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

3

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10

11 **Highlights**

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

16

17 **Keywords**

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

19

20

21

22 **Abstract**

23 The recycling of biomass is the cornerstone of sustainable development in the bioeconomy. In this  
24 context, digestates and composts from processed agricultural residues and biomasses are returned  
25 to the soil. Whether or not the presence of pathogenic microorganisms in these processed biomasses  
26 is a threat to the sustainability of the current on-farm practices is still the subject of debate. In this

27 review, we describe the microbial pathogens that may be present in digestates and composts. We  
28 then provide an overview of the current European regulation designed to mitigate health hazards  
29 linked to the use of organic fertilisers and soil improvers produced from farm biomasses and  
30 residues. Finally, we discuss the many factors that underlie the fate of microbial pathogens in the  
31 field. We argue that incorporating land characteristics in the management of safety issues connected  
32 with the spreading of organic fertilisers and soil improvers can improve the sustainability of biomass  
33 recycling.

34

### 35 **Introduction**

36 Human activities have been shaping the environment since the 1950s and have caused irreversible  
37 changes (Zalasiewicz, 2015). The earth has entered the Anthropocene, a new geological era in which  
38 the main driver of the evolution of the Earth is no longer geological forces but humans (Crutzen,  
39 2002; Crutzen & Steffen, 2003; Steffen et al., 2007). From now on, mitigating the impact of the  
40 human society on the environment and better management of energy and material flows are  
41 indispensable (Williams & Crutzen, 2013). Recycling is a major landmark in the path to sustainable  
42 human activities. Recycling requires the requalification of by-products, previously referred to as  
43 wastes, as resources to be valued. For example, in the bioeconomy, recycling of biomass (food left-  
44 overs, garden residues, farm effluents, crop residues), makes it possible to close the loop of used and  
45 consumed nutrients e.g. nitrogen and phosphorus, and organic carbon, and can result in the  
46 production of high added-value compounds in biorefineries (De Corato et al., 2018). The added-value  
47 of recycling residual biomass includes the production of energy, (e.g. biogas), organic soil improvers  
48 (compost) and fertilisers (digestates) for agricultural soils (Kumar Khanal et al., 2021). Several  
49 processes from the most low-tech, (e.g. direct spreading or composting) to the most high-tech, (e.g.  
50 anaerobic digestion and environmental biorefining), can be used to improve the reuse of these  
51 biomasses. The bioeconomy is therefore a credible way to achieve sustainable development  
52 objectives (Bogdanski et al., 2021) however its generalisation may involve health hazards, especially

53 through the food chain, due to the circulation of pathogens and other contaminants (WHO, 2018).  
54 Indeed, even though the agronomic benefits of composts and digestates are well documented, the  
55 dissemination of traces of heavy metals (Beggio et al., 2021), organic compounds and pathogens  
56 (Thakali & MacRae, 2021) are concerns that need to be properly assessed. In agroecology, these  
57 safety issues could compromise the sustainability of large-scale recycling of farm effluents through  
58 processing and spreading on the land (Dumont et al., 2013; Thakali & MacRae, 2021) unless health  
59 policies tackle the problem of the presence of pathogens in livestock effluents and their potential  
60 dissemination in the environment (Nag et al., 2021; Nag et al., 2022).

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

76

## 77 **1. Literature search methodology**

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

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

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

96

## 97 **2. Fate of farm effluents**

98

99 Farm effluents, mainly manure and slurry, are by far the biggest source of organic fertilisers spread  
100 on land. About half of the effluents come from farmyards and farm buildings while the rest is directly  
101 deposited on pastures by grazing animals. Slurries are liquid mixtures whose dry matter content  
102 ranges from 1% to 12.5% and is composed of faeces, urine, water and used bedding (Houot et al.,  
103 2014). Manures are predominantly composed of bedding mixed with the animal faeces and urine.

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

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

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

122 When mixed with straw or other plant residues, manure, the solid phase of slurries and the slurries  
123 themselves can be composted. Composting is a biological two-stage process in which the organic  
124 matter is stabilised in the presence of oxygen (Haug, 1993; Epstein, 1997; Bernal et al., 2009). During  
125 the active stage, aerobic degradation of organic matter increases the microbial biomass, produces  
126 heat: temperatures can rise to 70 °C. When readily biodegradable substrates are exhausted, the  
127 temperature decreases. Under mesophilic conditions, the maturation stage is characterised by the  
128 conversion of aromatic and aliphatic compounds into humic acids and by the biosynthesis of  
129 macromolecules. Composts are soil improvers and their application on land is beneficial for soil

130 quality and plant health (De Corato, 2020). They improve the structure and hydraulic properties of  
131 the soil (Rivier et al., 2022), supply nutrients to the soil (Duong et al., 2013), increase soil fertility and  
132 crop yield (Ayilara et al., 2020), and enable biocontrol of diseases (Ayilara et al., 2020).

