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Landscape Drivers and Social Dynamics Shaping Microbial Contamination Risk in Three Maya Communities in Southern Belize, Central America

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Abstract: Land transformation can have cascading effects on hydrology, water quality, and human users of water resources, with serious implications for human health. An interdisciplinary analysis is presented, whereby remote-sensing data of changing land use and cover are related to surface hydrology and microbial contamination in domestic use areas of three indigenous Maya communities in Belize, Central America. We asked whether a departure from traditional land-use patterns toward intensified use led to consequences for hydrology and microbial contamination of drinking water, and investigated how social factors in the three study communities may act to ameliorate human health risks associated with water contamination. We showed that a departure from traditional land use to more intensive cultivation and grazing led to significantly increased surface water runoff, and intensified microbial contamination of surface water sources sometimes used for drinking. Results further suggested that groundwater contamination was widespread regardless of land cover, due to the widespread presence of pit latrines, pigs, and cows on the landscape, and that human users were consistently subject to health risks from potential pathogens as a result. Given that both surface and groundwater resources were found to be contaminated, it is important that water distribution systems (piped water from tanks; shallow and deep wells) be monitored for *Escherichia coli* and treated when necessary to reduce or eliminate contaminants and protect public health. Results of interviews suggested that strengthened capacity within the communities to monitor and treat centralized drinking water sources and increase water treatment at the point of use could lead to reduced risk to water consumers.

Keywords: water management; microbial contamination; nutrient loading; land use/land cover change; Moho River; Southern Belize

1. Introduction

Throughout rural Latin America, millions of people, including many indigenous peoples, depend upon local streams and aquifers for their water, which is often supplied directly or through rudimentary storage and treatment systems [1]. These communities often receive inadequate access to clean and safe drinking water [2]. Lack of access to safe water has numerous negative consequences, including infant illness and mortality [3]. In 2015, only 65% of the population of Latin America and the

Caribbean were drinking water from "an improved source that is accessible on premises, available when needed and free from faecal and priority chemical contamination" [3]. A robust body of literature has examined the underlying causes of unequal access to safe drinking water in Latin America (see, e.g., [3–6]). Where rural communities are dependent on natural sources or rudimentary water systems (RWSs) for water access, it is critical to understand the environmental processes that determine the quality of surface and subsurface waters. Tropical streams in Latin America are complex systems shaped by natural and social processes and require interdisciplinary approaches that map dynamic changes to terrestrial landscapes and related resources. The terrestrial-aquatic nexus is tight, and land transformation can have cascading effects on hydrology, water quality, and human users of these resources by extension.

In Belize, Central America, several sources have documented the uneven access to safe water [7,8], emphasizing the importance of the southernmost Toledo District; the poorest in the country [9–11]. Toledo is home to the rural, indigenous communities most dependent on unimproved water systems. To date, we have found no published peer-reviewed studies of the factors shaping the quality of drinking water sources in rural southern Belize (but see [12–15]). Our paper builds off a growing literature addressing land use and land cover change in southern Belize and its impacts on ecosystem structure and function [15–17] to study effects of land use and cover change in southern Belize via interdisciplinary analysis of water quantity and quality. More specifically, we integrate remote-sensing analysis of changing land use and cover in the Moho River watershed (1975–2017) and make linkages between changing land cover and surface hydrology, potential sources of contamination, and indicators of actual microbial contamination in domestic use areas of three indigenous Maya communities.

At the most recent census (2010), rural Toledo was home to 25,434 people and one of the least densely settled regions of Central America, with only 18 persons per square mile [11]. Toledo is the district most likely to lack improved water and sewage facilities. Across Belize, 66% of homes have a flush toilet, but in Toledo only 28% do, mainly in urban areas [11]. Rural Toledo also has the lowest proportion of households with access to fixed bath or shower inside the dwelling and the highest proportion of households accessing water via rivers, streams, or creeks [11]. Though Belize met Millennium Development Goal target 7C ("By 2015, halve the proportion of people without sustainable access to safe drinking water and basic sanitation"), progress toward safe water access in rural Toledo lags [11]. One study of health in Toledo found a correlation between the absence of household sanitary facilities and human growth stunting [18].

The literature on land use, forest change, and stream conditions in tropical Latin America is vast (see, e.g., [19–21]), though Belize's streams have been understudied. To generalize, the literature shows that in rural Latin America, deforestation is caused by a complex ensemble of factors: roadway expansion, market opportunities, land ownership, and demographic shifts, including migration ([22,23]; regarding Belize: [24–27]). As social and economic changes drive deforestation or land-cover change, surface and groundwater sources are often degraded [28]. Changes to land use and land cover influence water runoff, which can serve as an important mechanism for delivery of contaminants, including disease-causing microbes, into drinking water sources via runoff and shallow groundwater flow to streams and other water sources [29]. Rapid advances in this research have come by integrating social and ecological research with remote sensing of land use and cover change (LUCC) (e.g., [30]). While previous works have adopted mixed methods to study the dynamics of forest change in northern [31] and southern Belize [25], none have considered water resources. Our paper is intended as a modest contribution to this lacuna. It provides, for the first time (to our knowledge), watershed-focused results on forest change along with data on hydrology, potential contamination sources, and drinking-water quality in the Maya communities. The literature on LUCC and water quality is expansive (for a review, see [32]). Yet very few studies have been conducted in Belize (but see [33,34]) and relatively few of studies conducted on LUCC and water quality in Latin America have focused on indigenous-use areas (an exception is the work of [15], this issue). We have found none that focus specifically on microbial contamination.

This research was initiated in 2009 at the request of leaders of one Maya community to examine change in their forests, leading to a pair of scientific papers [27,35]. Subsequently, Maya leaders from the three communities studied here asked us to expand our research to consider the conditions of their water supplies. This led us to ask whether changes in land-use patterns that had taken place over the previous three decades, and specifically a departure from traditional land-use patterns in the western part of the study area, led to cascading consequences for hydrology and the risk of microbial contamination of drinking water. We further investigated how social factors intrinsic and extrinsic to the three study communities may act to ameliorate risks associated with water contamination.

Land use and cover changes were documented using interpretation of satellite remote-sensing data. As an important driver of water contamination, hydrologic runoff was shown to increase after conversion of forest to cultivated or pasture lands, potentially increasing the risk of downstream contamination of source waters. Visually evident sources of microbial contamination (e.g., livestock presence near streams) were located and mapped to substantiate obvious contributors of fecal matter to water sources. Several indicators of fecal contamination were measured from known drinking-water sources on multiple occasions and at many locations in each village. Finally, the social and political dynamics of domestic water management were examined in three study communities through a combination of interviews and archival research to improve our understanding of risk factors. While our work is directed toward contributing to the sustainability of local indigenous land and water governance, the present paper offers no specific management recommendations.

2. Materials and Methods

2.1. Site Description

The study area is in southern Belize, Central America, within the 1286 km^2 Moho River watershed. The main channel of the Moho river flows into Belize from Guatemala, and is fed by two northern tributaries, Aguacate Creek and Blue Creek, which drain the karst fringe of the Maya mountains in Belizean territory. The hydrologic and agricultural context of the study watershed is affected by the underlying Cretaceous limestone geology, which is overlain by a sequence of younger (Tertiary) mudstone, sandstone, limestone, and conglomerates known as the Toledo Beds. Aguacate Creek and Blue Creek flow through limestone cave systems before meeting the mainstem in the middle reaches of the river network near the study communities (described below). The soils of southern Belize have been characterized by two major surveys [36,37]. The limestone foothills at the headwaters of the Temash and Moho produce shallow, quickly-draining clay soils of low to no agricultural value. The Toledo Beds dominating the mid and lower reaches produce two groups of soils: upland clay soils that are well-draining and highly fertile, and coastal plain clay soils that are poorly-draining and of more limited fertility. Finally, aqueous swamp soils reside among depressions and coastal mangroves of the river delta.

