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How can we improve understanding of faecal indicator dynamics in karst systems under changing climatic, population, and land use stressors? – Research opportunities in SW China

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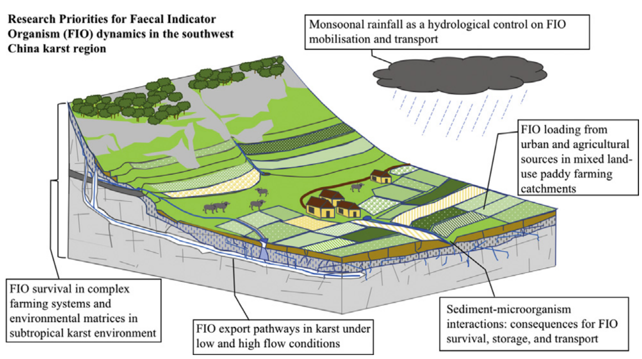
HIGHLIGHTS

- Gaps exist in our understanding of FIO dynamics in karst catchments.
- SW China represents a karst region exemplar for identifying research opportunities.
- Research needs identified by critical review and catchment surveys.
- Five priority themes for FIO research in karst terrain emerge.

GRAPHICAL ABSTRACT



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ABSTRACT

Human exposure to water contaminated with faeces is a leading cause of worldwide ill-health. Contaminated water can be transmitted rapidly in karst terrain as a result of the connectivity of surface and groundwater systems, high transmissivity of aquifers over large areas, and well-developed underground conduit systems. Faecal indicator organisms (FIOs) are the most widely-used indicator of faecal contamination and microbial water quality; however, the conceptualisation of FIO risk and associated sources, pathways, and survival dynamics of FIOs in karst landscapes requires a degree of modification from traditional conceptual models of FIO fate and transfer in non-karst systems. While a number of reviews have provided detailed accounts of the state-of-the-science concerning FIO dynamics in catchments, specific reference to the uniqueness of karst and its influence on FIO fate and transfer is a common omission. In response, we use a mixed methods approach of critical review combined with a quantitative survey of 372 residents of a typical karst catchment in the southwest China karst region (SWCKR) to identify emerging research needs in an area where much of the population lives in poverty and is groundwater dependent. We found that the key research needs are to understand: 1) overland and subsurface FIO export pathways in karst hydrology under varying flow conditions; 2) urban and agricultural sources and loading in mixed land-use paddy farming catchments; 3) FIO survival in paddy farming systems and environmental matrices in karst terrain; 4) sediment-FIO interactions and legacy risk in karst terrain; and 5) key needs for improved hydrological modelling and risk assessment in karst landscapes. Improved knowledge of

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these research themes will enable the development of evidence-based faecal contamination mitigation strategies for managing land and water resources in the SWCKR, which is highly vulnerable to climate change impacts on water supply and quality of water resources.

1. Introduction

Exposure to water resources contaminated with either human, livestock or wildlife faeces can pose a potential risk to human health (Kay et al., 2007). Disease (primarily gastroenteritis) caused by microbial pathogens from faecal matter disproportionally affects developing and rural regions, and is the second leading cause of child mortality under age five worldwide (Gall et al., 2015; WHO and UNICEF, 2014). Microbial pathogens can persist in a variety of catchment matrices including ground and surface waters, sediments, animal and human faecal matter, and soils (Alegbeleye et al., 2018; Sidhu et al., 2015). Faecal indicator organisms (FIOs), for example *E. coli*, are the most routinely used microbial compliance parameter for confirming faecal (though not necessarily pathogen) contamination of the environment, and their use in water quality legislation around the world demonstrates their widespread utility (Oliver et al., 2016). Quantification of FIOs is subsequently a key environmental management tool, serving as a direct indicator of faecal contamination levels of soil, water and other target media, and as a fundamentally important parameter in models that predict microbial pollution at larger spatial and temporal scales that cannot be easily monitored (de Brauwere et al., 2014). Understanding the sources of FIOs, and their associated fate and transfer processes, across multiple scales thus provides the underpinning evidence-base for informing effective management of microbial water quality in catchments (Bradford et al., 2013).

Karst catchments offer "distinctive hydrological pathways and landforms that arise from high rock solubility and well developed secondary (fracture) porosity" (Ford and Williams, 2007). These are further shaped by a range of climatological, biogeomorphological and biochemical controls (Phillips, 2016). For example, planes of weakness such as faults, fractures, and fissures are a necessary precursor, providing preferential flow pathways, and development of karstic landforms is highly rainfall dependent (Harmand et al., 2017). The preferential dissolution along existing planes of weakness, whose orientation is typically structurally controlled, is a positive feedback loop and results in extreme vertical and horizontal anisotropy of aquifer properties, such as hydraulic conductivity and transmissivity. Karst hydrology is characterised by infiltration of surface water into the groundwater system through sink holes and depressions, and rapid underground transport through conduit systems to springs (Gutiérrez and Gutiérrez, 2016). This results in high connectivity of surface and groundwater systems, and high transmissivity and connectivity of aquifers over large areas, which can lead to uncertainty in our underpinning understanding of hydrological functioning of karst systems (Hartmann et al., 2014). As a result, contaminated water can be rapidly transferred to points of human exposure, e.g. emerging at drinking water bores and springs or used for irrigation and domestic purposes. Managing microbial water pollution in karst landscapes therefore, presents a unique set of challenges, not least because of the extreme heterogeneity observed in aquifer properties.

