Environ Sci Pollut Res (2016) 23:4000–4012
DOI 10.1007/s11356-015-4943-3

REVIEW ARTICLE

Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes

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Received: 31 January 2015 / Accepted: 22 June 2015 / Published online: 8 July 2015

Abstract Antibiotic use in the early 1900 vastly improved human health but at the same time started an arms race of antibiotic resistance. The widespread use of antibiotics has resulted in ubiquitous trace concentrations of many antibiotics in most environments. Little is known about the impact of these antibiotics on microbial processes or "non-target" organisms. This mini-review summarizes our knowledge of the effect of synthetically produced antibiotics on microorganisms involved in biogeochemical cycling. We found only 31 articles that dealt with the effects of antibiotics on such processes in soil, sediment, or freshwater. We compare the processes, antibiotics, concentration range, source, environment, and experimental approach of these studies. Examining the effects of antibiotics on biogeochemical processes should involve environmentally relevant concentrations (instead of therapeutic), chronic exposure (versus acute), and monitoring of the administered antibiotics. Furthermore, the lack of standardized tests hinders generalizations regarding the effects of antibiotics on biogeochemical processes. We investigated the effects of antibiotics on biogeochemical N cycling, specifically nitrification, denitrification, and anammox. We found that environmentally relevant concentrations of fluoroquinolones and sulfonamides could partially inhibit denitrification. So far, the only documented effects of antibiotic inhibitions were at therapeutic doses on anammox activities. The most studied and inhibited was nitrification (25–100 %) mainly at therapeutic doses and rarely environmentally relevant. We recommend that firm conclusions regarding inhibition of antibiotics at environmentally relevant concentrations remain difficult due to the lack of studies testing low concentrations at chronic exposure. There is thus a need to test the effects of these environmental concentrations on biogeochemical processes to further establish the possible effects on ecosystem functioning.

Keywords Antibiotic · Biogeochemical processes · Environmentally relevant concentration · Microbial ecotoxicology

Responsible editor: Philippe Garrigues

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Background and purposes

Since their discovery in the 1930s, antibiotics have considerably improved human and animal health and agricultural yields. However, their overuse soon caused problems such as ineffectiveness on pathogenic bacteria (Finley et al. 2013) due to the accelerated development of antibiotic resistant bacteria. Moreover, most antibiotics are not metabolized and are released into the environment via urine and feces. Concentrations of antibiotics can reach several mg/kg of sediment, especially in aquatic ecosystems downstream of manufacturing plants (see review in Larsson 2014) or aquaculture farms (e.g., Rico et al. 2014). Even in other environments such as river water or sediment, antibiotics often occur at concentrations from ng/L to μg/L or μg/kg. These environmental concentrations are generally too low to inhibit bacterial activity. On the contrary, inhibitory concentrations (see definition in Nordberg et al. 2009) are often referred to as therapeutic concentrations (≥1 mg/L). The effects of environmentally relevant concentrations of antibiotics can fundamentally differ from therapeutic concentrations, enhancing bacterial communication or

transcription regulation (Andersson and Hughes 2014; Davies and Ryan 2012). While many unknowns concerning chronic exposure to low doses remain, there is concern that low doses may favor and sustain genes for antibiotic resistance in the environment (Allen et al. 2010; Kümmerer 2009b).

Although antibiotics have been detected in many environments, their ecological effects have been poorly investigated, particularly concerning non-target bacteria and their related ecological functions. Antibiotics target bacteria by preventing their growth (bacteriostatic) or killing them (bactericidal). Antibiotics can be natural (i.e., produced by microorganisms in their own habitat) or of synthetic origin. Most antibiotics target one of three bacterial functions: cell wall biosynthesis, protein synthesis, or DNA replication and repair. Some antibiotics affect a wide range of pathogens (broad spectrum) while others specifically target a group of bacteria (e.g., Gram-positive bacteria). At the level of an individual bacterium, antibiotic resistance can develop by (1) pumping out the antibiotic, (2) destroying the active compounds of the antibiotic, or (3) reprogramming (or camouflaging) the target structure (Schmieder and Edwards 2012). At the ecosystem level (i.e., soil, sediment, or water), the microbial community and physical habitat can respond to antibiotic exposure or modulate its effects, with large physicochemical differences between ecosystems and consequently strong differences in response to antibiotic exposure. Ecosystems such as soils are extremely physicochemically complex and dynamic, supporting high microbial diversity. Abiotic inactivation of the antibacterial molecules can occur before ever reaching bacteria by interactions with soil organo-mineral compounds (e.g., clays), organic matter (Conkle and White 2012), or reactions such as photodegradation or hydrolysis (Thiele-Bruhn 2003). A lack of antibiotic effect can be due to antibiotic physicochemical properties such as a strong sequestration and low bioavailability (Rosendahl et al. 2012) and thus depends on the type of environmental matrix (solid or liquid). The antibiotic tolerance of a bacterial community, or more specifically of a bacterial function, may involve a shift in the community structure (composition, richness, density) or depend on the spatial distribution of members of the community. Bacterial biofilms, made up of an aggregation of microbes of different species surrounded by extracellular polymers, are more tolerant than planktonic bacteria because polymers are resistant to penetration of toxic compounds (Campos et al. 2001; Stewart 2002). Furthermore, the more tolerant bacteria in the biofilm benefit from the exposure and become the dominant members of the community (Knapp et al. 2008), or tolerant bacteria may develop on the dead remains of the biofilm (Kotzerke et al. 2011; Demoling et al. 2009; Kleineidam et al. 2010; Reichel et al. 2013; Yergeau et al. 2012).

Natural microbial communities are characterized by a large functional redundancy, with multiple species able to carry out the same process. Functional redundancy can allow a process to continue during antibiotic exposure despite modifications in community structure. Bacterial communities can also adapt quickly on hourly or monthly time frames. Only a few studies have considered both the effects on antibiotic exposure on community structure and ecosystem functioning via process rates (Näslund et al. 2008; Roose-Amsaleg et al. 2013; Underwood et al. 2011; Wunder et al. 2013; Yamamura et al. 2014).

