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What on earth? The impact of digestates and composts from farm effluent management on fluxes of foodborne pathogens in agricultural lands

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Highlights

- Do pathogenic microorganisms in digestates and composts from processed farm effluents threaten sustainable agronomic recycling?
- What is the current EU regulation concerning the safety of farm organic fertilisers?
- Which factors can predict the survival of pathogens after land application?

Keywords

farm effluent management; digestates; composts; microbial pathogens; fate in soil; EU regulation

Abstract

The recycling of biomass is the cornerstone of sustainable development in the bioeconomy. In this context, digestates and composts from processed agricultural residues and biomasses are returned to the soil. Whether or not the presence of pathogenic microorganisms in these processed biomasses is a threat to the sustainability of the current on-farm practices is still the subject of debate. In this review, we describe the microbial pathogens that may be present in digestates and composts. We then provide an overview of the current European regulation designed to mitigate health hazards linked to the use of organic fertilisers and soil improvers produced from farm biomasses and residues. Finally, we discuss the many factors that underlie the fate of microbial pathogens in the field. We argue that incorporating land characteristics in the management of safety issues connected with the spreading of organic fertilisers and soil improvers can improve the sustainability of biomass recycling.

Introduction

Human activities have been shaping the environment since the 1950s and have caused irreversible changes (Zalasiewicz, 2015). The earth has entered the Anthropocene, a new geological era in which the main driver of the evolution of the Earth is no longer geological forces but humans (Crutzen, 2002; Crutzen & Steffen, 2003; Steffen et al., 2007). From now on, mitigating the impact of the human society on the environment and better management of energy and material flows are indispensable (Williams & Crutzen, 2013). Recycling is a major landmark in the path to sustainable human activities. Recycling requires the requalification of by-products, previously referred to as wastes, as resources to be valued. For example, in the bioeconomy, recycling of biomass (food left-overs, garden residues, farm effluents, crop residues), makes it possible to close the loop of used and consumed nutrients e.g. nitrogen and phosphorus, and organic carbon, and can result in the production of high added-value compounds in biorefineries (De Corato et al., 2018). The added-value of recycling residual biomass includes the production of energy, (e.g. biogas), organic soil improvers (compost) and fertilisers (digestates) for agricultural soils (Kumar Khanal et al., 2021). Several processes from the most low-tech, (e.g. direct spreading or composting) to the most high-tech, (e.g. anaerobic digestion and environmental biorefining), can be used to improve the reuse of these biomasses. The bioeconomy is therefore a credible way to achieve sustainable development objectives (Bogdanski et al., 2021) however its generalisation may involve health hazards, especially through the food chain, due to the circulation of pathogens and other contaminants (WHO, 2018). Indeed, even though the agronomic benefits of composts and digestates are well documented, the dissemination of traces of heavy metals (Beggio et al., 2021), organic compounds and pathogens (Thakali & MacRae, 2021) are concerns that need to be properly assessed. In agroecology, these safety issues could compromise the sustainability of large-scale recycling of farm effluents through processing and spreading on the land (Dumont et al., 2013; Thakali & MacRae, 2021) unless health policies tackle the problem of the presence of pathogens in livestock effluents and their potential dissemination in the environment (Nag et al., 2021; Nag et al., 2022).

Because the production of pathogen-free residual biomasses is not feasible, the sustainability of their agronomic recycling relies on controlling the fate of pathogens following land application. For this reason, after a brief overview of the current farm effluent management strategies and European regulations, we review the many factors and land characteristics that contribute to the fate of pathogens, either decay or persistence, following spreading of the processed farm effluents on the land. Because in practice, the most widely used processes are composting and anaerobic digestion, we focus on the application of composts and digestates on the land. We discuss whether or not the survival of pathogenic bacteria could reduce the relevance and sustainability of recycling farm effluents. We highlight the complex trade-offs between utilisation pathways, application practices, and soil and climate characteristics that need to be taken into consideration to avoid health hazards all along the food chain. This article reviews the scientific literature, as well as European regulations and standards. First, we describe the hazardous foodborne pathogens potentially circulating through farm effluents, and then review current EU regulations for dealing with health issues related to manure and slurry recycling. Finally, we discuss the complex interplay between the many factors that shape the fate of pathogens after land application.

1. Literature search methodology

In January 2022, several searches of the literature were conducted to identify relevant research and review papers in the Web of Science Core Collection. The search terms used for processes applied to farm effluents, were “nitrification AND denitrification AND slurry OR manure”, “compost* AND on-farm OR manure OR soil OR quality OR challenge* OR potential*, “anaerobic digestion or digestate AND state of the art OR processing OR agronomic”.

The search concerning human pathogens in soil covered the period 1995-2022, and used the following keywords: human pathogen* OR *Listeria monocytogenes* OR *Salmonella* OR *Escherichia coli* OR *Clostridium* OR *Campylobacter* OR *Criptosporidium* OR *Giardia* AND Soil AND fate OR survival OR decay OR factor OR parameter OR property OR characteristic. A total of 5 926 references were retrieved. Specific sets of references were constructed by implementing specific screens. For example, the term “field study or field studies” selected 608 references. Information on land application of composts and digestates (69 references) was collected using the following search terms: human pathogen* OR *Listeria monocytogenes* OR *Salmonella* OR *Escherichia coli* OR *Clostridium* OR *Campylobacter* OR *Criptosporidium* OR *Giardia* AND Soil AND fate OR survival OR decay OR factor OR parameter OR property OR characteristic AND compost OR digestate OR anaerobic digestion.

Title screening resulted in the selection of 600 references of which 163 were finally retained after reading he corresponding abstracts. The grey literature was searched for relevant EU regulations.

2. Fate of farm effluents

Farm effluents, mainly manure and slurry, are by far the biggest source of organic fertilisers spread on land. About half of the effluents come from farmyards and farm buildings while the rest is directly deposited on pastures by grazing animals. Slurries are liquid mixtures whose dry matter content ranges from 1% to 12.5% and is composed of faeces, urine, water and used bedding (Houot et al., 2014). Manures are predominantly composed of bedding mixed with the animal faeces and urine.

Their composition very much depends on the characteristics of the bedding and their dry matter varies from 19% to 62% (Houot et al., 2014).

Their fertilisation potential varies according to the type of animals bred, their age, feed, and other farming practices. Land application of manures and slurries is an ancient agricultural practice. As shown in Figure 1, nowadays, manures and slurries can be processed to add value to their recycling (Foged et al., 2011).

Raw slurries can be separated into solid/liquid phases. Specific nitrification/denitrification processes enable the reduction of the nitrogen content of raw slurries and/or the liquid phases (Bernet & Beline, 2009; Riano & Garcia-Gonzalez, 2014; Marti et al., 2020). The process relies on the management of oxygen content of activated sludge. Aerobic phases enable oxidation of ammonium into nitrite and finally into nitrate. When oxygenation stops, anoxic conditions drive reduction of nitrite and nitrate into dinitrogen. This process is appropriate when the nitrogen content of farm effluents exceeds actual plant fertilisation requirements in the fields available for land application in the area (Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources). At the end of the process, activated sludge and decantation waters can be used as fertilisers. Thermal dehydration, nitrogen stripping, catalytic nitrogen disposal, phosphorus precipitation, flocculation, filtration are other processes are seldom used for the treatment of slurries (Foged et al., 2011).

