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APPLIED RESEARCH

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Dynamics of soil properties and pathogen levels in Pacific white shrimp ponds during a production cycle: Implications for aquaculture management

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Abstract

This study examines the temporal and spatial variations in soil properties and microbial populations in Pacific white shrimp ponds throughout a production cycle in Phuket Province, southern Thailand, aiming to refine shrimp farming methodologies and enhance pond soil management. We collected soil samples from four ponds across two aquaculture sites at six different stages of the production cycle—Before Sediment Flushing (BSF), After Sediment Flushing (ASF), and during each month of the four-month cycle (M1 to M4). These samples were analyzed from both central and peripheral pond zones at three soil depths (0–5 cm, 5–10 cm, and 10–15 cm). The results indicated negligible variation in soil characteristics and microbial loads across all stages. Nevertheless, a significant finding was the fluctuation in levels of easily decomposable organic matter (EDOM), which is critical for maintaining soil and water quality and affects both shrimp growth and disease incidence. EDOM levels decreased to their lowest after ASF, then progressively increased, reaching a peak at M4 ($p < 0.05$). The study suggests that effective sediment flushing post-cultivation not

only mitigates the accumulation of deleterious residues, but also reduces the necessity for prolonged pond desiccation, thereby offering a sustainable strategy to maintain the ecological balance of shrimp ponds over successive farming cycles.

KEYWORDS
Litopenaeus vannamei, Pacific white shrimp, pond bottom soil, shrimp pond

1 | INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, often termed the whiteleg shrimp, is integral to global aquaculture and was the predominant species cultivated in 2022, constituting 89.6% of all marine and coastal-cultured crustaceans with a production yield of 6.8 million tons (FAO, 2024). Introduced in Thailand as a more disease-resistant alternative to the black tiger shrimp (*Penaeus monodon*), *L. vannamei* is renowned for its rapid growth rate and efficient cultivation. However, the cultivation of Vannamei shrimp in Thailand faces significant challenges because of a range of diseases that mirror those previously seen in black tiger shrimp. These diseases stem from a variety of pathogens, including viruses such as yellow head virus (YHV) (Klongklaew et al., 2021), white spot syndrome virus (WSSV) (Zhang et al., 2023), infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Hou et al., 2023), runt-deformity syndrome virus (RDS) (Yang et al., 2022), infectious myonecrosis virus (IMNV) (Wan et al., 2023), Taura syndrome virus (TSV) (Sappat et al., 2011), bacteria like acute hepatopancreatic necrosis disease (AHPND or EMS) caused by *Vibrio parahaemolyticus* (AHPND) (Boonyawiwat et al., 2017; Kumar et al., 2021), parasite like *Enterocytozoon hepatopenaei* (EHP) (Liu et al., 2018; Tangprasittipap et al., 2013), or a combination of these factors, such as white feces syndrome (WFS) caused by EHP, *Vibrio*, fungi, gregarine, etc. (Kumar et al., 2022). Factors such as suboptimal broodstock quality, inadequate farm management practices, and environmental deterioration within the pond ecosystems exacerbate these pathogenic challenges (Boyd & Phu, 2018; Macusi et al., 2022).

In earthen pond aquaculture, the bottom soil plays a crucial role as a reservoir for organic detritus, residual feed, and other waste products, which significantly influence both the health and functionality of the pond system (Hasibuan et al., 2023; Saraswathy et al., 2019; Shaughnessy et al., 2019). Maintaining water quality directly depends on the interaction between the bottom soil and the overlying water (Avnimelech & Ritvo, 2003; Boyd, 1995; Mahajan & Billore, 2014; Muralidhar et al., 2018; Prihutomo et al., 2016; Shafi et al., 2022; Wiyoto et al., 2016). Nonetheless, poor management practices, such as inadequate pond preparation, excessive feeding, and inefficient sediment removal, can lead to oxygen deprivation and the promotion of anaerobic conditions. These conditions are conducive to the formation of harmful reduced chemical compounds and the accumulation of nutrient-rich waste (Burducea et al., 2022; Kumararaja et al., 2018). Such developments not only degrade water quality but also encourage the proliferation of phytoplankton and bacterial pathogens, which result in stunted shrimp growth and elevated disease incidence, thus compromising the sustainability and productivity of aquaculture operations (Barik et al., 2018; Crane, 2019; Joyni et al., 2011).

This research examines the temporal and spatial variations in soil properties, bacterial populations, and pathogen concentrations across six distinct phases within a single Vannamei shrimp production cycle. These phases include pre- and post-sediment flushing (post-harvest and post-cleaning), as well as monthly assessments over a four-month period. By investigating these variations, accumulations, and distributions of different substances, the study seeks to illuminate the dynamics of shrimp farm management practices. The findings are expected to provide pragmatic strategies for enhancing shrimp farming operations, particularly through meticulous management of organic matter

residues and other critical substances in the pond environment. This careful regulation is intended to maintain these substances at beneficial levels, preventing harmful accumulations. Ultimately, this research strives to promote sustainable practices in shrimp farming and advance the understanding of ecological management in earthen aquaculture ponds, an area where significant knowledge gaps still exist.

2 | MATERIALS AND METHODS

2.1 | Area of study

We collected bottom soil samples from four different Pacific white shrimp ponds located within two aquaculture facilities in Phuket Province, Thailand. The initial facility, operating as a commercial shrimp farm, boasted two rectangular ponds, each encompassing an area of 4800 m^2. The Phuket Coastal Aquaculture Research and Development Center, the second facility, featured two square ponds, each measuring 800 m^2 (Figures 1 and 2).

2.2 | Shrimp culture management

In this study, Pacific white shrimp postlarvae were stocked at varying densities to compare high-density and low-density culture systems. The high-density culture, typical of commercial shrimp farms, used a stocking density of 167 postlarvae per square meter. By contrast, the Department of Fisheries (DOF) experimental ponds, representing low-density culture, were stocked at 50 postlarvae per square meter. This setup highlights a significant difference from ordinary commercial farms, where stocking densities generally exceed 100 postlarvae per square meter. The ponds underwent a drying process after cleaning, which took approximately two to three days; the commercial farm's ponds dried for 1 week, while the DOF's ponds dried for 2 weeks. The spring tide schedule primarily influenced this drying period at the commercial farm, which ranged from 0 to 15 days, before restocking the ponds with shrimp postlarvae.

Feeding protocols at the commercial farm involved four daily feedings supplemented with Artemia during the initial 1–40 days of shrimp cultivation management. The frequency of feedings increased to five times per day upon reaching 41 days of age. Starting at 1 month of age, we utilized feeding trays to adjust food quantities and monitor the shrimp's health. We initiated water changes when the shrimp reached 40 days, replacing 20 cm of water per pond each time. Subsequently, water changes were systematically conducted every 15 days throughout the rearing period to maintain optimal conditions. For shrimp harvesting, Department of Fisheries (DOF) ponds underwent a single harvest per cycle, whereas private shrimp farms implemented partial harvests.

2.3 | Pattern of pond bottom soil sample collection

Bottom soil samples were collected at six distinct stages during a Pacific white shrimp production cycle to assess variations in soil composition. These stages included:

1. Before sediment flushing (Immediately after shrimp harvest) (BSF)
2. After sediment flushing (Immediately after pond cleaning) (ASF)
3. The first month (M1)
4. The second month (M2)
5. The third month (M3)
6. The fourth month (M4) after stocking

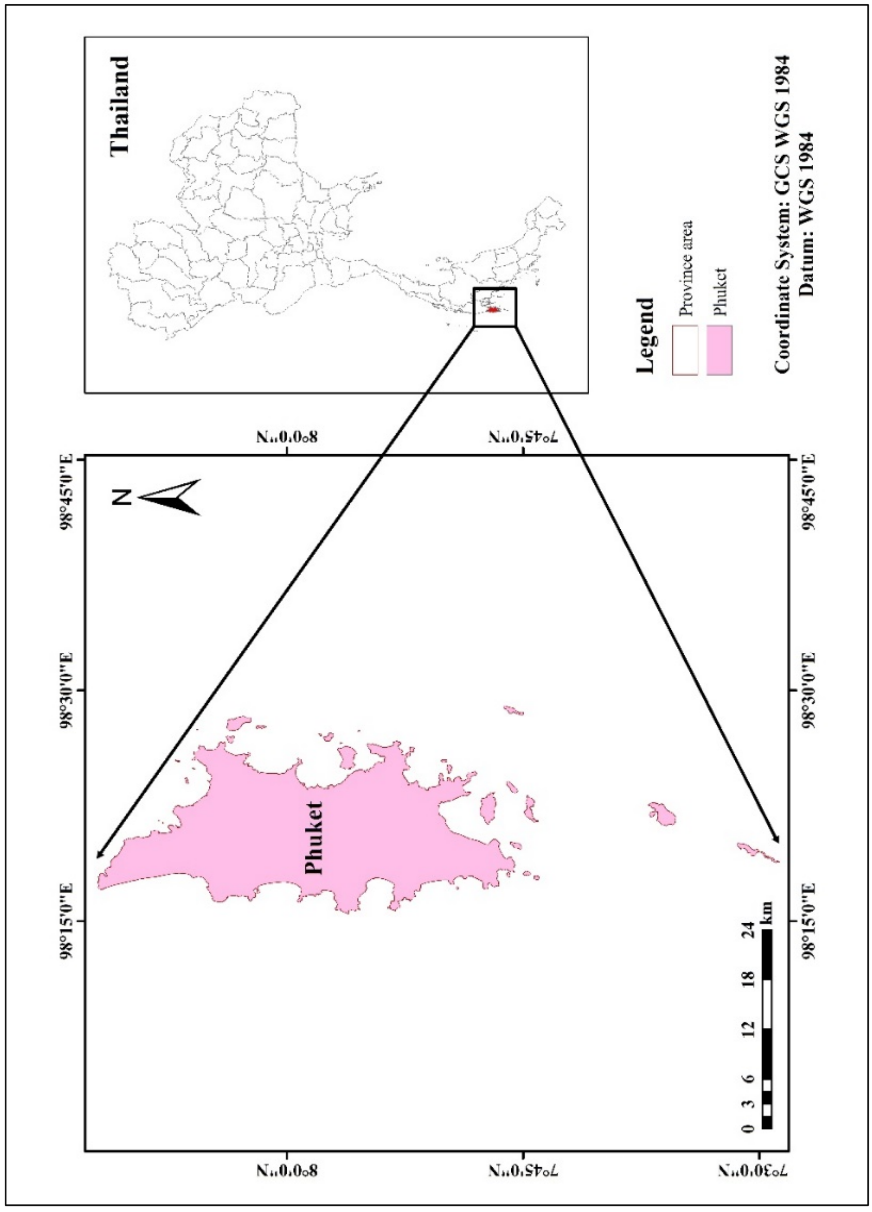

FIGURE 1 Map of Phuket Province, located in the Andaman region of southern Thailand.

