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Antibiotics and the Terrestrial Nitrogen Cycle: A Review

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Abstract The distribution, fate, and effects of human and veterinary antibiotics in the environment have been the subject of intense investigation for nearly two decades. Studies show that the structure and function of microbial communities in soil and sediment are modified by antibiotic exposure but the resulting impact on biogeochemical processes is poorly understood. This review summarizes the most recent data on the present use and physicochemical properties of human and veterinary antibiotics and provides an overview of their occurrence in soil and sediment. This is followed by an examination of the potential effects of antibiotics on microbial nitrogen turnover and methodological approaches to measuring the effects of antibiotics on nitrification and denitrification. Recent studies identified six major classes of antibiotics in soil and sediment, occurring at concentrations between ng·kg^{-1} and mg·kg^{-1}. Among these, tetracycline and fluorquinolone antibiotics are the most resistant to degradation and leaching and may accumulate to high concentrations (mg·kg^{-1}) in terrestrial environments. Less persistent compounds such as the sulfonamides are often detected at lower concentrations (ng·kg^{-1} to μg·kg^{-1}) but their occurrence is also reported more frequently. Only 26 studies were found that investigated the effects of antibiotics on microbial nitrogen cycling. Some antibiotics had no observable effect on nitrogen redox activity in soil and sediment while others appeared to increase or decrease rates of reaction. This lack of consensus could be attributed to a number of different variables including antibiotic dose, method of antibiotic application, variations in the microbial community structure, or method of quantification. We conclude by recommending that future studies adopt a more comprehensive approach to report on changes of the microbial community structure and function as well as the short- and long-term impacts of antibiotics on the accumulation and loss of nitrogen pollutants.

Keywords Antibiotics · Soil · Sediment · Nitrification · Denitrification · Nitrogen pollutants

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Introduction

Since their introduction in the early twentieth century, antibiotics have been proved enormously beneficial to human and animal health. Now used for variety of therapeutic, prophylactic, and growth promotion purposes, global antibiotic consumption has increased considerably. Antibiotic production presently exceeds 100,000 to 200,000 tons per year [1], and a growing proportion of these antibiotics are being administered to poultry and livestock raised in concentrated production facilities [2–5]. As antibiotic usage rises, so too does the risk of antibiotic contamination to the environment. As much as 90 % of the antibiotics being administered are excreted without being metabolized [6] and are poorly removed by wastewater treatment [7]. Consequently, active antibiotic compounds in wastewater, sewage sludge, and manure are

conveyed to terrestrial and aquatic ecosystems by a combination of disposal, discharge, and use as fertilizer amendments.

A large number of antibiotics have been detected in soil and sediment at concentrations ranging from ng·kg^{-1} to mg·kg^{-1} [5]. In general, these concentrations are considered therapeutic and are well below the minimum inhibitory concentrations (MICs) established by acute toxicity tests. Sub-lethal or therapeutic doses, however, can promote the development of antibiotic resistance in both target and non-target organisms [8] and have been found to affect the structure and function of ecologically important microbial communities [9].

Microbial communities in soil and sediment play a fundamental role in nutrient recycling and in mitigating global imbalances caused by human activity. This is particularly evident in the N cycle where inorganic fertilizer use, fossil fuel combustion, and N-fixation cultivation have generated a significant imbalance, depositing up to 140 Tg·yr^{-1} of reactive N species to terrestrial and aquatic environments [10]. The increase in reactive N species has contributed to a number of environmental and human health concerns [11–15]. Mitigation strategies include isolating organisms capable of converting reactive N to N_2 as well as maximizing natural recycling potential in affected watersheds. For example, wastewater treatment commonly includes nitrification and denitrification tanks to reduce the concentration of organic and inorganic N waste prior to being discharged into surface waters. The latter step of the reduction process, denitrification, reduces the eco-toxic compound nitrate (NO_3^-) to N_2 or nitrous oxide (N_2O gas) which are lost to the atmosphere. In agroecosystems, denitrification is a naturally occurring ecosystem service and is estimated to remove up to 22 % of applied N [16] and up to 51 % at the watershed scale [17]. Though microbial N processing is often regarded as a sink for ammonium (NH_4^+) and NO_3^-, it may also serve as a source of eco-toxic nitric oxide (NO) or N_2O. N_2 gas is the most common product of denitrification and is not associated with human health problems or environmental degradation, but up to 3.9 % of denitrification results in the production of N_2O [18], a powerful greenhouse gas [19] and the leading contributor to stratospheric ozone depletion [20]. NO is a component of smog and is a contributor to a number of human health concerns [21]. Although NO is considered a minor product of nitrification and denitrification, up to 0.75 % of applied NH_4^+-N fertilizers may be lost as NO [22]. Considerable advances have been made in nutrient management practices to promote high N use efficiency and to minimize non-point source NO_3^- and N_2O pollution. As antibiotics are introduced to soils, however, the resulting impact on microbial activity and N speciation may reduce the efficacy of these efforts.

Evidence that antibiotics affect the structure of microbial communities in soil, sediment, and sewage sludge is abundant. In a 2010 review, Ding et al. [9] identified 31 studies reporting the effects of 14 antibiotics on microbial communities in soil, sediment, and activated sludge. Reported changes include positive shifts in the ratios of fungi to bacteria and ammonia-oxidizing bacteria (AOB) to ammonia-oxidizing archaea (AOA), increased antibiotic resistance, decreased rates of bacterial growth, and temporal shifts in microbial diversity. Despite functional redundancies within the microbial community, structural changes resulting from exposure to antibiotics may also affect community function (e.g., rates of mineralization, nitrification, and denitrification) and therefore impact important ecosystem services in contaminated soil and sediment. Roose-Amsalag and Laverman [23] provide an excellent overview of the mechanisms that may contribute to these structural and functional changes. In this review, we focus on the effects of antibiotics on the biogeochemical N cycle in soil and sediment. We first briefly describe the occurrence and fate of antibiotics in the environment, concentrating on studies published since the last major review in 2009 [24]. In the second part of this paper, we discuss the effects of antibiotics on the microbial N cycle in soil, sediment, and wastewater sludge. In the final section, we discuss methodological approaches to investigating the effects of antibiotics on the microbial N cycle.

Occurrence of Antibiotics in Soil and Sediment

The occurrence of antibiotics in the terrestrial environment is well-documented. A number of substantial reviews published between 1999 and 2009 summarize research that reports upon the occurrence of antibiotic and antimicrobial compounds in soil and sediment [5, 6, 24, 25]. In addition to their application in human medicine, antibiotics are broadly dispensed for therapeutic, prophylactic, and growth promotion purposes in the livestock and poultry industries. Up to 90 % is excreted without being metabolized [4, 6], and recent studies have identified as many as 20 different antibiotic compounds in feces samples from swine, poultry, and livestock production facilities [26–29]. Hospital effluent and wastewater samples are also consistently found to contain a broad range of antibiotic compounds at low concentrations [30, 31]. When contaminated manure, sewage sludge, or polluted water are applied to agricultural soils, these residual antibiotic compounds and their degradation products are introduced to the terrestrial environment where they often persist and remain bioavailable [24]. Application of manure to agro-ecosystems is a common practice, particularly in regions where concentrated animal production occurs. In 2009, for example, over 15 million acres of US cropland were fertilized with manure, often in close proximity to livestock and poultry facilities [32]. This figure is likely to grow alongside organic crop production, which doubled between 1997 and 2005 [33]. Although empirical data are scarce, the proportion of cropland receiving manure fertilizers is presumed to be much larger in developing

countries where use of N fertilizers is rising dramatically [34]. While the occurrence of antibiotics in soil and sediment has been documented throughout the world [5, 24], the most recent studies have focused extensively on these regions where agricultural output and fertilizer use is on the rise.