When mixed with straw or other plant residues, manure, the solid phase of slurries and the slurries themselves can be composted. Composting is a biological two-stage process in which the organic matter is stabilised in the presence of oxygen (Haug, 1993; Epstein, 1997; Bernal et al., 2009). During the active stage, aerobic degradation of organic matter increases the microbial biomass, produces heat: temperatures can rise to 70 °C. When readily biodegradable substrates are exhausted, the temperature decreases. Under mesophilic conditions, the maturation stage is characterised by the conversion of aromatic and aliphatic compounds into humic acids and by the biosynthesis of macromolecules. Composts are soil improvers and their application on land is beneficial for soil quality and plant health (De Corato, 2020). They improve the structure and hydraulic properties of the soil (Rivier et al., 2022), supply nutrients to the soil (Duong et al., 2013), increase soil fertility and crop yield (Ayilara et al., 2020), and enable biocontrol of diseases (Ayilara et al., 2020).

Anaerobic digestion (AD) is another option for the management of manures and slurries. This biological process enables the production of biogas (methane and carbon dioxide) from organic matter in the absence of oxygen (Weiland, 2010). The two main processes, which require either mesophilic (37 °C to 42 °C) or thermophilic conditions (50 °C to 55 °C), are liquid AD and solid-state AD (Nasir et al., 2012; Andre et al., 2018). The process parameters influence the water content and the stability of the organic matter in the resulting digestate. Raw digestates can be further processed by composting, separation of the solid/liquid phase, drying of the solid phase, and nutrients in the liquid phase can be concentrated using membrane separation or evaporation (Fuchs & Drosg, 2013; Tambone et al., 2017; Tambone et al., 2019). Raw digestates as well as their solid and liquid fractions are of agronomic value as organic fertiliser (Tambone et al., 2010; Walsh et al., 2012).

Because composting and AD are currently the two main on-farm processes used for the management of manures and slurries, the following sections focus on the microbial hazards connected with composts and digestates used as soil improvers and organic fertilisers.

3. Hazardous foodborne pathogens potentially circulating through composts and digestates

Recent reports (Bohnel & Lube, 2000; Burtscher & Wuertz, 2003; Dharmasena & Jiang, 2018; Chiapetta et al., 2019) and comprehensive reviews (Bloem et al., 2017) suggest that pathogen loads generally decrease during anaerobic digestion and composting but that pathogens are still detected in digestates and composts. C*ryptosporidium parvum*, *Salmonella spp.* (including *S.* Typhi and *S.* paratyphi), *norovirus*, *Streptococcus pyogenes*, enteropathogenic *E. coli* (EPEC), *Mycobacterium spp.*, *Clostridium spp.*, *Listeria monocytogenes* and *Campylobacter coli* are of concern they may be able to survive anaerobic digestion (Nag et al., 2019; Planchon et al., 2020). Soil can represent a reservoir of human pathogens (Table 1), and whether or not large-scale organic fertilisation can modify the fluxes of these microorganisms in the biosphere and their persistence in soil is still the subject of debate. In the following sections, the focus on major foodborne pathogens (*Salmonella enterica*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Clostridium* spp.) and process indicators (*Escherichia coli*, *Enterococcus* spp.) is motivated by the safety issues they raise in the food chain, and by the comprehensive body of literature addressing their persistence in soil after application of organic fertilisers and soil improvers.

It is already clear that the fate of a bacterium upon its arrival in soil is species-specific and depends to a great extent on intrinsic characteristics (Hutchison et al., 2004; Girardin et al., 2005; Johansson et al., 2005; Reed-Jones et al., 2016; Roberts et al., 2016; Underthun et al., 2018). For example, phylogroup-dependent variation in *E. coli* prevalence confirmed differences in intraspecific fitness in soils with different ecological profiles (Dusek et al., 2018). Survival of three non-pathogenic *E. coli* and three attenuated *E. coli* O157:H7 isolates spread on manured experimental field plots confirmed the importance of the genotype (Sharma et al., 2019). Laboratory experiments led to similar conclusions concerning generic *E. coli* (Topp et al., 2003), non-O157 verotoxigenic *E. coli* (Bolton et al., 2011), *E. coli* O157:H7 (Hutchison et al., 2004; Ibekwe et al., 2014; Ma et al., 2014; Liu et al., 2015; Reed-Jones et al., 2016; Roberts et al., 2016), *L. monocytogenes* and *Yersinia pseudotuberculosis* (Sidorenko et al., 2006; Falardeau et al., 2018).

The following section recaps the microbial standards implemented in Europe to regulate these safety issues. We then discuss the factors that affect the persistence of pathogens in soil after land application.

4. EU regulation and microbial standards

This section addresses current safety rules, environmental regulations and specific regulations designed to mitigate health hazards involved in the management and recycling of farm manures and slurries in the European Union.

4.1 Environmental regulation

Composting and AD plants must comply with the EU environmental regulation Industrial Emission Directive 2010/75/EU (IED), the main EU instrument that regulates pollutant emissions from industrial installations, including biological waste treatment, cropping and livestock breeding. The IED aims to ensure a high level of protection of human health and the environment taken as a whole by reducing harmful industrial emissions across the EU. This directive relies on better application of Best Available Techniques (BAT) and on the integrated approach that accounts for the whole environmental performance of the plant including emissions into the air, water and soil, waste generation, use of raw materials, energy efficiency, noise, accident prevention, and restoration of the site upon closure. Directive 2012/18/EU of the European Parliament and of the Council of 4 July 2012 sets rules to control major accident hazards involving dangerous substances. EU member states are responsible for implementing these directives and may apply specific criteria in the industrial and farming sectors.

4.2 Safety rules

European Union REGULATION (EC) No 1069/2009 of 21 October 2009 lays down health rules for animal by-products and derived products not intended for human consumption and repeals Regulation (EC) No 1774/2002 (Animal by-products Regulation). Animal by-products are categorised in three specific groups according to the level of risks for animal and public health. This regulation defines rules for the management and disposal of animal by-products according to the category to which they belong. Farm effluents belong to category 2. Animal by-products in this category can be used as substrates for biogas production (anaerobic digestion) and/or composting. Direct spreading of raw farm effluents on the land is authorised. For these raw effluents, the regulation does not define any microbial safety criteria. However, when a risk of transmission of severe infectious disease is likely, competent authorities can specify new rules and/or prohibit further land spreading of the contaminated raw products.

A safety agreement is compulsory for AD and for composting plants that treat farm effluents. As specified in Annex V of the regulation (EU) No 142/2011 of 25 February 2011 (implementing Regulation (EC) No 1069/2009), these composts and digestates must comply to two types of microbial criteria: i) *E. coli* or *Enterococcacae* (n = 5, c = 1, m = 1 000, M = 5 000 in 1 g), ii) *Salmonella* sp. (absence in 25 g: n = 5; c = 0; m = 0; M = 0), with n = number of samples to be tested, m = threshold value for the number of bacteria (the result is considered satisfactory if the number of bacteria in all the samples does not exceed m), M = maximum value for the number of bacteria (the result is considered unsatisfactory if the number of bacteria in one or more samples is M or more), and c = number of samples the bacterial count which is tolerated between m and M (the sample still being considered acceptable if the bacterial count of the other samples is m or less). *E. coli* or *Enterococcacae* must be quantified during or directly after processing, in order to monitor the AD or composting process, whereas *Salmonella* sp. must be analysed on composite samples collected during storage of digestates or composts.

