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1 **Environmental risk assessment of antibiotics in agroecosystems: ecotoxicological effects on aquatic**
2 **microbial communities and dissemination of antimicrobial resistances and antibiotic**
3 **biodegradation potential along the soil-water continuum**

4

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21

22 **Abstract**

23 Antibiotics, have a wide application range in human and veterinary medicines. Being designed for
24 pharmacological stability, most antibiotics are recalcitrant to biodegradation after ingestion and can
25 be persistent in the environment. Antibiotic residues have been detected as contaminants in various
26 environmental compartments where they cause human and environmental threats, notably with
27 respect to the potential emergence and proliferation of antibiotic resistant bacteria. An important
28 component of managing environmental risk caused by antibiotics is to understand exposure of soil
29 and water resources to their residues. One challenge is to gain knowledge on the fate of antibiotics in
30 the ecosystem along the soil::water continuum, and on the collateral impact of antibiotics on
31 environmental microorganisms responsible for crucially important ecosystem functions. In this
32 context, the ANTIBIOTOX project aims at studying the environmental fate and impact of two
33 antibiotics of the sulfonamides class of antibiotics, sulfamethazine (SMZ) and sulfamethoxazole
34 (SMX).

35

36 **Keywords:** antibiotics; resistance gene; bacteria; biodegradation

37

38 **1. Environmental risks of antibiotics in agroecosystems**

39 Antibiotics (antimicrobials used to kill bacteria) are widely used in human medicine and in many
40 terrestrial and aquatic food animal production systems. Numerous antibiotics are recalcitrant to
41 degradation following ingestion and are excreted intact. Therefore, some antibiotics are widely
42 detected in terrestrial and aquatic matrices (Bottoni and Caroli 2015). In agroecosystems, antibiotic
43 residues can be transported from livestock production areas or from agricultural soils amended with
44 antibiotic-contaminated manure by surface runoff or infiltration, contaminating adjacent surface-
45 and groundwater resources (Boxall 2004). Once released in the environment, antibiotics may exert a
46 selection pressure favoring the emergence of bacteria harboring antimicrobial resistances (AMR)
47 even at low environmentally relevant concentrations (Gulberg et al. 2011). Indeed, the development
48 of antibiotic-resistant bacteria near confined animal feed operations (Chee-Sanford et al. 2001; Koike
49 et al. 2007; Sapkota et al. 2007), in soils treated with animal manures or in waste water treatment
50 plants (Gao et al. 2012) suggests that exposure to antibiotics can lead to the increase in abundance
51 of resistance genes in the environment (Heuer et al. 2011; Storteboom et al. 2010).

52 The World Health Organization has identified the increasing occurrence of AMR in human pathogens
53 as one of the most pressing contemporary global public health challenges. Due to the risks for
54 subsequent transfers of resistance factors to human pathogens an increasing reservoir of AMR
55 bacteria in the environment is of human health concern (Ashbolt et al. 2013; Bengtsson-Palme et al.,
56 2018). Promoting the 'One Health' concept, the United Nations and the European Commission
57 highlighted the need to develop approaches to better assess the risk for resistance selection in
58 natural microbial communities (Bengtsson-Palme and Larsson, 2016). In June 2017, the European
59 Commission adopted the new EU One Health Action Plan against AMR and the French Ministry of
60 Agriculture launched the new EcoAntibio national plan (2017-2021) with the objective of mitigating
61 the risk of AMR development. Managing the environmental risk of AMR is predicated on
62 understanding the exposure of soil and water resources to antibiotic residues, antibiotic-resistant
63 bacteria and antibiotic resistance genes (Blanco et al. 2009; Knapp et al. 2010; Pruden et al. 2013).
64 There is thus a need to identify environmental conditions that govern the fate of antibiotics in
65 agroecosystems including transfers along the soil-water continuum, as well as dissipation (e.g.
66 through biodegradation) and persistence (e.g. through retention due to sorptive processes) in the
67 different environmental compartments (Larsson et al. 2018). Although up to now, the major
68 concern about antibiotics is associated with the development and dissemination of resistance
69 mechanisms by bacteria (Heuer et al. 2011) and the implications of this process for both human and
70 animal health. Their sustained release to different environmental compartments and their bioactive
71 properties also raise serious issues about their impact on non-target organisms (Gonzales-Pleiter et
72 al. 2013). Briefly, and as recently underlined by Barra-Caracciolo et al. (2015) and Brandt et al. (2015),

73 much more knowledge concerning environmental emissions of antibiotics, their persistence,
74 degradation pathways and impacts on ecosystem health is required. Environmental risk assessments
75 should take into account terrestrial and aquatic microbial communities that undertake the main
76 ecosystem functions and services including the biodegradation of contaminants (Ghiglione et al.
77 2016).

