experimentally, it usually lowers CH_4 output by about 10–11%, but this depends on the

species and diet [25]. Anaerobic fungi produce both H₂ and formate, the latter of which is an under-characterized but important methanogenic substrate.

5.3 The hydrogen economy and functional ecology

The hydrogen economy in the rumen, which is the balance between processes that make H₂ and processes that use H₂, controls the amount of methane produced. High CH₄ systems have too much reductant available, which methanogens can easily capture. The goal of engineering interventions is to change the flow of electrons so that they go to different pathways (like propionate, butyrate, acetogenesis, or nitrate reduction) that compete for H₂. Dietary additives, including fatty acids, nitrates, and plant secondary metabolites, have been demonstrated to influence these flows; however, their impact on the overall microbiome and animal performance necessitates meticulous monitoring [25].

In addition to community composition, regulating functional gene activity and comprehending the role of lesser-known substrates such as formate and methylamines are essential for developing precise predictive models for methane mitigation.

5.4 Ways to change the microbiome

There are a few different ways that microbiome engineering works in ruminants:

Selective breeding for low-methane phenotypes, wherein host genetics are associated with stable, low-emission rumen microbial structures [22].

Microbial inoculation and transfaunation, which means moving rumen fluid from animals with low emissions to animals with high emissions to change how microbes work.

Feed-based modulation, such as dietary oils, tannins, seaweed extracts (*Asparagopsis taxiformis*), and nitrate supplementation, to reduce methanogenesis.

Phage therapy or engineered inhibitors that target certain methanogen clades or hydrogen producers without harming helpful fermenters.

Synthetic consortia design involves putting together groups of microbes that support propionate and succinate pathways, which makes them good competitors for H₂ with methanogens.

These methods are part of a larger trend in microbiome management: moving from interventions that change the composition of the microbiome to function-driven engineering that targets specific metabolic flows.

5.5 Measurement, modelling, and functional forecasting

Methane mitigation research increasingly depends on multi-omics integration—connecting metagenomes, metatranscriptomes, metabolomes, and flux modeling—to measure activity instead of abundance.

Transcriptomic data consistently surpass taxonomic data in forecasting methane yield, underscoring the necessity to monitor gene expression and metabolite outputs. Even though there have been improvements, the current databases for rumen microbes are still not complete, which makes it hard to accurately annotate methanogenic genes and pathways [25]. For global comparisons and accurate modelling of microbial activity, it will be important to build well-annotated reference genomes and standardised protocols for measuring methane [22,25].

5.6 Moving towards sustainable pastoral systems

Microbiome engineering gives pastoral farming a way to find a balance between being productive and being good for the environment. Targeted changes to rumen communities can lower methane emissions, make feed conversion more efficient, and improve animal health by optimising fermentation profiles. Combining microbial interventions with breeding, nutrition, and management creates a systems-based approach that makes animal performance match climate goals [22,25].

To sum up, the rumen microbiome is both a source of greenhouse gas emissions that can be controlled and a factor that affects productivity. The transition from enumerating microbial taxa to measuring functional activity and hydrogen flow signifies a pivotal advancement in methane mitigation science. Using tools from microbial and metabolic engineering, along with knowledge of ecology, can turn pastoral systems into ecosystems for production that are sustainable, low-emission, and high-efficiency.

One Health Bridge

The One Health framework acknowledges the interdependence of human, animal, and ecosystem health, with the microbiome serving as a central connecting axis. Microbial communities affect not only the physiology of individual hosts but also the spread of pathogens, nutrient cycles, and the stability of the environment. Microbiome engineering functions as a biomedical and ecological instrument to enhance resilience and sustainability within the human–animal–environment continuum [21,23].

6.1 Microbial ecosystems and interconnected health

Microbes facilitate critical biochemical interactions between living organisms and their surroundings. The microbiome regulates immunity, metabolism, and neuroendocrine communication in humans; in animals, it influences nutrient conversion efficiency, disease resistance, and productivity; and in soils and plants, it manages nitrogen fixation, carbon sequestration, and crop yield [1,7,21,23]. These microbial functions that work together make up the biochemical basis of One Health. Any kind of disruption, like soil degradation, too much use of antibiotics, or changes in the climate, has an effect on the whole system, including food security, zoonotic risk, and ecosystem balance.

6.2 Resistance to antibiotics and transmission between animals and humans

The dissemination of antimicrobial resistance genes (ARGs) constitutes a critical cross-domain challenge. The gut, soil, and water microbiomes act as places where ARGs can be stored and shared, allowing resistant bacteria to move between people, animals, and the environment [23]. Excessive use of antibiotics in

healthcare and agriculture expedites this gene flow. The World Health Organisation says that more than 60% of human pathogens come from animals. This is also true of recent microbiome-based studies that have found links between livestock and wildlife microbial communities and new infectious diseases like SARS-CoV-2 and MERS-CoV [23].

Microbiome-informed surveillance and stewardship—like metagenomic screening for resistance genes in animal production systems and wastewater—are necessary to stop new resistant pathogens from appearing and spreading.