Biogeochemical functioning of ecosystems relies largely on microbial activity, with ecosystem services such as nutrient cycling, organic matter production, and turnover or degradation of pollutants regulated by microbial metabolism (Ducklow 2008). In this mini-review, we investigated how environmentally relevant antibiotic concentrations affect biogeochemical functioning and thus disturb ecosystem processes. We were interested to know if antibiotics have environmental side-effects in addition to inducing and sustaining antibiotic resistance. Studies exploring effects of antibiotics on the microbial community structure and biomass will not be discussed here, only those investigating the side-effect on a biogeochemical process. We first briefly described the fate of antibiotics in the environment and the known microbiological effects of antibiotics and then provide an overview of the impact of antibiotics on biogeochemical processes. In the last section, we assessed the effects of antibiotics on the three major processes of the nitrogen cycle: nitrification, denitrification, and anammox. We discussed both acute and chronic antibiotic exposures, though we focused on the latter since it is more environmentally representative. We did not include tests directly conducted on purified enzymes, as the effect on enzyme activity is distinct from the effect of antibiotics on bacterial growth or life. We concluded that future studies should include both community analysis and process rate measurements, in order to establish a mechanistic relationship and explain the effects on biochemical processes mediated by antibiotic exposure.

Antibiotics in the environment

In the last 20 years, improved analytical capabilities have allowed the detection of antibiotic residues in virtually all natural habitats (Bell et al. 2013; Kümmerer 2009a; Thiele-Bruhn 2003). At least 1500 publications have addressed antibiotic molecules in the environment. Similarly, the spread of antibiotic resistance in hospitals and in the environment has been extensively discussed and studied with over 850 publications. Many of the studies report an inventory of antibiotic resistance genes (Nesme et al. 2014) and many address bacteria in aquatic environments (for review see Kümmerer 2009b) or soils (Gatica and Cytryn 2013). The resistance of bacterial species used as fecal indicators was also often investigated (Luczkiewicz

et al. 2013; Servais and Passerat 2009). However, despite the proliferation of environmental antibiotic studies in general, relatively few have considered the impact of antibiotics on "ecosystem health" (Gu 2014).

Microbiological effects of antibiotics in the environment

Regarding the effects of antibiotics in the environment, two aspects have to be considered: (1) the type of bacteria exposed to antibiotics in the environment and (2) the range of antibiotic concentrations to which these bacteria are exposed. While antibiotics can be lethal for pathogenic bacteria, little is known regarding their toxicity on microorganisms that are not the intended targets of a particular antibiotic (Nordberg et al. 2009). These non-target organisms comprise the majority of bacteria inhabiting natural environments. The vulnerability of bacteria to antibiotics is evaluated by measuring the minimum inhibitory concentration (MIC) (Andersson and Hughes 2014). A list of antibiotic MIC exists for bacteria and antibiotics of medical interest (European Committee on Antimicrobial Susceptibility Testing, data from the EUCAST MIC distribution website, last accessed 24 March 2015. http://www.eucast.org). Because more than 99 % of environmental bacteria are not able to be cultured (Amann et al. 1995), their MIC cannot be measured. Furthermore, MIC measures the acute lethal toxicity (Nordberg et al. 2009), the effect of high levels over short-term periods as revealed by mortality (Crane et al. 2006), of a single strain of a particular species. This strongly contrasts natural environments where chronic exposure occurs over long periods, sometimes representing a substantial portion of a microbial community's life-span, and toxicity is measured by observing mortality, growth, or reproduction (Nordberg et al. 2009).

Exposure to therapeutic levels of antibiotics can favor resistant phenotypes, representing a serious public health hazard. However, in environmentally relevant concentrations, vulnerable strains can continue to grow, though sometimes at a reduced rate (Andersson and Hughes 2014). Though some studies have extrapolated the effects of acute, high doses of antibiotics, there are likely non-linear thresholds and emergent behavior of sub-inhibitory doses in the environment. Sub-inhibitory doses of antibiotics can select for bacterial resistance *in vitro* originating from both enrichment of pre-existing antibiotic resistant bacteria and from selection of *de novo* resistant bacteria (Andersson and Hughes 2014). These doses further cause the acceleration and spread of resistance in bacteria affecting humans and animals. Sub-inhibitory levels of antibiotic on bacterial physiology can cause mutagenesis, virulence, biofilm formation, and horizontal gene transfer recombination, at least *in vitro*. Furthermore, environmentally relevant concentrations of antibiotics ranging from ng to μg/L or /kg could act as signaling molecules involved in quorum-sensing biofilm formation and virulence (Andersson and Hughes 2014).

Effects on biogeochemical processes

The consequences of antibiotics on biogeochemical processes are still not well-documented. To the present date (2015, March), we found 31 articles (Table 1) exploring effects of antibiotics on these processes. Identifying general patterns was complicated by the fact that there is no single standardized method to study the effect on biogeochemical processes. Nevertheless, a common way to study antibiotic effects on biogeochemical processes is to reproduce in the laboratory a simple system and then controlling several parameters, notably the antibiotic exposure. Depending on the nature of the environmental sample, different laboratory tests are used, such as batches (homogenization of the sample) or microcosms (intact soil or sediment incubation; Underwood et al. 2011; Yan et al. 2013). Mesocosms and field trials are less often used due to the complexity of their setup (Rosendahl et al. 2012) even though they tend to better reflect chronic exposure conditions in the environment. We found 12 studies from natural aquatic environments, 6 from wastewater treatment plants (WWTP), 8 from soils, and 5 on bacterial enrichments. Of these studies, 77 % included processes involved in the nitrogen cycle. The non-nitrogen-related studies involved sulfate-reduction (Hansen et al. 1992; Ingvorsen et al. 2003; Liu et al. 2014), pyrene degradation (Näslund et al. 2008), iron reduction (Thiele-Bruhn and Beck 2005; Toth et al. 2011), arsenic oxidation and reduction (Yamamura et al. 2014), methanogenesis (Conkle and White 2012; Fountoulakis et al. 2004; Liu et al. 2014), and acetate biodegradation kinetics (Wunder et al. 2013). We only found a few studies that examined the effect of antibiotics on several biogeochemical processes (Conkle and White 2012; Kotzerke et al. 2008; Kotzerke et al. 2011; Liu et al. 2014; Rosendahl et al. 2012; Toth et al. 2011).