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

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

146

### 147 **3. Hazardous foodborne pathogens potentially circulating through composts and digestates**

148 Recent reports (Bohnel & Lube, 2000; Burtscher & Wuertz, 2003; Dharmasena & Jiang, 2018;  
149 Chiapetta et al., 2019) and comprehensive reviews (Bloem et al., 2017) suggest that pathogen loads  
150 generally decrease during anaerobic digestion and composting but that pathogens are still detected  
151 in digestates and composts. *Cryptosporidium parvum*, *Salmonella* spp. (including *S. Typhi* and *S.*  
152 *paratyphi*), *norovirus*, *Streptococcus pyogenes*, enteropathogenic *E. coli* (EPEC), *Mycobacterium* spp.,  
153 *Clostridium* spp., *Listeria monocytogenes* and *Campylobacter coli* are of concern they may be able to  
154 survive anaerobic digestion (Nag et al., 2019; Planchon et al., 2020). Soil can represent a reservoir of  
155 human pathogens (Table 1), and whether or not large-scale organic fertilisation can modify the fluxes

156 of these microorganisms in the biosphere and their persistence in soil is still the subject of debate. In  
157 the following sections, the focus on major foodborne pathogens (*Salmonella enterica*, pathogenic  
158 *Escherichia coli*, *Listeria monocytogenes*, *Clostridium* spp.) and process indicators (*Escherichia coli*,  
159 *Enterococcus* spp.) is motivated by the safety issues they raise in the food chain, and by the  
160 comprehensive body of literature addressing their persistence in soil after application of organic  
161 fertilisers and soil improvers.

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

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

176

#### 177 **4. EU regulation and microbial standards**

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



181

182           4.1 Environmental regulation

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

195

196           4.2 Safety rules

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

206 is likely, competent authorities can specify new rules and/or prohibit further land spreading of the  
207 contaminated raw products.

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

221

#### 222 4.3 Rules for the marketing of fertilising products in the EU

223 Anaerobic digestion residues and composts may be placed on the market and used as organic  
224 fertilisers or soil improvers. Organic fertilisers are products originating from biomass and complying  
225 with EU Regulation 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying  
226 down rules on the making available on the market of EU fertilising products and amending  
227 Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003.  
228 Soil improvers are materials added to soil whose main function is to maintain or improve its physical  
229 and/or chemical and/or biological properties. Under these circumstances, they must comply with EU  
230 regulation 2019/1009 that lays down rules on the making available on the market of EU fertilising

231 products, organic fertilisers and organic soil improvers. Seven product function categories (PFC) and  
232 11 Component Material Categories (CMC) are listed in this document. Microbial criteria similar to  
233 the criteria regulating the animal by-products (see above) are implemented to control safety issues.  
234 EU member states may introduce their own national legislation to regulate their national market for  
235 organic fertilisers or soil improvers.  
236 However the legal EU requirements (*E. coli* or *Enterococcaceae* and *salmonella enterica*) may not fully  
237 capture all the fluxes of pathogens that circulate in organic fertilisers and soil improvers. Under some  
238 circumstances, this could lead to health and sustainability concerns (Nag et al., 2020). From a safety  
239 point of view, the main problem with on-farm management of biomass is understanding the fate of  
240 the pathogens following application on agricultural land. While rapid decay causes limited concern,  
241 long-term survival and/or transfer of the pathogens could have consequences in terms of fluxes of  
242 pathogens in the environment. It is therefore critical to clarify the factors and land characteristics  
243 that influence the fate of these microbial pathogens.

244

## 245 **5. Factors affecting the fate of pathogens after land spreading**

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

250

251 5.1 Persistence of pathogens in soil after land application of digestates and composts is dose  
252 dependent and species specific

253 Many studies that simulated the application of digestate to the land under laboratory conditions  
254 concomitantly investigated the persistence of several pathogens in the soil. One investigation of  
255 soil+cattle manure digestate reported that *Listeria* spp. was detected in the digestate at 10<sup>4</sup> CFU/g  
256 dry weight whereas *E. coli* and *Salmonella enterica* were not detected (Goberna et al., 2011). During

257 the first 30 days of incubation at 20 °C, the population of *Listeria* spp. was significantly higher in  
258 soil+digestate columns than in controls but subsequently, the differences were not significant and *L.*  
259 *monocytogenes* was never detected (Goberna et al., 2011). Gomez-Brandon et al. (2016) reported  
260 that although coliforms and *E. coli* were numerated from  $2 \cdot 10^2$  CFU/g to  $10^3$  CFU/g in cattle manure  
261 digestates and composts, after mixing with soil, their populations dropped during incubation at 22 °C  
262 and their presence was no longer detected after 60 days (Gomez-Brandon et al., 2016). Conversely,  
263 *C. perfringens* initially present at  $2 \cdot 10^3$  CFU/g was still detected after 60 days of incubation (Gomez-  
264 Brandon et al., 2016). When heavily spiked digestate made of household food residues was added to  
265 soil, *E. coli*, *S. Thyphimurium*, *C. tyrobutyricum*, *T. emersonii* and porcine parvovirus were reported to  
266 survive for at least 49 days, i.e., the duration of the experiment in a climate chamber, but *L.*  
267 *monocytogenes* was not detected at the end of the experiment (Johansson et al., 2005).