6.3 Microbiomes in the environment and the health of the planet

Microbiomes in the environment control the flow of nutrients around the world. Microbial communities in soil and water control the flow of carbon and nitrogen, affect greenhouse gas emissions, and affect soil fertility [21,23]. Changes in these systems can lead to climate feedback and lower agricultural productivity. Microbiome engineering can help restore ecosystem functions by encouraging beneficial microbial communities through sustainable farming, reforestation, and pollution bioremediation. Adding microbial interventions to programs for climate-smart agriculture and carbon sequestration is a big chance for One Health.

6.4 Bringing microbiome science into policy and practice

For One Health approaches to work, medicine, veterinary science, agriculture, and environmental management all need to work together. Microbiome data offer common indicators for assessing system health—like biodiversity indices, ARG prevalence, or carbon turnover rates—that can inform collaborative policy frameworks. For instance, cutting back on the use of antibiotics in animal feed not only lowers ARGs in the microbiomes of livestock, but it also lowers the number of resistant bacteria in human wastewater and agricultural soils [21,23]. Adding microbial ecology to food safety standards, climate policies, and wildlife management makes it easier for different sectors to get ready for global health threats.

6.5 Towards integrated microbiome management

Transdisciplinary microbiome stewardship that brings together genomics, data science, and policy will be needed for One Health to work in the future. This includes:

Making shared databases of microbes that connect isolates from humans, animals, and the environment;

Setting up networks for monitoring ARGs and zoonotic microbes together;

Using eco-friendly microbial technologies like biofertilizers, bioremediators, and engineered probiotics to make things more resilient;

Encouraging microbiome knowledge in medical and farming training.

To sum up, the microbiome is the biological link between One Health's medical, agricultural, and ecological systems because it lets microbes do the same things in all three areas. We can deal with antibiotic resistance, zoonotic emergence, and environmental degradation all at once by treating microbial communities as shared resources that need to be cared for, protected, and used in a way that is good for the environment. Microbiome engineering is therefore an essential field for creating a healthy, strong, and long-lasting planet [21,23].

Measurement & Methods (How We Know)

The swift growth of microbiome research and engineering is due to improvements in analytical tools that allow for detailed characterisation, modelling, and monitoring of microbial communities. It is important to understand "how we know" because accurate measurement determines causality, guides design, and makes sure that results can be repeated in both the lab and the field. This section describes the main molecular, ecological, and modelling methods that are used to study and engineer microbiomes in both human and agricultural systems [1–25].

7.1 Meta-omics technologies: from existence to function

Microbiome analysis has evolved from fundamental 16S rRNA inventories to extensive multi-omics platforms that clarify community structure, functional capacity, and activity.

Metagenomics reveals the taxonomic diversity and genetic capacity of microbial communities. It assists in discovering metabolic pathways for nutrient cycling, antibiotic resistance, and the synthesis of signalling molecules [1,11,12].

Metatranscriptomics captures active gene expression, elucidating ongoing metabolic processes, such as methanogenesis in rumen archaea or SCFA biosynthesis in the gut [22,25].

Metaproteomics and metabolomics quantify the actual enzymes and metabolites present, facilitating the comprehension of microbial influence on host physiology [14,15].

Bringing these layers together makes it clear that function, not composition, is what determines ecosystem outcomes. This means that interventions can focus on biochemical pathways instead of taxa.

7.2 Culturomics and the rebuilding of synthetic communities

Omics show diversity, but culturomics, which is the large-scale cultivation of microbial isolates in different environments, is still very important for testing and engineering [3,13,15]. Improvements in anaerobic culturing and microfluidics have made it possible to get back gut and soil species that were thought to be "unculturable" before. Once separated, microbes can be put back together into synthetic communities (SynComs) that mimic basic ecological functions in simpler systems.

In farming, SynComs of PGPR and AMF help plants take in more nutrients and deal with stress better [5,7,24].

In human health, characterised bacterial consortia emulate donor microbiota for secure, reproducible faecal microbiota transplantation alternatives [17,18].

SynCom methodologies integrate discovery and design through the evaluation of mechanistic hypotheses concerning cooperation, competition, and metabolic exchange.

7.3 Modelling how communities work: from GEMs to systems that change over time

Researchers can use genome-scale metabolic models (GEMs) to model how metabolic fluxes work within and between different types of microbes. GEMs anticipate nutrient dependencies, cross-feeding, and reactions to environmental alterations, facilitating the selection of compatible species in engineered consortia [11,15,17]. Static models are not able to fully capture dynamic ecological feedbacks. Because of this, dynamic community models and agent-based simulations are being used more and more to predict growth and metabolite trajectories over time.

Some important uses are:

Forecasting nutrient competition and hydrogen dynamics in rumen methanogenesis models [22,25].

Simulating the metabolism of bile acids and the production of short-chain fatty acids in human gut ecosystems [12,15].

Creating multi-strain inoculants that improve the cycling of nitrogen and phosphorus in soils [1,4,7].

Modelling converts intricate multi-species interactions into quantitative design principles for microbiome engineering.

7.4 Experimental systems: models for in vitro, in vivo, and ex vivo research

Different experimental systems give you different levels of control and realism:

In vitro microcosms simulate environmental conditions for soil, rhizosphere, or gut microbial communities under regulated inputs.

Ex vivo platforms, including gut-on-chip and root-on-chip devices, amalgamate host cells, fluid dynamics, and spatial gradients to replicate physiological environments [14].

