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Fecal indicator bacteria diversity and decay in an estuarine mangrove ecosystem of the Xuan Thuy National Park, Vietnam

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ABSTRACT

Mangroves are complex and dynamic ecosystems that are highly dependent on diverse microbial activities. In this study, laboratory experiments and field studies for fecal indicator bacteria (FIB) decay rates are carried out for the first time in the Xuan Thuy Mangrove Forest Reserve of Vietnam. Results show that there are significant differences in bacterial diversity in the water of mangrove areas that have been deforested compared to those which have been planted. The highest mean total coliform (TC) and *Escherichia coli* (EC) values were found in the natural mangroves (3,807 ± 2,922 and 964 ± 1133 CFU 100 ml^{-1}, respectively). The results indicated that the source of contamination and seasonal changes affect the abundance of fecal bacteria. These results were exceeding by far the safety guidelines for individual, non-commercial water supplies in most of the samples. In the planted mangrove sampling sites, the highest mean *Fecal streptococci* (FS) values of 1,520 ± 1,652 CFU 100 ml^{-1} were found. Microbial die-off rates were calculated over 5 days, and observed to be systematically higher for TC than for EC.

Key words: die-off rates, *E. coli*, FIB, mangrove

HIGHLIGHTS

- The microbial dynamics in aquatic systems have been studied.
- Worked on a range of aquatic ecosystems, most recently on the Red River estuary.
- Experience in this area will be invaluable in understanding the ecological interactions between nutrients, phytoplankton, and bacteria.
- Experienced in using models to combine hydrology with biogeochemistry to understand carbon cycles and transport.

INTRODUCTION

In general, anthropogenic activities, including human settlements, industrialization, agricultural, and aquacultural practices, contribute greatly to the degradation of water quality and safety (May *et al.* 2006). Fecal indicator bacteria (FIB) groups, such as total coliform (TC), *fecal coliform* (FC), and *Escherichia coli* (EC), are used commonly around the world as indicators of pathogen content to measure the health hazards in bathing and shellfish-harvesting waters (Thomann & Mueller 1987; Bordalo 1993; Sanders *et al.* 2005) and have been included in water quality standards in different parts of the world (European Union 75/44/EEC; E.C. 2006). However, little is known about the behavior of FIB in tropical environments, particularly in the mangrove forest, since most studies have been performed in temperate to cold locations. In particular, the potential microbiological risks associated with the consumption of contaminated aquaculture products in coastal systems lead to significant social and economic consequences (GESAMP 2001; Touron *et al.* 2007; Retnam *et al.* 2013).

Mangrove forests are situated at the boundary between land and sea in the subtropics and tropics (Feller *et al.* 2010). Some studies have reported that about one-third of mangroves around the world have already been lost over recent decades as a result of deforestation, urbanization, human settlements, agricultural, as well as aquacultural practices (Alongi 2002; Lotze *et al.* 2006; Feller *et al.* 2010; Peixoto *et al.* 2011; Van Lavieren *et al.* 2012; Suárez-Abelenda *et al.* 2014; Kauffman *et al.* 2017). Microbial function and dynamics are a key element of the mangrove ecosystem and are directly responsible for the productivity of the mangrove ecosystem (Bashan *et al.* 2000; Holguin *et al.* 2001). More depth in our understanding of

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mangrove ecosystems is necessary to formulate effective management policies (Touron *et al.* 2007). Therefore, it is important to monitor the presence of waterborne pathogens in the coastal and mangrove environments, not just to protect the people from infectious diseases, but also to protect the developing aquaculture industry.

The fate of microorganisms is influenced by the following factors: physical and chemical characteristics (Curtis *et al.* 1992; Solic & Krstulovic 1992), livestock grazing (Hartke *et al.* 2002), sunlight, and factors of predation and competition (Cornax *et al.* 1990; Rozen & Belkin 2001; Soller *et al.* 2010; Wu *et al.* 2016). Source-specific FIB die-off rates were examined because FIB from different sources can have different die-off rates (Sinton *et al.* 2002). EC die-off rate is important in many engineering applications, including outfall design for sewage disposal, environmental planning, and impact assessment (Chen *et al.* 2013). Moreover, identifying the source of fecal contamination can allow for polluted water bodies to be remediated.

Recent studies have now focused on the effects of pollutants on the microbiota of the mangrove ecosystem (Zhou *et al.* 2008; Gomes *et al.* 2011). Identifying the source of fecal contamination is important because the health risk associated with microbial pollution in recreational waters depends on the source of pollution (Soller *et al.* 2010). In this study, the Xuan Thuy mangrove was investigated for its water quality (enumeration of TC, EC, and *Fecal streptococci* (FS)). Understanding and quantifying the growth potential of bacteria in water are essential for a holistic approach to microbial risk assessment. The work reported herein had the following three objectives: (1) to estimate the die-off rates of FIB (TC and EC) in seawater collected from different mangrove forests (a natural mangrove forest, deforested mangrove forests that have been destroyed 5 years ago, and planted mangrove forest of 3–7 years old), (2) to provide information about the concentration of FIB in different mangrove forests, and (3) to identify the source of fecal contamination. Towards this goal, we investigated the bacterial dynamics and diversity of bacteria in water samples from protected and harvesting areas in the Xuan Thuy mangrove. The outcomes of this study may serve as a basis for understanding how mangrove ecosystems function and how they are likely to respond to human-induced stresses such as excessive deforestation.

