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Microbial faecal pollution of river water in a watershed of tropical Ethiopian highlands is driven by diffuse pollution sources

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ABSTRACT

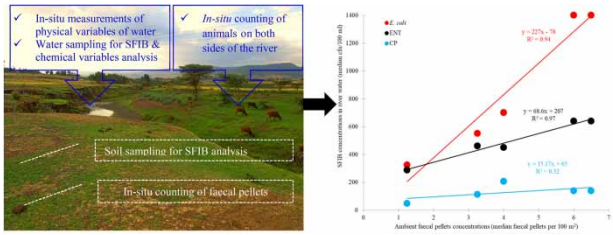
Tropical communities in the developing world depend heavily on riverine systems for their socioeconomic development. However, these resources are poorly protected from diffuse pollution, and there is a lack of quantitative information regarding the microbial pollution characteristics of riverine water, despite frequently reported gastrointestinal diseases. The aim of our study was to apply faecal taxation (i.e., faecal pellet counting in representative test areas to estimate the potential availability of diffuse pollution sources) in combination with a detailed microbiological faecal pollution analysis in a riverine environment to elucidate the importance of diffuse pollution. To realize this approach, ambient faecal pellets, a multiparametric data set for standard faecal indicator bacteria (SFIB), including *Escherichia coli*, *Clostridium perfringens* spores and enterococci from catchment soil and river water, and a number of riverine water physicochemical variables were analysed during a one-year cycle. We demonstrated that the abundance of ambient faecal pellets, which were consistently counted at reference sites in the catchment, was associated with faecal pollution in the river water. Water SFIB, dissolved oxygen, nutrients, conductivity and total suspended solids were strongly linked with the abundance of ambient faecal pellets in the river catchment, as demonstrated by principal component analysis (PCA). Elevated concentrations of SFIB in the riverine water in the absence of rainfall also suggested the direct input of faecal bacteria into the riverine water by livestock (e.g., during watering) and humans (e.g., during bathing). Statistical analyses further revealed that the microbiological water quality of the investigated riverine water was not influenced by SFIB potentially occurring in the soil. This study demonstrates the importance of diffuse faecal pollution sources as major drivers of the microbiological quality of riverine water in the Ethiopian highlands. In addition, the new successfully applied integrated approach could be very useful for developing predictive models, which would aid in forecasting riverine microbiological quality in tropical developing countries.

Key words: catchment landscape, diffuse pollution source, faecal indicator bacteria, faecal taxation, riverine water, soil

HIGHLIGHTS

- Faecal taxation could reveal diffuse drivers of standard faecal indicator bacteria (SFIB) in riverine water.
- Data from rainfall, soil and water SFIB and faecal pellet counts were integrated.
- Rainfall-induced runoff introduced catchment faecal pellets to the river water.
- SFIB densities in the riverine water strongly correlated with faecal pellet counts.
- Faecal pellets and rainfall-induced runoff were the dominant drivers of SFIB in the riverine water.

GRAPHICAL ABSTRACT



INTRODUCTION

River ecosystems make a significant contribution to socioeconomic development, species survival and the function of the natural environment (Savio *et al.* 2015; Koundouri *et al.* 2016). However, a substantial number of these river systems originate from highlands, where animals, including livestock, graze (Atickem & Loe 2013). These animals account for a significant number of faecal pellets in the watershed that increases the risk of river water contamination. Despite the fact that faecal pellets are the origin of faecal microbes, including pathogenic bacteria and viruses, there exist no studies in tropical countries attempting to explore the connection between watershed faecal pellets and microbiological quality of riverine water, despite the increasing number of livestock in the tropical watersheds (Strauch *et al.* 2009). While contamination of river waters has direct economic impacts on catchment communities via the loss of fisheries and restrictions on recreational uses, there is no information regarding the extent of watershed faecal pellets and faecal contamination in riverine water, although many communities in this environment are heavily dependent upon river catchment for many of their activities (Woldemichael *et al.* 2016). This lack of information not only limits our understanding regarding the type of management needed to intercept and mitigate this source of pollution but also our inability to apply appropriate measures suitable for mitigating waterborne diseases in the tropical areas. Consequently, there is a need to comprehensively investigate the contribution of watershed faecal pellets to microbiological quality in tropical waterways to create a reliable water quality management plan appropriate for catchments affected by faecal pellets.

Like many developing countries in tropical environments, Ethiopia is characterized by a high livestock population (54 million head of cattle and 25.5 million sheep), the majority of which are kept in traditional extensive production systems (Central Statistical Agency 2013). Owing to the existence of diverse riparian vegetation and water, many river catchments in Ethiopia have attracted a significant number of livestock, leading to the direct deposition of faecal materials on riparian land (Muirhead *et al.* 2005; Kay *et al.* 2018). Given that livestock faeces represent a serious concern to public health (Cox *et al.* 2005; Hutchison *et al.* 2005), understanding the fate of faecal materials deposited by grazing livestock on the riparian environment is of the utmost importance, especially to safeguard the growing population and economic development that are rapidly occurring in Ethiopia. Although riparian areas are characterized by vegetation known to intercept riparian contaminants, preventing contaminants from entering the waterways (Smith 1989; Quinn *et al.* 1993; Wilcock *et al.* 2009), in Ethiopia, riparian vegetation serves as pivotal grazing land, with a large area used for crop cultivation, and in the dry season, riparian areas are the only source of green pastures for livestock. Given that grazers destabilize riverbanks and reduce the quantity and quality of riparian vegetation, it is unclear to what extent disturbed riparian vegetation in tropical environments may prevent faecal-associated bacteria from entering the river water. Therefore, there is a need to understand how the microbiological quality of river water responds to the grazed riparian vegetation of tropical river systems to design an improved mechanism for preventing the occurrence of unnecessary conflict between pastoralists and watershed managers responsible for maintaining the health of the river ecosystem.

Standard faecal indicator bacteria (SFIB) originate from primary human and animal intestines and from secondary habitats, such as sediment and soil accumulators. These faecal microbes are used to gauge the presence and extent of faecal contamination (Byamukama *et al.* 2005), as well as in predicting pathogenic bacteria (Touron *et al.* 2007) and outbreaks of waterborne diseases (Colford *et al.* 2012) in the environment. Commonly applied SFIB include *Clostridium perfringens* (CP), *Escherichia coli* and enterococci (ENT) (Byamukama *et al.* 2000, 2005; Goshu *et al.* 2010; Mushi 2018). Well-established and certified standard procedures (ISO 2000a, 2000b, 2013) are used to detect and enumerate SFIB from waterways. The use of SFIB to completely gauge the microbiological safety of river water, whose riparian environment

serves a substantial number of grazing livestock and is influenced by faecal pellets, has not been implemented in the tropical environment, although waterborne diseases are frequently reported in this environment (Azage *et al.* 2015, 2017). Available information in the literature regarding the quality status of riverine water in Eastern Africa is exclusively related to the measurement of heavy metals (Woldemichael *et al.* 2016; Zinabu *et al.* 2019) and macroinvertebrates (Aschalew & Moog 2015; Desalene 2018), which do not infer the presence of faecal contamination or predict outbreaks of waterborne diseases.