78

79 **2. The case of Sulfonamides**

80 Among the antibiotics, the sulfonamides (SMs), one the oldest family on the market, are often
81 detected in aquatic and terrestrial matrices (Baran et al. 2006; Manzetti and Ghisi 2014; Deng et al.,
82 2018) because of the large volume used, high excretion rates, and relative persistence in soil (Brandt
83 et al. 2009). In a pioneering study, Kolpin et al. (2002) reported the detection of SMs in 80% of the
84 139 streams and rivers sampled in 1999 and 2000 across 30 states in the US. Since then SMs are
85 frequently detected in the environment (Hruska and Franek 2012) including rural streams and
86 connected rivers surrounding livestock production (Kolpin et al. 2002; Veach and Bernot 2011;
87 Bernot et al. 2013; Iglesias et al. 2014). It is noteworthy that sulfamethoxazole (SMX; used primarily
88 in human medicine) and sulfamethazine (SMZ; used primarily in food animal production) are among
89 the most often SM residues detected in various contaminations sources (waste water, digestate and
90 manure) and in the different compartments of the environment (soil, sediment, surface- and ground-
91 water) (Table 1). The repeated fertilization of cropland with animal manure containing veterinary
92 antibiotics (VAs) is one of the main contamination routes of surface water resources (Kemper 2008).
93 While their concentrations in surface water worldwide vary between a few and several dozen ng.L^{-1}
94 (Kolpin et al. 2002; Veach and Bernot 2011; Bernot et al. 2013; Iglesias et al. 2014; Matongo et al.
95 2015; Grenni et al. 2018), peak concentrations can sometimes reach several $\mu\text{g.L}^{-1}$ in different rivers
96 (Yargau et al. 2007; Peng et al. 2008; Lacina et al. 2012; Matongo et al. 2015). In France, a survey
97 performed in the Seine River revealed that SMX was detected in all samples, with a maximum
98 concentration of 544 ng.L^{-1} (Tamtam et al. 2008). In the US, a study showed that 20% of groundwater
99 samples were contaminated with SMX at concentrations of up to $1.0 \mu\text{g.L}^{-1}$ which are in the range of
100 pesticide concentrations observed in agricultural streams (Barnes et al. 2008). A recent review of the
101 maximum concentrations of the most frequently detected SMs in contamination sources and in
102 different environmental compartments including soils, surface and ground waters can be found in
103 Deng et al. (2018).

104 SMs are broad-spectrum bacteriostatic agents that inhibit the biosynthesis of folic acid through
105 competitive inhibition of dihydropteroate synthase, blocking the condensation of 6-hydroxymethyl-
106 7,8-dihydropteridine pyrophosphate to para-aminobenzoic acid (Petri 2001). Although known as
107 recalcitrant compounds, SMs can be metabolized, the most frequently detected metabolites being

108 the 3-amino-5-methylisoxazole and 2-amino-4,6-dimethylpyrimidine for SMX and SMZ, respectively
109 (Ricken et al. 2013) (Figure 1). Strains belonging to the genus *Rhodococcus*, known to co-metabolize
110 hydrocarbons, co-metabolically degrade SMX as well as other SMs such as sulfamethizole (Gauthier
111 et al. 2010; Larcher and Yargeau 2011). Other strains belonging to the class of γ -proteobacteria have
112 been shown to co-metabolically degrade various SMs (Zhang et al. 2012a; Zhang et al. 2012b; Mao et
113 al. 2018). The co-metabolic transformation or biodegradation of sulfonamide antibiotics by various
114 microorganisms has recently been reviewed (Deng et al. 2018; Chen and Xie 2018; Wang and Wang
115 2018).

116 Chronic or punctual exposure of natural soil microbial communities to SMs can enhance their
117 capacities to degrade this class of antibiotic. Indeed, repeated long-term treatment of agricultural
118 soil with SMZ resulted in an increase of SMZ mineralization capacities of autochthonous microbial
119 communities, at least partially due to the selection of a resistant bacterial population
120 (*Microbacterium* sp. strain C448) able to metabolize and grow at the expense of SMZ and other
121 sulfonamides (Topp et al. 2013). *Microbacterium* C448 mineralizes the benzylic ring of the molecule,
122 excreting the pyrimidine moiety as an end product of metabolism (Topp et al. 2013) (Figure1). Two
123 other *Microbacterium* strains, BR1 (Ricken et al. 2013) and SDZm4 (Tappe et al. 2013) were isolated
124 from a waste water treatment plant and from a lysimeter exposed to pig manure, respectively.
125 Biodegradation of SMs was subsequently reported in members of the phyla Proteobacteria
126 (*Pseudomonas* (Jiang et al. 2014), *Ochrobactrum* (Mulla et al. 2018) and *Acinetobacter* (Wang et al.
127 2018)) and Actinobacteria (*Microbacterium* (Ricken et al. 2013; Tappe et al. 2013; Topp et al. 2013);
128 *Arthrobacter* (Deng et al. 2016), *Gordonia* (Mulla et al. 2018), and *Leucobacter* (Reis et al. 2018)). SM
129 degradation of *Microbacterium* sp. BR1 is initiated by an *ipso*-substitution (Ricken et al. 2013)
130 followed by a ring cleavage in the downstream pathway (Ricken et al. 2015). The *sadABC* gene cluster
131 encodes two monooxygenases (SadA and SadB) and one flavine mononucleotide (FMN) reductase
132 (SadC). SadA and SadC initiate the transformation of SMs yielding to the release of 4-aminophenol.
133 This intermediate is then transformed by SadB and SadC to 1,2,4-trihydroxybenzene which is entirely
134 mineralized (Ricken et al. 2017). This gene cluster is nearly identical (between 97 and 99% similarity)
135 in the draft genomes of *Microbacterium* sp. C448 (Martin-Laurent et al. 2015), *Microbacterium* sp.
136 SDZm4 (Tappe et al. 2013) and *Arthrobacter* sp. D2 (Deng et al. 2016), suggesting that it might be
137 dispersed through horizontal exchange in the *Actinobacteria* phylum (Ricken et al. 2017). Overall, the
138 widespread distribution of sulfonamide-biodegrading bacteria presumably decreases the
139 environmental persistence of these antibiotics, and thus the selection pressure for resistance
140 development. Bioaugmentation of soil with *Microbacterium* sp. strain C448 decreased the
141 persistence of SMZ, indicating that bioremediation is an option for dealing with pollution hotspots
142 (Hirth et al. 2016).