Gnotobiotic animal models, which are organisms that are either germ-free or have a specific microbiota, make it easier to study how certain microbes work [11,15].

Field trials in agricultural and pastoral systems assess persistence, colonisation, and productivity effects in the context of natural variability [6,7,9,22].

A tiered design, from the lab to the field, makes sure that changes to the microbiome are still based on good science and are still important to the environment.

7.5 How to measure how long something lasts and how it gets set up

Because microbial establishment is important for successful interventions, measuring persistence has become a key part of microbiome engineering. According to ecological theory, establishment depends on Propagule Pressure (PP), Environmental Filtering (EF), and Biotic Interactions (BI) [2,10].

PP is measured by the inoculum dose, how often it is given, and how long it stays alive.

We monitor EF by checking pH, temperature, and gut nutrient levels.

We test BI using competition and cooperation assays, meta-omics co-occurrence analyses, and studies on how biofilms form.

Quantitative persistence data assist developers in enhancing the design of inoculants, assessing their likelihood of engraftment, and optimising field application strategies.

7.6 Tests in the field and ecological validation

In agriculture, microbiome-based products must show that they work well and can be used on a wide range of soils, climates, and crop types. Factorial field designs utilise soil chemistry, climatic data, and management practices to evaluate performance and reproducibility [5–9, 21, 24].

In human health research, randomised controlled trials (RCTs) assess colonisation, metabolite production, and immune markers to confirm the efficacy of probiotics, engineered strains, and particular consortia [12,15,17,18]. Standardised endpoints, such as microbial persistence, metabolite restoration, and barrier integrity, enable comparisons across studies and regulatory frameworks.

7.7 Integrating data and systems bioinformatics

Big microbiome projects now create terabytes of multi-omics data that need special computer programs to work with. Machine learning and network analysis can help find keystone species, core metabolic modules, and things that can predict changes in function. Cross-domain data integration—linking soil microbiomes to crop yields or gut microbiomes to clinical phenotypes—consolidates human and agricultural research within the One Health paradigm [21,23]. Making databases that can work together and open-access pipelines makes sure that results can be repeated and makes it easier to go from finding something to using it.

7.8 Summary

Measurement is the basis of engineering. It is now possible to move from descriptive microbiome ecology to actionable design thanks to multi-omics technologies, synthetic community building, and modelling frameworks that connect microbial activity to system function. Combining lab accuracy with field testing in ecosystems, such as the human gut, the rhizosphere, and the rumen, ensures that microbiome treatments are based on sound science, good for the environment, and likely to work [1–25].

Why Things Fail: Barriers to Translation

Microbiome-based treatments are not always effective outside of controlled settings, even though science has made a lot of progress. The transition from laboratory efficacy to real-world reliability remains inconsistent, both in human medicine and agriculture. Problems with the environment, technology, and rules that make it hard for microbes to grow, stay alive, and do their jobs all the time. To make microbiome engineering a reliable, scalable technology, we need to know what these barriers are [1–25].

8.1 Environmental barriers to establishment and survival

A primary issue is that introduced microbes, regardless of their potential benefits, frequently do not integrate into pre-existing complex ecosystems. Ecological theory delineates three interrelated factors that influence successful colonisation: Propagule Pressure (PP), Environmental Filtering (EF), and Biotic Interactions (BI) [2,10].

Insufficient propagule pressure: Many inoculants are used in doses that are too low to overcome random changes in population and the environment. A low population density raises the risk of early extinction, particularly when pH, oxygen, and nutrient levels are not stable.

Harsh or mismatched environments: Soil chemistry, temperature, moisture, host diet, or gut transit stress can kill off introduced microbes before they can join [6,7,8,12]. In the human gut, gastric acidity and bile salts kill a lot of oral probiotic cells before they can reach their target niche [14,18].

Negative biotic interactions: Established communities may outcompete newcomers for resources or space, release inhibitory substances, or even prey upon them. Antagonism, protozoal predation, or phage infection of introduced strains frequently diminishes persistence in both soil and gastrointestinal environments [2,10].

For translation to work, designs must take into account the local ecology. This can be done by using the best delivery methods, protective formulations, co-inoculation strategies, and timing with environmental changes to create new ecological niches.

8.2 Dependence on context and reproducibility

Field and clinical inconsistency continue to pose the most significant practical obstacle to adoption. A microbe or consortium that excels in one environment frequently underperforms in another due to context-dependent performance [1,4,6,8].

Soil pH, nutrient content, texture, and crop genotype have a huge effect on how microbes colonise and work in agriculture. Even small changes in temperature or in rainfall can change the rhizosphere microbiome [8,24]. Diet, genetics, medication use, and baseline microbiota composition contribute to personalised responses to probiotics or faecal microbiota transplantation in human health [12,20].

Because of this context dependence, there is no one-size-fits-all inoculant. Translation necessitates localised consortia, genotype-specific formulations, and adaptive validation across diverse environmental contexts. It

also shows that we need big, multi-site trials that look at how the environment changes instead of averaging it out.