Therefore, this study investigated the contribution of animals grazing on riparian vegetation to the microbiological quality of river water in the Upper Awash Basin, which is frequently prone to rainfall-induced runoff events. To fully understand this contribution, concentrations of SFIB from the river water were statistically compared with those of catchment soil and the abundance of ambient faecal pellets. We hypothesized that livestock faecal pellets in the watershed rather than riparian soil were responsible for the elevated faecal pollution in the water column of the riverine system, and SFIB of faecal pollution should exemplify this relationship. To date, there have surprisingly been no investigations regarding data linking ambient faecal pellets to the microbiological quality status of waterways in tropical countries such as Ethiopia. This gap in knowledge is surprising given the importance of safe freshwater to human development. However, numerous investigations have been performed on the contribution of catchment landscapes to the microbiological quality of riverine systems. Those investigations focused almost exclusively on the contribution of catchment soil (Mushi 2018), counts of catchment septic tanks (Verhougstraete *et al.* 2015) and sewage effluents (Xiao *et al.* 2018) to the microbiological quality of riverine water. To the best of our knowledge, only Kirschner *et al.* (2004) have published a paper on faecal taxation in stagnant ponds but not in riverine systems. To generate a comprehensive data set for hypothesis testing, the abundance of ambient faecal pellets and SFIB concentrations in the catchment soil and river water were separately measured over a 12-month faecal pollution study across five sites strategically spaced throughout the Upper Awash Basin in the Ethiopian Highlands.

MATERIALS AND METHODS

Study area and sampling site characteristics

The Awash River begins on a high plateau in the Central Ethiopian Highlands at an elevation of 3,000 m a.s.l. This river flows along the East African rift valley into the Afar triangle and terminates in Lake Abbe, a salt lake, over a stretch of 1,200 km (Dessu *et al.* 2016; Müller *et al.* 2016). The river has an annual flow of 4.9 billion cubic metres (Tadese *et al.* 2019) as a result of draining a catchment with a diverse land-use system, including forest, agricultural fields, urban areas and other areas (Müller *et al.* 2016). The Awash River Basin is divided into four distinct physical and socioeconomic zones: Upper Basin, Upper Valley, Middle Valley and Lower Valley (Taddese *et al.* 2003). Due to the topography of the area and the existence of diverse land-use systems and animals, including cattle, sheep, goats, donkeys, horses, mules, dogs, birds and cats, the head section of the Awash River (Figure 1) with an elevation ranging from 2,181 to 2,484 m a.s.l was selected for testing the hypotheses of this study. This environment is characterized by a mean annual rainfall ranging from 160 to 1,600 mm and an air temperature ranging from 2.5 to 25 °C. The Awash River experiences two prominent discharge peaks per year as a result of short (March–April) and long (June–September) rains. The soil texture of the catchment ranges from clay-loam to clay and to clay-loam-sand (Tolera *et al.* 2018). While catchment soils support diverse forest plants and food crops, river water serves a significant number of Ethiopians as a source of open bathing, in-stream washing, swimming, animal watering, crop irrigation and drinking water supply (Kebede *et al.* 2020). Despite the fact that these services are critical to the socioeconomic development of Ethiopian communities, river water is not protected from anthropogenic influences, such as livestock grazing in the riparian zones, sand excavation along the riverbanks and open defecation by catchment communities living close to the Upper Awash River. Furthermore, the quantity and quality of riparian vegetation is considerably reduced in the downstream direction along the river, resulting in soil erosion as signified by gullies in the catchment and carvings along the riverbanks (Figure 2). To investigate the contribution of catchment-associated faecal pellets to the microbiological quality of the river water, five sampling sites (AW1 to AW5) were selected within a 22 km stretch of the Upper Awash River Basin (Figures 1 and 2). Site AW1 is located close to the source of the Awash River. The catchment around this site is characterized by 5,000 ha of forest that supports diverse species of plants and wildlife (Kebede *et al.* 2020). Although there were no observable human settlements in this site, approximately 0.09 livestock per hectare are occasionally brought into this forest for grazing. Site AW2 was located 2 km downstream of site AW1, where the Worebo Stream joins the Awash River. This site is characterized by a limited number of human settlements (23 households) dispersed throughout the forest. The people in this area primarily work in small-scale crop production and approximately 0.54 livestock

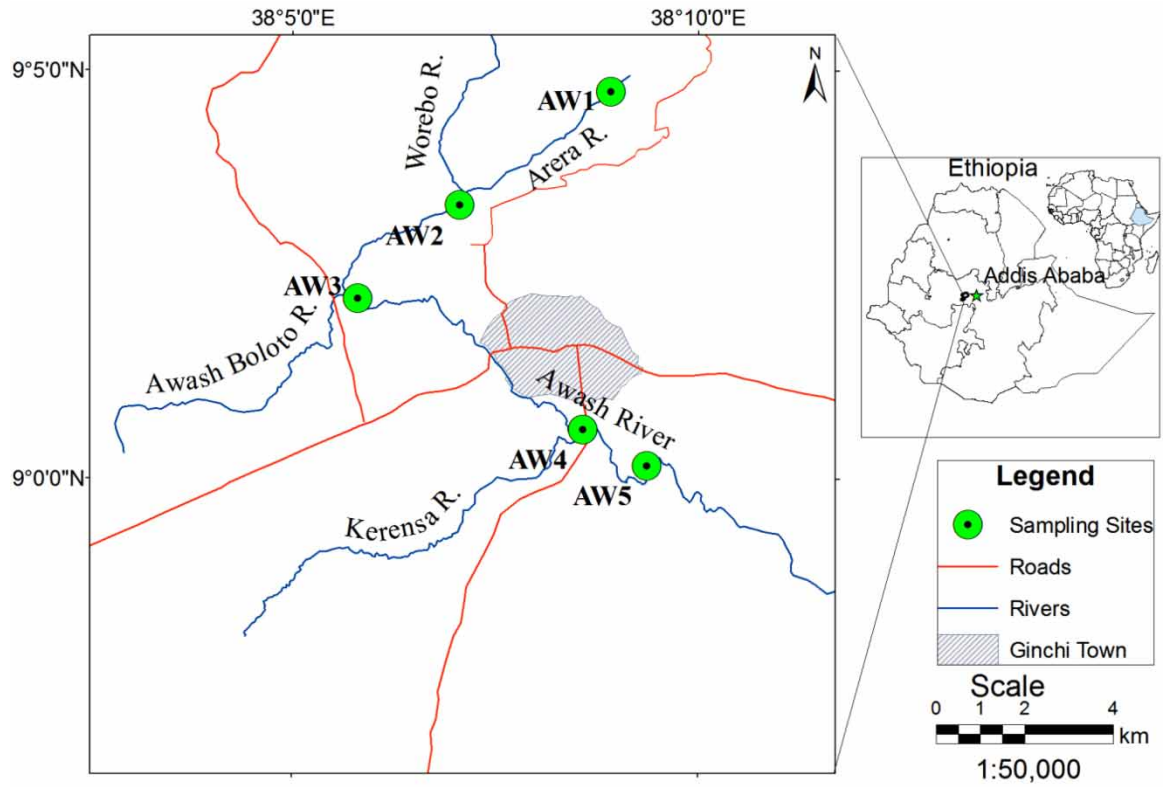


Figure 1 | Map of the Upper Awash River showing locations of the selected sampling sites where ambient faecal pellet abundance, SFIB concentrations and physicochemical variables were measured.

per hectare are observed under a free-range system in this area. A significant number of populations in this site lack sanitary facilities and therefore, practise open defecation. Sampling site AW3 was located after the confluence of the Boloto and Awash Rivers. This site is characterized by a highly disturbed riparian zone and pastureland that supports a significant number of livestock (1.56 livestock per hectare). Site AW4 was located downstream of Ginchi town, with >18,000 inhabitants. This site is influenced by wastewater released from a paper mill factory, livestock (4.52 livestock per hectare) grazing in riparian areas, in-stream activities (such as washing of domestic animals, clothes, vehicles and bathing) and open defecation. Site AW5 was located further downstream in a catchment influenced by rain-fed and irrigation-dependent farming systems, riverbank sand excavation and livestock (4.84 livestock per hectare in this site).