4.3 Rules for the marketing of fertilising products in the EU

Anaerobic digestion residues and composts may be placed on the market and used as organic fertilisers or soil improvers. Organic fertilisers are products originating from biomass and complying with EU Regulation 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. Soil improvers are materials added to soil whose main function is to maintain or improve its physical and/or chemical and/or biological properties. Under these circumstances, they must comply with EU regulation 2019/1009 that lays down rules on the making available on the market of EU fertilising products, organic fertilisers and organic soil improvers. Seven product function categories (PFC) and 11 Component Material Categories (CMC) are listed in this document. Microbial criteria similar to the criteria regulating the animal by-products (see above) are implemented to control safety issues. EU member states may introduce their own national legislation to regulate their national market for organic fertilisers or soil improvers.

However the legal EU requirements (*E. coli* or *Enterococcacae* and *salmonella enterica*) may not fully capture all the fluxes of pathogens that circulate in organic fertilisers and soil improvers. Under some circumstances, this could lead to health and sustainability concerns (Nag et al., 2020). From a safety point of view, the main problem with on-farm management of biomass is understanding the fate of the pathogens following application on agricultural land. While rapid decay causes limited concern, long-term survival and/or transfer of the pathogens could have consequences in terms of fluxes of pathogens in the environment. It is therefore critical to clarify the factors and land characteristics that influence the fate of these microbial pathogens.

5. Factors affecting the fate of pathogens after land spreading

This section focusses on the factors that need to be taken into account to predict the fate of the many allochthon pathogens able to persist in soils (Table 1). A fine-tuned understanding of the ecology of microbial pathogens could pave the way for the holistic management of safety issues in the framework of the 'One Health' paradigm.

5.1 Persistence of pathogens in soil after land application of digestates and composts is dose dependent and species specific

Many studies that simulated the application of digestate to the land under laboratory conditions concomitantly investigated the persistence of several pathogens in the soil. One investigation of soil+cattle manure digestate reported that *Listeria* spp. was detected in the digestate at 10^4 CFU/g dry weight whereas *E. coli* and *Salmonella enterica* were not detected (Goberna et al., 2011). During the first 30 days of incubation at 20 °C, the population of *Listeria* spp. was significantly higher in soil+digestate columns than in controls but subsequently, the differences were not significant and *L. monocytogenes* was never detected (Goberna et al., 2011). Gomez-Brandon et al. (2016) reported that although coliforms and *E. coli* were numerated from 2 10^2 CFU/g to 10^3 CFU/g in cattle manure digestates and composts, after mixing with soil, their populations dropped during incubation at 22 °C and their presence was no longer detected after 60 days (Gomez-Brandon et al., 2016). Conversely, *C. perfringens* initially present at 2 10^3 CFU/g was still detected after 60 days of incubation (Gomez-Brandon et al., 2016). When heavily spiked digestate made of household food residues was added to soil, *E. coli*, *S.* Thyphimurium, *C. tyrobutyricum*, *T. emersonii* and porcine parvovirus were reported to survive for at least 49 days, i.e., the duration of the experiment in a climate chamber, but *L. monocytogenes* was not detected at the end of the experiment (Johansson et al., 2005).

These laboratory experiments confirmed that, when present in composts and digestates, some pathogens can be detected in the soil after land application but the results depended on the bacterial species as well as on the original concentration of the pathogen. Unfortunately, survival data collected from amended microcosms under laboratory conditions are difficult to transpose to real field conditions (Cekic et al., 2017), even though such data are critical for the proper assessment of safety issues.

For this reason, field experiments are more informative and important conclusions were extracted from the available literature. First, field experiments confirmed a reduction in the populations of enteric microorganisms during the processing of farm effluents. Indeed, lower concentrations of coliforms, *E. coli* and enterococci were found in plots fertilised with digestates compared to plots fertilised with untreated dairy cattle slurry and manure (Saunders et al., 2012; Nolan et al., 2020) and in runoff (Nolan et al., 2020). However, target organisms were still detected 30 days after amendment, suggesting that more than one month is necessary for the organisms to become undetectable (Saunders et al., 2012; Nolan et al., 2020).

A two-year field study confirmed that the transfer of allochthon pathogens was correlated with the dose of organic fertiliser applied on the land (Gondim-Porto et al., 2016). In their study the number of coliforms, used as indicators of contamination, counted in plots that received low doses of digested sludge was similar to the number counted in control plots, while in plots that received higher doses, they were more abundant than in the control plots and were still detectable 24 months after land spreading (Gondim-Porto et al., 2016). Interestingly, the numbers of enterococci and *Clostridium* spores were significantly higher in the fertilised plots. This study confirmed that the fate of pathogens is dose and species dependent.

Other studies focussed on the transfer of pathogens from compost to soil. Increased concentrations of total thermotolerant coliforms was observed in experimental plots fertilised with non-spiked dairy manure composts but, 120 days after application, the differences between treated and control plots were no longer significant (Wind et al., 2018). Four years of monitoring experimental plots fertilised with various composts suggested limited transfers of pathogens if the organic fertilisers complied with the current French regulation NFU 44-051 (Brochier et al., 2012). This French standard defines microbial criteria for *Salmonella enterica* (absence in 1 g, or in 25 g for home vegetable gardens), helminth eggs (absence in 1.5 g) and process indicators (*E. coli*: 100/g; enterococci 1000/g). *Clostridium botulinum* in soil was shown to persist for several years following land spreading of spiked composts but, again, the results depended on the original concentration of the pathogen (10^3 CFU/g and 10^5 CFU/g) and on the dose of spiked compost added to the plots (Gessler & Bohnel, 2006). Similarly, C. *perfringens* was detected for 10 years after land spreading of compost made with swine manure (Scott et al., 2018). *Clostridium sporogenes* was detected for one year after land spreading of spiked bovine manure and sewage sludge composts whereas *Listeria innocua* was only detected in the first three months of the experiment (Girardin et al., 2005). However, in this experiment, the composts were also heavily spiked (10^5 CFU/g). Similarly, heavy spiking of poultry and bovine manure composts with an avirulent variant of *E. coli* O157:H7 (10^7 CFU/g) enabled the pathogen to survive for more than five months in the fertilised plots but the pathogen was not detected in control plots that were not spiked (Islam et al., 2004; Islam et al., 2005).

Overall, these laboratory and field experiments suggest that (i) populations of microbial pathogens are able to survive in the soil, (ii) the results are species-specific, and (iii) the results are dose-dependent. Finally, because the literature suggests that persistence is site specific, it is important to identify exactly which environmental factors affect the survival of pathogenic microorganisms after application, in order to determine the conditions that have the least impact on the soil and more globally on the environment. In the following section, we review the studies that addressed this issue.

5.2 Multiple extrinsic factors affect the persistence of pathogens in soil

Soil is a highly complex matrix comprising a mineral fraction, organic matter, and a liquid and gas phase. Soil is the habitat of many living organisms including bacteria, Archaea, fungi, viruses, protozoa, nematodes, microarthropods, earthworms, insects and insect larvae (Briones, 2018; Bunemann et al., 2018; Rabot et al., 2018). Agricultural soils are open systems that interact with water, air, vegetation, and animals and are also under strong anthropogenic pressure. In this section we review the many factors that have a critical impact on the fate of pathogens upon their arrival in the soil (Figure 2).