FIGURE 2 Sediment being flushed from a commercial Pacific white shrimp pond.

Soil samples were systematically collected from four separate Pacific white shrimp ponds using a hand-operated core sampler (Royal Eijkelkamp Company, The Netherlands, Cat. No. 04.15.SA) with a 5-cm diameter. Sampling was conducted in two distinct zones within each pond: Zone A at the center and Zone B along the periphery. At each depth level (0–5 cm, 5–10 cm, and 10–15 cm), samples were thoroughly extracted from a minimum of eight points around each pond to ensure thorough spatial representation. These individual samples were then combined to form a comprehensive composite sample for each depth and zone. Figures 2 and 3 depict the details of the centrally located drain system that equipped each of the four ponds.

2.4 | Soil preparation and analysis

Upon collection, wet soil samples were immediately sealed in polyethylene bags and chilled with ice to inhibit biochemical reactions such as oxidation and nitrification, which typically occur when submerged soils are exposed to air or dried. To ensure the accuracy of analytical results, it is crucial to promptly analyze the following bottom soil parameters upon arrival at the laboratory: sediment oxygen demand (SOD), ammonia-nitrogen, nitrite-nitrogen, total bacteria, total *Vibrio*, yellow and green colony-forming bacteria, *Vibrio harveyi*, *V. parahaemolyticus*, and *Enterocytozoon hepatopenaei* (EHP). This timely analysis is essential to prevent any alteration in the measured values because of the aforementioned reactions.

The parameters for soil and bacteria measured on a wet-weight basis were converted to a dry-weight basis using the soil moisture factor. This conversion involved multiplying the wet weight of the soil samples by the soil moisture

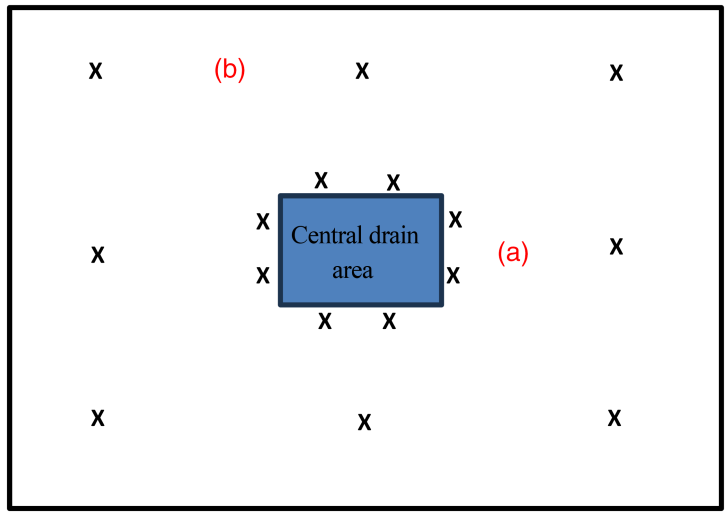

FIGURE 3 Bottom soil samples were collected from two zones in the shrimp ponds: (a) in the middle of the pond and (b) at the edge of the pond.

factor, as presented below. This adjustment is crucial for standardizing measurements across samples with varying moisture contents, ensuring comparability and accuracy in the analysis.

$$\text{Soil moisture factor} = \text{the weight of dry soil (g)} / \text{the weight of wet soil (g)},$$

where the weight of dry soil (g) is obtained by drying the wet soil at 105°C for 24 h.

Additional soil parameters, including organic matter, total carbon, total nitrogen, extractable phosphorus, exchangeable calcium (Ca), magnesium (Mg), and potassium (K), as well as extractable iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), soil pH, lime requirement, and soil texture, were quantified using dry soil samples. These samples were first air-dried at room temperature, finely ground using a wooden mortar, and then systematically sieved through ASTM E11 standard sieves with mesh sizes of No. 10 (2 mm), No. 35 (500 μm), and No. 120 (125 μm) to suit different analytical needs. Table 1 details the methods and references used to determine soil, bacteria, and parasite metrics.

The analysis of soil parameters was conducted within the laboratory of the Department of Fisheries Technology, situated in the Faculty of Agricultural Technology at Phuket Rajabhat University. Meanwhile, the assessment of bacterial and parasitic pathogens was carried out at the Aquatic Animal Disease Examination Unit, which is part of the Phuket Coastal Aquaculture Research and Development Center, under the purview of the DOF in Phuket, Thailand.

2.5 | Data analysis

The comprehensive dataset derived from various stages of a shrimp production cycle, including samples from multiple depths and zones within each stage, was subjected to a detailed statistical analysis. This analysis involved both one-way and two-way ANOVA tests to examine the differences among groups, supplemented by Duncan's new multiple range test as a Post hoc method to precisely identify these differences at a 95% confidence level. Prior to conducting the ANOVA, essential assumptions such as the normal distribution of data, equal variance across groups, homogeneity of variances, independence of samples, continuity of data, and random sampling were verified. For pathogen data, a natural logarithm (Ln) transformation was applied to normalize the distributions, ensuring the validity and reliability of the subsequent F-tests.

TABLE 1 Soil, bacteria, and parasitic pathogen parameters, methods, and references.

Soil, bacteria, and parasite parameters	Methods	References
1. Sediment oxygen demand (SOD)	Modified water BOD method	Department of Fisheries (2008)
2. Organic matter (OM)	Wet oxidation method (Walkley and Black)	Nelson and Sommers (1996)
3. Total carbon (TC)	Dry combustion	Nelson and Sommers (1996)
4. Total nitrogen (TN)	Kjeldahl method	Bremner (1996), Rutherford et al. (2008); Tan (2005)
5. Ammonia-nitrogen (NH_3-N)	3% NaCl extracting solution (Indophenol blue method)	Modified Chuan and Sugahara (1984), Maynard et al. (2008)
6. Nitrite-nitrogen (NO_2-N)	3% NaCl Extracting solution (Diazotization method)	Modified Chuan and Sugahara (1984), Strickland and Parsons (1972)
7. Extractable phosphorus	Bray II extracting solution	Tan (2005), Kuo (1996)
8. Exchangeable Ca, Mg, and K	NH_4OAc method	Tan (2005)
9. Extractable Fe, Mn, Cu, and Zn	DTPA method	Gambrell (1996), Loeppert and Inskeep (1996), Reed and Martens (1996); Tan (2005)
10. Soil pH	1:1 ratio of air-dried soil: water	Thomas (1996)
11. Lime requirement (LR)	Modified Adams-Evans method	Boyd (1995), Sims (1996)
12. Soil texture	Hydrometer method	Boyd (1995), Tan (2005); Kroetsch and Wang (2008)
13. Total bacteria	Spread plate method	Modified Steubing (1993), Maturin and Peeler (2001)
14. Total *Vibrio*, green and yellow colony-forming bacteria	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
15. *Vibrio harveyi*	Spread plate method	Modified Steubing (1993), Kaysner et al. (2004)
16. *V. parahaemolyticus* (AHPND/EMS)	Multiplex PCR	Modified Steubing (1993), Tinwongger et al. (2014)
17. *Enterocytozoon hepatopenaei* (EHP)	Real-time PCR (qPCR)	Liu et al. (2018)

Note: The Steubing (1993) method has been modified by incorporating a Vortex Mixer for 1 minute during soil extraction.

Multiple linear regression was performed to predict shrimp production using soil and pathogen data. Before running the model, the assumptions of multiple linear regression analysis must be checked as follows:

1. Model Errors: The errors should be normally distributed, independent, and have constant variance. This can be assessed by plotting the residuals against the estimated values of the dependent variable.
2. Linearity: The relationship between independent and dependent variables must be linear.
3. Outliers and Influential Observations: There should be no outliers or influential observations, which can be checked using Cook's distance.
4. No Multicollinearity: There should be no multicollinearity among the independent variables, which can be verified using the variance inflation factor (VIF).

Several methods were used for variable selection, including the coefficient of determination (R^2), Mallow's Cp statistic, and the Akaike Information Criterion (AIC). Additionally, sequential variable selection methods such as backward and stepwise selection were applied to identify the most predictive model (Kaps & Lamberson, 2009; Ramsey & Schafer, 2002).

All statistical procedures were performed using the SAS Education Analytical Suite 9.4, ensuring a high standard of accuracy and reliability in the findings.

3 | RESULTS AND DISCUSSION

3.1 | Shrimp production

Shrimp yield data from two farms are presented in Table 2, illustrating significant differences in productivity metrics. The two experimental ponds managed by the Department of Fisheries (DOF) reported an average yield of 6794 kg per hectare and an average feed conversion ratio (FCR) of 1.38. Conversely, the private farm, specializing in Pacific white shrimp, achieved a substantially higher average yield of 22,110 kg per hectare, albeit with a higher FCR of 2.14. The primary factor contributing to the discrepancy in yields between the farms is the varying stocking densities; higher stocking densities employed at the private farm resulted in increased yields per hectare but also led to less efficient feed conversion rates.

3.2 | Pond bottom properties and pathogen load

The variance of the shrimp pond floor properties and pathogen load in one production cycle was systematically organized into eight distinct groups, each categorically delineated based on the components or relationships within them. These groups are as follows:

3.2.1 | Organic substances in pond bottom soil

The organic matter content in pond soil is a crucial environmental factor that influences several aspects of pond ecology, including water quality, soil quality, bacterial communities, and the growth of aquatic animals both directly and indirectly (Chainark et al., 2024; Hou et al., 2022; Iber & Kasan, 2021; Saeed & EL-Gammal, 2009). To assess the presence and concentrations of organic substances and their dynamics in shrimp pond sediments, we measured several parameters: sediment oxygen demand (SOD) (used to estimate the amount of easily decomposable organic matter (EDOM); the portion of organic matter that directly impacts aquaculture. The SOD value is correlated with the

TABLE 2 Pond size and production of Pacific white shrimp reared in four ponds from two aquaculture facilities in Phuket Province, Thailand.

Ponds	Pond size (m^2)	Number of PL12s stocked	Stocking density (shrimp/m^2)	Survival rate (%)	Total feed used (kg)	Shrimp yield (kg)	Productivity (kg/hectare)	FCR
Pond F5 (DOF)	800	40,000	50	80	712	546	6825	1.30
Pond F6 (DOF)	800	40,000	50	81	782	541	6763	1.45
Average				**80.5**	**747**	**544**	**6794**	**1.38**
Pond 2 (Commercial farm)	4800	800,000	167	81	22,328	10,599	22,081	2.11
Pond 5 (Commercial farm)	4800	800,000	167	78	23,008	10,626	22,138	2.17
Average				**79.5**	**22,668**	**10,613**	**22,110**	**2.14**

Abbreviation: DOF = Department of Fisheries.