MATERIALS AND METHODS

Study site

The Xuan Thuy National Park is located in the Red River Delta, Northeast Vietnam and is a UNESCO biosphere reserve, typical of coastal ecosystems. The National Park was recognized as the first Ramsar site in Southeast Asia in 1989 and was designed as a national park in 2003. The coastal estuary mudflats are also a source of valuable seafood such as shrimp, crabs, fish, clams, and other species, as a well as migratory site for a number of birds, including some of the endangered species (black-faced spoonbill) that have been recorded in the IUCN Red Book. The National Park buffer zone is used for aquaculture and agricultural production, providing high income for local inhabitants.

The National Park is an estuarine wetland affected by tides, by water quality upstream of the Red River, and a number of sources of pollution in neighboring localities. The Red River is the main water source for the population in the North of Vietnam but also a large reservoir of wastewater from agricultural cultivation, urban waste, etc., reducing the amount of water available, especially in the Xuan Thuy mangrove forest. In addition, human activities in the buffer zone, such as agricultural cultivation, aquaculture, and fishing, have been causing significant impacts on natural ecological balance, as well as the quality of soil environment and regional water. Changing land-use patterns in the basin also lead to increased emissions of pollutants to the coastal environment (Tue *et al.* 2012).

Water sampling

Field sampling was conducted in the Xuan Thuy mangrove forest (Figure 1). The sampling frequency was carried out twice per year (rainy and dry seasons) during 2017 and 2018. A total of 11 sampling sites were selected in different locations in the three mangrove areas of the National Park: (1): sampling sites N1–N4 were chosen within the most developed and far from anthropogenic activity; (2) deforested mangrove forests that have been destroyed 5 years ago: sampling sites D1–D4 were characterized by aquaculture and rice farming activities; (3) planted mangrove forests of 3–7 years old: sampling sites P1–P3. 1,000 ml samplings were done at each of the selected sites and separately stored in sterile bottles. Then, they were immediately kept in an icebox until microbiological analyses were carried out within 4 h of collection. Temperature, pH, and total suspended solids (TSS) were measured *in situ* using a water quality probe WQC-22A (TOA, Japan), and conductivity (Cond) was determined using a conductivity meter (Hach, USA).