The 12 studies tested a total of 14 antibiotic families with 31 compounds displaying extremely different physicochemical properties that cause differences in target species and antibiotic mechanisms. In the following sections, we give a brief overview of the experimental procedure of exposure concentrations and experimental design to compare and review the effects of antibiotics on biogeochemical processes.

Across studies, the fate of the antibiotic substance during the experiment was often unconsidered, and only 40 % of the studies quantified the antibiotic concentration in the experimental medium to determine the effect on a certain process (Ahmad et al. 2014; Campos et al. 2001; Hansen et al. 1992; Hou et al. 2015; Kotzerke et al. 2008; Kotzerke et al. 2011; Liu et al. 2014; Rico et al. 2014; Roose-Amsaleg et al. 2013;

Table 1 List of observed effects of antibiotic on biogeochemical processes in environmental samples; A and C, mean acute and chronic effect, respectively; an exposure was designed as chronic if the duration of the exposure reached a value higher than the life-span of the targeted organisms and eventually if the level of exposure was low (ng to μg/L or /kg). WWTP designs wastewater treatment plant and S and C design single and continuous addition, respectively. Processes from the nitrogen cycle are in bold.

Microbial Process	Chronic/ acute	Antibiotic					Effects		Environment	Reference
		Molecule	Nominal concentration	Addition	Duration	Quantification (loss)	(approximate % changement)	Effective concentration		
Acetate biodegradation	C	Sulfamethoxazole, erythromycin, and ciprofloxacin (mixture)	0.33–3.33 μg/L	C	Weeks	No	No effect	-	Water/biofilm	Wunder et al. (2013)
Anammox	A	Tetracycline hydrochloride	100–1000 mg/L	S	Hours	No	Inhibition (30 %)	100 mg/L	WWTP	Fernandez et al. (2009)
Anammox	C	Tetracycline hydrochloride	50 mg/L	C	Months	No	Reversible inhibition (40 %)	50 mg/L	WWTP	Fernandez et al. (2009)
Anammox	A	Sulfathiazole	100–1000 mg/L	S	Hours	No	Inhibition (up to 50 %) decrease with the duration of exposure	450 mg/L	WWTP	Lotti et al. (2012)
Anammox	C	Sulfathiazole	100 and 500 mg/L	S	Weeks	No	Inhibition (final decrease of 50 %)	100 mg/L	WWTP	Lotti et al. (2012)
Anammox	A	Oxytetracycline	100–1000 mg/L	S	Hours	no	Inhibition (up to 20 %) decrease with the duration of exposure	200 mg/L	WWTP	Lotti et al. (2012)
Anammox	C	Oxytetracycline	100 and 500 mg/L	S	Weeks	No	Inhibition (final decrease of 25 %)	100 mg/L	WWTP	Lotti et al. (2012)
Anammox	A	Chloramphenicol	250–1000 mg/L	S	Hours	No	Inhibition (40 %)	250 mg/L	WWTP	Fernandez et al. (2009)
Anammox	C	Chloramphenicol	20 mg/L	C	Months	No	Irreversible inhibition (up to 80 %)	20 mg/L	WWTP	Fernandez et al. (2009)
Arsenate reduction	C	Tetracycline	50 mg/L	S	Days	No	Accelerated	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenate reduction	C	Lincomycin	50 mg/L	S	Days	No	Minor effect	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenate reduction	C	Erythromycin	50 mg/L	S	Days	No	Minor effect	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenate reduction	C	Chloramphenicol	50 mg/L	S	Days	No	No effect (aerobic), inhibition (anaerobic)	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenate reduction	C	Ampicillin	50 mg/L	S	Days	No	Partial inhibition	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenite oxidation	C	Tetracycline	50 mg/L	S	Days	No	Delayed	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenite oxidation	C	Lincomycin	50 mg/L	S	Days	No	Minor effect	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenite oxidation	C	Erythromycin	50 mg/L	S	Days	No	Minor effect	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenite oxidation	C	Chloramphenicol	50 mg/L	S	Days	No	Inhibition	50 mg/L	Sediment	Yamamura et al. (2014)
Arsenite oxidation	C	Ampicillin	50 mg/L	S	Days	No	Indeterminable	50 mg/L	Sediment	Yamamura et al. (2014)
Chemical oxygen demand removal	C	Ciprofloxacin, gentamicin, sulfamethoxazole, trimetoprim, vancomycin (mixture)	0.1–40 mg/L	C	Months	Extrapolation	Reversible inhibition (25 %)	30 mg/L	WWTP	Schmidt et al. (2012)

Table 1 (continued)

