Science of the Total Environment 955 (2024) 176874

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Fate of fluoroquinolones associated with antimicrobial resistance in circular periurban agriculture

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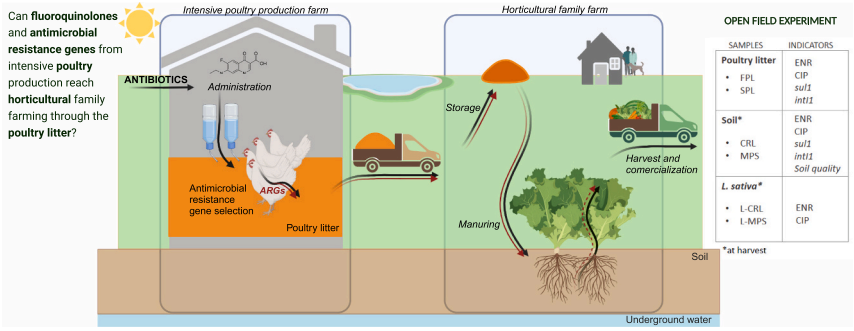
HIGHLIGHTS

- Fluoroquinolones and AMR pathway in circular periurban agriculture.
- In an open-field experiment stored poultry litter (PL) is used as a soil amendment.
- Lettuce accumulates enrofloxacin and ciprofloxacin from intensive animal husbandry.
- PL improves soil quality but increases *sul1* and *intI1* gene abundance.
- Soil amendment with PL contributes to fluoroquinolone pollution and AMR spread.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniela Maria Pampanin

Keywords:
Poultry litter
Horticulture
Soil
Fluoroquinolones
sul1
intI1

ABSTRACT

Animal antibiotic use contributes to antimicrobial resistance (AMR) in humans. While animal manure benefits soil fertility, it also acts as hotspot for antibiotic residues, antibiotic-resistant bacteria, and their genes. Amending soils with poultry litter is recognized as "magic" among horticulture farmers and it remains a common practice globally. However, this poses a risk especially in countries where prophylactic use of antibiotics is allowed. In Argentina, fluoroquinolones are used in this way besides being listed as essential medicines and classified as "watch" by the World Health Organization. Antibiotic selective pressure can favour AMR in the environment but the fate of antibiotic residues and AMR dissemination from these practices remains poorly understood. Our research addresses this gap with a biological model tracing fluoroquinolones from poultry to soil to lettuce and tracking anthropogenic AMR with the proposed biomarker genes *sul1* and *intI1*. Fresh poultry litter was stored for six months before application in a horticulture field experiment. The experiment included control and manured

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https://doi.org/10.1016/j.scitotenv.2024.176874
Received 12 June 2024; Received in revised form 19 September 2024; Accepted 9 October 2024
Available online 15 October 2024

plots where lettuce was cultivated till harvest. Enrofloxacin concentration was 7.3 μg/kg in fresh poultry litter, while its metabolite ciprofloxacin was 39.22 μg/kg after storage. Although no fluoroquinolones were detected in soils, lettuce from manured plots contained enrofloxacin and ciprofloxacin at 14.97 and 9.77 μg/kg, respectively, providing evidence of fluoroquinolone bioaccumulation in plants. Abundance of *sul1* and *intI1* in poultry litter was not affected by storage. Manured soils showed better soil quality than controls, but *sul1* gene abundance was 1.6 times higher, reaching 7.61 Log *sul1*/g soil. A less sensitive, but significant effect was registered for *intI1*. These findings show that static storage is insufficient to stop the transmission of antibiotics and AMR biomarkers from poultry to horticulture. Amending soil with industrial poultry litter contributes to pollution with these emergent contaminants and risks human antibiotic exposure through fresh vegetables.

1. Introduction

Antimicrobial resistance (AMR) poses a significant public health challenge by jeopardizing the ability to treat clinical infections (Murray et al., 2022). Addressing it requires a One Health approach, integrating human, animal, plant, and environmental health (Banerjee and van der Heijden, 2023). Agricultural industrialization has separated plant production -livestock feed- and livestock production (Modernel et al., 2016), disrupting nutrients and carbon cycling. Circular agriculture aims to close these cycles for sustainable soil use and align with Sustainable Development Goals (Schroeder et al., 2019). However, industrial livestock production often relies on non-therapeutic antimicrobials use, such as growth promotion and prophylaxis, contributing to the spread of AMR (Vågsholm et al., 2020). Antimicrobials select for antimicrobial resistance genes (ARG) in livestock (Tang et al., 2017), and can persist in manure in an active form (Quaik et al., 2020; Wang et al., 2014). Therefore, applying such manure to soils can inadvertently close the cycles for antimicrobials and ARG, which can be absorbed by plants (Barra Caracciolo et al., 2022) acquired by soil and endophytic bacteria (Hölzel et al., 2018; Scaccia et al., 2021).

Fresh vegetables can serve as ideal vectors for residual antimicrobials (Chen et al., 2024) and AMR transference to humans (Kläui et al., 2024), as they are often consumed raw or with minimal processing. Long-term dietary exposures to antimicrobials can lead to gut microbiome disruption, AMR development (Subirats et al., 2019), increased carcinogenic risks (Ben et al., 2022), and food allergies (Li et al., 2019), depending on the exposure levels.

