

CER: A microbiome-relay model for cross-kingdom RNA signalling and seed-stage epigenetic modulation in plants

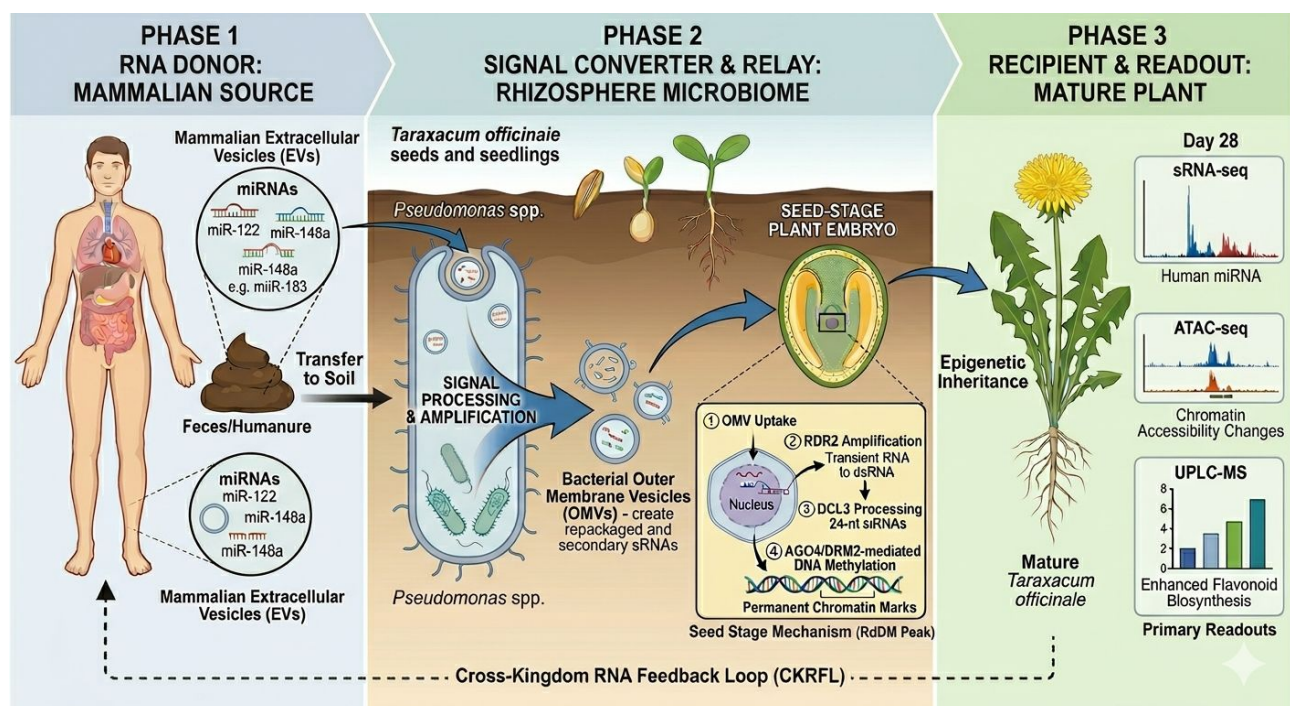
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Graphical abstract



Abstract

In this article, we present the Cross Epigenetic Recycling (CER) hypothesis, which postulates a microbiome-based channel for RNA information transfer between mammals and plants via the soil. The central novelty is the relay model: miRNAs from mammalian waste-derived extracellular vesicles (EVs) enter the soil and are taken up by rhizosphere bacteria - particularly *Pseudomonas spp.* with a documented OMV/sRNA system - which repackage and retransmit the signal to plant root cells via outer membrane vesicles (OMVs). The model relies exclusively on biologically documented intermediate steps, each with at least one clear precedent in the literature. The proposed mechanistic window is the seed stage: the plant embryo exhibits the highest RNA-directed DNA methylation (RdDM) activity of any plant tissue, and RDR2-dependent amplification can fix exogenous RNA signals as permanent chromatin marks. Recent studies confirm bidirectional RNA transfer between mammalian cells and bacteria via EVs (Gröger et al., 2026) and between bacteria such as e.g. *Pseudomonas* and plants (Ravet et al., 2025), collectively strengthening the biological realism of the relay model. Additionally, we propose five testable predictions, including a negative control, an experimental design using apomictic *Taraxacum officinale*, incorporating sRNA-seq, ATAC-seq, UPLC-MS metabolomics, and rhizosphere microbiome sequencing, to test the CER model and explore its potential for non-GMO precision RNA agriculture.

