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Synteny Patterns of Class 1 Integrons Reflect Microbial Adaptation and Soil Health in Agroecosystems

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Abstract

Mobile genetic elements such as integrons are key drivers of microbial evolution, enabling rapid adaptation to environmental pressures through the acquisition and rearrangement of gene cassettes. In this study, we explored the structural diversity and synteny of class 1 integrons (*intI1*) across a set of agroecosystem-related environments, including digestate, compost, and rhizosphere soils from wheat crops (*Triticum durum* and *T. aestivum*). Our results reveal distinct gene cassette architectures shaped by the origin of the samples: digestate harbored the most diverse and complex arrays, while compost displayed streamlined structures. Rhizosphere soils exhibited intermediate configurations, reflecting a dynamic balance between environmental exposure and host influence. Genes associated with resistance to antibiotics and heavy metals, such as *qacEΔ1* and *ebrA*, were differentially distributed, suggesting site-specific selective pressures. The observed patterns of cassette organization and diversity underscore the role of integron synteny as a molecular fingerprint of microbial adaptation. These findings position class 1 integrons as promising bioindicators of soil health and functional resilience, supporting a One Health approach to sustainable agriculture and microbial risk monitoring.

Keywords: *intI1*; resistome; mobilome; antimicrobial resistance; gene cassette

Academic Editor: Alvyra Šlepetienė

Received: 21 July 2025
Revised: 26 August 2025
Accepted: 28 August 2025
Published: 28 August 2025

Citation: Visca, A.; Costanzo, M.; Di Gregorio, L.; Nolfi, L.; Bernini, R.; Bevivino, A. Synteny Patterns of Class 1 Integrons Reflect Microbial Adaptation and Soil Health in Agroecosystems. *Agriculture* **2025**, *15*, 1833. https://doi.org/10.3390/agriculture15171833

1. Introduction

Soil health is a critical determinant of sustainable agricultural productivity and ecosystem resilience [1]. In recent years, the intricate relationships between soil microbiomes and environmental health have raised significant attention, particularly within the framework of the One Health approach [2]. This holistic paradigm emphasizes the interconnectedness of soil, plant, animal, and human health, advocating for integrated strategies to enhance agricultural sustainability and ecological stability [3]. Central to this approach is the understanding of genetic elements within soil microbiomes, such as integrons, which play central roles in microbial adaptability and resistance mechanisms [4].

Integrons, particularly those carrying the *intI1* gene, serve as genetic platforms that enable bacteria to acquire, exchange, and express gene cassettes [5,6]. These elements play a crucial role in microbial evolution, allowing bacteria to rapidly adapt to environmental stressors, including antibiotics and heavy metals [7]. Among the different classes, class

1 integrons represent fundamental components of the microbial mobilome, significantly influencing bacterial adaptation [8]. While integrons themselves lack inherent mobility, they are frequently associated with transposable elements and plasmids, facilitating their widespread dissemination across microbial communities [9]. Genomic studies estimate that class 1 integrons are present in approximately one out of every six sequenced bacterial genomes [10], with emerging evidence also suggesting their occurrence in non-bacterial taxa [11].

In clinical and environmental settings, class 1 integrons are of particular concern due to their tendency to accumulate antibiotic resistance genes (ARGs) within their cassette arrays [4,12,13]. This characteristic has positioned them as major drivers in the global spread of antimicrobial resistance. Their near-ubiquitous occurrence in the human gut microbiome results in high concentrations in wastewater systems [14], making them reliable indicators of anthropogenic influence on microbial ecosystems [15]. Moreover, their abundance in environmental samples strongly correlates with overall ARG levels, establishing them as valuable biomarkers for tracking resistance gene contamination [16].

The dual role of class 1 integrons, both as facilitators of microbial adaptation and as markers of human impact, highlights their importance in understanding antimicrobial resistance evolution across clinical and environmental settings [13,17–19]. Their ability to integrate diverse functional genes while maintaining mobility through plasmid and transposon associations makes them particularly efficient in promoting bacterial evolution under selective pressures [20], especially those induced by human activities. Consequently, studying integrons in soil environments is essential, given that soils serve as dynamic reservoirs of genetic diversity and hotspots for horizontal gene transfer [21]. However, despite their significance, the distribution and functional implications of *intI1* integrons across different soil types and agricultural amendments remain insufficiently explored [22].

Recent studies have emphasized the critical role of integrons in disseminating ARGs in both clinical and environmental contexts [5,12,23]. Research has demonstrated that integrons can harbor diverse gene cassettes conferring resistance to multiple antibiotics, presenting substantial challenges for public health and agricultural management [8]. For example, investigations have highlighted their contribution to antimicrobial resistance spread in environmental microbiomes, reinforcing their potential as reservoirs of resistance genes [15]. Similarly, studies in agricultural soils have revealed their prevalence, suggesting that human activities, such as manure and compost application, may significantly influence their distribution and diversity [24–26].