In Buenos Aires peri-urban area, intensive poultry husbandry supplies poultry litter to horticultural farms that provide fresh vegetables to 15 million people (https://www.ambadata.gob.ar/). Poultry litter, rich in nutrients (Sokolowski et al., 2024), is widely used as a soil amendment despite the use of antimicrobials in livestock and 50 % prevalence of multidrug-resistant *Enterobacteriaceae* (Prack McCormick et al., 2023). Argentina's Ley 27,680 and Resolution 445/2024 aim to limit antimicrobials use in livestock, banning growth promotion and some antibiotics reserved for human infections (e.g. polymyxins), but do not yet address fluoroquinolones, which are critical for human medicine (World Health Organization, 2023, 2016) yet still used prophylactically in poultry. Enrofloxacin, administrated to poultry via drinking water, metabolizes into active ciprofloxacin (Trouchon and Lefebvre, 2016), an antimicrobial reserved for human use. Residues from both fluoroquinolones are prevalent in local poultry litter, with concentrations ranging from 810 to 3175 μg/g, and accompanied mainly by tylosin and salinomycin (Alonso et al., 2024; Teglia et al., 2017). Environmental surveillance has detected fluoroquinolones in river samples near cities (Mastrángelo et al., 2022; Teglia et al., 2019) and in wildlife (Blanco et al., 2023), but no research has traced their fate in horticulture or their potential transfer to fresh produce.

Monitoring antimicrobials and ARG spread in the environment requires effective biomarkers and collaborative efforts. The ARG *sul1* is a frequently used anthropogenic AMR biomarker with relevance for One Health due to its high abundance, prevalence, and mobility (Gillings et al., 2014; Nardelli et al., 2012; Wang et al., 2023). It is prevalent in animal manure and soils exposed to antimicrobials, making it valuable for monitoring AMR in horticulture (Fahrenfeld et al., 2014; Subirats et al., 2021). Additionally, *intI1*, an integron-integrase, is increasingly recognized as a proxy for anthropogenic pollution (Knecht et al., 2023; Gros et al., 2023). Its environmental presence often correlates with ARGs, as integrons facilitate rapid bacteria adaptation by incorporating new genes (Gillings et al., 2014). Combining *intI1* analysis with ARGs monitoring can reveal ARG mobility in environmental systems (Franklin et al., 2024). In Argentina, surveillance efforts have detected ARGs, including *sul1,* and *intI1* in bacteria isolated from soil (Nardelli et al., 2012; Pellegrini et al., 2022) and vegetables (González et al., 2017), but these studies focus on cultivable bacteria, which account for only 1.4–14.1 % of the total soil microbial community (Janssen et al., 2002). The lack of culture-independent methods highlights the need for more comprehensive monitoring approaches.

Despite the importance of understanding AMR's environmental spread, particularly in horticulture, there is limited knowledge about the fate of antimicrobials and ARGs after manure application, especially in Latin America. This study aims to trace the pathway of enrofloxacin, its metabolite ciprofloxacin, and the selected AMR indicators *sul1* and *intI1*, from poultry litter to soil and/or lettuce in a peri-urban agricultural setting. We hypothesise that the application of poultry litter as a soil amendment facilitates the transfer of antimicrobials (specifically enrofloxacin and its metabolite ciprofloxacin) and AMR indicators from poultry litter to the soil and subsequently to lettuce. This process potentially contributes AMR dissemination through the food chain and the environment, raising concerns about human exposure to AMR and the sustainability of nutrient cycling in circular agriculture systems.

2. Materials and methods

In this study, we conducted two primary phases: a contextual analysis, involving satellite imaging to map the distribution of poultry litter heaps within a chosen horticultural area on the peri-urban of Buenos Aires, and the subsequent field experiment designed to test our hypotheses under controlled conditions (Fig. 1).

2.1. Density of poultry litter heaps

An approximation of the distribution of poultry litter heaps in the horticultural area of Villa San Luis and La Capilla in Florencio Varela, southern green belt of Buenos Aires (Argentina), was obtained through satellite images extracted from Google Earth Pro. The count of poultry litter heaps was conducted based on imagery from September 18, 2018, covering an area of 1900 ha in Villa San Luis and 7197 ha in La Capilla. In a first step, the location of poultry litter heaps was identified during visits to farmers (documented in Sokolowski et al., 2024), georeferenced and correlated with satellite images. Secondly, based on the morphology of these known heaps, the additional heaps were identified directly on the images. Lastly, during subsequent farm visits, on-site verification was carried out to confirm the presence of heaps identified through the satellite imagery. Poultry litter heaps density was determined using QGIS 3.22 with a grid of 500 × 500 m. The horticultural area in both localities was delimited and considered for the calculation of poultry litter heaps density.

2.2. Site description, field experiment and sample collection

The experiment was conducted in the field of the Faculty of Agricultural Sciences at Universidad Nacional de Lomas de Zamora (34° 47' South Latitude, 58° 26' West Longitude) in Buenos Aires, Argentina. The area has a humid temperate climate with an average annual temperature of 22.3 °C and an isohydric rainfall regime, with an average annual rainfall of 1050 mm (SMN, 2023). The soil is a silty clay loam, moderately well-drained, and with a very gentle slope. The pH is neutral to slightly acidic throughout the soil profile, and the total organic matter content is 2.73 % from 0 to 25 cm in the surface horizon (Sokolowski et al., 2024).

The experimental design was a randomized complete block design with two fertilization treatments and three replicate experimental units (plots) per treatment. The vegetable crop selected was lettuce (*Lactuca sativa* L). Each plot had an area of 4 m^2 and the planting density was 12.5 plants per m^2. The fertilization treatments assessed were "control (CRL)" and "manured poultry soils (MPS)". In CRL plots, plants only received nutrients available in the soil, without external fertilization. In MPS plots, the soil was fertilized with 13.6 kg of dry poultry litter one day