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

274

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

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

291 Other studies focussed on the transfer of pathogens from compost to soil. Increased concentrations  
292 of total thermotolerant coliforms was observed in experimental plots fertilised with non-spiked dairy  
293 manure composts but, 120 days after application, the differences between treated and control plots  
294 were no longer significant (Wind et al., 2018). Four years of monitoring experimental plots fertilised  
295 with various composts suggested limited transfers of pathogens if the organic fertilisers complied  
296 with the current French regulation NFU 44-051 (Brochier et al., 2012). This French standard defines  
297 microbial criteria for *Salmonella enterica* (absence in 1 g, or in 25 g for home vegetable gardens),  
298 helminth eggs (absence in 1.5 g) and process indicators (*E. coli*: 100/g; enterococci 1000/g).  
299 *Clostridium botulinum* in soil was shown to persist for several years following land spreading of  
300 spiked composts but, again, the results depended on the original concentration of the pathogen ( $10^3$   
301 CFU/g and  $10^5$  CFU/g) and on the dose of spiked compost added to the plots (Gessler & Bohnel,  
302 2006). Similarly, *C. perfringens* was detected for 10 years after land spreading of compost made with  
303 swine manure (Scott et al., 2018). *Clostridium sporogenes* was detected for one year after land  
304 spreading of spiked bovine manure and sewage sludge composts whereas *Listeria innocua* was only  
305 detected in the first three months of the experiment (Girardin et al., 2005). However, in this  
306 experiment, the composts were also heavily spiked ( $10^5$  CFU/g). Similarly, heavy spiking of poultry  
307 and bovine manure composts with an avirulent variant of *E. coli* O157:H7 ( $10^7$  CFU/g) enabled the

308 pathogen to survive for more than five months in the fertilised plots but the pathogen was not  
309 detected in control plots that were not spiked (Islam et al., 2004; Islam et al., 2005).

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

317

## 318 5.2 Multiple extrinsic factors affect the persistence of pathogens in soil

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

326

### 327 5.2.1. Soil characteristics

#### 328 Abiotic properties

329 The abiotic properties of the soil influence the survival of pathogenic bacteria. Many laboratory  
330 studies (Table 2) have shown that low pH is detrimental to the survival of *Listeria monocytogenes*  
331 (Dowe et al., 1997; Locatelli et al., 2013). Similar results have been reported for enteric pathogens

332 such as *Escherichia coli* and *Salmonella enterica* (Bolton et al., 2011; Erickson et al., 2014; Ma et al.,  
333 2014; Wang et al., 2014<sup>b</sup>).

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

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

341 Survival of *E. coli* O157:H7 has been shown to be negatively correlated with electric conductivity (Ma  
342 et al., 2012; Erickson et al., 2014; Ma et al., 2014; Yao et al., 2015), free Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> (Wang et al.,  
343 2014<sup>a</sup>; Yao et al., 2015). Another factor identified as being beneficial for survival of *L. monocytogenes*  
344 and *Yersinia pseudotuberculosis* is a high concentration of exchangeable cations measured by cation  
345 exchange capacity (Sidorenko et al., 2006; Locatelli et al., 2013).

346

#### 347 Biotic characteristics

348 The soil microbiome is a key environmental factor and has a major impact on allochthonous  
349 microorganisms. Soil microorganisms develop complex networks of interactions that may lead to  
350 exclusion of allochthon microorganisms through exploitation, competition, and interference (Tan et  
351 al., 2015; Stubbendieck & Straight, 2016). Indeed, while populations of allochthon pathogenic  
352 bacteria decrease over time in the soil, in sterilised soil, they generally increase (Dowe et al., 1997;  
353 Jiang et al., 2002; Ishii et al., 2010; McLaughlin et al., 2011; Locatelli et al., 2013; Moynihan et al.,  
354 2013). Of course, removing all soil microorganisms is neither very informative nor realistic. A gradual  
355 decrease in soil biodiversity reflects real conditions more accurately. Experimental alteration of the  
356 balance of soil microbial communities was found to be correlated with enhanced survival of *E. coli*  
357 (van Elsas et al., 2007; van Elsas et al., 2012; Xing et al., 2019) and *L. monocytogenes* (Vivant et al.,

358 2013<sup>a</sup>). Beyond diversity, phylogenetic composition and community structure play a determining role  
359 in the exclusion of pathogens, probably because specific communities develop inhibition through a  
360 combination of exploitation, competition, and antibiosis (Vivant et al., 2013<sup>a</sup>; Spor et al., 2020; Ma et  
361 al., 2013).

362 Comparison of the survival of *S. enterica*, *E. coli* and *L. monocytogenes* in soil microcosms with  
363 contrasting edaphic characteristics confirmed that the main factor explaining the decay rate of these  
364 pathogens was the composition of the soil microbial community (Ma et al., 2013; Moynihan et al.,  
365 2015). Conversely, introducing the pathogen *E. coli* O157:H7 (Yao et al., 2014) or *L. monocytogenes*  
366 (Spor et al., 2020) altered soil microbial diversity. All these studies underline the complexity of the  
367 interactions between microbial communities, allochthon pathogens, and other soil characteristics  
368 (Ibekwe et al., 2014; Moynihan et al., 2015; Weller et al., 2015; Falardeau et al., 2018).