Keywords: cross-kingdom RNA signalling, extracellular vesicles, soil microbiome, epigenetic inheritance, RNA-directed DNA methylation, *Taraxacum officinale*, plant epigenome, CER relay

1. Introduction

For millennia, agriculture has rested on a simple intuition: organic waste from humans and animals fertilizes the soil, which in turn produces healthier plants. Modern agronomy has explained this relationship primarily in chemical terms focusing on the metabolism of nitrogen, phosphorus, and potassium. However, the discovery of cross-kingdom RNA interference (RNAi) in pathogen-host interactions, such as between *Botrytis cinerea* and tomato, suggests an additional informational dimension to this exchange [1]. *Botrytis cinerea* exports sRNAs packaged in extracellular vesicles (EVs) that are taken up by plants, where they hijack host AGO/RISC complexes and suppress defense genes within 24–72 hours [1]. More broadly, bacterial RNA secreted in extracellular vesicles (evRNA) is now documented in numerous pathogens - from *Pseudomonas aeruginosa* to *Salmonella* and *Listeria* - with a demonstrated capacity to directly regulate eukaryotic gene expression [26]. Importantly, this cross-kingdom RNA communication is bidirectional. Recent studies show that human colon cells secrete EVs containing miR-192-5p, which are taken up by gut bacteria and alter their physiology, while bacterial EVs induce transcriptomic changes in human epithelial cells [29]. Likewise, Ravet et al. (2025) demonstrated that *Arabidopsis* plants expressing sRNAs directed against *Pseudomonas syringae* can suppress bacterial pathogenesis - a process termed Antibacterial Gene Silencing (AGS) [40]. Plant-derived sRNAs (non-vesicular, EV-packaged, and free dsRNA) were recovered inside bacterial cells, proving that *Pseudomonas spp.* actively take up sRNA from the extracellular environment and respond with measurable transcriptomic changes. This bidirectional plant–bacterium RNA exchange provides direct mechanistic precedents for key steps of the CER relay model. If functional RNA signals can be transmitted to plants, a fundamental question arises: could other organisms, including humans, do the same through pathways not yet observed? The phenomenon of environmental RNAi (eRNAi) - sequence-specific regulation of gene expression - is well established in pathogenic contexts but likely has far broader evolutionary implications [11]. Bacterial delivery of nucleic acids to eukaryotic cells via OMVs and secretion systems is now a recognized biological mechanism [32], and while plant miRNAs have been shown to influence the mammalian gut microbiome (though this remains controversial) [34], the reverse direction - transfer of mammalian miRNAs to plants, directly or via the soil microbiome - has not yet been described. Given the evolutionary timescale, humans and plants have coexisted for millennia in ecosystems where organic waste containing exosomal miRNAs was routinely returned to the soil, creating sustained selective pressure for mechanisms capable of sensing and responding to these signals. In this article, we propose the microbiome-centric Cross Epigenetic Recycling (CER) hypothesis. Rather than requiring direct transfer of mammalian RNA into plant cells, the model posits an indirect relay in which the soil microbiome acts as a biological converter and signal transducer: human fecal miRNAs reach rhizosphere bacteria (particularly *Pseudomonas spp.*), which alter their gene expression and community composition before retransmitting secondary signals (sRNAs and/or metabolites) that modulate the plant epigenome and metabolome. A central feature of the hypothesis is the proposed mechanistic window

- the seed stage - during which the plant embryo displays the highest RNA-directed DNA methylation (RdDM) activity of any tissue, allowing RDR2-dependent amplification to convert transient RNA signals into stable chromatin marks. Below, we detail the proposed mechanism, compare it with the *Botrytis cinerea* model, present five falsifiable predictions including a negative control, and describe an experimental design using apomictic *Taraxacum officinale*. For terminological clarity, we define three concepts used throughout this paper: (1) CER (Cross Epigenetic Recycling) - the main testable hypothesis regarding the influence of human fecal miRNAs on the plant epigenome and metabolome via the soil microbiome; (2) the relay model - the indirect mechanism in which the soil microbiome serves as signal converter and transmitter (an integral component of CER); and (3) CKRFL (Cross-Kingdom RNA Feedback Loop) - a broader, speculative closed-loop concept (human-soil-plant-human), discussed as a direction for future research, verifying CER.

2. Cross-kingdom RNA signalling: from *Botrytis cinerea* to the CER relay model

Botrytis cinerea provides the closest biological precedent for the CER hypothesis. This fungal pathogen produces sRNAs (20–27 nt, peaking at 24 nt) that are processed by DCL1/2 enzymes, packaged into extracellular vesicles, and taken up by plant cells via endocytosis [1,2]. Once inside the plant, fungal siRNAs - including Bc-siR37 - load onto host AGO1/RISC complexes and suppress defense genes such as WRKY7 and PMR6/FEI2 within 24–72 hours of infection [1, 3]. Deep sequencing and qPCR confirm accumulation of fungal sRNAs in plant tissues, while silencing the fungal BcAGO gene increases plant susceptibility, supporting a causal role for RNA transfer [4]. Although the direct causality of some observed effects remains debated and requires more rigorous controls [4], the *Botrytis* system establishes a key proof of concept: sRNAs packaged in extracellular vesicles can cross kingdom boundaries and reprogram gene expression in the recipient organism. The CER relay model adapts this principle to a mutualistic, soil-mediated context and introduces three key structural differences. First, it reverses and extends the direction of transfer: rather than pathogen→plant, CER proposes a human→soil→plant pathway in which rhizosphere bacteria serve as an active biological converter. Plant miRNAs are already known to reshape rhizosphere bacterial communities [5, 6]; CER postulates the reverse direction. Second, the bacterial intermediary adds an amplification and repackaging layer: species of *Pseudomonas* and *Bacillus* take up environmental miRNAs and generate secondary sRNA signals that are delivered to plant root cells via OMVs - a mechanism directly demonstrated in both the bacterium→fungus direction [35] and the plant→bacterium direction [40]. *Pseudomonas aeruginosa* provides the best-characterised molecular precedent for bacterial OMV-mediated sRNA secretion and delivery to eukaryotic cells [15, 16]; however, the CER relay model is not mechanistically dependent on this single species. *P. aeruginosa* is acknowledged to occur at low frequencies in agricultural soils under standard conditions [18, 19], and the model explicitly encompasses other common rhizosphere taxa with documented OMV systems. *Pseudomonas fluorescens* and *Pseudomonas putida* are abundant, well-established plant-growth-promoting rhizobacteria that colonise roots of diverse crop species [47], and the *Pseudomonas* genus as a whole is characterised by versatile extracellular vesicle production and RNA secretion capacity [20]. The experimental design (Section 5.4) does not presuppose which taxon mediates the relay: 16S rRNA sequencing of the rhizosphere before and after fecal application will empirically identify which species respond