EDOM, or easily oxidized material (EOM)) (Avnimelech et al., 2004), organic matter (OM), total carbon (TC), and total nitrogen (TN). The SOD values ranged from 1678 to 9987 mg O_2/kg, with an average of 5505 ± 1781 mg O_2/kg, as shown in Table 3. The lowest SOD value was observed immediately after sediment flushing (ASF), with values increasing steadily throughout the culture period (Suplee & Cotner, 1996), returning to pre-flushing levels (BSF) by the third month (M3) ($p < 0.05$). By contrast, the concentrations of OM, TC, and TN showed no significant changes across different sampling points, remaining relatively stable ($p > 0.05$), as indicated in Figures 4 and 5.

An analysis of the horizontal distribution of organic substances across different regions (middle vs. edge) and sediment depths (0–5 cm, 5–10 cm, and 10–15 cm) within the shrimp pond indicated consistent SOD values regardless of location and depth during each observation stage ($p > 0.05$), suggesting a uniform distribution of EDOM throughout the pond. However, SOD values were typically higher in the central area compared with the periphery at equivalent depths across all phases (Table 4). Similar patterns were observed with other organic matter parameters, where no statistically significant differences in TC were found among areas and depths throughout the shrimp culture cycle ($p > 0.05$). OM and TN levels also showed no significant variation between the two areas and three depths in all six phases, except for the ASF and second month (M2) phases. During these phases, the 0–5 cm soil depth at the pond's periphery displayed lower OM and TN values compared with all three soil depth levels at the pond's center ($p < 0.05$) (Table 4).

The findings of this study suggest that flushing the pond bottom sediment is an effective method for removing residual organic substances (the EDOM portion determined by SOD) from previous crops. This process necessitates thorough implementation across the entire pond, particularly at depths where organic substances tend to accumulate, which may vary depending on the soil's physico-chemical properties, as indicated in Tables 3 and 4. It is imperative that this operation be carried out responsibly to ensure environmental sustainability and maintain the ecological balance of the pond.

The critical shrimp culture practice of sediment flushing, the first step in shrimp pond preparation, influenced not only organic substances but also other soil and pathogen parameters. This practice improved the quality of pond bottom soil after sediment removal (ASF) compared with other stages. The decrease in some soil parameters, such as ammonia-nitrogen was correlated with the reduction in organic substance content because of sediment flushing. This parameter is a component of organic substances, possibly forming complex structures (Giacalone et al., 2005) or being absorbed onto soil particle surfaces (Borisover & Davis, 2015). Similarly, the quantity of pathogens was also correlated with the amount of organic matter; as the organic substance decreased, the pathogen quantity also decreased (Burford et al., 1998). However, after the ASF stage, the concentrations of various substances and pathogen quantities such as SOD, ammonia-nitrogen, and pathogen parameters in the pond bottom soil increased again (statistical and practical significance), reaching the highest levels in the 3rd month (M3) and the 4th month (M4) of the grow-out period, with most values returning to the BSF level. Proper sediment flushing requires thorough washing to effectively reach and cleanse the lower soil levels, where organic matter is prone to deep penetration because of varying absorption, exchange, or infiltration characteristics of the soil, which differ by soil type and vary across farms. Research indicates that EDOM levels in the lower soil strata (10–15 cm) do not significantly differ from those in the upper layers (Table 4). Nevertheless, inadequate washing or the absence of an efficient central drainage system can result in excessive accumulation and deeper penetration of substances than observed in other areas. This can lead to a myriad of issues, including deterioration of soil and water quality, inhibited shrimp growth, and increased disease outbreaks, underscoring the importance of complete and effective sediment flushing to prevent the accumulation of residual organic substances (Akazawa & Eguchi, 2017; Boyd & Phu, 2018).

Pond drying, a critical component of pond preparation, facilitates the exposure of organic and reduced substances in the sediment to air, which promotes their aerobic degradation and transformation into oxidized forms or carbon dioxide. This process significantly reduces the residual organic matter from previous farming cycles and aids in managing sediment quality (Kumararaja et al., 2018). If sediment flushing is performed effectively after shrimp harvesting, the duration of pond drying may be shortened or even deemed unnecessary, unless it is specifically required to eliminate sediment-borne pathogens or adjust the pH of the pond sediment (Chumnanka et al., 2015).

TABLE 3 Mean (±standard deviation) comparison of soil properties concentrations, bacteria, and parasite quantities collected from two areas (pond edge and central area) at three soil depths (0–5, 5–10, and 10–15 cm) for each area of four Pacific white shrimp ponds at six different stages of one Pacific white shrimp production cycle.

Soil, bacteria, and parasite parameters	BSF	ASF	M1	M2	M3	M4
1. Sediment oxygen demand (mg O_2 /kg dry soil)	6561 ± 1520[a]	3829 ± 928[c]	5132 ± 1626[b]	5230 ± 1517[b]	6009 ± 1707[ab]	6267 ± 1872[a]
2. Organic matter (%)	4.08 ± 1.81	4.33 ± 2.03	4.35 ± 1.95	4.12 ± 1.55	4.72 ± 2.28	4.64 ± 2.21
3. Total carbon (%)	2.36 ± 1.07	2.24 ± 1.01	2.17 ± 1.02	2.25 ± 1.02	2.06 ± 1.01	2.12 ± 1.05
4. Total nitrogen (%)	0.21 ± 0.09	0.22 ± 0.10	0.22 ± 0.10	0.21 ± 0.08	0.24 ± 0.11	0.23 ± 0.11
5. Ammonia-nitrogen (mg/kg)	12.37 ± 11.36[b]	10.55 ± 9.89[c]	12.63 ± 14.06[b]	15.20 ± 9.50[b]	17.31 ± 10.82[ab]	26.00 ± 28.89[a]
6. Nitrite-nitrogen (mg/kg)	0.104 ± 0.176	0.108 ± 0.113	0.088 ± 0.104	0.114 ± 0.156	0.087 ± 0.123	0.164 ± 0.227
7. Extractable phosphorus (mg/kg)	100 ± 113	111 ± 110	96 ± 95	112 ± 124	104 ± 115	106 ± 118
8. Exchangeable calcium (mg/kg)	2229 ± 1441	2454 ± 1376	3021 ± 1829	2704 ± 1766	3219 ± 1937	3453 ± 2030
9. Exchangeable magnesium (mg/kg)	1496 ± 295	1360 ± 467	1494 ± 585	1471 ± 391	1412 ± 470	1385 ± 395
10. Exchangeable potassium (mg/kg)	249 ± 212	289 ± 207	277 ± 221	250 ± 242	303 ± 238	414 ± 226
11. Extractable iron (mg/kg)	383 ± 406	252 ± 326	284 ± 252	194 ± 149	257 ± 244	257 ± 245
12. Extractable manganese (mg/kg)	15.93 ± 8.24	13.97 ± 11.87	16.16 ± 10.57	13.43 ± 8.31	13.39 ± 7.12	14.63 ± 14.07
13. Extractable zinc (mg/kg)	5.53 ± 3.04	5.17 ± 3.34	5.50 ± 2.91	4.02 ± 1.57	4.42 ± 2.07	4.71 ± 1.98
14. Extractable copper (mg/kg)	1.78 ± 2.03	1.71 ± 2.25	2.10 ± 2.25	2.02 ± 2.17	1.96 ± 2.29	2.60 ± 3.12
15. Soil pH	4.45 ± 1.92	5.06 ± 2.01	4.98 ± 1.91	4.59 ± 1.77	4.83 ± 2.03	5.24 ± 1.93
16. Lime requirement (kg $CaCO_3$ /hectare)	8537 ± 8430	6883 ± 8236	5864 ± 6841	7172 ± 7409	7594 ± 7992	5618 ± 7376
17. Sand (%)	43 ± 15	46 ± 17	43 ± 16	48 ± 16	43 ± 13	44 ± 15
18. Silt (%)	31 ± 13	29 ± 12	29 ± 10	26 ± 10	32 ± 9	27 ± 10
19 Clay (%)	26 ± 5	26 ± 8	28 ± 7	27 ± 7	27 ± 7	29 ± 8
20. Total bacteria (cfu/g) (Ln-transformed data)	9.73 ± 1.63[a]	8.53 ± 1.56[b]	8.64 ± 1.20[b]	9.46 ± 1.40[ab]	9.05 ± 1.57[ab]	9.42 ± 1.94[ab]
21. Total *Vibrio* (cfu/g) (Ln-transformed data)	3.45 ± 3.98	1.97 ± 3.04	2.40 ± 2.72	3.55 ± 3.71	3.16 ± 3.30	3.35 ± 3.80
22. Yellow colony-forming bacteria (cfu/g) (Ln-transformed data)	3.17 ± 3.79	1.71 ± 2.76	2.37 ± 2.68	3.17 ± 3.59	3.14 ± 3.32	3.23 ± 3.71
23. Green colony-forming bacteria (cfu/g) (Ln-transformed data)	2.75 ± 3.40	1.33 ± 2.63	0.70 ± 1.56	1.64 ± 2.94	1.16 ± 1.96	1.46 ± 2.28
24. *Vibrio harveyi*	ND	ND	ND	ND	ND	ND

TABLE 3 (Continued)

Soil, bacteria, and parasite parameters	BSF	ASF	M1	M2	M3	M4
25.*V. parahaemolyticus* (AHPND)	ND	Detected in the topsoil (0–5 cm) of one pond	ND	Detected throughout two pond floors	ND	ND
26. *Enterocytozoon hepatopenaei* (EHP)	ND	ND	ND	ND	ND	ND

Note: 1. Different superscript lowercase letters horizontally indicate statistical difference ($p < 0.05$). 2. ND = non-detected.

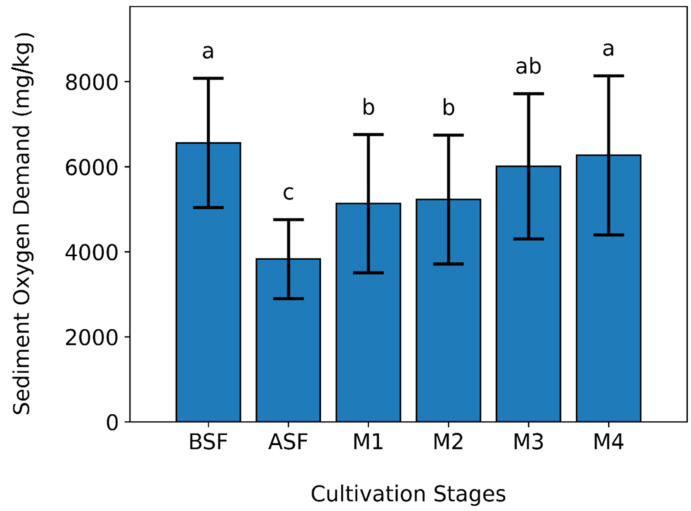

FIGURE 4 Differences in mean sediment oxygen demand in the bottom soil of Pacific white shrimp, *Litopenaeus vannamei*, ponds at six different farming stages ($p < 0.05$).