The conceptualisation of FIO risk and associated sources, pathways, and survival dynamics of FIOs in karst landscapes requires a degree of modification from traditional conceptual models of FIO fate and transfer in non-karst systems. While a number of reviews have provided detailed accounts of the state-of-the-science concerning FIO dynamics in catchments (Cho et al., 2016; Kay et al., 2007; Oliver et al., 2016), specific reference to the uniqueness of karst and its influence on FIO fate and transfer is a common omission. Given that 25% of the world's population are dependent on karst water resources for drinking (Hartmann et al., 2014) it is critical that karst catchments are not overlooked when considering landscape-scale drivers of microbial water pollution. The general framework of source-pathway-receptor used to conceptualise FIO risk is valid for karst, but the processes that control FIO dynamics through this continuum are likely to be very different. Thus, findings of FIO dynamics in non-karst systems provide valuable comparative data but may offer limited transferability due to the fundamental differences in hydrology upon which varying land management approaches may be implemented (Bonacci et al., 2009).

The southwest China karst region (SWCKR) is one of the largest continuous karst zones in the world and represents 20% of the land area in China (Cao et al., 2015). The SWCKR is characterised by well-developed karst geology, high intensity precipitation events, population pressure, intensive agriculture and animal husbandry, and the highest national poverty rates, which have all been identified as causes of increased microbial contamination risk (Balbus and Embrey, 2002; Curriero et al., 2001; Dangendorf et al., 2002; Guo et al., 2009; Howard et al., 2003; Luffman and Liem, 2014). This combination of factors makes this geographical area a high priority for considering the challenges and opportunities of FIO research needs in karst terrain. Thus, the aims of this critical review are to identify research needs for advancing our understanding of FIO fate and transfer in the SWCKR, and to extrapolate from this region to identify a broader, more generic set of research priorities needed to better define our understanding of karst-related FIO behaviour.

2. The southwest China karst region: an exemplar

In rapidly-developing countries such as China, there is high vulnerability to climate change impacts on water supply and quality. Understanding the fate and transfer of FIOs and their pathways of exposure to human populations (e.g. via drinking water, crop irrigation, household food preparation) is therefore important in areas of China where water sources are vulnerable and prone to contamination, e.g. karst landscapes. However, this urgent need to explore microbial pollution issues in the SWCKR is driven by more than climate-change concerns. The Chinese Ministry of Agriculture (2018) stipulated that there must be zero growth of chemical fertiliser consumption by 2020, thus the world's top producer of rice and wheat will need to secure nutrient inputs from alternative sources other than mineral fertilisers. The anticipated response will be a significant increase in the use of organic fertiliser, which is currently under-utilised, to help boost agricultural output (Chadwick et al., 2015). Organic fertilisers such as livestock manures will contribute higher microbial loading to land and when coupled with changing climatic drivers that promote microbial transfer (e.g. increased frequency of storm events) the risk of faecal contamination of water sources is likely to increase. The Environmental Quality Standards for Surface Water (EQSSW) (GB3838-2002) are used in China as a regulatory framework. However, there are suggestions that these standards have struggled to keep pace with rapid economic growth and limited awareness of environmental protection, and that strategic amendments are needed to establish national water quality criteria to support the revision of water quality standards (Zhao et al., 2018).

Rice is a staple crop and major export of provinces in southwest China, and China is the leading global producer of rice (Peng et al., 2009; Wang, 2016). The land-levelling, irrigation, and drainage practices that underpin paddy rice farming differ fundamentally from typical Western agriculture (Sprague, 1975), yet studies of FIO pollution in paddy rice regions are scarce relative to other forms of agriculture.

Burgeoning demand for meat and dairy in the last 20 years has seen intensive livestock farming grow to be responsible for the majority of point source faecal pollution in China, with little or no manure management and an estimated 30–70% of liquid manure discharged directly to waterways (Gao et al., 2014; Norse and Ju, 2015; Strokal et al., 2016). Agriculture and livestock farming are hence likely to be major and increasing sources of microbial contamination of watercourses, but there is currently limited data of sufficient spatial coverage to evidence this. Such a combination of stressors on both the environment and the population place the rural communities of the SWCKR amongst the most vulnerable to contaminated water supply issues (Tao and Xin, 2014).

3. FIO fate and transfer in the SWCKR: the emergence of priority research needs

In response to growing awareness of the challenge of karst-related FIO pollution, we have identified five key themes and a series of research priorities that would support a critical advance in our knowledge of FIO behaviour in the SWCKR, as related to key processes and features of this terrain (Fig. 1). These have emerged from a critical review of the literature and through a quantitative questionnaire of 312 local farmers and 60 community members from across seven villages in a mixed-land use catchment (the Houzhai catchment) in Guizhou Province, in the centre of the SWCKR. The survey sought views on approaches to manure and farm management, fertiliser application and catchment water resources.

3.1. Theme #1: patterns of FIO transfer through karst hydrological pathways

In the field of groundwater contamination, many processes relating to FIO fate and transfer, particularly those that enhance transport such as conduit flow, (e.g. Fig. 1:1,4) are poorly understood (Bradford and Harvey, 2017). Gaps in understanding can be attributed to the complexity of processes governing FIO transport, the activation of different hydrological pathways under varying flow conditions, and the heterogeneity of natural karst systems.