However, while extensive research has focused on integrons in clinical and aquatic environments, their diversity, structural organization (synteny), and ecological significance in soil ecosystems remain understudied [27]. Organic and inorganic fertilization, along with various soil amendments, can contribute to the spread of integrons in agricultural soils, leading to an increase in ARGs and antibiotic-resistant bacteria (ABR) in the soil environment [28]. Recent works highlighted that rhizoplane microbiomes are particularly rich in integron gene cassettes, with each crop species hosting a unique set of these gene cassettes, suggesting that they could play a crucial role in specific plant–bacterial interactions [29]. To date, no studies have examined the genetic diversity and structural organization of both integrons and the gene cassette pools in agricultural soils, particularly at the full-sequence level, and the potential impact of soil amendments on the crop soil environment. Traditional approaches relying on short-read sequencing and PCR-based surveys have provided limited insights, often restricting analyses to the detection of specific resistance genes while overlooking broader integron architecture and evolutionary dynamics. The emergence of long-read sequencing technologies [30], such as Oxford Nanopore, now offers unprecedented opportunities to overcome these limitations. These advancements enable

the reconstruction of complete integron structures, uncovering gene arrangement patterns that may correlate with functional traits and environmental adaptations.

These innovative techniques can be leveraged to provide future research with deeper insights into the ecological and evolutionary roles of integrons in soil systems. Ultimately, this will inform strategies to mitigate the spread of antimicrobial resistance in agricultural and natural environments.

In this context, the present study aims to investigate the genetic diversity and structural organization of *intI1*-associated gene cassettes in various soil improvers and agricultural soils. By employing nanopore sequencing, samples from compost, digestate, and rhizosphere soils associated with different cultivars of *Triticum durum* and *Triticum aestivum* have been analyzed. The findings reveal distinct patterns of gene cassette distribution, highlighting the genetic variability and structural stability of *intI1* integrons across different environmental matrices. Furthermore, these results underscore the potential roles of these genetic elements in enhancing microbial adaptability and resistance, thereby contributing to the broader understanding of soil health and microbiome dynamics.

This study provides valuable insights into the genetic architecture of *intI1* integrons in soil environments, emphasizing their significance in sustainable agricultural practices and ecosystem resilience. By elucidating the factors influencing integron diversity and dissemination, our research contributes to the development of integrated strategies for promoting soil health and mitigating the spread of resistance genes within agricultural systems.

2. Materials and Methods

2.1. Samples Collection

The samples used in this study were collected during different sampling campaigns conducted in 2024 year. General information about the samples are reported in Table 1. The digestate samples were derived from the solid fraction of three anaerobic digestates (side streams from food industry, municipalities, and agriculture). The compost samples originated from the composting process of food waste collected from a canteen. Both the compost and digestate sampling procedure consisted in collecting the material from five different spots. Once carried in the laboratory, the five sampling points were mixed and homogenized before proceeding to DNA extraction. The rhizospheric soil samples were collected from four different regions across Italy. Sampling was performed by collecting soil from four points selected in proximity to the roots of *Triticum durum* and *Triticum aestivum* plants.

Table 1. List of samples analyzed. The first column refers to the ID of the sample and the second and third columns refer to the type of sample and the classification, respectively. The last column refers to the source of the sample.

Sample ID	Type of Sample	Classification	Source
sample 01	Digestate	Soil improver	Side streams from food industry, municipalities and agriculture
sample 02	Digestate	Soil improver	Side streams from agriculture
sample 03	Digestate	Soil improver	Side streams from food industry and agriculture
sample 04	Compost	Soil improver	Food wastes
sample 05	Soil	Rhizosphere	*T. aestivum* cv. Taylor rhizosphere (north Italy)
sample 06	Soil	Rhizosphere	*T. aestivum* cv. Providence rhizosphere (north Italy)
sample 07	Soil	Rhizosphere	*T. durum* cv. Platone rhizosphere (center Italy)
sample 08	Soil	Rhizosphere	*T. durum* cv. Shrekan rhizosphere (south Italy)