Antibiotics in Soil

A search of scientific databases yielded 20 studies reporting on the occurrence of antibiotics in soil since 2009. Among these, 15 were conducted on field sites in East Asia where animal manure, wastewater, or contaminated surface water were applied to the soil. A total of 36 different antibiotic compounds from six different antibiotic classes were quantified. The median and maximum concentrations reported for each antibiotic are shown in Table 1 alongside the average frequency of detection, region of study, and potential antimicrobial source. The most frequently investigated compounds (≥50 % of studies) include oxytetracycline (OXY), tetracycline (TET), chlortetracycline (CTC), ciprofloxacin (CIP), norfloxacin (NOR), and enrofloxacin (ENR). Sulfonamides were investigated in fewer studies but these and tetracycline antimicrobials were the most frequently detected (up to 100 %). Notably, no recent studies have investigated the occurrence of the medically important β-lactams group. Among the antibiotics tested, seven were detected at least once at concentrations in excess of 1 $mg{\cdot}kg^{-1}$: CTC (12.9 $mg{\cdot}kg^{-1}$), OTC (1.41 $mg{\cdot}kg^{-1}$), TET (1.01 $mg{\cdot}kg^{-1}$), flumequine (FLE, 1.33 $mg{\cdot}kg^{-1}$), CIP (5.6 $mg{\cdot}kg^{-1}$), ENR (1.35 $mg{\cdot}kg^{-1}$), and NOR (2.16 $mg{\cdot}kg^{-1}$). Maximum concentrations for the remaining antibiotics ranged from 0.007 $\mu g{\cdot}kg^{-1}$ (anhydrotetracycline, ATC) to 898 $\mu g{\cdot}kg^{-1}$ (ofloxacin, OFL), though the median concentration for most of the antibiotics tested rarely exceeded 100 $\mu g{\cdot}kg^{-1}$. Minimum concentrations were reported for 20 of the 36 antibiotics investigated (not shown), and all but CTC were <5 $\mu g{\cdot}kg^{-1}$ and some as low as 20 $ng{\cdot}kg^{-1}$. Several of these studies also reported detection of antibiotics below the limits of quantification (LOQ), indicating that our knowledge about the extent to which antibiotics persist at trace levels in soils is limited by analytical capabilities.

Antibiotics in Sediment

The occurrence of antibiotics in sediment is reported in 11 recent studies (Table 2). The majority of these sampled sediments in high-intensity agricultural regions such as the Pearl and Yangtze River basins in southern China where wastewater discharge and agricultural runoff are significant sources of antibiotic contamination. Among the 35 antibiotics that were investigated, five were not detected in any sediment sample, and the concentrations of five additional antibiotics were below quantification limits. Tetracycline, sulfonamide, and select fluoroquinolone antibiotics were the most frequently researched compounds, appearing in as many as nine individual studies. Three antibiotics whose concentrations exceed 1 $mg{\cdot}kg^{-1}$ in soil were also detected at concentrations exceeding 1 $mg{\cdot}kg^{-1}$ in sediment. These include CTC (1.01 $mg{\cdot}L^{-1}$), NOR (1.14 $mg{\cdot}L^{-1}$), and OFL (1.56 $mg{\cdot}L^{-1}$). Overall, the median concentration of antibiotics in sediments (0.2–54.6 $\mu g{\cdot}kg^{-1}$) is lower than those in soil (0.23–157 $\mu g{\cdot}kg^{-1}$).

Fate of Antibiotics in Soil and Sediment

Once they have entered the terrestrial environment, the fate of antibiotics is largely governed by their physicochemical properties (Table 3) and interactions with the environmental matrix. In terrestrial environments, antibiotics with high octanol-water partitioning coefficients (K_{ow}) values and large sorption coefficients (K_d) tend to sorb strongly to the soil matrix and hence are poorly mobile. The tetracycline class of antibiotics exemplifies this behavior. Their sorption coefficients range from 400 to 1620 $L{\cdot}kg^{-1}$ (see Table 3), and they are rarely found to migrate beyond upper 10 cm of the soil column [84]. Poor mobility and long half-lives provide opportunity for fluoroquinolones (120–2310 days) and tetracyclines (400–1620 days) to accumulate over time, likely accounting for the frequency at which these antibiotics are detected in soils at concentrations in excess of 500 $\mu g{\cdot}kg^{-1}$, especially where manure applications are frequent. Since both sorb strongly to soil and sediment particles, comparably high concentrations of fluoroquinolones and tetracyclines are also observed in sediment. Sulfonamides are among the most frequently detected antibiotic compounds in both soil and sediment but their low K_d values (0.6–4.9) render these compounds highly mobile. In combination with low half-lives (max $t_{½}$ = 21.3 days), sulfonamides do not show the same tendency to accumulate and are infrequently detected at concentrations beyond 50 $\mu g{\cdot}kg^{-1}$ in soil or 5 $\mu g{\cdot}kg^{-1}$ in sediment.

Effects of Antibiotics on the Terrestrial Nitrogen Cycle

The Nitrogen Cycle

The N cycle is a global biogeochemical cycle in which N flows between atmospheric, aqueous, and terrestrial reservoirs. Microbial activity in soil and sediment drives a significant portion of the bulk cycle, converting organic N into plant available forms (NH_4^+ and NO_3^-) and reducing excess inorganic N to gasses (N_2 and N_2O) that escape to the atmosphere, completing the cycle (Fig. 1). NH_4^+ accumulates in soil as a result of mineralization, N fixation (legumes), direct deposition from the atmosphere, or by application of inorganic fertilizers containing NH_4^+ salts, e.g., $(NH_4)_2SO_4$. NH_4^+ strongly sorbs to the negatively charged surfaces of soil minerals and

Table 1 Median and maximum concentrations of antibiotics detected in soil (μg·kg^{-1})