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

373

#### 374 5.2.2. Site-specific features affect persistence

375 The literature affirms that many site-specific features shape the fate of pathogens after the  
376 application of organic fertilisers and soil improvers to the land. A recent paper reported the results of  
377 a large-scale study conducted from 2011 to 2015 at 12 experimental sites in three geographical  
378 regions in the United States (Sharma et al., 2019). The study investigated the survival of several  
379 genotypes of *E. coli* after application of four artificially contaminated organic amendments. The  
380 statistical analysis of 324 survival profiles confirmed the complexity of the factors that influence  
381 pathogen survival in the soil. In order to predict survival time, farming practices (type, amendment,  
382 spreading methods, initial dose of pathogens, conventionally managed versus organic agriculture),



383 weather and geography have to be considered at the same time. These factors have been shown to  
384 be more important than the amendment and the spreading method, and some combinations can  
385 enable pathogens to survive for more than three months.

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

392 Other field studies focussed on possible correlations between multiple factors and the incidence of  
393 pathogens in soil (Park et al., 2013; Strawn et al., 2013<sup>a</sup>, 2013<sup>b</sup>; Park et al., 2014, 2015; Weller et al.,  
394 2015, 2016, 2020; Dusek et al., 2018). These studies suggest that certain locations are environmental  
395 reservoirs with a high incidence of pathogens. It may be possible to predict these at-risk locations  
396 using a complex combination of landscape and meteorological factors as discussed below. All these  
397 studies underline the multifactorial dimension of the prevalence and fate of pathogens in soil. The  
398 following sections address these factors individually.

399

#### 400 Landscape features, topography

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

406 Similarly, increased likelihood of detection of *Listeria monocytogenes* has been reported in vegetable  
407 fields located close to pastures, forests, grasslands, scrubland, water bodies and wetlands (Strawn et

408 al., 2013<sup>a</sup>; Chapin et al., 2014; Weller et al., 2016; Harrand et al., 2020)). River flooding was a factor  
409 that increased the prevalence of *L. monocytogenes* in soil samples (Linke et al., 2014).  
410 Slope was identified as another relevant feature for increased likelihood of detection of *L.*  
411 *monocytogenes* in vegetable fields (Chapin et al., 2014).

412

413 Climate / seasonality

414 Under given soil conditions, the persistence of pathogens depends on the season and the weather.  
415 Interestingly, Weller et al. (2015) found increased levels of *L. monocytogenes* after a rainfall event  
416 and following irrigation in fields planted with spinach (Weller et al., 2015). Similar correlations  
417 between rainfall and the occurrence of *L. monocytogenes* were identified in several wooded areas  
418 and on vegetable farms (Ivanek et al., 2009; Strawn et al., 2013<sup>b</sup>; Pang et al., 2017; Harrand et al.,  
419 2020), and between rainfall and detection of *S. enterica* (Strawn et al., 2013<sup>b</sup>) and generic *E. coli*  
420 (Park et al., 2014). This is consistent with the fact that soil moisture, available stored soil water and  
421 the soil drainage class all affect detection of the two pathogens (Strawn et al., 2013<sup>b</sup>; Weller et al.,  
422 2016). Microcosm experiments with soils adjusted to 20% and 40% water content, and contaminated  
423 with *S. enterica*, led to opposite conclusions in one out of the three soil types tested, probably due to  
424 interactions between soil texture, moisture and microbiota activities (Erickson et al., 2014). However,  
425 no correlation was found between climatic factors and the presence of Shiga toxin-producing  
426 *Escherichia coli* (Strawn et al., 2013<sup>b</sup>). In laboratory soil microcosm experiments, a positive  
427 correlation between soil moisture and the survival of *E. coli* and *E. coli* O157:H7 was reported  
428 (Ohtomo et al., 2004; Habteselassie et al., 2008) while drought periods reduced the number of *E. coli*  
429 detected (Ishii et al., 2010). However, a meta-analysis of reports on the survival of *E. coli* and *E. coli*  
430 O157:H7 in land spread with manure suggested longer survival when manure was applied to dry soil  
431 (Park et al., 2016). A longitudinal field study partly confirmed these results (Sharma et al., 2019).