We have found no peer-reviewed scientific studies of the hydrology, ecology, or environmental conditions on the Moho river (but see [38]). The Moho River has a sub-tropical climate, with mean monthly temperatures (measured from 1985–1989 within the study areas) ranging from 22 to 29 °C, and annual precipitation ranging from 2296 to 3812 mm delivered in relatively distinct wet (June–December) and dry (January–May) seasons [39]. More than 80% of annual precipitation in southern Belize occurs during the wet season [38]. The study area is prone to hurricane strikes; the last hurricane to affect the study area was Hurricane Iris in October 2001.

The three communities studied within the watershed were Santa Cruz, Aguacate, and Jalacté (Table 1). These villages reflect customary, indigenous Maya communities, studied intensively by anthropologists (see, e.g., [40,41]), ethno-historians [42], and geographers [40,43]. Economic life in these rural communities is organized on a household basis, with livelihoods produced through a blend of agricultural production, hunting, and sale of labor power in urban areas. Notwithstanding some minor differences in social history, for present purposes the villages are comparable in critical respects:

they share the same climate, soil, hydrology (the Moho watershed) and historical-cultural land and water use patterns. The principal differences concern recent (post-1981) expressions of landscape change (a factor we have analyzed elsewhere) and the presence/absence of centralized water systems. Historical land cover in the watershed was largely dominated by tropical broadleaf forest, including the mid-reaches where the study communities are located. The forests near the communities have long been managed on a communal, usufructory basis by the indigenous Q'eqchi' and Mopan people who use the land to support agrarian, household-based livelihoods [40,44].

Two sub-basins of the Moho River watershed were used to test for relationships between runoff and changing LUCC. The first ("Catchment A") is 660 km^2 of the hilly terrane in the southeastern corner of the Guatemalan Petén and also encompasses three villages in southern Belize (see Figure 1). Due in part to an influx of Maya refugees from Guatemala, land use in Catchment A has changed over the past three decades from primary forest to a mix of "non-customary" pasture and short-fallow cultivation. The adjacent catchment ("Catchment B") drains 206 km^2 of similar terrain in southern Belize. Here, Maya farmers have produced livelihoods for over a century with few changes made to land-use practices. Under "customary" management, they clear a patch of secondary forest, burn it to manage fertility and weeds, and allow an average 8.2-year fallow period before recultivation [35]. Land cover is a dense mosaic of succession states [26]. The water sampling, stream surveys, interviews, village discussions, were conducted in 2016–2018.

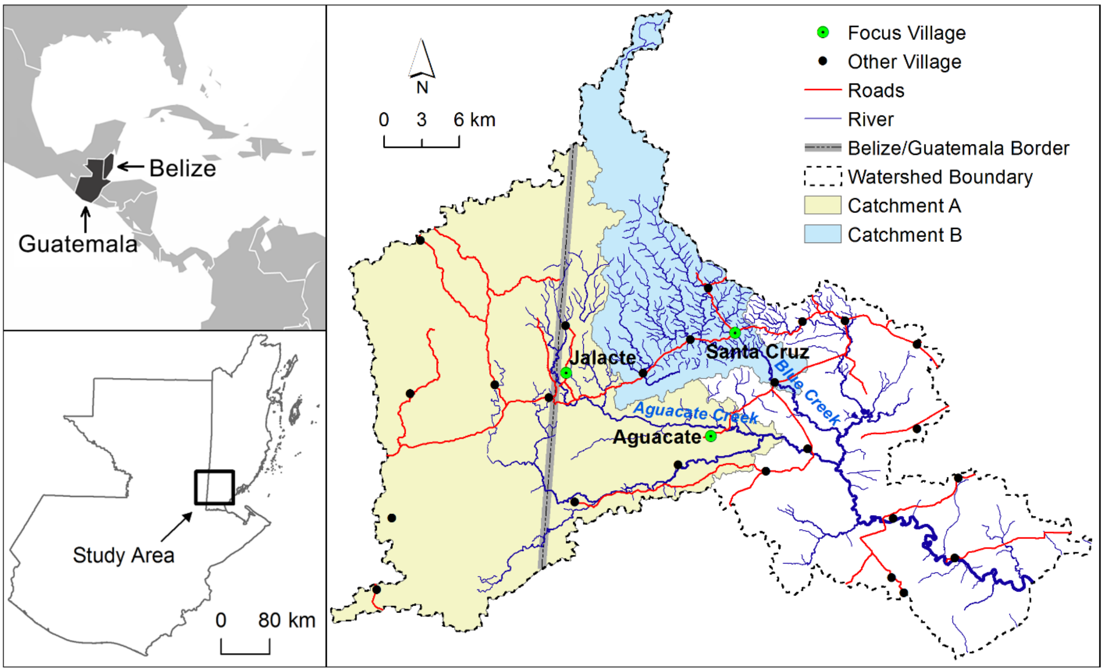

Figure 1. Locator map showing the study watershed (Moho River watershed), the villages studied, and the locations of catchments used for hydrologic analysis.

Table 1. Summary of population, water system, and other pertinent statistics for each of the three study communities. Household and population data were taken from the 2010 census [10]. Data on principle water access was drawn from interviews, with number of respondents indicated.

Village	Households (#)	Population (#)	Dist. to Paved Highway. (km)	Interviews (#)	Water Pumps (as of Year)	Rudimentary Water System	Year of Installation	Principal Water Access	Secondary Water Access	Tertiary Water Access
Aguacate	64	369	16.5	12	0 (1984) 7 (1995) 4 (2018)	Yes	ca 2006	Rudimentary water system (10 respondents)	hand pumps (India Mark II)	stream
Jalacté	119	769	1.5	9	0 (1990) 1 (1995) 4 (2018)	No	N/A	Private wells (5 respondents) hand pumps (India Mark II) (4 respondents)	cisterns or rainwater	stream
Santa Cruz	67	386	0	12	8 (1990) 8 (1995) 8 (2018)	Yes	ca 2004	Rudimentary water system (9 respondents) private wells (5 respondents)	hand pumps (India Mark II)	stream

2.2. Land Use and Cover Analysis

Remote sensing images were obtained from the online Landsat archive of the U.S. Geological Survey [45]. To avoid issues due to vegetation phenology, all images were taken from a 10-week window during the height of the dry season, a time when Maya farmers cut and dry forest patches in preparation for burning and planting. After extensive searching on the Landsat Archive, 12 images were selected with minimal cloud cover (Table S1). The Landsat images have a spatial resolution of 30 m. The bounding area shown in the main plot of Figure 1 has a size of 1749 pixel rows and 2057 pixel columns (52.5 km × 61.7 km) on the remote-sensing images. To carry out remote-sensing image classification, training and test data were randomly selected and verified through visual interpretation of the Landsat images and comparing Google Earth images as well as with field data.

Five land-cover types were interpreted from the Landsat images: road/built-up area, cultivation, vegetation, water, and cloud (for some years). The road/built-up area class included both roads and the built-up areas of settlements (towns and villages). Roads and settlements were difficult to differentiate in the remote-sensing images for our study area and thus were combined. Cultivation refers to cultivated areas where Maya farmers cleared the forest and planted crops. The vegetation class consisted primarily of forest, but may also include grass. For the five classes, the signatures of cloud and water were substantially different and could be easily interpreted with little confusion. The signatures of road/built-up area, cultivation, and vegetation were more difficult to differentiate. In previous studies [27,35] of relatively small and simple study areas, a binary classification of "cultivated area" and "vegetation" was used. For this relatively more complex and larger study area, a two-step classification approach was developed while integrating some of the classification techniques used in the earlier work. The first and most difficult step was to extract road and built-up areas. The second step was to extract cultivated areas and vegetation.