8.3 Uncertainty about function: composition does not equal performance

Another common problem is the tendency to think that the composition of microbes is the same as their function. Research consistently demonstrates that analogous taxa can exhibit divergent behaviours across various hosts or soils, whereas distinct communities may fulfil equivalent functions owing to functional redundancy [1,7,22,25]. For instance, total methanogen abundance does not consistently forecast methane yield in ruminants; activity-level assessments (metatranscriptomics) provide significantly greater insight [22,25]. Similarly, in the gastrointestinal tract, the metabolism of short-chain fatty acids (SCFAs) and bile acids relies on active gene expression rather than the presence of particular genera [12,15].

Translational programs that depend only on 16S profiles or DNA-based markers might not see real functional dynamics. It is therefore necessary to combine multi-omics and metabolite-level readouts in order to predict efficacy.

8.4 Limitations in technology and methods

Genome editing and synthetic biology have made great strides, but there are still gaps in the tools available, especially for organisms that aren't models.

In the human microbiome, only a limited number of commensals are currently suitable for precise genetic manipulation, primarily due to ineffective DNA delivery and robust restriction–modification systems [11,15].

In agriculture, there are not many field-ready tools to monitor microbial activity in situ; many useful strains are still uncultured or poorly documented [1,7,9].

Dynamic modelling frameworks that forecast microbial fluxes in real time remain computationally demanding and data-intensive [15,17].

Scaling microbiome interventions is still hard because there aren't good ways to engineer and track community function.

8.5 Problems with formulation, stability, and deployment

Microbial products need to stay alive and work properly during manufacturing, storage, and use in order to be successful in business. Some common problems are:

Short shelf life or being sensitive to heat and dryness.

Not being able to use fertilisers, pesticides, or medicines at the same time.

Insufficient carriers or delivery modes can lead to uneven distribution in soil or host environments [6,7,9].

Biofilm-based formulations, nanocarriers, and polymer encapsulation are some new ideas that could help organisms live longer and spread more easily [9,14]. However, these ideas are more expensive and harder to regulate.

8.6 Safety, rules, and public support

Safety worries are a major reason why genetically modified or live microbial therapeutics are not more widely used. Regulatory agencies categorise engineered probiotics and live biotherapeutic products (eLBPs) as biologic drugs necessitating comprehensive GMP production, pharmacokinetic assessment, and containment systems to avert horizontal gene transfer [18,19,20]. In agriculture, long registration processes and overlapping jurisdictions slow down the approval of new products, especially in the European Union [7].

Another limitation is how the public sees things. People are often afraid of genetic engineering, even in helpful microbes. This shows how important it is to be open about what you're doing, do thorough risk assessments, and label everything. Ethical issues, like ecological spillover, gene transfer to wild populations, and effects on biodiversity, need to be dealt with before they happen.

8.7 Data, reproducibility, and standardisation

Microbiome research produces vast, diverse datasets, frequently gathered using inconsistent protocols. Differences in sequencing depth, analysis pipelines, and metadata make it hard to compare studies. The lack of standardised endpoints, including uniform definitions for "engraftment," "persistence," or "function restoration," hinders meta-analyses and evidence-based regulation [12,15,17,18]. For reproducibility and regulatory confidence to be possible, there must be harmonised data standards, reference materials, and open-access databases.

8.8 Barriers to the economy and infrastructure

In many areas, especially in developing agricultural economies, high production costs, a lack of cold-chain storage, and few extension services make it hard to get microbial products. In medicine, the high cost of clinical trials for eLBPs and engineered probiotics is still too high, which is holding back commercialisation even though there is strong preclinical data [9,18]. For fair deployment, it will be very important to build a decentralised production and testing infrastructure.

8.9 Recap

Microbiome interventions do not fail due to the lack of microbial potential, but rather because ecological complexity, functional uncertainty, and logistical constraints are inadequately acknowledged. To get past these problems, ecology, systems biology, engineering, and policy need to come together. To be successful in the future, we need to design for persistence (PP–EF–BI balance), measure function instead of composition, fill in genetic and formulation gaps, and make sure that safety, regulatory, and social

frameworks work together across sectors. Microbiome engineering can only provide consistent, scalable benefits for human and agricultural systems by incorporating these lessons [1–25].

Solution Playbooks

This section summarises useful, evidence-based ways to turn microbiome research into reliable, scalable changes that can help people and crops. Each playbook connects design decisions to measurable outcomes and references techniques and examples from Papers 1–25.

9.1 Design for persistence: dose, timing, niche, and delivery

Engineer for establishment (PP–EF–BI). To increase the chances of engraftment, adjust Propagule Pressure (dose, frequency), match Environmental Filtering (niche resources, pH, temperature, host diet), and control Biotic Interactions (competition, antagonism, predation/fage) [2,10].

Choose delivery that works with the environment. To make sure that cells get to and live in the target (gut segment, rhizosphere, endosphere), use protected formats (encapsulation, lyophilised seed coats, gels) and site-specific release [7,10,14,18].

Time with interruptions or windows. Dose around antibiotic pulses, fertiliser events, irrigation/rain, or plant phenology to open niches and reduce competitive exclusion [2,6,8,10,14].

Explicitly measure establishment. Instead of just presence, look at persistence (colonisation over weeks to months), proliferation, and function (metabolites, pathway activity) [2,10,11,15,18,25].