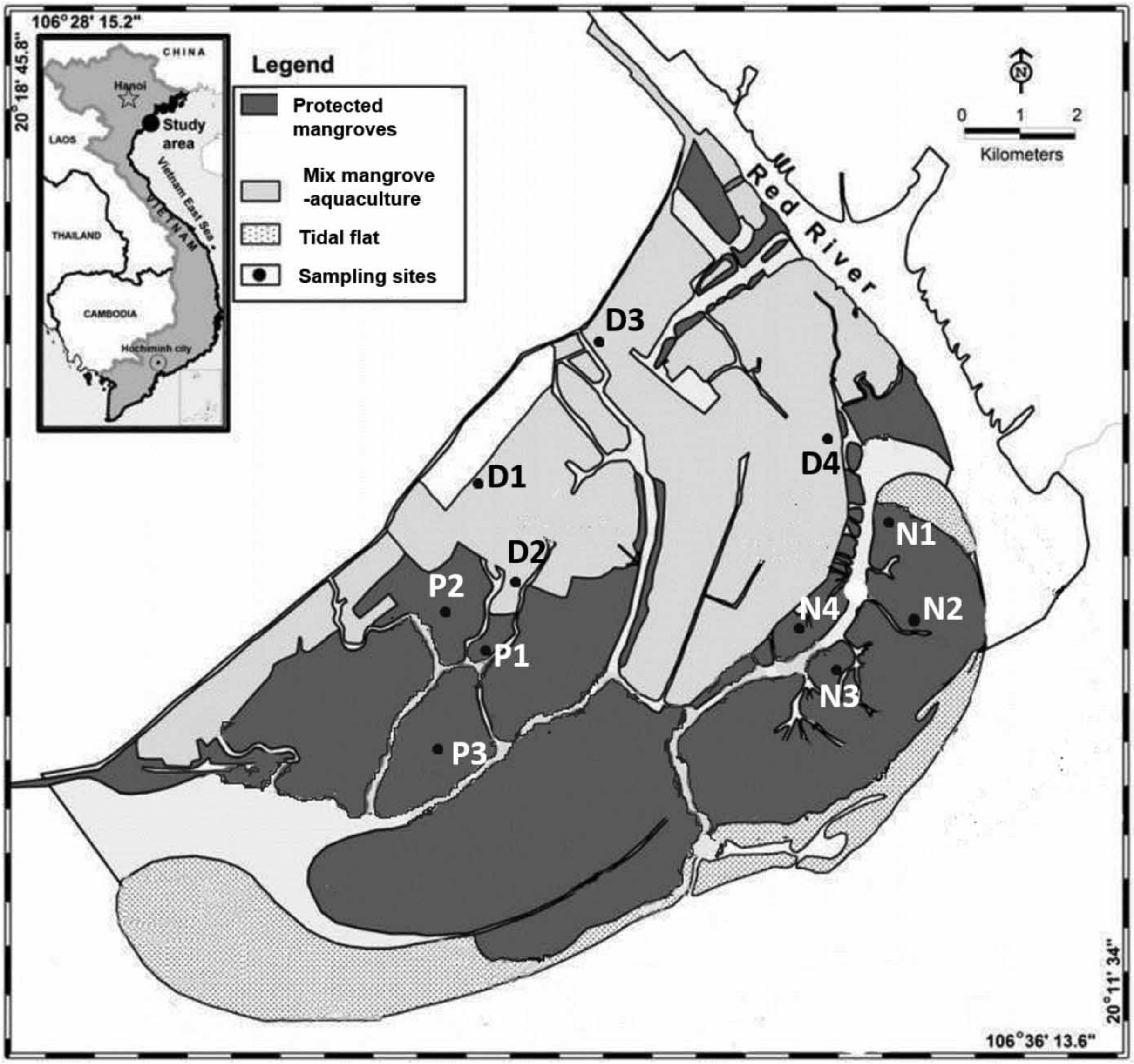

Figure 1 | Map of the Xuan Thuy National Park and the sampling sites. The cross-system sampling sites are assigned as N: natural mangroves; P: planted mangroves; D: deforested mangroves (modified from Tue *et al.* 2012).

Laboratory analysis

The bacteria were grown in different media for each type: EC and TC (3 M Petrifilm™ EC/Coliform Count Plate, USA), FC (C-EC Agar, Biolife, Italy), and FS (Slanetz–Bartley medium, Biocorp). For EC and TC, the plates were incubated at 37 °C for 24 h; for FC, the plates were incubated at 44.5 °C for 24 h; and for FS, the plates were incubated at 44 °C for 48 h.

The number of colonies (EC, FC, and TC) was determined using a Colony Counter CL-560 (Sibata, Japan). The number of bacteria was expressed as colony forming units per 100 ml (CFU 100 ml^{-1}). All experiments were carried out in three trials with subsequent statistical treatment.

Source of microbiological contamination

The estimation of the source of fecal contamination was determined by the FC/FS ratio (Geldreich 1976). It indicates contamination from human (FC/FS >4), domestic animal (FC/FS between 0.1 and 0.6), and wild animal (FC/FS <0.1) sources. The interval <4.0 to >0.7 was considered as a mixed contamination.

Die-off rates

At all stations, the second series of samples was collected in the same way for the determination of FIB die-off rates over time. For each station, 1,000 ml of sample were incubated in duplicates in glass bottles at *in situ* temperature and in the dark for 5 days. For the estimation of die-off rates, samples were collected from the incubations every day for 5 days ($T0$, $T1$, $T2$, $T3$, $T4$, $T5$) to determine the decrease in FIB numbers.

The die-off rates of TC and EC were estimated by fitting an exponential equation to bacterial abundances measured over time. The equations were expressed as first-order decay in the general form as:

$$C_t = C_0 e^{(-kt)}$$

where C_t is the number of bacteria at elapsed time t, C_0 is the initial number of bacteria, k is the decay rate constant (d^{-1}), and t is the elapsed time in days.

All the variables were determined using the biphasic exponential decay option in the SigmaPlot V13 (SPSS) software.

Statistical analyses

All of the other statistical analyses were performed with XLSTAT (v. 2014). Pearson's correlation was used to test the relationships between variables. Wilcoxon's non-parametric test was used to test for significant differences between variables, and the Kruskal–Wallis test was used to test differences between stations and seasons as the data were non-normally distributed even after normalization. When a significant difference was observed, a posteriori Dunn's all-pairwise test was used. Significance is determined as $p<0.05$.

RESULTS AND DISCUSSION

Physical parameters

The physical parameters determined on all water samples between sites are shown in Table 1. There was no significant difference in pH between the sites, although there was a significant difference between sampling seasons ($p<0.05$). Generally, the pH increases during the dry season and decreases during the rainy season, similar results for seasonal variations as a critical factor that affects pH were presented by Hanrahan *et al.* (2003). The highest pH value (7.6 ± 0.1) was observed in deforested mangrove sites, whereas the lowest was recorded from the natural mangrove sites (7.0 ± 0.2), similar to that reported previously due to the effects of deforestation (Bosire *et al.* 2003). The trends in salinity (Table 1) were more or less similar to that of the pH. The salinity was slightly higher in the deforested mangrove forest ($1.2 \pm 0.4\%$) compared to the natural mangrove forest ($0.9 \pm 0.4\%$). Salinity levels were significantly different between sites (Kruskal–Wallis, $p<0.05$).