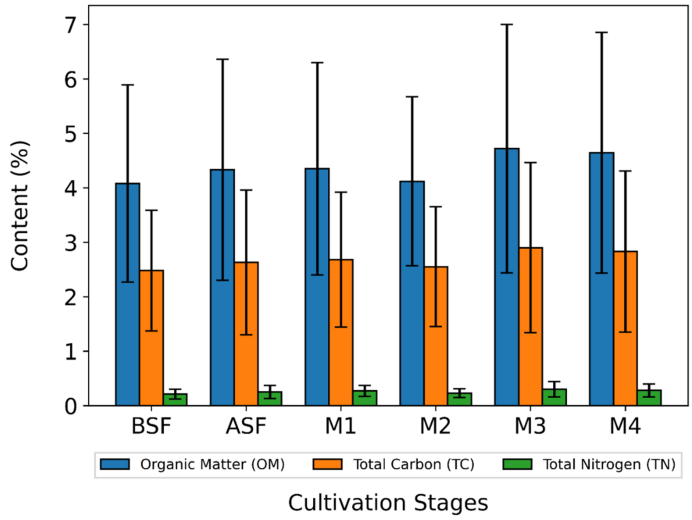

FIGURE 5 Mean organic matter (OM), total carbon (TC), and total nitrogen (TN) in the bottom soil of Pacific white shrimp, *Litopenaeus vannamei*, ponds at six different farming stages ($p > 0.05$).

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TABLE 4 Comparison of mean (±standard deviation) soil properties and bacterial count of four Pacific white shrimp ponds from two areas of the pond bottom (middle and edge of the pond) at three depth levels (0–5, 5–10, and 10–15 cm) during six rearing stages within one production cycle.

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
1. Sediment oxygen demand (mg O_2/kg)	BSF	6784 ± 767	7001 ± 2061	6747 ± 2369	6049 ± 1180	5973 ± 419	6811 ± 2102
	ASF	3594 ± 728	4295 ± 817	4537 ± 147	2827 ± 811	3596 ± 1112	4125 ± 964
	M1	5106 ± 1063	5527 ± 1394	5741 ± 1630	4045 ± 1068	4508 ± 1554	5351 ± 1705
	M2	5666 ± 1614	5572 ± 1567	6469 ± 1045	3789 ± 961	4534 ± 1330	5866 ± 2732
	M3	7136 ± 1182	6202 ± 1153	6420 ± 1386	4694 ± 986	4654 ± 1817	6367 ± 1809
	M4	8232 ± 1037	6597 ± 1533	6540 ± 2234	4841 ± 1869	5172 ± 1977	6803 ± 1959
2. Organic matter (%)	BSF	4.23 ± 1.71	4.66 ± 1.31	3.76 ± 2.87	2.86 ± 1.11	4.64 ± 1.31	4.34 ± 2.48
	ASF	4.43 ± 1.96^{ab}	5.32 ± 1.40^{ab}	6.36 ± 2.42^{a}	1.67 ± 0.63^{c}	3.29 ± 0.97^{bc}	4.92 ± 0.77^{ab}
	M1	3.93 ± 1.52	4.91 ± 0.98	5.69 ± 2.67	2.19 ± 0.71	4.01 ± 1.65	5.35 ± 2.17
	M2	4.08 ± 1.29^{a}	4.69 ± 1.27^{a}	5.37 ± 1.18^{a}	1.96 ± 0.25^{b}	3.71 ± 1.20^{ab}	4.91 ± 1.55^{a}
	M3	4.60 ± 2.24	4.53 ± 2.55	5.27 ± 1.95	3.29 ± 2.75	4.24 ± 2.13	6.37 ± 2.28
	M4	4.73 ± 1.08	4.67 ± 2.23	6.07 ± 2.69	2.43 ± 0.91	4.02 ± 2.31	5.94 ± 2.39
3. Total carbon (%)	BSF	2.13 ± 1.10	2.05 ± 0.79	2.47 ± 1.19	2.40 ± 1.61	2.24 ± 1.01	2.86 ± 1.14
	ASF	2.08 ± 1.08	2.25 ± 0.82	2.55 ± 1.12	2.40 ± 1.78	1.96 ± 0.92	2.22 ± 0.67
	M1	1.83 ± 0.77	2.20 ± 0.69	2.36 ± 1.31	2.12 ± 1.49	2.03 ± 1.12	2.45 ± 1.15
	M2	1.96 ± 0.78	2.14 ± 0.76	2.33 ± 0.61	2.44 ± 1.90	2.24 ± 1.30	2.40 ± 0.95
	M3	1.83 ± 1.08	1.95 ± 1.19	2.19 ± 0.93	1.60 ± 0.87	2.08 ± 1.13	2.74 ± 1.15
	M4	2.12 ± 0.64	1.94 ± 1.12	2.33 ± 1.26	1.98 ± 1.37	1.91 ± 1.28	2.44 ± 1.19
4. Total nitrogen (%)	BSF	0.21 ± 0.08	0.23 ± 0.07	0.19 ± 0.14	0.15 ± 0.06	0.23 ± 0.07	0.22 ± 0.12
	ASF	0.22 ± 0.10^{ab}	0.27 ± 0.07^{ab}	0.32 ± 0.12^{a}	0.09 ± 0.03^{c}	0.17 ± 0.05^{bc}	0.25 ± 0.04^{ab}
	M1	0.20 ± 0.08	0.25 ± 0.05	0.29 ± 0.14	0.11 ± 0.04	0.20 ± 0.08	0.27 ± 0.11
	M2	0.21 ± 0.06^{a}	0.23 ± 0.06^{a}	0.27 ± 0.06^{a}	0.10 ± 0.01^{b}	0.19 ± 0.06^{ab}	0.25 ± 0.08^{a}
	M3	0.23 ± 0.11	0.23 ± 0.13	0.27 ± 0.10	0.17 ± 0.14	0.21 ± 0.11	0.32 ± 0.11
	M4	0.24 ± 0.05	0.24 ± 0.11	0.30 ± 0.13	0.12 ± 0.04	0.20 ± 0.12	0.30 ± 0.12

TABLE 4 (Continued)

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
5. Ammonia-nitrogen (mg/kg)	BSF	14.92 ± 14.57	19.54 ± 16.64	19.31 ± 9.92	4.22 ± 1.52	6.19 ± 3.63	10.06 ± 9.91
	ASF	8.87 ± 4.92^{abc}	17.48 ± 9.95^{ab}	21.24 ± 15.81^{a}	1.64 ± 0.84^{c}	6.12 ± 2.24^{bc}	7.93 ± 3.23^{bc}
	M1	9.81 ± 6.02	16.46 ± 10.70	29.21 ± 27.19	3.79 ± 2.67	6.66 ± 3.07	9.86 ± 7.11
	M2	18.24 ± 8.15^{ab}	20.46 ± 10.57^{ab}	25.25 ± 11.62^{a}	6.19 ± 2.41^{c}	9.25 ± 1.39^{bc}	11.79 ± 3.02^{bc}
	M3	22.08 ± 3.23^{ab}	24.63 ± 10.83^{a}	28.17 ± 14.30^{a}	7.54 ± 4.40^{c}	9.56 ± 4.59^{bc}	11.87 ± 4.31^{bc}
	M4	50.96 ± 43.30^{a}	43.43 ± 34.16^{ab}	38.40 ± 22.57^{ab}	4.26 ± 2.88^{b}	7.77 ± 3.97^{b}	11.18 ± 4.16^{b}
6. Nitrite-nitrogen (mg/kg)	BSF	0.244 ± 0.292	0.046 ± 0.051	0.041 ± 0.041	0.228 ± 0.272	0.044 ± 0.052	0.019 ± 0.020
	ASF	0.133 ± 0.147	0.081 ± 0.072	0.069 ± 0.086	0.182 ± 0.199	0.104 ± 0.091	0.079 ± 0.058
	M1	0.131 ± 0.131	0.080 ± 0.111	0.049 ± 0.065	0.145 ± 0.161	0.075 ± 0.086	0.046 ± 0.065
	M2	0.173 ± 0.205	0.064 ± 0.095	0.055 ± 0.085	0.249 ± 0.274	0.083 ± 0.095	0.060 ± 0.050
	M3	0.096 ± 0.100	0.060 ± 0.065	0.042 ± 0.042	0.062 ± 0.255	0.062 ± 0.063	0.028 ± 0.027
	M4	0.306 ± 0.136	0.085 ± 0.090	0.084 ± 0.085	0.419 ± 0.435	0.060 ± 0.063	0.034 ± 0.037
7. Extractable phosphorus (mg/kg)	BSF	120 ± 128	87 ± 86	56 ± 59	204 ± 201	91 ± 86	44 ± 34
	ASF	104 ± 108	85 ± 86	65 ± 68	185 ± 186	134 ± 123	97 ± 84
	M1	95 ± 102	104 ± 104	51 ± 49	170 ± 154	97 ± 90	57 ± 43
	M2	104 ± 113	98 ± 101	58 ± 58	217 ± 214	142 ± 138	54 ± 34
	M3	140 ± 149	100 ± 114	72 ± 70	204 ± 186	76 ± 50	34 ± 4
	M4	162 ± 159	109 ± 110	41 ± 32	196 ± 189	90 ± 77	38 ± 10
8. Exchangeable calcium (mg/kg)	BSF	2667 ± 1401	2378 ± 1750	1315 ± 1008	2961 ± 1813	2645 ± 1702	1411 ± 680
	ASF	2931 ± 1390	2427 ± 1799	1587 ± 1095	3184 ± 1094	2436 ± 1499	2162 ± 1606
	M1	3862 ± 1459	3413 ± 2748	1597 ± 756	3677 ± 1634	3150 ± 2235	2429 ± 1679
	M2	3413 ± 1937	2819 ± 2122	1758 ± 1157	3307 ± 1905	2892 ± 2219	2036 ± 1661
	M3	4089 ± 2263	3274 ± 2281	2639 ± 2054	3983 ± 2183	3434 ± 2084	1893 ± 684
	M4	5363 ± 1224	3781 ± 2397	1998 ± 1074	4191 ± 2152	3336 ± 2554	2050 ± 914