Microbial Process	Chronic/ acute	Antibiotic					Effects		Environment	Reference
		Molecule	Nominal concentration	Addition	Duration	Quantification (loss)	(approximate % changement)	Effective concentration		
Denitrification	C	Ciprofloxacin	10–50 mg/L	S	Weeks	Yes, biotransformation	Inhibition (25 %)	50 mg/L	Enrichment cultures	Liu et al. (2014)
Denitrification	C	Sulfamethoxazole	1.2 μg/L–0.5 g/L	S	Weeks	No	Inhibition (47 %)	1.2 μg/L	Groundwater	Underwood et al. (2011)
Denitrification	C	Sulfamethazine	0.01–1 mg/L	S	Days	Yes	Inhibition of nitrate removal (17 %) and nitrite production (82 %)[a]	0.01 and 1 mg/L, respectively	Groundwater	Ahmad et al. (2014)
Denitrification	C	Chlortetracycline	0.01–1 mg/L	S	Days	Yes, no degradation	Inhibition of nitrate removal (15.4 %) and nitrite production (31 %)[a]	1 and 1 mg/L, respectively	Groundwater	Ahmad et al. (2014)
Denitrification	C	Flumequine	0.1 μg/L–50 mg/L	C	Weeks	Yes (62 %)	Inhibition (41 %)	50 mg/L	Sediment	Yan et al. (2013)
Denitrification	C	Sulfamethoxazole	0.2 μg/L–50 mg/L	C	Weeks	Yes (93 %)	Inhibition (39 %)	50 mg/L	Sediment	Yan et al. (2013)
Denitrification	C	Vancomycine	0.3 μg/L–0.2 mg/L	C	Weeks	No	No effect	-	Sediment	Yan et al. (2013)
Denitrification	C	Tetracycline	0.9 μg/L–7 mg/L	C	Weeks	Yes (92 %)	No effect	-	Sediment	Roose-Amsaleg et al. (2013)
Denitrification	A	Sulfamethazine	0.05–100 μg/L	S	Hours	Yes	Inhibition (20–30 %)	50 ng/L	Sediment	Hou et al. (2015)
Denitrification	A	Erythromycin	1 mg/L	S	Hours	No	Inhibition (≈37 %)	1 mg/L	Sediment	Costanzo et al. (2005)
Denitrification	A	Clarithromycin	1 mg/L	S	Hours	No	Inhibition (≈37 %)	1 mg/L	Sediment	Costanzo et al. (2005)
Denitrification	A	Ciprofloxacin	0.1–1000 μg/L	S	Hours	No	No effect	-	Sediment	Costanzo et al. (2005)
Denitrification	A	Amoxicillin, clavulanic acid	1 mg/L	S	Hours	No	No effect	-	Sediment	Costanzo et al. (2005)
Denitrification	A	Amoxicillin	1 mg/L	S	Hours	No	Inhibition (≈37 %)	1 mg/L	Sediment	Costanzo et al. (2005)
Denitrification	C	Tetracycline	1–1000 μg/kg	S	Days	No	No effect	-	Soil	Conkle and White (2012)
Denitrification	C	Sulfamethoxazole	1–1000 μg/kg	S	Days	No	Inhibition	500 μg/kg	Soil	Conkle and White (2012)
Denitrification	C	Ciprofloxacin	1–1000 μg/kg	S	Days	No	No effect	-	Soil	Conkle and White (2012)
Denitrification	C	Sulfadiazine	10 and 100 mg/kg	S	Weeks	Yes (66–100 %)	Inhibition (≈70 %)	10 mg/kg	Soil	Kotzerke et al. (2008)
Denitrification	C	Difloxacin, sarafloxacin	7–12 μg/kg	S	Month	Yes	No effect	-	Soil	Rosendahl et al. (2012)
Denitrification	C	Difloxacin	1–100 mg/kg	S	Weeks	Yes	Inhibition (50 %)	10 mg/kg	Soil	Kotzerke et al. (2011)
Denitrification	A	Chloramphenicol	100–2000 mg/L	S	Hours	No	Inhibition (19–41 %)[b]	100 mg/L	Soil	Murray and Knowles (1999)
Iron reduction	C	Sulfapyridine or oxytetracycline	0.02–500 mg/kg	S	Days	Yes	Inhibition (10 %)[a]	ED 10=0.003–7.35 μg/g	Soil	Thiele-Bruhn and Beck (2005)
Iron reduction	C	Sulfadimethoxin	25–200 μg/kg	S	Weeks	No	Inhibition (>95 %)	25 μg/kg	Soil	Toth et al. (2011)
Iron reduction	C	Monensin	10–100 μg/kg	S	Weeks	No	Reversible inhibition	10–100 μg/kg	Soil	Toth et al. (2011)

Table 1 (continued)