The maximum measurements for conductivity (2.8 Sm^{-1}, Table 1) were recorded in deforested mangrove forests, whereas conductivity measurement was lowest in natural mangrove forests (0.8 Sm^{-1}, Table 1). High conductivity levels recorded in deforested mangrove forests can be attributed to human activities (Lang'at 2009; Thompson *et al.* 2012), especially in polluted waters from aquaculture farms due to an increase in the major ions (Afolabi *et al.* 2000; Obasohan & Agbonlahor 2010) because of the utilization of inorganic fertilizer. Furthermore, similar effects in tree canopy cover on these differences in physical characteristics were achieved in the work of Sjöling *et al.* (2005a, 2005b) and Emoyoma *et al.* (2020).

Table 1 | Ranges and mean values of physicochemical variables

	pH			**Salinity (%)**			**Conductivity (Sm^{-1})**		
Variables	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**
Deforested mangroves	7.1	7.7	7.6 ± 0.1	0.7	1.7	1.2 ± 0.4	1.3	2.8	2.1 ± 0.7
Planted mangroves	7.0	7.6	7.3 ± 0.3	0.5	1.5	1.0 ± 0.4	1.0	2.6	1.6 ± 0.6
Natural mangroves	7.0	7.3	7.0 ± 0.2	0.4	1.5	0.9 ± 0.4	0.8	2.6	1.6 ± 0.7

SD, standard deviation.

FIB abundance

Spatial and temporal variations of bacteria in different locations (natural, planted, and deforested mangrove forests) in the Xuan Thuy mangrove forest have been presented in Figures 2 and 3. The results showed that the observed values exceeded the recommended water quality standards for individual, non-commercial water supplies in Vietnam (20 EC CFU 100 ml^{-1} and 150 TC CFU 100 ml^{-1}) (MoH 2009). For example, the values (mean ± SD) of TC, EC, and FS were 3,350 ± 2,865; 711 ± 831; and 1,266 ± 1,268 CFU 100 ml^{-1}, respectively. The highest mean TC and EC values were found in the natural mangrove forest (3,807 ± 2,922 and 964 ± 1,133 CFU 100 ml^{-1}, respectively), whereas a minimum was recorded in deforested mangrove forest (1,886 ± 1,754 and 314 ± 227 CFU 100 ml^{-1}, respectively) (Figure 2). In contrast with the planted mangrove sampling sites, the highest mean FS values of 1,520 ± 1,652 CFU 100 ml^{-1} was found, whereas minimum values were observed in deforested mangrove forest with 1,000 ± 661 CFU 100 ml^{-1} (Figure 2). Additionally, there was a negative correlation between physicochemical parameters and the level of fecal bacteria, especially with the salinity parameters (Table 1). A similar study was conducted on the Persian Gulf coastal (Iran) by Karbasdehi *et al.* (2017) showed that indicator bacteria density increases with a decrease in salinity. From the results of the present study, it can be seen that in deforested mangrove forests, the presence of FIB is the lowest in the studied sites. The most FIB contaminated sample was taken from natural mangrove forests in this study, as an eco-tourism forest circuit (Thanh & Yabar 2015). A comparable survey from seashore waters in Zanzibar (Tanzania) reported the occurrence of FIB exceeding guidelines in a touristic site (Moynihan *et al.* 2012).

The fecal indicator density ranges (100–9,700 CFU 100 ml^{-1}; 0–3,400 CFU 100 ml^{-1}; and <1–4,400 CFU 100 ml^{-1} for TC, EC, and FS, respectively) are similar to other data. For example, the range abundances of TC and EC were 600–2,200 and 79–3,300 CFU 100 ml^{-1}, respectively, in the Persian Gulf (Iran) (Karbasdehi *et al.* 2017), on the Georgian coast of the Black Sea (590–2,900 and 470–1,000 CFU 100 ml^{-1}, respectively) (Janelidze *et al.* 2011). On the contrary, the fecal indicator density reported in this study was lower compared to a study in the Güllük Bay, Aegean Sea (Turkey) that reported a range of 30–2,160,000 CFU 100 ml^{-1}; 1–14,600 CFU 100 ml^{-1}; and 1–21,100 CFU 100 ml^{-1} for TC, EC, and FS, respectively (Kalkan & Altug 2015).