5.2.1. Soil characteristics

Abiotic properties

The abiotic properties of the soil influence the survival of pathogenic bacteria. Many laboratory studies (Table 2) have shown that low pH is detrimental to the survival of *Listeria monocytogenes* (Dowe et al., 1997; Locatelli et al., 2013). Similar results have been reported for enteric pathogens such as *Escherichia coli* and *Salmonella enterica* (Bolton et al., 2011; Erickson et al., 2014; Ma et al., 2014; Wang et al., 2014[b]).

Soil texture is another important factor. High clay content promotes survival whereas sand does not (Ma et al., 2011; Locatelli et al., 2013; Wang et al., 2018; Jechalke et al., 2019). Field experiments confirmed that pathogen types and abundance were higher in clay soil than in loam and loamy-sand types of soil (Obayomi et al., 2019). In addition, the mineral composition of the clay itself can affect pathogen survival (Brennan et al., 2014; Cai et al., 2018).

High concentrations of organic matter, total carbon and total nitrogen promote pathogen survival in soil (Franz et al., 2008; Yao et al., 2013).

Survival of *E. coli* O157:H7 has been shown to be negatively correlated with electric conductivity (Ma et al., 2012; Erickson et al., 2014; Ma et al., 2014; Yao et al., 2015), free Fe_2O_3 and Al_2O_3 (Wang et al., 2014[a]; Yao et al., 2015). Another factor identified as being beneficial for survival of *L. monocytogenes* and *Yersinia pseudotuberculosis* is a high concentration of exchangeable cations measured by cation exchange capacity (Sidorenko et al., 2006; Locatelli et al., 2013).

Biotic characteristics

The soil microbiome is a key environmental factor and has a major impact on allochthonous microorganisms. Soil microorganisms develop complex networks of interactions that may lead to exclusion of allochthon microorganisms through exploitation, competition, and interference (Tan et al., 2015; Stubbendieck & Straight, 2016). Indeed, while populations of allochthon pathogenic bacteria decrease over time in the soil, in sterilised soil, they generally increase (Dowe et al., 1997; Jiang et al., 2002; Ishii et al., 2010; McLaughlin et al., 2011; Locatelli et al., 2013; Moynihan et al., 2013). Of course, removing all soil microorganisms is neither very informative nor realistic. A gradual decrease in soil biodiversity reflects real conditions more accurately. Experimental alteration of the balance of soil microbial communities was found to be correlated with enhanced survival of *E. coli* (van Elsas et al., 2007; van Elsas et al., 2012; Xing et al., 2019) and *L. monocytogenes* (Vivant et al., 2013[a]). Beyond diversity, phylogenetic composition and community structure play a determining role in the exclusion of pathogens, probably because specific communities develop inhibition through a combination of exploitation, competition, and antibiosis (Vivant et al., 2013[a]; Spor et al., 2020; Ma et al., 2013).

Comparison of the survival of *S. enterica*, *E. coli* and *L. monocytogenes* in soil microcosms with contrasting edaphic characteristics confirmed that the main factor explaining the decay rate of these pathogens was the composition of the soil microbial community (Ma et al., 2013; Moynihan et al., 2015). Conversely, introducing the pathogen *E. coli* O157:H7 (Yao et al., 2014) or *L. monocytogenes* (Spor et al., 2020) altered soil microbial diversity. All these studies underline the complexity of the interactions between microbial communities, allochthon pathogens, and other soil characteristics (Ibekwe et al., 2014; Moynihan et al., 2015; Weller et al., 2015; Falardeau et al., 2018).

Interestingly, disturbance of the physical habitat during invasion of the soil by a pathogen can lead to changes in autochthon microbial communities and finally affect pathogen survival (Spor et al., 2020). Similarly, the effect of global warming on soil microbiome could also affect the control of pathogens in soil ensured by ecosystem services (French et al., 2009).

5.2.2. Site-specific features affect persistence

The literature affirms that many site-specific features shape the fate of pathogens after the application of organic fertilisers and soil improvers to the land. A recent paper reported the results of a large-scale study conducted from 2011 to 2015 at 12 experimental sites in three geographical regions in the United States (Sharma et al., 2019). The study investigated the survival of several genotypes of *E. coli* after application of four artificially contaminated organic amendments. The statistical analysis of 324 survival profiles confirmed the complexity of the factors that influence pathogen survival in the soil. In order to predict survival time, farming practices (type, amendment, spreading methods, initial dose of pathogens, conventionally managed versus organic agriculture), weather and geography have to be considered at the same time. These factors have been shown to be more important than the amendment and the spreading method, and some combinations can enable pathogens to survive for more than three months.

A meta-analysis of the results of 70 published studies on the survival of *E. coli* in soil after application of contaminated organic additives, confirmed the complexity of the factors that underlie the fate of pathogens introduced in the soil. The variability of experimental results can be partly explained by the genetic diversity in the same bacterial species and partly by the diversity of environmental and soil and climate characteristics. All these intrinsic (pathogen characteristics) and extrinsic (environmental characteristics) factors influence the fate of pathogens (Park et al., 2016).

Other field studies focussed on possible correlations between multiple factors and the incidence of pathogens in soil (Park et al., 2013; Strawn et al., 2013[a], 2013[b]; Park et al., 2014, 2015; Weller et al., 2015, 2016, 2020; Dusek et al., 2018). These studies suggest that certain locations are environmental reservoirs with a high incidence of pathogens. It may be possible to predict these at-risk locations using a complex combination of landscape and meteorological factors as discussed below. All these studies underline the multifactorial dimension of the prevalence and fate of pathogens in soil. The following sections address these factors individually.

Landscape features, topography

The prevalence of pathogens depends on landscape features. A large-scale study targeting 1,428 soil samples showed that land use patterns alter the probability of detecting *E. coli* (Dusek et al., 2018). *E. coli* was most often isolated in pastures, followed by in forests (Park et al., 2013, 2014, 2015; Dusek et al., 2018). Conversely, the incidence of *E. coli* in cultivated land was lower. Interestingly, proximity to a stream and/or forest also increased the likelihood of detection (Dusek et al., 2018).

Similarly, increased likelihood of detection of *Listeria monocytogenes* has been reported in vegetable fields located close to pastures, forests, grasslands, scrubland, water bodies and wetlands (Strawn et al., 2013[a]; Chapin et al., 2014; Weller et al., 2016; Harrand et al., 2020)). River flooding was a factor that increased the prevalence of *L. monocytogenes* in soil samples (Linke et al., 2014).

Slope was identified as another relevant feature for increased likelihood of detection of *L. monocytogenes* in vegetable fields (Chapin et al., 2014).