Microbial Process	Chronic/ acute	Antibiotic					Effects		Environment	Reference
		Molecule	Nominal concentration	Addition	Duration	Quantification (loss)	(approximate % changement)	Effective concentration		
Methanogenesis	C	Ciprofloxacin	10–100 mg/L	S	Weeks	Yes, no biotransformation	Inhibition (40 %)	80 mg/L	Enrichment cultures	Liu et al. (2014)
Methanogenesis	A	Sulfamethoxazole	10–400 mg/L	S	Days	No	Inhibition	IC50>400 mg/L	Enrichment cultures	Fountoulakis et al. (2004)
Methanogenesis	A	Ofloxacin	10–400 mg/L	S	Days	No	Inhibition	IC50=334 mg/L	Enrichment cultures	Fountoulakis et al. (2004)
Methanogenesis	C	Sulfamethoxazole	1–1000 μg/kg	S	Days	No	Stimulation (30 %)	500 μg/kg	Soil	Conkle and White (2012)
Methanogenesis	C	Tetracycline	1–1000 μg/kg	S	Days	No	No effect	-	Soil	Conkle and White (2012)
Methanogenesis	C	Ciprofloxacin	1–1000 μg/kg	S	Days	No	Inhibition (25 %)	1 μg/kg	Soil	Conkle and White (2012)
Nitrification	A	Colistin	0.3–300 mg/L	S	Hours	No	No effect on nitrite oxidation	-	Mixed culture	Bressan et al. (2013)
Nitrification	A	Colistin	0.3–300 mg/L	S	Hours	No	Inhibition of ammonia-oxidation (correlation)	0.3–300 mg/L	Mixed culture	Bressan et al. (2013)
Nitrification	C	Chloramphenicol	10–250 mg/L	C	Weeks	Yes	No effect	-	Mixed culture	Campos et al. (2001)
Nitrification	C	Oxytetracycline hydrochloride	10–250 mg/L	C	Weeks	Yes	Inhibition (50 %)	250 mg/L	Mixed culture	Campos et al. (2001)
Nitrification	A	Ofloxacin or sulfamethoxazole	2–10 mg/L	S	Hours	No	Inhibition (75 and 60 %)	6 mg/L for each	Pure culture	Dokianakis et al. (2004)
Nitrification	C	Sulfadiazine	10 and 100 mg/kg	S	Weeks	Yes (66–100 %)	Inhibition (≈25 %)	100 mg/kg	Soil	Kotzerke et al. (2008)
Nitrification	C	Oxytetracycline	12.5–75 mg/L	S	Week	No	Inhibition (50 %)[a]	EC 50=8.60–26.96 mg/L	Water	Klaver and Matthews (1994)
Nitrification	A	Erythromycin	1–267 mg/L	S	Days	No	Inhibition (72 %)	>20 mg/L	WWTP	Alighardashi et al. (2009)
Nitrification	C	Ciprofloxacin, gentamicin, sulfamethoxazole, trimetoprim, vancomycin (mixture)	0.1–40 mg/L	C	Months	Extrapolation	Complete inhibition	40 mg/L	WWTP	Schmidt et al. (2012)
Nitrification	A	Tetracycline	50 or 200 mg/L	S	Hours	No	Complete inhibition	200 mg/L	WWTP	Katipoglu-Yazan et al. (2013)
Nitrification	A	Erythromycin	50 or 200 mg/L	S	Hours	No	Complete inhibition	50 mg/L	WWTP	Katipoglu-Yazan et al. (2013)
Nitrification	C	Enrofloxacin	1–1000 μg/L	C	Weeks	yes (70–98 %)	Inhibition	NOEC[a] bacterial and archaeal ammonia oxidizers to enrofloxacin 10 and 1 μg/L, respectively	Freshwater	Rico et al. (2014)
Nitrification	C	Sulfadimethoxin	25–200 μg/kg	S	Weeks	No	Inhibition (60 %)	200 μg/kg	Soil	Toth et al. (2011)

Table 1 (continued)

Microbial Process	Chronic/ acute	Antibiotic					Effects		Environment	Reference
		Molecule	Nominal concentration	Addition	Duration	Quantification (loss)	(approximate % changement)	Effective concentration		
Nitrification	C	Monensin	10–100 μg/kg	S	Weeks	No	No effect	-	Soil	Toth et al. (2011)
Nitrification	C	Difloxacin, sarafloxacin	7–12 μg/kg	S	Month	Yes	No effect	-	Soil	Rosendahl et al. (2012)
Nitrification	C	Difloxacin	1–100 mg/kg	S	Weeks	Yes	Inhibition (≈30 %)	10 mg/kg	Soil	Kotzerke et al. (2011)
Nitrification	C	Ciprofloxacin	1–50 mg/kg	S	Weeks	No	Stimulation (20 %)	1 mg/kg	Soil	Cui et al. (2014)
Organic carbon removal	A	Tetracycline	50 or 200 mg/L	S	Hours	No	Inhibition (25 and 71 %)	50 and 200 mg/L	WWTP	Katipoglu-Yazan et al. (2013)
Organic carbon removal	A	Erythromycin	50 or 200 mg/L	S	Hours	No	Inhibition (22 and 26 %)	50 and 200 mg/L	WWTP	Katipoglu-Yazan et al. (2013)
PAH (Pyrene) degradation	C	Ciprofloxacin	20–2000 μg/L	S	Month	No	Inhibition (66 %)	1000 μg/L	Sediment	Näslund et al. (2008)
Sulfate reduction	C	Oxytetracycline, oxolinic acid, flumequine	400, 100, 100 mg/kg	S	Months	Yes dissipation	Reversible inhibition (90 %)	400, 100, 100 mg/kg	Sediment	Hansen et al. (1992)
Sulfate reduction	A	Chloramphenicol + streptomycin	20 and 100 mg/L	S	Days	No	Inhibition (90 %)	20 mg/L chloramphenicol +100 mg/L streptomycin	WWTP	Ingvorsen et al. (2003)
Sulfate reduction	C	Ciprofloxacin	10–80 mg/L	S	Weeks	Yes, no biotransformation	Reversible inhibition	10–80 mg/L	Enrichment cultures	Liu et al. (2014)

[a] Calculated by the authors

[b] Depending on soil type tested

Rosendahl et al. 2012; Thiele-Bruhn and Beck 2005; Yan et al. 2013). Regardless the antibiotic family, these studies revealed considerable loss of antibiotics over the experiment. In the environment, antibiotic degradation includes diverse processes such as biodegradation, evaporation, and sorption to organic matter. For studies where it was reported, antibiotic concentrations decreased by 62 to 100 % during the experiments: 66–100 % in soil (Kotzerke et al. 2008), 62–93 % in sediment (Yan et al. 2013), and 64–98 % in water (Rico et al. 2014). Although antibiotics have low volatilities and do not tend to bioaccumulate, they show variable adsorption capacities and decay rates (half-lives from hours to months and even to years in a solid matrix). Sorption coefficients of the 14 antibiotics ranged from high (oxolinic acid) to low (sulfamethoxazole) in the 12 studies. Continuous addition of antibiotic could compensate for the antibiotic degradation observed in all studies. Otherwise, studies should consider both nominal and measured antibiotic concentrations to determine whether the degradation rates observed in the laboratory and semi-field experiments provide sufficient antibiotic exposure.

We assessed whether acute or chronic toxicity was studied though it was rarely explicitly mentioned (see Table 1). We considered the duration of the experiment, the type of antibiotic supply, and the antibiotic concentration of the exposure.