Antibiotic class	Antibiotic	# of studies	Region[a] (# of studies)	Potential sources (# of studies)	Med.	Max.	Mean. Freq. (%)	Reference
Amphenicol	*Chloramphenicol*	1	Ch (1)		–	22.3	–	[35]
Fluoroquinolone	*Ciprofloxacin*	10	Ch (7), Sp (1), Bz (1), In (1)	SM (4), CM (3), PL (2), WW (1), MD (1), U (1)	101.5	5600	70.5	[26–28, 36–42]
	Difloxacin	3	Ch (3)	SM (2), CM (2), PL (1), WW (1)	21.5	21.5	6.0	[27, 39, 42]
	Enrofloxacin	9	Ch (6), Bz (1), My (1), Tk (1)	PL (4), SM (3), CM (3), MM (1), WW (1)	87	1347.6	65.9	[26–29, 37, 38, 41–43]
	Fleroxacin	2	Ch (2)	MM (1), WW (1)	–	559	7.0	[42, 43]
	Flumequine	1	My (1)	PL (1)	114.5	1331	–	[29]
	Lomefloxacin	4	Ch (4)	SM (1), CM (1), PL (1), MM (1), WW (2)	13.7	93.6	69.0	[28, 39, 41, 42]
	Norfloxacin	10	Ch (6), Sp (1), Bz (1), In (1) My (1)	PL (3), CM (2), WW (3), SM (1), MM (1), U (1)	21.5	2160	66.0	[26–29, 35, 36, 39–42]
	Ofloxacin	7	Ch (5), Sp (1), In (1)	SM (3), CM (2), PL (1), WW (2), MD (1), U (1)	93.5	898	80.3	[27, 28, 36, 37, 39, 40, 42]
	Sarafloxacin	1	Ch (1)	MM (1), WW (1)	–	9.06	6.0	[39]
Ionophore	*Lasalosid*	1	Dk (1)	U (1)	–	nd	–	[44]
	Monensin	1	Dk (1)	U (1)	–	0.0004	–	[44]
	Narasin	1	Dk (1)	U (1)	–	nd	–	[44]
	Salinomycin	1	Dk (1)	U (1)	–	0.0022	–	[44]
Macrolide	*Erythromycin*	5	Ch (4), My (1)	WW (3), SM (1), CM (1), PL (1)	4.4	7.2	11.0	[28, 29, 35, 39, 42]
	Josamycin	1	Ch (1)	WW (1)	–	nd	–	[42]
	Roxithromycin	3	Ch (3)	SM (1), CM (1), PL (1), WW (2), U (1)	49.2	96.3	42.5	[28, 39, 42]
	Spriamycin	2	Ch (2)	MM (1), WW (2)	–	1.12	0.8	[39, 42]
	Tylosin	4	Ch (3), My (1)	PL (1), MM (1), WW (2)	23	679	29.9	[28, 29, 39, 42]
Sulfonamide	*Sulfachlorpyridazine*	1	Ch (1)	SM (1), CM (1), PL (1)	–	52.9	100.0	[28]
	Sulfadiazine	7	Ch (6), My (1)	SM (2), CM (2), PL (2), WW (3), MM (1), U (1)	21.5	85.5	22.5	[27–29, 39, 41, 42, 45]
	Sulfadimethoxine	4	Ch (3), US (1)	CM (2), SM (1), PL (1), WW (1), U (1)	26	40.4	82.0	[28, 41, 42, 46]
	Sulfadimidine	1	Ch (1)	SM (1), CM (1), PL (1)	–	177.9	65.4	[28]
	Sulfamerazine	2	Ch (2)	WW (1), U (1)	–	93.5	52	[41, 42]
	Sulfameter	1	Ch (1)	WW (1), U (1)	–	120.4	87	[41]
	Sulfamethazine	6	Ch (4), US (1), K (1)	SM (2), WW (2), CM (1), MM (1), U (1)	3.2	74	77.8	[27, 35, 39, 41, 46, 47]
	Sulfamethoxazole	6	Ch (4), Sp (1), US (1)	SM (2), CM (2), WW (2), PL (1), MM (1)	19.3	54.5	17.5	[28, 36, 39, 41, 45, 46]
	Sulfamonomethoxine	3	Ch (3)	SM (1), CM (1), MM (1), WW (2)	2.79	5.37	0.8	[27, 39, 42]
	Sulfapyridine	2	Ch (2)	SM (1), CM (1), MM (1), WW (1)	2.91	5.11	98.2	[27, 39]
	Sulfathiazole	1	K (1)	SM (1)	0.23	0.38	100.0	[47]
	Sufisoxazole	1	Ch (1)	WW (1	–	nd	–	[42]
Tetracycline	*Anhydrotetracycline*	1	US (1)	U (1)	–	0.007	–	[48]
	Chlortetracycline	9	Ch (6), Tk (1), US (1), K (1)	SM (4), CM (3), MM, (2) PL (1), WW (1)	102.3	12900	77.5	[27, 28, 37, 41, 43, 45–47, 49]
	Doxycycline	5	Ch (4), My (1)	SM (2), CM (2), PL (2), MM (2), WW (1)	157	728	100.0	[27–29, 39, 49]
	Oxytetracycline	11	Ch (6), Sp (1), Tk (1), US (2), K (1)	SM (4), CM (3), MM (2), U (2), PL (1), WW (1)	40.6	1410	75.5	[27, 28, 36, 37, 41, 43, 45–49]

Table 1 (continued)

Antibiotic class	Antibiotic	# of studies	Region[a] (# of studies)	Potential sources (# of studies)	Med.	Max.	Mean. Freq. (%)	Reference
	Tetracycline	10	Ch (6), Sp (1), US (2), K (1)	SM (4), CM (3), U (2), PL (1), MM (1), WW (1)	105	1010	69.6	[27, 28, 35–37, 41, 46–49]

The mean reported frequency of detection is calculated from studies that explicitly report rate of detection. Individual studies may list more than one potential antibiotic source

SM swine manure, *CM* cattle manure, *PL* poultry litter, *MM* mixed manure, *WW* wastewater discharge, *MD* manufacturing discharge, *U* unspecified manure, *nd* not detected

[a] China (Ch), Malaysia (My), Korea (K), Turkey (Tk), India (In), Spain (Sp), United States (US), Denmark (Dk), Brazil (Bz)

soil organic matter (SOM) and is therefore resistant to leaching, but it may be lost by surface runoff, plant uptake, biological nitrification, or annomox reactions. NO_3^- that is produced via nitrification or directly added to soils via inorganic fertilizers, e.g., KNO_3, is susceptible to a number of losses. These include plant uptake, assimilation into microbial tissue, leaching, and biological denitrification. In the following sections, we will briefly review the biology of nitrification and denitrification, followed by an examination of the effects antibiotics and antimicrobials have on these processes.

Nitrification

Nitrification is a general term used to describe naturally occurring NH_4^+ oxidation reactions. The most common oxidation pathway leads to the production of NO_3^- via the intermediate product, NO_2^-. Studies of chemoautotrophic nitrifying organisms such as *Nitrosomonas europaea* describe the $NH_4^+ \rightarrow NO_2^-$ oxidation as a two-step enzymatic process (see Eqs. 1 and 2) catalyzed by ammoniamonoxygenase (AMO) and hydroxylamine oxidoreductase (HAO), respectively [85]:

$$NH_4^+ + O_2 + H^+ \overset{AMO}{\rightarrow} NH_2OH + H_2O \quad (1)$$

$$NH_2OH + H_2O \overset{HAO}{\rightarrow} NO_2^- + 5H^+ \quad (2)$$

The resulting intermediate product, NO_2^-, is rapidly oxidized to NO_3^-. Chemoautotrophic nitrifiers of the genus *Nitrobacter and Nitrosomonas* express the nitrite oxidoreductase (NOR) enzyme, which facilitates a second oxidation reaction ($NO_2^- \rightarrow NO_3^-$) to provide energy for cell growth [86, 87]. Although considered secondary to autotrophic AOA and AOB in most soils, a number of heterotrophic nitrifiers have also been isolated. These include the gram-negative bacteria *Alcaligenes faecalis* and *Pseudomonas aeruginosa* [88, 89]. The mechanisms for heterotrophic nitrification are poorly understood, and the process yields insufficient energy to support heterotrophic cell growth [86]. Heterotrophic nitrification has been also reported to include alternate redox pathways following the initial $NH_4^+ \rightarrow NH_2OH$ oxidation step. These include oxidation of NH_2OH to NO or N_2O (nitrifier nitrification) and reduction of NO_2^- to N_2O (nitrifier denitrification) [89, 90].

Effects of Antibiotics on Nitrification

A literature search identified a total of 13 studies that investigated the effects of 19 different antibiotics, antimicrobials, and antibiotic mixtures on nitrification in soil, wastewater sludge, or pure culture (Table 4). Inhibition is often deemed the most probable effect of antibiotics on nitrification, but this hypothesis is ineffectually supported by the present studies. Among 19 antibiotics and antimicrobials investigated, fewer than half

Table 2 Minimum and maximum concentrations of antibiotics detected in sediment ($\mu g \cdot kg^{-1}$)