To separate road/built-up areas from cultivation and vegetation we first classified the remote-sensing images into five classes: road/built-up area, cultivation, vegetation, water, and cloud. We tried two classifiers: maximum likelihood classification (MLC) implemented using the MATLAB discriminant analysis [46], and support vector machine (SVM) implemented through the LIBSVM MATLAB tool [47]. The overall classification accuracies ranged between 85 and 93% for MLC, and between 91 and 96% for SVM; while comparable, the classes of road/built-up area, cultivated area, and vegetation were not well discriminated from one another. Thus, the class maps were further processed to extract the road/built-up area class only. For SVM classification, we obtained 12 class maps for the 12 years. We then stacked these 12 maps together and compared the stack with the Landsat images and Google Earth images. Through experimentation, we found that if a pixel was classified as road/built-up area at least seven times out of the 12 class maps, it was, in reality, road/built-up area. Further manual editing of road/built-up area was carried out based on the digital road map as well as Google Earth images. To remove salt-and-pepper effect caused by very-fine-scale spatial patterning, a median filter was applied to remove patches smaller than 5 × 5 pixels from larger areas of homogenous land cover. The area of road/built-up area accounts for 0.5–1.2% of the whole study area during the years of 1975–2017.

The second step was to classify the remote-sensing images into four classes: Water, Cloud, Vegetation, and Disturbed land (including road/built-up area and cultivation). The two classifiers, MLC and SVM, returned quite similar classification accuracies, ranging from 96 to 98% for MLC and from 96 to 99% for SVM. The SVM classification tended to overestimate cultivation area for this region. Thus, further processing was based on the results of MLC. Similar to Step 1, a median filter was applied, and patches smaller than 5 × 5 pixels were removed. The classified maps were then integrated with the road/built-up area from Step One to create the final class map.

In this study, we were interested in the bidirectional land cover changes between cultivation and forest. Detailed changes between these two land cover types are shown in Table 2, along with the user accuracy for both land cover types. Based on [48,49], the change between cultivation and forest cannot be explained by classification error. In other words, land cover change actually occurred during these years. Results in Table 2 further confirm the shifting cultivation pattern in the study area. Each year, there are substantial losses and gains both in cultivation and forest and in most years, the cultivation gain is greater than cultivation loss, and forest loss is greater than forest gain.

Table 2. Land cover change between cultivation and forest for all years studied. The Cultivation and Forest columns show the percent area classified as each in the study area. Classification accuracies (% of all pixels correctly classified) for cultivation and forest classes are shown next. Cultivation loss is the percent change from cultivation to forest in a given time step. Cultivation gain is the percent change to cultivation due to deforestation. Forest loss is the percent of forest changed to cultivation, and forest gain is the percent of forest gained as reforestation from cultivation in a given time step.

Year	Cultivation (%)	Forest (%)	Cultivation Accuracy (%)	Forest Accuracy (%)	Cultivation Loss (%)	Cultivation Gain (%)	Forest Loss (%)	Forest Gain (%)
1975	2.0	97.2	87.7	98.0	-	-	-	-
1987	9.5	88.4	97.0	97.6	68.1	453.3	9.2	1.4
1993	3.2	94.9	93.6	98.2	82.9	16.3	1.8	8.9
1994	2.6	96.0	89.1	99.9	80.4	61.3	2.1	2.7
1995	3.8	94.9	99.4	98.0	87.3	131.1	3.6	2.4
1996	6.9	91.8	95.0	99.0	75.3	157.5	6.3	3.0
1998	9.8	88.6	94.7	99.4	58.4	101.7	7.6	4.4
1999	7.0	91.6	96.9	98.3	70.0	41.6	4.6	7.8
2000	15.3	83.2	97.4	99.8	27.9	145.3	11.2	2.1
2001	9.3	89.2	95.6	99.9	54.2	15.0	2.8	10.0
2011	13.4	85.1	95.2	97.8	40.3	84.5	8.8	4.2
2017	20.4	78.1	95.4	98.5	28.0	79.8	12.6	4.4

To further analyze the spatiotemporal trend, we examined land-cover change across a west-east axis. Cultivation area was summarized by pixel column to create transects of percent routine cultivation, a method used in our previous studies [27]. Routine cultivation refers to planting crops on the same plot for multiple consecutive years and is characteristic of a non-traditional land-use practice. The percent of routine cultivation (y_i) by pixel column in the raster map was calculated by dividing the number of pixels in the routine cultivation class in each column by the total number of pixels in each column as shown in Equation (1). In practice, we averaged the y_i for clusters of the 20 pixel columns to create a smoother profile (Y_j).

$$\begin{aligned} y_i &= \tfrac{c_i}{n_i}, i = 1, 2, \ldots, 2057 \\ Y_j &= \tfrac{1}{20} \sum_{k=1}^{20} y_{20(j-1)+k}, j = 1, 2, \ldots, 103 \end{aligned} \quad (1)$$

where c_i is the number of pixels classified as routine cultivation in pixel column$_i$, n_i is the number of pixels in pixel column$_i$, y_i is the percent routine cultivation of pixel column i, and Y_i is the average of y_i per each cluster of 20-pixel columns. The last bin was aggregated based on 17 columns as $20 \times 102 + 17 = 2057$.

2.3. Hydrologic Analysis

The study catchments were delineated from a 30-m resolution digital elevation model derived from Shuttle Radar Topography Mission (SRTM) data to distinguish areas with customary versus non-customary land-use practices. Hydrological tools in QGIS (version 3.2) were used to delineate the sub-catchments and estimate their areas. Daily discharge data for the period from 1982 to 2013 were secured from the Belize National Meteorological Service (NMS) at Blue Creek (Catchment A) and Jordan (Catchment B) villages. Average monthly precipitation data for the region were secured for the same time period from the same data source. Runoff ratios (C) were calculated, equivalent to the annual river discharge (Q), normalized by area (A) and precipitation (P):

$$C = \frac{Q}{[A \times P]} \times 100$$

We hypothesized that: (1) runoff ratios would be higher under non-customary management; and (2) percent forest cover would be a primary correlate to the runoff ratio. Student's *t*-tests were used to make pairwise comparisons between Catchments A and B across the entire time series, and to test for significant within-catchment temporal trends between the periods of early (1982–1995) and late (1997–2012) parts of the time series.

2.4. Visual Inspection for Contamination Sources along Streams and Rivers

Streams and rivers serve as conduits for fecal microbes (including potential pathogens) and other sources of pollution originating from upstream in the watershed and along the banks and, at the same time, serve as drinking water sources for residents of the study area. Streams and rivers were traversed on foot and by canoe to map potential sources of fecal contamination and other pollutants that could be hazardous to human health. Both direct and indirect sources were characterized and mapped. Direct sources included livestock entry points into streams and rivers and laundry-wash sites along river banks. Livestock presence was confirmed by the presence of pigs, cows, horses, and other animals along or in streams and rivers, or from hoof prints. Residents of the study communities frequently wash their laundry by hand along the margins of rivers and streams. This activity can be a source of fecal contamination from cloth diapers, as well as chlorine (direct observation by P.C.E.), nitrates, and phosphates [50]. Indirect sources of pollution included areas where agricultural activity or grazing led to clearing of natural vegetation within the riparian buffer. Riparian vegetation can filter contaminants (manure, nutrients, agrochemicals) moving overland or through shallow groundwater to the stream channel (reviewed in [51]). The absence of vegetation, therefore, may result in increased risk of contamination. We noted areas where riparian forests were either missing entirely (i.e., pastures or corn up to the river bank), or were very thin (i.e., pastures or corn were obvious from the stream channel through a thin line of natural vegetation, usually less than 20 feet).