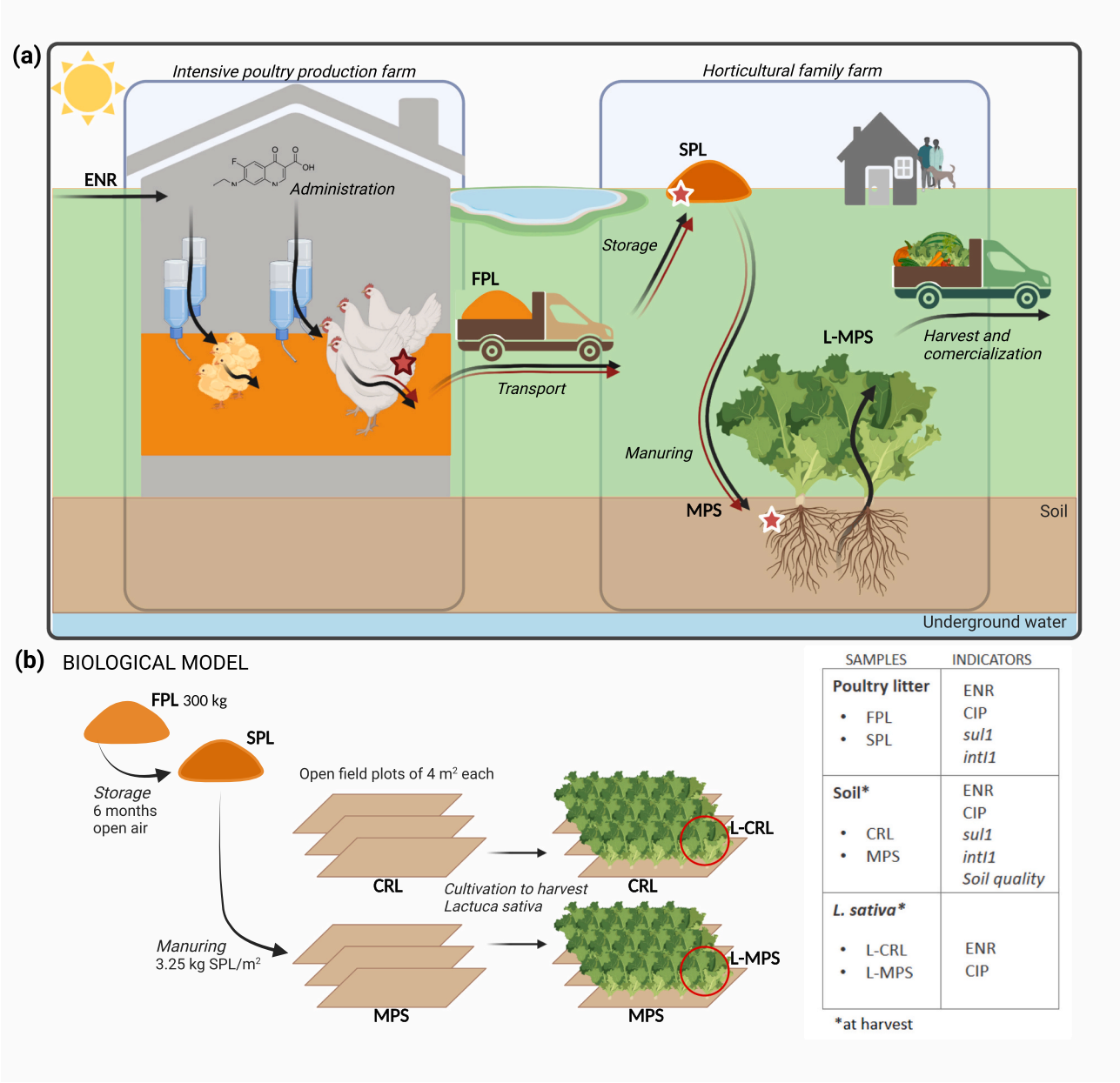

Fig. 1. Transmission of Antimicrobials and Antimicrobial Resistance from Industrial Poultry Production to Horticultural Family Farming. (a) hypothesized transmission of antimicrobials and dissemination of antimicrobial resistance genes (ARG) from industrial poultry production to horticultural family farming. Several antimicrobials, including enrofloxacin (ENR) are administered to poultry in industrial settings. Fresh poultry litter (FPL), containing antimicrobials and AMR biomarkers, enters the horticultural family farms where it is stored as a heap in the open air and bare soil. Stored poultry litter (SPL) is applied as fertilizer to the soil (MPS) in the fields where vegetables (L-MPS) intended for human consumption are grown. Arrows indicate the hypothesized flow of antibiotics and AMR biomarkers, and red stars represent areas that may act as hotspots for ARG selection. (b) biological model diagram and workflow of the field experiment. Fresh poultry litter (FPL) from local industrial poultry production is stored as a heap in an open-air environment for six months. The resulting stored poultry litter (SPL) is then utilized as fertilizer in a horticulture field experiment, with control (CRL) plots lacking poultry litter and manured (MPS) plots using poultry litter. Lettuce (L-CRL and L-MPS) is grown in these plots until harvest for human consumption. The experiment assesses the potential transmission of enrofloxacin (ENR) and ciprofloxacin (CIP) from poultry production to vegetables, the abundance of *sul1* and *intI1* genes, and chemical and biological indicators of soil quality.

before lettuce implantation. All plots were irrigated with drinking quality water added to the total rainfall of 248.8 mm.

Soil and lettuce samples were collected at lettuce harvest, 82 days after implantation, at the end of the winter season. One soil sample composed of 16–20 subsamples was collected from each plot using a soil auger at a depth of 0 to 10 cm. Samples were sieved through a 2000 μm mesh and divided into three portions. One was preserved at −20 °C for fluoroquinolones quantification and DNA extraction, the second one at 4 °C for soil biological quality, and the third one air-dried at room temperature for soil chemical quality analysis. One lettuce-leaves sample composed of eight subsamples was collected from each plot. Lettuce leaves were rinsed following the recommendation for leafy vegetables for human consumption, and stored at −20 °C until fluoroquinolones quantification.

2.3. Poultry litter origin and storage

To prepare the poultry litter treatment, fresh poultry litter (FPL) was obtained from an intensive poultry production farm located in Buenos Aires, Argentina. It was used as bedding for the production of five broiler flocks. To resemble local practices of poultry litter storage at horticultural farms, the litter was stored in the open air and on bare soil for six months. The initial heap had a conical shape, with a base of 1.30 cm, a height of 90 cm, and a weight of approximately 300 kg. Three temperature sensors were placed within the heap, 25 cm from the centre and 20 cm deep, to record temperature variations during storage. At six months, the stored poultry litter (SPL) was used for the open field fertilization experiment performed in this study (see Section 2.1).