Antibiotic class	*Antibiotic*	# Studies	Region[a] (# of studies)	Med.	Max.	Reference
Amphenicol	*Chloramphenicol*	1	Ch (1)	–	<LOQ	[50]
	Florfenicol	1	Ch (1)	–	<LOQ	[50]
	Thiamphenicol	1	Ch (1)	–	<LOQ	[50]
Fluoroquinolone	*Ciprofloxacin*	1	Ch (1)	16.6	197	[50, 51]
	Difloxacin	1	Ch (1)	–	nd	[51]
	Enrofloxacin	4	Ch (3), US (1)	4.84	137	[27, 50–52]
	Fleroxacin)	1	Ch (1)	6.69	6.69	[51]
	Lomefloxacin	3	Ch (3)	2.78	29	[27, 51, 53]
	Norfloxacin	6	Ch (6)	26.6	1140	[27, 50, 51, 53–55]
	Ofloxacin	8	Ch (7), Sp (1)	54.6	1560	[27, 36, 50, 51, 53–55]
	Sarafloxacin	1	Ch (1)	–	nd	[51]
Ionophore	*Lasalosid*	1	Dk (1)	–	nd	[44]
	Monensin	1	Dk (1)	–	nd	[44]
	Salinomycin	1	Dk (1)	–	7E-04	[44]
	Narasin	1	Dk (1)	–	4E-04	[44]
Macrolide	*Erythromycin*	5	Ch (5)	14.8	385	[27, 51, 53–55]
	Roxithromycin	5	Ch (5)	3.42	302	[50, 51, 53–55]
	Spriamycin	1	Ch (1)	61.9	61.9	[55]
	Tylosin	1	Ch (1)	–	nd	[27]
Sulfonamide	*Sulfachlorpyridazine*	1	US (1)	–	nd	[52]
	Sulfadiazine	6	Ch (6)	1.27	83.9	[27, 50, 51, 53–55]
	Sulfadimethoxine	3	US (2), Ch (1)	0.2	0.2	[46, 51, 52]
	Sulfamerazine	3	Ch (2), US (1)	1.44	2.47	[50–52]
	Sulfamethazine	9	Ch (6), US (2), K (1)	2.87	248	[27, 46, 47, 50–55]
	Sulfamethizole	1	USA (1)	–	nd	[52]
	Sulfamethoxazole	9	Ch (5), US (2), K (1), Sp (1)	0.52	7.86	[27, 36, 46, 47, 50–54]
	Sulfamonomethoxine	2	Ch (2)	1.55	1.86	[27, 51]
	Sulfapyridine	2	Ch (2)	3.71	9.12	[50, 51]
	Sulfaquinoxaline	1	Ch (1)	0.54	0.959	[50]
	Sulfathiazole	5	Ch (4), US (1)	2.06	5.94	[47, 50–52, 55]
	Sulfisoxazole	1	Ch (1)	1.71	1.71	[51]
Tetracycline	*Chlorotetracycline*	6	Ch (4), US (2)	10.5	1010	[27, 46, 47, 50, 52, 53]
	Doxycycline	3	Ch (3)	14.6	444	[27, 50, 53]
	Oxytetracycline	9	Ch (5), US (2), K (1), Sp (1)	41.5	214	[27, 36, 46, 47, 50, 52–55]
	Tetracylcine	9	Ch (5), US (2), K (1), Sp (1)	42	94.79	[27, 36, 46, 47, 50, 52–55]

Antibiotics whose concentration were below the limits of quantification (LOQ) are indicated as < LOQ

nd none detected

[a] China (Ch), Malaysia (My), Korea (K), Spain (Sp), United States (US), Denmark (Dk)

(9) show that the antibiotic or antimicrobial tested inhibited nitrification and the minimum inhibitory concentration (MIC) ranged from 200 $\mu g\ kg^{-1}$ (sulfadimethoxine, SDM) [95] to 200 $mg\ kg^{-1}$ (TET) [103]. Based on their low sorption coefficients (Table 3), sulfonamide antibiotics are likely to be the most bioavailable, which may account for the low inhibitory concentration of SDM relative to more sorptive species like CTC and TET. Although this claim is poorly supported by the MIC of other sulfonamides, a fair comparison is difficult because the lowest tested concentrations of the other sulfonamides were 2 $mg\ kg^{-1}$ (sulfadiazine, SDZ) and 4 $mg\ kg^{-1}$ (sulfamethoxazole, SMX).

Among the remaining antibiotics, the following five had no observable effect on nitrification: CTC, difloxacin (DIF), monensin (MON), ivermectin (INV), and chloramphenicol (CPH). That nitrification rates were not significantly modified at either low ($\mu g \cdot kg^{-1}$) or high ($mg \cdot kg^{-1}$) therapeutic concentrations for some antibiotics do not conclusively show that the

Table 3 Usage and physicochemical properties of select antibiotics

Antibiotic class	Antibiotic	Usage	pKa	Log K_{ow}	K_d ($L \cdot kg^{-1}$)	Half-life in soil (days)
Fluoroquinolone	*Ciprofloxacin*	Human Health, Veterinary [56]	6.09[a], 6.82[b] [57]	0.28	61,000 [58]	2310 ± 1155 [59]
	Enrofloxacin	Veterinary [56]	6.27[a], 8.3[b] [58]	1.1 [58]	260–6000 [58]	n/a
	Norfloxacin	Human Health [56]	6.40[a], 8.68[b] [60]	−1.0 [61]	n/a	1155 [59]
	Ofloxacin	Human Health [56]	5.97[a], 8.28[b] [58]	0.35 [58]	310 [58]	1386 ± 434 [59]
Macrolide	*Erythromycin*	Human Health[i], Veterinary [56]	8.88[a], 12.44[b] [62]	3.06 [62]	n/a	360 [63]
	Roxithromycin	Human Health[i] [56]	8.80[a], 12.45[b] [64]	2.75 [65]	n/a	>> 120 [66]
	Tylosin	Veterinary [56]	7.50[c] [67]	3.5 [58]	129.5 (est.) [68]	8.3 [66]
Sulfonamide	*Sulfachlorpyridazine*	Veterinary [56]	1.87[d], 5.45[e] [67]	0.31 [69]	09–1.8 [70]	21.3 [71]
	Sulfadiazine	Human Health[k], Veterinary [56]	2.01[d], 6.15[e] [72]	−0.092 [72]	2.0 [73]	12–18 [74]
	Sulfamethazine	Veterinary [75]	2.65[d], 7.65[e] [58]	0.89 [58]	0.6–3.1 [58]	18.6 [71]
	Sulfamethoxazole	Human Health[k] [56]	1.97[d], 5.70[e] [76]	0.89 [76]	n/a	9–18.3 [77]
	Sulfamonomethoxine	Veterinary [56]	1.98[d], 5.96[e] [78]	0.70 [69]	n/a	n/a
	Sulfathiazole	Veterinary [79]	2.01[d], 7.11[e] [67]	0.05 [69]	4.9 [58]	n/a
Tetracycline	*Chlortetracycline*	Human Health[k], Veterinary [56]	3.3[f], 7.44[g], 9.27[h] [80]	−0.36 [80]	794 [81]	25.9–30.8 [82]
	Doxycycline	Human Health[k], Veterinary [56]	3.02[f], 7.97[g], 9.15[h] [67]	0.02 [69]	n/a	533 ± 23 [59]
	Oxytetracycline	Human Health[k], Veterinary [56]	3.3[f],7.3[g], 9.1[h] [83]	1.22 [83]	680–1030 [84]	30.2–41.3 [82]
	Tetracycline	Human Health[k], Veterinary [56]	3.32[f], 7.78[g], 9.58[h] [67]	1.30 [69]	400–1620 [58]	578 [59]

n/a data not available

[a] Carboxyl group

[b] Protonated amino group

[c] Basic dimethylamine group

[d] Basic amine group

[e] Acidic amide group

[f] Tri-carbonyl group

[g] Dimethylamine group

[h] β-diketone

[i] Critically important antibiotic

[k] Highly important antibiotic

nitrifying community was unaffected. For example, Luis Campos et al. [91] observed no change in net nitrification in soils treated with either 10–250 $mg \cdot L^{-1}$ CPH or lower doses (<100 $mg \cdot L^{-1}$) of OTC but suggested that shifts in the ratio of ammonia-oxidizing bacteria (AOB) to ammonia-oxidizing archaea (AOA) may account for the lack of apparent response. Kotzerke et al. [99] proposed a similar explanation, stating that the contributions of fungal and archaeal nitrification may be sufficient to regulate net nitrification when AOB are inhibited. Although one study concludes that AOB are more important in N-rich soils [104], others tend to support the hypothesis that AOA are able to regulate nitrification when AOB are compromised. It has also been reported that AOA outnumber and likely outperform AOB [105].