Quantification of ambient faecal pellets and sampling of environmental matrices (water and soil, see detailed descriptions below) at each of the selected sampling sites was performed once a month from March 2017 to February 2018 during the time of year when a mixture of rainy and dry patterns was evident. Over the entire investigation period, 12 samples of ambient faecal pellets, soil and water were obtained from each of the selected sampling sites (AW1–AW5), comprising 60 samples per variable.

Faecal taxation: quantification of faecal pellet abundance

On-site quantification of animal faecal pellets was performed by a method modified from a previously published procedure developed by Kirschner *et al.* (2004). Briefly, a quadrant 20×20 m in area was established on the left and right riparian environments, which represented the immediate surroundings of each of the selected sampling points (Figure 1). Faecal pellets were counted manually in each of the established quadrants and were expressed as faecal pellets per 100 square metres. Of note, faecal pellets detected in the designed quadrats around the selected site were counted irrespective of their source, nature, age or shape.

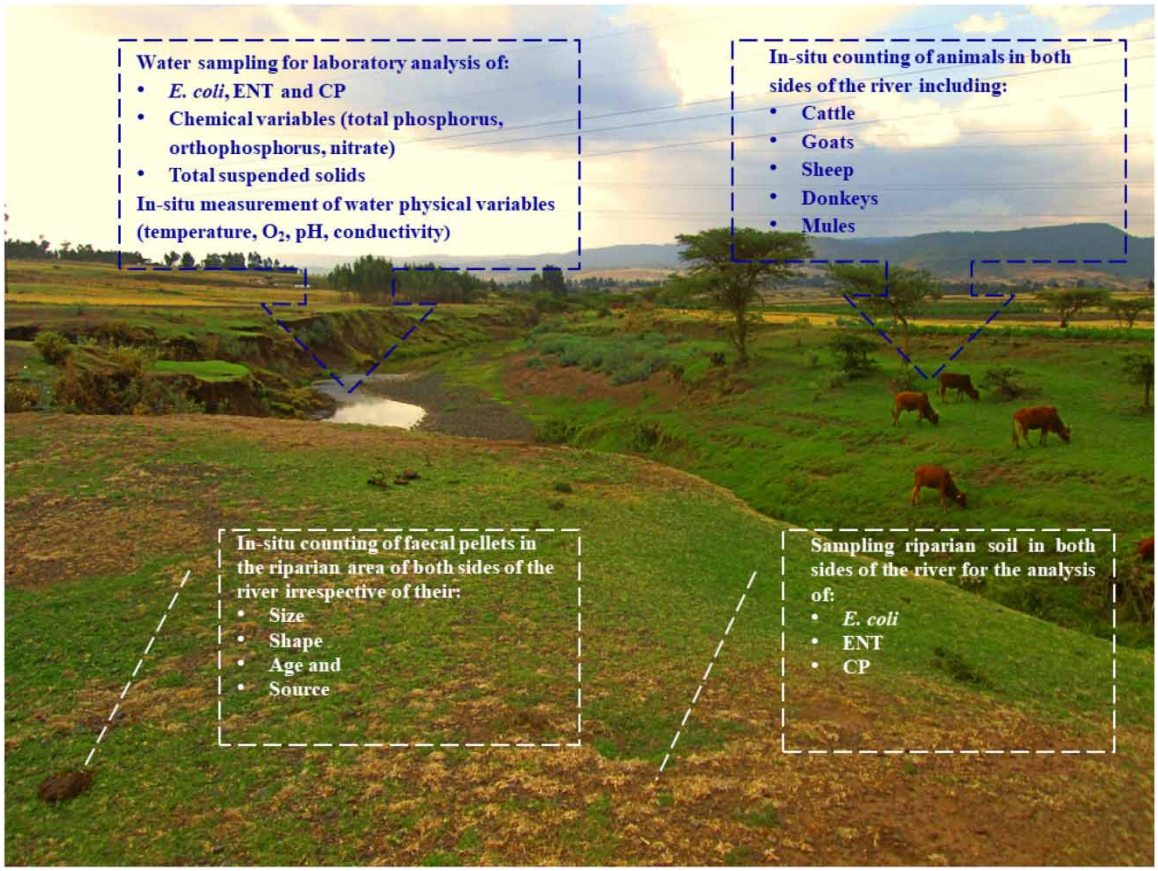


Figure 2 | A section of Upper Awash River showing landscape characteristics and anthropogenic activities. Arrow boxes illustrate variables measured in the riparian environment (the number of animals, faecal pellet abundance and soil SFIB), as well as in the river water (water SFIB, physicochemical parameters). This image was taken from site AW5 by Kebede, G. (co-author) during the sampling campaign of 2018. ENT, enterococci; CP, *Clostridium perfringens*.

Sampling water and soil for SFIB analyses

Water samples were aseptically collected from a depth of 30 cm below the surface of the river water using 1 L sterile glass bottles (APHA 2000), while soil samples from the corresponding riparian environment were obtained using a protocol described by Byamukama *et al.* (2005). Briefly, soil auger was used to obtain the first 15-cm layer of the integrated soil sample core from 10 randomly selected spots within a 20-m radius of the catchment of the water-sampling site. Soil samples were placed into sterile plastic bags using sterile spoons. Of note, the cross contamination of the soil samples from different sites was avoided by using a fresh sterile spoon for each site to obtain an approximately 10 cm^3 aliquot from the outer part of the soil core, which was not in contact with the auger. Water and soil samples were stored separately at ~4 °C before being transported to the Ambo University Laboratory in Ethiopia for SFIB quantification. In the laboratory, soil samples were immediately analysed according to the procedure of Byamukama *et al.* (2005). Briefly, 10 g of the homogenized composite soil was added to 100 ml sterile distilled water before being shaken and sonicated for 1 min. The mixture was allowed to sit for 1 h to allow suspended particles to settle. Different volumes of the supernatant water from the processed soil samples (10^{-4} to 10 ml) and river water samples (10^{-3} to 100 ml) collected from each of the selected sampling points were separately filtered through Whatman cellulose nitrate membrane filters with 0.45 μm pore-size and 47 mm diameter (Sartorius, Vienna, Austria) and analysed for *E. coli*, CP and ENT content using the standard methods described by Farnleitner *et al.* (2010). Of note, multiparametric faecal pollution indicators were applied in this study to obtain a robust measure of microbiological faecal pollution in riverine water, as established for East African environments (Byamukama *et al.* 2005; Goshu *et al.* 2010). Filters containing bacterial cells from soil-related supernatant water and river water samples were separately transferred to Chromocult Coliform Agar (CCA; Merck, Darmstadt, Germany), m-Enterococcus agar (Merck) and

Fluorocult-tryptose sulphite cycloserine (F-TSC; VWR Prolabo Chemicals, Darmstadt, Germany), which contained selective nutrients for *E. coli*, ENT and CP, respectively. Incubation of the inoculated agar plates was performed under the temperature and duration of time specified by the agar manufacturers. After incubation, FIB colonies were identified according to the agar manufacturer's protocol, in which dark blue colonies on CCA were scored as *E. coli* (Byamukama *et al.* 2000), while fluorescing black colonies growing on F-TSC plates (Byamukama *et al.* 2005) and round pink to dark maroon-coloured colonies on mENT plates were scored as CP and ENT, respectively. Of note, the detection limit (DL) for the considered faecal indicator bacteria in this study was 10 cfu/g for soil and 1 cfu/100 ml for water as determined according to the formula described by Frick *et al.* (2018). The numbers of *E. coli*, ENT and CP colonies were separately quantified from their respective plates and were expressed as colony-forming units per 100 ml or gram for water or soil samples, respectively.