143

144 In addition to their crucial role in the dissipation of pollutants including SMs, natural microbial
145 communities in soil and water are responsible for a range of ecosystem functions and services.
146 However, ecotoxicological effects of antibiotics and notably of SMs on microbial communities are still
147 understudied, and there is a crucial need to investigate their impact on biogeochemical processes to
148 further assess their possible adverse effects on ecosystem functioning (Roose-Amsaleg and Laverman
149 2016). This is notably the case for surface waters such as small rivers draining agricultural areas
150 including livestock production farms or crop fields amended with manure. In these lotic ecosystems,
151 most of the microbial activities are assumed by benthic communities which develop on hard and soft
152 immersed substrates and which are composed of both phototrophic (mainly algae and
153 cyanobacteria) and heterotrophic (mainly bacteria, fungi, and protozoa) microorganisms.
154 Pharmaceutical accumulation in these microbial assemblages has been occasionally reported (Writer
155 et al. 2013; Wunder et al. 2016; Huerta et al. 2016). For instance, SMX tends to progressively
156 accumulate in river biofilms (Corcoll et al. 2015). Exposure of aquatic microbial communities to
157 antibiotics spiked at chronic concentration in microcosm was also found to induce resistance
158 strategies at community level including microbial degradation of antibiotics (Vila-Costa et al., 2017).
159 Environmental exposure to antibiotics potentiates the environmental reservoir of antibiotic
160 resistance increasing the threat to both human and animal health (Wright 2010; Ashbolt et al. 2013;
161 Finley et al. 2013; Berendonk et al. 2015). One can distinguish two kinds of resistance to
162 sulfonamides. On the one hand, 3- of 6-bp duplication within *sulA*, the chromosomal gene encoding
163 for dihydropteroate synthase, was shown to confer resistance to sulfonamides to *Streptococcus*
164 *pneumoniae* (Lopez *et al.*, 1987; Maskell *et al.* 1997). On the other hand, sulfonamide resistance
165 involves the acquisition of an additional dihydropteroate synthase (i.e. *sul1*, *sul2* and *sul3*) on a
166 mobile genetic element that is not inhibited by this class of antibiotics and that bypass the inhibition
167 of the enzyme encoded by the chromosomal genes. The *sul* resistance genes, located within
168 integrons (Type 1 integrase) and carried on conjugative plasmids, are widely distributed in SM-
169 resistant bacteria (Heuer et al. 2011). These genes have been detected in various aquatic ecosystems
170 (e.g. Pei et al. 2006; Pruden et al. 2007; Luo et al. 2010; McKinney et al. 2010; Stoll et al. 2012). In
171 addition, zinc was found to increase the abundance of *sul* resistance genes in intestine of weaned
172 pigs fed with high dietary zinc supplementation as an alternative to antibiotics (Vahjen et al. 2015).
173 This suggests that heavy metals can co-select antibiotic resistances by exerting a selection pressure
174 favorable to the maintenance of mobile mosaic gene structures harboring both heavy metal and
175 antibiotic resistances in the environment (Vahjen et al. 2015). Nevertheless, there are relatively few
176 studies documenting their dispersal and survival from different sources of contaminations in
177 agroecosystems microbiota, particularly with respect to the terrestrial to aquatic continuum.

178 Ecotoxicological risks and effects of SMs in agroecosystems are also largely understudied. Recently, a
179 few papers proposed different types of usage pattern-based exposure to prioritize antibiotics to be
180 considered in environmental risk assessment (ERA) (Di Nica et al. 2015; Menz et al. 2015). These
181 approaches are based on the calculation of the risk quotient (RQ) as the ratio of predicted
182 environmental concentration (PEC) over the predicted no effect concentration (PNEC). RQ values
183 permit to predict the environmental risk for each antibiotic in the phase II of environmental risk
184 assessment. However, besides *a priori* ERA of SMs including toxicological test on very sensitive
185 species such as *Anabaena*, very few studies report their ecotoxicological impact on microbial
186 communities. The first studies dealing with the effects of SMs on aquatic microbial communities
187 focused on bacteria from activated sludge (Xia et al. 2012; Collado et al. 2013). SMX was found to
188 shift bacterial community structure but did not modify microbial functions such as organic carbon
189 and nitrogen removal. However, SMs induced large shifts in the transcriptome of microbial
190 communities in the gene categories related to N, P and C cycling (Yergeau et al. 2012). A negative
191 exponential relationship between denitrifying gene abundance and SMZ concentrations was
192 observed in estuarine and coastal sediments (Hou et al. 2015). This decrease in the presence of SMZ
193 led to a reduction of denitrification rates. A mechanistic model to interpret the toxic effects of SMs
194 on nitrification in activated sludge system was proposed (Huang et al. 2016). Effects of SM exposure
195 on fungi and algae co-existing with bacteria in aquatic biofilms are still unknown. In soil, microbial
196 communities exposed to SMs experienced an overall reduction in the bacteria/fungi biomass ratio
197 suggesting that fungi consumed nutrients released by bacteria killed by the antibiotic (Gutierrez et al.
198 2010; Pinna et al. 2012). This response might also occur to microbial communities in antibiotic-
199 contaminated river ecosystems but may be modulated by the difference in proportion of bacteria,
200 algae and fungi among different benthic substrata (cobbles, sediment, leaves) (Artigas et al. 2009;
201 Mora-Gomez et al. 2016). Simple additive effects occur with binary mixtures of SMs (SMZ and SMX)
202 on the growth inhibition of the freshwater green algae *P. subcapitata* (Yang et al. 2008). Diatoms and
203 cyanobacteria were found to be more sensitive to pharmaceutical mixtures including SMs than to
204 single antibiotics (Van der Grinten et al. 2010; Perron and Juneau 2011). Few studies have assessed
205 how changes in microbial composition and activity of river biofilms in response to antibiotic exposure
206 could lead to perturbation of ecosystem functions like nutrient cycling.