432 Temperature is another key environmental factor influencing pathogen survival, which is facilitated  
433 at low temperatures as long as the temperatures are above freezing (Ivanek et al., 2009; Ishii et al.,

434 2010; Saunders et al., 2012; Farhangi et al., 2013; Strawn et al., 2013<sup>b</sup>; Underthun et al., 2018).  
435 Better survival of *Listeria spp.* and *L. monocytogenes* has been documented in winter/early spring  
436 compared to in other periods of the year (Girardin et al., 2005; Strawn et al., 2013<sup>b</sup>; Chapin et al.,  
437 2014). However, in a yearlong experiment of cover crops in artificially contaminated experimental  
438 plots, temperature was not among the factors that significantly affected the survival of *L. innocua*  
439 (Reed-Jones et al., 2016).  
440 Seasonality was also observed to have an impact on the persistence of *E. coli* and *S. enterica* in field  
441 experiments and led to conflicting results depending on the year the experiment was performed and  
442 on other environmental parameters (Ishii et al., 2010; Reed-Jones et al., 2016; Sarr et al., 2020).  
443 Other studies of the presence of *E. coli* O157:H7 in soil amended with various manures and other  
444 organic fertilisers, demonstrated better survival in autumn than in spring but temperature and  
445 rainfall appeared to have a limited effect on the survival of this pathogen (Oliveira et al., 2012;  
446 Sharma et al., 2019). Conversely, a meta-analysis of survival data in the literature clearly identified  
447 water content and temperature as factors that play an important role in the survival of *E. coli*  
448 O157:H7 in soil (Park et al., 2016).

449

### 450 5.2.3. Farming practices must be taken into account

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

454

#### 455 Organic farming

456 The high biodiversity found in organic farming can improve the biotic control of pests (Crowder et al.,  
457 2010) and pathogens (Jones-Dias et al., 2016; Jones et al., 2019<sup>a</sup>, 2019<sup>b</sup>). However, results in the  
458 literature vary with the type of soil. In experiments with cattle manure incorporated in the soil, the  
459 prevalence of *E. coli* O157:H7 was higher in conventional than in organic soils in three out of five

460 combinations, whereas *S. enterica* survival was similar in soils under the two management systems  
461 (Franz et al., 2005). However, a follow-up study with more soil samples failed to provide evidence for  
462 significant differences in the survival rate of *E. coli* 157:H7 between organic and conventionally  
463 managed soils (Franz et al., 2008). In a multi-year survey to compare the effect of the type of organic  
464 amendments on the survival of pathogenic *E. coli*, survival time was longer in conventional than  
465 organic farms, but the results varied with the year, the type of amendment and other temporal  
466 factors (Sharma et al., 2019). Topological analysis of *E. coli* O157:H7 survival in a selection of 32 US  
467 organic and conventionally managed soils originating from two contrasting states (California and  
468 Arizona) showed shorter survival times in organic soils than in conventional soils but this result was  
469 strain- and location-dependent (Ibekwe et al., 2014). In fact, one strain of *E. coli* O157:H7 survived  
470 better in organic soils collected from one site, while survival in soils collected from another site was  
471 similar, regardless of the soil management practices (Ma et al., 2012, 2013). This dataset suggests  
472 that soil management can affect the survival of *E. coli* O157:H7 but differences between organic and  
473 conventional soils are highly location- and strain-dependent. Overall, these results tend to show that  
474 the persistence of pathogenic bacteria is lower in organic farms than conventional farms, but other  
475 environmental and soil factors modulate this difference. For this reason, large-scale surveys are  
476 required to produce more experimental data to better predict the fate of pathogens and to compare  
477 health risks in organic and conventionally managed farms.

478

479 Mode of application of organic fertilisers

480 Solid digestates with more than 18% total solids are typically applied to the soil surface and need to  
481 be incorporated into the soil to prevent the emission of odours, whereas digestates with less total  
482 solids can be either spread on the soil surface or injected to the subsurface (Crolla et al., 2013). The  
483 fate of pathogens varies depending on the method of application of digestate. Subsurface  
484 incorporation has been shown to delay pathogen decay compared to surface application (Hutchison  
485 et al., 2004; Alegbeleye & Sant'Ana, 2020), but other authors found no significant difference between

486 subsurface and surface application (Saunders et al., 2012). A laboratory study investigated the impact  
487 of application (soil incorporation versus surface application) on the survival of various pathogens.  
488 The study was carried out using two soils (sandy loam and loamy clay) amended with either pig slurry  
489 stored in a lagoon or cattle manure and sludge taken from a wastewater treatment plant, and  
490 artificially contaminated with a cocktail of pathogens (*S. enterica*, *L. monocytogenes*, *C. jejuni*, *C.*  
491 *perfringens*, *coliphages*). A rapid decline of *C. jejuni* was observed within seven days whereas *S.*  
492 *enterica* and *L. monocytogenes* survived longer (Roberts et al., 2016). Interactions between organism  
493 x management practice x soil were significant suggesting that pathogen decay is affected by  
494 management, but the risk of persistence also depends on other farm characteristics, land use  
495 patterns and on the microorganism considered (Roberts et al. 2016). The study by Sharma et al.  
496 (2019) confirmed that the survival of *E. coli* following field fertilisation depended on the mode of  
497 application but their results also depended on other temporal factors including soil management,  
498 the type of amendment used, soil characteristics and climate.