2.2. DNA Extraction, Library Preparation, and Sequencing

For each sample, material was collected from five distinct subsampling points, which were subsequently pooled and thoroughly homogenized in the laboratory to ensure representativeness of the sampling spot. From the homogenized material, 0.25 g aliquots were randomly collected for DNA extraction. Three independent technical extraction replicates were performed using the DNeasy PowerSoil Pro kit (Qiagen Inc., Germantown, MD, USA), following the manufacturer's instructions. Extracted DNA was quantified with a Nanodrop spectrophotometer to assess both DNA concentration (ng/µL) and purity (260/280 and 260/230 ratios). In addition, DNA integrity and fragmentation were verified on 1% agarose gel. Each DNA replicate was subsequently used as template for PCR amplification, and the resulting amplicons were sequenced in three independent technical sequencing replicates to ensure robustness and reproducibility of the analysis. Library construction was carried out using PCR amplification performed on 25 ng of DNA from each sample, using the primers *intI1*_FW: (5′-GGCATCCAAGCAGCAAG-3′) and *intI1*_RV: (5′-AAGCAGACTTGACCTGA-3′), which are complementary to the 5′ and 3′ conserved segments of class 1 integrons, respectively [31]. Amplification was conducted with Taq polymerase (Bioline, Meridian Bioscience, Cincinnati, OH, USA), and the PCR products were verified by gel electrophoresis on a 1% agarose gel. The PCR amplicons for each sample were purified using AMPure beads (Beckman Coulter, Indianapolis, IN, USA), and approximately 45 ng of purified product was utilized for library preparation. Libraries were constructed using the Rapid Barcoding Kit 24 (SQK-RBK 114.24) according to the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). Sequencing was performed on a Flongle Flow Cell (R10.4.1) using the Mk1B device (Oxford Nanopore Technologies, Oxford, UK) overnight. Basecalling and demultiplexing were carried out using MinKNOW software (v23.08.9) integrated with Dorado software (v0.8.2).

2.3. Bioinformatic and Statistical Analyses

Each sample was obtained by averaging the results of three biological replicates and three technical replicates. Raw sequencing reads generated via Oxford Nanopore Technologies and processed through basecalling were used for downstream bioinformatic analysis. For each sample, consensus sequences of the *intI1* gene were generated using Minimap2 [32] for read mapping and Racon (https://github.com/isovic/racon; accessed on 14 July 2025) for polishing.

Consensus sequences were annotated with Prokka [33] to identify open reading frames and functional gene content. Gene cassette architectures were visualized using Clinker [34], allowing comparative analysis of gene content and synteny across samples.

Statistical analyses and graphical visualizations were performed in RStudio (version 2025.05.1+513), employing the tidyverse (https://www.tidyverse.org/; accessed on 14 July 2025) and Rtsne [35] packages. Additional analyses on gene presence/absence, clustering, and dimensionality reduction (PCA and t-SNE) were conducted to explore functional diversity among samples.

3. Results

3.1. Gene-Cassette Signatures

The distribution analysis of resistance gene cassettes across different environmental samples revealed clear ecological partitioning of genetic determinants (Figure 1). Digestate samples emerged as hotspots for diverse resistance mechanisms, particularly notable for their enrichment in mobile genetic elements including transcriptional regulators (*tra*) and insertion sequences (*IS6*, *IS1595*). In these samples, the ebrA multidrug efflux pump gene was detected uniquely, together with multiple pentapeptide repeat proteins (PRPs)

associated with fluoroquinolone resistance [36]. These elements co-occurred with complete sets of antibiotic resistance genes.