9.2 Design of consortia: splitting up work and steering the spectrum

Design functions, not lists of strains. Map target pathways, such as SCFA or bile-acid remodelling in the gut and N fixation/P /P/P solubilization/ISR in crops, and group them with other guilds, such as producers, cross-feeders, and "scaffold" colonisers [1,4,5,12,15,24].

Take advantage of cooperative ecologies. To keep communities stable, use cross-feeding and metabolic handoffs. Don't let one strain take over and break down function when stressed [3,5,13,15].

Use co-culture to start chemistry. Use co-cultured *Streptomyces* to make silent BGCs and expand antifungal spectra; adjust the dose to find the right balance between promoting growth and stopping it [13].

Protect the spectrum. Prefer narrow-spectrum antagonism (lipopeptides, phenazines, AMPs) to kill pathogens without hurting helpful ones; guide community outputs (like propionate in low-CH₄ rumen types) [5,9,13,16,25].

9.3 Materials and devices: make sure that delivery and measurement work

Target and protect. Use enteric coatings, pH/enzymatic triggers, hydrogels, and microcapsules for oral eLBPs/probiotics. For rhizosphere/endosphere colonisation, use biofilm-aware carriers or seed coatings [7,14,16,18].

Before men/field, model. Utilise organoids, gut-on-chip, and root-on-chip to evaluate colonisation, barrier effects, and dosing regimens within authentic gradients [14].

Put the intervention into action. Incorporate biosensors and noninvasive sampling to monitor spatiotemporal function (SCFAs, bile acids, pH, inflammatory cues) and engraftment kinetics in vivo and in field plots [14,16].

Co-design with formulation. Make sure that nanocarriers are safe and useful by weighing the pros and cons of their use (benefits vs. toxicity/regulatory burden) [7,9,14].

9.4 Precision engineering: genomes, circuits, and control

Choose a manageable chassis and add it to the toolbox. Use species that already have parts (like *Bacteroides*, *E. coli* Nissle, *Lactococcus*, and *Saccharomyces*) while you work on ways to deliver and edit non-models (like conjugation, phage vectors, integrases, and CRISPRi/CRISPR) [11,15,17,18].

Set up therapeutic functions. Use strains that release cytokines and peptides, break down toxins and metabolites (like ammonia and Phe), or change the immune and metabolic axes. Use biosensor-gated expression to lower the burden and off-targets [11,15,17–20].

Make safety a part of the plan. Use kill switches, auxotrophy, and genomic insulation to lower HGT. Also, choose chassis and payloads that have good immunogenicity. Plan PK/PD and shedding profiles ahead of time [18–20].

Use metabolism models to plan communities. Use GEMs and L-D-B-T cycles to pick the smallest, most cooperative groups. When time-series data backs them up, use dynamic models [11,15,17].

9.5 Field-aware programs: check where life happens

Create trials that take place at more than one site and last for more than one season. Capture variation across soil types, climates, genotypes, and management practices; report contextual metadata to facilitate generalisation [1,4–9,21,24].

Be in line with agronomy and animal husbandry. Combine with IPM, fertiliser plans, irrigation, and pasture composition (e.g., clover ratios); in ruminants, incorporate breeding for low-CH₄ phenotypes with dietary and microbial modulation [7–9,21,22,25].

Use endpoints that focus on function first. In crops: nutrient use efficiency, yield stability, pathogen load, micronutrient biofortification; in humans: barrier integrity, metabolite restoration, immune markers—not just 16S shifts [5,7,12,15,17,24,25].

Plan for manufacturing and shipping ahead of time. Make sure that GMP/cGMP, cold-chain or shelf-stable formats, and compatibility with standard equipment (seeders, sprayers, and clinical dosing) are all in place [7,9,18].

9.6 Decision trees and checklists: making success happen

Persistence triage (PP–EF–BI).

If the dose is low, increase the inoculum or the dose frequency to improve carrier survival.

If the niche doesn't fit, add prebiotics or substrates, change the timing or placement, and pre-adapt the strains.

If biotic antagonism is present, add facilitators or keystones, give physical protection, or use blockers that target phage [2,10].

Consortium builder. • Set target functions and readouts, map them to guilds, choose the fewest strains for division of labour, simulate with GEMs, check cross-feeding and stability, and make a plan for co-delivery [5,11,15,17].

Human eLBP path. • Choosing a chassis, parts, and circuits (with biosensor gates and biocontainment), materials for GI transit, a PK/PD plan, phase-appropriate endpoints (engraftment and function), and a GMP and regulatory dossier [14,17–20].

Deployment in agriculture. • Site assessment (soil, climate, genotype, management) → pilot SynCom vs single-strain comparison → dose/formulation optimisation → seasonal timing → multi-site validation with function-first metrics → farmer-ready SOPs [1,4–9,21,24,25].

Summary

Microbiome engineering that works is not just one new idea; it is the combination of ecology-aware persistence design, consortia logic, materials-enabled delivery and measurement, precision genetic tools, and real-world validation. Using these playbooks and reporting standardised, function-first endpoints turns the promise of the lab into real-world effects in medicine and farming [1–25].