Measuring physicochemical and environmental variables

On each sampling occasion, the measurements of water temperature (°C), dissolved oxygen (mg/L), pH and electrical conductivity (μS/cm) in the river water were performed *in situ* using a multiparameter probe (Hach HQ 40d, USA) after calibration according to the manufacturer's instructions. Analyses of total phosphorus (mg/L), orthophosphates (mg/L), nitrates (mg/L) and total suspended solids (mg/L) were performed at the laboratory following standard procedures described by the American Public Health Association (APHA 2000). Rainfall (mm/day) and air temperature data covering the entire study period for the investigated environment were obtained from the nearest recording rain gauge of the Holeta Agricultural Research Center under the Ethiopian Agricultural Research Institute (EIAR) located in Ginchi town (see Figure 1).

Statistical assays

SFIB concentrations below the detection limits (DL) were retained to allow for the comparison of SFIB concentrations between sample matrices. SFIB data were $\log_{10}$-transformed without the addition of 1 and were presented as the median, 5th percentile and 95th percentile. Statistical analysis was performed using SPSS for Windows (SPSS Inc., 16.0, Chicago, IL, USA) utilizing non-parametric tests because assumptions of normality were not met. Specifically, the Kruskal–Wallis test was used to compare SFIB concentrations across distinct rainfall situations (heavy, light and period of little or no rainfall). Four-day cumulative rainfall data around the time of sampling were used to explore their relationship with ambient faecal pellet abundance and concentrations of SFIB. The association of faecal pollution (SFIB, ambient faecal pellets) and physicochemical characteristics of riverine water was established using PCA coupled with varimax rotation and Kaiser normalization. To understand the patterns displayed by microbial pollution characteristics in river water over a period of 1 year, SFIB concentrations from every sample collected in the Awash River and Euclidian distance built in PRIMER V7 software (PRIMER-E Ltd, Ivybridge, UK) were used to compute non-metric multidimensional scaling (nMDS) ordination. A simple linear regression was used to investigate the relationship between rainfall and water SFIB, water SFIB and faecal pellets, and faecal pellets and rainfall data using Sigma Plot version 2.01. Spearman's rank correlation was used to investigate the extent of the relationship between soil and water SFIB. Of note, multiple correlations performed using Spearman's correlation algorithm in this investigation were Bonferroni-adjusted to minimize type I errors. Reference concentrations for *E. coli*, ENT and CP reported previously (Tiefenthaler *et al.* 2009; Verhougstraete *et al.* 2015; Mushi 2018) for river water were used to establish the extent of faecal pollution in the investigated river system, as no established guidelines for acceptable levels of SFIB in river water for the studied environment existed. For all statistical tests performed, significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Ambient faecal pellet abundance varies significantly with sampling date

We quantified faecal pellets across 12 sampling dates spanning 1 year in 10 quadrants distributed in the riparian area along the sides of the Awash River channel. Ambient faecal pellets were consistently detected in the examined quadrants throughout the sampling campaign irrespective of land-use type, quadrant location or sampling date. Despite the fact that livestock were observed in the riparian environments across the sampling sites, irrespective of the sampling periods, the ambient faecal pellet abundance reflected rainfall patterns of the investigated environment. Across sampling months, the highest median faecal pellets, primarily from livestock (2–8 faecal pellets/100 m^2), were observed in months with little or no rainfall, such as October, November, December and January, while the lowest medians (<2 faecal pellet/m^2) were detected during the months of July to September during heavy rainfall (Table 1). As faecal pellets are known to accommodate a substantial

Table 1 | Dynamics of faecal pellet abundance (faecal pellets/100 m^2) in the riparian environment of the reference sampling sites

Year of study	Sampling month	Sampling sites					Median values
		AW1	AW2	AW3	AW4	AW5	
2017	March	5	7	13	6	3	6
	April	7	4	6	8	10	7
	May	3	4	5	5	4	4
	June	3	2	5	4	6	4
	July	1	1	1	1	2	1
	August	1	1	3	7	1	1
	September	1	0.5	2	1	2	1
	October	0.25	2	12	2	6	2
	November	1	4	13	8.5	8	8
	December	0.75	2.5	9	11	7	7
2018	January	1.5	4	6	8	4	4
	February	2	5	10	9	1	5

The colour-scaled heatmap represents the spatiotemporal data and median values of ambient faecal pellet abundance enumerated within the reference sites of the studied Upper Awash River through all time points. Red represents the most abundant, and blue represents the least abundant. Please refer to the online version of this paper to see this table in colour: https://doi.org/10.2166/wh.2021.269.

number of SFIB (Farnleitner *et al.* 2010; Vierheilig *et al.* 2013) and potential pathogens of human health concern (Gerba & Smith 2005), their presence in the catchment increases the risk of river water contamination because it is well known that rivers are the recipients of catchment-associated pollutants (Zhou *et al.* 2012). Ambient faecal pellet abundance has been successfully used as a surrogate for estimating the abundance of wildlife species in many terrestrial environments around the world (Forsyth *et al.* 2011; Rouco *et al.* 2016). However, this is the first study showing that riparian areas of a riverine system were contaminated with a significant abundance of ambient faecal pellets that were consistently detected but substantially varied with respect to time as a result of potential washout when faeces are exposed to precipitation. The presence of this faecal parameter along the riverine system suggests that in the presence of rainfall events or soil erosion, river water could become extremely contaminated with faecal-associated bacteria. Therefore, faecal pellets detected in the riparian areas of the examine riverine system could be an additional parameter for the assessment of water pollution risk and prediction of riverine faecal pollution, as well as potential outbreaks of gastrointestinal diseases.

Faecal pellet abundance is strongly correlated with SFIB concentrations in the river water

The concentrations of SFIB exceeded the standards set by the World Health Organization (WHO 2003) for safe recreational water in all examined water samples, allowing for pooling of SFIB data for further microbiological characterization of the Awash River. According to the faecal classification of the river water (Kirschner *et al.* 2009), the concentrations of SFIB in the Awash River (Table 2) ranged from moderate to strong faecal pollution, and the detection rate and abundance of SFIB recovered from the analysed river water reflected the occurrence and abundance of ambient faecal pellets. There was a significant association between ambient faecal pellet abundance and the concentration of SFIB quantified from the analysed river samples (Figure 3). According to previous studies (Farnleitner *et al.* 2010; Vierheilig *et al.* 2013), livestock faecal matter may contain a much greater load of SFIB than the background concentration (*E. coli*: 1.4 $\log_{10}$ MPN·100 mL^{-1}, ENT: 1 $\log_{10}$ MPN·100 mL^{-1}, CP: 1.3 MPN · 100 mL^{-1}) of riverine water (Tiefenthaler *et al.* 2009; Verhougstraete *et al.* 2015; Mushi 2018) as SFIB concentrations significantly increase when livestock faecal matter deposited in the river. Indeed, the concentrations of *E. coli* (2.4–3.7 log cfu/100 ml), ENT (2.0–4.1 log cfu/100 ml) and CP (1.8–2.1 log cfu/100 ml) quantified in the investigated river water (Table 2) were substantially higher than the background values. Given that SFIB can persist in faeces deposited in the environment (Sinton *et al.* 2007), it is clear that this persistence behaviour increases the probability of SFIB reaching riverine water, especially when there are no intercepting barriers,