Two composite samples of poultry litter were collected. FPL composite sample was collected when the heap was built and SPL at the end of storage. Samples were preserved at −20 °C for fluoroquinolones quantification and DNA extraction. The general characterization of FPL and SPL is presented in Table 1.

2.4. Fluoroquinolones quantification

The Fluoroquinolones ENR and CIP were quantified in poultry litter, soil and harvested lettuce samples, which were sent to the Food Technology Institute, National Institute of Agricultural Technology (INTA, Argentina) for analysis. A solid-phase extraction (SPE) followed by liquid chromatography coupled to single quadrupole mass spectrometry (UPLC-MS) was used. Analytical standards for the analytes of interest were purchased from Sigma-Aldrich, Enrofloxacin VETRANALTM and Ciprofloxacin VETRANALTM with ≥95 % purity. Strata-X 200 mg/3 mL cartridges (Phenomenex) were used for solid-phase extraction.

Samples were treated as follows, 5 g were weighed into a 50 mL PP tube where 50 mL of a methanol:glacial acetic acid mixture (99:1 *v*/v) were subsequently added. The suspension was vortexed for 1 min to homogenize, sonicated for 5 min and then centrifuged for 15 min at 3000*g*. The supernatant was quantitatively transferred to a flask and evaporated to dryness using a nitrogen stream at 40 °C. The dried extract was reconstituted in 10 mL of milli-Q water, sonicated for 5 min, and passed through a Strata-X cartridge preconditioned with 6 mL of methanol and 6 mL of milli-Q water. After washing with 1 mL of 5 % methanol in water and drying with 2 min purge, the analytes were eluted with 2 mL of ethanol:ethyl acetate mixture (50:50 v/v). This final extract was dried with nitrogen stream at 40 °C and reconstituted in 1 mL of mobile phase for UPLC-MS analysis.

The chromatographic analysis was performed on a Waters Acquity UPLC instrument equipped with a Waters SQD single quadrupole mass detector. Selected ion monitoring (SIM) mode was used in quantitative analysis. Mass spectrometer acquisition was performed by electrospray ionization (ESI) in positive mode, using retention time and abundance of the following ions: *m/z* 36 and m/z 316 for enrofloxacin; m/z 332, m/z 288 for ciprofloxacin as identification criteria. A 2.5 μm, 2.1 × 150 mm XBridge BEH C18 column was used. The flow rate was 0.25 mL/min and the injection volume was 20 μL. A binary gradient elution program with mobile phases A (acetonitrile with 0.1 % formic acid) and B (water with 0.1 % formic acid) was employed as follows: from 0 to 3 min, isocratic with 10 % A; from 3 to 10 min, 10–60 % A; from 10 to 12 min, 60–95 % A; from 12 to 15 min, isocratic with 95 % A; from 15 to 17 min, 95–10 % A; and from 17 to 25 min, isocratic with 10 % A. The column oven and autosampler temperatures were 25 °C and 15 °C, respectively.

For quantification, matrix-matched calibration curves were prepared for each compound and each matrix, with 5 concentration levels: 3, 5, 10, 15, and 25 ng/kg. The calibration curves were processed together with the samples, reagent blanks and matrix blanks. The calculated limit of detection (LOD) was 1 ng/kg for enrofloxacin and ciprofloxacin, while the limit of quantification (LOQ) was 3 ng/kg for both analytes.

2.5. DNA extraction and real-time quantitative PCR

DNA was extracted from poultry litter samples from FPL and SPL and soil samples from CRL and MPS plots using the DNeasy PowerSoil Kit from Qiagen (Cat. No. 12888–50). The extraction was performed according to the manufacturer's instructions. Real-time quantitative PCR (qPCR) assays were performed for the detection and quantification of sequences specific for 16S rRNA, *sul1* and *intI1* genes. The selected primers can be found in Supplementary Table 1.

qPCR assays were performed as in (Adelowo et al., 2018) with minor modifications. In this case, SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, CA, USA) was used on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad, CA, USA). Technical replicates were three per sample and the final reaction mixture volume was 10 μL. Cycling conditions were 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s.

To normalize for different extraction, amplification efficiencies, and changes in bacterial abundance, the relative abundance of target genes was calculated by dividing the copy number of each gene by the copy number of 16S rRNA. The qPCR raw data for *16S* rRNA gene*, sul1, intI1* was deposited in FigShare doi:10.6084/m9.figshare.25800118.

2.6. Soil chemical and biological indicators of soil quality

The following soil chemical properties were included: pH, soil organic carbon (SOC), extractable phosphorus (EP) and total nitrogen (TN). pH was measured in a 1:2.5 (soil: deionized water) weight ratio, using a Hanna pH 211 microprocessor with the Hanna HI1331B pH electrode and an Orion Conductivity meter (Model 120) with the Orion conductivity cell 012010, respectively. SOC was determined according to the procedure proposed by Walkley and Black (Jackson, 1964). EP was determined using the extraction method proposed by Bray & Kurtz, according to NORMA-IRAM (2010). NT was determined following the Semi-Micro Kjeldahl method according to SAMLA (2004).

The biological properties included were soil microbial activity studied through soil basal respiration (SBR) and biomass through

Table 1
Characterization of fresh and stored poultry litter.

	pH	EC (dS/m)	Moisture (%)	Ashes (%)	TOM (%)	TP (%)	TN (%)	$N\text{-}NH_4^+$ (g/kg)
FPL	8.02	7.19	42.51	14.04	85.96	2.04	1.52	0.497
SPL	9.71	3.20	67.62	27.34	72.66	2.15	1.38	0.098

pH: potential Hydrogen (1:10); EC: electrical conductivity (1:10); TOM: total organic matter; TP: total phosphorus, NT: total nitrogen.

substrate-induced respiration (SIR), both based on CO_2 release. CO_2 release was determined by the alkali absorption method followed by titration (Zibilske, 1994). SIR was measured by the method adapted by Horwath and Paul (1994). This method is based on the addition of an easily degradable substrate which triggers microbial respiration. The substrate used was glucose 1 % added by fumigation. 20 g of soil are incubated for 6 h at 22 °C. A tube with 25 mL of 0.025 N NaOH was placed in each of the containers with the soil samples. The titration was performed using 0.025 N HCl.