Karst aquifers respond differently to rainfall compared to typical granular aquifers, due to rapid responses in discharge along high velocity flow paths, high connectivity over long distances, activation of different hydrological pathways under low and high flow conditions, and variable epikarst thickness (Fig. 1:1,3,4) (Bakalowicz, 2005; Fu et al., 2016a). Under the hydrological conditions induced by heavy rainfall, FIOs and other potential contaminants are transported more rapidly by subsurface transport through the soil profile due to enhanced unsaturated and saturated zone flow (McCarthy and McKay, 2004; Savoy, 2007). In karst systems, overland flow is normally activated when precipitation exceeds a threshold where the epikarst zone becomes saturated (Zhang et al., 2011). Integration of geophysical, hydrometric, and hydrogeochemical methods has recently shown notable changes in flow connectivity between hillslopes and landscape depressions in cockpit karst, which is a major karst landform in the SWCKR, with slow, fracture dominated flow during dry periods shifting to rapid recharge of conduits from hillslope flow during heavy rainfall (Chen et al., 2017b).

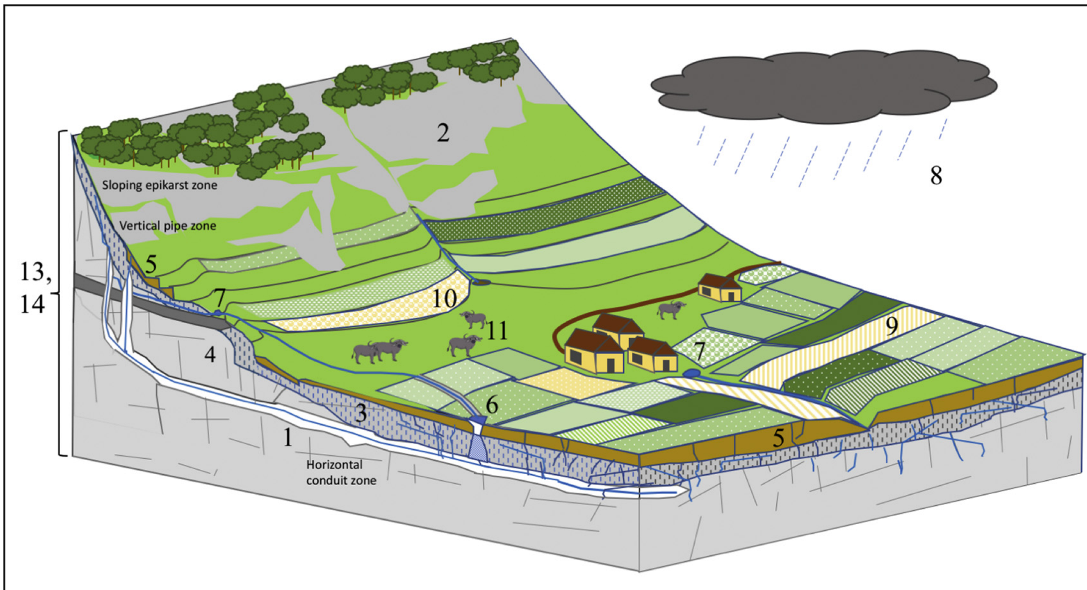
Moderate or light rainfall may also result in transport of FIOs to groundwater through subsurface flow pathways. Hillslope soils in the SWCKR are thin (typically <30 cm), and characterised by high rock fragment content, making them prone to rapid infiltration. Depression soils are thicker (up to 2.0 m) and more homogenous, but with high clay content and underlain by fissured and fractured epikarst, making them also prone to preferential flow path development (Chen et al., 2017a; Hu et al., 2015; Zhang et al., 2011). Under conditions of continuous moderate rainfall (e.g. 25 mm/day) vertical leaching of FIOs through finer, more porous soils is reduced (but still significant) relative to poorly drained soils that exhibit macropores, and leaching to groundwater at >3 m depth has been observed within <1 day in soil prone to preferential transport (Aislabie et al., 2001; Gagliardi and Karns, 2000; Krog et al., 2017).

During low flow or dry periods, FIOs (and other contaminants) may be stored in the epikarst zone. The importance of the epikarst (Fig. 1:3) as a storage component in the karst system and release of stored water following storm events is well established (e.g. Aquilina et al., 2006; Fu et al., 2016b). More broadly the vadose zone is recognised as a potential reservoir for microbial storage between mobilising precipitation events (Gotkowitz et al., 2016; Tafuri and Selvakumar, 2002). The variation in thickness of the epikarst and soil zones between hillslopes and depressions means there is likely to be large spatial variation in potential for FIO storage and filtering. Furthermore, karst systems have been identified as permanent reservoirs of viable but non-culturable *E. coli* even when culturable *E. coli* became undetectable at water sources (Petit et al., 2018). The implications of this for FIO survival and storage in different components of the karst system are largely unknown and require investigation to advance our understanding and enable representation of FIO storage and transfer.