(Continues)

TABLE 4 (Continued)

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
9. Exchangeable magnesium (mg/kg)	BSF	1556 ± 432	1560 ± 247	1438 ± 325	1216 ± 212	1543 ± 119	1666 ± 307
	ASF	1722 ± 656^{a}	1590 ± 381^{a}	1548 ± 290^{a}	726 ± 168^{b}	1161 ± 180^{ab}	1413 ± 255^{a}
	M1	1482 ± 638	1728 ± 468	1473 ± 674	885 ± 158	1509 ± 414	1886 ± 757
	M2	1591 ± 283	1651 ± 376	1659 ± 199	1002 ± 241	1304 ± 267	1621 ± 557
	M3	1645 ± 460	1430 ± 573	1430 ± 464	931 ± 247	1447 ± 458	1593 ± 493
	M4	1540 ± 330	1342 ± 461	1567 ± 448	1022 ± 175	1271 ± 331	1567 ± 468
10. Exchangeable potassium (mg/kg)	BSF	493 ± 69^{a}	187 ± 191^{bc}	131 ± 191^{c}	403 ± 77^{ab}	250 ± 248^{bc}	31 ± 8^{c}
	ASF	400 ± 240	288 ± 275	143 ± 203	289 ± 39	346 ± 146	271 ± 283
	M1	529 ± 92^{a}	199 ± 270^{bc}	84 ± 113^{c}	334 ± 78^{abc}	412 ± 214^{ab}	101 ± 122^{c}
	M2	559 ± 63^{a}	292 ± 287^{ab}	146 ± 235^{b}	216 ± 156^{b}	258 ± 272^{ab}	32 ± 5^{b}
	M3	608 ± 55^{a}	262 ± 266^{bc}	165 ± 223^{bc}	408 ± 91^{ab}	318 ± 235^{bc}	60 ± 10^{c}
	M4	642 ± 83^{a}	453 ± 269^{abc}	259 ± 246^{bc}	424 ± 35^{abc}	532 ± 57^{ab}	178 ± 229^{c}
11. Extractable iron (mg/kg)	BSF	150 ± 113^{bc}	338 ± 108^{bc}	859 ± 641^{a}	70 ± 62^{c}	261 ± 163^{bc}	617 ± 441^{ab}
	ASF	188 ± 132^{b}	343 ± 106^{ab}	694 ± 622^{a}	15 ± 6^{b}	114 ± 94^{b}	155 ± 26^{b}
	M1	201 ± 161	323 ± 99	565 ± 452	38 ± 19	221 ± 124	355 ± 154
	M2	140 ± 76^{bc}	198 ± 57^{bc}	389 ± 207^{a}	54 ± 63^{c}	111 ± 70^{bc}	272 ± 112^{ab}
	M3	124 ± 94	297 ± 225	444 ± 346	30 ± 6	207 ± 76	440 ± 285
	M4	90 ± 41^{c}	281 ± 254^{abc}	532 ± 316^{a}	79 ± 84^{c}	144 ± 71^{bc}	417 ± 222^{ab}
12. Extractable manganese (mg/kg)	BSF	20.02 ± 14.06	18.86 ± 10.64	14.11 ± 0.97	9.75 ± 6.97	12.85 ± 0.75	19.98 ± 6.03
	ASF	17.20 ± 12.88	25.13 ± 20.08	18.94 ± 6.97	3.63 ± 1.45	10.03 ± 6.43	8.92 ± 3.11
	M1	16.90 ± 12.83	17.29 ± 15.33	19.45 ± 10.13	8.09 ± 5.60	17.82 ± 12.46	17.38 ± 7.60
	M2	13.01 ± 8.15	15.18 ± 11.84	16.35 ± 10.47	11.62 ± 12.23	11.06 ± 5.53	13.38 ± 2.29
	M3	13.90 ± 8.91	13.00 ± 9.47	15.69 ± 7.07	5.44 ± 1.47	14.02 ± 4.52	18.30 ± 4.89
	M4	13.53 ± 7.99	10.44 ± 5.80	29.71 ± 28.74	6.32 ± 3.40	9.71 ± 2.90	18.06 ± 10.33

TABLE 4 (Continued)

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
13. Extractable zinc (mg/kg)	BSF	5.71 ± 2.53	4.23 ± 1.80	6.15 ± 3.54	4.09 ± 2.03	4.78 ± 1.81	8.25 ± 5.01
	ASF	4.12 ± 2.15	4.38 ± 2.05	6.50 ± 3.07	4.22 ± 2.40	8.05 ± 6.42	3.73 ± 0.65
	M1	4.91 ± 3.78	5.91 ± 4.43	5.72 ± 3.30	3.29 ± 1.68	6.01 ± 2.29	7.15 ± 0.81
	M2	4.19 ± 2.29	4.28 ± 2.72	3.82 ± 1.36	3.76 ± 1.41	3.70 ± 0.65	4.38 ± 1.17
	M3	3.76 ± 1.59	3.84 ± 1.44	5.36 ± 4.07	3.32 ± 1.30	4.20 ± 1.14	6.06 ± 1.04
	M4	4.28 ± 1.64	3.82 ± 2.27	6.72 ± 2.75	3.99 ± 1.81	3.97 ± 0.56	5.51 ± 1.47
14. Extractable copper (mg/kg)	BSF	1.71 ± 1.09	1.75 ± 1.70	0.90 ± 1.13	4.05 ± 3.82	1.75 ± 1.19	0.54 ± 0.61
	ASF	1.32 ± 1.05	1.10 ± 0.97	0.76 ± 1.33	3.85 ± 4.45	2.33 ± 2.17	0.91 ± 1.03
	M1	3.13 ± 3.19	2.27 ± 1.92	1.03 ± 1.66	3.51 ± 3.69	1.86 ± 0.53	0.84 ± 0.67
	M2	2.37 ± 1.77	1.81 ± 1.49	1.15 ± 1.95	3.87 ± 3.82	2.31 ± 1.92	0.60 ± 0.27
	M3	2.35 ± 2.31	2.77 ± 4.29	1.45 ± 1.37	3.25 ± 2.74	1.46 ± 0.28	0.51 ± 0.53
	M4	4.87 ± 4.61	2.96 ± 4.14	1.06 ± 1.30	3.98 ± 3.91	1.92 ± 1.17	0.84 ± 0.55
15. Soil pH	BSF	5.92 ± 1.27^{a}	3.63 ± 1.11^{b}	3.27 ± 2.29^{b}	6.56 ± 1.22^{a}	4.52 ± 1.70^{ab}	2.78 ± 0.58^{b}
	ASF	5.36 ± 1.38^{ab}	3.78 ± 1.03^{b}	3.42 ± 2.23^{b}	7.72 ± 0.22^{a}	5.69 ± 1.94^{ab}	4.42 ± 1.68^{b}
	M1	6.23 ± 0.87^{ab}	4.21 ± 1.24^{c}	3.58 ± 2.46^{c}	7.45 ± 0.35^{a}	5.24 ± 1.14^{bc}	3.21 ± 0.43^{c}
	M2	5.58 ± 1.01^{ab}	4.12 ± 1.12^{ab}	3.34 ± 1.83^{b}	6.22 ± 1.70^{a}	5.16 ± 2.24^{ab}	3.17 ± 0.33^{b}
	M3	6.16 ± 1.31^{ab}	4.30 ± 2.29^{bc}	3.96 ± 2.27^{bc}	7.35 ± 0.21^{a}	4.54 ± 0.97^{bc}	2.71 ± 0.36^{c}
	M4	7.10 ± 0.66^{a}	5.27 ± 2.12^{a}	3.35 ± 1.24^{b}	7.01 ± 0.60^{a}	5.49 ± 1.46^{a}	3.22 ± 0.68^{b}
16. Lime requirement (kg $CaCO_3$/hectare)	BSF	1619 ± 3238^{b}	9989 ± 6640^{ab}	$16{,}239 \pm 11051^{a}$	558 ± 741^{b}	6771 ± 5038^{ab}	$16{,}044 \pm 5686^{a}$
	ASF	2930 ± 3441^{b}	$10{,}325 \pm 6168^{ab}$	$16{,}350 \pm 11142^{a}$	0 ± 0^{b}	2902 ± 3554^{b}	8789 ± 9389^{ab}
	M1	866 ± 1211^{c}	6306 ± 4543^{bc}	$14{,}091 \pm 9530^{a}$	0 ± 0^{c}	2038 ± 2449^{c}	$11{,}886 \pm 1515^{ab}$
	M2	1535 ± 2325^{b}	7338 ± 5251^{ab}	$14{,}538 \pm 10193^{a}$	1172 ± 1924^{b}	5753 ± 7334^{ab}	$12{,}696 \pm 4516^{a}$
	M3	1200 ± 2400^{c}	$10{,}044 \pm 7853^{ab}$	$12{,}611 \pm 10080^{ab}$	0 ± 0^{c}	4744 ± 3251^{bc}	$16{,}966 \pm 3030^{a}$
	M4	0 ± 0^{b}	5358 ± 8257^{ab}	$13{,}421 \pm 8209^{a}$	0 ± 0^{b}	2316 ± 2684^{b}	$12{,}611 \pm 5400^{a}$

(Continues)

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TABLE 4 (Continued)

