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Antibiotics and antibiotic resistance genes in anaerobic digesters and agroecosystems

28/11/2023

Dr. Andrea Visca

Antibiotics

Antibiotics are medicines used to prevent and treat bacterial infections. They can act both by killing or inhibiting the bacterial growth.

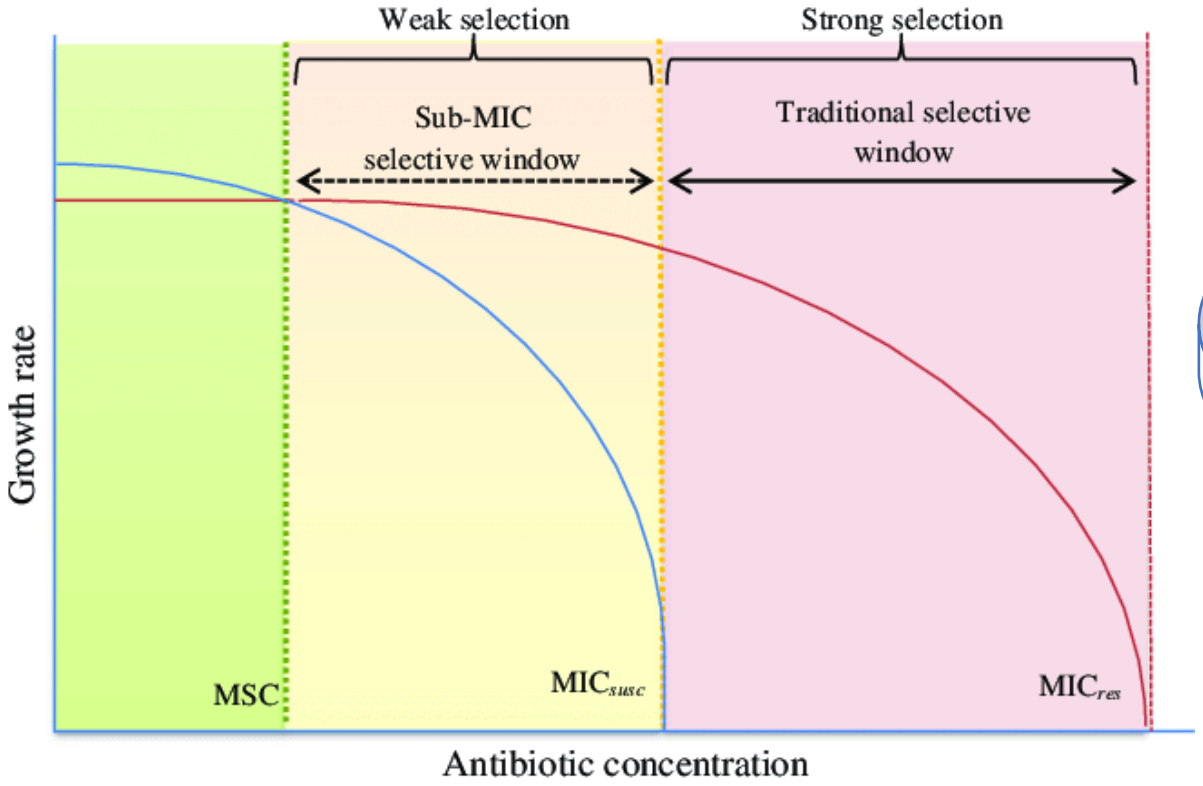
Antibiotics have been used for millennia to treat infections, although until the last century or so people did not know the infections were caused by bacteria.

The word 'antibiotics' was first used in 1939 by the Ukrainian-American inventor and microbiologist Selman Waksman, who in his lifetime discovered over 20 antibiotics.

Antibiotics have raised growing concerns due to extensive **environmental contamination resulting from their excessive use in both human and veterinary domains.**

Minimum Inhibitory Concentration (MIC)

In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical (i.e., antibiotic), which prevents visible in vitro growth of bacteria or fungi.