207

208 **3. The ANTIBIOTOX project**

209 Within this context, ANTIBIOTOX ('Fate of antibiotics and associated resistance genes in
210 agroecosystems: ecotoxicological risk for functional microbial communities of receiving river
211 systems) is an innovative project funded by the French ANR (Agence National de Recherche; contract
212 n° ANR-17-CE34-0003, 2018-2022) which aims to develop a multidisciplinary approach combining

213 chemistry, biochemistry, environmental microbiology, bacterial genetics, molecular biology and
214 ecotoxicology to (i) study transcriptomic and proteomic regulation during the time-course of SMs
215 biodegradation in *Microbacterium* sp. C448, (ii) characterize the biodegradation pathways and
216 estimate the ecotoxicological impact of microbial biodegradation of SMs in aquatic and terrestrial
217 compartments of agroecosystems, (iii) monitor the dissemination of SM-resistant and SM-degrading
218 bacteria and genes from agricultural soil to adjacent water resources, (iv) estimate the impact of SM
219 residues (and transformation products) on the abundance of resistant and degrading microbes and
220 microbial functions in the aquatic ecosystem and (v) communicate and promote results as well as
221 contribute to higher education programs in order to disseminate the main outcomes of this research
222 program. ANTIBIOTOX is focused on two SMs, namely sulfamethoxazole and sulfamethazine which
223 are used in human medicine and in food animal production. For example, 20% of the veterinary
224 antibiotics (147 tons of active substance per year) sold in France are in the SM class (ANSES 2015).
225 Sulfonamides are frequently detected in various aquatic environments (Deng et al., 2018). Overall,
226 this multidisciplinary project is expected to provide new insights in the ecotoxicological impact of
227 veterinary/human antibiotic residues on functional microbial diversity along the soil-water
228 continuum.

229

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236

237 **References**

238 ANSES (2015) Rapport annuel Médicaments vétérinaires contenant des antibiotiques en France en
239 2014

240 Artigas J, Romaní AM, Gaudes A, Muñoz I, Sabater S (2009) Benthic structure and metabolism in a
241 Mediterranean stream: from biological communities to the whole stream ecosystem function.
242 *Freshwater Biol* 50: 2025-2036

243 Ashbolt NJ, Amézquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH,
244 Heberer T, Lawrence JR, Larsson DGJ, McEwen SA, Ryan JJ, Schönfeld J, Silley P, Snape JR, Van den
245 Eede C, Topp E (2013) Human health risk assessment (HHRA) for environmental development and
246 transfer of antibiotic resistance. *Environ Health Persp* 121:993-1001

247 Baran W, Sochacka J, Wardas WAA (2006) Toxicity and biodegradability of sulfonamides and products
248 of their photocatalytic degradation in aqueous solutions. *Chemosphere* 65: 1295-1299

249 Barnes KK, Koplín DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB (2008) A national reconnaissance
250 of pharmaceuticals and other organic wastewater contaminants in the United States — I)
251 Groundwater. *Sci Tot Environ* 402: 192-200

252 Barra Caracciolo A, Topp E, Grenni P (2015) Pharmaceuticals in the environment: Biodegradation and
253 effects on natural microbial communities. A review. *J Pharm Bio Anal* 106: 25-36

254 Batt AL, Kostich MS, Lazorchak JM (2008) Analysis of ecologically relevant pharmaceuticals in
255 wastewater and surface water using selective solid-phase extraction and UPLC-MS/MS. *Anal Chem.*
256 50: 5021-5030

257 Bengtsson-Palme J, Larsson JDG, 2016. Concentrations of antibiotics predicted to
258 select for resistant bacteria: Proposed limits for environmental regulation. *Environ Int* 86: 140-149

259 Bengtsson-Palme J, Kristiansson E, Larsson DGJ, 2018. Environmental factors influencing the
260 development and spread of antibiotic resistance. *FEMS Microbiol Rev* 1: 42

261 Berendonk TU, Manaia CM., Merlin C, Fatta-Kassinos D, Cytryn E, Walsh F, Burgmann H, Sorum H,
262 Norstrom M, Pons MN, Kreuzinger N, Huovinen P, Stefani S, Schwartz T, Kisand V, Baquero F,
263 Martinez JL (2015) Tackling antibiotic resistance: the environmental framework. *Nat Rev Micro* 13:
264 310-317

265 Bernot MJ, Smith L, Frey J (2013) Human and veterinary pharmaceutical abundance and transport in
266 a rural central Indiana stream influenced by confined animal feeding operations (CAFOs). *Sci Total*
267 *Environ* 445–446: 219-230

268 Blanco G, Lemus J A, Grande J (2009). Microbial pollution in wildlife: Linking agricultural manuring
269 and bacterial antibiotic resistance in red-billed choughs. *Microbial Environ Res* 109: 405–412

270 Brandt K K, Sjøholm OR, Krogh K A, Halling-Sørensen B, Nybroe O (2009) The environmental release
271 and fate of antibiotics. *Environ Sci Technol* 43: 2963-2968.

272 Brandt KK, Amézquita A, Backhaus T, Boxall A, Coors A, Heberer T, Lawrence JR, Lazorchak J,
273 Schönfeld J, Snape JR, Zhu YG, Topp E (2015) Ecotoxicological assessment of antibiotics and other
274 compounds with antimicrobial properties: a call for improved consideration of microorganisms.
275 *Environ Int* 85:189-205

276 Bottoni P, Caroli S (2015) Detection and quantification of residues and metabolites of medicinal
277 products in environmental compartments, food commodities and workplaces. A review. *J Pharm Bio*
278 *Anal* 106: 3-24

279 Boxall ABA (2004) The environmental side effects of medication: How are human and veterinary
280 medicines in soils and water bodies affecting human and environmental health? *EMBO Reports* 5:
1110-1116

281 Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-Jeanjean N, Mackie RI (2001) Occurrence and
282 Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine
283 Production Facilities. *Appl Environ Microbiol* 67: 1494–1502

284 Chen J, Xie S (2018) Overview of sulfonamide biodegradation and the relevant pathways and
285 microorganisms. *Sci Tot Environ* 640-641: 1465-1477

286 Chunhui Z, Liangliang W, Xiangyu G, Xudan H (2016) Antibiotics in WWTP discharge into the Chaobai
287 river, Beijing. *Arch Environ Prot.* 42: 48-57