In addition to providing resiliency when AOB are compromised, some studies have shown that population growth among AOA [106] or dose-related shifts in the fungi to bacteria ratio [107] is stimulated by some antibiotics. These types of shifts may partially explain stimulated nitrification, an outcome that was observed in soils treated with NOR (1 $mg \cdot kg^{-1}$) [74], bacitracin (BAC, 100 $mg \cdot kg^{-1}$), and a mixture of BAC, MON, and INV(0.1–100 $mg \cdot kg^{-1}$) [96]. In the latter treatment, a large shift in the AOA:AOB was correlated to accelerated nitrification observed in short-term soil mesocosms 7 and 30 days after receiving a 100-$mg \cdot kg^{-1}$ dose [96]. In an associated field experiment where lower doses (0.1–10 $mg \cdot kg^{-1}$) were applied, stimulation did not become evident until a year after the initial antibiotic application. AOA:AOB ratios were not shown for the field soils, but the delayed (1 year) response at lower doses suggests that changes in the microbial community may simply proceed more slowly when exposed to lower concentrations.

Stimulation was also observed in soil microcosms treated with CIP [92] and NOR [74]. In these experiments, nitrification was stimulated only at the lowest doses tested (1 $mg \cdot kg^{-1}$). At higher concentrations (>5 and >100 $mg \cdot kg^{-1}$, respectively), CIP and NOR inhibited nitrification. The apparent

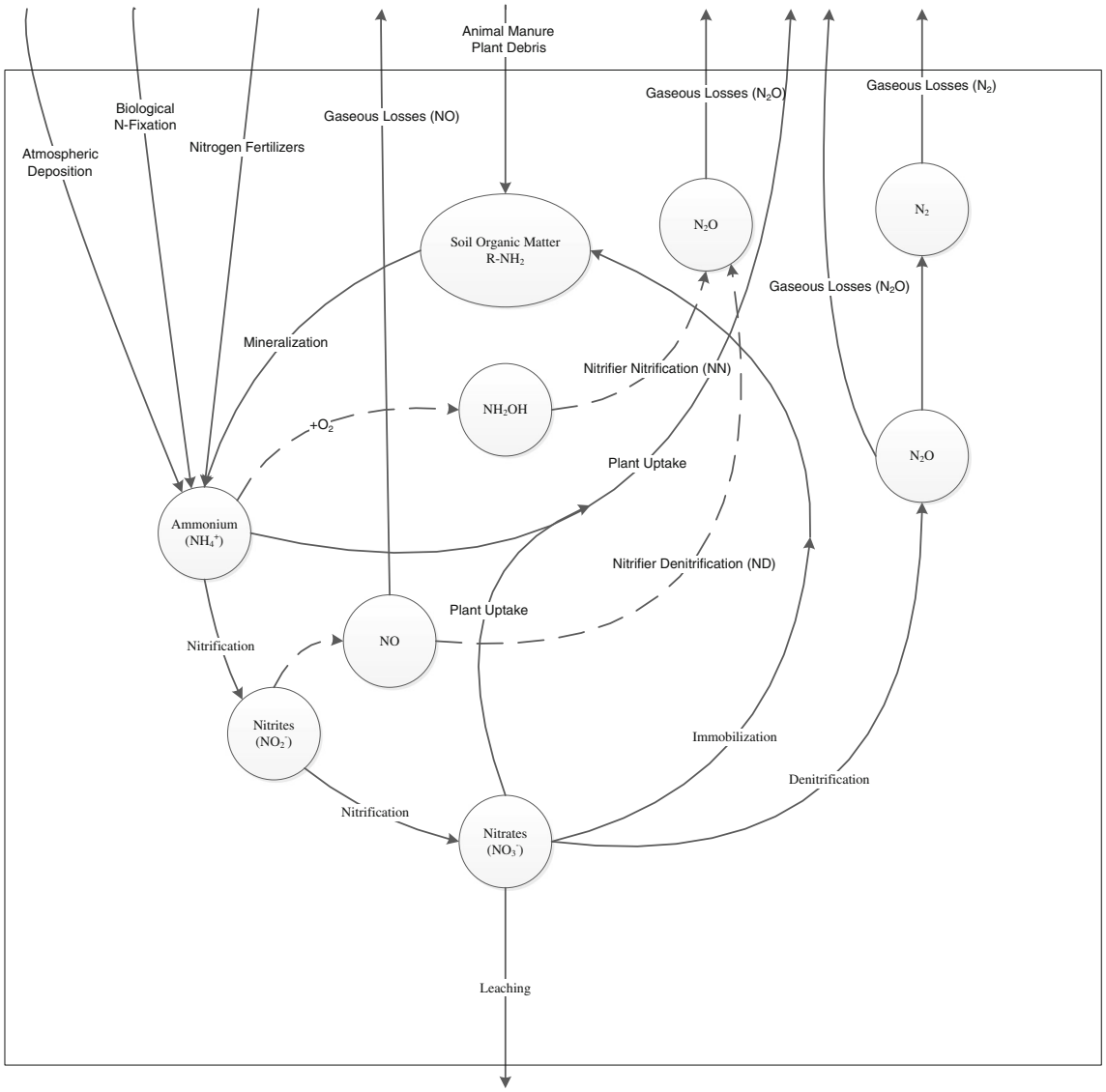

Fig. 1 Biogeochemical nitrogen cycle

disagreement at different doses is characteristic of hormesis, a J-shaped dose response in which low doses of a toxin stimulate response and high doses are inhibitory [108], though hormesis has never been explicitly studied for complex microbial communities such as those occurring in soils.

Presently, changes to microbial communities are the dominant hypotheses proposed to explain why nitrification is unchanged or even stimulated in some soils or sewage sludge following exposure to antibiotics. Positive shifts in the AOA:AOB ratio, for example, illustrate functional redundancy in the soils that may compensate for reduced AOB activity leading to no observed effect. Alternately, if AOA outperform AOB, a shift in the AOA:AOB ratio may accelerate nitrification in some soils following exposure to antibiotics. These changes do provide a potential explanation for stimulated nitrification where antibiotic exposure occurs, but they do not satisfactorily explain how the same dose of a single antibiotic can yield different results when applied to different media. For example, Louvet et al. [97] evaluated the effect of 0.1–20 mg·L^{-1} erythromycin (ERY) on nitrification in two different activated sludge materials. In the first sludge (Nancy), nitrification was inhibited (>10 mg·kg^{-1}), an observation corroborated by Katipoglu-Yazan et al. [103]. When Louvet et al. applied the same treatment to a different sludge (Epinal), however, a stimulatory effect was observed. Disagreement between these results may point to the role of the endemic microbial community in determining its response to antibiotic exposure. Though the sludges were obtained from the same region, the Nancy sludge was prepared with a biofilm on sand whereas the Epinal sludge was prepared in an oxidation ditch with no settling. These two sludge-forming environments may favor different groups of nitrifying organisms whose responses to ERY are sufficiently unique that stimulation is observed in one and inhibition in the other.

Table 4 List of observed effects of antibiotic on nitrification rate and nitrification potential[a] in soil and wastewater sludge