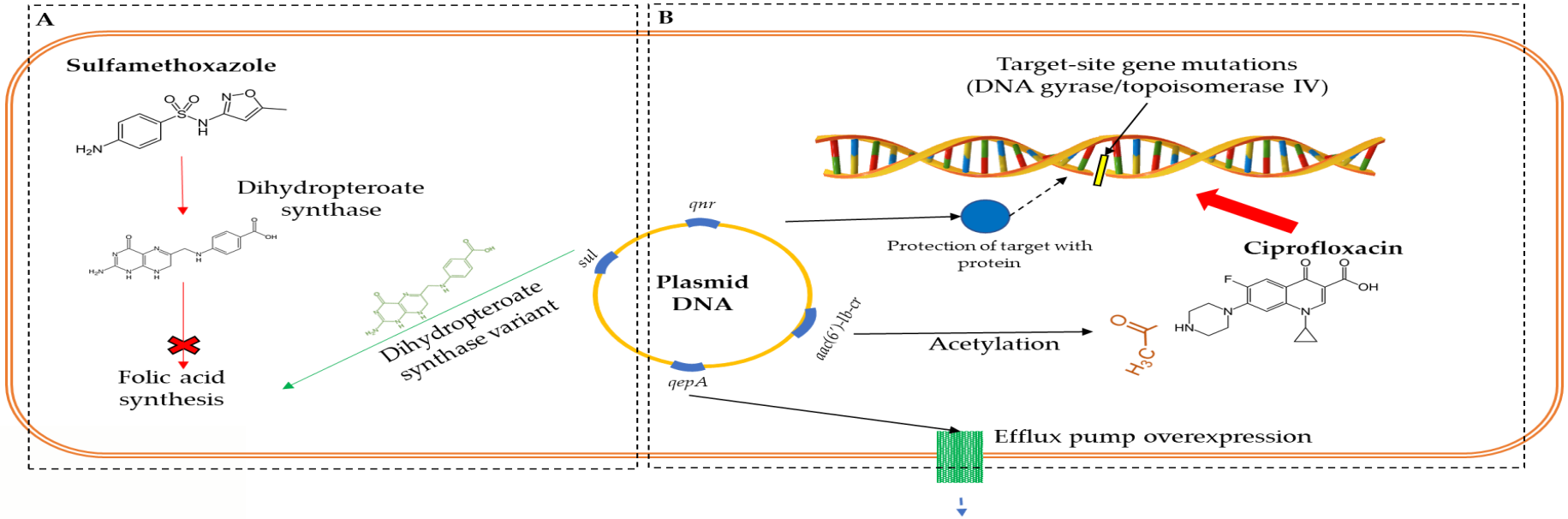


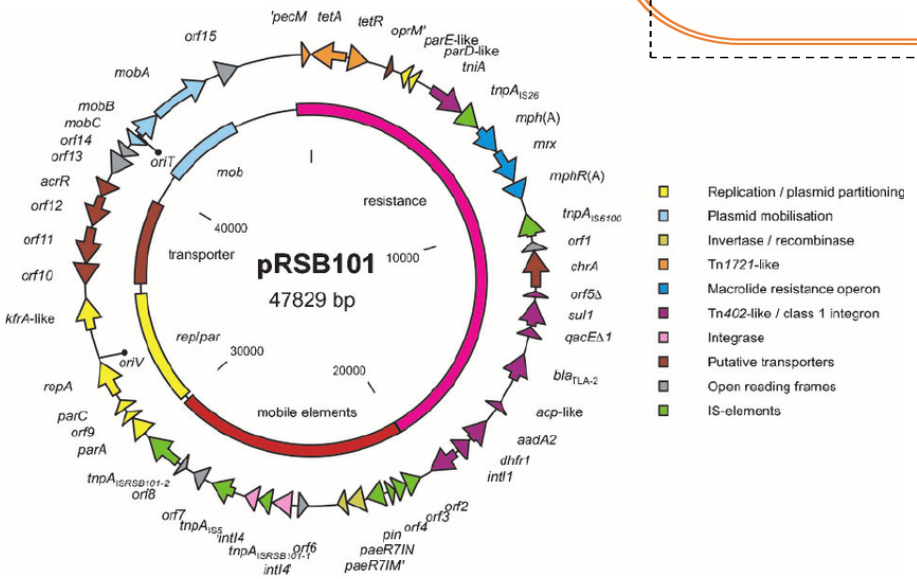
Antibiotics are only partially metabolized, the great % is excreted through faeces and reach, eventually, the environment

Antibiotics enters the environment at sub-MIC concentration, meaning they will not have the biocidal effect they are meant for

Antibiotic resistance

Antibiotic resistance refers to the ability of a microorganism to resist at the action of the antimicrobial compound.