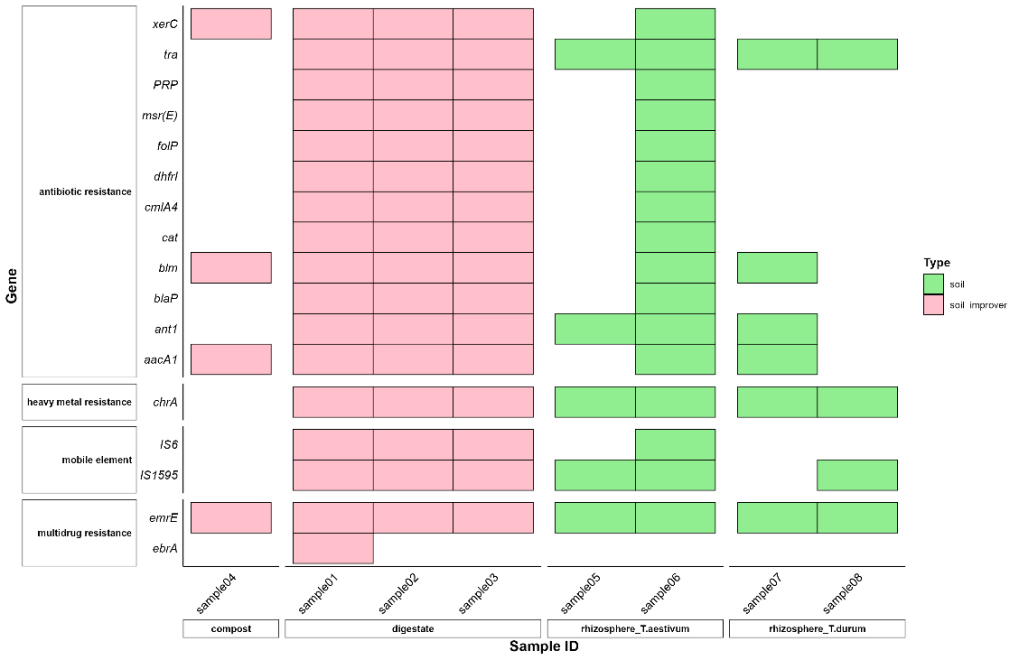

Figure 1. Tile plot representing the distribution of gene in the *intI1* cassette among the samples. The genes are clustered according to a macro-category (antibiotic resistance, heavy metal resistance, mobile element, and multidrug resistance). Pink rectangles refer to soil improver samples (compost and digestate) and green rectangles refer to soil samples (rhizospheres).

In contrast, compost samples exhibited a markedly reduced resistome complexity, dominated by core antibiotic resistance genes such as aminoglycoside-modifying enzymes (*aacA1* and *ant1*) but largely lacking associated mobile genetic elements.

Rhizosphere samples from both wheat varieties displayed intermediate complexity profiles, with several distinctive features. Heavy metal resistance genes, particularly *chrA*, showed significant enrichment in these samples, potentially reflecting selection pressures from agricultural amendments or soil geochemistry.

The comparative analysis revealed quantitative variations between *T. aestivum* and *T. durum* rhizospheres. While both wheat-associated microbiomes contained largely the same set of resistance genes, *T. aestivum* samples exhibited higher relative abundances of *folP* and *dhfrI*, whereas *T. durum* samples showed elevated levels of *chrA* and certain heavy metal resistance genes (Figure 2). These differences suggest cultivar-specific modulation of microbial communities rather than distinct gene content.