Antibiotic class	Antibiotic	Concentration ($mg \cdot kg^{-1}$ or $mg \cdot L^{-1}$)	Effect	Media[b]	Application method	Experimental duration	Reference
Amphenicol	*Chloramphenicol*	10–250	No Effect	Mixed culture[4]	Antibiotic solution	Weeks	[91]
Avermectin	*Invermectin*	0.1–10	No Effect	Soil (field)[4]	Antibiotic solution	Weeks/years	[91]
Fluoroquinolone	*Ciprofloxacin*	1–50	Inhibition (>5 $mg \cdot kg^{-1}$) Increased (1 $mg \cdot kg^{-1}$)	Soil microcosm[1]	Antibiotic solution	Hours	[92]
	Difloxacin	0.007–0.012	No effect	Soil microcosm[1]	Contaminated manure	Weeks	[93]
	Norfloxacin	1–200	Stimulation (1 $mg \cdot kg^{-1}$) inhibition (>100 $mg \cdot kg^{-1}$)	Soil microcosm[2]	Antibiotic solution	Weeks	[74]
	Ofloxacin	2–10	Inhibition	Pure culture[4]	Spiked media	Hours	[94]
Ionophore	*Monensin*	0.010–0.100	No effect	Soil microcosm[3]	Spiked manure	Weeks	[95]
		0.1–10	No effect	Soil (field)[4]	Antibiotic solution	Weeks/years	[96]
		100	No effect	Soil microcosm[4]		Weeks	
Macrolide	*Erythromycin*	0.1–20	Inhibition (10 $mg \cdot L^{-1}$) stimulation (10 $mg \cdot L^{-1}$)	WW sludge[4]	Spiked wastewater	Hours	[97]
		1–267	Inhibition (>20 $mg \cdot L^{-1}$)	WW sludge[4]	Spiked wastewater	Days	[98]
	Virginiamycin	1.5–500	Inhibition (>15 $mg \cdot kg^{-1}$)	Soil microcosm[4]	Antibiotic solution	Days	[99]
Mixed	*(Bac/Mon/Inver)*	0.1–10	Stimulation (>1 year)	Soil (field)[4]	Antibiotic solution	Weeks/years	[96]
		100	Stimulation	Soil microcosm[4]		Weeks	
Organoarsenic	*Roxarsone*	1.5–500	Inhibition (>150 $mg \cdot kg^{-1}$)	Soil microcosm[4]	Antibiotic solution	Days	[100]
Polymyxin	*Colistin*	0.3–300	Inhibition (ammonia oxidation)	Mixed culture[4]	Antibiotic solution	Hours	[101]
Polypeptide	*Bacitracin*	0.1–10	No effect	Soil (field)[4]	Antibiotic solution	Weeks/years	[91]
		100	Increased	Soil microcosm[4]		Weeks	
		1.5–500	No effect	Soil microcosm[4]	Antibiotic solution	Days	[100]
Sulfonamide	*Sulfadiazine*	10 and 100	Inhibition (100 $mg \cdot kg^{-1}$)	Soil microcosm[1]	Spiked manure	Weeks	[99]
		4	Inhibition	Soil microcosm[1]	Spiked manure	Weeks	[102]
	Sulfadimethoxine	0.025–0.200	Inhibition (200 $\mu g \cdot kg^{-1}$)	Soil microcosm[3]	Spiked manure	Weeks	[95]
	Sulfamethoxazole	2–10	Inhibition	Pure culture[4]	Spiked media	Hours	[94]
Tetracycline	*Chlortetracycline*	0.0003–0.03	No effect	Soil microcosm[3]	Spiked manure	Weeks	[103]
	Oxytetracycline	10–250	Inhibition	Mixed culture[4]	Antibiotic solution	Weeks	[91]
	Tetracycline	50 and 200	Inhibition (200 $mg \cdot kg^{-1}$)	WW sludge[4]	Antibiotic solution	Hours	[103]

[a] Nitrification potential measures accumulation of NO_2^- when NO_2^- to NO_3^- oxidation step is inhibited

[b] Method used for quantification: [1] chlorate inhibition (nitrification potential), [2] NO_3^--N, [3] NO_2^--N, [4] $NO_2^- + NO_3^-$

Denitrification

Denitrification is a naturally occurring process in which NO_3^- is sequentially reduced to N_2 gas (Eq. 3):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO\ (g) \rightarrow N_2O\ (g) \rightarrow N_2\ (g) \quad (3)$$

Denitrifying organisms include a diverse group of bacteria, fungi, and archaea [109], but the majority of denitrification is attributed to heterotrophic anaerobes. The best-studied denitrifying bacteria include *Paracoccus denitrificans* and *Pseudomonas stutzeri* [110]. Each stage of the denitrification process is facilitated by one or more membrane-bound enzymes including: NAR (NO_3^- reductase), NIR (NO_2^- reductase), NOR (NO reductase), and NOS (N_2O) reductase [111]. Although N_2 is the dominant denitrification product (>95 %), some fraction is lost as free NO or N_2O.

Effects of Antibiotics on Denitrification

The effects of 18 different antibiotics on denitrification have been investigated, and the results vary considerably among the different solid matrices and concentrations tested (Table 5). Inhibition was reported in soil, sediment, and/or groundwater treated with the following 12 antibiotics: BAC, amoxicillin (AMO), clarithromycin (CLA), CTC, ERY, FLE, gentamicin (GTC), narasin (NAR), SDZ, SMZ, SMX, and vancomycin (VAN). In sediment, Costanzo et al. [113], Yan et al. [115], Laverman et al. [120], and Roose-Amsaleg et al. [119] measured the effects of eight different antibiotics on denitrification rate. Inhibition relative to the control was observed for seven of these antibiotics, but none at a concentration <1000 $\mu g \cdot L^{-1}$ except where SMZ (0.05–100 $\mu g \cdot L^{-1}$) was applied [116]. Because SMZ has very low sorption coefficient in comparison to most of the other antibiotics tested, what appears to be greater sensitivity to this antibiotic may simply be a reflection of bioavailability. On the other hand, SMX is equally mobile and was only observed to inhibit denitrification in sediment at concentrations in excess of 57.5 $mg \cdot L^{-1}$ [115]. Because the antibiotic agencies and physicochemical properties of SDZ and SMX are comparable, a 1000-fold difference between their reported MICs is unexpected, but there are a number of experimental dissimilarities that may account for it. For example, Yan et al. [115] conducted a series of flow-through reactor experiments in which the input solution containing 0.24, 2.1, 11, or 57,500 $\mu g \cdot L^{-1}$ SMX was continuously supplied over a period of weeks, and steady-state denitrification was measured from the ratio of effluent to influent $NO_2^- + NO_3^-$. Significant inhibition was observed only at the 57,500-$\mu g \cdot L^{-1}$ dose, leading the authors to suggest that chronic exposure to therapeutic doses may promote SMX resistance. Resistance is less likely to develop in short-term experiments following a single antibiotic dose, which may explain why Hou et al. [116] were able to observe inhibition in sediments 1–8 h after they were treated with lower doses of SDZ (0.05–100 $\mu g \cdot L^{-1}$). On the other hand, the effects of SMX were not investigated for any dose between 11 and 52,500 $\mu g \cdot L^{-1}$, and future studies conducted within this range may identify inhibitory concentrations of SMX that are more consistent with the results of short-term studies. Furthermore, the resistance hypothesis does not explain why therapeutic concentrations of SMX and SMZ inhibited denitrification in groundwater studies even when the antibiotic was continuously supplied [117, 118]. A total of 7 antibiotics (3 sulfonamide, 1 β-lactam, 1 aminoglycoside, 1 ionophore, and 1 polypeptide) inhibited denitrification in soils, while several others were reported to stimulate denitrification, particularly at ultra-low ($ng \cdot kg^{-1}$ or $ng \cdot L^{-1}$) concentrations. For example, SMX inhibited denitrification in groundwater at 1.2 $\mu g \cdot L^{-1}$ [118] but accelerated NO_3^- reduction in flow-through column experiments (1 $ng \cdot L^{-1}$), and the effect was amplified over time [112].