In the Xuan Thuy mangrove forest, the results showed no significant difference between sampling locations for TC, EC, and FS density (Kruskal–Wallis test, $p > 0.05$). In addition, while EC did not display any seasonal pattern, TC showed significant seasonal variation (Kruskal–Wallis test, $p < 0.05$) (Figure 3). In the entire Xuan Thuy mangrove forest, the lowest mean TC density was 2,715 ± 2,776 CFU 100 ml^{-1} during the rainy season, whereas the highest mean was recorded during the dry season with 5,256 ± 2,329 CFU 100 ml^{-1} (Figure 3(a)). Moreover, this pattern was similar in all other regions. For example, the lowest mean TC density of 1,340 ± 1,397 CFU 100 ml^{-1} during the rainy season, whereas the highest mean during the dry season of 3,250 ± 2,333 CFU 100 ml^{-1} in the deforested mangrove forest (Figure 3(b)). Similar results were observed in planted mangrove forests, with the lowest mean TC density of 1,450 ± 1,398 CFU 100 ml^{-1} in the rainy season and the highest

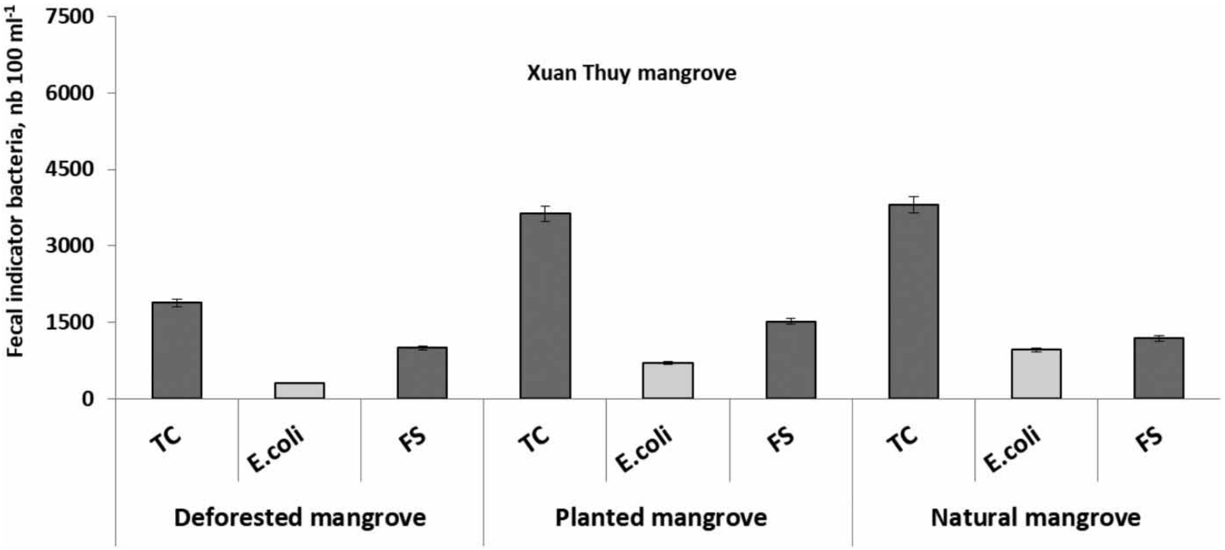

Figure 2 | Spatial variation of bacteria in different locations in the Xuan Thuy mangrove forest.