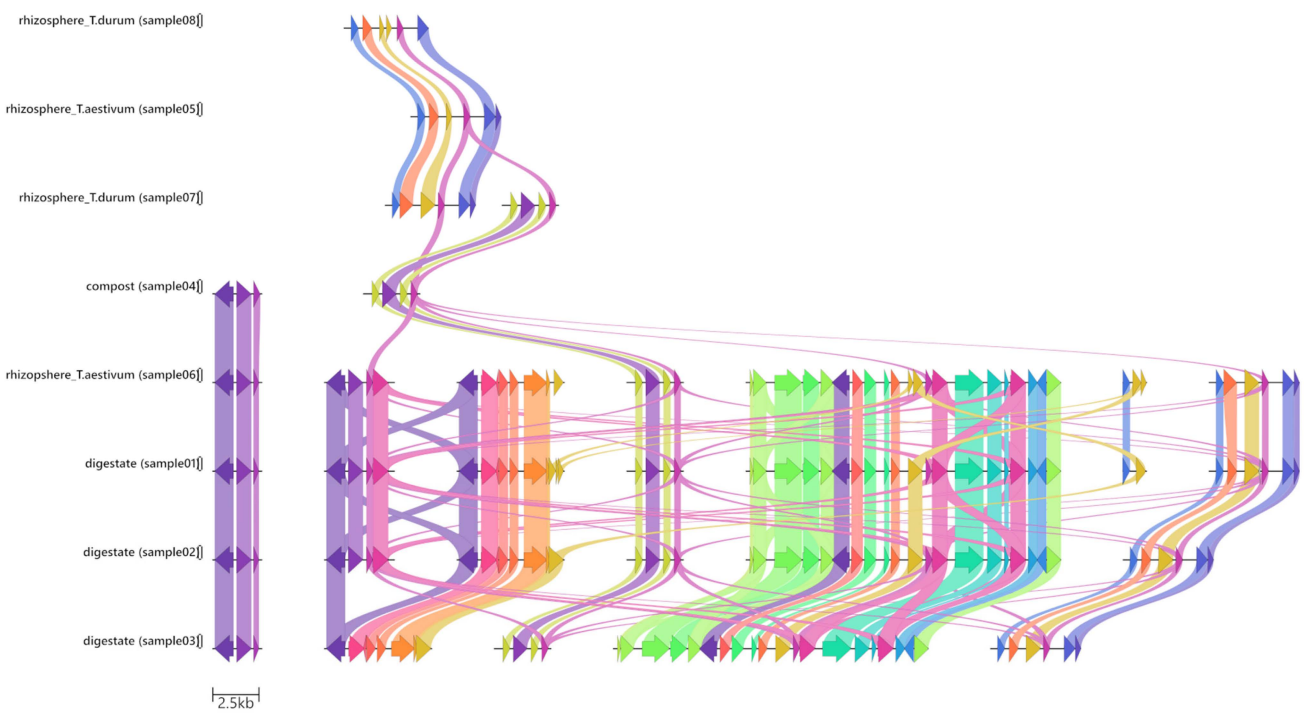

Figure 2. Comparative gene cluster visualization of *intI1*-associated cassettes across eight environmental samples using Clinker. Each row represents a sample (sample01 to sample08), and arrows indicate individual open reading frames (ORFs), color-coded by sequence similarity (violet: tyrosine recombinase; magenta: dihydropteroate synthase; orange: chloramphenicol efflux transporter; yellow: streptomycin 3′-adenyltransferase; green: ABC-F type ribosomal protection protein; turquoise: pentapeptide repeat protein; red: beta-lactamase). Homologous genes are connected across samples with shaded regions. Gene orientation is indicated by arrow direction. The horizontal scale bar indicates gene cluster length in base pairs. A complete annotated version of the figure is provided in Supplementary Materials (Figure S1).

3.2. Gene-Cassette Synteny

The structural organization and gene content of class 1 integrons across the eight environmental samples are presented in Figure 2. Clinker-based visualization of the gene cassettes revealed clear differences in both gene composition and synteny among the samples. Digestate samples (samples 01–03) displayed the most diverse and extensive integron architectures. These gene cassettes harbored multiple resistance determinants spanning several functional classes, including antibiotic resistance genes (e.g., *aadA* and *sul1*), multidrug efflux systems (e.g., *qacEΔ1*), and elements linked to heavy metal resistance. The structural organization within these samples also showed evidence of gene duplications and insertions, suggesting active recombination and gene acquisition processes, consistent with the high microbial turnover and selective pressure typically found in digestate environments. Sample04 (compost) featured the shortest and least complex integron structure. Its gene cassette contained fewer functional annotations and lacked many of the resistance genes observed in digestate samples.

Soil samples associated with *Triticum aestivum* (samples 05 and 06) showed intermediate integron complexity. While some resistance genes were shared with digestate samples (e.g., *sul1* and *qacEΔ1*), their overall gene content and synteny were less diverse.

Soil samples from *Triticum durum* (samples 07 and 08) displayed cassette structures that were also intermediate in complexity, but with distinct gene arrangements compared to those in *T. aestivum*. Notably, one of the *T. durum* samples contained a unique gene

combination including a heavy metal resistance determinant not found in the other samples, indicating possible site-specific or crop-specific pressures.

Across all samples, a core region of conserved genes was observed, including the *intI1* integrase and *qacEΔ1* gene, reinforcing the structural stability of the integron scaffold.

3.3. Machine Learning Clustering

The combined t-SNE and PCA/k-means clustering analyses (Figure 3) provided a detailed view of the distribution of integron-associated gene cassettes across the different environments (digestate, compost, and wheat rhizosphere samples from *T. durum* and *T. aestivum*). The two approaches, although based on different mathematical principles, converged on consistent biological patterns. In both visualizations, digestate samples clearly separated from the other groups, reflecting their high variability and the presence of complex and heterogeneous cassette arrays. Compost samples, by contrast, formed a compact cluster in both t-SNE and PCA space, highlighting their genetic uniformity and the reduced cassette diversity likely resulting from composting processes. Rhizosphere samples occupied an intermediate position between these two extremes: in t-SNE they appeared as partially overlapping but distinguishable sub-clusters according to wheat species, while in PCA they were distributed along the first principal component axis, which explained most of the variance and distinguished them from both digestate and compost. The k-means partitioning (silhouette score: 0.76) further confirmed the robustness of these groupings, defining three main clusters corresponding to digestate, compost, and rhizosphere environments.