Effects of Antibiotics on NO and N_2O Emissions

Eco-toxic NO and N_2O gases are minor products of nitrification and denitrification. N_2O is produced by bacteria, archaea, and some fungi in soil and sediment as a byproduct of nitrification or as free intermediates of denitrification. Under anoxic conditions, the predominant pathway is via the sequential reduction: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O$. Although a portion of N_2O produced in soil and sediment will be consumed by bacteria able to use it as a terminal electron acceptor [121, 122], some will ultimately be diffused to the surface and lost to the atmosphere. Because N_2O is a potent greenhouse gas and can reduce stratospheric ozone, the flux of N_2O from soil and sediment is of significant interest. However, the impact of terrestrial antibiotics on N_2O emissions from soil and sediment has scarcely been addressed. In fact, only 2 studies were found that explicitly investigate this topic. Both observed a rise N_2O flux in soils treated with sub-therapeutic doses of antibiotics. Hou et al. [116] tested the effects of SMZ (0.05–100 $\mu g \cdot L^{-1}$) and reported an increase in N_2O flux by as much as 300 % (>50 $\mu g \cdot L^{-1}$ SMZ) within 8 h of exposure. Because the increase in N_2O flux coincided with inhibited denitrification, the authors propose that antibiotics may more strongly inhibit N_2O reduction to N_2 than N_2O production itself, resulting in an increased N_2O:N_2 production ratio [116]. DeVries et al. [112] proposed a similar conclusion upon observing a threefold increase in N_2O flux in soils amended with 1–1000 $ng \cdot kg^{-1}$ NAR after a 3-day incubation. Alternately, increased denitrification, which was reported for 4 antibiotics in soil and groundwater, may also increase N_2O flux without an associated shift in the N_2O:N_2 ratio and ought to be investigated in future studies. NO is also produced in small quantities during nitrification and is a free intermediate of denitrification. Though it is a major component of smog, no studies

Table 5 List of observed effects of antibiotic on denitrification rate or potential

Antibiotic class	Antibiotic	Concentration ($mg \cdot kg^{-1}$ or $mg \cdot L^{-1}$)	Effect	[a]Media	Application method	Experimental duration	Reference
Aminoglycoside	*Gentamicin*	1E-6–0.001	Inhibition (1–10 $ng \cdot kg^{-1}$) Stimulation (>100 $ng \cdot kg^{-1}$)	Soil[1]	Antibiotic Solution	Days	[112]
β-Lactam	*Amoxycillin*	1.0	Inhibition	Sediment[2]	Antibiotic solution	Hours	[113]
Fluoroquinolone	*Ciprofloxacin*	0.0001–1.0	No effect	Sediment[2]	Antibiotic solution	Hours	[113]
		0.001–1.0	No effect	Soil[2]	Antibiotic solution	Days	[114]
	Difloxacin	0.007–0.012	No effect	Soil[2]	Contaminated Manure	Weeks	[93]
		1–100	Inhibition (500 $\mu g \cdot kg^{-1}$)	Soil[2]	Spiked manure	Weeks	[99]
	Flumequine	0.00014–52.5	Inhibition (52,500 $\mu g \cdot L^{-1}$)	[+]Sediment FTR[3]	Antibiotic solution	Weeks	[115]
Ionophore	*Narasin*	1E-6–0.001	Inhibition (>5 days) stimulation (1–4 days)	Soil[1]	Antibiotic solution	Days	[112]
Macrolide	*Clarithromycin*	1.0	Inhibition	Sediment[2]	Antibiotic solution	Hours	[113]
	Erythromycin	1.0	Inhibition	Sediment[2]	Antibiotic solution	Hours	[113]
Polypeptide	*Bacitracin*	1.5–500	Inhibition	Soil[1]	Antibiotic solution	Days	[100]
Sulfonamide	*Sulfadiazine*	10–100	Inhibition (10 $\mu g \cdot kg^{-1}$)	Soil[2]	Spiked manure	Weeks	[99]
		1E-6–0.001	Inhibition (>100 $ng \cdot kg^{-1}$) Stimulation (1–10 $ng \cdot kg^{-1}$)	Soil[1]	Antibiotic solution	Days	[112]
		4.0	No effect	Soil[2]	Spiked manure	Weeks	[102]
	Sulfamethazine	0.00005–0.100	Inhibition	Sediment[4]	Antibiotic solution	Hours	[116]
		0.01–1	Inhibition (>0.01 $mg \cdot L^{-1}$)	Groundwater[3]	Antibiotic solution	Days	[117]
	Sulfamethoxazole	0.00024–57.5	Inhibition (57,500 $\mu g \cdot L^{-1}$)	[+]Sediment FTR[3]	Antibiotic solution	Weeks	[115]
		0.0012–500	Inhibition (1.2 $\mu g \cdot L^{-1}$)	Groundwater[3]	Antibiotic solution	Weeks	[118]
		1E-6–0.001	Inhibition 10 $ng \cdot kg^{-1}$) stimulation (1, 1000 $ng \cdot kg^{-1}$)	Soil[1]	Antibiotic solution	Days	[112]
		1E-6	Stimulation	Groundwater[1]	Antibiotic solution	Days	[112]
		0.001–1.0	Inhibition (500 $\mu g \cdot kg^{-1}$)	Soil[2]	Antibiotic solution	Days	[114]
Tetracycline	*Chlortetracycline*	0.01–1	Inhibition (1 $mg \cdot L^{-1}$)	Groundwater[3]	Antibiotic solution	Days	[117]
	Tetracycline	2–128	No effect	[+]Sediment FTR[1]	Antibiotic solution	Weeks	[119]
		0.001–1.0	No effect	Soil[2]	Antibiotic solution	Days	[114]
Other	*Roxarsone*	1.5–500	None	Soil[1]	Antibiotic solution	Days	[100]
		0.00029–0.187	None	[+]Sediment FTR[3]	Antibiotic solution	Weeks	[115]
		0.001–1.0	Inhibition (1000 $\mu g \cdot L^{-1}$)	[b]Sediment FTR[1]	Antibiotic solution	Days	[120]
	Virginiamycin	1.5–500	None	Soil[1]	Antibiotic solution	Days	[100]

[a] Method used for quantification: [1] NO_3^-, [2] Acetylene Block, [3] $NO_2^- + NO_3^-$, [4] Isotopic Enrichment [15] N-NO_3^-

[b] Sediment flow through reactor

have investigated the effects of antibiotics on NO flux from soil or sediment.

Overview of Current Measurement Methodology

The results of these investigations allow few broad conclusions regarding the effects of antibiotics and antimicrobials on nitrification and denitrification. Total consensus is not expected because individual antibiotics target different types of organisms and vary in their efficacy. Inconsistent results among antibiotics of the same class or for a single antibiotic compound, however, likely are influenced by methodological differences. Konopka et al.'s [96] two investigations illustrate this point well. In their short-term study, 100 mg·kg^{-1} of BAC/MON/ROX stimulated nitrification in soil mesocosms but lower doses (0.1–10 mg·kg^{-1}) had the same effect in field soils, but it was not observed until 1 year after the initial application. Had the field study been terminated after a few weeks, the authors would have reported that the lower doses have no effect, which highlights the need for a higher degree of consistency in terms of antibiotic dose and experimental duration. The results of individual experiments may also be influenced by natural variations in soil or sediment composition or the use of nutrient amendments. For example, Konopka et al. [96] reported increased nitrification and a positive shift in the AOA:AOB ratio in a loamy soil 7 days after it was dosed with 100 mg·kg^{-1} BAC. In contrast, Banerjee et al. [100] reported no effect within 5 days after applying a comparable dose to a silty loam. An accompanying fatty acid methyl ester (FAME) profile analysis indicated that there was no significant change in the microbial community [100]. Notable differences between the two studies include the soil properties and the use of N fertilizer amendments. The organic carbon (OC) content of the soil used by Banerjee et al. was higher (3.9 vs. 2.5%), and the soil was amended with $(NH_4)_2SO_4$ to help promote nitrification. Higher OC may enhance the role of heterotrophic nitrifiers, and if these organisms are less sensitive to BAC than autotrophic AOB, there may be less opportunity for AOA to take a more prominent role in nitrification. Alternately, amending the soil with $(NH_4)_2SO_4$ stimulates all nitrifying activity, and the resulting growth may compensate for any negative impacts that BAC may have on one or more individual groups of organisms.