Duration of the experiments: short-term versus long-term effects

One of the first studies investigating the effect of antibiotics on a functional trait (denitrification) was short-term, on the scale of hours (Costanzo et al. 2005). Short exposure time is problematic for slow-growing bacteria. Generation times of known cultivated ammonia-oxidizing bacteria vary from 8 to 138 h with a common value of 20 h (Prosser 1989) and the doubling time of anammox bacteria is 9 days (Strous et al. 1999). Under optimal conditions, the doubling time for denitrifiers varies between 1.5 and 1.9 h (*Paracoccus denitrificans* and *Pseudomonas Stutzeri*; Carlson and Ingraham, 1983). However, the doubling time of heterotrophic microorganisms in soils is~9 days (Baath et al. 1988). Acute toxicity tests regularly last hours rather than days or weeks. The duration of the exposure of colistin used by Bressan et al. (2013) on ammonia-oxidizing bacteria was 5 h. Schmidt et al. (2012) already stated that "inhibition of cell division or protein biosynthesis may be observed only after test duration of several days." The importance of incubation time was also shown in the "Substrate Induced Respiration (SIR)" test of Thiele-Bruhn and Beck (2005); no effect was observed after 4 h, whereas respiration was inhibited by both oxytetracycline and sulfapyridine after 24 h. Short-term experiments that are shorter than the growth rate of the targeted bacteria, therefore, explore the effect of the antibiotic on enzyme activity. Studies regarding short-term effects (Bressan et al. 2013; Costanzo et al. 2005; Hou et al. 2015; Katipoglu-Yazan et al. 2013) can be contrasted to long-term effect studies: from days (Ahmad et al. 2014; Alighardashi et al. 2009; Conkle and White 2012; Fountoulakis et al. 2004; Hou et al. 2015; Ingvorsen et al. 2003; Thiele-Bruhn and Beck 2005; Yamamura et al. 2014), weeks (Campos et al. 2001; Cui et al. 2014; Klaver and Matthews 1994; Kotzerke et al. 2008; Kotzerke et al. 2011; Lotti et al. 2012; Rico et al. 2014; Roose-Amsaleg et al. 2013; Toth et al. 2011; Underwood et al. 2011; Wunder et al. 2013; Yan et al. 2013), months (Fernandez et al. 2009; Hansen et al. 1992; Näslund et al. 2008; Rosendahl et al. 2012), to a year (442 days by Schmidt et al. 2012). Conducting experiments for extended time periods is difficult because degradable antibiotics, as well as nutrients, can be quickly consumed unless continuously added (Alighardashi et al. 2009).

Supply of antibiotic

In most studies (25 out of 31), the antibiotic was added once or several times (Campos et al. 2001; Fernandez et al. 2009; Rico et al. 2014; Schmidt et al. 2012). Only three studies continuously supplied the antibiotic (Wunder et al. 2013, Yan et al. 2013; Roose-Amsaleg et al. 2013). With a single antibiotic addition, the exposure might be insufficient and a lack of effect can be confounded with a lack of exposure of the antibiotic due to degradation. Among the 24 studies using a single addition of the antibiotic, 8 quantified the antibiotic. The chronic exposure cannot be ascertained in the other cases. In order to be sure of the exposed and effective antibiotic levels, these should be measured over the course of the experiment.

Level of exposure

Most studies investigating the effect on biogeochemical process used an antibiotic exposure which is therapeutic (mg/L or mg/kg), thus higher than those found in polluted environments. These high concentrations of antibiotic could be used as a positive control in the experiments, but a larger range of antibiotic concentrations, including environmentally relevant ones, should be tested. However, over half of the studies (17 of 31) exclusively tested those therapeutic levels which do not reflect environmental exposures. Inhibitive effects were almost always observed in those studies but were sometimes reversible (Hansen et al. 1992) or temporary (Kotzerke et al. 2011). Among the five antibiotics tested at 1 mg/L by Costanzo et al. (2005), three inhibited (erythromycin, clarithromycin, amoxicillin), whereas two (ciprofloxacin and the mixture amoxicillin and clavulanic acid) did not inhibit denitrification. Similarly, Campos et al. (2001) did not observe

any inhibition for one among two antibiotics (chloramphenicol) on nitrification even when applied at 10–250 mg/L. However, as explained by Alighardashi et al. (2009), the use of high levels of antibiotic is appropriate when batch experiments are carried out, notably on wastewater.

As a conclusion given by Alighardashi et al. (2009), long-term "laboratory-scale" studies using low antibiotic concentrations should be complemented by short-term "batch" experiments that use large doses. Researchers should be aware of what level of antibiotic exposure best suits their research question: acute or chronic in order to give relevant results. Effects of environmentally relevant concentrations on processes need further study, with particular attention to incubation period, the antibiotic of interest, and the environmental matrix.

Studies on the effects of cocktails or mixtures of antibiotics on biogeochemical processes are even scarcer but of growing concern (Ingvorsen et al. 2003; Schmidt et al. 2012; Wunder et al. 2013). The positive synergistic effect of an antibiotic mixture on the model organism *Vibrio Fischeri* has been demonstrated by Backhaus et al. (2000). Although the low doses of each antibiotic were not toxic individually, the mixture was strongly toxic. A study carried out with a combination of environmentally relevant concentrations (μg/L) showed that the simultaneous exposure to three antibiotics (sulfamethoxazole, ciprofloxacin, and erythromycin) had no effect on the degradation of acetate (Wunder et al. 2013). The two other studies tested therapeutic concentrations of antibiotic mixtures and observed expected inhibition. Schmidt et al. (2012) explored the effect of a mixture of ciprofloxacin, gentamycin, sulfamethoxazole, trimethoprim, and vancomycin (0.1–40 mg/L) on chemical oxygen demand and nitrification. The chosen experimental setup for their study simulated conditions of a wastewater treatment plant; nitrification was completely inhibited at 40 mg/L. They concluded that typical conditions in a treatment plant would not result in suppression of nitrogen removal. Similarly, Ingvorsen et al. (2003) showed a strong inhibition (90 %) of sulfate reduction after a high dose of chloramphenicol and streptomycin, 20 and 100 mg/L, respectively.