2.7. Statistical analyses

To compare levels of fluoroquinolones, *sul1*, *inti1,* and soil quality indicators between two groups or treatments, unpaired two-tailed t-Student tests were performed, except when normal distribution could not be assumed. For relative abundance of *sul1*, TN and SIR, non-parametric Mann-Whitney tests were performed. The correlation between soil quality indicators and emergent contaminants was calculated using Pearson's correlation coefficients (Pearson r). The statistical programs GraphPad Prism 8.0.1 and InfoStat (2008) were used for the graphs and the tests. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Poultry litter use intensity in peri-urban horticulture

The district of Florencio Varela, located in peri-urban Buenos Aires, plays a significant role in vegetable production. Within this district we delineated an horticultural area of approximately 4379 ha, and identified a total of 341 poultry litter heaps, with 106 in Villa San Luis and 235 in La Capilla. This translates to a density of 1 poultry litter heap per 13 ha across the entire horticultural area, 1 per 15 ha in Villa San Luis, and 1 per 12 ha in La Capilla.

3.2. Detection and transmission of fluoroquinolones: from poultry to lettuce

When analysing poultry litter, in FPL, ENR was detected at a concentration of 7.3 μg/kg DW while CIP was not detected at this time point. However, in SPL, CIP was detected at a concentration of 39.22 μg/kg DW while ENR was below the detection limit. Fluoroquinolones were not detected in soil from either the CRL plots or MPS plots. However, when analysing lettuce samples, every L-MPS showed measurable concentrations of both fluoroquinolones, totalling a content of 24.73 ± 7.10 μg/kg (Fig. 2). These concentrations were significantly higher than those found in L-CRL ($p < 0.05$).

3.3. Changes in the absolute and relative abundance of biomarker sul1 in poultry litter and soils

The average abundance of the 16S rRNA was 10.29 and 8.97 log copies per gram of dry-weight poultry litter or soil, respectively. In poultry litter, the abundance did not show significant variation with the storage (Fig. 3a). In soil, the abundance did not vary significantly between CRL plots and MPS plots (Fig. 3d).

Regarding *sul1,* FPL and SPL contained 9.91 and 9.40 Log *sul1* copies per g of poultry litter respectively (Fig. 3b), corresponding to relative abundances of −0.22 and −1.06 Log *sul1* per 16S rRNA copy. Although a trend was found, the abundance of this gene did not significantly diminish with poultry litter storage. In CRL soils, the absolute abundance of *sul1* was 4.74 log *sul1* copies per g soil, equivalent to a relative abundance of −4.51 Log *sul1* per 16S rRNA copy. The absolute abundance increased significantly in MPS to up to 7.61 Log *sul1* copies per g soil due to amendment with SPL (Fig. 3e). The relative abundance followed the same trend, increasing to up to −0.94 Log *sul1* per 16S rRNA copy. However, this difference was not significant.

Regarding *intI1* in FPL, the absolute abundance was 7.57 Log *intI1* copies per g poultry litter (Fig. 3c), corresponding to a relative abundance of −2.57 Log *intI1* per 16S rRNA copy. The copy number of this gene did not significantly change between FPL and SPL which contained −2.47 Log *intI1* per 16S rRNA copy. In soils, the absolute abundance of *intI1* in MPS plots was 6.88 Log *intI1* copies per g soil, slightly and significantly higher than in CRL plots (6.61 Log *intI1* copies per g soil) (Fig. 3f). The relative abundance of *intI1* in MPS plots was −1.64 Log *intI1* per 16S rRNA copy, slightly but not significantly higher than in the CRL plots (−2.64 Log *intI1* per 16S rRNA copy).

3.4. Correlation between soil quality indicators and emergent contaminants

The use of SPL resulted in significant changes in physical-chemical and biological indicators of soil quality in MPS, compared to CRL plots (as shown in Table 2). The MPS plots had a pH closer to neutrality and higher availability of phosphorus. They also showed a higher SOC content and higher soil microbial activity, measured by SBR.

The correlation analysis revealed associations between the relative abundance of specific genes in soil and soil quality indicators, as well as the presence of fluoroquinolones in lettuce (Table 3). *sul1* showed a significant positive correlation with a more neutral soil pH, higher

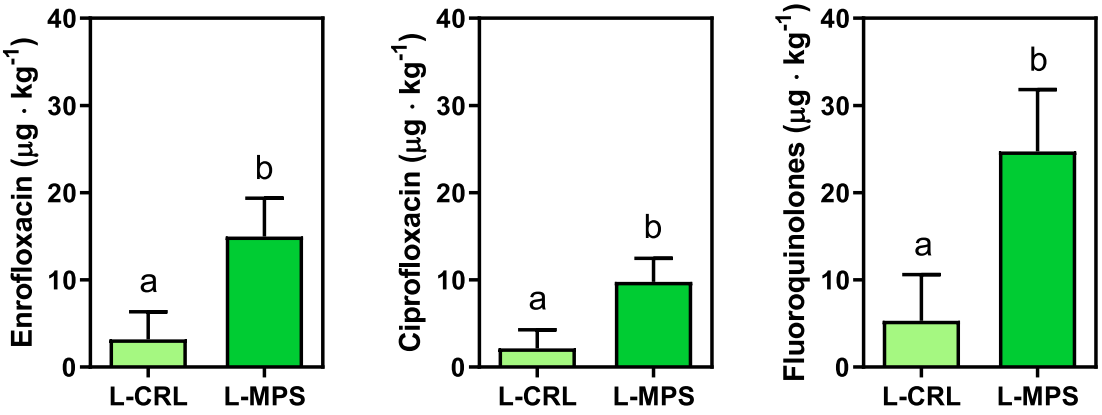

Fig. 2. Detection of fluoroquinolones in lettuce at harvest from control and manured soil. Enrofloxacin, ciprofloxacin and their total content increased in lettuce leaves as a consequence of soil amendment with stored poultry litter from intensive animal husbandry. Antimicrobial content is expressed as μg of antimicrobials per kilogram of fresh lettuce leaves. Different letters indicate significant differences between groups as determined by the one-tailed t-student test ($p < 0.05$). L-CRL ($n = 3$), lettuce samples from CRL plots; L-MPS ($n = 3$), lettuce samples from MPS plots. Error bars indicate standard deviations.