288 Chitescu CL, Oosterink E, De Jong J, Stolker AAM (2012) Accurate mass screening of pharmaceuticals
289 and fungicides in water by U-HPLC-Exactive Orbitrap MS. *Anal Bioanal Chem.* 403: 2997e3011.
290 <https://doi.org/10.1007/s00216-012-5888-8>

291 Collado N, Buttiglieri G, Marti E, Ferrando-Climent L, Rodriguez-Mozaz S, Barcelo D, Comas J,
292 Rodriguez-Roda I (2013) Effects on activated sludge bacterial community exposed to
293 sulfamethoxazole. *Chemosphere* 93: 99-106

294 Corcoll N, Casellas M, Huerta B, Guasch H, Acuña V, Rodriguez-Mozaz S, Serra-Compte A, Barcelo D,
295 Sabater S (2015) Effects of flow intermittency and pharmaceutical exposure on the structure and
296 metabolism of stream biofilms. *Sci Tot Environ* 503-504: 159-170

297 Deng Y, Li B, Zhang T (2018) Bacteria that make a meal of sulfonamide antibiotics: blind spots and
298 emerging opportunities. *Environ Sci Technol* 52: 3854–3868

299 Deng Y, Mao Y, Li B, Yang C, Zhang T (2016) Aerobic degradation of sulfadiazine by *Arthrobacter* spp.:
300 kinetics, pathways and genomic characterization. *Environ Sci Technol* 50: 9566-9575

301 De Vries SL, Zhang P (2016) Antibiotics and the terrestrial nitrogen cycle: a review. *Curr Pollution Rep.*
302 2: 51-67

303 de Weert J, Smedes F (2015) Passive sampling van grondwater. Een trial in peilbuizen van het
304 provinciaal meetnet van de provincie Noord-Brabant. Deltares, Delft, the Netherlands. Report no
305 210074-000-BGS-0009, 1-44

306 Di Nica V, Menabelli L, Azimonti G, Finizio A (2015) RANKVET: a new ranking method for comparing
307 and prioritizing the environmental risk of veterinary pharmaceuticals. *Ecol Indic* 52: 270-276

308 Finley RL, Collignon P, Larsson DGJ, McEwen SA, Li XZ, Gaze WH, Reid-Smith R, Timinouni M, Graham
309 DW, Topp E (2013) The scourge of antibiotic resistance: the important role of the environment. *J Clin*
310 *Infect Dis* 57: 704-710

311 Gao P, Munir M, Xagorarakis I (2012) Correlation of tetracycline and sulfonamide antibiotics with
312 corresponding resistance genes and resistant bacteria in a conventional municipal wastewater
313 treatment plant. *Sci Tot Environ* 421: 173-183

314 Gauthier H, Yargeau V, Cooper DG (2010) Biodegradation of pharmaceuticals by *Rhodococcus*
315 *rhodochrous* and *Aspergillus niger* by co-metabolism. *Sci Tot Environ* 408: 1701-1706

316 Ghiglione JF, Martin-Laurent F, Pesce S (2016) Microbial ecotoxicology: an emerging discipline facing
317 contemporary environmental threats. *Environ Sci Pollut Res* (2016) 23: 3981–3983

318 González-Pleiter M, Gonzalo S, Rodea-Palomares I, Leganés F, Rosal R, Boltes K, Marco E, Fernández-
319 Piñas F (2013) Toxicity of five antibiotics and their mixtures towards photosynthetic aquatic
320 organisms: Implications for environmental risk assessment. *Water Res* 47: 2050-2064

321 Grenni P, Ancona V, Caraciollo AB (2018) Ecological effects of antibiotics on natural ecosystems: a
322 review. *Microchem J.* 136: 25-39

323 Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson, DI (2011). Selection of
324 Resistant Bacteria at Very Low Antibiotic Concentrations. *PLoS Pathog* 7(7): e1002158

325 Gutierrez IR, Watanabe N, Harter T, Glaser B, Radke M (2010) Effect of sulfonamide antibiotics on
326 microbial diversity and activity in a Californian Mollic Haploxyeralf. *J Soils Sediments* 10: 537–544

327 Heuer H, Schmitt H, Smalla K (2011) Antibiotic resistance gene spread due to manure application on
328 agricultural fields. *Curr Opin Microbiol* 14: 236–243

329 Hirth N, Topp E, Dörfler U, Stupperich E, Munch JC, Schroll R (2016) An effective bioremediation
330 approach for enhanced microbial degradation of the veterinary antibiotic sulfamethazine in an
331 agricultural soil. *Chem Biol Technol Agric* 3: 29.

332 Hou L, Yin G, Liu M, Zhou J, Zheng Y, Gao J, Zong H, Yang Y, Gao L, Tong C (2015) Effects of
333 sulfamethazine on denitrification and the associated N₂O release in estuarine and coastal sediments.
334 *Environ Sci Technol* 49: 326-333

335 Hruska K, Franek, M (2012) Sulfonamides in the environment: a review and a case report. *Veterinari
336 Medicina.* 57: 1–35

337 Huang X, Feng Y, Hu C, Xia X, YU D, Zou X (2016) Mechanistic model for interpreting the toxic effects
338 of sulfonamides on nitrification. *J Haz Mat* 305: 123-129

339 Huerta B, Rodríguez-Mozaz S, Ponsatí L, Acuña V, Sabater S, Barceló D (2016) Determination of a
340 broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water
341 treatment plant-impacted river. *Sci Tot Environ* 540: 241-249

342 Iglesias A, Nebot C, Vazquez BI, Miranda JM, Abuin CFM, Cepeda A (2014) Detection of veterinary
343 drug residues in surface waters collected nearby farming areas in Galicia, North of Spain. *Environ Sci
344 Pollut Res* 21: 2367-2377