2.5. Microbial Testing

The bacterium *Escherichia coli* has been used worldwide as an indicator of fecal pollution based on the following criteria: (1) it is always present in the feces of humans and other mammals in large numbers, whether one is healthy or sick; (2) it does not multiply when it leaves the body and enters water, (3) it dies slowly when shed in feces, but survives in water longer than the bacteria that cause typhoid fever, cholera, and dysentery; and (4) it is relatively easy to detect [52]. However, recent studies have shown that after being released from warm-blooded animals through fecal droppings that *E. coli* can attach to soil, sand, sediment, or algae and, under certain conditions, survive and replicate for long periods of time in the non-enteric environment [53,54]. This caveat notwithstanding, the World Health Organization (WHO) continues to promote *E. coli* as an indicator of the microbial quality of drinking water because it provides "evidence of recent faecal contamination" (p. 57), and "[. . .] provides a high degree of assurance because of their large numbers in polluted waters" (p. 148), [55]. Therefore, *E. coli* was used as an indicator of fecal bacteria in the current study and risk levels assessed based on published guidelines by the WHO (see below).

Water samples were collected from a variety of drinking-water sources used by community members in Santa Cruz, Jalacté, and Aguacate villages on three occasions: (1) 31 December 2016 to 5 January 2017; (2) 29 October to 12 November 2017, and (3) 16 June to 24 June 2018. Sampling was conducted to maximize the number of sources tested to get an integrated picture of contamination risk on a village-by-village basis over the study period. Repeat samples were also collected to assess the temporal consistency of results. Water sources included natural stream and river habitats near farms and domestic-use areas, wells, and piped water from RWSs.

One-hundred milliliter water samples were collected in sterile Whirlpack bags with care not to contaminate any part of the mouth or interior of the bags through contact with fingers, pipe ends, or other objects. Samples were placed on ice in the dark in coolers immediately after collection until they could be analyzed, typically within 48 h. Two simple tests for the presence and abundance of

E. coli were used to establish disease risk to human consumers of the water (see [56] for detailed methods). The IDEXX Colilert Presence/Absence test was performed in a glass tube containing a sterile dried nutrient powder suitable for *E. coli* growth. The test indicates the presence or absence of *E. coli* bacteria in a 10-mL water sample, which is confirmed if the incubated sample fluoresces under ultraviolet light. The 3M Petrifilm *E.coli*/Coliform Count Plate test was used to count individual *E. coli* bacteria in 1-mL samples of water. The Petrifilm test consists of a flat, 7.5 × 10-cm rectangle with a bottom layer coated with sterile dried nutrients to support bacterial growth. Both tests require incubation, which was completed at 98.6° F for 10 to 18 h in a temperature-controlled egg incubator. The number of *E. coli* colonies that grew within this incubation period were counted and compared to health-risk charts published by the UN Human Settlements Programme and the WHO [56,57] (Table 3).

Results from the three sampling events were pooled to compare mean *E. coli* counts between source types in all villages combined (piped water from centralized systems, hand pumps, hand-dug shallow wells, rain water vats, creeks/rivers) and between villages. The *E. coli* counts for repeated sites were averaged for the comparison of sources and villages. Because data were non-normally distributed, a non-parametric Kruskal–Wallace test was used to test for significant differences between the medians of different groups based on rank-order statistics. A Dwass, Steel, Critchlow-Fligner (DSCF) non-parametric multiple comparison procedure was used to assess the significance of differences between pairwise rankings of *E. coli* counts. Significance was judged at the p <0.5 level. Analyses of *E. coli* responses were carried out in SAS version 9.4.

Table 3. Correspondence between *Escherichia coli* presence from the Colilert test, *E. coli* counts from the Petrifilm test, and WHO disease risk categories [56,57].

Level of *E. coli*	WHO Disease Risk Level [a]	Colilert Flouresence	Petrifilm # Colonies	MSF Action
<1 in 10 mL	Low	-	0	Consume as is
1–10 in 10 mL	Moderate	+	0	Treat if possible
1–10 in 1 mL	High	+	1–10	Must be treated
>10 in 1 mL	Very High	+	>10	Reject or thoroughly treat

2.6. Social Factors

Our description of the social factors surrounding water use and governance draws from three methods: long-term participant observation of water use and governance in the communities; literature review and archival research at the National Archives of Belize and the National Archives in Kew, London; and 40 interviews with residents of each community, primarily male heads of households, as well as key informants who are involved in water system management.

3. Results

3.1. Land Use and Cover Analysis

Land cover changed dramatically in the western portion of the study area from 1975 to 2017 (Figure 2). The change was particularly evident on the Guatemala side of the watershed starting in the early 1980s when a substantial increase in deforestation took place, potentially associated with land transformation toward intensive perennial cultivation and away from traditional patterns of shifting cultivation (e.g., with an 8-year fallow period). The pattern in intensified cultivation was indicated by an increase in the number of years that individual pixels were classified as cultivated land (i.e., "routine cultivation"; Figure 3). The pattern in intensive cultivation with no fallow period expanded eastward into the Belizean part of the watershed over the study period. The proportion of area under routine cultivation increased considerably from 1975 to 2017 on the Guatemala side. By contrast, on the Belize side, the percentage of land under regular cultivation remained relatively consistent, except a spike on the far eastern side of the study area (right end of Figure 4).

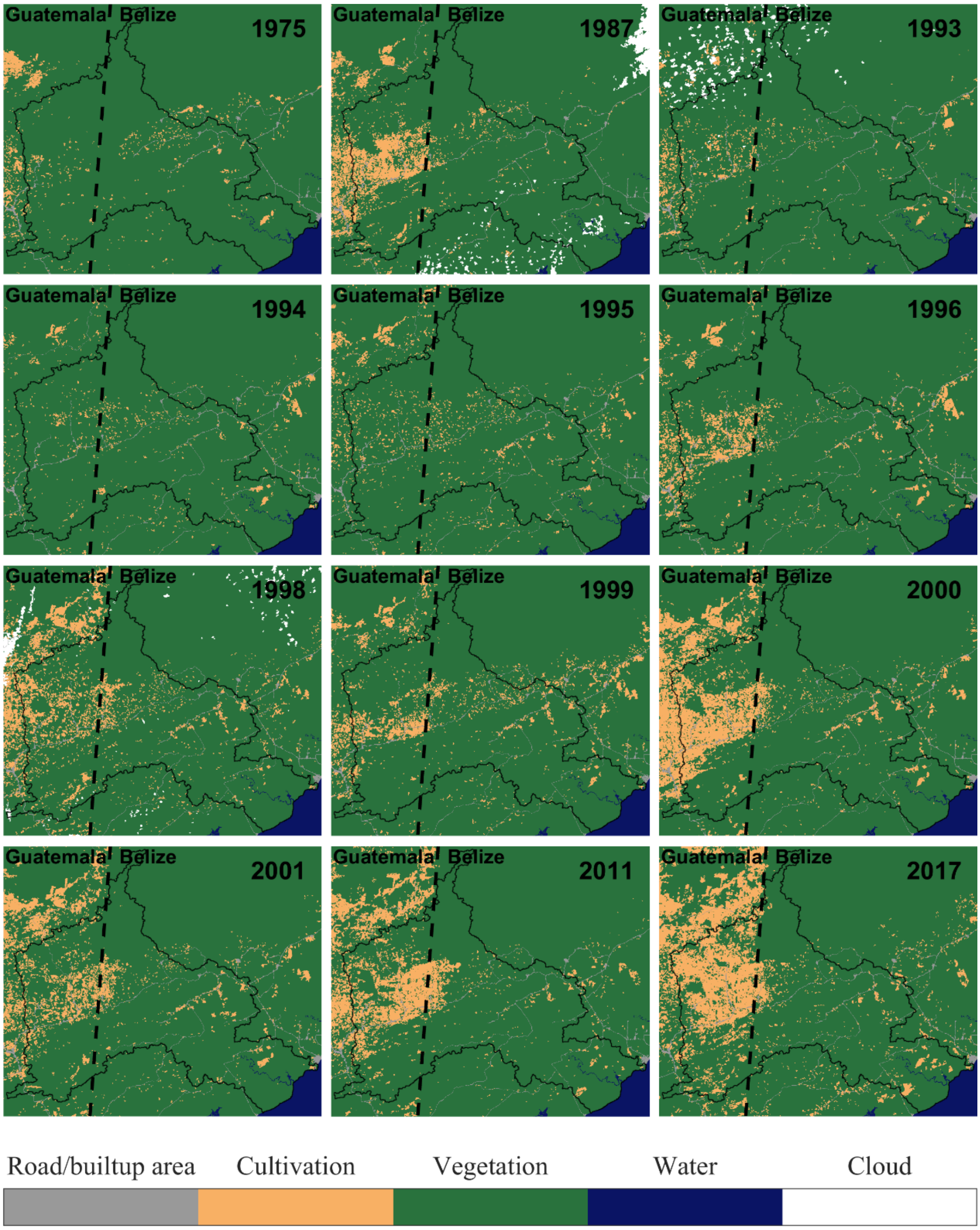

Figure 2. Time series of land use and cover change (LUCC) classification results showing an increase in intensive cultivation starting in Guatemala and spreading into the Belizean portion of the Moho River watershed.