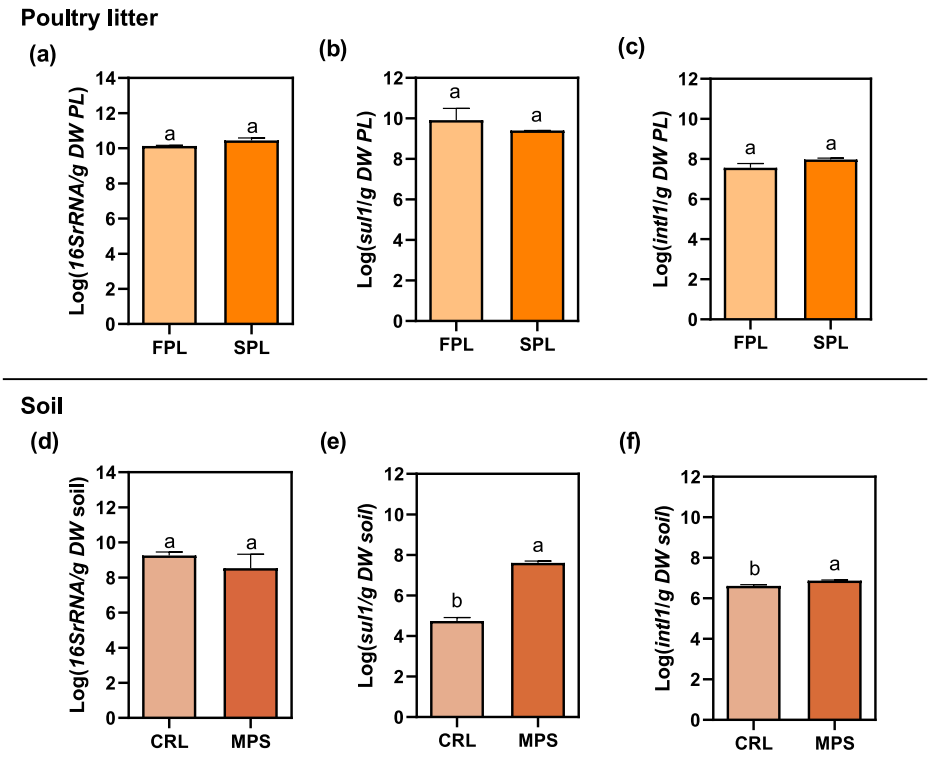

Fig. 3. Abundance of 16S rRNA gene and antimicrobial resistance biomarker genes *sul1* and *intI1* in poultry litter (FPL and SPL) and soil (CRL and MPS). (a, d) Abundance of 16S rRNA gene in poultry litter (PL) and soil was not affected by storage nor by manuring ($p > 0.05$). (b-c) Abundance of *sul1* and *inti1* genes was not affected by poultry litter storage. (e-f) Abundance of *sul1* and *inti1* genes increased as a consequence of soil amendment with stored poultry litter from intensive animal husbandry ($p < 0.05$). Gene abundance is expressed as a Log of gene copy number per gram of dry-weight poultry litter (a-c) or soil (d-f). Different letters indicate significant differences between groups as determined by the two-tailed t-student test or Mann-Whitney test ($p < 0.05$). FPL ($n = 2$), fresh poultry litter; SPL ($n = 2$), stored poultry litter; CRL ($n = 3$), soil from control plots; MPS ($n = 3$), soil from manured plots. Error bars indicate standard deviations.

Table 2
Physical-chemical and biological indicators of soil quality.

	pH	TN (%)	EP (m/kg)	SOC (%)	SBR (mg CO_2/kg/day)	SIR (mg CO_2/kg/h)
CRL	6.26a	0.21a	75.38a	1.99a	56.34a	25.37a
MPS	6.59b	0.28a	257.85b	2.36b	124.28b	29.86a

pH: potential hydrogen, TN: total nitrogen, EP: extractable phosphorus, SOC: soil organic carbon, SBR: soil basal respiration, SIR: substrate-induced respiration (0.5 % glucose). Different letters indicate significant differences between groups as determined by the two-tailed t-student test or Mann-Whitney test ($p < 0.05$).

Table 3
Pearson correlation matrix between soil quality indicators and emergent contaminants.

Indicator		*intI1*	*sul1*	ENR	CIP
Physical-chemical	Ph	0.81	**0.94**[a]	0.54	0.55
	TN	**0.89**[a]	**0.95**[a]	0.56	0.57
	EP	**0.91**[a]	**0.90**[a]	0.42	0.43
	SOC	**0.84**[a]	**0.93**[a]	0.54	0.56
Biological	SBR	**0.84**[a]	**0.93**[a]	0.51	0.52
	SIR	0.67	0.44	0.27	0.26
	16S rRNA	−0.67	−0.65	−0.28	−0.30
Emergent contaminant	*intI1*	1	**0.96**[a]	0.68	0.68
	sul1		1	0.73	0.73
	ENR			1	**1.00**[a]
	CIP				1

[a] Bold letters indicate a significant correlation between variables ($p < 0.05$).

availability of soil nutrients (TN and EP), higher SOC content and higher SBR. *IntI1*, in turn, showed a similar but less strong pattern with a significant positive correlation with TN, EP, SOC, and SBR. The strongest correlation found for both ENR and CIP was a positive correlation with *sul1* (Pearson $r = 0.73$), although it was not significant.