345 Jiang B, Li A, Cui D, Cai R, Ma F, Wang Y (2014) Biodegradation and metabolic pathway of
346 sulfamethoxazole by *Pseudomonas psychrophila* HA-4, a newly isolated cold-adapted
347 sulfamethoxazole-degrading bacterium. *Appl Microbiol Biotechnol* 98: 4671-4681

348 Knapp CW, Dolfing J, Ehlert PAI, Graham DW (2010) Evidence of Increasing Antibiotic Resistance
349 Gene Abundances in Archived Soils since 1940. *Environ Sci Technol* 44: 580–587

350 Kemper N (2008) Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol Indicat* 8: 1-
351 13

352 Koike S, Krapac IG, Oliver HD, Yannarell AC, Chee-Sanford J, Aminov RI, Mackie RI (2007) Monitoring
353 and Source Tracking of Tetracycline Resistance Genes in Lagoons and Groundwater Adjacent to
354 Swine Production Facilities over a 3-Year Period. *Appl Environ Microb* 73:4813–4823

355 Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT (2002) Response to
356 Comment on “Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S.
357 Streams, 1999–2000: A National Reconnaissance”. *Environ Sci Technol* 36: 1202-1211

358 Lacina P, Zenatova P, Vavrova M (2012). The assessment of contamination of selected river streams
359 in the Czech Republic by human and veterinary drug residues with liquid and gas chromatography.
360 *Fresenius Environ Bull* 21: 3318-3324

361 Larcher S, Yargeau V (2011) Biodegradation of sulfamethoxazole by individual and mixed bacteria.
362 *Appl Microbiol Biotechnol* 91: 211-218

363 Larsson DGJ, Andremont A, Bengtsson-Palme J, Brandt KK, de Roda Husman AM, Fagerstedt P, Fick J,
364 Flach C-F, Gaze WH, Kuroda M, Kvint K, Laxminarayan R, Manaia CM, Nielsen KM, Plant L, Ploy M-C,
365 Segovia C, Simonet P, Smalla K, Snape J, Topp E, van Hengel AJ, Verner-Jeffreys DW, Virta MPJ,
366 Wellington EM, Wernersson A-S. 2018. Critical knowledge gaps and research needs related to the
367 environmental dimensions of antibiotic resistance. *Environ Int* 117:132-138.

368 Lopez P, Espinosa M, Greenberg B, Lacks SA (1987) Sulfonamide resistance in *Streptomyces*
369 *pneumoniae*: DNA sequence of the gene encoding dihydropteorate synthase and characterization of
370 the enzyme. *J. Bacteriol.* 169: 4320-43-26.

371 Lundström SV, Östma M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson
372 KM, Tysklind M, Flach CF, Larsson DGJ (2016) Minimal selective concentrations of tetracycline in
373 complex aquatic bacterial biofilms. *Sci Tot Environ* 553: 587-595

374 Luo Y, Mao D, Rysz M, Zhou Q, Zhang H, Xu L, Alvarez PJJ (2010) Trends in antibiotic resistance genes
375 occurrence in the Haihe River, China. *Environ Sci Technol* 44: 7220-7225

376 Manzetti S, Ghisi R, 2014. The environmental release and fate of antibiotics. *Marine Pollut Bull* 79: 7-
377 15

378 Mao F, Liu X, Wu K, Zhou C, Si Y (2018) Biodegradation of sulfonamides by *Shewanella oneidensis*
379 MR-1 and *Shewanella* sp. strain MR-4. *Biodegradation* 29, 129-140

380 Martin-Laurent F, Marti R, Waglechner N, Wright GD, Topp E (2014) Draft genome sequence of the
381 sulfonamide antibiotic-degrading *Microbacterium* sp. strain C448. *Genome Announcements*.
382 doi:10.1128/genomeA.01113-13

383 Maskell JP, Sefton AM, Hall LMC (1997) Mechanism of sulfonamide resistance in clinical isolates of
384 *Streptococcus pneumoniae*. *Antimicrob Agents Chemother.* 41: 2121-2126

385 Matongo S, Birungi G, Moodley B, Ndungu P (2015) Occurrence of selected pharmaceuticals in water
386 and sediment of Umgeni River, KwaZulu-Natal, South Africa. *Environ Sci Pollut Res* 22: 10298-10308

387 McKinney CW, Loftin KA, Meyer MT, Davis JG, Pruden A (2010) Tet and sul antibiotic resistance genes
388 in livestock lagoons of various operation type, configuration, and antibiotic occurrence. *Environ Sci*
389 *Technol* 44: 6102-6109

390 Menz J, Schneider M, Kümmerer K (2015) Usage pattern-based exposure screening as a simple tool
391 for the regional priority-setting in environmental risk assessment of veterinary antibiotics: A case
392 study of north-western Germany. *Chemosphere* 127: 42-48

393 Mora-Gómez J, Freixa A, Perujo N, Barral-Fraga L (2016) In *Aquatic Biofilms: Ecology, Water Quality*
394 *and Wastewater Treatment* (Romaní AM, Guasch H and Dolors M, Balaguer, eds). Caister Academic
395 Press

396 Mulla SI, Hu A, Sun Q, Li J, Suanona F, Ashfaq M, Yu C-P (2018) Biodegradation of sulfamethoxazole in
397 bacteria from three different origins. *J Environ Manag* 206: 93-102

398 Pei R, Kim SC, Carlson KH, Pruden A (2006) Effect of River Landscape on the sediment concentrations
399 of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Res* 40: 2427-2435

400 Peng X, Tan J, Yu Y, Wang Z (2008) Multiresidue determination of fluoroquinolone, sulfonamide,
401 trimethoprim, and chloramphenicol antibiotics in urban waters in China. *Environ Toxicol Chem* 27:
402 73-79