Climate / seasonality

Under given soil conditions, the persistence of pathogens depends on the season and the weather. Interestingly, Weller et al. (2015) found increased levels of *L. monocytogenes* after a rainfall event and following irrigation in fields planted with spinach (Weller et al., 2015). Similar correlations between rainfall and the occurrence of *L. monocytogenes* were identified in several wooded areas and on vegetable farms (Ivanek et al., 2009; Strawn et al., 2013[b]; Pang et al., 2017; Harrand et al., 2020), and between rainfall and detection of *S. enterica* (Strawn et al., 2013 [b]) and generic *E. coli* (Park et al., 2014). This is consistent with the fact that soil moisture, available stored soil water and the soil drainage class all affect detection of the two pathogens (Strawn et al., 2013[b]; Weller et al., 2016). Microcosm experiments with soils adjusted to 20% and 40% water content, and contaminated with *S. enterica*, led to opposite conclusions in one out of the three soil types tested, probably due to interactions between soil texture, moisture and microbiota activities (Erickson et al., 2014). However, no correlation was found between climatic factors and the presence of Shiga toxin-producing *Escherichia coli* (Strawn et al., 2013[b]). In laboratory soil microcosm experiments, a positive correlation between soil moisture and the survival of *E. coli* and *E. coli* O157:H7 was reported (Ohtomo et al., 2004; Habteselassie et al., 2008) while drought periods reduced the number of *E. coli* detected (Ishii et al., 2010). However, a meta-analysis of reports on the survival of *E. coli* and *E. coli* O157:H7 in land spread with manure suggested longer survival when manure was applied to dry soil (Park et al., 2016). A longitudinal field study partly confirmed these results (Sharma et al., 2019).

Temperature is another key environmental factor influencing pathogen survival, which is facilitated at low temperatures as long as the temperatures are above freezing (Ivanek et al., 2009; Ishii et al., 2010; Saunders et al., 2012; Farhangi et al., 2013; Strawn et al., 2013[b]; Underthun et al., 2018). Better survival of *Listeria spp.* and *L. monocytogenes* has been documented in winter/early spring compared to in other periods of the year (Girardin et al., 2005; Strawn et al., 2013[b]; Chapin et al., 2014). However, in a yearlong experiment of cover crops in artificially contaminated experimental plots, temperature was not among the factors that significantly affected the survival of *L. innocua* (Reed-Jones et al., 2016).

Seasonality was also observed to have an impact on the persistence of *E. coli* and *S. enterica* in field experiments and led to conflicting results depending on the year the experiment was performed and on other environmental parameters (Ishii et al., 2010; Reed-Jones et al., 2016; Sarr et al., 2020). Other studies of the presence of *E. coli* O157:H7 in soil amended with various manures and other organic fertilisers, demonstrated better survival in autumn than in spring but temperature and rainfall appeared to have a limited effect on the survival of this pathogen (Oliveira et al., 2012; Sharma et al., 2019). Conversely, a meta-analysis of survival data in the literature clearly identified water content and temperature as factors that play an important role in the survival of *E. coli* O157:H7 in soil (Park et al., 2016).

5.2.3. Farming practices must be taken into account

Farming practices have a direct and/or indirect impact on the survival of allochthon pathogens. For example, distribution of *E. coli* in soil aggregates differs according to land use and farming history, which could modify decay rates (Kravchenko et al., 2013).

Organic farming

The high biodiversity found in organic farming can improve the biotic control of pests (Crowder et al., 2010) and pathogens (Jones-Dias et al., 2016; Jones et al., 2019[a], 2019[b]). However, results in the literature vary with the type of soil. In experiments with cattle manure incorporated in the soil, the prevalence of *E. coli* O157:H7 was higher in conventional than in organic soils in three out of five combinations, whereas *S. enterica* survival was similar in soils under the two management systems (Franz et al., 2005). However, a follow-up study with more soil samples failed to provide evidence for significant differences in the survival rate of *E. coli* 157:H7 between organic and conventionally managed soils (Franz et al., 2008). In a multi-year survey to compare the effect of the type of organic amendments on the survival of pathogenic *E. coli*, survival time was longer in conventional than organic farms, but the results varied with the year, the type of amendment and other temporal factors (Sharma et al., 2019). Topological analysis of *E. coli* O157:H7 survival in a selection of 32 US organic and conventionally managed soils originating from two contrasting states (California and Arizona) showed shorter survival times in organic soils than in conventional soils but this result was strain- and location-dependent (Ibekwe et al., 2014). In fact, one strain of *E. coli* O157:H7 survived better in organic soils collected from one site, while survival in soils collected from another site was similar, regardless of the soil management practices (Ma et al., 2012, 2013). This dataset suggests that soil management can affect the survival of *E. coli* O157:H7 but differences between organic and conventional soils are highly location- and strain-dependent. Overall, these results tend to show that the persistence of pathogenic bacteria is lower in organic farms than conventional farms, but other environmental and soil factors modulate this difference. For this reason, large-scale surveys are required to produce more experimental data to better predict the fate of pathogens and to compare health risks in organic and conventionally managed farms.

Mode of application of organic fertilisers

Solid digestates with more than 18% total solids are typically applied to the soil surface and need to be incorporated into the soil to prevent the emission of odours, whereas digestates with less total solids can be either spread on the soil surface or injected to the subsurface (Crolla et al., 2013). The fate of pathogens varies depending on the method of application of digestate. Subsurface incorporation has been shown to delay pathogen decay compared to surface application (Hutchison et al., 2004; Alegbeleye & Sant'Ana, 2020), but other authors found no significant difference between subsurface and surface application (Saunders et al., 2012). A laboratory study investigated the impact of application (soil incorporation versus surface application) on the survival of various pathogens. The study was carried out using two soils (sandy loam and loamy clay) amended with either pig slurry stored in a lagoon or cattle manure and sludge taken from a wastewater treatment plant, and artificially contaminated with a cocktail of pathogens (*S. enterica*, *L. monocytogenes*, *C. jejuni*, *C. perfringens, coliphages*). A rapid decline of *C. jejuni* was observed within seven days whereas *S. enterica* and *L. monocytogenes* survived longer (Roberts et al., 2016). Interactions between organism x management practice x soil were significant suggesting that pathogen decay is affected by management, but the risk of persistence also depends on other farm characteristics, land use patterns and on the microorganism considered (Roberts et al. 2016). The study by Sharma et al. (2019) confirmed that the survival of *E. coli* following field fertilisation depended on the mode of application but their results also depended on other temporal factors including soil management, the type of amendment used, soil characteristics and climate.

Soil solarisation

Soil solarisation may be an effective way to reduce the populations of pathogens initially present in organic amendments (Barbour et al., 2002; Wu et al., 2009). Solarisation after spreading of digestate on the land has been shown to significantly modify the structure and abundance of soil microbial communities (Fernandez-Bayo et al., 2017).

Conclusion

The presence of human pathogens in organic fertilisers and soil improvers can cause safety problems all along the food chain. The EU regulation sets out rules to mitigate the risk of transfer of pathogens from organic fertilisers and soil to the food system. When processed, materials to be applied on land must comply with microbial rules. Nevertheless, the presence of pathogenic bacteria in composts and digestates and their persistence in the soil after spreading is documented. Because soils are so complex, it is difficult to predict the behaviour of pathogenic microorganisms after application, both abiotic and biotic soil characteristics are extrinsic factors that determine the fate of human pathogens in soil.

The persistence of pathogens depends on the chemical composition, texture and physical structure of the soil. The diversity and community structure of the soil microbiota are critical factors that tend to limit the persistence of allochthon species. These effects can limit the invasion process and lead to the disappearance of newly arrived microorganisms.