to miRNA input signal and expand in abundance, providing an unbiased, data-driven identification of the active bacterial intermediary. Third, the proposed mechanistic window is the seed stage, during which the plant embryo exhibits the highest RNA-directed DNA methylation (RdDM) activity of any tissue [21]. RDR2-dependent amplification at this stage can convert transient exogenous RNA signals into stable, heritable chromatin marks [22], making even low-abundance environmental miRNAs potentially sufficient to induce lasting epigenetic changes. The final step of the relay - uptake of microbiome-derived secondary signals by the plant and their translation into epigenetic and metabolic changes, including modulation of lncRNA expression and flavonoid biosynthesis pathways - serves as the primary readout of the proposed experimental design [4,21].

3. RNA bioavailability in the rhizosphere: a reappraisal

The stability of exogenous RNA in soil has long been viewed as a fundamental barrier to environmental RNA signalling hypotheses. This consensus, however, rests on methodologically limited data. Commercial RNA extraction kits typically recover little or no RNA from rhizosphere soil, not because of rapid enzymatic degradation but due to strong adsorption of nucleic acids to clay minerals. In contrast, optimized CTAB-based protocols with high-ionic-strength buffers recover high-quality RNA even from clay-rich agricultural soils [37, 38]. As a result, the apparent instability of RNA in soil largely reflects a detection artefact rather than true molecular lability, implying that the bioavailable RNA pool in the rhizosphere is substantially larger and more persistent than previously assumed. Independent evidence reinforces this reappraisal. Soil metatranscriptomics shows that adsorption to mineral particles is the dominant stabilisation mechanism for environmental RNA [43], with bacterial rRNA exhibiting half-lives exceeding 200 days at low temperatures [44]. RNA-viromics of agricultural soils further reveals a diverse and actively cycling pool of environmental RNA molecules [45], undermining the assumption that exogenous RNA is rapidly eliminated upon entering the soil matrix. In the CER model, miRNA availability is additionally enhanced by three protective mechanisms: packaging within OMVs/EVs shields RNA from extracellular RNases [7]; adsorption to mineral and organic soil particles increases persistence [8]; and rapid uptake by rhizosphere bacteria minimises exposure time in the extracellular environment. The protective role of EVs is directly documented in feces: Koga et al. (2011) showed that human fecal miRNAs encapsulated in exosomes resist RNase digestion and remain detectable after incubation in fecal homogenates, whereas free miRNAs degrade rapidly [42]. Although mammalian miRNAs are known to occur in feces and be taken up by gut bacteria [34, 41], their transfer to plants via soil remains undescribed, yet the mechanisms outlined above suggest its biological feasibility. Importantly, sequence-specific RNAi effects can be induced by exogenous RNAs at concentrations as low as 0.03 ng/μL [13], and applied dsRNA has been shown to penetrate plant tissues and trigger DNA methylation via the RdDM pathway [10]. The quantitative plausibility of this scenario is assessed in the following section.

3.1 Signal quantity across relay steps: a conservative feasibility estimate

A recurring objection to environmental RNA signalling hypotheses concerns the absolute quantity of signal molecules available at each relay step. We address this by constructing a conservative order-of-magnitude

estimate for the CER pathway under the proposed experimental conditions (1g lyophilized fecal material per kg of soil, seed-stage application).

Step 1 - Source abundance. Human fecal samples contain detectable miRNA in both free and EV-encapsulated forms, with the EV-packaged fraction resistant to RNase digestion even under prolonged incubation at 37°C [42]. Liu et al. (2016) demonstrated that miRNAs are abundant in human fecal samples and present within extracellular vesicles, with concentrations sufficient to enter bacteria and regulate their gene transcripts [41]. Although absolute copy numbers per gram of human feces have not been standardised across studies, the detectable and biologically active concentrations reported in both works are consistent with an estimated input of 10^6 - 10^8 EV-encapsulated miRNA molecules per gram of fecal material - a range we treat as the starting point of the estimate.

Step 2 - Soil persistence. As detailed in Section 3, adsorption to clay minerals is the dominant stabilisation mechanism for environmental RNA, with bacterial rRNA half-lives exceeding 200 days at low temperatures [44], and soil metatranscriptomics confirming a substantially larger and more persistent environmental RNA pool than previously assumed [43]. We conservatively assume that 1-10% of input EV-encapsulated miRNA remains biologically available at the rhizosphere interface within the first days of soil contact, yielding an estimated 10^4 - 10^7 accessible molecules per gram of soil.