499

#### 500 Soil solarisation

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

505

#### 506 **Conclusion**

507 The presence of human pathogens in organic fertilisers and soil improvers can cause safety problems  
508 all along the food chain. The EU regulation sets out rules to mitigate the risk of transfer of pathogens  
509 from organic fertilisers and soil to the food system. When processed, materials to be applied on land  
510 must comply with microbial rules. Nevertheless, the presence of pathogenic bacteria in composts  
511 and digestates and their persistence in the soil after spreading is documented. Because soils are so

512 complex, it is difficult to predict the behaviour of pathogenic microorganisms after application, both  
513 abiotic and biotic soil characteristics are extrinsic factors that determine the fate of human  
514 pathogens in soil.

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

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

537

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539

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1115 **Legend of Figures and tables**

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1117 Figure 1. Schematic of the main pathways leading to the spreading of raw and processed farm  
1118 effluents on agricultural land.

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1120 Figure 2. Schematic of the complex interactions between factors that affect the fate of pathogens  
1121 upon their arrival in the soil.

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1123 Table 1. References reporting the detection of human pathogens in soil.

1124

1125 Table 2. Site characteristics, climatic conditions for laboratory studies of the survival of human  
1126 pathogens in soils.

1127

1128 Table 1. References reporting the detection of human pathogens in soil.

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

		Heijnsbergen et al., 2016
Mycetes		
<i>Sporotrix schenckii</i>	Invasive Mycosis	Ramirez-Soto et al., 2018
<i>Rhizopus and Mucor</i>	Invasive Mycosis	Mousavi et al., 2018
<i>Aspergillus</i> ,	Mycotoxin production	Nguyen et al., 2017
<i>Fusarium</i>	Mycotoxin production	Nguyen et al., 2017; Vogelgsang et al., 2019
<i>Penicillium</i>	Mycotoxin production	Nguyen et al., 2017; Dombink-Kurtzman & McGovern, 2007; Elmholt, 2003
Eucaryotes		
<i>Cyclospora cayetanensis</i>	Nausea, diarrhoea, fatigue	Chacin-Bonilla, 2008; Giangaspero et al., 2015
<i>Giardia duodenalis</i>	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
<i>Cryptosporidium</i> 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

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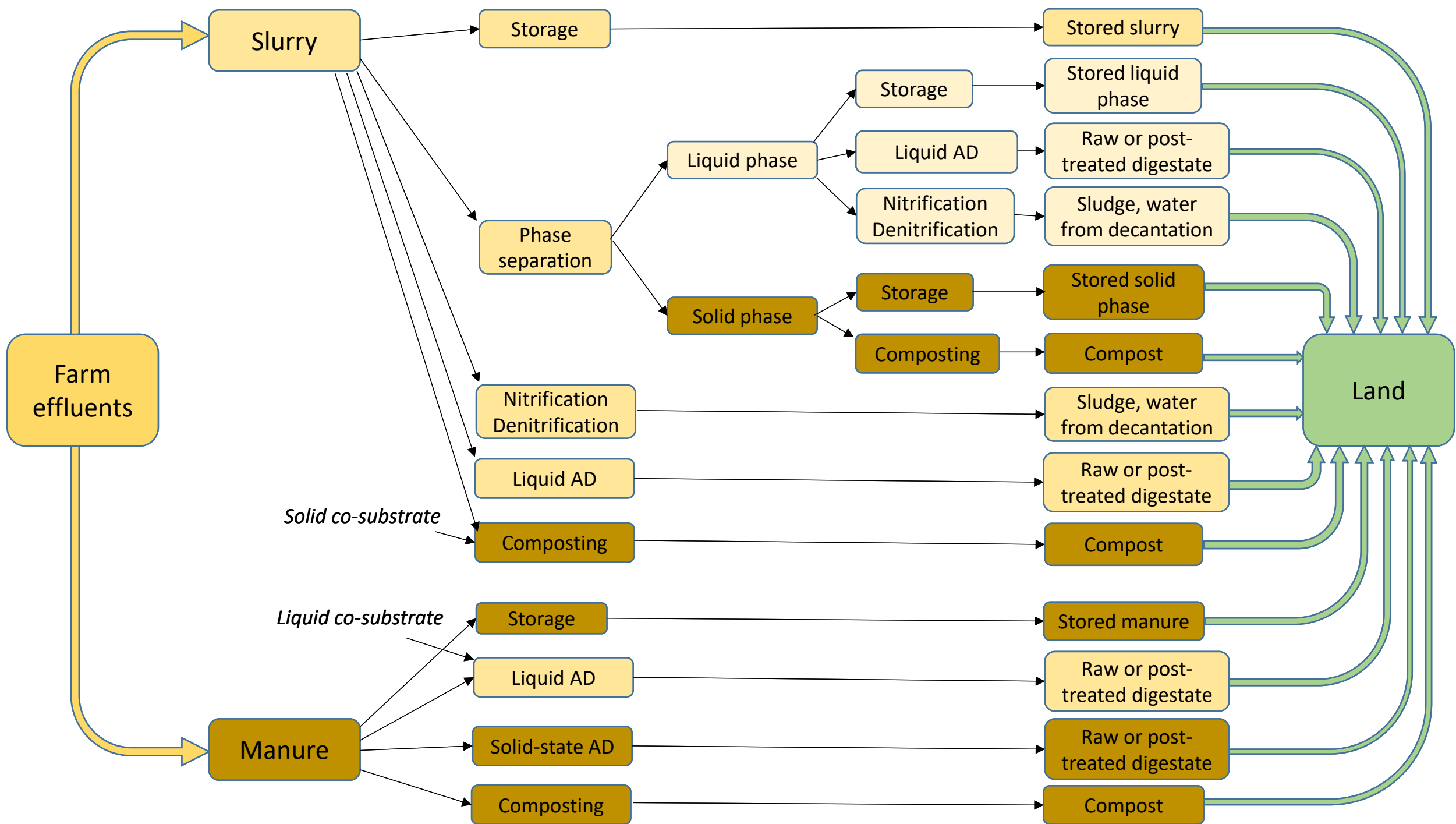
Table 2. Site characteristics, climatic condition for lab survival studies of human pathogens in soils.