Table 2 | SFIB concentrations in the riverine water during heavy rainfall, light rainfall and the period of no rainfall

Rainfall intensity	Statistics	SFIB concentration (log_{10} cfu/100 ml)		
		E. coli	ENT	CP
Heavy rainfall	5% percentile	2.4	2.3	1.5
	Median	3.7	4.1	2.1
	95% percentile	4.8	5.1	4.3
	n	38	38	38
Light rainfall	5% percentile	1.7	2.1	1.5
	Median	2.8	3.6	2.1
	95% percentile	3.8	4.1	3.2
	n	14	14	14
Little or no rainfall	5% percentile	np	np	np
	Median	2.4	2.0	1.8
	95% percentile	np	np	np
	n	8	8	8
Kruskal–Wallis H-test	*p*-value	0.001	<0.001	0.053

CP, *Clostridium perfringens* spores; ENT, enterococci; *n*, the number of samples; np, calculation not possible. See Figure 6 for the definition of heavy, light and little or no rainfall categories.

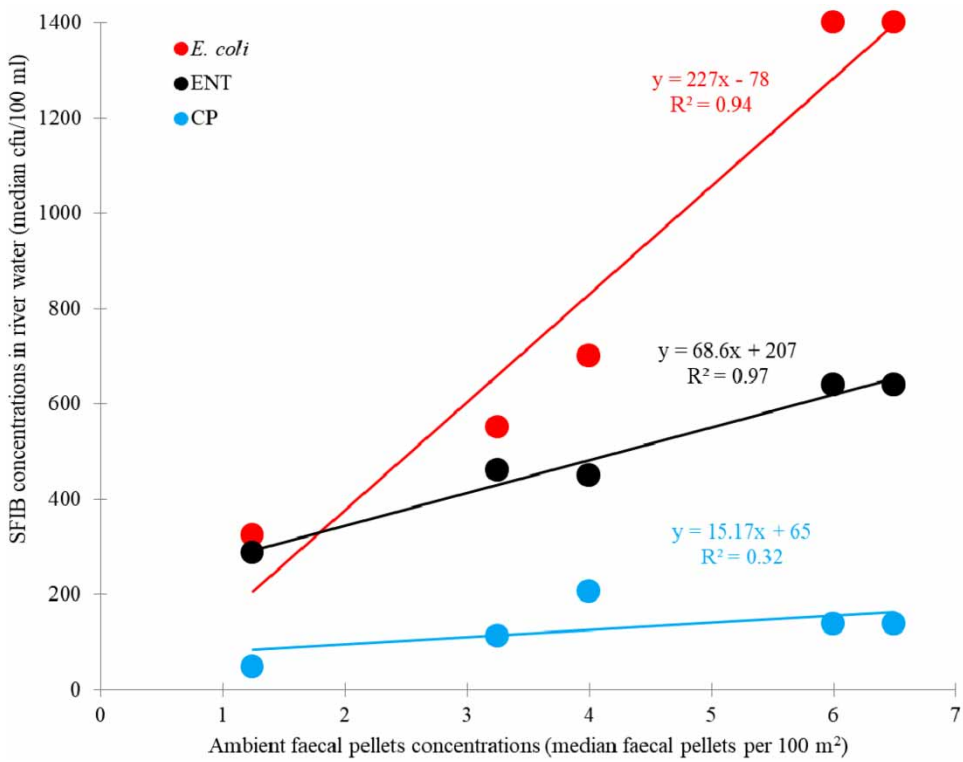


Figure 3 | Simple linear regression curves showing the comparison between median concentrations of SFIB and counts of faecal pellets. Data of faecal pellets and SFIB for each site were separately pooled and median quantified before testing. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.269.

such as vegetation. Certainly, the investigated riversheds were located in an environment characterized by disturbed riparian vegetation and compacted riparian soil, which facilitated rapid wash-off of animal faeces into the waterways by rainfall-induced runoff (Figure 2). This observation was supported by the fact that faecal pellet abundance in the rivershed was significantly but negatively correlated with rainfall data (Figure 4), suggesting that rainfall-induced runoff was a primary agent transporting ambient

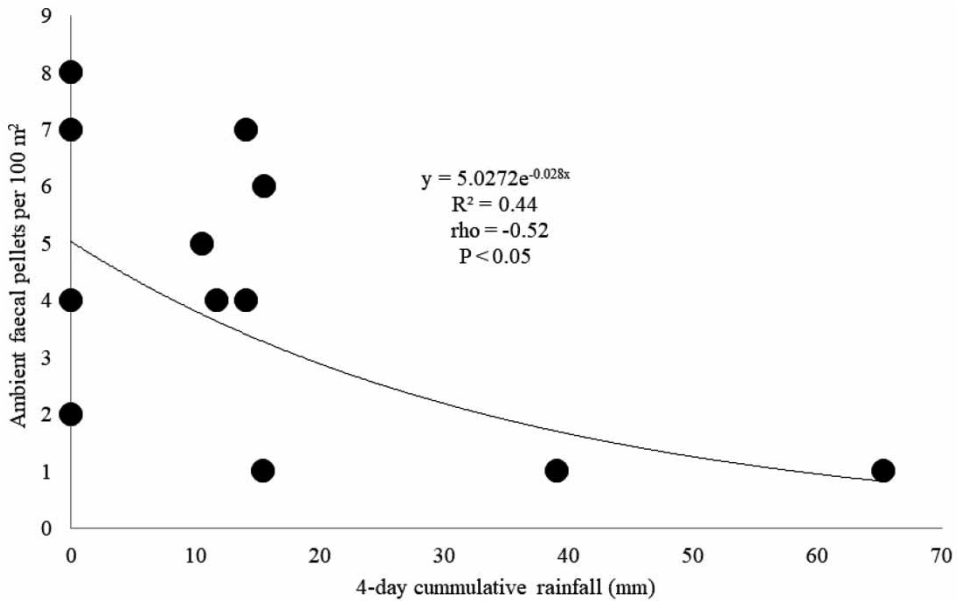


Figure 4 | Simple exponential curve and Spearman's correlation coefficient (*r*) for comparison of four-day cumulative rainfall (mm) and concentrations of faecal pellets (number of faecal pellets/100 m^2) in the investigated riverine system. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.269.

faecal pellets to the riverine water rather than individual cells of pellet-associated SFIB. Although the microbiological contribution of catchments to riverine water quality has been previously documented (Verhougstraete *et al.* 2015; Mushi 2018; Xiao *et al.* 2018), this is the first report to demonstrate a strong association between catchment faecal pellets and rainfall data, although riversheds have been used for decades as vital ecosystems for sustaining human life. These correlative results provide further evidence that ambient faecal pellets may control the microbial pollution characteristics of the studied riverine system, and riparian vegetation failed to prevent faecal pellets from entering the river water, likely due to heavy grazing by livestock. Since faecal pellets are known to associate with enteropathogenic microorganisms, such as *Cryptosporidium parvum* and *Salmonella typhi*, it is clear that their entrance into the river water is a threat to public health, especially considering that communities living in the studied catchment use the river as a source of drinking water and for irrigating crops. This situation can place the community at risk of contracting waterborne diseases. Indeed, a significant number of reports regarding frequent instances of waterborne outbreaks in Ethiopian communities have been published (Azage *et al.* 2015, 2017), and these communities use this type of water for domestic purposes. As a result, to restore the microbiological quality of the Upper Awash River, appropriate methods to protect riparian areas from grazing livestock need to be established, and riparian areas should be replanted with appropriate vegetation. Furthermore, pastoral best management practices should be implemented in the studied rivershed.