Step 3 - Bacterial uptake. Ravet et al. (2025) demonstrated that *Pseudomonas syringae* actively takes up extracellular sRNAs from the plant apoplast, with sRNA recovered inside bacterial cells and producing measurable transcriptomic changes [40]. Although Ravet et al. (2025) measured uptake in the plant apoplast rather than bulk soil, the sRNA concentrations at which uptake was documented fall within the same order of magnitude as the rhizosphere concentrations estimated above, supporting the plausibility of analogous uptake under soil conditions. We therefore consider bacterial uptake of environmental miRNA at this range to be biologically plausible, while acknowledging that quantitative uptake efficiency by rhizosphere *Pseudomonas* spp. under soil conditions remains to be measured directly - which is precisely what the pilot module (Section 5.2) is designed to establish.

Step 4 - RNAi threshold at the seed. Sequence-specific RNAi effects have been demonstrated at exogenous RNA concentrations as low as 0.03 ng/ μ L [13], and root-applied dsRNA persists systemically in seedlings for at least 30 days [14]. The seed stage, with its peak RdDM activity and RDR2-dependent amplification, lowers the effective threshold further by converting even trace exogenous signals into stable chromatin marks [21, 22]. Under the conservative estimate above, miRNA concentrations at the seed surface are likely to approach or exceed the documented RNAi threshold, provided that EV integrity is maintained during soil transit - an assumption directly tested in the pilot module.

This estimate does not demonstrate that CER operates in nature; it demonstrates that the proposed experimental doses are not obviously insufficient. The pilot module (Section 5.2) will provide the first empirical data to replace these estimates with measured values.

Aspect	<i>Botrytis cinerea</i> (documented)	CER (microbiome-centric)
Direction	Fungus → plant	Human → soil → plant
Transport	OMV / endocytosis	Waste (OMV?) → bacteria (up- take/metabolites)
Dose / time	High / acute (24–72 h)	Low / chronic (weeks, seed-stage)
Effect	Defense suppression	Epigenome/metabolome shift
Validation	HIGS, sequencing	Dandelion test + pathogen-inacti- vated, size-fractionated feces

Table 1 - Comparison of *Botrytis cinerea* vs. CER (hypothetical model)

4. Hypothesis and predictions

The CER hypothesis posits that miRNA signals derived from human feces can be amplified by the rhizosphere microbiome and induce lasting changes in the plant epigenome and metabolome, particularly during the seed stage. To test this hypothesis, we propose the following five falsifiable predictions. In all predictions below, "human feces" refers to the pathogen-inactivated, size-fractionated preparation described in Section 5.1.

1. Application of size-fractionated, pathogen-inactivated human feces (retaining the EV-encapsulated and protein-associated RNA fraction) to *Taraxacum officinale* seeds will produce measurable changes in the plant epigenome (e.g., via bisulfite sequencing) compared with controls (water or RNA-depleted fecal fraction).
2. Exposure to human feces will significantly alter rhizosphere microbiome composition (assessed by 16S rRNA and ITS sequencing) relative to controls.
3. Exposure to human feces during the seed stage will induce more persistent and substantial changes in the plant epigenome and metabolome than a single acute application at later developmental stages.
4. Human miRNAs will be detectable in rhizosphere bacteria and/or plant tissues following application of human feces.
1. Negative (falsifying) prediction: Application of RNA-depleted, pathogen-inactivated fecal fraction will not result in statistically significant changes in the plant epigenome or metabolome compared with water-treated controls, thereby disproving functional RNA transfer.

5. Materials and Methods (Proposed)

5.1. Collection and Standardization of Human Samples

Feces will be collected from 20–30 healthy adult volunteers following ethics committee approval and immediately frozen at -80°C . To inactivate pathogens while preserving EV-encapsulated RNA integrity, samples will be subjected to low-speed centrifugation to remove cellular debris, followed by sterile filtration (0.45 μm to remove bacteria, retaining EVs) or low-dose UV inactivation at a fluence validated not to degrade small RNAs. Samples will then be lyophilized and reconstituted in RNase-free water prior to application. RNA content will be quantified on a subset using fluorometry (Qubit), with qPCR of selected miRNAs (miR-122, miR-148a) serving as internal reference markers. Controls will include pathogen-inactivated fecal fraction treated with RNase (RNA-depleted) and pathogen-inactivated fecal fraction without RNase treatment.

5.2. Pilot Module - miRNA Stability and Uptake Test

Prior to the main *Taraxacum officinale* experiment, a pilot module will replace the order-of-magnitude estimates presented in Section 3.1 with directly measured values. Specifically, it will quantify three parameters central to the mass-balance analysis: (a) the fraction of EV-encapsulated miRNA remaining biologically available after 7 and 14 days in experimental soil; (b) the uptake efficiency of human miRNAs by rhizosphere bacteria under soil conditions; and (c) whether recovered miRNA concentrations approach the 0.03 ng/ μL threshold at which sequence-specific RNAi has been demonstrated [13]. Selected miRNAs (miR-122, miR-148a) will be spiked into sterilized soil under two conditions: (1) sterilized soil without bacterial