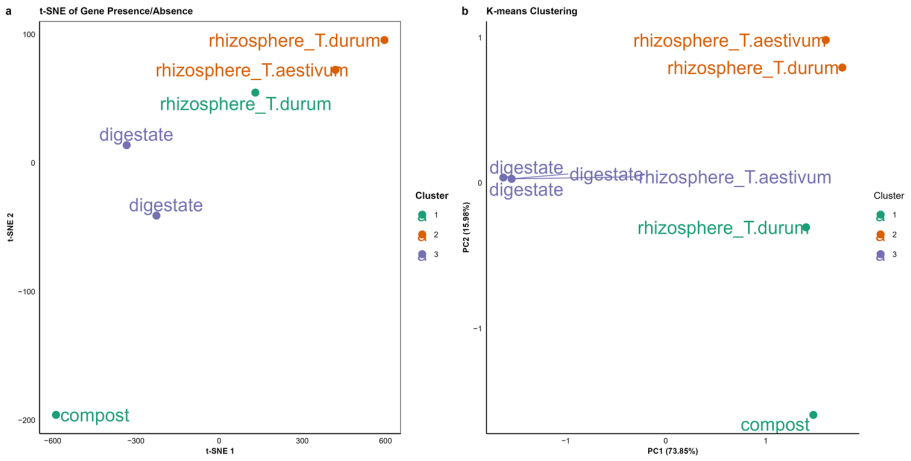

Figure 3. (**a**) t-distributed Stochastic Neighbor Embedding (t-SNE) plot based on gene presence/absence profiles of eight environmental samples. Color indicates k-means cluster assignment. Sample labels are positioned near each point to identify origin. The two axes represent the t-SNE components, providing a non-linear projection of high-dimensional genetic data into a 2D space that preserves local similarity relationships between samples. (**b**) Principal Component Analysis (PCA) plot of gene presence/absence matrix across eight environmental samples. Points are colored by k-means cluster membership. Axes correspond to the first two principal components (PC1 and PC2), which capture the majority of variance in gene cassette composition. Sample labels are shown next to each point. PC1 explains 73.85% of the total variance, while PC2 accounts for 15.98%.

Together, these analyses underscore that digestate represents a hotspot of integron diversity, compost displays the most streamlined and homogeneous profiles, and rhizosphere soils show intermediate and crop-modulated features. The convergence of two independent dimensionality reduction methods strengthens the confidence in these ecological patterns and illustrates how sample origin and management practices leave a recognizable genomic imprint on integron architecture.

4. Discussion

The comprehensive analysis of class 1 integron architecture across agricultural environments presented in this study reveals fundamental insights into how microbial genetic adaptation reflects and influences soil health within the One Health framework. The distinct synteny patterns observed in digestate, compost, and wheat rhizosphere soils demonstrate how anthropogenic inputs create selective landscapes that shape microbial evolutionary trajectories, with significant implications for sustainable agriculture and antimicrobial resistance mitigation [37–39].

The presence of folate pathway inhibitors (*folP* and *dhfr1*) in rhizospheres suggests these microbial communities may face particular selective pressures from sulfonamide and trimethoprim compounds [40].

For instance, in digestate we identified complex cassette arrays harboring multiple β-lactamases and efflux pumps, illustrating how anaerobic digestion environments act as hotspots for the assembly of resistance determinants. By contrast, compost samples showed simplified arrays largely limited to aminoglycoside resistance genes, reflecting the selective pressures imposed by thermal treatment. In wheat rhizospheres, the enrichment of heavy metal resistance determinants such as *chrA* provides a concrete example of how past agrochemical use can leave a genetic footprint that persists in plant-associated microbiomes. These examples highlight how specific agricultural practices translate into recognizable integron signatures, making them powerful molecular sentinels of environmental history.

The striking genetic complexity found in digestate samples presents both opportunities and challenges for soil health management. On one hand, the abundance of mobile genetic elements (transcriptional regulators, *IS6*, and *IS1595*) and diverse resistance cassettes (*ebrA*, PRP proteins, and multiple β-lactamases) reflects the dynamic microbial ecology of anaerobic digestion systems. These systems naturally select for microorganisms capable of horizontal gene transfer and rapid adaptation to changing substrates. However, when applied to agricultural soils, these genetic elements may facilitate the spread of resistance determinants to indigenous soil microorganisms [41]. The presence of complete resistance operons in digestate-associated integrons suggests these environments maintain functional gene expression networks, potentially enhancing their role as antimicrobial resistance reservoirs. This finding aligns with recent studies showing persistent integron activity in manure-treated soils, though the nanopore sequencing approach applied in this study provides unprecedented resolution of these genetic structures [42].

Compost samples exhibited dramatically different integron profiles, characterized by reduced cassette diversity and minimal mobile elements. This pattern likely results from the combined effects of thermal inactivation during composting [43] and the more stable microbial communities that develop in mature compost [44,45]. Composting processes, particularly thermophilic phases, have been shown to significantly reduce the abundance of mobile genetic elements, including integrons and associated gene cassettes, due to selective pressure and microbial turnover under high-temperature conditions [46]. This likely reflects the selective elimination of complex genetic assemblies under high-temperature conditions, while basic resistance determinants may be retained. The preservation of core resistance genes (*aacA1* and *ant1*) despite this simplification suggests these determinants may be chromosomally encoded or associated with more stable genetic elements. From a soil health perspective, compost's streamlined integron architecture may represent a lower-risk amendment, and its enriched microbial diversity could potentially improve some functional benefits to soil ecosystems [3,47,48].