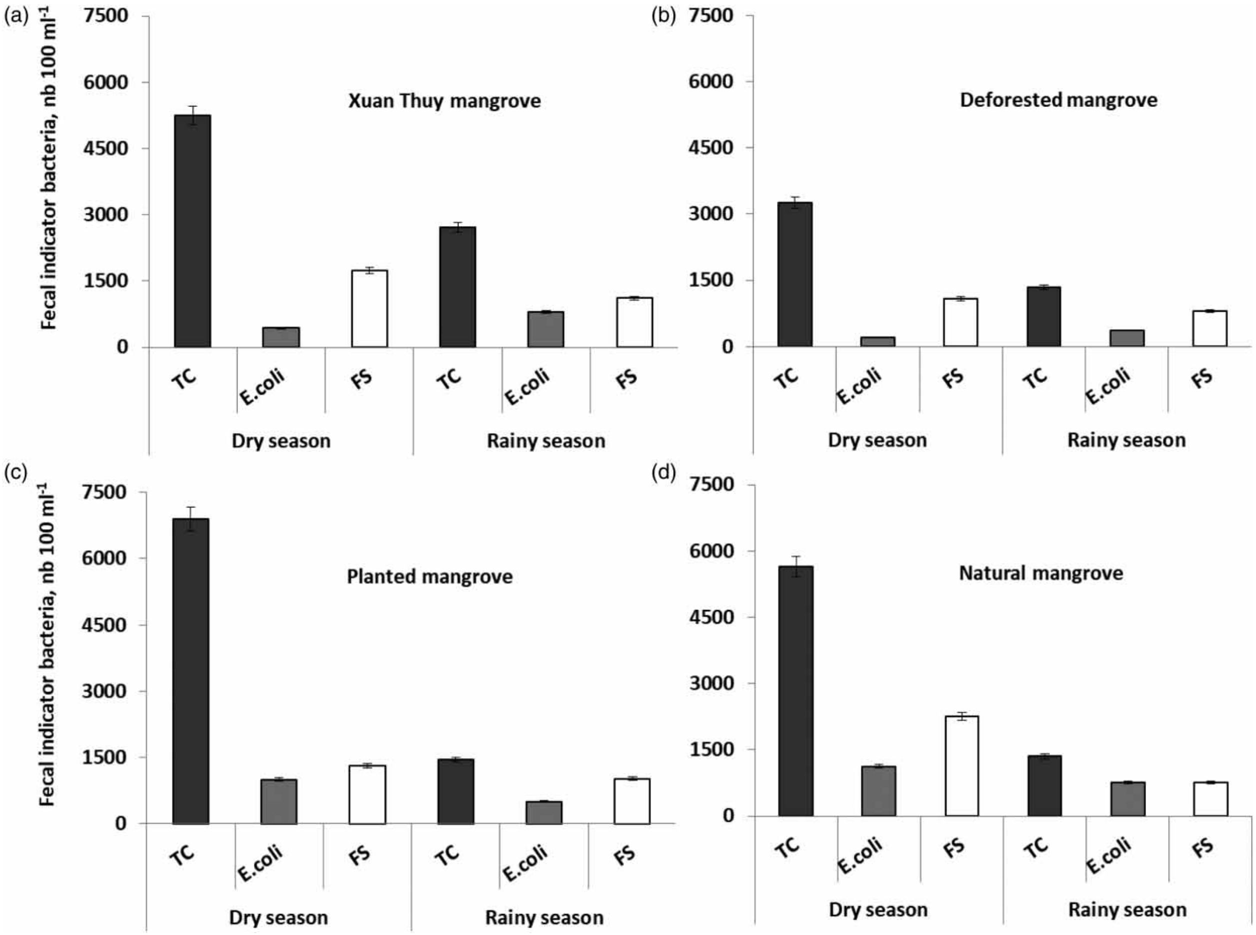

Figure 3 | Temporal variations of bacteria density during the sampling period in the Xuan Thuy mangrove forest.

mean of 6,900 ± 1,687 CFU 100 ml^{-1} in the dry season (Figure 3(c)), and the mean TC was higher during the dry season (5,650 ± 2,096 CFU 100 ml^{-1}) compared to the rainy season (1,542 ± 1,835 CFU 100 ml^{-1}) in natural mangrove forest (Figure 3(d)). This may be influenced by increased precipitation, where rain water dilutes the water system and disperses microbiological contamination (Cunha *et al.* 2005; Azalea *et al.* 2010; Park *et al.* 2016). This result was in contrast to those reported by Grisi & Gorlach-Lira (2010), Fan *et al.* (2015), and Barbirato *et al.* (2016), which suggest that the density of bacteria was higher in the rainy season than in the dry season. Accordingly, the increase of bacterial density in the rainy season can be attributed to the increasing flow of wastewater, then the bacteria were washed away from the soil and sand into the coastal waters (Krometis *et al.* 2007; Pronk *et al.* 2006, 2007). Therefore, depending on the hydrodynamic characteristics, dispersed bacteria in the aquatic environment leads to different FIB levels that may reflect the seasonal dependence of the FIB in the water. The abundance of coliform bacteria in the study sites demonstrate a level of bacterial pollution of public health concern. Therefore, people who use contaminated water for consumption or recreation are at high risk of waterborne diseases since FIB is closely linked to waterborne microbial diseases (Djuikom *et al.* 2006; Bastaraud *et al.* 2020).

The FC/FS ratio

The use of FC/FS ratio was first suggested by Geldreich *et al.* (1964). In recent years, this ratio has been used to indicate the common source of fecal contamination in water (Geldreich & Kenner 1969; Feachem 1975; Howell *et al.* 1996; Csuros & Csuros 1999; Sankararamakrishnan & Guo 2005). The data show that the FC/FS ratio >4 is a manifestation of fecal bacteria that is derived mainly from human waste. Values <0.7 suggest fecal contamination from others. The FC/FS ratio 0.7–4.0 points to pollution from humans and animals (mixed).

The values of the FC/FS ratio recorded in this study are a result of various activities (humans and animals) in the region (Table 2). For example, water sampled from deforested mangroves with an average FC/FS ratio of <0.7 (0.3 ± 0.3 and 0.3 ± 0.04 for the dry and rainy seasons, respectively) showed fecal contamination from non-human sources (Table 2). Similar results were found in the rainy season, the values of FC/FS ratio from planted and natural mangroves were 0.03 ± 0.05 and 0.3 ± 0.3, respectively, these values <0.7 also reflected the fecal contamination from other than human sources (Table 2). Whereas during the dry season, the majority (70%) of the samples from planted and natural mangroves had FC/FS values ranging from 0.7 to 4.0, which was an indication of mixed contamination (Table 2).

The highest FC/FS ratio (4.5) was recorded in planted mangrove forests in the dry season which is an indication of human contamination, possibly because of the defecation of farmers along the shoreline from time to time. On the contrary, in the rainy season, the only obvious source of fecal contamination in this site was wildlife (it had FC/FS values of <0.1) (Coyne & Howell 1994), as number of bird species in Xuan Thuy Ramsar sites was more than three-fold compared with other Ramsar sites–there were up to 40,000 migratory birds staying in mangrove forest during migration (AWCF 2018; Nguyen *et al.* 2021), and this region was a typical conservation area of wetland ecosystems with many rare wildlife.