inoculum, to measure miRNA persistence and stability independently of biological uptake (parameter a); and (2) sterilized soil inoculated with *Pseudomonas fluorescens* SBW25, with or without dandelion seedlings, to quantify bacterial uptake efficiency and assess whether recovered concentrations approach the RNAi threshold (parameters b and c). Both conditions will be monitored at 7 and 14 days using qPCR and sRNA-seq. To overcome the well-documented limitations of commercial kits in clay-rich soils [37, 38], an optimized CTAB phenol-chloroform extraction with high-ionic-strength NaP buffer (500 mM, pH 5.8) will be used [38]. This protocol, pre-registered on OSF, significantly improves RNA recovery by reducing clay-mineral adsorption and is essential for reliable detection of low-abundance environmental miRNAs. After 7 days of incubation with pathogen-inactivated, size-fractionated human feces and seedlings, rhizosphere bacteria will be isolated and their sRNA profiles sequenced to detect matches to human miRNAs. Threshold assessment (parameter c) will be derived from quantitative comparison of measured miRNA concentrations in rhizosphere extracts against the 0.03 ng/μL benchmark reported by Zheng et al. [13], using the same CTAB-extracted fractions.

5.3. *Taraxacum officinale* Experiment (Main)

Taraxacum officinale seeds will be sown in a sterilized peat-sand mixture under controlled growth-chamber conditions (22°C, 16/8 h photoperiod, 60% humidity). To establish a rhizosphere microbiome capable of mediating the CER relay, sterilized substrate will be inoculated with a standardized microbial community derived from agricultural soil via dilution-to-extinction, preserving native diversity while controlling inoculum dose. In parallel experimental arms, a defined inoculum of *Pseudomonas fluorescens* (strain SBW25 or equivalent PGPR strain with documented OMV capacity) will be used to test relay efficiency under controlled bacterial conditions. The presence and abundance of *Pseudomonas* spp. in each pot will be confirmed by 16S rRNA sequencing prior to fecal application (D0), defined here as the day of sowing (BBCH 00), to ensure that a relay-competent bacterial intermediary is established in the rhizosphere before the RNA signal is introduced. *Taraxacum officinale* was selected as the test plant because many populations reproduce via apomixis (clonal seeds without genetic recombination), providing an ideal system for isolating environmentally induced epigenetic effects that cannot be confounded by genetic variation. Prior studies have shown that environmental stress induces heritable DNA methylation changes in apomictic dandelions that are transmitted to offspring and produce measurable phenotypic shifts [24, 25]. Immediately after sowing (seed stage, BBCH 00), soil will be amended with standardized human fecal samples or controls (water or RNA-depleted, pathogen-inactivated fecal fraction). Plants will be grown for 28 days – a duration supported by evidence that root-applied dsRNA persists and remains detectable systemically in ash seedlings for at least 30 days [14]. A positive control consisting of synthetic dsRNA/siRNA targeting a defined plant marker gene (e.g., GFP in transgenic lines or an endogenous gene, per SIGS 2024–2025 protocols) will run in parallel to confirm uptake sensitivity and functionality under soil conditions, independent of the main treatment variable.

5.4. Omics Analysis

At the end of the 28-day growth period, plants and rhizosphere soil will be harvested for multi-omics profiling:

- sRNA-seq: Small-RNA profiles of plant roots, leaves, and rhizosphere bacteria will be generated to identify human miRNAs and their derivatives. Candidate bacterial sRNAs will be analysed for potential plant mRNA targets using IntaRNA and RNAhybrid (accounting for secondary structure), with experimental validation by dual RNA-seq or RIP-seq [28].
- ATAC-seq: Chromatin accessibility in plant tissues will be assessed to detect epigenetic changes induced by fecal application.
- Metabolomics (UPLC-MS): Secondary metabolite profiles (particularly flavonoids) will be quantified in leaves.
- Rhizosphere microbiome profiling: Bacterial and fungal community composition and diversity will be analysed by 16S rRNA and ITS sequencing. As a complementary module, rhizosphere viral metatranscriptomics (viromics) is recommended: RNA sequencing of the viral fraction with phage filtering (IMG/VR, RefSeq viral databases) will distinguish human, bacterial, and phage-derived sRNA signals, preventing misattribution of phage RNA activity to human miRNA modulation [26, 39].

5.5. Statistical Analysis

Differential expression of RNA-seq data will be performed with DESeq2 and edgeR. Epigenetic and metabolomic datasets will be analysed using ANOVA, PCA, and hierarchical clustering. Multi-omics integration methods will be applied to identify correlations across the plant microbiome, epigenome, and metabolome.

6. CER v3.0 Scheme: Hypothetical Time Cascade (Seed Stage)

The following timeline outlines a proposed mechanistic sequence for the CER relay model during the critical seed stage of plant development:

- D0 (BBCH 00, $\sim 10^2$ cells): Size-fractionated, pathogen-inactivated human feces retaining approximately 10^6 – 10^8 EV-encapsulated RNA molecules per gram of applied material are introduced to the soil (see Section 3.1 for derivation; these values are preliminary and require experimental validation).
- D1–D3 - Uptake and Initial Processing: Human miRNAs are rapidly taken up by rhizosphere bacteria, where they may undergo limited amplification by bacterial RNA-dependent RNA polymerases (predicted ~ 10 -fold; requires confirmation). Acquired human miRNAs can also function as RNA sponges within the bacterial sRNA network, sequestering endogenous bacterial sRNAs and thereby derepressing multiple target mRNAs - allowing a single miRNA signal to propagate through the entire bacterial regulatory network without direct RNA amplification [30].
- D3–D10 - Signal Amplification and Epigenetic Recording: Rhizosphere bacteria that have processed human miRNAs generate secondary sRNA signals and metabolites delivered to germinating seed cells via bacterial OMVs - the core relay step of the CER model. During early germination, the embryo exhibits the highest