Both land use practices and environmental degradation in the SWCKR may enhance overland and subterranean transport of FIOs to receiving waters. Overland flow is likely to be enhanced due to the increased bare rock associated with prevalent karst rocky desertification and reduced opportunity for filtration through the thin or absent soil profile (Fig. 1:2) (Ben-Hur et al., 2011; Fu et al., 2015). Dry land cropping on sloping land, induced by population pressure forcing expansion of crop lands, can lead to manure application to land that is more vulnerable to overland flow following rainfall, and in turn more susceptible to promoting rapid *E. coli* export or infiltration and storage in the epikarst during low-flow periods (Fig. 1:10). Low natural vegetation cover due to land clearance, livestock grazing, and rocky desertification may also reduce FIO and other contaminant attenuation (Jiang et al., 2014). The land-use practices and soil-rock profile in the SWCKR also have the potential to enhance sub-surface transport of FIOs to conduits and thus reduce storage in the soil and epikarst zones. For example, saturated soils, thin soil profiles, and fractured bedrock (Fig. 1:5) have been shown to increase infiltration rates and groundwater recharge, and reduce soil water retention (Appels et al., 2015; Rathay et al., 2017; Unc and Goss, 2003).

3.1.1. Research priority A: characterisation of overland and subsurface FIO export pathways in karst hydrology under varying flow conditions

Characterising how FIO flux is apportioned to both subsurface and overland flow hydrological pathways under varying hydrological conditions in karst is essential for understanding the dynamics of FIO export from this specific type of terrain. Such characterisation requires improved quantification of temporal FIO transfer via different pathways, together with a more detailed appreciation of the key mechanisms by which FIOs are transferred from sources to receiving water bodies, and the role of key storage components in the karst system. Accurate quantification of FIO export via different pathways also needs to account for variability across land-use types, soil type and depth, and degree of karstification. Advances in interdisciplinary working are key to delivering on this research priority and there are current 'Critical Zone Observatory' projects in China that are integrating tools and techniques to better understand karst hydrology to ensure sustainability of soil and water ecosystem services (e.g. Chen et al., 2017b). For example, geophysical surveys can inform on structure and hydraulic conductivity profiles of karst, helping to visualise the architecture of this complex subterranean environment (Fig. 1:5); hydrochemistry and isotopic data can characterise different water sources such as freshly infiltrated surface water and stored epikarst water (Zhang et al., 2018), and if combined with FIO concentrations, would allow an interpretation of likely FIO pathways. Furthermore, high resolution discharge and physical and chemical water properties are becoming more widely available,



1	Well-developed conduit network and fractured bedrock resulting in rapid flow paths and heterogeneous aquifer properties;
2	Karst rocky desertification and vegetation clearance potentially altering overland and subterranean flow regimes, increasing erosion, and enhancing contaminant transport to streams;
3	Epikarst zone of variable thickness and permeability, providing variable water retention, buffering, and filtration capacity;
4	Complex flow connectivity between hillslope and depressions in cockpit karst, involving both fast and slow flow pathways and impermeable marl and silicate layers (aquitards);
5	Variable soil structure; thin and rocky soils on hillslopes contrast with thicker, clay rich soils in valley depressions and may have implications for filtering of FIOs during infiltration of surface water;
6	Sinkholes, commonly used as rubbish repositories, providing a mechanism for direct introduction of surface water, suspended sediments, and contaminants into the groundwater system;
7	Springs where groundwater from conduits or epikarst directly enters the surface water system are used as drinking and domestic water sources;
8	Heavy and episodic rainfall due to the semi-monsoonal climate is likely to be a key driver of FIO export, particularly when combined with rapid discharge responses due to the karst hydrology;
9	Paddy agriculture in depressions involves intensive abstraction, irrigation, and drainage of water containing manure (potentially inadequately treated) and fertilisers;
10	Dryland cropping on sloping land, often with thin soil cover, also uses manure as a fertiliser. New commercial farming practices are being introduced and there is an anticipated increase in manure application (this also applies to depressions);
11	Animals graze freely and are used for meat (and increasingly) dairy production in small household scale farms as well as large intensive farming facilities, and to assist in farm labour;
12	Urban sources such as open or poorly managed toilets, compost, and rubbish heaps in close proximity to waterways;
13	Karst mineralogy results in high pH in karst waters, while humid-subtropical conditions and high nutrient levels may increase FIO and pathogen survival rates;
14	The combination of karst hydrology and extremes of flow regimes may result in different sediment dynamics to non-karst terrain and non-monsoonal climates, and more generally sediment-microorganism interactions and consequences for survival and transport of FIOs are poorly understood.

Fig. 1. Features of karst terrain and land-use practices in the SWCKR relevant to faecal contamination and health risk that are poorly understood. The numbered processes are referred to in the text in bold.

providing a potentially powerful method of inferring FIO behaviour through comparative and multidisciplinary data analysis (Frank et al., 2018; Hartmann et al., 2014).

3.1.2. Research priority B: classifying FIO C-Q dynamics in the karst terrain

Programmes of baseline monitoring coupled with high-resolution sampling during precipitation events of varying magnitude are required to understand how land-use, catchment size, and karst hydrology act in combination to moderate the effects of rainfall events on FIO export (Fig. 1:8). Characterising concentration–discharge (C-Q) relationships and hysteresis during storm events in karst catchments is challenging owing to their short-lived nature, but this type of dataset can prove invaluable. Analysis of C-Q relationships gives understanding of the contribution of proximal and distal sources, and source amount and depletion (Bowes et al., 2015). Classifying FIO C-Q event dynamics according to different typologies of storm (e.g. intensity, duration), and according to season or antecedent conditions would provide insight into the interactions and relative importance of event characteristics associated with agricultural and seasonal variables. Finally, nested catchment studies would allow determination of the importance of scaling effects on FIO concentrations and flux in karst catchments. However, site selection will inevitably be challenging due to the complexity of subterranean hydrological pathways and associated 'leakiness' of such catchments, and thus require an accurate surface and subsurface catchment model to guide the design of such studies.