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
17. Sand (%)	BSF	41 ± 16	37 ± 16	37 ± 8	58 ± 18	42 ± 13	44 ± 13
	ASF	40 ± 13^{b}	37 ± 12^{b}	34 ± 8^{b}	71 ± 15^{a}	48 ± 18^{b}	44 ± 14^{b}
	M1	37 ± 11^{b}	37 ± 15^{b}	32 ± 10^{b}	65 ± 14^{a}	47 ± 12^{b}	41 ± 11^{b}
	M2	44 ± 12	38 ± 15	41 ± 9	68 ± 17	51 ± 17	46 ± 7
	M3	42 ± 10^{bc}	32 ± 13^{c}	35 ± 10^{bc}	61 ± 11^{a}	49 ± 7^{ab}	42 ± 6^{bc}
	M4	41 ± 15^{b}	34 ± 17^{b}	32 ± 10^{b}	65 ± 9^{a}	48 ± 10^{ab}	43 ± 11^{b}
18. Silt (%)	BSF	30 ± 16	33 ± 17	37 ± 14	24 ± 15	29 ± 13	30 ± 11
	ASF	33 ± 12	31 ± 10	36 ± 8	15 ± 9	27 ± 11	32 ± 15
	M1	32 ± 10	31 ± 12	35 ± 9	18 ± 8	29 ± 10	31 ± 10
	M2	27 ± 9	29 ± 11	30 ± 10	15 ± 10	24 ± 12	29 ± 9
	M3	28 ± 11	34 ± 10	34 ± 9	34 ± 9	28 ± 11	31 ± 6
	M4	29 ± 13	29 ± 13	32 ± 8	17 ± 7	26 ± 9	28 ± 8
19. Clay (%)	BSF	29 ± 3^{a}	30 ± 3^{a}	26 ± 8^{a}	18 ± 3^{b}	29 ± 2^{a}	26 ± 4^{a}
	ASF	28 ± 3^{a}	32 ± 2^{a}	30 ± 6^{a}	14 ± 6^{b}	25 ± 8^{a}	24 ± 5^{a}
	M1	31 ± 3^{a}	32 ± 4^{a}	34 ± 2^{a}	17 ± 6^{c}	25 ± 4^{b}	28 ± 2^{ab}
	M2	29 ± 3^{a}	34 ± 6^{a}	29 ± 6^{a}	17 ± 7^{b}	25 ± 6^{a}	26 ± 3^{a}
	M3	30 ± 2^{ab}	34 ± 4^{a}	31 ± 10^{ab}	19 ± 5^{c}	24 ± 5^{bc}	27 ± 3^{ab}
	M4	31 ± 3^{a}	34 ± 7^{a}	37 ± 9^{a}	19 ± 4^{b}	27 ± 5^{ab}	30 ± 5^{a}
20. Total bacteria (cfu/g) (Ln-transformed data)	BSF	10.30 ± 2.11	9.36 ± 1.22	8.87 ± 0.86	10.71 ± 1.96	10.18 ± 1.83	8.95 ± 1.63
	ASF	9.44 ± 1.32^{ab}	8.15 ± 0.50^{b}	6.30 ± 0.89^{c}	10.09 ± 0.78^{a}	8.90 ± 1.41^{ab}	8.33 ± 1.35^{ab}
	M1	8.48 ± 0.94	8.79 ± 1.22	7.42 ± 1.01	9.18 ± 1.40	8.98 ± 0.66	8.98 ± 1.61
	M2	10.42 ± 0.48	9.15 ± 1.59	7.88 ± 2.31	10.11 ± 0.42	9.66 ± 0.58	9.57 ± 1.08
	M3	10.68 ± 0.85^{a}	8.27 ± 1.66^{bc}	8.14 ± 1.21^{c}	10.28 ± 0.78^{ab}	8.83 ± 0.79^{abc}	8.07 ± 1.97^{c}
	M4	11.21 ± 1.39^{a}	8.60 ± 2.11^{ab}	8.55 ± 1.58^{ab}	11.03 ± 1.54^{a}	9.11 ± 1.25^{ab}	8.04 ± 1.93^{b}

TABLE 4 (Continued)

Soil and bacteria parameters	Rearing stages	Middle (cm)			Edge (cm)		
		0–5	5–10	10–15	0–5	5–10	10–15
21. Total *Vibrio* (cfu/g) (Ln-transformed data)	BSF	4.84 ± 5.60	1.65 ± 1.92	1.08 ± 2.16	5.50 ± 4.75	3.95 ± 4.60	3.65 ± 4.22
	ASF	4.46 ± 3.21	0 ± 0	0.55 ± 1.11	3.68 ± 4.27	1.87 ± 3.73	1.29 ± 2.58
	M1	4.26 ± 2.91	1.95 ± 2.66	0.55 ± 1.11	3.83 ± 3.25	1.16 ± 1.34	2.64 ± 3.63
	M2	7.68 ± 0.43^{a}	3.31 ± 4.01^{b}	1.55 ± 3.10^{b}	7.26 ± 1.31^{a}	0 ± 0^{b}	1.48 ± 2.95^{b}
	M3	7.30 ± 0.94^{a}	2.41 ± 2.78^{bc}	0 ± 0^{c}	5.54 ± 3.77^{ab}	3.18 ± 2.43^{bc}	0.55 ± 1.09^{c}
	M4	8.11 ± 1.30^{a}	0 ± 0^{c}	0.56 ± 1.11^{c}	5.80 ± 4.03^{ab}	3.66 ± 3.13^{bc}	1.99 ± 3.97^{bc}
22. Yellow colony-forming bacteria (cfu/g) (Ln-transformed data)	BSF	4.62 ± 5.34	1.55 ± 1.80	0.54 ± 1.08	5.11 ± 4.62	3.68 ± 4.25	3.52 ± 4.07
	ASF	4.27 ± 3.02	0 ± 0	0 ± 0	3.19 ± 3.71	1.50 ± 3.01	1.29 ± 2.58
	M1	4.12 ± 2.82	1.95 ± 2.66	0.55 ± 1.11	3.81 ± 3.21	1.16 ± 1.34	2.60 ± 3.63
	M2	7.31 ± 0.59^{a}	3.31 ± 4.01^{b}	0 ± 0^{b}	6.91 ± 1.26^{a}	0 ± 0^{b}	1.48 ± 2.95^{b}
	M3	7.40 ± 1.24^{a}	2.41 ± 2.78^{bc}	0 ± 0^{c}	5.46 ± 3.72^{ab}	3.05 ± 2.48^{bc}	0.55 ± 1.09^{c}
	M4	7.92 ± 1.56^{a}	0 ± 0^{c}	0.56 ± 1.11^{c}	5.71 ± 3.99^{ab}	3.50 ± 3.18^{bc}	1.70 ± 3.39^{bc}
23. Green colony-forming bacteria (cfu/g) (Ln-transformed data)	BSF	4.32 ± 4.99	1.38 ± 1.61	0.54 ± 1.09	4.86 ± 4.10	3.26 ± 3.97	2.15 ± 2.86
	ASF	2.22 ± 3.18	0 ± 0	0.55 ± 1.11	3.43 ± 4.00	1.80 ± 3.60	0 ± 0
	M1	1.77 ± 2.31	0 ± 0	0 ± 0	1.90 ± 2.60	0 ± 0	0.54 ± 1.08
	M2	3.55 ± 4.10^{a}	0 ± 0^{b}	1.55 ± 3.10^{b}	4.77 ± 3.36^{a}	0 ± 0^{b}	0 ± 0^{b}
	M3	3.60 ± 2.68^{a}	0 ± 0^{b}	0 ± 0^{b}	2.77 ± 1.93^{a}	0.62 ± 1.23^{b}	0 ± 0^{b}
	M4	3.86 ± 2.88^{a}	0 ± 0^{b}	0 ± 0^{b}	3.63 ± 2.63^{a}	1.28 ± 1.48^{ab}	0 ± 0^{b}

Note: Different superscript lowercase letters horizontally indicate statistical difference ($p < 0.05$).

Properly conducted sediment flushing allows ponds to be immediately reused for subsequent cycles without the risk of substance accumulation over time.

The assessment of organic substance content in pond bottom soil, both directly and indirectly, involves parameters such as SOD, OM, TC, and TN. While SOD specifically measures the rate at which aerobic bacteria consume oxygen in decomposing organic matter, the other parameters quantify both readily degradable organic matter and more complex, resistant forms such as humus and lignin (Avnimelech et al., 2004; Smagin et al., 2018). These methods often incorporate both organic and inorganic components within their analytical scope. Nonetheless, the total measurements do not accurately reflect the levels of perishable organic matter, such as uneaten food, feces, and plankton detritus, which significantly influence soil and water quality, as well as pathogen populations in the pond (Avnimelech et al., 2004; Chainark et al., 2024; Joyni et al., 2011). Consequently, the resulting data on organic content often appear inconsistent or variable, complicating the assessment of organic matter accumulation in the pond bottom (Table 3, Figure 5). Contrarily, SOD measurements have demonstrated more distinct variations in organic matter content across six rearing stages compared with the total quantifications, highlighting its sensitivity to changes in fresh organic matter in the pond environment (Figure 4).

3.2.2 | Inorganic nitrogen distribution

The dynamics of inorganic nitrogen, particularly ammonia-nitrogen and nitrite-nitrogen, were closely monitored across different stages of a production cycle in a shrimp pond. The highest mean concentration of ammonia-nitrogen was documented during the M4 stage of the rearing period. Meanwhile, the lowest mean value was observed in the ASF stage ($p < 0.05$) (Table 3). The variation in ammonia-nitrogen concentration during a single production cycle of Pacific white shrimp was similar to SOD, with the lowest value found after pond cleaning (ASF) and then increasing throughout the culture period. For nitrite-nitrogen, averages across the six stages remained statistically similar ($p > 0.05$).

Upon analyzing the horizontal distribution of ammonia-nitrogen across two areas at three depth levels within each stage of a shrimp production cycle, it was observed that the central area of the pond exhibited higher concentrations, particularly at the depth of 0–5 cm ($p < 0.05$) as shown in Table 4. This pattern of nitrogen compound distribution, which aligns with findings by Lu et al. (2016), is attributed to the accumulation of nitrogen-rich easily decomposable organic matter (nitrogen-rich EDOM), such as uneaten feed and shrimp feces, predominantly in the pond's middle. This accumulation is further facilitated by the action of aerators, which concentrate organic deposits in the central area (Yuvanatemiya et al., 2011).

Examining the vertical distribution of ammonia in the pond floor revealed no significant variances among different depth levels in each region ($p > 0.05$) (Table 4); however, ammonia tended to accumulate more at greater depths in both areas. This observation contrasts with the findings of Lu et al. (2016), who reported higher concentrations of ammonium-nitrogen in the upper sediment layers (0–6 cm) compared with deeper subsoil layers (7–60 cm) in freshwater aquaculture settings. Different cultural practices between freshwater fish and marine shrimp aquaculture might account for this discrepancy, suggesting that sediment dynamics can significantly vary across different aquaculture systems.

For nitrite-nitrogen, the horizontal distribution pattern of mean concentrations across various stages of the production cycle was statistically similar at all sampled points ($p > 0.05$). Although the mean values did not statistically differ ($p > 0.05$) (Table 4), nitrite-nitrogen tended to be higher in the top soils compared with subsoils in both areas of the pond floor. This elevation in nitrite-nitrogen concentration can likely be traced back to the higher oxygen levels in the upper soil layers, thanks to the diffusion of dissolved oxygen from the aerated water, which normally increases with the grow-out period, reaching the sediment surface. These conditions were conducive to nitrification, facilitating the conversion of ammonia to nitrite, predominantly at the soil-water interface, which is characterized by a thin oxidized layer on the surface. This observation elucidates the nuanced interplay between oxygen availability,

location within the pond, and the consequent nitrogen transformations, with potential implications for pond management practices.