In the natural environment, multiple antibiotics are present as mixtures. Individual antibiotic applications reveal potential mechanisms of inhibition, whereas to determine realistic effects on processes, mixtures should be investigated. In the future, studies regarding this mixture and possible synergistic effect should be considered (notably using existing concepts for the prediction of mixture toxicities; Backhaus et al. 2000). Studying multiple antibiotics at environmentally relevant concentrations as well as the possible role of other contaminants (e.g., metals or pesticide) should be tested.

Effect of antibiotics on nitrogen cycle

The following sections specifically examine the three most studied biogeochemical processes in the nitrogen cycle: nitrification, anammox, and denitrification. They represent distinct processes in regard to metabolism and microbes. Bacterial denitrification is almost always facultative (Zumft 1997) in contrast to anammox and denitrification. Although denitrifiers form a phylogenetically diverse group (Philippot and Hallin 2005) spread among three kingdoms, microorganisms involved in nitrification or anammox have much lower phylogenetic diversity. This difference might affect their vulnerability to antibiotic exposure, with multi-species communities showing higher resilience to antibiotics (Näslund et al. 2008). Furthermore, both nitrifiers and anammox bacteria have single cellular organizations entailing different responses to antibiotic exposure. Nitrification is carried out in two steps by both Gram-negative bacteria (Kowalchuk and Stephen 2001) and *Archaea* (Treusch et al. 2005) during ammonia oxidation and only by Gram-negative bacteria during nitrite oxidation (Spieck and Lipski 2011). *Archaea* are less sensitive to antibiotics than bacteria due to differences in cell envelopes and metabolic processes (Shen et al. 2013). For example, the ammonia oxidizing archaea *Nitrososphaera viennensis* is resistant to streptomycin, kanamycin, ampicillin, and carbenicillin (Tourna et al. 2011). Similarly, the anammox bacteria, all members of the order Planctomycetales (Strous et al. 1999), are known for their unique membrane lipids. Furthermore, planctomycetes do not have murein in their cell walls, a target of several antibiotics, and are thus resistant towards these compounds (Claus et al. 2000). Furthermore, the anammox process is carried out within an internal organel, the anammoxosome, which may protect the activity from the effects of antibiotics. Among the studies testing antibiotics on N transformations, 13 publications studied the effect of antibiotics on nitrification, 12 on denitrification, and 2 on anammox.

Nitrification (13 studies—16 antibiotics)

Nitrifiers have been used to determine the effect of antibiotics on nitrification. The ISO 9509 norm tests toxicity by assessing the inhibition of nitrification of activated sludge microorganisms (Juliastuti et al. 2003). Nitrifiers are highly sensitive to inhibitory compounds (including antibiotics) in aerobic environments. Furthermore, nitrifiers are used as biosensors to detect the toxicity of molecules present in wastewater treatment plants (Alighardashi et al. 2009; Carucci et al. 2006).

Out of the 13 studies, 9 showed an effect of antibiotics out of which only four (four antibiotics) did not inhibit nitrification, confirming the high sensitivity of nitrifiers at therapeutic antibiotic concentrations. There was no measured inhibition (1) of soil nitrifiers in the studies involving antibiotic-spiked

manure added to soil (Toth et al. 2011; Rosendahl et al. 2012) with monensin and difloxacin, respectively; (2) of mixed nitrifying cultures by chloramphenicol (Campos et al. 2001); and (3) of ammonia oxidizers from a mixed culture of nitrifying bacteria by colistin (Bressan et al. 2013). The latter study observed that nitrite-oxidizing bacteria were more susceptible to colistin than ammonia oxidizers. Except for the physicochemical behavior of the antibiotic, Campos et al. (2001) explained the lack of inhibition by a shift in ammonia-oxidizing bacteria (AOB) versus ammonia-oxidizing archaea (AOA). This is in agreement with Schauss et al. (2009) who calculated via modeling an EC50 strongly higher for AOA compared to AOB for sulfadiazine.

Among the studies showing an effect, only three tested both environmentally relevant and therapeutic concentrations (Rico et al. 2014; Toth et al. 2011; Schmidt et al. 2012). Rico et al. (2014) carried out a chronic exposure of tropical freshwater to a fluoroquinolone (enrofloxacin) in a microcosm. An inhibitive effect of this antibiotic above 100 μg/L was demonstrated whereas they measured no observed effect concentration (NOEC see Nordberg et al. 2009 for definition) of 10 and 1 μg/L from bacterial and archaeal ammonia-oxidizer abundance, respectively. They actually observed a relation between nitrification activity and an antibiotic exposure at 1000 μg/L, which is above environmentally relevant concentration. They explained this by the "high resilience of the whole water–sediment microbial community and a fast recovery from antibiotic exposure." Toth et al. (2011) measuring only the production of NO_2^- on soil chronically exposed to manure spiked with a sulfonamide (sulfadimethoxin) observed inhibition of ammonia oxidation until 200 μg/kg.

To conclude, 14 out of the 16 tested antibiotics inhibited nitrification in the environment at therapeutic levels: sulfadiazine, oxytetracycline, ofloxacin, sulfamethoxazole, erythromycin, ciprofloxacin, gentamicin, colistin, trimethoprim, vancomycin, sulfadimethoxin, enrofloxacin, difloxacin, and tetracycline. The inhibition of such therapeutic antibiotic concentrations could entail severe efficiency loss from 25 % (sulfadiazine, in soils at 100 mg/kg; Kotzerke et al. 2008) to the complete inhibition of nitrification (all in WWTP, see the antibiotic mixture of Schmidt et al. 2012; tetracycline or erythromycin see in Katipoglu-Yazan et al. 2013). Overall, environmental relevant concentrations of fluoroquinolones alone or in mixtures were able to inhibit nitrification rates in environmental samples, but the majority of the studies tested therapeutic levels.