3.2. Theme #2: understanding point and diffuse sources of FIOs under changing farming regimes

Agriculture in the SWCKR is undergoing substantial change, with subsistence farming giving way to the expansion of commercial crops, dairy, and livestock farming that is driven by changing demands and economic development (Chadwick et al., 2015; Fu et al., 2010). Hence, there are a range of farm scales and practices across the SWCKR, all of which may contribute diffuse and point sources of FIOs (Fig. 1:9,10,11).

3.2.1. Diffuse pollution from SWCKR agriculture

Manure is currently used extensively to fertilise both paddy and dryland crops in the SWCKR: of 298 farmers in the Houzhai survey, 44% used some combination of animal and human manure and 60% were unaware of the potential for pathogens to be present in these materials. Dual nitrate isotope analysis has indicated that manure is the major source of nitrate in receiving waters in the dry season, and a significant source during the wet season (Yue et al., 2015). Under the national regulations on synthetic fertiliser application, whereby zero growth of chemical fertiliser application is permitted after 2020, manure application is likely to increase as farmers will need to source more nutrients from organic fertiliser. Optimum management of manure applied to crops is limited by labour availability, which probably leads to suboptimum pathogen inactivation due to inability to follow best management practice (Chadwick et al., 2015; Smith and Siciliano, 2015).

The abstraction, flood irrigation, and drainage practices that underpin paddy rice farming in the SWCKR result in the release of large volumes of irrigation water containing residual organic and inorganic fertilisers into the karst hydrological system. This may result in increased FIO fluxes into aquifers during key periods in the agricultural calendar, resulting in a higher risk of exposure to downstream users if irrigation waters emerge at springs (Fig. 1: 7). Manure application and irrigation practices can influence the survival rates and transport to aquifers of *E. coli* and selected pathogens in agricultural systems: flood irrigation practices have been shown to result in greater leaching and transport of *E. coli* and *Campylobacter* through the vadose zone into groundwater compared with spray irrigation (Close et al., 2008; Weaver et al., 2016), and it is well established that drainage methodology impacts discharge water quality and paddy soil salinity (Jafari-Talukolaee et al., 2015; Manjunatha et al., 2004). Transport of bacteria via macropores is generally enhanced in wet and poorly-drained soils, and fissures and fractures in the epikarst, which underlies both paddy and dryland cropping systems in the SWCKR, providing the potential for rapid flow paths (Aislabie et al., 2001; Unc and Goss, 2003). Dryland cropping is practiced on hillslopes and valley floors during the dry season and transport of FIOs from dryland cropping to ground and surface water may be enhanced by irrigation with flooded channels and rocky desertification. Grazing livestock also present a potential diffuse source risk of FIO pollution, which is highly-relevant to this region where grazing cattle and water buffalo have open access to surface waters. Studies that have assessed baseline faecal contamination levels or captured FIO emergence over events in karst agricultural settings have found the presence of grazing ruminant livestock and direct defecation by cattle into surface streams to be important controls on FIO levels (Laroche et al., 2010; Reischer et al., 2008; Smolders et al., 2015).

3.2.2. Point sources of FIOs in the SWCKR: urban contamination and livestock farms

The use of open toilets directly discharging untreated waste into waterways and disposal of waste potentially containing sewage, or organic waste which attracts scavenging animals, in sink holes, rivers, or in heaps close to riverine banks are common practices in impoverished rural areas of the SWCKR. This is likely to result in urban areas and households contributing large FIO loads to catchments (Fig. 1:6,12). Intensive livestock farms, in particular the farmyard areas, also represent point and diffuse source risks for microbial contamination of downstream water resources. Even where livestock waste management is practiced, runoff from animal-housing surfaces and leakage or rainfall-induced overflow from slurry pits may cause severe microbial water quality impairment of receiving water bodies (e.g. Edwards et al., 2008; Mallin and Cahoon, 2003). The impervious surfaces and artificial drains of Chinese farm enterprises may therefore facilitate efficient transfer of microbial pollutants.

3.2.3. Research priority C: characterisation of urban and agricultural sources of FIOs and quantification of loading to receiving waters in mixed land-use paddy farming catchments

Characterising the risk of FIO loss from urban and household sewage sources, and from different farm typologies and varying scales of farm systems typical of the SWCKR, would provide a useful conceptual framework upon which to devise more targeted management and mitigation strategies for FIO pollution. Combining social sciences with natural sciences presents a clear opportunity to assess how risk of FIO pollution relates to management of both farm animal waste and human sewage and how socio-economics might impact on management (Oliver et al., 2009). For example, mapping of urban point sources could be assisted by surveys on household and village waste management practices, and quantification of loading from urban areas could be established by key outlet or downstream monitoring points. There is currently little quantitative information to support FIO risk assessment of paddy and dryland cropping, small and large scale livestock farms, subsistence farms, and emerging large scale commercial farms. Generating relevant data to support a risk assessment of different farming typologies would require a combination of plot, field and farm scale experiments, FIO audits and baseline monitoring to quantify FIO loss from different agricultural systems under high and low flow conditions. As this develops, future opportunities could also capitalise on the integration of microbial source tracking (Devane et al., 2018), targeted in a Chinese context.