3.2.3 | Extractable phosphorus distribution

The distribution of extractable phosphorus content across different rearing stages, areas, and depths showed no significant variation ($p > 0.05$) (Table 3). The range of extractable phosphorus values spanned from 2 to 433 mg/kg dry soil, averaging at 105 ± 111 mg/kg dry soil. These data point towards a high retention capability of the soil for phosphorus, which may be present as inorganic or organic phosphate, originating from various sources within or external to the pond (Reddy et al., 1999). The uniform distribution pattern of phosphorus adsorbed in the bottom soil was akin to the distribution observed for SOD, reflecting a relatively consistent distribution across the pond (Table 4).

3.2.4 | Exchangeable bases

The levels of exchangeable bases (calcium, magnesium, and potassium) remained consistent throughout all stages of a single shrimp cultivation cycle (Table 3). This indicates that each shrimp farming cycle does not alter the number of exchangeable bases in the pond bottom soil.

Observing the horizontal distribution pattern, it emerged that calcium concentrations did not differ among areas and depths in all culture stages within one cultivation cycle ($p > 0.05$) (Table 4). However, calcium tended to be greater in the topsoil, with the highest values located in the upper soil stratum, and average values diminishing as the depth increased (Table 4). The distribution pattern of calcium is possibly an outcome of the utilization of large volumes of liming materials (Thunjai et al., 2004), which are predominantly spread across the pond bottom's surface without undergoing tilling.

In the case of magnesium, no specific horizontal distribution was identified in all six culture stages ($p > 0.05$), but during the ASF stage, the mean values at the 0–5 cm depth level at the pond's periphery were lower than all three depth levels of the pond's central areas (Table 4).

Regarding potassium, most concentrations were high at the surface soil level, decreasing as depth increased, especially in the middle of the pond. While the edges of the pond showed a similar trend to the middle, the pattern was not as clearly defined (Table 4). The accumulation at the upper soil level might result from exchanges occurring between the top bottom soil and the overlaying seawater or brackish water, or perhaps from residual food or waste containing potassium. Therefore, a higher concentration of potassium is observed at the upper soil level compared with lower levels.

The implications of exchangeable base concentrations (primary cations) in pond soils on pond water quality, plankton population, and the yield of cultivated organisms remain somewhat obscure. Yet, it is generally posited that elevated concentrations of calcium, magnesium, and potassium are advantageous (Silapajarn et al., 2004).

3.2.5 | Extractable trace elements

Throughout the six stages of a single shrimp farming cycle, the concentrations of extractable trace elements, including iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu), exhibited no noteworthy variations ($p > 0.05$) (Table 3).

However, the horizontal distribution across three depth levels highlighted some distinct patterns among these elements. Manganese, zinc, and copper showed no significant variance across regions and depth levels throughout a single production cycle ($p > 0.05$) (Table 4), reflecting a fairly consistent distribution throughout the pond. Meanwhile, iron presented a different pattern, showcasing its minimum concentrations at the surface layer (0–5 cm) and

subsequently escalating as the depth increased, reaching its peak in the 10–15 cm layer ($p < 0.05$) (Table 4). Furthermore, iron content tended to be higher in the central zone compared with the peripheral zone ($p > 0.05$).

Similar to exchangeable bases, the significance of trace elements in an extractable form within earthen pond aquaculture remains a topic of uncertainty, aside from the potential toxicity they might introduce when substantially released in environments with exceedingly low pH (Brady & Weil, 2008).

3.2.6 | Soil pH and lime requirement

Soil pH values in all six stages of one cultivation cycle ranged from 2.00 to 7.92, with an average value of 4.86 ± 1.91. The average pH values showed no statistically significant differences across all six stages ($p > 0.05$) (Table 3). This can be explained by the limited time for pond preparation, which took approximately 2 weeks and did not significantly affect pyrite oxidation in the bottom soil. Even when clay minerals cracked, and the subsoil was exposed to air, the impact remained minimal. However, when examining the horizontal distribution pattern of soil pH at different areas and depths in the ponds, we observed that both areas had the highest average soil pH values at the uppermost level (0–5 cm), with pH values decreasing as depth increased (Table 4, Figure 6). This finding aligns with De Queiroz et al., (2004) research. This pattern can be attributed to the lime requirement, which ranged from 0 to 25,000 kg $CaCO_3$/hectare, with an average of 6944 ± 7663 kg $CaCO_3$/hectare. The lime requirement exhibited a similar pattern to the soil pH values across all six stages, increasing with depth as soil pH values decreased (Table 4, Figure 7). This situation arises from the predominance of acidic sulfate soil or potential acid sulfate soil in the ponds' soil composition, which results from the presence of pyrite. Pyrite oxidation generates sulfuric acid, leading to highly acidic soil with a very low pH (Brady & Weil, 2008).

Lime is typically applied to the soil surface without tilling, effectively neutralizing the acidic upper layer, while the deeper layers remain acidic and may release acids, particularly during the drying stage when they are exposed to air (Nimrat et al., 2008; Yuvanatemiya et al., 2011). This selective neutralization is supported by research from De Queiroz et al., (2004), which demonstrated that agricultural limestone application significantly increases soil pH only in the top 8 cm, with the greatest effect in the 0–4 cm layer. This study revealed that ponds with a long history (over

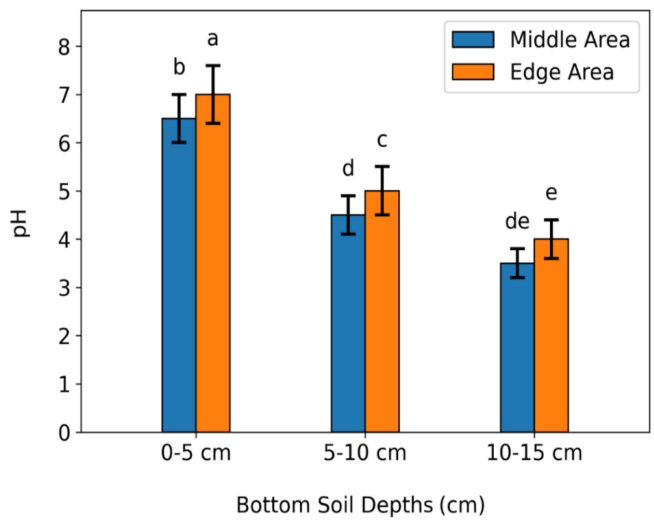

FIGURE 6 Differences in mean soil pH between the middle area and the edge area across three depth levels (0–5, 5–10, and 10–15 cm) throughout a production cycle of Pacific white shrimp ponds ($p < 0.05$).

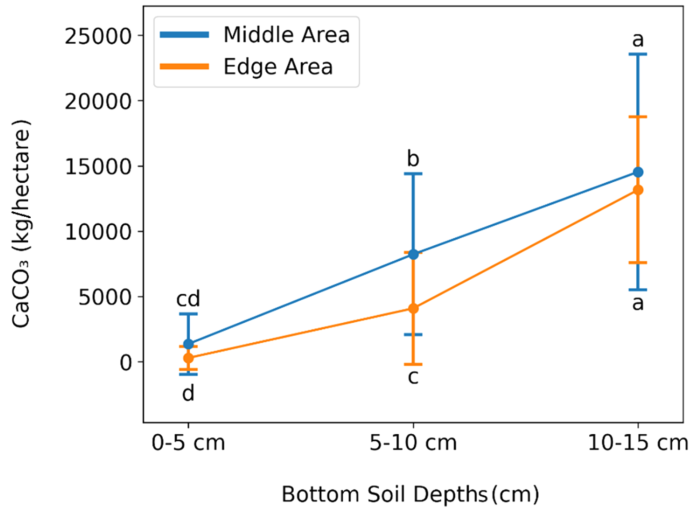

FIGURE 7 Differences in mean lime requirement between the middle area and the edge area across three depth levels (0–5, 5–10, and 10–15 cm) throughout a production cycle of Pacific white shrimp ponds ($p < 0.05$).

20 years) of lime application still showed acidic conditions at lower soil levels. To address this issue comprehensively, it is recommended to periodically till the pond bottom soil using a disk harrow, as this practice not only helps distribute lime more evenly but also enhances soil respiration and accelerates the degradation of organic matter, providing a more permanent solution to soil acidity problems (Avnimelech & Ritvo, 2003; Boyd, 1995; Boyd et al., 2010; Boyd & Phu, 2018).

3.2.7 | Soil texture

The percentages of sand, silt, and clay particles in the pond soil disclosed no significant differences across all six rearing stages ($p > 0.05$) (Table 3), indicating that white shrimp farming within a production cycle had no noticeable impact on soil texture. The average percentages of sand, silt, and clay in the pond soil were 44%, 29%, and 27%, respectively.

Regarding horizontal distribution, when comparing central and edge areas at three depth levels during different culture stages, it was found that sand percentages were higher in the pond's edge area at the 0–5 cm depth level, while clay percentages were lower at the corresponding depth and area ($p < 0.05$) (Table 4). Clay particles were more abundant in the middle area of the pond than in other areas ($p < 0.05$). Larger particles settle near the pond's edge, while circular water currents generated by wind or an aerator carry away smaller particles, leading to their accumulation in the central area. The topsoil in the edge pond area contained more sand particles than the subsoil (Shafi et al., 2022). Nevertheless, the soil profile of the middle pond area showed no significant difference in the distribution of sand particles.

The percentage of silt in the soil profile of the middle pond area did not show significant differences across all areas and depth levels throughout the six rearing stages (Table 4). This consistency suggests that the silt content remained stable across the entire pond during the farming cycle, indicating uniform sediment characteristics throughout the production process.

The soil texture of the Department of Fisheries (DOF) ponds was predominantly classified as clay loam and loam, displaying a finer substrate texture compared with the private farm ponds, which consisted mostly of sandy clay loam soil. Research by Pine et al. (2018) suggests that shrimp tend to grow better in sandy clay loam soil than in

clay loam soil. All ponds contained more than 20% clay, satisfying the minimum requirement for aquaculture pond construction as outlined by Boyd (1995). Notably, the soil texture in these ponds remained relatively consistent across all six rearing stages of one production cycle, indicating stable sediment characteristics conducive to aquaculture.

3.2.8 | Bacterial and parasitic pathogens

Changes in the quantity of bacteria and other pathogens in the soil of Pacific white shrimp ponds over six phases in one cultivation cycle revealed that most bacterial variables did not show statistically significant differences across different cultivation phases ($p > 0.05$), except for total bacteria, which significantly decreased after the ASF stage ($p < 0.05$) (Table 3). However, the average values of all bacterial variables tended to decrease after the ASF phase and then increase as the cultivation stages progressed. The pattern of change was similar to that of SOD and ammonia-nitrogen values, where the quantities reached their lowest after the ASF phase and then increased with the cultivation time, peaking mostly in the M4 and the BSF stages (Table 3).