Case Snapshots (Optional concise vignettes)

10.1 Engineered commensal for metabolic and IBD indications

Issue. Dysbiosis, characterised by SCFA/bile-acid imbalance and barrier dysfunction, induces inflammation and metabolic dysregulation.

Intervention. Engineered *Lactococcus lactis* to secrete IL-10 or GLP-1; engineered *Escherichia coli* to degrade phenylalanine (PKU) or ammonia (hyperammonemia); biosensor-gated expression to minimise burden and off-target effects.

Readouts. ↓ Inflammatory markers, better glycemia/lipids, target metabolite depletion, barrier integrity assays; colonisation kinetics (short residency means re-dosing).

Notes for design. Choosing the right chassis and parts, biocontainment (kill switches/auxotrophy), HGT mitigation, PK/PD planning, and GMP manufacturing. [11,15,17–19]

10.2 Defined groups to bring back colonisation resistance

Issue. Wide dysbiosis after antibiotics or in rCDI; variability with FMT.

Action. Defined donor-inspired consortia (e.g., 7–11 strains) optimised for SCFA/propionate production, pathogen inhibition, and immune activation.

Readouts. Engraftment (strain-level tracking), restoring metabolites (SCFAs/bile acids), and clinical endpoints (rCDI cure and adjunct oncology responses).

Notes on the design. Division of labour, cross-feeding, spectrum steering, materials-enabled delivery, phase-appropriate endpoints, and a safety dossier. [12,15,17,18]

10.3 Co-cultured *Streptomyces* for crop protection and growth

Issue. Single-strain biocontrols have narrow spectra and don't always work well in the field.

Intervention. Co-culturing rhizosphere *Streptomyces* (MDJK11+MDJK44) to activate dormant BGCs, broaden antifungal activity (e.g., *Fusarium* spp.), and improve P/N solubilisation and phytohormone profiles.

Readouts. Smaller lesion areas on leaves, more growth of roots and seedlings at the right dilution, and LC-MS metabolite enrichment (antibiotics and plant hormones).

Notes on the design. Dose is important (high dilution promotes growth; low dilution stops it), spectrum trade-offs (less anti-*E. coli*), and media/field context (OSMAC). [13]

10.4 Low-CH₄ ruminotype: what it is and what it aims for

Issue. Enteric methane is a significant greenhouse gas and energy loss; data on abundance alone cannot predict emissions.

Intervention. Choose low-CH₄ ruminotypes that are rich in propionate-linked bacteria (like *Quinella*, *Fibrobacter*, and *Prevotella*) and change the activity of methanogens (not just their number). Utilise diet (nitrate, lipids, seaweed extracts), microbial transfaunation, or synthetic consortia to divert H₂ from methanogenesis.

Readouts: lower CH₄ yield, metatranscriptomic methanogenesis signatures, VFA profiles, and animal performance.

Notes on design. Keep an eye on hydrogen and formate flows; connect them to breeding and pasture management; and make functional databases better for prediction. [22,25]

10.5 Consortia versus single strains in stressed crops

Issue. Single PGPR often doesn't work well in soils that are low in P, alkaline, sandy, or dry.

Intervention. Consortia (PGPR + AMF/endophytes) that combine N-fixation, P-solubilization, siderophores, and ISR; tomato and grain systems have better yield and nutrient uptake under stress than single strains.

Readouts. Pathogen load, micronutrient biofortification, P/N uptake, and yield stability.

Notes on design. Tailoring for specific sites, making sure the product lasts a long time and can be delivered to seeds and soil, and validating it at multiple sites. [4,5,24]

10.6 eLBP clinical reality check

Issue. Early engineered LBP trials demonstrate brief residency (~48 h) and variable efficacy; approximately half of the concluded trials report insufficient activity.

Intervention. Re-dose schedules, rethinking the chassis, biosensor-gated circuits to make things easier, and organs-on-chips to reduce risk before human trials.

Readouts. Engraftment, shedding, target function, safety, and cGMP-grade consistency.

Notes on design. Teach doctors and patients; use function-first endpoints to build regulatory evidence. [17,18]

Synthesis: Design Principles

The main point of Papers 1–25 is clear: reliable microbiome interventions happen when ecology, engineering, and measurement work together. The following principles turn that agreement into design rules that can be used.

11.1 Function first, not taxa first

Focus on pathway activity and metabolite goals (like SCFAs, bile-acid remodelling, and hydrogen sinks) instead of the presence of certain genera. Focus on metatranscriptomics, metabolomics, and functional assays to help with design and goals [1,12,14,15,22,25].

11.2 Design for longevity with PP–EF–BI

Plan for Propagule Pressure (dose/frequency), Environmental Filtering (niche, timing, disturbance), and Biotic Interactions (competition, antagonism, facilitation/predation) as co-determinants of establishment.

Use decision trees to find out what went wrong and choose levers like re-dosing, adding resources, protection, or helpers [2,10].

11.3 Tune every intervention to the situation

Expect the site, season, genotype, management, and baseline community to affect the results. Localise consortia and protocols; test them in trials or cohorts that happen at more than one site and in more than one season before scaling up [1,4–9,21,24].

11.4 Use consortia logic and division of labour to build

Use complementary guilds (producers, cross-feeders, scaffold colonisers) to cover target functions; exploit co-culture to activate silent BGCs; steer spectrum to spare beneficials and hit pathogens; avoid single-strain dominance [3,5,9,13,15,24].