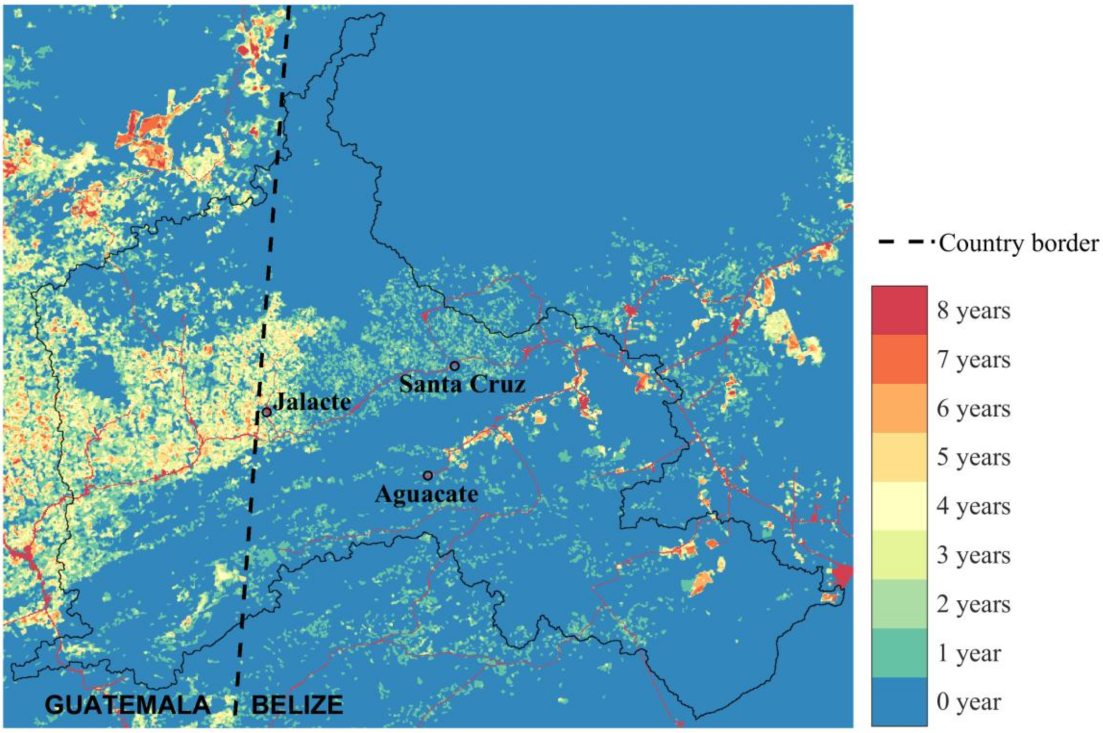

Figure 3. The frequency of land used for routine cultivation for the eight years of 1993–1996 and 1998–2001. While data for year 1997 was excluded due to heavy cloud cover, the time series is treated as continuous for this calculation. Land on the Guatemala side experiences more return cultivation, and land on the Belize side experiences less return use. Return cultivation or return use is defined as farmers cultivating an area that has been cultivated in prior consecutive years.

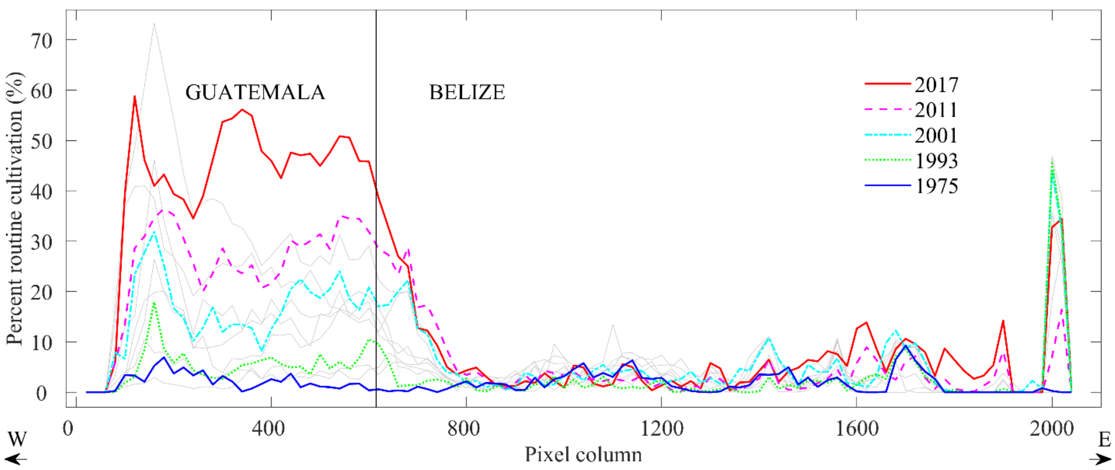

Figure 4. Percentage of land under routine cultivation in the Moho River watershed for all the 12 years shown in Table S1. Years 1975, 1993, 2001, 2011, 2017 are highlighted with colors and line types. Light gray lines show results for the other seven years.

We further divide the watershed into two sub-regions by the Belize/Guatemala border: Moho watershed of Belize; Moho watershed of Guatemala. The percent of land under routine cultivation for each sub-region was calculated (Figure 5). The proportion of routine cultivation was consistent across years for the Belize sub-region. In contrast, the routine cultivation proportion increased substantially for the sub-region in Guatemala over the same time period. A peculiarity in the pattern is 1987 where the routine cultivation in Guatemala sub-region is substantially higher than before and after 1987, due to expanded migrant population pressure due to the civil war of Guatemala [27].