4. Discussion

This study reveals that using stored poultry litter from intensive animal husbandry as a soil amendment, a wide spread practice in Buenos Aires periurban horticulture, introduces fluoroquinolones to vegetables and increases *sul1* and *inti1* gene abundance in horticultural soils. These findings highlight the risks of using poultry litter from intensive animal husbandry as it may increase human plant-dietary exposure to critically important antimicrobials and contribute to the spread of AMR in the open environment and towards the clinical settings.

4.1. Implications of antibiotic use in animals for antibiotic contamination

Fluoroquinolones are often found in poultry litter, with 74 % enrofloxacin and 42 % ciprofloxacin occurrences detected by Alonso et al. (2024) in Argentina. This is due to enrofloxacin's widespread use in intensive animal husbandry, poor gut absorption, and excretion as active enrofloxacin and ciprofloxacin (Trouchon and Lefebvre, 2016). Levels detected in this study, though lower than in fresh litter at poultry farms (810–3175 μg/kg) or after staking and piling treatment (Alonso et al., 2024; Teglia et al., 2017), were sufficient to be absorbed by lettuce, indicating that recommended poultry litter treatments are not enough to prevent downstream pollution.

While the fluoroquinolone concentrations in lettuce are within acceptable daily intake limits for enrofloxacin, i.e. 50 g lettuce portion is

equivalent to 5.5 % of the ADI (Food and Agriculture Organization of the United Nations, 1997), they contribute to overall dietary exposure. Recent studies in Argentina have found high levels of enrofloxacin in other food products, suggesting that total dietary exposure might be higher than estimated. Particularly, Teglia et al. (2021) detected toxicological levels of enrofloxacin (350 μg/kg) in commercialized dried eggs. If enrofloxacin use is not restricted, revising MRLs for unassessed food items would be needed, as long-term dietary exposure can affect human gut microbiome, increase AMR and allergic reactions (Ben et al., 2022; Li et al., 2019; Subirats et al., 2019).

Notably, fluoroquinolones were detected in lettuce but not in soil samples 82 days after poultry litter incorporation, likely due to sorption, plant absorption and degradation. Soil matrix complexity —including texture, organic matter and cation exchange capacity— can increase antimicrobial sorption and make their extraction and measurement challenging (Jechalke et al., 2014; Leal et al., 2013). In 13 Brazilian soils with varying properties, fluoroquinolone sorption was very high (Kd ⩾ 544 L kg^{-1}), capturing at least 84 % of the applied amount (Leal et al., 2013). Lettuce absorbs the available fluoroquinolones from the soil solution, likely accumulating them in plant tissues and thus reducing their soil availability, a process akin to metal remediation strategies (Nnaji et al., 2023). Additionally, increased microbial activity in MPS plots may aid in the degradation of antibiotics (Blau et al., 2019). Overall, using lettuce as a proxy for soil contamination with fluoroquinolones is worth exploring, as it offers a simpler matrix for evaluation and directly implicates human health.

With regard to the open environment, enrofloxacin was found in wild Andean condors from Patagonia (Blanco et al., 2023), raising concerns about bioaccumulation in the local terrestrial food web as they present higher bioaccumulation capacity than sulphonamides, tetracyclines and macrolides (Hu et al., 2023).

4.2. Antimicrobial resistance dissemination in horticulture in the conceptual framework of One Health

AMR and ARG pollution in horticulture remain emerging areas of study in Argentina, as in most Latin American countries. This is the first study to assess *sul1* gene abundance as an anthropogenic-AMR biomarker in environmental DNA using a culture-independent method. In our clayey soil, *sul1* and *intI1* levels in CRL plots aligned with global reports from intermediate impacted soil (Fig. 3e-f) (Blau et al., 2019; Chessa et al., 2016; Stiborova et al., 2021). While *intI1* levels were similar between poultry litter and soil samples, *sul1* was several orders of magnitude higher in poultry litter than in CRL soil (−1.06 versus −4.51 Log *sul1* per 16S rRNA copy). Log *sul1* abundance increased by 1.6-fold after poultry litter application, reaching levels of a highly polluted soil (Bengtsson-Palme et al., 2023), highlighting its sensitivity as an anthropogenic pollution biomarker in soils. In contrast, changes in *intI1* gene abundance, while significant, do not seem to be as effective a biomarker in soil as for water (Franklin et al., 2024) however the presence of *sul1* coupled with *inti1* might indicate high ARG mobility capacity. Poultry litter showed higher *sul1* abundance than manure from grass-fed cows (Chessa et al., 2016) and pig farms (Blau et al., 2019), but it was comparable to poultry litter from Canada (Subirats et al., 2021).

The staking storage system used did not reduce *sul1* abundance (Fig. 3b), although it eliminates *E. coli* harbouring *sul1* by day 7 (Okada et al., 2024), emphasizing the usefulness and need for culture-independent methods in surveillance and composting development. Successive poultry litter applications could further elevate ARGs levels in soil, making it important to monitor horticultural farms with long-term use, due to potential ARG transfer from soil to vegetables (Wang et al., 2022).

Poultry litter application may create hotspots for AMR selection and dissemination. The incorporation of organic matter and nutrients to the soil enhances soil quality and microbial activity (Table 2), which benefits vegetable production (González et al., 2010; Milicia et al., 2023; Prack McCormick et al., 2022; Tejada et al., 2006). However, combined with antibiotics, ARG, mobile genetic elements, these conditions promote the survival of resistant bacteria and horizontal genetic transfer between microbial populations, including unrelated species (Cycoń et al., 2019; Wellington et al., 2013). The presence of fluoroquinolones, ARGs, and class 1 integrons in horticulture raises concerns about human AMR exposure. In Latin America and Caribbean, fluoroquinolone-resistant *Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aureoginosa* are among the 10 deadliest pathogen-drug combinations, with fluoroquinolone-resistant *E. coli* increasing >5 % annually (Naghavi et al., 2024).