Headwater catchments offer considerable scope for informing on the importance of different land use types contributing to FIO loading of receiving waters. Headwaters can constrain the number and variety of farm units and thus provide an effective approach to isolate the effects of different farming practices relative to larger catchment areas, which are inherently more complex. They provide an ideal location to perform field and farm scale experiments tracing FIO transport through the soil and epikarst profiles into groundwater with, for example, soil water sampling and drains, in hillslope and depressions under different farming and irrigation practices, and rainfall conditions. However, larger catchment-scale studies can provide opportunities too: monitoring of water quality at catchment and sub-catchment outlets combined with spatial analysis of the contributing areas of different land use should deliver important understanding of the contributions of urban, agricultural, and forested land to catchment FIO export (Winter et al., 2011).

Surveys on timing, method, type, and quantity of manure application, and timing of agricultural phases allow identification of potential sources and guide spatial and temporal baseline monitoring, through informing on likely higher risk periods such as during manure application and paddy discharge. Such surveys would also reveal farmer knowledge that can be applied in controlling FIO loading. For example, in the Houzhai survey 58% of farmers surveyed did not think that their farming activities held any consequence for downstream users of water, and 60% said they did not know how water moves through the environment. This strongly suggests that knowledge exchange by scientists and/or education of farmers and local residents could help improve understanding of the links between farm practice and water quality.

3.3. Theme #3: FIO survival in paddy farming systems and the wider karst system

3.3.1. Paddy farming

Survival of FIOs in the shallow aquatic systems of paddy fields is likely to be influenced by a complex mix of physical, chemical, and microbial conditions as observed in other aquatic environments (Hassard et al., 2017). *E. coli* and total coliform concentrations in paddies, where recycled waste water is used for irrigation, fluctuate widely throughout the growing season, attributed to dilution, settling, and re-agitation of sediments (An et al., 2007; Jang et al., 2013). The precise timing and duration of paddy rice cropping varies by region, but manure typically resides in stagnant water for up to several months (Fig. 1:9) (Toan et al., 1997). Survival times of *E. coli* in aquatic environments vary from 11 h for a 95% population reduction (T_{95}) in anaerobic reduced conditions to 260 days in sterile filtered river water for a 99% reduction (T_{99}) (Flint, 1987; Lisle, 2016; Personné et al., 1998). The shallow and stagnant nature of paddy fields is likely to result in: (i) temperature and UV fluctuations as the rice grows; (ii) variable redox conditions in soil and water as decomposition of organic matter proceeds under a range of different drainage regimes; and (iii) elevated nutrient concentrations and altered microbial communities due to the addition of inorganic fertilisers and pesticides (Takai and Kamura, 1966; Yagi and Minami, 1990). Many of these factors influence survival of *E. coli* in water: extremes of temperature and pH diminish survival rates, as does UV light and the presence of grazing microbes, while nutrient availability may increase survival rates (Gagliardi and Karns, 2000; González, 1995; Jamieson et al., 2004; McFeters and Stuart, 1972; Pachepsky et al., 2014; Rozen and Belkin, 2001).

3.3.2. FIO survival in environmental matrices in karst geological settings

The high solubility of the carbonate minerals causes karst aquifer waters to have elevated levels of dissolved carbonates and a high pH (Fig. 1:13) (Ford and Williams, 2007). For example, in the region where the Houzhai survey was conducted, typical of the SWCKR, the pH of catchment waters ranges from 7.1–8.7 (Li et al., 2010). FIO survival studies in water have not found a consistent relationship between pH and *E. coli* survival rates, with temperature generally reported to be the most important factor (Blaustein et al., 2013; John and Rose, 2005). However, survival of *E. coli* declines under high pH conditions in manure and soil, with the enterohaemorrhagic *E. coli* O157:H7 growing at low pH in soil, manure or water (van Elsas et al., 2011). High pH values (>7.8) have also been found to significantly diminish survival rates of viral and bacterial pathogens in groundwater (John and Rose, 2005). Meta-analysis on the controls of *E. coli* and pathogen survival in the soil-water continuum is hampered by lack of complete reporting of the physical, chemical, and biological characteristics of the matrix in survival studies, as the importance of this has only recently been appreciated (Franz et al., 2014). In the environment generally, there are gaps in understanding around transfer between soil, water and rock matrices, the effects of autochthonous microbial community structure, and survival enhancement mechanisms such as sediment storage (van Elsas et al., 2011; Engström et al., 2015). Understanding the extent to which FIO transfer occurs between these matrices is necessary to predict persistence in watercourses and waterbodies, as these cause substantial variance in the suitability for FIO survival. In addition, the presence of naturalised *E. coli* populations in soil growing over the summer period and capable of surviving winter has been observed in tropical, sub-tropical, and temperate environments, but has not been investigated specifically in karst (Ishii et al., 2006). The existence of naturalised *E. coli* populations does not undermine *E. coli* as an indicator of faecal contamination, but does have implications for *E. coli* sampling strategies and how results are interpreted.