Antibiotics that affect the structure and function of the soil or sediment microbial community may also alter nitrification pathways or denitrification product ratios. Where this occurs, standard methods for quantifying nitrification may not accurately measure the nitrification rate in soils exposed to antibiotics or capture shifts in the N_2O:N_2 ratio. Nitrification is most commonly measured by monitoring the size of the reactant (NH_4^+) or product (NO_2^-/NO_3^-) pools over time. Under nitrifying conditions, the NH_4^+ pool will be reduced over time, and nitrification rate is taken as $\Delta NH_4^+/\Delta t$. NH_4^+ can be extracted from soil and sediment with a concentrated salt solution (2 M KCl) and quantified colorimetrically. The indophenol blue method [123] is most common and can be performed manually or by automated flow injection analysis. Since autotrophic oxidation to NO_3^- via NO_2^- is the dominant nitrification pathway in soil and sediment [124], nitrification rates determined by the product pools are measured by quantifying the accumulation of $NO_2^- + NO_3^-$ over time. Both are easily extracted from soil and sediment into aqueous solution and can subsequently be quantified by a number of reliable and inexpensive colorimetric methods, e.g., cadmium reduction [125, 126]. Under some conditions, NO_3^- may undergo rapid reduction to N_2O or N_2 (denitrification) and preclude reliable measurements of nitrification from the combined $NO_2^- + NO_3^-$ pool. In these circumstances, an inhibitor such as sodium chlorate can be added to the soil to prevent the oxidation of NO_2^- to NO_3^-. When inhibitors are used, the measurement is called potential nitrification and is determined from the increase of NO_2^- over time [127].

Alternate nitrification pathways that affect the concentration of NO_2^- and NO_3^- are not captured by these methods. For example, nitrifier nitrification (NN), nitrifier denitrification (ND), and annamox each influence the size of the NO_2^- pool. NN lowers NO_2^- production rate by oxidizing NH_2OH to N_2O and the latter (ND and annamox) consume NO_2^-. Assuming no change to total nitrification, an increase in the ratio of any of these pathways to complete oxidation ($NH_4^+ \rightarrow NO_3^-$) will cause the nitrification rates to be underestimated when the $NO_2^- + NO_3^-$ pool is used for quantification. If the shift is significant, the apparent reduction in nitrification rate may even be reported as inhibition. Similarly, if antibiotics reduced the contributions of NN, ND, and annamox to total nitrification, the $NO_2^- + NO_3^-$ pool would increase in size and cause the nitrification rate to be overestimated and reported as stimulation. In order to avoid over/underestimation of nitrification rate, we recommend that determination of NO and N_2O flux be included in future studies evaluating the effects of antibiotics on nitrification rate.

The most common methods for quantifying the effects of antibiotics on denitrification rate include monitoring the depletion of NO_3^- over time under anaerobic conditions and the acetylene block method. In the latter method, acetylene gas is added to gas-tight sample vials to inhibit reduction of N_2O to N_2, and denitrification rate is determined from the concentration of N_2O in headspace [128]. NO_3^- measurements often require destructive sampling, so the acetylene block method is better suited to evaluate changes on shorter time scales, i.e., hours vs. days. Since denitrification follows a linear pathway, neither method is prone to over/underestimating denitrification as a result of changes to the microbial community but they also do not provide a coincident measure of the N_2O:N_2 or NO:N_2 flux ratios. Where NO_3^- is used as a metric, N_2O flux

is not considered at all. In the latter, NO is not quantified, and acetylene inhibits the reduction of N_2O to N_2 which will mask shifts in the N_2:N_2O ratio that may result from antibiotic exposure. Furthermore, both of these methods are conducted under fully anaerobic conditions to prevent nitrification from adding to the NO_3^- pool during the measurement period. This may be realistic for sediment, but denitrification in soils is more often limited to anaerobic hotspots that develop in micropore spaces and rarely occurs in complete isolation from nitrification. It may therefore be more relevant in soils to use stable ^{15}N methods to quantify nitrification and denitrification rates.

Stable ^{15}N isotopic tracers have the advantage that they can capture process-rate changes in nitrification and denitrification under conditions favoring coupled nitrification-denitrification. For example, the isotope dilution method uses a ^{15}N-KNO_3 enrichment, and nitrification rate (μg N g^{-1} soil d^{-1}) may be calculated from ^{15}N-NO_3^- dilutions according to the equations 1–11 of Kirkham and Bartholomew [129]. Denitrification rate (μg N g^{-1} soil d^{-1}) can also be determined from this enrichment using the ratios of $^{28}N^2$, $^{29}N_2$, and $^{30}N_2$ in headspace [130]. These methods can be paired with a ^{15}N-NH_4^+ enrichment to concurrently measure organic N mineralization rates, which have not previously been measured in soils treated with antibiotics. Because they allow for quantification of the cumulative effects of the antibiotic on reaction rate and the resultant accumulation of N_2O and NO_3^-, combining these measurements may be particularly relevant under fluctuating soil moisture conditions or when changes to the soil/sediment microbial community are probable.

Conclusions and Prospects

Current data indicate that the biogeochemical N cycle may be altered by environmentally relevant concentrations of antibiotics. Of the processes evaluated, nitrification appears less sensitive to antibiotics than denitrification at therapeutic doses (<mg·kg^{-1}). Although mg·kg^{-1} concentrations have been reported in wastewater and wastewater sludge where inhibition of either process may reduce overall wastewater treatment efficiency, there remains inadequate information regarding the effects at sub-therapeutic concentrations to conclusively evaluate the risk to ecosystem function in aquatic and terrestrial environments. As limits of detection have improved, it has become evident that a number of antibiotics are present in soils at concentrations in the low ng·kg^{-1} range, and thus, there is a clear need to examine a broader range of concentrations when testing for effects on N processing. Where environmentally relevant concentrations have been evaluated, the sulfonamide group appears to have the greatest potential to significantly affect microbial N cycling. Although this partially is due to the fact that the sulfonamides have been the most frequently tested antibiotics, the associated risk is enhanced by their high mobility in soil and sediment and the apparent sensitivity of both nitrifiers and denitrifiers to concentrations as low as 1 or 1 ng·L^{-1}.

The number of studies exploring the impacts of antibiotics on biogeochemical N cycling has notably increased in recent years; yet, there are a number of substantial weaknesses highlighted by this review. Like Roose-Amsalag and Laverman [23], we find that there is a distinct lack of consistency among different studies in terms of antibiotic dose, substrate, method by which nitrification and/or denitrification are measured, and the duration of the experiment. The result is that comparisons between individual studies are difficult, if not impossible. Second, all of the research summarized here focuses on process rates and with little or no regard to the measurable outcome of process-related change such as the accumulation of eco-toxic NO_3^-, NO, and N_2O. Furthermore, common methodological approaches to quantify process rates may over/underestimate the effects of antibiotics on a given process where the size of the N-pool used for quantification is affected by changes to the redox pathway.

Addressing these concerns will require a more systematic and comprehensive approach to future investigations. Recommendations include establishing a standardized set of antibiotic doses that include sub-therapeutic concentrations (<μg kg^{-1}) and including testing antibiotics from the β-lactams group. Where the effects of antibiotics on process rate are evaluated, e.g., nitrification or denitrification, more comprehensive measurement tools should be considered to avoid either (1) over/underestimating the effects of antibiotic exposure or (2) masking the accumulation of eco-toxic compounds. For example, nitrification measurements can be modified to include NO and N_2O flux measurements. In addition to providing relevant information about the effects of the antibiotic on these fluxes, including these measurements may also afford a more accurate determination of the effects of antibiotics on nitrification. Where possible, isotopic tracer studies can be substituted for the acetylene block methods to allow simultaneous measurements of denitrification and N_2O flux. Furthermore, a combination of isotope dilution techniques can be combined to study the effects of antibiotics on coupled nitrification-denitrification in soils, which would allow net effect of antibiotics on the resulting accumulation of N_2O and leachable NO_3^- in soils to be effectively determined. Although isotopic tracer studies are more expensive and time-consuming than the other methods discussed (e.g., mineral N diffusions for ^{15}N analysis require a 1–3-week incubation [131]), these may be well-suited for long-term investigations. There is evidence that the effects of antibiotic exposure may not be evident for as long as 1 year after initial exposure, highlighting the need for future studies to include multi-year investigations in which antibiotics applications are replicated over time or delivered continuously.

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Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to declare.

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