Climate, season, local weather and landscape features further influence the persistence of pathogens in the field and their transfer to the environment. This information should be integrated into farm management practices, especially the application of organic fertilisers and soil improvers. Adapting practices to the specificities of each plot could mitigate the occurrence and persistence of pathogens. Practices that maximise biodiversity tend to maximise the control of pathogens. Two complementary ways to manage these health issues are processing the biomass to reduce pathogen levels and managing surface application to minimise pathogen persistence, particularly as a function of climatic conditions. Given the deep interconnection of all the factors and phenomena that drive the ecology of pathogenic microorganisms, effective management of health risks requires a multidisciplinary and interdisciplinary approach under the ‘One Health’ paradigm. Although indicators of sustainability have been developed (Rocchi et al., 2021; Espinoza, 2021), they currently do not include health hazards due to pathogenic bacteria. To include safety issues in the assessment of sustainability, the current strategy to evaluate impacts on the quality of the environment and on human health due to emissions of organic compounds and heavy metals during land spreading of composts and digestates needs to be adapted to include biological hazards. This will require the inclusion of all the factors detailed in the present review to ensure the accurate assessment of the fate of pathogens in agricultural soils. Although challenging, proper spatialization of the fate of pathogens is indispensable to capture the weight of local features.

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Legend of Figures and tables

Figure 1. Schematic of the main pathways leading to the spreading of raw and processed farm effluents on agricultural land.

Figure 2. Schematic of the complex interactions between factors that affect the fate of pathogens upon their arrival in the soil.

Table 1. References reporting the detection of human pathogens in soil.

Table 2. Site characteristics, climatic conditions for laboratory studies of the survival of human pathogens in soils.

Table 1. References reporting the detection of human pathogens in soil.

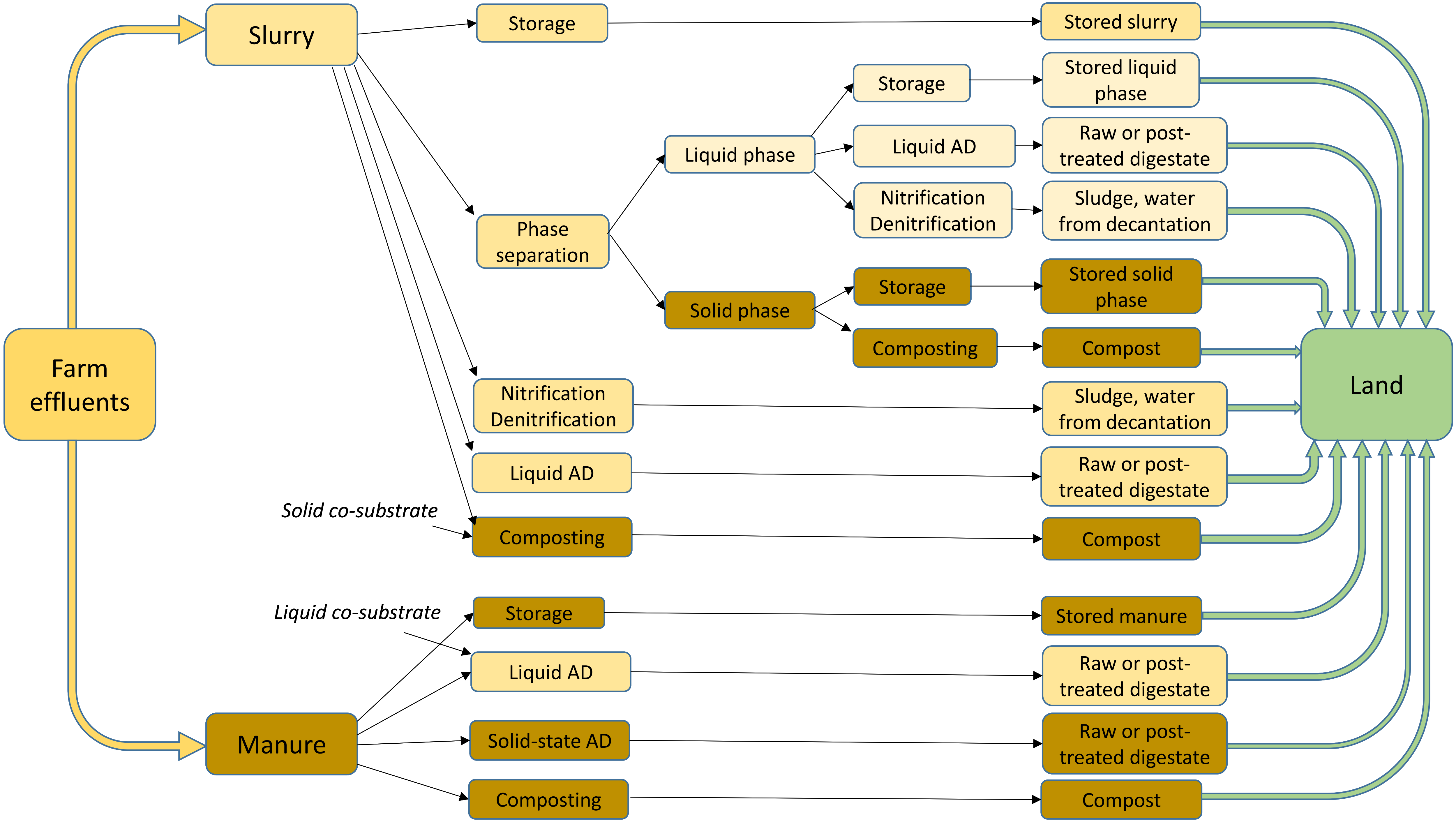
Name	Infection	Reference
	Bacteria	
Listeria monocytogenes	Listeriosis	Dowe et al., 1997; Nightingale et al., 2004; Ivanek et al., 2006; Fox et al., 2009; Sauders et al., 2012; Locatelli et al., 2013; Vivant et al., 2013[b]; Linke et al., 2014
Salmonella enterica	Gastroenteritis	Ceuppens et al., 2015
Escherichia coli pathotypes	-Gastroenteritis - Hemolytic Uremic Syndrome	Ceuppens et al., 2015; Somorin et al., 2016; Somorin et al., 2018
Campylobacter jejuni	Gastroenteritis	Bronowski et al., 2014; Brown et al., 2004; Ceuppens et al., 2015
Bacillus cereus	Toxin production and gastroenteritis	Arnesen et al., 2008; Ceuppens et al., 2013
Clostridium perfringens	Toxin production and gastroenteritis	Kim et al., 2004; Voidarou et al., 2011
Clostridium botulinum	Neurotoxin production	Baumgardner, 2012
Clostridium difficile	Diarrhoea	Janezic et al., 2016; Knight & Riley, 2019; Rodriguez et al., 2019
Proteus sp.	Nosocomial infections	Drzewiecka, 2016
Acinetobacter spp.	Nosocomial infections	Adewoyin & Okoh, 2018; Al Atrouni et al., 2016
Burkholderia cepacia	Pneumonia and septicaemia	Denet et al., 2017
Pseudomonas aeruginosa	Nosocomial infections	Denet et al., 2017; Colinon et al., 2013, Deredjian et al., 2014
Stenotrophomonas maltophilia	Nosocomial infections	Denet et al., 2017; Deredjian et al., 2016
Clostridium tetani	Tetanus	Kim et al., 2004
Mycobacterium avium complex	- Lung Infections - Intestinal infections	Walsh et al., 2019
Mycobacterium tuberculosis	Tuberculosis	Walsh et al., 2019
Bacillus anthracis	Anthrax	Jensen et al., 2003
Legionella pneumophila	Lung infections	van Heijnsbergen et al., 2014; van