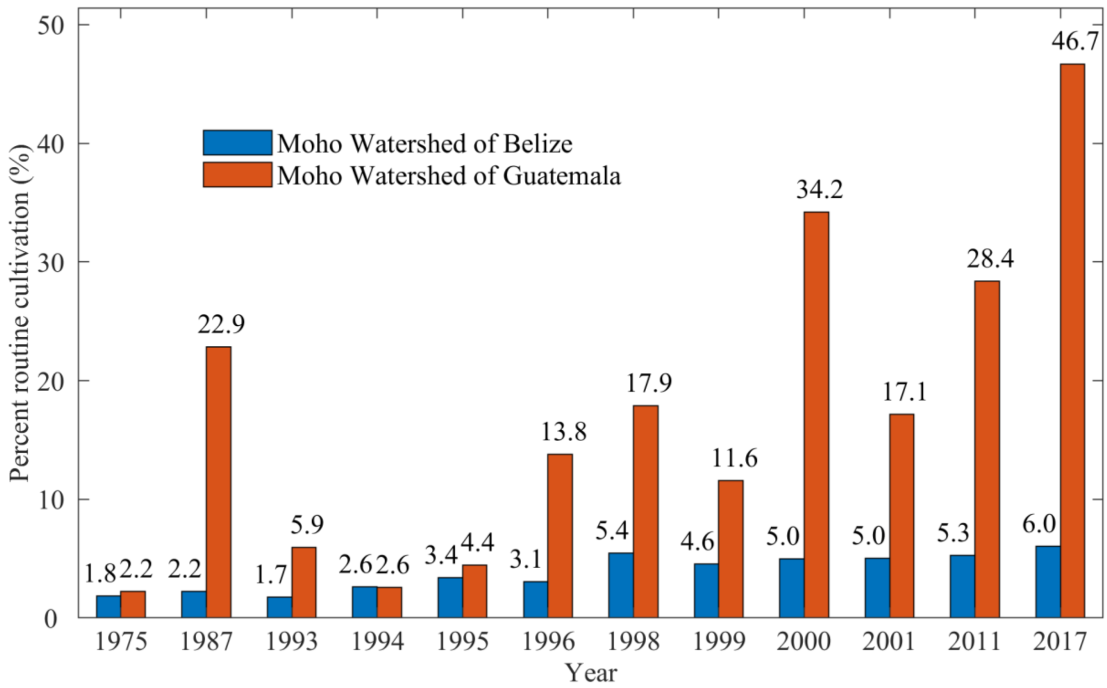

Figure 5. Percentage of area under routine cultivation in Moho River watershed separated by the Belize/Guatemala border.

3.2. Hydrologic Analysis

To investigate the relationship between land cover change and runoff, we compared river discharge, precipitation, and land use data from 1982 to 2013 in two adjacent catchments of the Moho River watershed (see Figure 1). Catchment A in the western part of the study area was strongly affected by an increase in routine cultivation, while catchment B remained under traditional cultivation with long fallow periods. We tested three hypotheses about the runoff coefficient (C): (1) runoff in catchment A (C_A) is greater than runoff in catchment B (C_B); (2) runoff in catchment A (C_A) will increase over the study period, while runoff in catchment B (C_B) will remain stable; and (3) the changes in natural vegetation cover will correlate with observed changes in runoff coefficient.

After verifying that runoff data were approximately normally distributed, a Student's *t*-test showed that runoff in catchment A was significantly greater than in catchment B ($t = 6.56$, degrees of freedom (df) = 40, $p < 0.0001$) (Figure 6A), confirming our first hypothesis. Our results suggest an approximate 50–100% greater runoff in catchment A than catchment B (95% confidence interval). Regression analysis of the runoff coefficients against time for catchments A and B over three decades (Figure 6B) showed a highly significant change in runoff over the study period for catchment A (adjusted $R^2 = 0.43$; $p < 0.001$) and no significant change in runoff for catchment B through time (adjusted $R^2 = 0.11$; $p = 0.57$), thus supporting our second hypothesis. The observed differences in runoff ratio corresponded closely to changes in percent routine cultivation over the entire study period and through time (Figure 6C,D). To evaluate the degree to which the change in runoff ratios in catchment A were related to changes in land use, we regressed runoff ratio versus percent natural vegetation in the sub-catchment and found that percent vegetation accounted for 57% of the observed variation in runoff ratio (adjusted $R^2 = 0.57$; $p < 0.01$) (Figure S1).

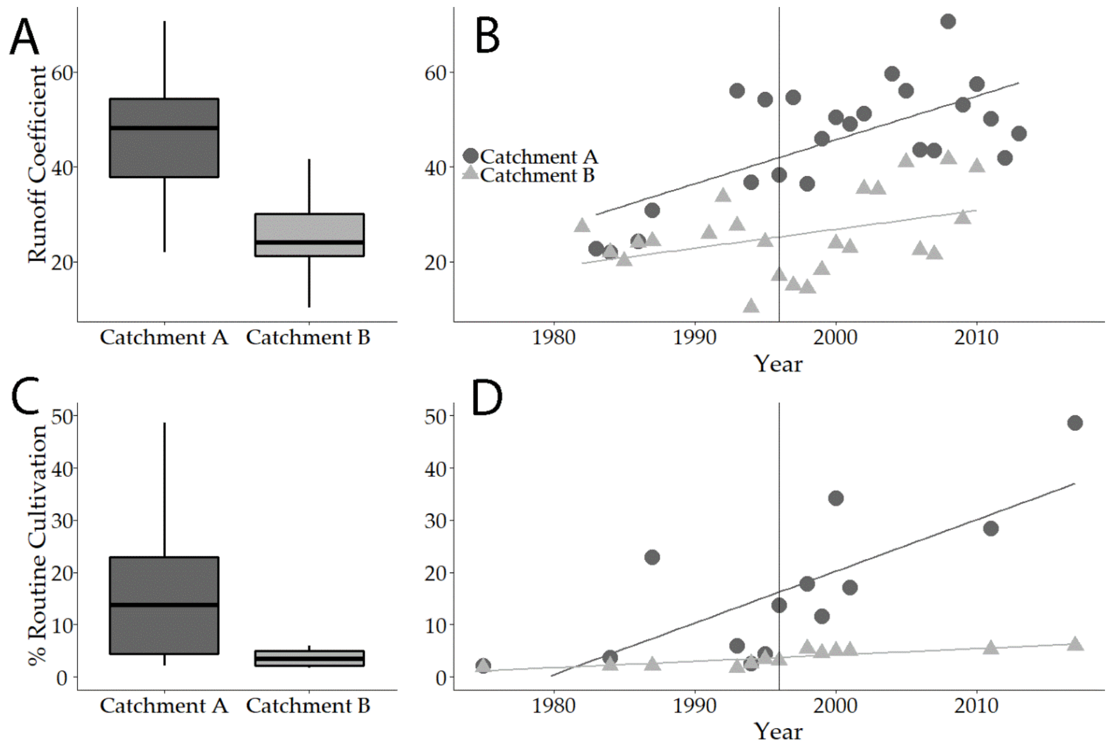

Figure 6. (**A**) Boxplot of runoff data in adjacent catchment areas A and B of the Moho watershed; (**B**) Runoff coefficient over time (1987–2012); (**C**) Boxplot of routine cultivation data in catchments A and B; and (**D**) Percentage of landscape under routine cultivation over time (1975–2017).

3.3. Visual Inspection for Contamination Sources along Streams and Rivers

A total of 13.5 km of river were paddled in canoes while mapping human activities (Table 4), and each of the villages was traversed extensively on foot. Two hundred and six locations along the river where human activities were apparent were mapped in total. Thin or absent riparian buffer was the most commonly observed activity usually in association with either cultivation, grazing, or domestic settlements. Laundry and wash sites were common where rivers passed through villages (Figure 7), particularly in Jalacté where 11 laundry sites were mapped along the river where it passed through the village. Many of these laundry sites contained multiple wash stones and were being actively used at the time of mapping. The presence of pigs and horses was pervasive in all three communities; so much so that we assume that all rivers and creeks running through a community were being accessed regularly by these animals. Cattle production was observed taking place near rivers and creeks in Jalacté and Aguacate. The most commonly used sanitation system in the study communities were unlined and unventilated pit latrines that were in open communication with the groundwater.

Table 4. Summary of human-activity mapping results. The table shows the length of river assessed and the density of various types of activities along that reach. The term "buffer" refers to riverside forests.

Village	Length (km)	Activities (#/km)	No Buffer (#/km)	Thin Buffer (#/km)	Laundry/Wash (#/km)	Livestock (#/km)	Road Access (#/km)
Santa Cruz	3.2	10.5	1.8	4.6	2.2	0.9	1.2
Jalacté	2.1	30.5	7.3	13.1	6.8	1.9	1.5
Aguacate	8.2	12.9	3.2	8.1	0.6	0.4	0.6

Figure 7. Photographic examples of thin riparian buffer (**A**), laundry sites (**B**), no riparian buffer (**C**), and no riparian buffer with livestock access (**D**).