RNA-directed DNA methylation (RdDM) activity of any plant tissue, with CHH methylation levels exceeding those in nearly all other tissues studied [21]. Exogenous RNA signals are amplified by the plant's RDR2 polymerase, which generates dsRNA that is processed by DCL3 into 24-nt p4-siRNAs. These siRNAs guide AGO4 and DRM2 to establish sequence-specific DNA methylation [22]. The signal spreads locally through plasmodesmata and systemically via the phloem to distant organs, including germline cells [36]. In contrast to a simplistic mitotic-amplification model (which does not occur spontaneously), this RdRP-centric mechanism converts transient miRNAs into stable chromatin marks that are perpetuated through subsequent cell divisions. The seed stage therefore serves as an optimal window for CER not because RNA replicates mitotically, but because the embryo provides a biochemically primed environment for efficient RdDM-mediated signal fixation.

- D10–D28 - Microbiome Boost and Metabolic Effects: Human miRNA-induced changes in rhizosphere microbiome composition and activity are predicted to drive production of specific bacterial metabolites. These metabolites may upregulate plant lncRNAs (e.g., ELENA1) and MYB transcription factors, potentially enhancing flavonoid biosynthesis; the magnitude of such effects remains to be determined experimentally.
- D28 - Mature Plant: The resulting epigenetic and metabolic changes become permanent and are ready for comprehensive multi-omics analysis.

7. Discussion

The CER hypothesis offers a novel framework for cross-kingdom communication, extending it beyond pathogenic interactions. By highlighting the seed stage and signal amplification through proliferating embryonic cells and the rhizosphere microbiome, it directly addresses the frequent objection that environmental RNA concentrations are too low to exert biological effects [9]. More broadly, CER fits within the emerging paradigm of environmental RNAi (eRNAi) as an evolutionarily conserved mechanism that spans pathogenic, mutualistic, and purely environmental interactions [11, 32]. A central open question remains whether uptake of exogenous RNA reliably leads to functional gene silencing; data from fungal models show this link is not automatic [12], making rigorous experimental validation of CER essential.

7.1. Biological Significance and Applications

While plant miRNAs are known to regulate rhizosphere bacteria and mammalian miRNAs modulate the gut microbiome [34], the reverse direction - mammalian miRNA transfer to plants via the soil microbiome - remains undescribed. This project aims to provide the first experimental data on mammalian-to-plant RNA transfer via the soil microbiome. Root application is a practical delivery path: dsRNA applied to roots of woody seedlings is taken up, transported systemically to above-ground tissues, and remains detectable for at least 30 days post-application [14], supporting the feasibility of this route under the 28-day experimental window proposed here. Sequence-specific RNAi can be triggered at concentrations as low as 0.03 ng/μL [13], suggesting that fecal miRNA doses are biologically plausible once they reach target cells intact. Confirmation of CER would reshape our view of human-plant interactions and open pathways for personalized agriculture

and biofortification. A particularly powerful application arises from integrating the CER relay with Antibacterial Gene Silencing (AGS) [40]: plants already use extracellular sRNAs to reprogram *Pseudomonas* transcriptomes; synthetic sRNAs supplied externally could achieve the inverse — crop-specific, reversible reprogramming of rhizosphere bacteria that then relay signals back to the plant via OMVs. This creates a new category of intervention - precision RNA agriculture - in which synthetic sRNA sequences precisely dictate the molecular message delivered by native soil bacteria, offering control unattainable with live biofertilizers. The approach is inherently non-GMO: the plant genome remains unmodified, bacterial strains are native residents, and the RNA signal is transient and biodegradable. This profile aligns with regulatory preferences for biological agents and may accelerate market approval compared with transgenic methods.

7.2. Alternative Explanations and Confounding Factors

Four principal confounds require consideration when interpreting positive results. First, human feces contains numerous non-RNA bioactive components - hormones, bile acids, short-chain fatty acids, and bacterial metabolites - which may potentially be capable of independently modifying plant epigenetic marks via yet non-described molecular pathways; the RNA-free feces control (Prediction 5) directly isolates the RNA-specific contribution. Second, fecal application will inevitably restructure the rhizosphere microbiome regardless of RNA content, potentially altering plant gene expression through microbiome-derived phytohormone analogues [23]; multi-omics integration across treatment arms will statistically decouple microbiome restructuring from miRNA-specific signalling. Third, short sRNA sequences can match human miRNAs by chance, generating false positives; stringent alignment filters (zero seed-region mismatches, ≥ 10 reads coverage) and viromics cross-referencing [39] mitigate this risk. Finally, the current protocol tests epigenetic changes in the directly treated F0 generation only and makes no claim regarding heritable transmission - that question is reserved for a subsequent F1 experiment in apomictic offspring.