3.3.3. Research priority D: FIO survival in paddy farming systems and environmental matrices in karst terrain

Replicated and tightly controlled multi-factorial experiments would allow better understanding of the mechanisms of FIO survival in paddy farming systems and in the soil, water, and rock matrices of the karst environment. Microcosm experiments investigating FIO survival in (i) typical karst waters, with high pH and elevated dissolved carbonate levels, (ii) paddy waters throughout the course of planting cycles, (iii) typical SWCKR cropland soils, manures, and slurries; and (iv) different components of the soil-water-rock matrix, are therefore required as they are missing from the existing evidence-base on FIO persistence. The use of multifactorial laboratory experiments would also help to inform on how FIOs respond under climate change scenarios, where simultaneous variation of multiple factors (e.g. temperature, pH, light) will regulate competitive outcomes. Such experiments need also to recognise the importance of interactions across multiple pollutant hazards to account for relative competitiveness and opportunities for FIOs to flourish or perhaps survive more optimally, such as in the presence of higher nutrient concentrations. Intrinsic complexity across a range of spatial and seasonal scales will add further challenges to this important research need.

3.4. Theme #4: sediment-FIO interactions and legacy risk in karst terrain

The combination of karst hydrology and extremes of flow regimes may result in different sediment dynamics to non-karst terrain and non-monsoonal climates (Fig. 1:14). Precipitation events, particularly heavy and episodic, can rapidly increase the delivery of surface sediments and entrained substances via runoff (potentially containing contaminants) to surface waters and aquifers (Leigh et al., 2013). This is particularly marked in aquifers with low buffering capacity, typical of many karst aquifers, particularly those with well-developed conduit systems (Mahler and Lynch, 1999; Toran et al., 2006). Studies monitoring FIO concentrations in both karst and non-karst catchments after storm events have attributed the high suspended sediment levels to both surface runoff and resuspension of streambed stores, and some modelling of *E. coli* concentrations in receiving waters of catchments now incorporates stream bed stores (e.g. Garcia-Aljaro et al., 2017; Kim et al., 2010; Morasch, 2013). However, modelling of the physical mechanisms of deposition and resuspension, along with other transport mechanisms involved in FIO transport in catchments more generally still requires development (Porter et al., 2017).

FIO transport, storage, and survival can be influenced by sediment associations due to sorption to particles providing a transport mechanism, increasing the availability of nutrients, and providing shelter from predation, UV, and extremes of temperature when deposited in stream beds (Garzio-Hadzick et al., 2010; Jamieson et al., 2004). Higher *E. coli* concentrations in streambed sediments have been linked to direct access of livestock to streams, diffuse runoff from agriculture, and human and animal point sources (Fig. 1:6,9,10,11,12) (Bragina et al., 2017; Davies-Colley et al., 2004). However, the relationship between FIOs, pathogens, and sediment particles appears complex and highly variable and the association of *E. coli* in the water column with suspended sediment appears to vary widely between sites and hydrological conditions. For example, studies in different karst settings have reported a range of values for the degree of association of FIOs with sediment, from non-significant to 100%, but generally increasing under high flow conditions (e.g. Mahler et al., 2000; Pronk et al., 2006). FIOs have been found to attach preferentially to specific particle size ranges and mineralogy, though results vary between studies, and maximum *E. coli* load in streams in karst regions following precipitation has been observed to coincide with maximum turbidity, TOC, and a relative increase in smaller sediment particles (Foppen and Schijven, 2005; Jeng et al., 2005; Pronk et al., 2007). Particulate-associated faecal bacteria within karst conduits can persist for up to several months demonstrating the potential for accumulating sediment in underground cave systems of karst terrain to represent a legacy risk and reservoir for possible future contamination (Ward et al., 2016).

3.4.1. Research priority E: develop core understanding of sediment-microorganism interactions for FIO survival, storage, and transport in karst systems

Research on sediment provenance through sediment microbial fingerprinting techniques and residence time of sediments in different 'reservoirs' within the karst system, combined with microcosm experiments on survival of FIOs in sediment and empirical data on FIO concentrations in sediment reservoirs are required. This will allow improved modelling of sediment transport and FIO associated storage and transport. In addition, simultaneous sampling of *E. coli* concentrations in sediment and the water column under varying flow regimes will allow verification and improvement of modelling of sediment dynamics and associated *E. coli* transport and storage in catchments (Pandey et al., 2016). Field studies of FIO concentrations in sediment components of the karst system such as river beds, lakes, and underground conduits combined with studies on the factors influencing attachment of FIOs to particles, such as sediment mineralogy, particle size and cell characteristics (Wyness et al., 2018), will provide insight into the importance of sediments in promoting legacy FIO pollution in karst catchments, i.e. the ability of sediments to prolong the risk of FIO presence in a catchment system.