403 Petri W (2001) In: Goodman and Gilman's the Pharmacological Basis of Therapeutics, 10th ed.;
404 Hardman J G, Limbird L E, Eds.; McGraw-Hill: New York. pp. 1171-1188

405 Perron MC, Juneau P (2011) Effect of endocrine disrupters on photosystem II energy fluxes of green
406 algae and cyanobacteria. *Environ Res* 111: 520-529

407 Pinna MV, Casraldi P, Deiana P, Pusino A, Garau G (2012) Sorption behavior of sulfamethazine on
408 unamended and manure-amended soils and short-term impact on soil microbial community.
409 *Ecotoxicol Environ Safety* 84: 234-242

410 Pruden A, Messner N, Pereyra L, Hanson RE, Hiibel SR, Reardon KF (2007) The effect of inoculum on
411 the performance of sulfate-reducing columns treating heavy metal contaminated water. *Water Res*
412 41: 904-914

413 Pruden A, Larsson DGJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S,
414 Silley P, Snape JR, Topp E, Zhang T, Zhu YG (2013) Management Options for Reducing the Release of
415 Antibiotics and Antibiotic Resistance Genes to the Environment. *Environ Health Perspect* 121: 878-
416 885

417 Reis AC, Cvanarova M, Liu Y, Lenz M, Hettich T, Kolvenbach BA, Corvini PFX, Nunes OC (2018)
418 Biodegradation of sulfamethoxazole by a bacterial consortium of *Achromobacter denitrificans* PR1
419 and *Leucobacter* sp. GP. *Appl Microbiol Biotechnol* 102: 10299-10314

420 Ratsak C, Guhl B, Zühlke S, Delschen T (2013) Veterinärantibiotikarückstände in Gülle und Gärresten
421 aus Nordrhein-Westfalen. *Environ Sci Europe*. 25:7. <http://www.enveurope.com/content/25/1/7>

422 Ricken B, Corvini PF, Cichocka D, Parisi M, Lenz M, Wyss D, Martínez-Lavanchy PM, Müller JA,
423 Shahgaldian P, Tulli LG, Kohler HP, Kolvenbach BA (2013). *Ips*-Hydroxylation and Subsequent
424 Fragmentation: a Novel Microbial Strategy To Eliminate Sulfonamide Antibiotics. *Appl Environ*
425 *Microbiol* 79: 5550-5558

426 Ricken B, Fellmann O, Kohler HP, Schäffer A, Corvini PFX, Kolvenbach BA (2015) Degradation of
427 sulfonamide antibiotics by *Microbacterium* sp. strain BR1 – elucidating the downstream pathway.
428 *New Biotechnol* 32: 710-715

429 Ricken B, Kolvenbach BA, Bergesch C, Benndorf D, Kroll K, Strnad H, Vlcek C, Adaixo R, Hammes F,
430 Shahgaldian P, Schäffer A, Kohler HPE, Corvini PFX (2017) FMNH₂-dependent monooxygenase initiate
431 catabolism of sulfonamides in *Microbacterium* sp. strain BR1 subsisting on sulfonamide antibiotics.
432 *Scientific Reports* 7 : 15783.

433 Roose-Amsaleg C, Laverman AM (2016) Do antibiotics have environmental side-effects? Impact of
434 synthetic antibiotics on biogeochemical processes. *Environ Sci Pollut Res* 23: 4000-4012

435 Sapkota AR, Curriero FC, Gibson KE, Schwab KJ (2007) Antibiotic-Resistant Enterococci and Fecal
436 Indicators in Surface Water and Groundwater Impacted by a Concentrated Swine Feeding Operation.
437 *Environ Health Perspect* 115: 1040–1045

438 Stoll C, Sidhu JPS, Tiehm A, Toze S (2012) Prevalence of clinically relevant antibiotic resistance genes
439 in surface water samples collected from Germany and Australia. *Environ Sci Technol* 46: 9716-9726

440 Storteboom H, Arabi M, Davis JG, Crimi B, Pruden A (2010) Tracking antibiotic resistance genes in the
441 South Platte River basin using molecular signatures of urban, agricultural, and pristine sources.
442 *Environ Sci Technol* 44: 7397–7404

443 Tamtam F, Mercier F, Le Bot B, Eurin J, Dinh QT, Clément M, Chevreuil M (2008) Occurrence and fate
444 of antibiotics in the Seine River in various hydrological conditions. *Sci Total Environ* 393 : 84-95

445 Tappe W, Herbst M, Hofmann D, Koepchen S, Kummer S, Thiele B, Groeneweg J (2013) Degradation
446 of Sulfadiazine by *Microbacterium lacus* Strain SDZm4, Isolated from Lysimeters Previously Manured
447 with Slurry from Sulfadiazine-Medicated Pigs. *Appl Environ Microbiol* 79: 2572-2577

448 Topp E, Chapman R, Devers-Lamrani M, Hartmann A, Martin-Laurent F, Marti R, Sabourin L, Scott A,
449 Sumaraha M (2013) Accelerated Biodegradation of Veterinary Antibiotics in Agricultural Soil
450 following Long-Term Exposure, and Isolation of a Sulfamethazine-degrading *Microbacterium* sp. *J*
451 *Environ Qual* 42: 173-178

452 Vahjen W, Pietruszynska D, Starke IC, Zentek J (2015) High dietary zinc supplementation increases
453 the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs. *Gut*
454 *Pathog* 23: 1-7

455 Van der Grinten E, Pikkemaat MG, Van den Brandhof E-J, Stroomberg GJ, Kraak MHS (2010)
456 Comparing the sensitivity of algal, cyanobacterial and bacterial bioassays to different groups of
457 antibiotics. *Chemosphere* 80: 1-6

458 Veach AM, Bernot MJ (2011) Temporal variation of pharmaceuticals in an urban and agriculturally
459 influenced stream. *Sci Total Environ.* 409, 4553-4563