11.5 The therapy includes materials and delivery.

Combine biology with encapsulation, enteric coatings, hydrogels, biofilm-aware carriers, and site-specific triggers to get cells and cargos to the right place; use organoids/gut-on-chip and sensors for preclinical screening and in vivo monitoring [14,16,18].

11.6 Safety-by-design in precision engineering

Choose a manageable chassis and add tools for non-models, like conjugation/phage vectors and CRISPRi/CRISPR. Encode biosensor-gated circuits, kill switches, auxotrophy, and genetic insulation to reduce HGT and burden; strategise PK/PD, shedding, and immunogenicity from the outset [11,15,17–20].

11.7 Measure what matters (standardised, function-first endpoints)

Report engraftment/persistence, pathway activity/metabolites, barrier integrity/immune markers (human), and nutrient-use efficiency/yield stability/pathogen load/biofortification (agriculture). Align metadata and protocols to facilitate comparison and regulation [5,7,12,15,17,18,24].

11.8 Make a model before you mix

Use GEMs and L-D-B-T cycles to narrow down the number of small, cooperative communities. When time-series data supports them, use dynamic models. In rumen studies, simulate hydrogen/formate flows to forecast methane results [11,15,17,22,25].

11.9 Be in line with management, husbandry, and agronomy

Include microbes in fertility plans, IPM, irrigation, and pasture composition. For ruminants, combine microbial strategies with breeding for low-CH₄ phenotypes and diet design [7–9,21,22,25].

11.10 From the start, plan for how easy it will be to make and how it will be regulated.

Design for stability, shelf life, compatibility, and production that meets cGMP/GMP standards. For eLBPs, consider products as drugs with evidence appropriate for each phase; for agricultural biologics, expect limitations on regional registration and logistics [7,9,18–20].

11.11 Customisation and categorisation

Take into account the baseline microbiota, diet, genotype, medications (for people), and soil, variety, and climate (for farming). Employ patient or site stratification and customised consortia/dosing to diminish variance and enhance effect sizes [6,8,12,15,20,24].

11.12 One Health management

Keep an eye on ARGs, zoonotic risks, and biogeochemical effects. Make sure that datasets and policies for people, animals, and the environment work together so that improvements in one area don't cause problems in another [21,23].

In conclusion, microbiome engineering is effective when interventions prioritize functional outcomes, are designed for robust establishment, are contextually adapted, and employ rigorous measurement, while ensuring safety, manufacturability, and One Health stewardship are integrated throughout [1–25].

Future Directions

To turn microbiome science into reliable treatments, we need to close gaps in tools, standardise function-first endpoints, and make sure that ecology-aware design is part of every stage of development. The priorities below bring together ideas for moving forward in human health, farming, and pastoral systems.

12.1 Large-scale genome editing in situ

Transition from ex vivo strain editing to in situ modulation of resident communities utilising phage- and conjugation-based vectors, payload-stabilizing integrases, and CRISPR/CRISPRi systems tailored for non-model commensals [11,15,17,18]. Create modular delivery "kits" (phages that work with a wide range of hosts, conjugative elements that work with a wide range of hosts, and anti-restriction parts) and standard quality control for editing in complex microbiomes that are on-target and off-target [11,18].

12.2 Engineering that works in many kingdoms and networks

Expand beyond bacteria to include fungi, archaea, protists, and phages: create consortia that manage the division of labour across kingdoms (for example, AMF–PGPR nutrient handoffs; fungal–bacterial disease suppression; phages that change competitive landscapes) [1,5,7,13,24]. In the rumen, combine archaeal and protozoal modules to actively control the hydrogen/formate economy that makes methanogenesis happen [22,25].

12.3 Standardised clinical and field endpoints that take activity into account

Establish function-first endpoints (engraftment/persistence; SCFAs, bile-acid species; barrier integrity; immune markers; nutrient-use efficiency; yield stability; CH₄ yield) with standardised metadata and SOPs to ensure comparability of results across trials and regions [5,7,12,15,17,18,24,25]. Focus on metatranscriptomics/metabolomics and flux proxies rather than just composition, especially for reducing methane and metabolic indicators [12,22,25].

12.4 Default decision tools for establishment: PP–EF–BI

Make Propagule Pressure–Environmental Filtering work in real life. Biotic interactions are a standard part of every intervention, with tools like dose/re-dose calculators, disturbance-timing schedulers, and checklists for antagonism, predation, and facilitation all put together in useful design software for lab and field teams [2,10].

12.5 Materials that provide and assess function

Develop stimuli-responsive encapsulation, biofilm-aware carriers, and region-specific release systems for gut and root niches. Combine these with ingestible or implantable biosensors and organ-on-chip platforms to reduce the risk of dosing and dynamics before human or field deployment [14,16,18]. In crops, standardise seed-coat and soil gel formulations that co-deliver microbes and substrates to pre-match niches [7,14,24].