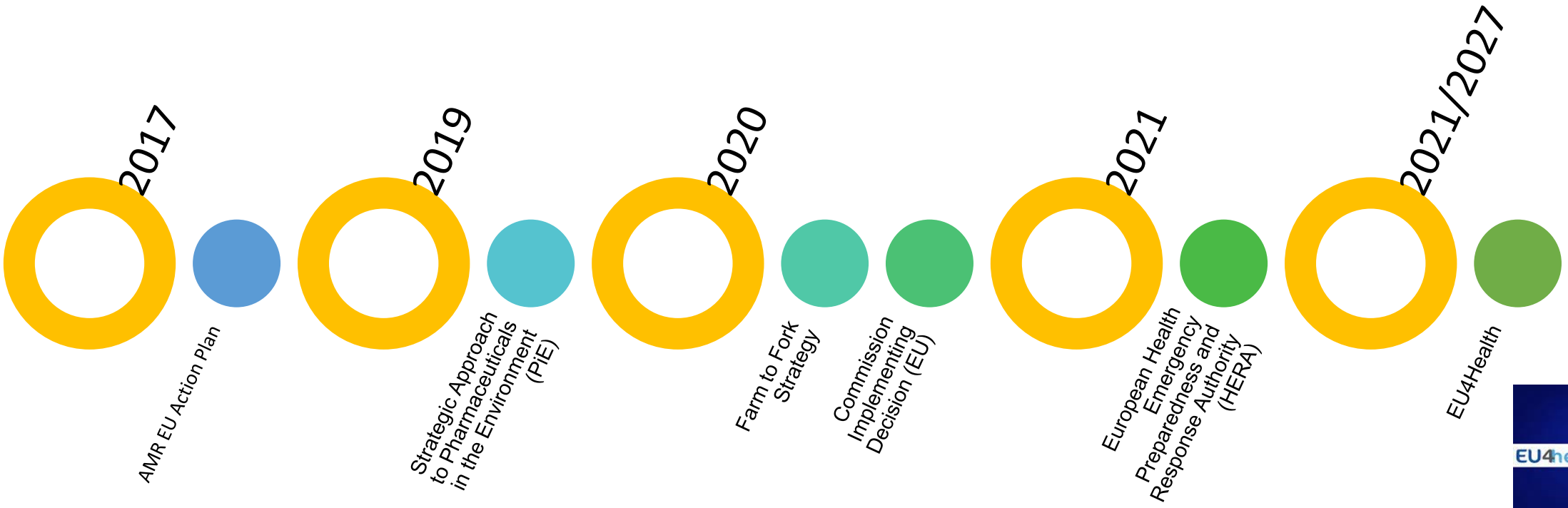




Plasmids can include the so-called “integron cassette”, which can capture mobile genetic elements thanks to the site-specific recombination mediated by an integron-integrase (*int*I). In this way, they can accumulate several cassettes, including the ones responsible for antibiotic resistance genes.

The menace of antibiotic resistance

Antibiotic resistance (AMR) is thought to be responsible for 33,000 fatalities in the EU each year. It is also predicted to cost the EU € 1.5 billion in healthcare costs and lost productivity per year. Since the implementation of the 2017 AMR EU Action Plan, significant advancements have been made to bolster the EU's approach to combatting AMR.



One Health

One Health is an approach that recognizes that the health of people is closely connected to the health of animals and our shared environment. One Health is not new, but it has become more important in recent years. This is because many factors have changed interactions between people, animals, plants, and our environment.

More people live in close contact with wild and domestic animals, both livestock and pets. Close contact with animals and their environments provides more opportunities for diseases to pass between animals and people.

The earth has experienced changes in climate and land use, such as deforestation and intensive farming practices. Disruptions in environmental conditions and habitats can provide new opportunities for diseases to pass to animals.

Agroecosystems

Agroecosystems are defined as ecosystems where human intervention intentionally alters the biota composition, specifically land areas devoted to crops and livestock, substituting the natural flora and fauna of the area to varying degrees.

Agriculture has a significant impact on biodiversity, through a variety of mechanisms:

1) as a political and economic instrument, by means of commodity prices or subsidies;
2) as a production technology using pesticides, fertilizer, and soil disturbance;
3) as a biological process resulting in habitat fragmentation and species invasions.

The expansion of agriculture has transformed landscapes into mosaics of managed and unmanaged ecosystems, resulting in habitat loss and fragmentation for many species of flora and fauna

Organic fertilizers

Organic fertilizers are considered as an alternative to synthetic ones as utilizing locally available nutrients' sources aligns with the circular economy criteria.

Organic amendments introduce antibiotics

Antibiotic *abuse* for ensuring/increasing livestock productivity

As a key element of the European Green Deal, the Farm to Fork strategy, the European Commission intends to achieve a 50% reduction in nutrient losses by 2030, all while preserving soil fertility at current levels. This effort is expected to lead to, at a minimum, a 20% decrease in the use of synthetic fertilizers and an increase in organic fertilizers.

Intensive farming

Antibiotic spread in the environment

Anaerobic digestion

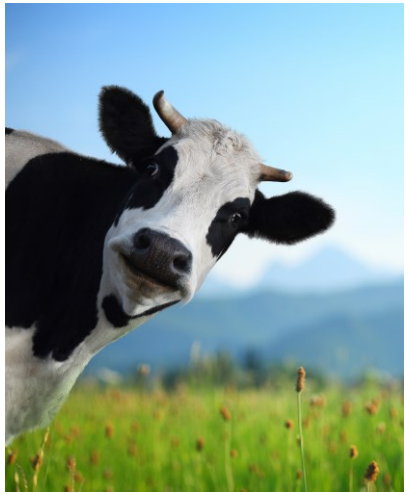
The utilization of manure for anaerobic digestion (AD) is an effective strategy that offers multiple benefits as it enables an effective management of organic waste while reducing the environmental impacts.