Our results show that, during both seasons in deforested mangrove forests, the source of fecal contamination comes from livestock waste and food processing (aquatic products such as fish and shellfish produced). Likewise, it was previously reported that the significant bacterial contamination exceeding safety levels was described from the fisheries (Selvam *et al.* 2020) and shellfish (Mok *et al.* 2018).

Die-off rates of TCs and EC

The die-off rates of TC and EC were calculated over 5 days. Our TC and EC die-off rates ranged from a minimum of 0.17 d^{-1} to a maximum of 0.87 d^{-1} for EC and from 0.17 to 1.13 d^{-1} for TC, with a mean of 0.87 ± 0.19 d^{-1} for EC and 1.13 ± 0.31 d^{-1} for TC. Similar to the values reported for sub-tropical systems, for example, in the coastal water around Hong Kong, Chan *et al.* (2015) also found values of between 0.85 and 1.50 d^{-1} for TC die-off rates, and Menon *et al.* (2003) reported values ranging from 0.11 to 0.66 d^{-1} in the Belgian coastal waters.

We observed systematically higher die-off rates for TC than for EC (Table 3). For example, TC die-off rates ranged from 0.45 to 0.89 d^{-1} with a mean (± SE) of 0.58 ± 0.20 d^{-1} higher than EC die-off rates between 0.17 and 0.58 d^{-1} with a mean (± SE) of 0.37 ± 0.17 d^{-1} at deforested mangrove forests; TC die-off rates ranged from 0.49 to 1.09 d^{-1} with a mean (± SE) of 0.47 ± 0.32 d^{-1} higher than EC die-off rates between 0.35 and 0.75 d^{-1} with a mean (± SE) of 0.46 ± 0.13 d^{-1} at planted mangrove forests; TC die-off rates ranged from 0.17 to 1.13 d^{-1} with a mean (± SE) of 0.57 ± 0.26 d^{-1} higher than EC die-off rates between 0.17 and 0.87 d^{-1} with a mean (± SE) of 0.47 ± 0.24 d^{-1} at natural mangrove forests (all pairwise comparisons

Table 2 | Origin of bacteriological pollution

	Deforested mangroves		**Planted mangroves**		**Natural mangroves**	
Site FC/FS ratio	**Dry**	**Rainy**	**Dry**	**Rainy**	**Dry**	**Rainy**
Min–Max	0–0.7	0.2–0.3	0.2–4.5	0–0.1	0–3.6	0.1–0.7
Mean ± SD	0.3 ± 0.3	0.3 ± 0.04	1.1 ± 1.5	0.03 ± 0.05	1.3 ± 1.2	0.3 ± 0.3
Origin	Non-human	Non-human	Mixed	Non-human	Mixed	Non-human

SD, standard deviation.

Table 3 | Average (± SE) die-off rates for TC and EC (k, d^{-1}) in the Xuan Thuy mangrove forest

	Deforested mangrove		**Planted mangroves**		**Natural mangroves**	
Decay rate (d^{-1})	**Ave**	**SE**	**Ave**	**SE**	**Ave**	**SE**
TC	0.58	0.20	0.47	0.32	0.57	0.26
EC	0.37	0.17	0.46	0.13	0.47	0.24

The values were calculated for the first 4 days of the incubation.

$p < 0.05$, Wilcoxon test). This is consistent with reports on EC as having higher survival rates compared to other bacteria (Carillo *et al.* 1985; Jiminez *et al.* 1989; Winfield & Groisman 2003).

It has generally been accepted that FIB do not survive for very long in seawater. In this study, we also observed a rapid decrease in EC and TC in both seasons over the 5-day incubation. However, FIB decline was less abrupt in the rainy season, whereas in the dry season, the differences decreased sharply (Figure 4). The mean T90 (the time taken for 90% of organisms to die) for TCs and EC was about 90 and 120 h, respectively. Our diurnal T90 values are close to those reported by other studies, for example, Mattioli *et al.* (2017), working in Half Moon Bay (USA), observed the mean T90 of 96 h for EC,