		Heijnsbergen et al., 2016
Mycetes		
Sporotrix schenckii	Invasive Mycosis	Ramirez-Soto et al., 2018
Rhizopus and Mucor	Invasive Mycosis	Mousavi et al., 2018
Aspergillus,	Mycotoxin production	Nguyen et al., 2017
Fusarium	Mycotoxin production	Nguyen et al., 2017; Vogelgsang et al., 2019
Penicillium	Mycotoxin production	Nguyen et al., 2017; Dombrink-Kurtzman & McGovern, 2007; Elmholt, 2003
Eucaryotes		
Cyclospora cayetanensis	Nausea, diarrhoea, fatigue	Chacin-Bonilla, 2008; Giangaspero et al., 2015
Giardia duodenalis	Nausea, diarrhoea, stomach ache	Olson et al., 1999; Barwick et al., 2003; Wilkes et al., 2009; Orlofsky et al., 2013; Balderrama-Carmona et al., 2014
Cryptosporidium spp.	Diarrhoea, stomach ache vomiting	Koken et al., 2013; McLaughlin et al., 2013; Orlofsky et al., 2013; Balderrama-Carmona et al., 2014; Hong et al., 2014; Barwick et al., 2000
Helminthes	Intestinal disease	Amoah et al., 2017
Virus		
Virus	Gastroenteritis, hepatitis, poliomyelitis	Rzezutka & Cook, 2004

Table 2. Site characteristics, climatic condition for lab survival studies of human pathogens in soils.

Bacterial species	Soil Type	Incubation	Period of detection	Reference
Pathogenic bacteria found in food				
Listeria monocytogenes	* Sandy brown grassland soil with clay addition	* 15 °C, humidity set at 65% of field capacity	* 96 days	Brennan et al., 2014
	* Sandy soil, silty-sandy soil, silty-clayish soil	* 25 to 30 °C	* more than 32 days	Dowe et al., 1997
	* Soil land spread with bovine manure	* 5 °C, 15 °C to 21 °C	* 43, 21 and 21 days respectively	Jiang et al., 2004
	* Brown forest soil	* 20 to 22 °C	* 2 days	Sidorenko et al., 2006
	* 100 soils representative of soils found in France	* 20 °C, humidity set at 80% of field capacity	* from 0 to 84 days	Locatelli et al., 2013
	* Forest soil	* 25 °C to 30 °C	* less than 7 days	McLaughlin et al., 2011
	* 12 soils sampled in Ireland	* 10 °C	* 110 days	Moynihan et al., 2015
	* Silty-sandy soil, silty-clayish soil spread with biosolids	* 14 days of cycles of 10 h at 30 °C (day) and 14 h at 20 °C (night)	* from 7 to 90 days	Roberts et al., 2016

Salmonella enterica	* Sandy brown grassland soil with clay addition	* 15 °C, humidity set at 65% of field capacity	* 40 to 96 days	Brennan et al., 2014
	* 12 soils sampled in Ireland	* 10 °C	* 110 days	Moynihan et al., 2015
	* Silty-sandy soil, silty-clayish soil spread with biosolids	* 25 °C humidity set at 40% and 20% of field capacity	* from 14 to 210 days	Roberts et al., 2016
	* 3 soils in the USA	* 15 °C	* from 15 to 18 weeks	Erickson et al., 2014
	* 4 soils with manure applied	* 20 °C, 16 h daylight	* from 30 to 58 days	Franz et al., 2005
	* Sandy and silty soils spread with pig and poultry manure and planted with lettuce	* climatic chamber (13 h daylight at 22 °C to 24 °C) and 11 h dark, at 15 °C to 18 °C)	* more than 40 days	Jechalke et al., 2019
	* Silty soil spread with poultry fertiliser	* 3 water contents; incubation at 20 °C and 30 °C	* less than 49 days (unfertilised) More than 91 days (fertilised)	Shah et al., 2019
	* Sandy and silty soils	* 20 °C and 30 °C	* 168 days	Underthun et al., 2018
Escherichia coli pathotypes	* Sandy brown grassland soil with clay addition	* 15 °C, humidity set at 65% of field capacity	* 40 to 96 days	Brennan et al., 2014
	* 12 soils sampled in Ireland	* 10 °C	* 110 days	Moynihan et al., 2015
	* Silty-sandy soil, silty-clayish soil spread with biosolids	* 25 °C humidity set at 40% and 20% of field capacity	* from 14 to 180 days	Roberts et al., 2016
	* 3 soils in the USA	* 15 °C	* from 15 to 18 weeks	Erickson et al., 2014
	* 4 soils spread with manure	* 3 water contents; incubation at 20 °C and 30 °C	* from 8 to 58 days	Franz et al., 2005
	* Sandy and silty soils	* 20 °C and 30 °C	* 56 days sandy soil; 224 days silty-sandy soil	Underthun et al., 2018
	* Silty-clayish soil	* 10 °C	* more than 5 weeks	Williams et al., 2007
	* Sandy and clayish soils	* 20 °C	* several months	Bolton et al., 2011
	* 6 soils in the USA	* 10 °C, humidity set at 60% of field capacity	* from 18 to 98 d	Ma et al., 2014
	* 3 types of soil	* 10 °C	* from 50 to 120 d	Ma et al., 2011
Campylobacter jejuni	* Silty-sandy soil, silty-clayish soil spread with biosolids	* 25 °C humidity set at 40% and 20% of field capacity	* 7 days	Roberts et al., 2016
Clostridium perfringens	* Silty-sandy soil, silty-clayish soil spread with biosolids	* 25 °C humidity set at 40% and 20% of field capacity	* from 14 to 210 d	Roberts et al., 2016
Clostridium botulinum	* experimental field spread with compost artificially contaminated with spores	* field experiment	* more than 939 d	Gessler & Bohnel, 2006
Clostridium difficile	* Silty-sandy soil, silty soil	* experimental containers applied in the field	* more than 450 d	Xu et al., 2016