Crops' rhizospheres are considered hotspots of ARGs, being characterized by intense microbial activity and nutrient exchange [49]. These ARGs can then be transferred to other microorganisms, potentially impacting both plant health and human health through the

food chain. The intermediate complexity observed in wheat rhizosphere soils reveals how crop-specific factors modulate integron ecology. The enrichment of heavy metal resistance genes (particularly *chrA*) in both *T. aestivum* and *T. durum* rhizospheres likely reflects historical exposure to metal-containing fertilizers or pesticides [50,51]. Although metal-based inputs are not explicitly recorded in the management practices, the enrichment of heavy metal resistance genes such as *chrA* in rhizosphere samples may reflect historical applications of metal-containing agrochemicals or long-term indirect selection driven by intensive fertilization and pesticide use. This supports the hypothesis that anthropogenic pressures, even if undocumented in current practices, may contribute to the persistence and selection of resistance determinants in agroecosystems. More interestingly, the differential abundance of folate pathway inhibitors (*folP* and *dhfrI*) between samples suggests crop-specific selection pressures, possibly mediated through root exudate chemistry or microbiome interactions [52]. These findings extend recent work on plant-mediated selection of soil resistomes by demonstrating how subtle variations in crop genotype can influence integron architecture at the gene cassette level [53].

In this context, crop exudates represent a crucial but often underestimated driver of integron dynamics. Root exudates supply carbon sources, secondary metabolites, and signaling molecules that can create localized selective pressures, thereby favoring microorganisms harboring integrons with adaptive gene cassettes [54]. For instance, organic acids and phenolic compounds released by roots can indirectly shape integron composition by altering microbial competition or by modulating horizontal gene transfer rates [55,56]. Similarly, soil amendments such as digestate or compost not only introduce exogenous microorganisms carrying integrons but also modify soil physicochemical properties (e.g., pH, organic matter, or redox conditions), which in turn affect the selective landscape for integron maintenance and cassette acquisition [39]. These direct and indirect pathways highlight the importance of integrating both plant and amendment effects when assessing how agricultural practices influence integron ecology.

Indeed, the machine learning analyses provided robust validation of these patterns, with clear separation between amendment types and more nuanced differentiation between rhizosphere samples [57]. The tight clustering of compost samples in both t-SNE and PCA space reinforces its genetic homogeneity, while digestate samples formed a distinct cloud reflecting their greater variability [58]. Rhizosphere samples occupied an intermediate position with partial overlap between wheat species, visually confirming the crop-specific effects observed in the synteny analysis [59]. These computational approaches not only support the structural findings but also provide a framework for classifying unknown samples based on their integron profiles [57].

The conservation of core integron features (*intI1* and *qacEΔ1*) across all environments despite their divergent cassette content speaks to the remarkable evolutionary stability of these genetic platforms. This conservation suggests integrons maintain basic functional requirements while allowing considerable flexibility in their adaptive gene content. From an ecological perspective, this makes them ideal sentinels for monitoring anthropogenic impacts on soil microbiomes [15], as their core architecture provides a consistent reference point for comparing acquired resistance determinants across environments.

These findings have several important implications for sustainable agriculture under the One Health paradigm. First, they highlight the need for differential risk assessment of organic amendments based on their integron profiles [60,61]. While digestate provides valuable nutrients, its potential as an antimicrobial resistance vector may warrant additional treatment steps or restricted use in sensitive areas [3]. Second, the crop-specific effects we observed suggest that plant genotype selection could be leveraged to manage soil resistomes [62], potentially through breeding programs that favor microbiome traits

associated with reduced horizontal gene transfer. Finally, the conserved features identified could form the basis for new monitoring tools to track antimicrobial resistance spread in agricultural systems.

The integration of these structural findings with the One Health approach reveals critical connections between agricultural practices, soil microbiome resilience, and public health. Soil health cannot be considered in isolation from the genetic potential of its microbial communities, particularly when those communities harbor mobile resistance determinants. This occurs because soil microbial communities are highly responsive to the chemical, physical, and biological changes induced by agricultural practices. Different inputs (such as compost, digestate, synthetic fertilizers, or manure) alter nutrient availability, pH, moisture, and organic matter composition, which in turn select for specific bacterial taxa. Some of these taxa are more likely to carry mobile genetic elements like integrons, which can capture and transfer ARGs between bacteria. When agricultural inputs favor bacteria that carry these elements, the soil effectively becomes a reservoir of mobile resistance determinants.