7.3. Implications for Organic Agriculture: The Informational Dimension of Humanure

The CER hypothesis highlights a possibility for a previously unrecognized informational layer in historical organic agriculture. Human excrement ("humanure") has served as fertilizer across civilizations, yet classical agronomy focused solely on its chemical content. CER proposes that fecal miRNAs, relayed through the soil microbiome, could also modulate plant metabolomes in a donor-specific manner (health, diet, microbiome). For millennia, crops grown near human settlements experienced a continuous stream of mammalian RNA signals, generating sustained selective pressure for receptive mechanisms. Modern industrial agriculture has largely severed this flow through sewage treatment and pasteurization. If CER is biologically realistic, this interruption may have subtle but cumulative effects on soil microbiomes and plant quality - effects invisible to purely chemical analyses. Synthetic fertilizers and pesticides further erode rhizosphere diversity (including key plant growth-promoting rhizobacteria (PGPR) such as *Pseudomonas* spp.), potentially disrupting RNA-mediated signalling and contributing to reduced nutritional and health-promoting qualities in conventionally grown produce. These implications remain speculative until the core CER mechanism is confirmed, yet they highlight the broader ecological relevance of this research for understanding differences between organic and industrial agriculture.

7.4. The Soil Microbiome as a Biological Transducer of RNA Signals

The most innovative aspect of CER is not the plant epigenetic outcome itself, but the soil microbiome's role as an active biological transducer. Unlike earlier environmental RNA hypotheses that assumed direct donor-to-recipient transfer, CER posits an indirect four-step relay: (1) human exosomal miRNAs enter soil via feces; (2) rhizosphere microbes (particularly *Pseudomonas* spp.) take them up and alter their transcriptome or community structure; (3) the microbiome generates secondary sRNAs or metabolites that transduce the original signal; and (4) these secondary signals reach the plant and modulate its epigenome and metabolome. This architecture solves the central mechanistic challenge by replacing direct mammalian-to-plant transport with a chain of individually documented steps [11, 12, 13, 14, 27, 32, 40, 46]. Plant secretion of extracellular sRNA into the rhizosphere is well established [46], and Ravet et al. (2025) have directly shown that *Pseudomonas syringae* internalizes both vesicular and free plant sRNAs, triggering sequence-specific gene silencing inside the bacterium [40]. Conversely, bacterial OMV-packaged sRNAs can load onto eukaryotic RISC complexes and regulate host genes [27]. Thus, the rhizosphere microbiome functions as an information filter: it stabilizes, interprets, and repackages fecal RNA into signals that plants can read. Soil is thereby transformed from a passive chemical reservoir into a dynamic biological processor of inter-kingdom information - a conceptual shift analogous to the gut-brain axis.

7.5. An Evolutionary Perspective: RNA as a Carrier of Ecosystem Information

No prior framework has integrated the individually documented segments of cross-kingdom RNA exchange into a coherent ecological model. CER proposes precisely this: a closed feedback loop connecting human waste, soil microbiome, plant epigenome, and human consumption (human → soil microbiome → plant → human via gut-microbiome effects). Although each segment is documented separately, the integration itself is novel. Over millennia of organic agriculture, organic waste containing exosomal miRNAs was routinely returned to cultivated soil, creating sustained selective pressure that could have fine-tuned microbial and plant receptivity to mammalian RNA signals. CER would therefore describe not a novel phenomenon but an ancient, unrecognized mechanism disrupted only by modern wastewater systems and synthetic inputs. We tentatively term this broader concept the Cross-Kingdom RNA Feedback Loop (CKRFL). It remains speculative and requires experimental confirmation but it offers a natural extension: once *Taraxacum officinale* experiments yield positive signals, the next logical step is to test whether exposed plants produce metabolites that, upon human consumption, influence the gut microbiome - thereby closing the informational loop.

7.6. *Pseudomonas aeruginosa* as a Candidate RNA Signal Converter (Speculative Extension)

While Section 2 emphasises that the CER relay is not mechanistically restricted to any single taxon, *Pseudomonas aeruginosa* nonetheless emerges as the most tractable candidate for initial experimental validation of the relay mechanism; all points in this section remain speculative and require direct testing. Meta-analyses confirm that *P. aeruginosa* is rare in pristine soils but strongly associated with human- and animal-derived organic matter [18], making it ecologically relevant to humanure-amended systems. It possesses a well-documented OMV/sRNA system capable of delivering functional RNA to eukaryotic cells [15, 16, 26],

and recent work demonstrates that related *Pseudomonas* species internalise plant sRNAs and undergo sequence-specific gene silencing [40]. The reverse direction is also established: mammalian fecal miRNAs in EVs are taken up by gut bacteria and alter their physiology [29, 41]. *P. aeruginosa* furthermore colonises the rhizosphere of diverse plant species and interacts directly with root and seed tissues via metabolic and signalling molecules [18, 23], positioning it ideally for the seed-stage window of CER. Limited soil persistence may even confer a built-in safety feature: the bacterium delivers its OMV payload during early germination and is then outcompeted by the native microbiome. A focused *in vitro* verification programme is proposed: (1) sRNA-seq of *P. aeruginosa* OMVs after exposure to human miRNAs; (2) uptake assays of labelled OMVs by *Taraxacum officinale* roots; and (3) profiling of plant sRNAs following OMV treatment to identify complementary target sequences - all under biosafety (BSL-2) protocols, given the opportunistic pathogenic potential of *P. aeruginosa* in immunocompromised individuals [17].