3.4. Microbial Testing

One hundred and thirty-seven water samples were analyzed for *E. coli* at 114 locations: 68 in the first sampling event (Jan. 2017), 46 in the second event (October 2017), and 23 in the third event (Jun. 2018) (Table 5). The third event included samples from Aguacate and Santa Cruz villages only. Forty-five samples were collected from local streams and rivers, 35 from shallow hand-dug wells, 30 from hand-pumped wells, 22 from water pipes connected to centralized water systems, and 5 from rainwater catchment systems.

Drinking from creeks and rivers posed a high public health risk in all villages. Of all creek and river samples analyzed, 89% ($n = 40$ of 45) fell into "high" or "very high" WHO disease risk categories, and only two sources—both springs—were free of *E. coli* in Santa Cruz village during the 2017 wet season sampling event. Significant differences existed between stream and river *E. coli* counts between villages (chi-squared = 18.07, df = 2, $p < 0.0001$). Specifically, stream and river water near Jalacté Village contained significantly greater *E. coli* mean densities than in either Aguacate or Santa Cruz, which were statistically indistinguishable from each other in pairwise comparison. This finding is consistent with our hypothesis that land conversion from traditional uses to routine cultivation and grazing would lead to higher rates of water contamination. However, this finding is only well supported for surface water, not groundwater (see below).

Forty-eight percent ($n = 33$) of other drinking water sources (pipes, wells, pumps, rainwater) fell into the low-risk category indicating the absence of detectable *E. coli* in samples (after averaging repeat samples at a single location). It is notable that, except for rainwater, all these sources originate from groundwater. A further 33% of sampled locations ($n = 23$) fell in the high-risk category, followed by 17% ($n = 12$) in moderate risk, and only one sample (1.4%) in the very-high-risk category. The comparison between villages showed no significant difference in *E. coli* counts from the groundwater sources (chi-squared = 2.57, df = 2, $p = 0.28$).

There were significant differences between source types when data were analyzed for all villages combined (chi-squared = 11.24, df = 2, $p < 0.01$). Pairwise comparisons indicated that shallow hand-dug wells were significantly more contaminated than either piped or pumped water (DSCF > 3.65; $p < 0.05$).

The only rainwater system sampled drained a corrugated metal roof to two ~1000-gallon vats and was found to have undetectable levels of *E. coli* on two sampling events.

Our repeat sample data allow us to only make general observations about temporal variability in contamination levels, because of low replication. Repeat samples were taken at three creek sites, one water pipe each in Aguacate and Santa Cruz, eight hand pumps, one rainwater system, and five shallow-well sites. Repeat samples at the sampled creeks were consistent insofar as their *E. coli* counts and associated risk levels remained high. After an initial sample showing high risk of disease in the Santa Cruz piped water system, the community superchlorinated the system (cleansed the tank and pipes with chlorine) and this successfully brought the risk level to "low". In Aguacate, the piped water remained safe to drink with low-risk levels. Hand-pump and shallow-well samples tended to be quite variable, with the status changing in both directions from moderate to low risk and vice versa and in two cases from high to low risk. The variability in these shallow groundwater sources suggests that complex groundwater dynamics may be in play; a factor not well accounted for by our methodology.

Table 5. Summary of *E. coli* contamination results in drinking water, rivers, and streams. Associated risk levels (see Table 3) for disease transmission from drinking each water source are shown in the middle four columns. L, M, H, and VH stand for Low, Medium, High, and Very High risk levels respectively.

Village	Drinking Water			Risk				River/Stream Water		
	# Locations	*E. coli* Present	Average Colonies (#)	L	M	H	VH	# Locations	*E. coli* Present	Average Colonies (#)
Santa Cruz	26	12	1.24	14	2	10	0	24	22	7.4
Jalacté	21	13	1.98	8	4	8	1	9	9	17.11
Aguacate	22	11	0.55	11	6	5	0	12	12	5.29

3.5. Social Factors

The poor quality of drinking water in rural Toledo is a long-standing concern. In one of the earliest extant colonial reports mentioning water quality, from 1894, Assistant Colonial Surgeon Frederic Keys reported that "the general health" of people in southern Belize "has not been on the whole satisfactory", placing blame on unsanitary water [9]. During the colonial era, rural Belize had essentially no improved water systems. The Colonial Report for British Honduras in 1936 makes no mention of any improved water systems in rural Belize and characterizes methods of disposing of human waste as "extremely faulty" [58]. Our interview data and a systematic review of the literature (including anthropological studies) suggest that very little progress was made to address water quality prior to Belize's political independence in September 1981. The cultural characteristics of the Q'eqchi' and Mopan Maya are the object of a vast anthropological literature, including numerous ethnographic descriptions of customary land use in southern Belize (see, e.g., [26,36,40,41,43,59,60]). Much of this literature analyses the distinctive livelihood practices employed by Maya communities. This anthropological literature has given little attention to the social factors determining access to clean drinking water. In this section, we draw upon this literature, as well as archival research and our interview data. At the time of Belize´s independence (and as is the case now), the mortality rate from water-borne illnesses in rural Toledo was substantially higher than the rest of the country. One study [61] reported that "In 1980, the mortality rate was estimated to be 9.5/1000 for Toledo and 5.5/1000 for the country as a whole" (p. 3) and cited an unpublished 1977 study of five Maya communities that found that "91% of the 812 persons from which stool specimens were obtained had at least one intestinal parasite" (p. 3). The poor quality of water provision in rural Toledo, therefore, has long been recognized as an important social problem.

Our interview subjects and the record show that efforts to improve access to clean water in rural Toledo have unfolded in two broad phases. In the first phase (ca 1985–1998), several externally-funded programs built latrines and hand pumps in rural Maya communities. Whereas in 1984, there were no RWSs in the Maya communities of southern Belize and only "one hand-pump for every 296 persons" (p. 4), by 1996, 36 villages were drawing water from 189 hand pumps ([61,62]). To improve efficiency of installation and repairs, the Government of Belize selected a standard hand pump model, the India

Mark II. Where they were installed, the India Mark II hand pumps superseded collection of water from streams, natural springs, and hand-dug wells; where rudimentary water supply systems were installed (as in Aguacate and Santa Cruz) they have fallen into disuse, except when the system breaks. Literature on the development of improved water systems in the Moho watershed is limited, but one consultant's report ([63], written ca 1989 for United Nations International Children's Emergency Fund (UNICEF), US Agency for International Development (USAID) and the Government of Belize (GoB)) on the state of rural water supply documented "widespread lack of access to potable water and [. . .] sanitary waste disposal [. . .] the most important factors in the high rate of infant mortality in the area" (p. 2). The study noted that Jalacté's water supply (river and springs) was too limited to meet demand and was in "poor" sanitary condition (p. 63). There were no wells in Jalacté at that time. Santa Cruz had eight wells and sanitary conditions were rated "OK." The report does not give details on the basis for these judgments. The first RWS in a rural Maya community was installed in San Antonio in 1996. After the 1998 election of the People's United Party a raft of new RWSs were constructed in rural Toledo.