Denitrification (12 studies—14 antibiotics)

Denitrification comprises four enzymatic steps involving heterotrophic, facultatively anaerobic microorganisms. However, the effect of antibiotics on the first step (i.e., nitrate reduction and nitrite production) was primarily studied while nitrous oxide production was rarely measured (Hou et al. 2015 and Roose-Amsaleg et al. 2013). Acute or chronic effects of several antibiotics on denitrification were studied using therapeutic levels (Costanzo et al. 2005; Murray and Knowles 1999; Kotzerke et al. 2008, 2011). Recently, environmentally relevant antibiotic concentrations have been tested in eight studies. Denitrification rates were inhibited at environmentally relevant doses of sulfamethoxazole (sulfonamide) in soils (Conkle and White 2012; 500 μg/kg) and groundwater (Underwood et al. 2011; 1.2 μg/L), whereas nitrate reduction rates were not affected in river sediments (Yan et al. 2013; 10 μg/L). An accumulation of nitrite was observed at low concentrations of sulfamethoxazole in the sediment (Yan et al. 2013), suggesting a negative effect of this antibiotic on the microbial community reducing nitrite to nitrous oxide and nitrogen gas. This supports the theory that a less diverse community is more vulnerable than a diverse one. The reduction of oxidized nitrogen species further to gaseous from is carried out by fewer organisms than nitrate reduction (Zumft 1997). Among the other antibiotics tested at environmentally relevant concentrations, only sulfamethazine was able to inhibit denitrification at environmentally relevant concentrations (Ahmad et al. 2014, groundwater, 10 μg/L; Hou et al. 2015, sediment, 50 ng/L). All this suggests that sulfonamides (sulfamethoxazole and sulfamethazine) could represent a risk for the ecosystem health at levels detected in natural habitats as a widespread, broad-spectrum antibiotic with a low sorption potential (Conkle and White 2012). The denitrification inhibitions measured ranged from 17 to 82 % but never completely blocked the denitrification process.

Anammox (two studies—four antibiotics)

Bacteria responsible for the anammox process express several typical cellular traits such as (1) single- or double-membrane-bounded compartments separating their chromosome from the remainder of the cytoplasm and (2) peptidoglycan-free cell walls (Strous et al. 1999) able to adapt to antibiotics. We would expect anammox bacteria to be more resistant to antibiotics due to their different cell structure, though this depends on the mode of action of the antibiotic (e.g., they are insensitive to ampicillin; Strous et al. 1999).

Two studies (Fernandez et al. 2009; Lotti et al. 2012) investigated the effect of antibiotics on the anammox process exploring both acute and chronic effects of four different antibiotics (oxytetracycline, tetracycline hydrochloride, sulfathiazole, and chloramphenicol). Only therapeutic concentrations (100-1000 mg/L) were tested in WWTP sludges. The anammox process was inhibited in all the assays regardless of the duration of exposure.

Anammox plays an important role in WWTPs as well as in natural environments (e.g., Dalsgaard et al. 2005). Further study should therefore focus on high-risk areas such as recipients of animal farm wastewater or runoff, aquaculture facilities using antibiotics, WWTP receiving hospital wastewater, and antibiotic synthesis facilities.

Conclusions and perspectives

Data on the sources, fate, and effects of antibiotics in the environment indicate that antibiotics may alter several biogeochemical cycles. In addition to being a threat to public health, antibiotics represent a threat to ecosystem functioning and health, even if their toxic effect could often be buffered in complex and diverse ecosystems. Regarding the existing bibliography, antibiotics such as fluoroquinolones or sulfonamides appear as the most noxious compounds among all families of antibiotics. However, these molecules were also the most tested, biasing this preliminary conclusion. Furthermore, among the different microbial processes in nitrogen cycling, nitrification and anammox appear to be less sensitive to antibiotic exposure (sensitivity at therapeutic concentrations) than denitrification.

In addition to "ecosystem health," there is a concern regarding the impact of antibiotics on ecosystem services delivered by environmental bacteria. Though the data gathered in this mini-review do not allow identifying certain molecules to inform usage recommendations, they clearly demonstrate the importance of further investigation of the effects of antibiotics on biogeochemical processes at environmentally relevant concentrations. It should be noted that from a regulatory perspective, environmental effects of pharmaceuticals are not considered in any way in practice. It is crucial to consider the antibiotic impacts on environments such as the accumulation of nitrite in aquatic environments or of nitrous oxide (a strong greenhouse effect gas) due to a possible inhibition of nitrification or denitrification. In watersheds, wetlands, or wastewater treatment plants with clear management priorities regarding nitrogen pollution, small effects of antibiotics on nitrogen removal might be very important. So far, establishing the effect of environmentally relevant antibiotic concentrations on processes is not standardized and results are contradictory (see Table 1).

A more standardized approach testing the effect of environmentally relevant concentrations of antibiotic at chronic levels (simulating *in situ* conditions) on more or less sensitive biogeochemical functions is needed to allow comparison between studies. We propose that future studies investigating the exposure of antibiotics on biogeochemical processes include chronic exposure, either via continuous supply of antibiotics or repeated additions. Furthermore, experimental design in aquatic systems can involve a mixed-batch setup mimicking environmental conditions. The effect of antibiotics in soil or sediments should preferably be carried out in microcosm, mesocosm, or field studies. In the latter, a survey of the administered compounds is recommended to rank antibiotics regarding their increasing ecological impact to allow the prioritization of regulation depending on different usages. Besides the type of antibiotic, other biogeochemical processes should be considered, in addition to N cycling processes, such as those involved in metal transformation or degradation of pollutants. Furthermore, case studies investigating the effect of antibiotics on biogeochemical processes should determine the effect of these pharmaceuticals at a larger scale, involving a comprehensive interdisciplinary approach, including hydrology (transport), chemistry (nutrients, pollutants, and pharmaceuticals), and biology (process rates and involved organisms).

Acknowledgments The authors acknowledge the constructive comments of four journal reviewers and Ben Abbott for proofreading the paper.

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