Animal manure goes as input to the anaerobic digester

The AD takes place in a meso (or thermo) philic environment for 45-90 days

Biogas is produced as first product

Digestate is the waste of AD, which can still be used as organic fertilizers due to its nutritional value

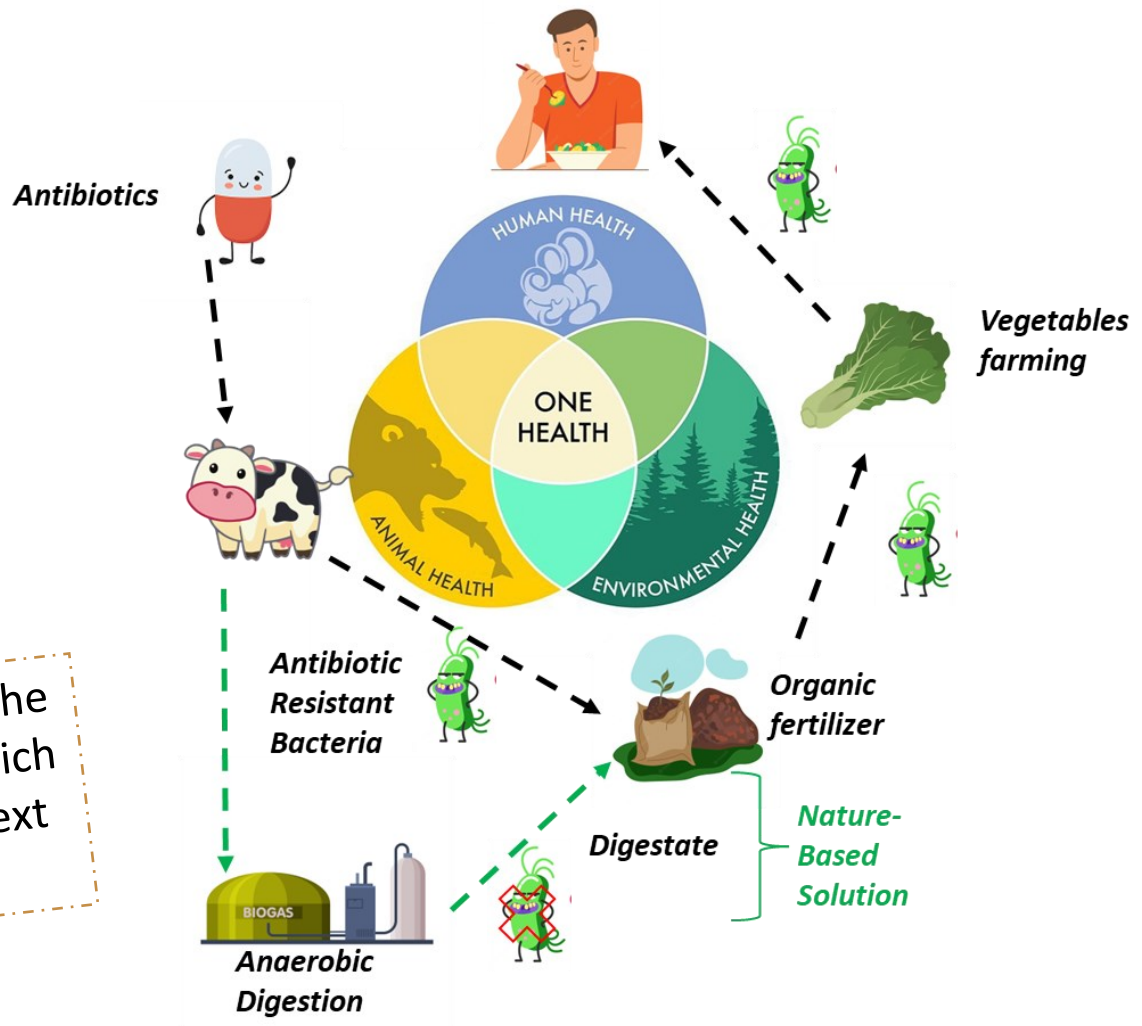


Digestate as organic fertilizer

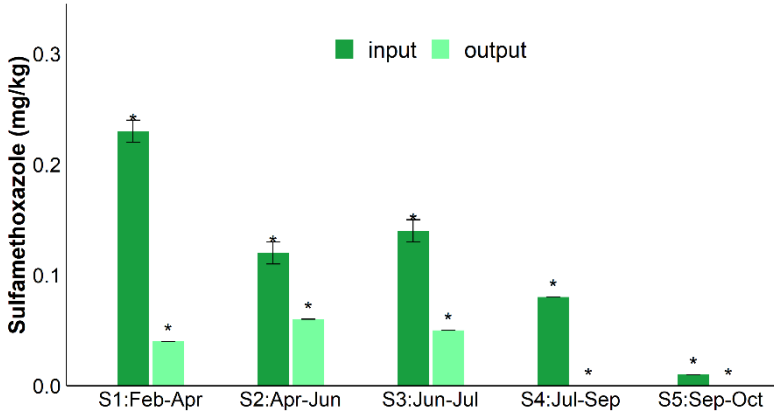
Can the use of digestate as a fertilizer significantly mitigates the spread of resistance genes in the agroecosystem?