3.5. Theme #5: hydrological modelling and risk assessment in karst terrain

3.5.1. Hydrological modelling

Accurate numerical simulation of the hydrological system is fundamental to quantifying FIO catchment fluxes and predicting microbial water quality under changing system stressors such as land-use, abstraction, and rainfall (Porter et al., 2017). An accurate hydrological model can help to predict FIO flux at monitoring points, quantify FIO loading from land-use types, evaluate the role and importance of different hydrological pathways in exporting FIOs, and determine concentrations and fluxes of FIOs in catchments under changing system stressors (Cho et al., 2016). This level of predictive power is currently not achievable for most karst regions, due to a need for further development of karst specific modelling capability and the heterogeneous hydrogeological context (Zhang et al., 2017; Hartmann et al., 2014). Modelling techniques in karst can be split into lumped, distributed and hybrid semi-distributed models. Generally, the more sophisticated semi-distributed and distributed models are more capable of simulating spatial variation, which is necessary for prediction of FIO concentrations and fluxes at different points in the catchment hydrological network. However, these modelling techniques are currently limited by lack of detailed calibration datasets or high resolution data on hydraulic properties (Goldscheider and Drew, 2007; Hartmann et al., 2014). Regionalisation of karst models is limited by the coarse scale of available datasets, lack of information on hydraulic properties, and subsurface catchment boundaries that are generally unmapped and different to surface watersheds (Hartmann et al., 2014). Southwest China has experienced a warming trend since 1960, with increasing frequency of droughts and heatwaves (Lian et al., 2015; Piao et al., 2010). The likelihood of further reduction in water resource availability due to climate change in this region and many other developing countries adds further urgency to the need for accurate modelling capability for prediction of water quantity and quality (Green et al., 2011; Hartmann, 2013).

3.5.2. Risk assessment

The range of environments and contaminants that pose a risk to health or ecosystems means that standardising a framework for risk assessment is a challenge, and many different approaches have yielded informative results (e.g. Dimitriou et al., 2008; Doerfliger et al., 1999; Hu et al., 2005; Neshat and Pradhan, 2015; Wang et al., 2012). A useful precursor to risk assessment would be an analysis of the regulatory framework in China set against existing frameworks for catchment-scale control of water quality (e.g. the EU Water Framework Directive, US EPA Clean Water Act). Risk assessments typically build on combinations of hydrological modelling, spatial datasets, and contaminant-specific information to identify areas at risk of hazardous contamination levels. Assessment of risk posed by microbial contaminants from faecal matter in the SWCKR needs to recognise that microbes are living organisms, interact with surfaces, and that exposure to a small dose due to rapid flow pathways can cause infection (Hunt and Johnson, 2017). Thus risk assessment for microbial contaminants is subject to the uncertainty in source apportionment, hydrological modelling, and spatial datasets, and from gaps in understanding of the behaviour of FIOs (and to an even larger extent, human pathogens). With additional resource, investigation of pathogen-indicator relationships in a Chinese context would be useful. Risk assessments not developed specifically for karst have limited transferability due to the assumption that diffuse infiltration serves as an attenuation mechanism for contaminants before reaching the groundwater system (Goldscheider, 2005). Several karst specific risk assessments have been developed and tested (e.g. Andreo et al., 2006; Goldscheider, 2005; Neukum et al., 2008), with the Pan-European Approach being the latest iteration containing karst and contaminant specific frameworks. However, due to the inherently multi-facetted nature of risk assessments, the question of how they should be validated remains a challenge (Andreo et al., 2006).

3.5.3. Research priority F: development of karst hydrological modelling with empirical data and integration with risk assessment

Progression of hydrological modelling and risk assessment in karst catchments requires detailed empirical data to refine conceptual understanding of the components of the karst system and calibrate karst specific models. In particular, evaluation of the role of different hydrological pathways and hydrological drivers of FIO mobilisation and transport, refinement of sediment storage and transport modelling, quantification of loading from land-use types, and data constraining FIO survival rates in the environment will allow progress in conceptual modelling of FIO dynamics in karst catchments (see Research Priorities A–E). Development and calibration of specific models requires accurate delineation of the surface and groundwater systems through tracer tests, high resolution data on hydrological properties, and measurement of FIO concentrations at key monitoring points during low- and high-flow conditions to calibrate land-use weightings. Incorporation of improved understanding of FIO export through karst hydrological pathways and loading from different land-use categories into catchment risk assessment will subsequently allow improvement of a conceptual model to underpin risk assessment in karst terrain. For regions where diffuse pollution is a major contributor, this could involve developing karst specific adaptations for FIO routing from source to receiving water body using catchment risk assessments such as SCIMAP (Porter et al., 2017). Validation of the performance of such risk assessments will then require robust empirical data to assess model predictions, as with hydrological modelling in the karst terrain.

4. Conclusion

The population of the SWCKR is highly vulnerable to water contamination issues, with mitigation challenged with a combination of climatic conditions, population pressures, and land-use regimes that can strongly influence FIO behaviour. Despite this, very little research has investigated FIO fate and transfer in this distinctive landscape that represents 20% of China's land mass. With growing interest in the relationship between environment and human health, and changing regulations that are expected to lead to increased use of organic manures as nutrient sources, a number of critical knowledge gaps associated with FIO dynamics in karst catchment systems have become apparent and require research attention. Some of the challenges that need addressing are achievable in the short term; others need more substation investment or co-ordination but over time will convert to exciting research opportunities. The research needs we identify will support improved knowledge of FIO fate and transfer in the SWCKR, and karst environments more generally. Delivering good quality data for these research needs would ultimately help to reduce the risk of human exposure to faecal contamination in these distinctive environments through improved risk assessment, modelling and better informed decision-making. In the context of the SWCKR, the high population density, direct dependence of communities on the land for food and water, and need for close proximity of water supplies to villages needs to be born in mind when designing research programmes. Thus, effective knowledge exchange and consultation with communities is also required in order to provide pragmatic recommendations when using scientific outcomes to guide policy or management practice.

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Conflict of interest

The authors declare no conflict of interest.

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