12.6 High-throughput strain and tool discovery for non-models

Speed up culturomics, directed evolution, and part libraries (promoters/RBS/terminators, recombineering helpers, RM bypass) for anaerobes and slow-growing organisms that are currently limiting therapeutic and agronomic options [11,15,17]. Make "starter kits" for priority genera (*Bacteroides*, *Firmicutes*, rhizosphere *Bacillus*/*Pseudomonas*, *Streptomyces*) with checked editing and delivery workflows [11,13,15].

12.7 Minimal consortia guided by models

Employ GEMs and Learn-Design-Build-Test cycles to refine minimal, collaborative consortia that achieve metabolite objectives with inherent stability (cross-feeding, niche complementarity), progressing to dynamic models featuring time-series data (e.g., bile-acid remodelling, hydrogen sinks) [11,15,17,22,25].

12.8 Pipelines for personalisation and stratification

Make stratification a regular part of your work. For example, match chassis/consortia to baseline microbiota, diet, genotype, medications (for people), soil type, climate, cultivar/variety (for agriculture) to increase effect sizes and lower variance [6,8,12,15,20,24]. Integrate adaptive dosing and response-guided re-formulation into protocols, acknowledging brief residency in certain chassis [18].

12.9 Rumen methane: from taxa to control theory

Consider methane mitigation as the regulation of electron flow: combine diet (nitrate, lipids, seaweed), selective microbial transfers, and engineered sinks (propionate/acetogenesis modules) with real-time CH₄/VFA sensing and activity-aware models; incorporate breeding for low-CH₄ phenotypes and pasture management [22,25].

12.10 Surveillance and stewardship for One Health

Set up cross-sector networks that keep an eye on ARGs, zoonotic risks, and biogeochemical effects on people, livestock, wildlife, soils, and waters. Connect environmental actions (like biofertilizers and bioremediators) to public health metrics and climate outcomes using the same data standards and policy frameworks [21,23].

12.11 Development that is safe by design and ready for regulation

Use biocontainment (kill switches, auxotrophy), genetic insulation to limit HGT, and PK/PD + shedding plans as the default. Treat eLBPs like drugs with phase-appropriate evidence and cGMP manufacturing from the start [17–20]. For agricultural biologics, expect regional registration and design quality to last longer on the shelf and work well in the field [7,9].

12.12 Infrastructure, cost, and access

Put money into decentralised manufacturing, formulations that don't need a cold chain, and extension services so that the benefits reach places with few resources. In medicine, lower trial costs are achieved by using organoid/organ-on-chip screening and adaptive designs that use functional biomarkers as early decision gates [14,18].

Prospects. The next wave of microbiome engineering will be defined by in situ programmability, multi-kingdom consortia, and standardised, function-centric endpoints—all executed through PP–EF–BI-aware design, materials that both deliver and measure, and regulatory-ready, safety-by-design development. If these priorities are met, living medicines and agricultural biologics can become everyday, dependable tools for protecting the environment, food security, and human health [1–25].

Conclusions

The reviewed papers (1–25) across human health, agriculture, and pastoral systems converge on a fundamental proposition: microbiome engineering is effective when designed for functionality, durability,

and contextual relevance. First, function-first targeting—measuring and directing pathway activity and metabolites (e.g., SCFAs, bile acids, hydrogen/formate sinks)—is superior to taxa-first approaches that depend solely on composition [1,12,14,15,22,25]. Second, establishment is engineered, not assumed: success depends on balancing Propagule Pressure, Environmental Filtering, and Biotic Interactions (PP–EF–BI) with the right dose, timing, niche matching, and protection/formulation to achieve durable engraftment and performance in complex resident communities [2,10,14,18]. Third, context matters—things like soil type, climate, host genotype, baseline microbiota, diet, and management all affect results. That's why multi-site, multi-season validation and stratified, locally tuned designs are necessary for reliability and scale [1,4–9,12,21,24].

In the realm of human health, there is a transition from empirical probiotics and faecal microbiota transplantation (FMT) to engineered live biotherapeutics and specified consortia, driven by genome and metabolic engineering, materials-enabled delivery, and functional endpoints. The translation process now relies on tools for non-model commensals, in situ editing and delivery, biocontainment, good manufacturing practices (GMP) manufacturing, and phase-appropriate clinical evidence [11,14–20]. In agriculture, single-strain inoculants are being replaced by consortia and co-culture strategies that provide nutrient cycling, stress tolerance, and biocontrol with greater strength, as long as they are made to last and fit with agronomy and the environment [4–8,13,24]. In pastoral systems, methane mitigation is progressing as a control-of-electron-flow challenge: activity-aware models and interventions that divert reductants from methanogenesis offer potential improvements in climate impact and feed efficiency [22,25]. One Health must connect these areas in order to manage microbiomes that are important for therapy response, food security, and biogeochemical cycles while also lowering the risk of zoonotic diseases and antimicrobial resistance [21,23].

The way forward is practical and shared: standardise function-first endpoints, make PP–EF–BI the default design triage, pair biology with materials and devices that both deliver and measure, expand toolkits for non-models (including in situ vectors), and build regulatory-ready pipelines from day one [2,7,9,11,14–20,24,25]. If these principles are consistently implemented, living medicines and agricultural biologics can achieve predictability, scalability, and safety, transforming microbiome science into a standard, dependable practice that enhances health outcomes, stabilizes yields, and promotes climate stewardship [1–25].

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