Rainfall is strongly correlated with microbial pollution characteristics of the riverine water

Faecal pollution characteristics in the investigated river water substantially varied with respect to sampling months, with the highest peaks of SFIB values detected from March to May 2017, from July to September 2017 and in February (2018), which were the months that coincided with rainfall events in the investigated area, while the lowest peaks were observed in the months of October, December and January 2017 when there was very little or no rainfall (Figure 5(a)). To better understand the robustness of the association between observed SFIB concentrations and four-day cumulative rainfall, nMDS analysis using Euclidean distance and log-transformed values of SFIB from monthly samples was performed, and an informative nMDS plot was generated (Figure 6). The analysis showed three distinct groups of sampling dates (groups 1, 2 and 3) that were significantly different from each other at $p < 0.05$ based on the Kruskal–Wallis test. A closer look at this nMDS plot revealed that the generated groups of sampling dates were governed by rainfall intensity. Group 1 consisted of samples collected during the period of light rainfall (10–40 mm) events, group 2 included water samples collected during the period of little or no rainfall (<10 mm) and group 3 was exclusively represented by samples collected during the time when there was heavy rainfall (>40 mm). This systematic clustering of faecal pollution from the sampling sites according to rainfall intensity further suggested that the

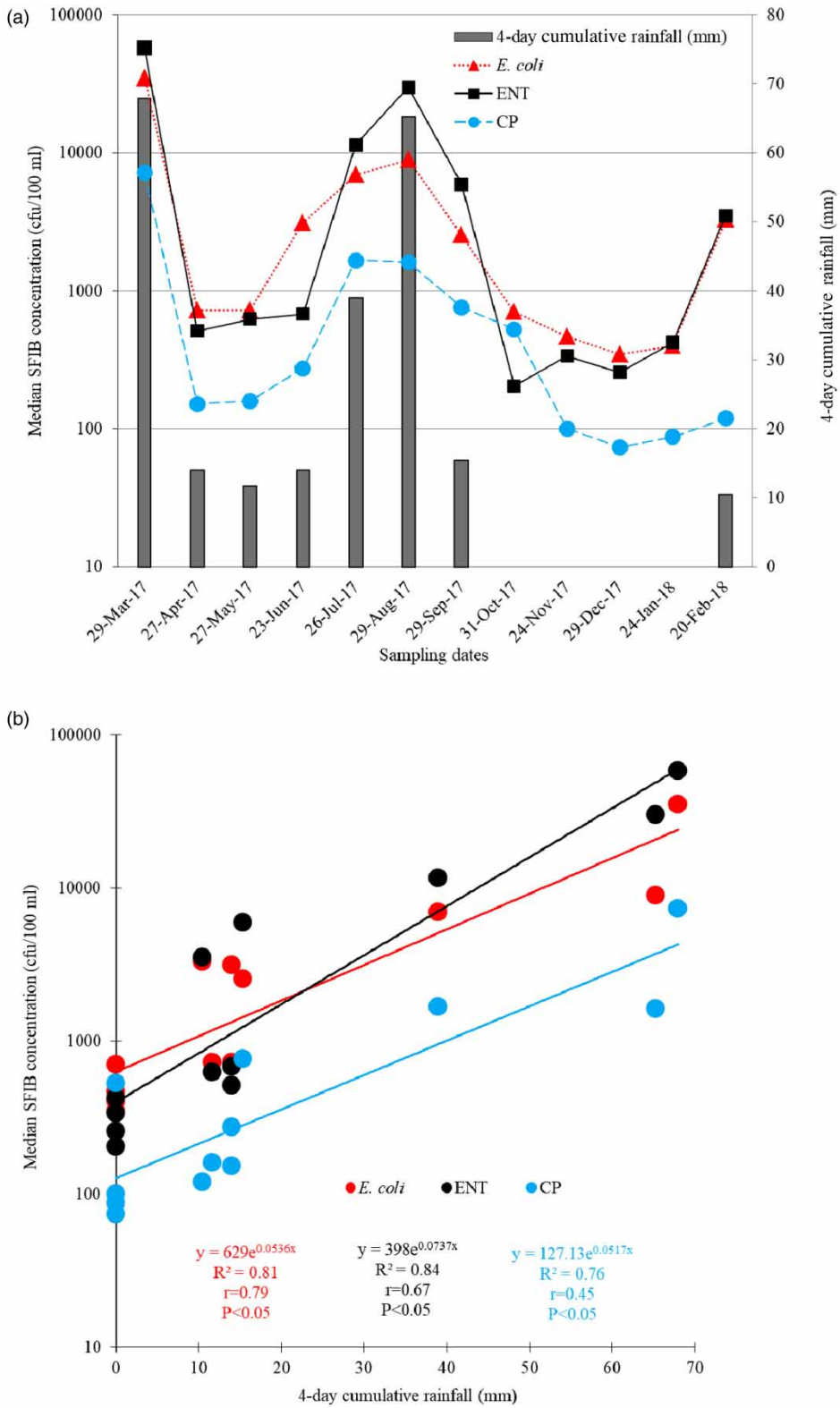


Figure 5 | Covariations of water SFIB (median values) and rainfall (four-day cumulative rainfall) with respect to sampling time (March 2017 to February 2018) (a), and simple exponential curves for the dependence of water SFIB concentrations on four-day cumulative rainfall (b) as detected in the investigated environment during the 1-year survey. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wh.2021.269.

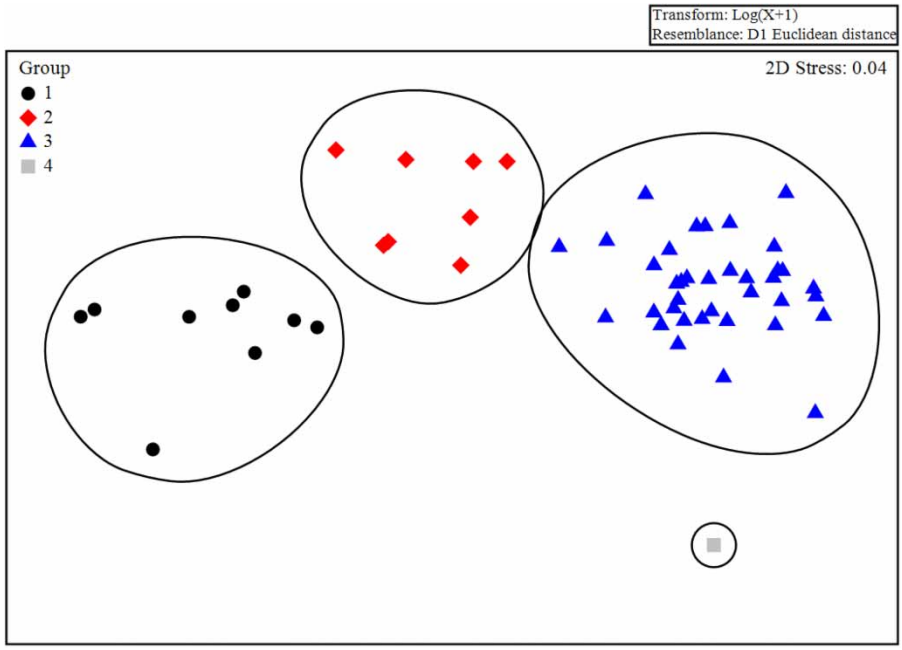


Figure 6 | Non-metric multidimensional scaling (nMDS) plots based on the Euclidian distance matrix for the concentrations of SFIB measured in water samples that taken from the Upper Awash River. Groups 1, 2 and 3 represent samples collected during the period of light rainfall (10–40 mm), very little or no rainfall (<10 mm) and heavy rainfall (>40 mm), respectively. These groups were statistically distinct from each other according to the applied Kruskal–Wallis test ($p < 0.05$; $n = 60$). Group 4 had a single point and was regarded as an outlier.