Farm effluents
Slurry
Storage
Stored slurry
Phase separation
Liquid phase
Storage
Stored liquid phase
Liquid AD
Raw or post-treated digestate
Nitrification Denitrification
Sludge, water from decantation
Solid phase
Storage
Stored solid phase
Composting
Compost
Nitrification Denitrification
Sludge, water from decantation
Liquid AD
Raw or post-treated digestate
Solid co-substrate
Composting
Compost
Manure
Liquid co-substrate
Storage
Stored manure
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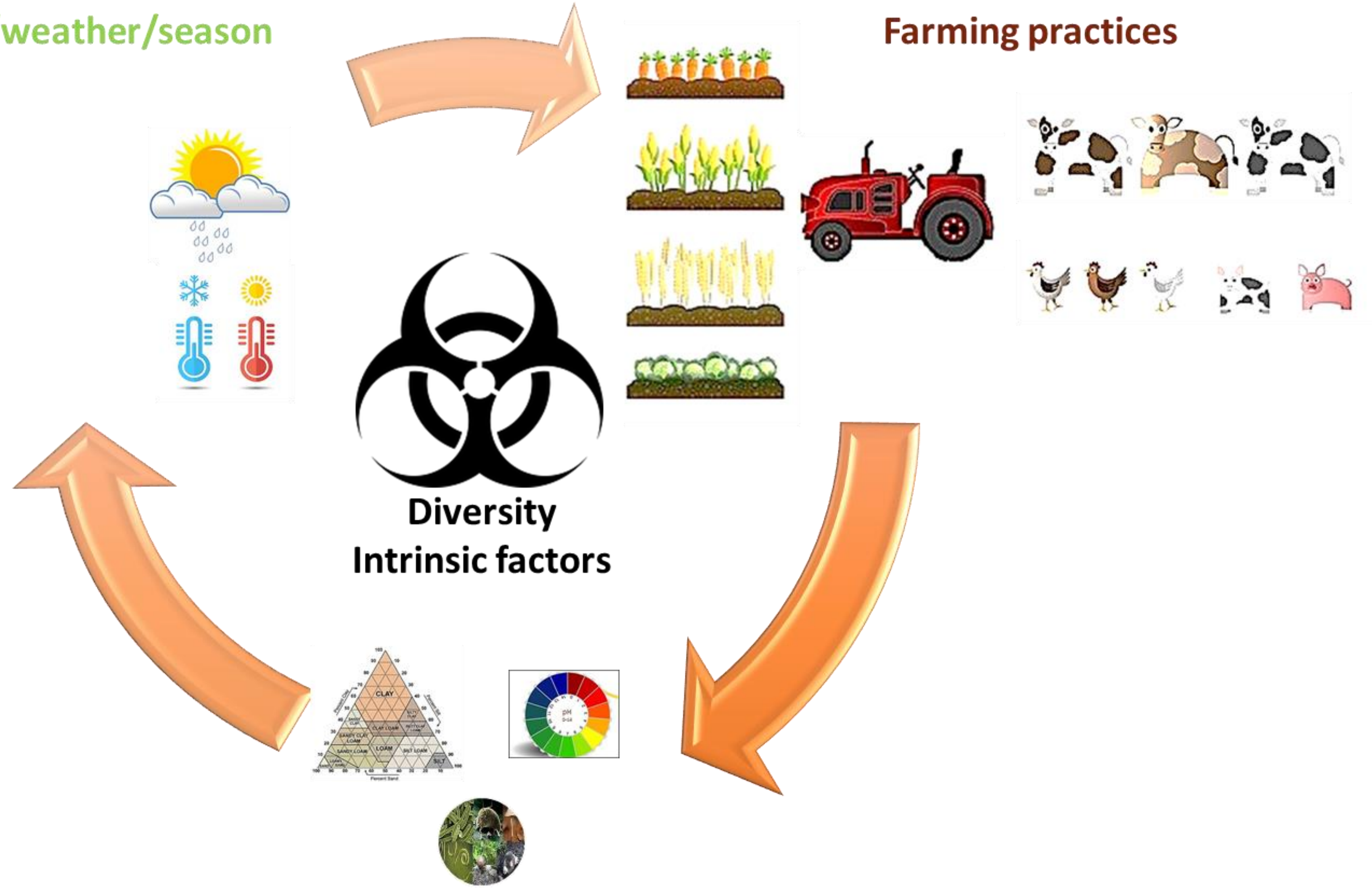
Raw or post-treated digestate
Solid-state AD
Raw or post-treated digestate
Composting
Compost
Land

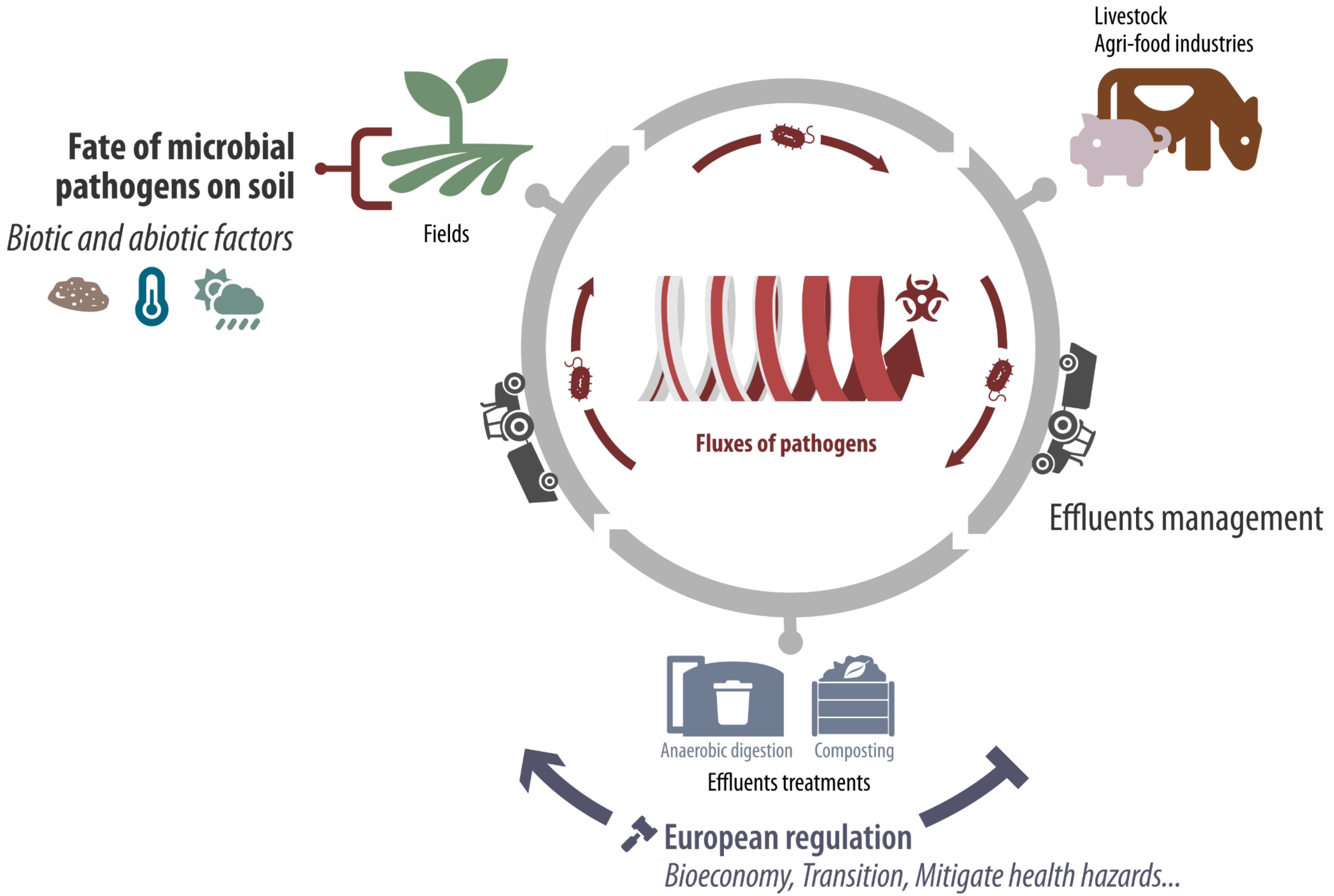


Climate/weather/season

Farming practices

Diversity
Intrinsic factors

Soil characteristics



Fate of microbial pathogens on soil

Biotic and abiotic factors

Fields

Livestock
Agri-food industries

Fluxes of pathogens

Effluents management

Anaerobic digestion

Composting

Effluents treatments

European regulation

Bioeconomy, Transition, Mitigate health hazards...

› **Current trend of increasing circular patterns for transition toward bioeconomy**