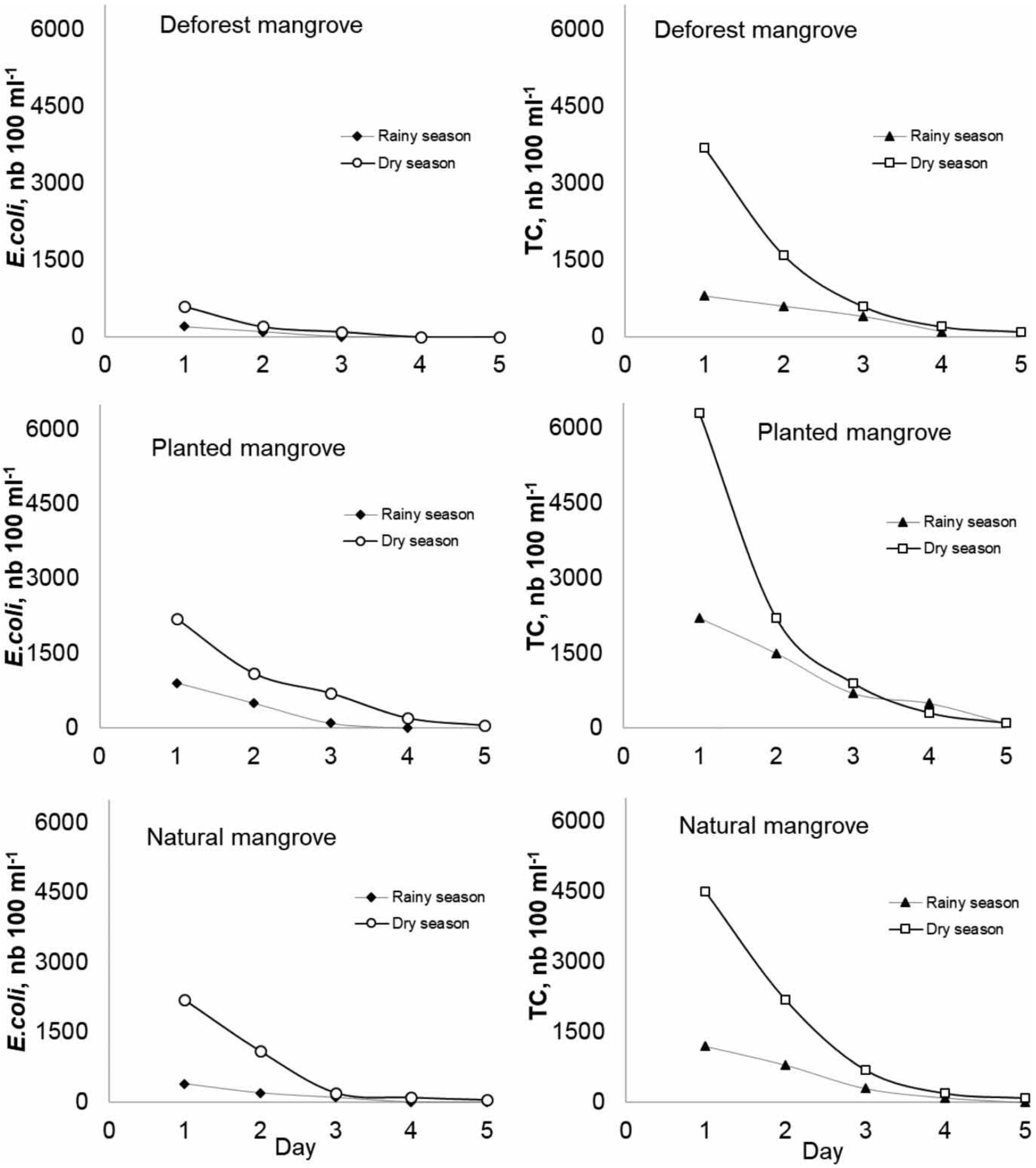

Figure 4 | Effect of time (days) on the abundance of bacteria in different locations in the mangrove forest.

and Troussellier *et al.* (2004) also reported the apparent good survival of FIB in Senegal River estuary (African coast) waters with a long residence time (96–120 h). However, the die-off rate was slower in our study than in other studies such as Rodrigues *et al.* (2011) (T90: 12–13.2 h), Schumacher (2003) (T90: 42 h), and Yalcin & Muhammetoglu (2011) (T90: 17–88 h). It is likely that lower salinity in our study contributed to this difference, their data showed a 10- to 40-fold higher level of salinity. In addition, pH is generally believed to be the major factor controlling bacterial die-off rates (Davies *et al.* 1995), and our results also show lower pH values than their data.

CONCLUSIONS

In this study, we assessed the abundance of FIB in different mangrove environments in the Xuan Thuy Mangrove Forest and evaluated the influence of warm-blooded animals, humans, and livestock on the fecal contamination of water in mangrove forests. The results indicated that the source of contamination and seasonal changes affect the abundance of fecal bacteria. The highest mean TC and EC values were found in the natural mangrove forests, whereas minimum values were observed in deforested mangrove forests. Overall, the bacterial density in the dry season was higher than in the rainy season in the entire Xuan Thuy mangrove forest. This may be influenced by increased precipitation, where rainwater dilutes the water system and disperses microbiological contamination. In addition, many pollution sources were identified in this study. The majority of the samples (70%) from planted and natural mangroves had FC/FS values ranging from 0.7 to 4.0, indicating mixed contamination (eco-tourism forest circuit, farmer defecation, and migratory birds staying). In contrast, the source of fecal contamination in deforested mangrove forests comes from livestock waste and food processing. We confirmed that all sites had fecal coliform concentrations exceeding the regulation limit for individual, non-commercial water supplies. This poses a danger for people using contaminated water for consumption or recreation who are at high risk of waterborne diseases since FIB is closely linked to waterborne microbial diseases. The study area deserves further monitoring and risk assessment.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict.

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