460 Vila-Costa M, Gioia R, Aceña J, Pérez S, Casamayor EO, Dachs J (2017) Degradation of sulfonamides as
461 a microbial resistance mechanism. *Water Res* 115: 309–317

462 Wang J, Wang S (2018) Microbial degradation of sulfamethoxazole in the environment. *Appl*
463 *Microbiol Biotechnol* 102: 3573-3582

464 Wang S, Hu Y, Wang J (2018) Biodegradation of typical pharmaceutical compounds by a novel strain
465 *Acinetobacter* sp. *J Environ Manag* 217:240–246

466 Wright GD (2010) Antibiotic resistance in the environment: a link to the clinic? *Curr Opin Microbiol*
467 13: 589–594

468 Writer JH, Antweiler RC, Ferrer I, Ryan JN, Thurman EM (2013) In-stream attenuation of neuro-active
469 pharmaceuticals and their metabolites. *Environ Sci Technol* 47: 9781-9790

470 Wunder DB, Bosscher VA, Cok RC, Hozalski RM (2011) Sorption of antibiotics to biofilm. *Water Res*
471 45: 2270-2280

472 Xia S, Jia R, Feng F, Xie K, Li H, Jing D, Xu X (2012) Effect of solids retention time on antibiotics
473 removal performance and microbial communities in an A/O-MBR process. *Bioresource Technol* 106:
474 36-43

475 Yang LH, Ying GG, Su HC, Stauber JL, Adams MS, Binet MT (2008) Growth-inhibiting effects of 12
476 antibacterial agents and their mixtures on the freshwater microalga *Pseudokirchneriella subcapitata*.
477 *Environ Toxicol Chem* 27: 1201-1208

478 Yargau V, Lopata A, Metcalfe C. 2007. Pharmaceuticals in the Yamaska River, Quebec, Canada.
479 Service de l'étude du milieu aquatique. 1985. La Yamaska, une rivière à rajeunir. Ministère de
480 l'environnement du Québec, Direction générale de l'assainissement de l'eau, Direction des études du
481 milieu aquatique, Québec.

482 Yergeau E, Sanschagrin S, Waiser MJ, Lawrence JR, Greer CW (2012) Sub-inhibitory concentrations of
483 different pharmaceutical products affect the meta-transcriptome of river biofilm communities
484 cultivated in rotating annular reactors. *Environ Microb Report*, 4: 350-359

485 Zhang WW, Wen YY, Niu ZL, Yin K, Xu DX, Chen LX (2012a) Isolation and characterization of
486 sulfonamide-degrading bacteria *Escherichia* sp. HS21 and *Acinetobacter* sp. HS51. *World J Microbiol*
487 *Biotechnol* 28: 447-452

488 Zhang WW, Xu D, Niu ZL, Yin K, Liu P, Chen LX (2012b) Isolation and characterization of *Pseudomonas*
489 sp. DX7 capable of degrading sulfadoxine. *Biodegradation* 23: 431-439

490

491

492 **Table 1.** Occurrence of sulfamethoxazole and of sulfamethazine in different environmental matrices
 493 (soil, sediment, surface- and ground-water) and in various sources of contamination (waste water,
 494 digestate and manure)

Antibiotic	Matrice	Place ^a (#of studies)	Concentrations ^{b,c}	Reference	
Sulfamethoxazole	Soil	Ch (4), Sp (1), US (1)	19.3 ^b	De Vries and Zhang, 2016	
	Sediment	Ch (5), US (2), K (1), Sp (1)	0.5 ^b	De Vries and Zhang, 2016	
		Surface water	It	1.8-2.4 ^c	Grenni et al., 2018
			It	68 ^c	Grenni et al., 2018
			Ca	578 ^c	Yargau et al., 2007
	US	150 ^c	Kolpin et al., 2002		
	Ground water	Nt(3)	50-75 ^c	Chitescu et al., 2012	
	Waste water	US (7)	98-2200 ^c	Batt et al., 2008	
	Digestate	Ge	880 ^b	Ratsak et al., 2013	
Manure	Ge	nd	Ratsak et al., 2013		
Sulfamethazine	Soil	Ch (4), US (1), K (1)	3.2 ^b	De Vries and Zhang, 2016	
	Sediment	Ch (6), US (2), K (1)	2.9 ^b	De Vries and Zhang, 2016	
	Surface water	US	6 ^c	Bernot et al., 2013	
		US	20 ^c	Kolpin et al., 2002	
	Ground water	Nt (2)	21 ^c	de Weert and Smedes, 2015	
	Waste water	Ch	382 ^c	Chunhui et al., 2016	
	Digestate	Ge	160 ^b	Ratsak et al., 2013	
	Manure	Ge	700 ^b	Ratsak et al., 2013	

495 ^aChina (Ch), Germany (Ge), Korea (K), Netherland (Nt), Spain (Sp), United States (US); ^bin $\mu\text{g}\cdot\text{kg}^{-1}$; ^cin $\text{ng}\cdot\text{L}^{-1}$

496

497 **Figure Caption**

498 **Figure 1.** Overview of the main microbial biodegradation pathways of SMX and SMZ (co-metabolic
499 pathways: brown arrows; metabolic pathways: black arrows). 1: 3-amino-5-methylisoxazole, 2: 4-
500 aminobenzenesulfonamide, 3:4-aminothiophenol, 4: aniline, 5: 4-aminophenol, 6: 2,4-
501 dihydroxyphenol, 7: hydroquinone, 8: N-hydroxymethyl-SMX, 9: N-hydroxyacetyl-SMX, 10: 4-
502 hydroxybenzenesulfonic acid, 11: 3-hydroxylamine-amino-5-carboxyl, 12: 4-hydroxy-N-(5-methyl-3-
503 isoxazole)benzene-1-sulfonamide, 13: 4-aminobenzenesulfonic acid. A: 2-amino-4,6-
504 dimethylpyrimidine.