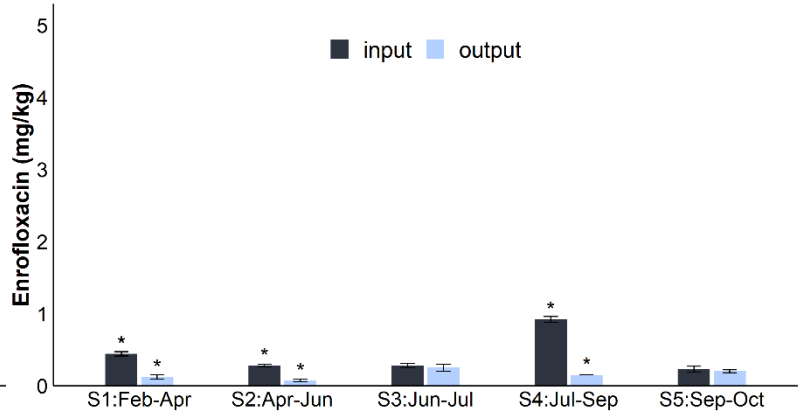
Digestate it is itself devoid (or nearly devoid) of these emerging contaminants, such as antibiotics and Antibiotic Resistance Genes (ARGs).

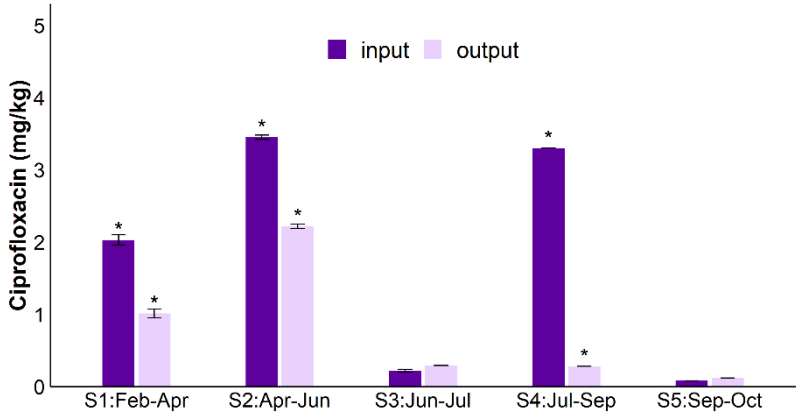
Furthermore, digestate is obtained from the AD process of agrozootechnical waste which increases its economic value in the context of circular economy.



Antibiotic and ARGs removal during anaerobic digestion: case studies







ARGs removal

R_{sul1} (%)	$R_{aac\text{-}(6')\text{-}Ib\text{-}cr}$ (%)	R_{intI1} (%)
-	96.2	-
97.2	85.8	95.8
98.3	72.3	98.8
50.6	94.9	73.9
97.6	99.9	97.0
64.0 ± 24.0%	90.0 ± 5.0%	69.0 ± 22.0%

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Effects of Sulfamethoxazole on the Microbial Community Dynamics During the Anaerobic Digestion Process

Valentina Mazzurco Miritana[1], Giulia Massini[1,2], Andrea Visca[1], Paola Grenni[1], Luisa Patrolecco[3], Francesca Spataro[3], Jasmin Rauseo[3], Gian Luigi Garbini[1], Antonella Signorini[2], Silvia Rosa[2] and Anna Barra Caracciolo[1]*

antibiotics
MDPI

Article

Effects of Ciprofloxacin Alone or in Mixture with Sulfamethoxazole on the Efficiency of Anaerobic Digestion and Its Microbial Community

Valentina Mazzurco Miritana [1,2], Luisa Patrolecco [3,*], Anna Barra Caracciolo [2,*], Andrea Visca [2], Flavia Piccinini [1], Antonella Signorini [1], Silvia Rosa [1], Paola Grenni [2], Gian Luigi Garbini [2], Francesca Spataro [3], Jasmin Rauseo [3] and Giulia Massini [1,2]

antibiotics
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Article

Anaerobic Digestion and Removal of Sulfamethoxazole, Enrofloxacin, Ciprofloxacin and Their Antibiotic Resistance Genes in a Full-Scale Biogas Plant

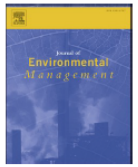
Andrea Visca [1], Anna Barra Caracciolo [1,*], Paola Grenni [1], Luisa Patrolecco [2], Jasmin Rauseo [2], Giulia Massini [3], Valentina Mazzurco Miritana [1,3] and Francesca Spataro [2]