In the second phase of efforts to improve access to clean water in rural Toledo (ca 1998–present) numerous Maya communities received RWSs which use a fuel-driven engine to pump water from an aquifer into a concrete storage tank. The systems were installed by the Belize Government with financing from the USAID, the Inter-American Development Bank, and UNICEF. They are managed and maintained by Village Water Boards (VWBs), which generally lack the technical capacity to maintain the systems ([8], interviews). For instance, although RWSs are supposed to integrate chlorine via chlorine pump, the pumps are often broken or missing (interviews). The cost of maintaining the RWSs is prohibitive for these villages. Water use is neither generally metered, nor are water rates standardized by volume ([8], interviews). In 1992, an environmental organization (Belize Centre for Environmental Studies) "undertook a country-wide survey of institutions and businesses doing water testing to evaluate capability for comprehensive water quality analysis" ([64], p. 1). They found an urgent need for a water lab and training. "No laboratory in Belize checks enough parameters to determine the true quality of the water. The three most active, best equipped government [. . .] laboratories in the country [. . .] that do the most extensive water testing are the Water and Sewage Authority (WASA), Public Health Bureau (PHB), and the Fisheries Department (FIS)" (p. 6). While water quality testing facilities have improved modestly in Belize, testing in rural Toledo—a responsibility of the Ministry of Health—remains irregular and limited. The VWBs report that they are often unaware of whether the Ministry tests water [interviews]. When a RWS is known to be contaminated, VWBs are responsible for sanitation by "hyperchlorination", but typically lack access to the chemicals and necessary training to execute the task.

4. Discussion

Taken together, our results provide evidence for plausible causal linkages between land-use change to routine cultivation/grazing, increased surface-water runoff, and microbial contamination of surface water that are used by study communities for drinking and other uses. We identified numerous potential sources of microbial contamination on the landscape, including pit latrines and the persistent presence of pigs and other mammalian livestock. These sources of fecal contamination (and the disease-causing microbes they may carry) are likely to be introduced into surface water via runoff, and into groundwater from pit latrines at any time of year and from livestock feces through infiltration following rain events. We found no evidence to support the assertion that land-use change caused increased *E. coli* contamination of shallow groundwater sources. Non-significant differences in *E. coli* counts from groundwater sources affected by traditional and non-traditional land use suggest that the transition from one to the other does not automatically translate to increased rates of groundwater contamination. Rather, groundwater contamination appeared to be widespread regardless of land cover. Because many drinking water sources derive from groundwater, human water users in the study communities were likely to be consistently exposed to health risks from unclean drinking water.

Even in the communities with RWSs, our data suggest that, where systems are not routinely treated with chlorine, piped water posed some risk to human health. It is important to note that our study focused on water at the source; contamination in the process of transporting and storing it prior to use (i.e., [29,65]) was not studied.

Previous work suggests possible directions for future efforts to refine our understanding of contaminant sources and factors driving high temporal variability in the *E. coli* signal. For instance, previous studies of microbial contamination of surface water in rural communities found that livestock feces are often the dominant source of contamination in these settings [66,67]. Recent advances in microbial source tracking (reviewed in [68]) that allow for attribution of bacterial sources to humans versus livestock could allow the primary source(s) of contamination within the study communities to be identified and mitigated. The temporal inconsistency in *E. coli* counts observed in our study may result from inconsistent management of water systems, climatic factors, or fine particulate matter in water samples. Wright [69] showed that fecal indicator bacteria in rural communities became concentrated through the dry months and peaked during the transitional period from dry to wet season when fecal matter on the land surface presumably was mobilized by overland flows and infiltrated to shallow groundwater. Seasonal aspects of microbial contamination in southern Belize may follow a similar pattern. In addition, indicator bacteria such as *E. coli* are often associated with particulate matter (e.g., clays, organic fractions) in streams and rivers. Mechanical disturbance by high flow events leads to increased suspension of sediment-borne bacteria and increased bacterial densities in the water column, even in the absence of spatially proximate fecal inputs from humans or livestock [70,71]. Fine sediment was abundant on river beds near Aguacate and Jalacté (P.C.E. personal observation) suggesting this as another potential source of contamination for consumers of stream and river water.

The present conditions of water quality in the study communities are a consequence of multiple intersecting factors. We hypothesized that changing land use was the most important factor. We found that declining forest cover, particularly evident in the Moho watershed in eastern Guatemala, contributed to increased runoff and surface water contamination. The decreasing forest cover is not only a consequence of social and economic factors. Local informants emphasize the changes wrought by Hurricane Iris (October 2001). However, there was no correlation between land use and groundwater contamination as measured through well water. Whether under customary or non-customary land use, the landscape appears to have sufficient densities of latrines and domestic animals to cause persistent fecal contamination throughout the settled portions of the watershed. Thus, from a watershed perspective, forest clearing has evident consequences for runoff, but not for the presence or absence of fecal contamination of local water systems. This helps explain why Santa Cruz village, which draws less water from sections of the Moho watershed that originate in Guatemala, also has contaminated water.

Therefore, the most critical factor shaping the quality of drinking water in the villages is not land-use practices, as we had hypothesized, but the presence and maintenance of efficient and water storage and delivery systems. Poor water quality can result from the complexities and costs involved with maintaining the RWSs in Santa Cruz and Aguacate, and the absence of an improved water system in Jalacté. The construction of improved water systems in the early 2000s has not resolved the problem of clean water access. Our interviews suggested that the RWSs may be too complex and expensive for the VWBs to adequately maintain. This is consistent with three key findings of a 2007 study by the Interamerican Development Bank (IDB), the key points of which are quoted at length below ([8], p. 15).

- "The rural water sector in Belize lacks a clear strategy. The sector needs a strategy that sets out the Ministry's plan for expanding water services to underserved areas and for introducing sewerage and improved sanitation systems to larger villages. [. . .] The institutional framework governing the sector [. . .] lacks key policies and regulations [. . .]."
- "VWBs are not financially sustainable. Many of the VWBs are not able to recover the costs of providing service, and few, if any, are able to make the necessary capital expenditures. As a result,

VWBs are dependent on unreliable subsidies from the GoB. Their weak financial performance is due to several factors, including the fact that the tariffs are below the cost of providing service (Ministry of Labor, 2012), and there is no transparent structure that governs how water tariffs are set."

- "The governance structures of the VWBs are weak. The Village Councils Act 2003 does not ensure that members of the VWBs have adequate technical skills, nor does it have provisions to ensure that members actively participate and that the VWBs can meet quorum. It also fails to protect the selection of VWB members from political interference—members only serve three-year terms and can be removed at will."

The underlying causes of these indicators of inefficient and ineffective governance as described by the IDB are the poverty of the communities and low state capacity, two qualities that have defined the region since the colonial era [72]. This is not to say, however, that the challenge of providing safe drinking water systems is static. The social dynamics surrounding water provision will continue to evolve. These problems facing VWBs are likely to become more severe in the future as climate change causes greater unevenness of precipitation events [73], hence more frequent periods of flooding and drought—particularly worrying for those communities like Jalacté, where people are entirely dependent on stream and groundwater access.

The challenges of maintaining centralized water systems have led many to emphasize the importance of treatment at the point of use (i.e., [74,75]). Point-of-use treatment may also help protect consumers in the study communities until centralized water systems are managed to provide drinking water that is consistently free of contamination. In the interim, community members and advocates may consider cost-effective options for water treatment at the point of use including chemical disinfection (i.e., chlorine, iodine) [76], boiling [76], solar water disinfection (e.g., [77,78]), disinfection with low-power ultraviolet light emitting diodes [79], fiber membranes [80], or filter media [76].

While specific management recommendations are beyond the scope of this paper, there are at least four urgent areas for further research. First, deeper investigation into the relationships between poverty and state capacity and water management may lead to obvious pathways to safer water provisioning. Second, microbial source tracking to identify the primary originators of fecal contamination on the landscape (i.e., livestock vs. humans) could help communities focus on management responses to abate the same. Third, an improved understanding of landscape hydrology as it relates to land cover and geology could yield insights into potential sources of improved groundwater quality i.e., (deep confined aquifers) and provide information about the role of forests and seasonality. Linking groundwater observations to mechanistic landscape hydrology models (i.e., [81]) could provide a means to link landscape-change information with groundwater dynamics. Finally, delivery of support services and training for the maintenance of RWSs, inclusive of water treatment, monitoring of contaminants, and business management skills, may be warranted in communities with central water systems.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/10/11/1678/s1, Table S1: List of LANDSAT images used in the analysis, Figure S1: Comparison of runoff ratio versus percent vegetation cover.

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