Bacterial species	Soil Type	Incubation	Period of detection	Reference
Pathogenic bacteria found in food				
<i>Listeria monocytogenes</i>	* 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	

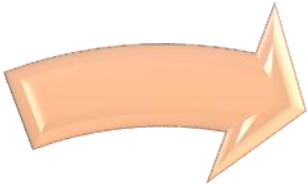
<i>Salmonella enterica</i>	<ul style="list-style-type: none"> <li>* Sandy brown grassland soil with clay addition</li> <li>* 12 soils sampled in Ireland</li> <li>* Silty-sandy soil, silty-clayish soil spread with biosolids</li> <li>* 3 soils in the USA</li> <li>* 4 soils with manure applied</li> <li>* Sandy and silty soils spread with pig and poultry manure and planted with lettuce</li> <li>* Silty soil spread with poultry fertiliser</li> <li>* Sandy and silty soils</li> </ul>	<ul style="list-style-type: none"> <li>* 15 °C, humidity set at 65% of field capacity</li> <li>* 10 °C</li> <li>* 25 °C humidity set at 40% and 20% of field capacity</li> <li>* 15 °C</li> <li>* 20 °C, 16 h daylight</li> <li>* climatic chamber (13 h daylight at 22 °C to 24 °C) and 11 h dark, at 15 °C to 18 °C)</li> <li>* 3 water contents; incubation at 20 °C and 30 °C</li> <li>* 20 °C and 30 °C</li> </ul>	<ul style="list-style-type: none"> <li>* 40 to 96 days</li> <li>* 110 days</li> <li>* from 14 to 210 days</li> <li>* from 15 to 18 weeks</li> <li>* from 30 to 58 days</li> <li>* more than 40 days</li> <li>* less than 49 days (unfertilised) More than 91 days (fertilised)</li> <li>* 168 days</li> </ul>	<ul style="list-style-type: none"> <li>Brennan et al., 2014</li> <li>Moynihan et al., 2015</li> <li>Roberts et al., 2016</li> <li>Erickson et al., 2014</li> <li>Franz et al., 2005</li> <li>Jechalke et al., 2019</li> <li>Shah et al., 2019</li> <li>Underthun et al., 2018</li> </ul>
<i>Escherichia coli</i> pathotypes	<ul style="list-style-type: none"> <li>* Sandy brown grassland soil with clay addition</li> <li>* 12 soils sampled in Ireland</li> <li>* Silty-sandy soil, silty-clayish soil spread with biosolids</li> <li>* 3 soils in the USA</li> <li>* 4 soils spread with manure</li> <li>* Sandy and silty soils</li> <li>* Silty-clayish soil</li> <li>* Sandy and clayish soils</li> <li>* 6 soils in the USA</li> <li>* 3 types of soil</li> </ul>	<ul style="list-style-type: none"> <li>* 15 °C, humidity set at 65% of field capacity</li> <li>* 10 °C</li> <li>* 25 °C humidity set at 40% and 20% of field capacity</li> <li>* 15 °C</li> <li>* 3 water contents; incubation at 20 °C and 30 °C</li> <li>* 20 °C and 30 °C</li> <li>* 10 °C</li> <li>* 20 °C</li> <li>* 10 °C, humidity set at 60% of field capacity</li> <li>* 10 °C</li> </ul>	<ul style="list-style-type: none"> <li>* 40 to 96 days</li> <li>* 110 days</li> <li>* from 14 to 180 days</li> <li>* from 15 to 18 weeks</li> <li>* from 8 to 58 days</li> <li>* 56 days sandy soil; 224 days silty-sandy soil</li> <li>* more than 5 weeks</li> <li>* several months</li> <li>* from 18 to 98 d</li> <li>* from 50 to 120 d</li> </ul>	<ul style="list-style-type: none"> <li>Brennan et al., 2014</li> <li>Moynihan et al., 2015</li> <li>Roberts et al., 2016</li> <li>Erickson et al., 2014</li> <li>Franz et al., 2005</li> <li>Underthun et al., 2018</li> <li>Williams et al., 2007</li> <li>Bolton et al., 2011</li> <li>Ma et al., 2014</li> <li>Ma et al., 2011</li> </ul>
<i>Campylobacter jejuni</i>	<ul style="list-style-type: none"> <li>* Silty-sandy soil, silty-clayish soil spread with biosolids</li> </ul>	<ul style="list-style-type: none"> <li>* 25 °C humidity set at 40% and 20% of field capacity</li> </ul>	<ul style="list-style-type: none"> <li>* 7 days</li> </ul>	<ul style="list-style-type: none"> <li>Roberts et al., 2016</li> </ul>
<i>Clostridium perfringens</i>	<ul style="list-style-type: none"> <li>* Silty-sandy soil, silty-clayish soil spread with biosolids</li> </ul>	<ul style="list-style-type: none"> <li>* 25 °C humidity set at 40% and 20% of field capacity</li> </ul>	<ul style="list-style-type: none"> <li>* from 14 to 210 d</li> </ul>	<ul style="list-style-type: none"> <li>Roberts et al., 2016</li> </ul>
<i>Clostridium botulinum</i>	<ul style="list-style-type: none"> <li>* experimental field spread with compost artificially contaminated with spores</li> </ul>	<ul style="list-style-type: none"> <li>* field experiment</li> </ul>	<ul style="list-style-type: none"> <li>* more than 939 d</li> </ul>	<ul style="list-style-type: none"> <li>Gessler &amp; Bohnel, 2006</li> </ul>
<i>Clostridium difficile</i>	<ul style="list-style-type: none"> <li>* Silty-sandy soil, silty soil</li> </ul>	<ul style="list-style-type: none"> <li>* experimental containers applied in the field</li> </ul>	<ul style="list-style-type: none"> <li>* more than 450 d</li> </ul>	<ul style="list-style-type: none"> <li>Xu et al., 2016</li> </ul>