Antibiotic resistance genes spread in agroecosystem: a predictive study

Contents lists available at ScienceDirect

Journal of Environmental Management

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



Antibiotics and antibiotic resistance genes in anaerobic digesters and predicted concentrations in agroecosystems

Andrea Visca [a], Jasmin Rauseo [b,*], Francesca Spataro [b], Luisa Patrolecco [b], Paola Grenni [a], Giulia Massini [c], Valentina Mazzurco Miritana [a,c], Anna Barra Caracciolo [a]

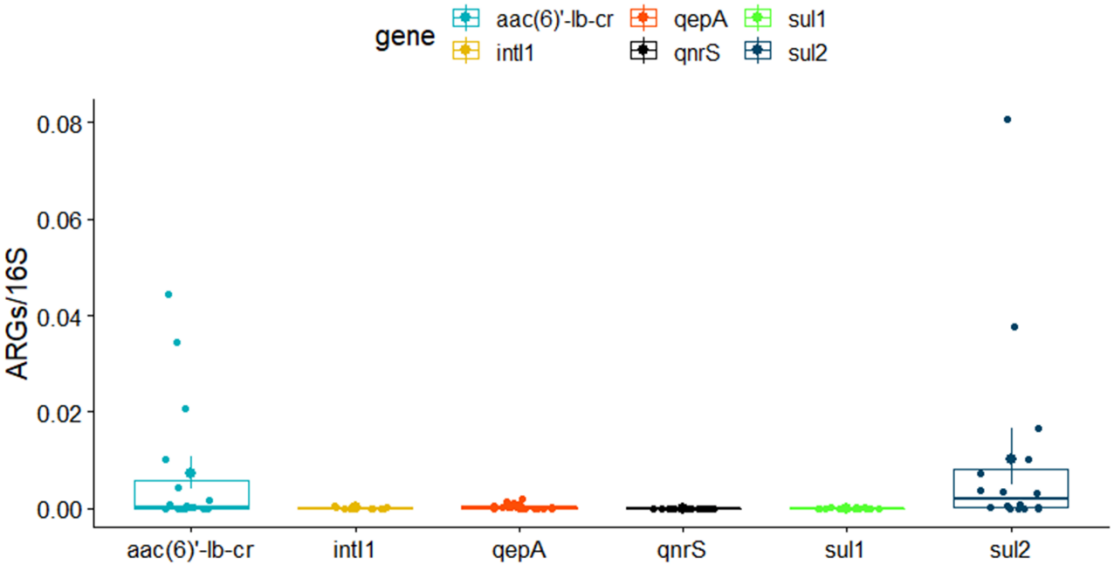
			Digester 1
Estimated concentrations (0.25%/ha)	SMX (μg/kg)	In	0.25
		Out	0.07
	CIP (μg/kg)	In	3.7
		Out	2.1
Estimated concentrations vs PNEC	SMX (ng/L)*	In	385.2
		Out	2.47
	CIP (ng/L)*	In	5508.2
		Out	67.38

Values estimated assuming an average In density of 1.5 kg/L (Achkari-Begdouri and 2015) and a treatment of 0.25% manure/digestate per hectare.

[a] Le Page et al. (2017).

[b] Mortimer et al. (2020).

The overall averages of ARGs and MGE found in this work were very low, and comparable with environmental background values in pristine soils (Nguyen et al., 2020), except in the case of the *aac-(6)'-Ib-cr* and *sul2* genes.



Post-treatment of digestate to further remove ARGs

Introduction of additional steps before and after the anaerobic digestion process (Gurmessa et al., 2021) makes digestate a suitable solution for application in agroecosystems as organic fertilizers.

Contents lists available at ScienceDirect

Bioresource Technology

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

Post-digestate composting shifts microbial composition and degrades antimicrobial resistance genes

Biyensa Gurmessa [a,*], Vesna Milanovic [a], Ester Foppa Pedretti [a], Giuseppe Corti [a], Amanda J. Ashworth [b], Lucia Aquilanti [a], Ilario Ferrocino [c], Maria Rita Corvaglia [c], Stefania Cocco [a]