4.3. The complexity of closing cycles when antibiotics are being used

Closing nutrient and organic carbon cycles is essential for the sustainable use of soils (Schroeder et al., 2019). In our experiment, poultry litter increased lettuce yield by 32.9 % compared to the unfertilized control and by 5.8 % compared to chemical fertilizer (Milicia et al., 2023). In one of the main poultry-producing regions of Argentina, poultry litter could replenish most nutrients removed by maize, wheat, sorghum, rice, and soybean harvest, reducing the need for chemical fertilizers by 72–109 % for P, 22–43 % for N and 44–83 % for K (Gange, 2016). However, the presence of antimicrobial residues and ARG in poultry litter poses significant risks that jeopardize the sustainability of nutrient cycling, compromising other Sustainable Development Goals.

Our findings highlight the urgent need to reduce antimicrobials use in intensive poultry production and improve the management for the storage and application of poultry litter to reduce the spread of antimicrobials and AMR. Auto-thermal treatment (staking and turning) at poultry farms reduces antibiotic mass by 67 % but doesn't lower fluoroquinolones to safe levels (Alonso et al., 2024). Improved composting can reduce up to 90 % of fluoroquinolones (Esperón et al., 2020) and reduce some indicator ARG, though *sul1* remains persistent (Subirats et al., 2021). Moreover, composting to eliminate antimicrobial residues requires time, infrastructure and control, which are not necessarily available in the socio-economic context of local horticulture.

Argentina has advanced in AMR prevention through the National Action Plan and Ley 27,680, which follows a "One Health" approach. In 2024, Resolution 445/2024 further restricted antimicrobials use in animal production, banning their use for growth promotion and reserving critical drugs for human medicine. A recent study identified seven major challenges in tackling AMR, including cultural factors, fragmented governance, and lack of awareness (Allel et al., 2024). Based on our results, evaluating restrictions on enrofloxacin use is recommended, as banning administration through drinking water in the U.S. was followed by reduced ciprofloxacin AMR in humans.

4.4. Limitations of the study

It is important to acknowledge that the sample size of this study was relatively small, and it was conducted in only one soil type, which is relevant for horticulture in the southern peri-urban area of Buenos Aires. Generalizing these results to the entire horticultural belt would require considering different soil types, growing seasons, manure sources and crop types (e.g. leafy vs. fruit or root vegetables). Both fluoroquinolones we detected in one CRL plot. We attribute this effect to fluoroquinolones runoff or leaching from MPS plots due to their high soil-water migration (Parente et al., 2019) or through dissolved organic matter-mediated transport (Gbadegesin et al., 2022). Longitudinal studies that track changes in antimicrobials and ARG over time in response to different management practices may be particularly valuable in identifying effective mitigation strategies.

5. Conclusion

Storage and use of poultry litter is generalized in periurban

horticulture within the green belt of Buenos Aires. Residues from fluoroquinolones administered in intensive poultry production can be transmitted to vegetables through soil amendment with poultry litter. Therefore, consumers of fresh vegetables produced in this area are likely to be exposed to antibiotics through their diet. However, the levels of exposure and the risks for the human gut microbiome are yet to be assessed. Poultry litter must be considered a source of ARG and *intI1* and the application of stored poultry litter to the soil in circular agriculture contributes to soil pollution with these emergent contaminants. Soil amendment with poultry litter stimulates soil microbial activity, as it represents a source of nutrients and organic matter. The combination of selective agents as the antimicrobials, with ARG and mobile genetic elements in the context of soil microbial activity stimulation could in turn benefit Horizontal Genetic Transfer of AMR determinants between environmental and pathogenic bacteria. Therefore, while amended plots may present improved soil quality and productivity, they may concurrently act as hotspots for the selection of bacteria carrying AMR genes. This phenomenon could potentially contribute to the dissemination of AMR in the environment, thereby heightening the risk to human health. Finally, the dual role of manure in agricultural productivity and AMR propagation calls for the design of more integrated approaches that can balance these conflicting aspects to safeguard both agricultural sustainability and public health.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.176874.

CRediT authorship contribution statement

Barbara Prack McCormick: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Camila A. Knecht:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Ana Clara Sokolowski:** Writing – review & editing, Methodology. **Pablo Martín Palladino:** Writing – review & editing, Methodology. **Dante Emanuel Rojas:** Writing – review & editing, Methodology. **Diego Sebastián Cristos:** Writing – review & editing, Methodology. **Hernan J. Rivera:** Writing – review & editing, Methodology. **Carola Gonçalves Vila Cova:** Writing – review & editing, Visualization, Methodology. **Javier De Grazia:** Writing – review & editing, Methodology. **Hernán A. Rodriguez:** Writing – review & editing, Methodology. **Pablo Tittonell:** Writing – review & editing, Conceptualization. **Daniela Centrón:** Writing – review & editing, Writing – original draft, Conceptualization. **Monica B. Barrios:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the grants PICT [PICT-2021-I-INVI-00787] from “Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación”, Argentina, and LomasCyT [FCA-43 Res. No 868/2017] from “Universidad Nacional de Lomas de Zamora”, Argentina, given to BPMcC. BPMcC and CAK were recipients of a CONICET postdoctoral grant from CONICET (Argentina). The funding sources had no involvement in the collection, analysis or interpretation of data; in the writing of the report; nor in the decision to submit the article for publication.

We are grateful to the “Faculty of Agricultural Sciences at Universidad Nacional de Lomas de Zamora” for providing the field for this study and helping with fieldwork, particularly to the Horticulture Chair.

Data availability

The raw data is available to download from doi: 10.6084/m9.figshare.25800118.

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