These results showed that different agricultural inputs create distinct integron ecosystems [63] and provide a scientific basis for developing more nuanced soil amendment policies that balance fertility benefits with antimicrobial resistance mitigation. This is particularly important because soil-borne integrons and ARGs can transfer beyond the soil environment, reaching plant-associated bacteria, entering food chains, or moving into water systems, thereby contributing to the global spread of antimicrobial resistance. From a One Health perspective, understanding how agricultural practices shape microbial communities is therefore critical to designing evidence-based management strategies that sustain soil fertility while minimizing public health risks.

Finally, a methodological consideration concerns the use of nanopore sequencing for full-length integron reconstruction. While nanopore reads have historically been associated with higher error rates compared to short-read technologies, recent advances in chemistry and basecalling have substantially improved accuracy, with our dataset achieving quality scores consistently above Q20. In addition, the high sequencing depth obtained in this study (5–10 Gb/samples), together with stringent polishing and reference-based annotation, ensured reliable reconstruction of cassette arrays. Although some degree of annotation uncertainty can never be entirely excluded, the combination of depth, updated chemistry, and rigorous downstream processing provides strong confidence that the observed synteny patterns reflect genuine biological structures rather than sequencing artefacts. Future studies could further benefit from hybrid approaches, but the present results already demonstrate the robustness of nanopore sequencing for integron profiling.

5. Conclusions

This study provides a comprehensive and integrative overview of the structural and functional diversity of *intI1*-associated gene cassettes across multiple environmental matrices, leveraging long-read sequencing, functional annotation, and robust integrative analyses. By characterizing full-length *intI1* integrons from soil improvers and rhizosphere soils, we demonstrate that the composition and synteny of gene cassettes are powerful reflections of environmental histories, anthropogenic inputs, and selective pressures. The pronounced variability, such as the exceptionally high gene diversity observed in digestate samples compared with the conserved, streamlined profiles in compost, underscores the complex ecological roles of integrons as dynamic adaptive platforms that can rapidly respond to environmental change. Rhizosphere samples display intermediate profiles, highlighting their sensitivity to both plant-associated and external environmental drivers.

These findings emphasize that monitoring mobile genetic elements like *intI1* is not only crucial for understanding microbial resilience but also essential for assessing the potential

for resistance gene dissemination. Our results provide strong evidence that integron architecture is directly influenced by land management practices, offering a concrete link between agricultural inputs and the functional potential of soil microbiomes. By framing these insights within a One Health perspective, this study highlights the necessity of integrating microbial genomic surveillance into agroecosystem management as a proactive measure for sustainable crop productivity and environmental stewardship.

Importantly, we propose that *intI1* synteny represents a promising potential bioindicator of soil health, capable of complementing classical functional metrics such as enzymatic activity, nutrient cycling, and crop performance. Future experimental frameworks should rigorously test this potential, but our work lays a strong foundation for microbiome-informed interventions aimed at simultaneously enhancing soil functionality and mitigating ecological and public health risks. In sum, the integration of genomic insights with land management strategies offers a pathway to more precise, evidence-based approaches for sustainable and resilient agroecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15171833/s1, Figure S1: Comparative gene cluster visualization.

Author Contributions: Conceptualization, A.V.; methodology, A.V., L.N., M.C. and L.D.G.; software, A.V., L.N., M.C. and L.D.G.; validation, A.V.; formal analysis, A.V.; investigation, A.V.; resources, A.B.; data curation, A.V., M.C. and L.D.G.; writing—original draft preparation, A.V.; writing—review and editing, L.N., M.C., L.D.G., R.B. and A.B.; visualization, A.V., M.C. and L.D.G.; supervision, R.B. and A.B.; project administration, A.B.; funding acquisition, A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Horizon Europe Programme of the EU under grant agreement N. 101112855 (DeliSoil project, Delivering safe, sustainable, tailored and societally accepted soil improvers from circular food production processes for boosting soil health), the European Union Next-GenerationEU (Agritech National Research Center, Piano Nazionale di Ripresa e Resilienza (PNRR)—Missione 4 Componente 2, Investimento 1.4—D.D. 1032 17/06/2022, CN00000022), and the Italian Ministry of Agriculture, Food Sovereignty and Forestry (SOIL-HUB2 project, Creazione di una rete italiana di competenze per la gestione sostenibile del suolo, D.M. MASAF n 672924 of 20-12-24). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Data Availability Statement: Data will be made available upon request.

Acknowledgments: The authors wish to thank their colleagues at ENEA for their valuable contribution to this work by providing samples of digestate and compost used in the study.

Conflicts of Interest: The authors declare no conflicts of interest.

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