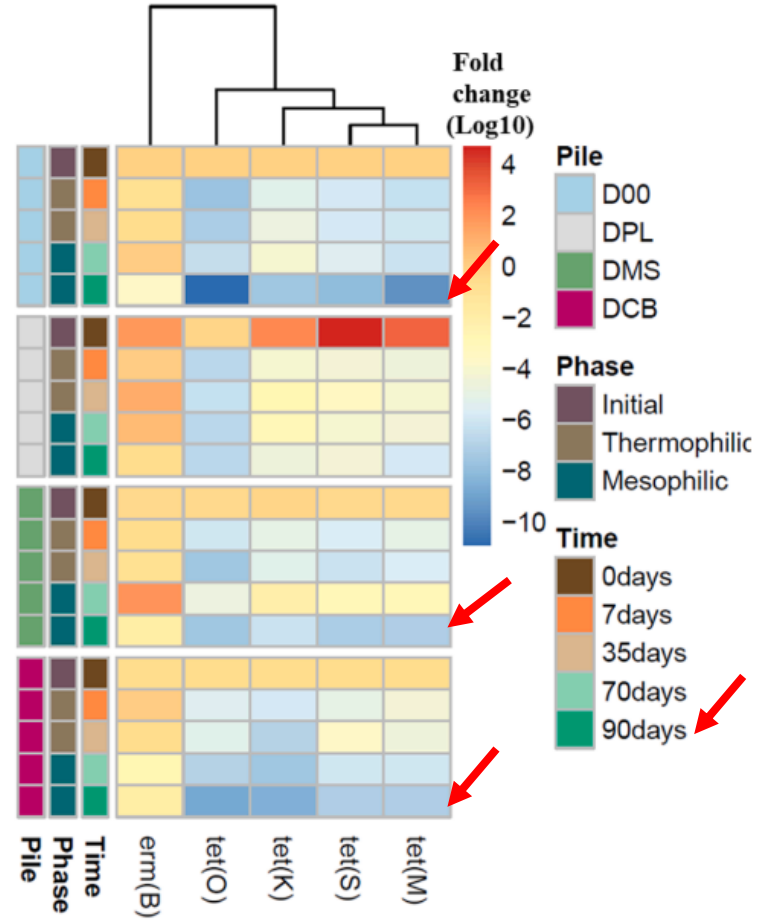
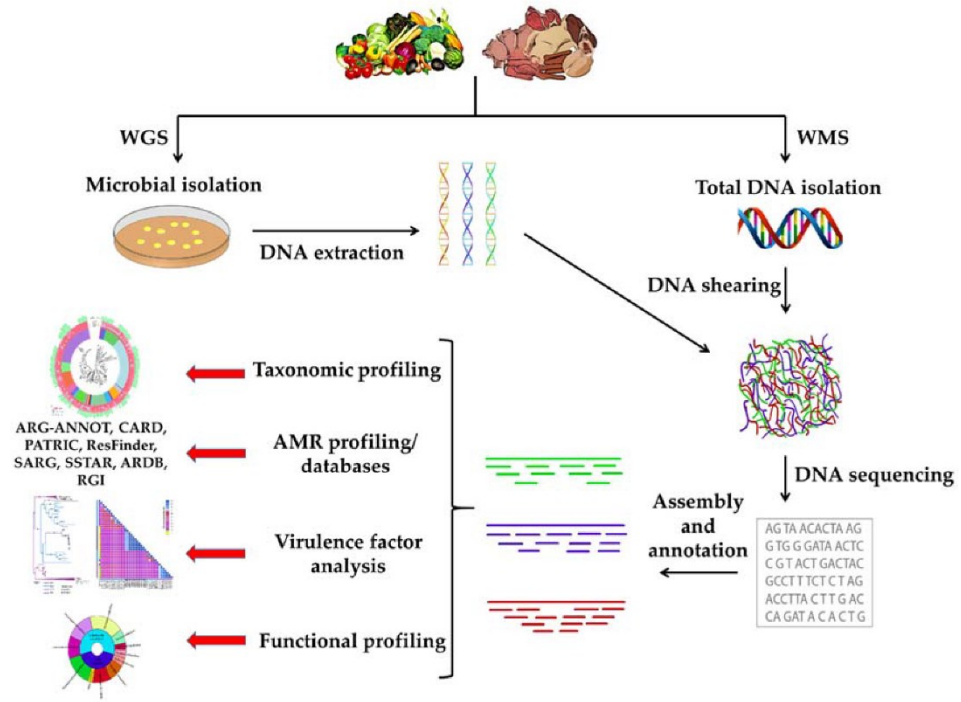


Fig. 2. Dynamics of ARGs in compost piles, expressed as log10 of fold change, during the composting period. Negative and positive results of the log fold change indicate reduction and enrichment, respectively.