microbiological quality status of water in the Upper Awash River was a consequence of the transportation of faecal pellets from the catchment to the riverine water via rainfall-induced catchment runoff. This observation agreed with the strong covariation of SFIB in the riverine water with rainfall data over time (Figure 5(b)). Upon subjecting these data to Spearman's correlation test, rainfall was significantly ($p < 0.05$) correlated with *E. coli* ($r = 0.79$), CP ($r = 0.45$) and ENT ($r = 0.67$) concentrations (Figure 5(b)), suggesting that transport behaviour and the fate of SFIB in the studied environment were similar. Such a strong correlation not only corroborates findings observed elsewhere (St Laurent & Mazumder 2014; Leight *et al.* 2016; García-Aljaro *et al.* 2017; Leight & Hood 2018) but also strongly supports the outbreak of waterborne diseases after heavy rainfall in Ethiopia (Azage *et al.* 2015, 2017) and other locations in the world, such as the USA (MacKenzie *et al.* 1994) and Canada (Hrudey *et al.* 2003). Given the importance of the Awash River to the Ethiopian population and the frequent occurrence of waterborne diseases, multivariate data generated from this study could be used to generate appropriate models, such as multivariate Bayesian regression modelling (Seis *et al.* 2018), for forecasting the evolution of waterborne diseases in Ethiopia to provide early warning of outbreaks and facilitate the public health response to moderate an impending outbreak. However, from a managerial point of view, protection of river water from faecal pollution associated with rainfall events combined with improved *in situ* or *ex situ* treatment of water will have the greatest societal impact because the Awash River is among the resources that are highly used for various socioeconomic developments in Ethiopia.

Faecal pollution characteristics of riverine water do not reflect soil SFIB

SFIB data for water samples were all above the DL, whereas those for soil samples were below the DL for 7% (*E. coli*), 17% (ENT) and 10% (CP) of the total samples. Although SFIB concentrations in the catchment soil were significantly different ($p < 0.05$) from those in riverine water, >48% of the 60 water samples exhibited SFIB concentrations higher than those detected in the soil samples, in contrast to that reported elsewhere (Mushi 2018). However, this result may be explained by the transport of entire portions of faecal pellets to the river water by rainfall-induced catchment runoff (Figure 4) without significant infiltration into the soil. While SFIB were consistently detected in river water at a substantial concentration throughout the entire sampling period, soil SFIB, including *E. coli*, ENT and CP, could not be detected in 7, 20 and 16% of the sampling dates, respectively. As a result, there were no statistical correlations of SFIB concentrations between soil and water samples (Table 3). While this observation provides additional support for faecal pellets being a dominant

Table 3 | Spearman's correlation coefficients (*r*) for pairwise comparison of SFIB in water, between water and soil and in the soil matrix

	Water			Soil	
	E. coli	**ENT**	**CP**	**EC**	**ENT**
Water					
E. coli	1				
ENT	**0.83**	1			
CP	**0.59**	**0.57**	1		
Soil					
EC	0.29	0.35	0.23	1	
ENT	0.07	0.23	0.07	−0.12	1
CP	0.18	0.21	0.3	−0.24	0.35

Values in bold are significant at a Bonferroni corrected value of $p < 0.0017$. For abbreviations, refer to Table 2.

source of observed faecal pollution in riverine water (Figures 3 and 4), it is not in line with what was previously reported in the literature in which SFIB concentration in the catchment soil was the main cause of faecal contamination in waterways (Fujioka *et al.* 1999; Desmarais *et al.* 2002). These contrasting findings can be explained by differences in environmental conditions existing among locations in which the previous and present works were conducted. For instance, Fujioka *et al.* (1999) and Desmarais *et al.* (2002) performed their surveys in warmer and humid environments that may support the survival of SFIB in the soil matrix. In contrast, the current study was conducted in a highland environment characterized by air temperatures ranging from 2.5 to 25 °C in which the growth and survival of SFIB in the soil of this environment is unlikely because some of the soil samples were completely free of SFIB, and the correlation between soil SFIB with air temperature was insignificant ($r < 0.1$). Furthermore, if soil does represent a source of SFIB in the studied environment, statistical correlations among soil SFIB are expected. However, this was not the case for this study (Table 3). Additionally, no obvious relationship was detected between faecal pellets and soil SFIB ($r = 0.23$, $p > 0.05$). Consequently, the soil SFIB detected in the Ethiopian environment seem to represent a natural and more isolated background, which apparently does not affect the indication capacity of the SFIB used.

The finding that riparian faecal pellets rather than soil were the dominant cause of elevated faecal pollution in the studied riverine water was further substantiated by PCA performed using riparian faecal pellets, SFIB and physicochemical characteristics of riverine water (Table 4). Three components (PC1, PC2 and PC2) with a cumulative explained variance of 71% were extracted. PC1 (41%) was related to strongly positively correlated with *E. coli*, CP, ENT, ambient faecal pellets, total suspended solids, orthophosphates and total phosphorus, while negatively correlated with dissolved oxygen. PC2 (19%) contained temperature and conductivity, while PC3 (11%) included pH, nitrates and total suspended solids. PC1 was conditionally associated with the 'rainfall-induced catchment runoff' component since it revealed parameters that were significantly influenced during rainfall events. The occurrence of physicochemical variables in a component with faecal pellets and SFIB seems logical, as faecal pellets contain elevated SFIB and organic substances that reduce dissolved oxygen in the riverine water during decomposition (Zhu *et al.* 2011), whereas organic substances increase suspended solids in the riverine water. Furthermore, Monaghan *et al.* (2007) reported that animal faecal pellets contain a considerable amount of nutrients, including orthophosphate and phosphorus, which become elevated upon contact with riverine water. On the other hand, PC2 and PC3 might be related to biogeochemical processes occurring in the river water and may constitute the biogeochemical component. For instance, the temperature is known to have a large influence on the transformation of chemical substances that define the conductivity of river water, while total suspended solids are partly composed of microbial biomass that is capable of performing nitrification to produce nitrate at a specified pH (de Boer *et al.* 1991). Thus, PCA applied in this study could further support that the microbiological and physicochemical variables applied in this study are suitable for the optimization of river monitoring and to better trace anthropogenic and natural changes along river waters.