7.7. Limitations and Challenges

The CER hypothesis, while mechanistically grounded, relies on extrapolation and contains speculative elements. Key limitations include: (a) absence of direct evidence for mammalian-to-plant RNA transfer (though bacterial uptake of plant sRNAs is now proven [40]); (b) methodological challenges in soil RNA detection, largely overcome by optimized CTAB protocols [38]; (c) risks of pathogen carry-over and antibiotic resistance from human feces, necessitating rigorous pathogen inactivation and ethical oversight; (d) variability in human sample RNA content, addressed by quantitative standardization; (e) uncertainty regarding bacterial amplification cascades and metabolite-to-lncRNA signalling, which require validation; (f) potential confounding by soil RNA viruses and bacteriophages, mitigated by viromics filtering [39]; and (g) the possibility that other EV-packaged small RNA classes present in human feces - including tRNA-derived fragments (tRFs), which transfer between cells and organisms via exosomes and regulate gene expression in recipient cells [31] - may contribute independently to or confound observed epigenetic effects, necessitating comprehensive sRNA-seq profiling beyond miRNA-focused analysis. Statistical power calculations indicate that the proposed design (60-150 plants) is adequately powered for moderate epigenetic effects; blinding and randomization will further strengthen validity. All limitations must be explicitly addressed in pre-registered protocols.

7.8. Future Research Directions

Priority areas include identifying effector miRNAs, testing additional crop species, and optimizing application protocols. Use of AGO/RdDM mutants will confirm molecular pathways. Positive *Taraxacum* results should trigger direct relay validation (sequencing of bacterial sRNAs after human miRNA exposure) and, ultimately, closed-loop CKRFL studies examining metabolite effects on the human gut microbiome.

7.9. Biosafety, Risk Considerations and Ethical Framework

Transgenerational epigenetic reprogramming via precision RNA agriculture demands proactive biosafety governance. Two core risks are identified: (1) off-target sequence effects, mitigated by mandatory Bioinformatic Safety Screening (BSS) against human, livestock, and beneficial-insect genomes using

miRBase, RNAhybrid, and BLAST; and (2) signal persistence in the food chain, addressed by the transient nature of RNA (epigenetic marks, not persistent RNA, are the output) plus routine harvest-time sRNA monitoring. The entire framework operates within non-GMO regulatory pathways (native bacteria, transient RNA, unmodified plant genome) and should be evaluated under biological-agent rather than GMO legislation. Early consultation with EFSA and EPA is recommended. Large-scale deployment of exogenous RNA in soil environments could additionally exert selective pressure on microbial communities, potentially fostering RNA-degrading or resistant phenotypes, with secondary risks including shifts in microbiome composition, altered plant–microbe signalling, and indirect effects on soil fauna or viromes. These ecological risks can be minimized through: (a) minimal effective (picomolar–nanomolar) doses, (b) seasonal rotation of RNA sequences, (c) RNase-treated negative controls in every trial, (d) longitudinal metagenomic/metatranscriptomic monitoring, and (e) initial restriction to contained experimental plots. All applications remain conditional on stepwise validation of the CER mechanism, with long-term ecological impact assessment as a mandatory component of any future development pathway.

7.10. Potential Applications Beyond Agriculture

Validated CER relay logic could extend to environmental biotechnology, particularly rhizosphere-mediated soil remediation. Synthetic sRNAs targeting native *Pseudomonas* spp. could enhance degradation of microplastics or pesticide residues without modifying plant genomes. This repurposes the bacterial intermediary as a primary effector rather than a signal relay. Any such extension remains conditional on core CER validation and would require separate, dedicated studies.

8. Conclusions

Inspired by the cross-kingdom RNAi mechanism of *Botrytis cinerea*, the microbiome-centric Cross Epigenetic Recycling (CER) model proposes that exosome-protected human RNA can modulate plant epigenomes and metabolomes through a rhizosphere microbiome relay. By targeting the seed stage - where RNA-directed DNA methylation activity peaks - and harnessing signal amplification in both proliferating embryonic cells and soil bacteria, CER directly addresses the longstanding challenge of low environmental RNA concentrations. The experimental framework using apomictic *Taraxacum officinale* provides a practical, falsifiable test of this hypothesis. Successful validation would open new avenues for research on environmental RNAi and its transformative applications in sustainable, non-GMO precision RNA agriculture and crop biofortification. Proactive attention to methodological, biosafety, and ethical challenges remains essential to uphold the scientific rigor and credibility of this work.

Additional Information

During the preparation of this manuscript, the authors used Claude (Anthropic) and Grok (xAI) as AI-assisted writing tools for text drafting, stylistic revision, and internal consistency checking. All scientific content,

hypotheses, experimental design, and conclusions were conceived, developed, and verified by the authors. The authors take full responsibility for the integrity of the work as published.

Author Contributions

All authors contributed equally to this work, including the conception of the CER hypothesis and relay model, development of the theoretical background, experimental design, and manuscript preparation. All authors reviewed the final manuscript and approved it for submission.

Conflict of Interest

The authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. A patent application related to this work has been filed (P.455121, UPRP, March 18, 2026).

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