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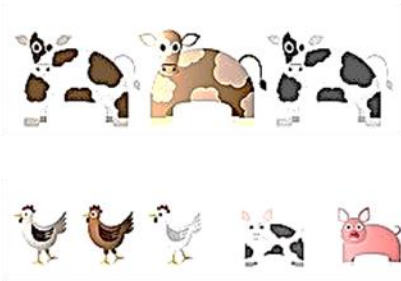
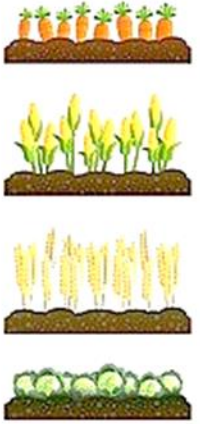




Climate/weather/season



Farming practices



Diversity  
Intrinsic factors



Soil characteristics

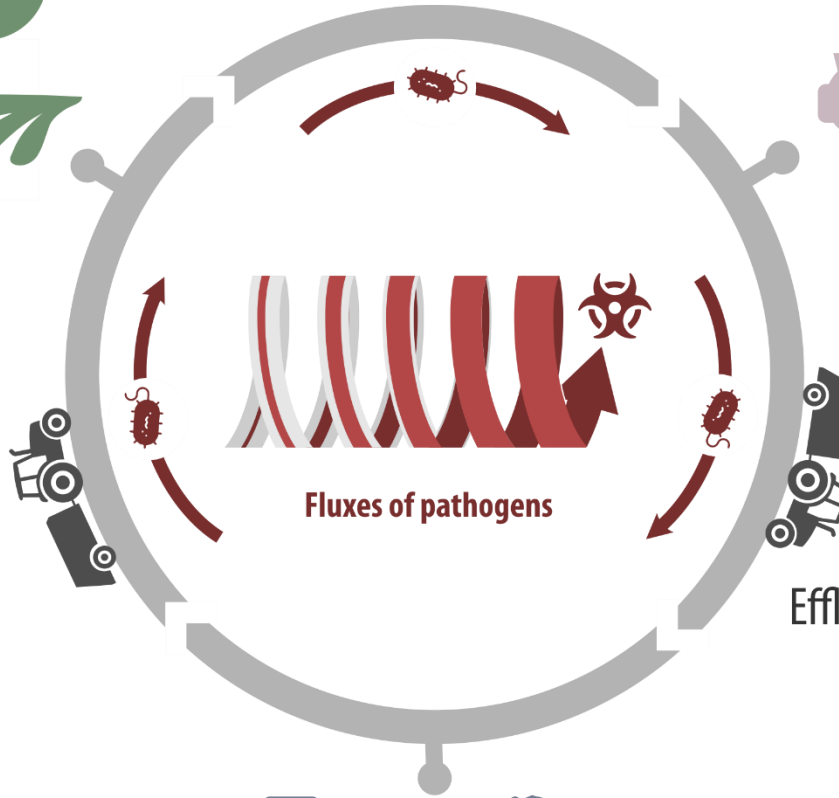
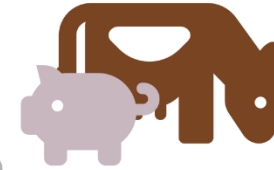


**Fate of microbial pathogens on soil**  
*Biotic and abiotic factors*



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**