SFIB successfully detected faecal pollution in river water

The present study demonstrates that SFIB, which is accepted worldwide for water quality monitoring, primarily originate from faecal materials in the tropical highlands of Ethiopia, as supported by multiple lines of evidence (Figure 3, Tables 2

Table 4 | PCA outcomes for the analysed faecal-associated parameters and physicochemical data demonstrating that principal component one (PC1) represents variables highly influenced by rainfall-induced runoff, while PC2 and PC3 represent biogeochemical components

	Correlation coefficients (*r*)		
Parameter	**PC1**	**PC2**	**PC3**
Temperature	0.09	**0.84**	0.16
pH	−0.21	0.12	**0.60**
Dissolved oxygen	**− 0.66**	0.13	−0.04
Conductivity	−0.39	**0.78**	−0.22
Total phosphorus	**0.89**	0.01	0.18
Orthophosphate	**0.89**	0.03	−0.17
Nitrate	0.22	−0.33	**0.81**
Total suspended solids	**0.54**	−0.15	**0.65**
E. coli	**0.93**	−0.04	−0.02
ENT	**0.89**	−0.09	0.08
CP	**0.69**	0.27	0.15
Faecal pellet	**0.82**	0.12	−0.19
Explained variance (%)	41	19	11

Values in bold are significant at $p < 0.05$.

and 3). Their detection in riverine water should be interpreted beyond doubt as contamination linked to faecal matter (Figure 4). This evidence supports previous studies conducted in tropical East African countries (Byamukama *et al.* 2005; Goshu *et al.* 2010), although a different study design was applied. Unlike previous studies, for the first time, this study applied a robust investigational approach involving catchment characteristics (soil and faecal pellets) and SFIB to understand the source and extent of faecal pollution in tropical riverine water. The strong relationship of SFIB with faecal pellet counts observed in this study not only justifies the hypotheses described in this study but also indicates the robustness of the multiple faecal indicator approach in identifying faecal pollution in riverine waters. Interestingly, SFIB respond consistently and in a comparable manner to riverine faecal contamination incidences irrespective of differences in ecological traits and whether temporal (the focus of this study) or spatial scales were considered. On the other hand, the strong correlation of CP, which is known for its inability to multiply in the environment due to the absence of conducive growth factors, with *E. coli* and ENT (Table 3) provides further evidence that these SFIB may not grow significantly in the riverine water of the studied environment. Taken together, SFIB are not only an easy and reliable approach that can be applicable in low resource settings to indicate faecal pollution but also provide a complete picture regarding faecal pollution characteristics in water in particular and environments in general. SFIB and faecal pellets represent a very useful combination not only in water quality monitoring but also in understanding in what situations SFIB cannot indicate the presence of faecal pollution in the tropical environment, especially in environments characterized by elevated temperature and humid conditions suitable for initiating their growth (Fujioka *et al.* 1999; Desmarais *et al.* 2002). Of note, despite being contaminated by faeces, the investigated watershed deserves further investigation to elucidate the presence of human-relevant microbial pathogens.

Rainfall is not the only transport agent of ambient faecal pellets into the river water

Although this study reported rainfall-induced runoff as the primary transporter of faecal pellets into the studied riverine water in the Ethiopian highlands (Figure 4), statistical removal of rainfall-related SFIB data from the overall data set suggested that rainfall was not the only mechanism through which faecal pellets entered the water. Data collected during the period of no rainfall for all sites indicated that SFIB concentrations of *E. coli* (median: $\log_{10}$ 2.4 cfu/100 ml), ENT ($\log_{10}$ 2.0 cfu/100 ml) and CP ($\log_{10}$ 1.8 cfu/100 ml) exceeded the reference concentrations (Verhougstraete *et al.* 2015; Mushi 2018) irrespective of the location of the sampling points. We speculated that direct input of faecal matter during watering of livestock, in-stream activities of people (bathing and washing of domestic animals, clothes and vehicles), sand excavation (as observed at sites AW4 and AW5) and sewage effluents (observed at site AW4) may have contributed to the observed SFIB concentrations

in the studied riverine environment during the period of no rainfall. It is important to note that these anthropogenic influences, with the subsequent adverse effects on the human and environmental health, are not unusual in developing countries (Byamukama *et al.* 2000, 2005; Goshu *et al.* 2010). Therefore, the public health risks associated with waterborne transmission of microbial pathogens and the environmental impacts resulting from in-stream human activities and raw sewage discharges need to be addressed through the provision of appropriate education to the communities in the Awash catchment by the respective department and installing efficient wastewater treatment plant combined with adequate monitoring programs in order to improve quality of riverine water and prevent health risks (Cox *et al.* 2005) to the Ethiopian communities. On the other hand, faecal pellets from the catchment could be transferred by livestock to the riverine water through their feet when visiting the river for watering and/or by human feet when visiting the river for bathing, swimming or washing cars, animals and clothes. However, further study on the possibility of these mechanisms in transferring faecal pellets to river water is needed to obtain a complete picture regarding the methods in which faecal pellets enter the riverine system so that a reliable managerial plan for this river can be established. Of note, specific animal species associated with the observed faecal pellets in the riverine landscape and the possibility that human faecal matters contributed to elevated SFIB in the riverine water were not determined in this study, although diverse ruminant animals and human habitats were observed in the catchment of the studied riverine system. Future studies using reliable microbial source tracking tools will be required to determine specific sources of the observed ambient faecal pellets in the investigated riverine system and to identify specific faecal source groups. This identification will allow the development of efficient and cost-effective remediation efforts in the catchment, such as the restriction of livestock numbers or the improvement of wastewater collection and treatment and will allow the efficiency of such measures to be evaluated (Reischer *et al.* 2006; Mayer *et al.* 2016, 2018; Kirschner *et al.* 2017). However, for immediate action, water and environmental managers would need to protect livestock from entering the river catchment by establishing appropriate protection measures, such as fences, encouraging zero-grazing and providing off-stream watering. More importantly, these mitigation measures should be practised in parallel with the restoration of riparian vegetation to limit soil erosion and filter faecal pellets during rainfall-induced runoff. This initiative will restore the quality of riverine water, reduce the cost of treating riverine water for drinking purposes and protect public health from waterborne diseases.

CONCLUSIONS

- This study demonstrated that the Upper Awash River located in the Ethiopian Highlands is characterized by an appreciable number of ambient faecal pellets, resulting from the presence of diverse animals in the riparian environments on both sides of the river channel.
- Rainfall plays a dominant role in the introduction of catchment faecal pellets into the Upper Awash River water, indicated by a strong negative correlation between rainfall and catchment faecal pellets.
- The strong general response of SFIB abundance in the water column to catchment faecal pellets irrespective of land-use diversity and elevation of the sampling sites suggests that catchment faecal pellet abundance is a dominant driver of microbiological pollution characteristics of the studied riverine water.
- Livestock (during watering), humans (bathing and washing of domestic animals, clothes and vehicles) and sewage effluents may have directly contributed faecal bacteria into the river water, as levels of SFIB in the river remained above standard levels in the absence of rainfall.
- Soil SFIB densities showed no association with microbial pollution characteristics of riverine water or origin from faecal pellets (no correlation between faecal pellets and soil SFIB), suggesting that the soil was not responsible for the heavy faecal pollution detected in the investigated riverine water but rather is a source of background SFIB in the studied environment.
- The correlation of catchment faecal pellets with physical and chemical characteristics of the riverine water indicates that faecal pellets are an essential variable to consider when monitoring the quality of riverine water.
- The novel integrated approach applied herein identified faecal pellet abundance as a primary driver of the microbiological faecal pollution characteristics of Awash riverine water in the Ethiopian highlands.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

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