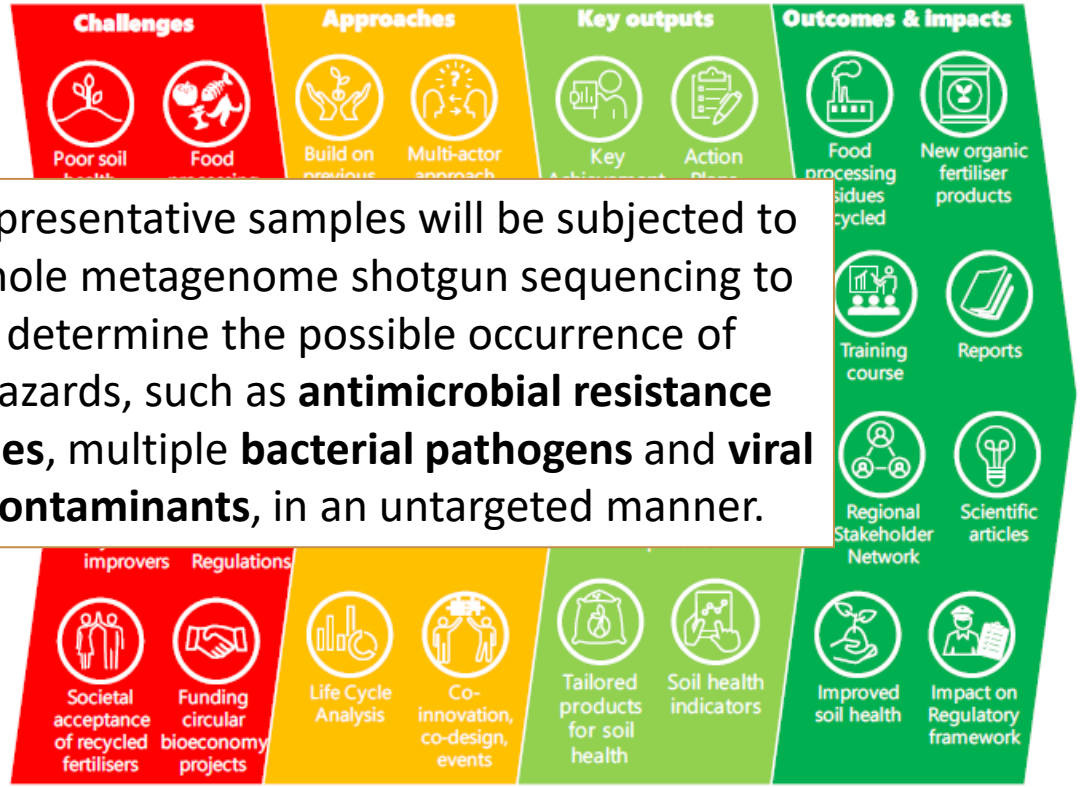
Tailored soil improvers

DeliSoil

Identify, quantify and manage regional food processing residues streams through sustainable and high-quality standardized valorization processes to obtain soil improvers.



DeliSoil's **overall goal** is to contribute to the European Union's Mission **"A Soil Deal for Europe"**, by improving the sustainability of food systems and enhancing soil health. This will be achieved by developing improved recycling and processing solutions for food industry residues, to produce safe, sustainable, tailored and societally accepted soil improvers[1] in the EU, in collaboration with the EU Soil Observatory (EUSO) and other projects.

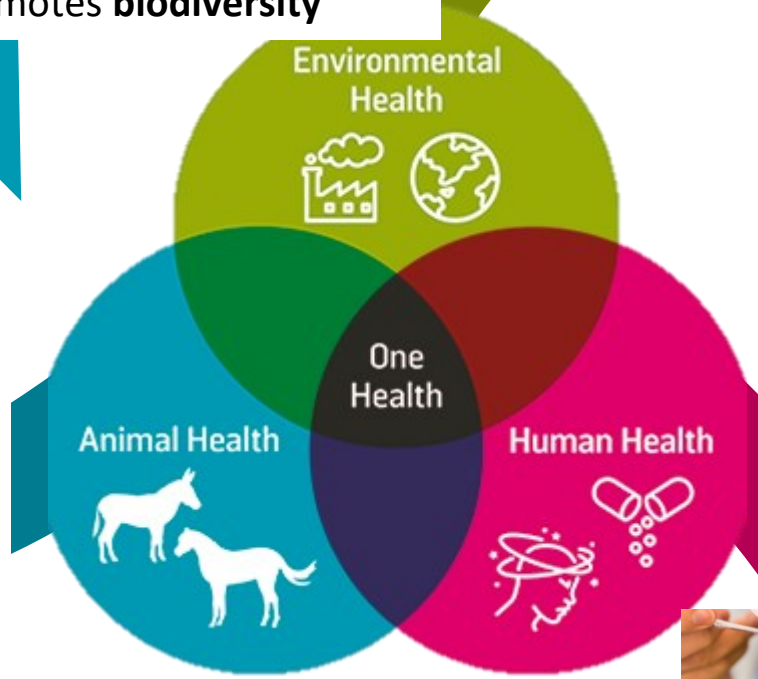


Representative samples will be subjected to whole metagenome shotgun sequencing to determine the possible occurrence of hazards, such as **antimicrobial resistance genes**, multiple **bacterial pathogens** and **viral contaminants**, in an untargeted manner.

Figure 1.1.a Overview of DeliSoil's approaches, key outputs, outcomes & impacts to meet the Mission's challenges.

Take home message

The use of **digestate** as an organic fertilizer **protects** the health of the environment and promotes **biodiversity**



Ensuring a One Health approach is essential for progress to **anticipate, prevent, detect and control diseases** that spread between animals and humans

Antibiotics and **resistance genes** present in manure have been found to **decrease** during anaerobic digestion