During the analysis of bacterial distribution at different locations and depths within the same rearing stage, several observations were noted. In the BSF phase, there were no significant differences in the quantity of bacteria across all four parameters at any point within the pond ($p > 0.05$) (Table 4). From the ASF stage through to the M4 stage, the highest bacterial counts were consistently observed at the soil surface level (0–5 cm) at both locations, with bacterial quantities diminishing with increasing depth. This pattern correlates with the concentration of organic substances in the soil profile. Despite these variations in depth, the bacterial counts at the 0–5 cm depth did not show significant differences between the two locations ($p > 0.05$), as presented in Table 4.

The quantity of bacteria in aquaculture pond soil correlates directly with the presence of easily oxidized material (EOM), as demonstrated by Avnimelech and Ritvo (2003) and Chainark et al. (2024). Bacteria, more abundant than fungi in pond sediments, play a crucial role in organic matter decomposition (Dai et al., 2021). Henze et al. (2002) noted that during aerobic decomposition, approximately 50% of metabolized organic matter converts into bacterial cells. Consequently, an increase in organic substances leads to a proportional rise in bacterial quantities, a relationship further supported by Burford et al. (1998). This interplay is crucial for maintaining the ecological balance within aquaculture ponds.

V. harveyi, a significant bacterial pathogen affecting penaeid shrimp, was not detected at any stage of the study. Nonetheless, *V. parahaemolyticus*, responsible for acute hepatopancreatic necrosis disease (AHPND), was identified in the soil at a depth of 0–5 cm in one pond following sediment flushing. This pond underwent disinfection with chlorine during preparation, and *V. parahaemolyticus* was detected again throughout the bottom of two ponds during the 2nd month (M2) grow-out period, as indicated in Table 3. Antibiotic and disinfectant were applied to the ponds. The concentration of *V. parahaemolyticus*, as measured in natural log (Ln)-transformed colony-forming units per gram (cfu/g), was significantly higher in the middle area of the AHPND-afflicted pond at 8.22 ± 2.14 cfu/g, compared with 4.79 ± 4.24 cfu/g in ponds not afflicted by AHPND (Chainark et al., 2024). Additionally, *E. hepatopenaei* (EHP), a microsporidian parasite linked to EHP disease, was not detected at any point during the cultivation cycle, as documented in Table 3.

3.2.9 | Results of the two-way analysis of variance

A two-way analysis of variance was conducted to determine if there were differences in shrimp pond bottom soil properties and bacterial load under varying conditions, including two different stocking densities and six different stages of the production cycle, as well as the interaction between these two factors, as depicted in Table 5. The findings are as follows:

TABLE 5 Results of a two-way analysis of variance for Pacific white shrimp bottom soil properties and bacterial abundance under two different stocking densities (low: 50 PL12s/m^2 and high: 167 PL12s/m^2) and six different farming stages (before sediment flushing (BSF), after sediment flushing (ASF), and during each month of the four-month cycle (M1 to M4)).

	Densities			Cultivation stages							Interaction (density*stage)
Parameters	Low	High	*p*-values	BSF	ASF	M1	M2	M3	M4	*p*-values	*p*-values
1. Sediment oxygen demand (mg/kg)	4922^{b}	6086^{a}	<0.0001	6561^{a}	3829^{c}	5132^{b}	5230^{b}	6009^{a}	6267^{a}	<0.0001	<0.0001
2. Organic matter (%)	3.62^{b}	5.13^{a}	<0.0001	4.08	4.33	4.35	4.12	4.72	4.64	0.7744	0.0960
3. Total carbon (%)	1.47^{b}	2.92^{a}	<0.0001	2.36	2.24	2.17	2.25	2.06	2.12	0.7743	0.9932
4. Total nitrogen (%)	0.18^{b}	0.26^{a}	<0.0001	0.21	0.22	0.22	0.21	0.24	0.23	0.7975	0.0951
5. Ammonia-nitrogen (mg/kg)	15.82	15.54	0.9122	12.37^{b}	10.55^{b}	12.63^{b}	15.20^{b}	17.31^{ab}	26.00^{a}	0.0087	0.0235
6. Nitrite-nitrogen (mg/kg)	0.03^{b}	0.19^{a}	<0.0001	0.104	0.108	0.088	0.114	0.087	0.164	0.3579	0.9648
7. Extractable phosphorus (mg/kg)	22^{b}	187^{a}	<0.0001	100	111	96	112	104	106	0.9752	0.9572
8. Exchangeable calcium (mg/kg)	1465^{b}	4228^{a}	<0.0001	2229	2454	3021	2704	3219	3453	0.0638	0.6524
9. Exchangeable magnesium (mg/kg)	1257^{b}	1620^{a}	<0.0001	1496	1360	1494	1471	1412	1385	0.7929	0.2569
10. Exchangeable potassium (mg/kg)	247^{b}	347^{a}	0.0080	249	289	277	250	303	414	0.1104	0.8523
11. Extractable iron (mg/kg)	261	281	0.6690	383	252	284	194	257	257	0.3415	0.7434
12. Extractable manganese (mg/kg)	11.85^{b}	17.32^{a}	0.0012	15.93	13.97	16.16	13.43	13.39	14.63	0.8764	0.4012
13. Extractable zinc (mg/kg)	4.07^{b}	5.71^{a}	<0.0001	5.53	5.17	5.50	4.02	4.42	4.71	0.1882	0.2457
14. Extractable copper (mg/kg)	0.76^{b}	3.31^{a}	<0.0001	1.78	1.71	2.10	2.02	1.96	2.60	0.7130	0.9822
15. Soil pH	4.23^{b}	5.49^{a}	<0.0001	4.45	5.06	4.98	4.59	4.83	5.24	0.6837	0.8877
16. Lime requirement (kg $CaCO_3$ /hectare)	9310^{a}	4579^{b}	0.0002	8537	6883	5864	7172	7594	5618	0.7642	0.7512
17. Sand (%)	35^{b}	54^{a}	<0.0001	43	46	43	48	43	44	0.6883	0.7312
18. Silt (%)	36^{a}	20^{b}	<0.0001	31	29	29	26	32	27	0.4545	0.9327
19 Clay (%)	27	27	0.5566	26	26	28	27	27	29	0.5499	0.7105
20. Total bacteria (cfu/g) (Ln-transformed data)	8.65^{b}	9.63^{a}	<0.0001	9.73^{a}	8.53^{c}	8.64^{bc}	9.46^{a}	9.05^{abc}	9.42^{ab}	0.0094	<0.0001
21. Total *Vibrio* (cfu/g) (Ln-transformed data)	1.68^{b}	4.28^{a}	<0.0001	3.45	1.97	2.40	3.55	3.16	3.35	0.4131	0.0476
22. Yellow colony-forming bacteria (cfu/g) (Ln-transformed data)	1.47^{b}	4.13^{a}	<0.0001	3.17	1.71	2.37	3.17	3.14	3.23	0.4025	0.1204
23. Green colony-forming bacteria (cfu/g) (Ln-transformed data)	1.10^{b}	1.92^{a}	0.0306	2.75	1.33	0.70	1.64	1.16	1.46	0.0569	<0.0001

Note: Different superscript lowercase letters within each factor (density and cultivation stage) indicate a statistically significant difference horizontally.

Stocking density

High stocking density in shrimp ponds (167 PLs/m^2) markedly elevates soil properties and microbial levels compared with low density (50 PLs/m^2). Under these conditions, key parameters such as SOD, OM, TC, TN, NO_2^--N, extractable P, and exchangeable Ca, Mg, and K, as well as levels of Fe, Mn, Zn, Cu, soil pH, sand content, and all bacterial measures were significantly higher, as presented in Table 5. The greater accumulation of inputs such as feed, fertilizers, chemicals, vitamins, and minerals, along with the waste production of uneaten feed and excreta from aquatic animals, is responsible for this increase. According to Burducea et al. (2022), higher nutrient and waste loads intensify soil and microbial parameters in high-density ponds compared to those with lower densities.

Under high stocking density, certain soil variables, such as lime requirement and silt particle content, show lower averages compared to those in low-density ponds, as shown in Table 5. This trend can be attributed to the more acidic conditions found in the soil of low-density ponds, typically DOF ponds, especially at lower soil depths. By contrast, private shrimp farming ponds, which are often older and subject to continuous use, undergo regular liming during preparation and throughout the farming process. This treatment results in a higher soil pH and a reduced necessity for lime in these ponds. Moreover, variables such as ammonia-nitrogen and clay particle content appear to be unaffected by changes in stocking density, culture stage, or their interaction, indicating a stable presence irrespective of farming intensity.

Culture stage

In most instances, soil variables and microbial levels remained statistically consistent across different culture stages, with the exceptions of SOD, NH_3-N, and total bacterial count. These variables decreased following the sludge injection stage, subsequently increased throughout the culture stages, and peaked in the third or fourth month, just prior to another sludge injection. This pattern aligns with the accumulation and subsequent reduction of organic matter in the pond—increasing as the culture progresses and decreasing during pond cleaning phases. These dynamics are corroborated by the results of a one-way analysis of variance (ANOVA) detailed in Table 3, illustrating significant fluctuations in these specific parameters at defined stages of the culture cycle.

Interaction

Most soil and bacterial parameters did not exhibit significant interactions between stocking density and culture stage, with notable exceptions such as SOD, ammonia-nitrogen, and several bacterial parameters, as indicated in Table 5. When an interaction is detected between factors, it suggests that the effects of these factors are interdependent, negating the need to consider each factor's main effect independently. Instead, evaluating the average outcomes of the combined treatments becomes more relevant. Despite this, observations indicated that the treatment combinations resulting in the highest averages generally corresponded closely to or were influenced by the main effects of both density and culture stage, indicating a strong alignment between these factors and their individual contributions to the variables measured.

3.2.10 | Predictive model for white shrimp yield

Nineteen soil variables and four pathogen variables were included in the variable selection process to develop a multiple linear regression equation for predicting the yield of white shrimp in earthen ponds. The analysis selected nine soil variables: TC, NH_3-N, P, pH, Mg, Fe, Mn, sand, and silt, along with one bacterial variable, Ln-green colony-forming bacteria ($p < 0.001$), for predicting white shrimp yield. The model achieved an R^2 of 0.897 and C(p) = 1.0943, as shown below:

$$\begin{aligned}\text{White shrimp yield (kg)} = {} & 16695 + 1522 \cdot \text{TC} - 31 \cdot \text{NH}_3 - \text{N} + 14 \cdot \text{P} + 737 \cdot \text{pH} + 1.17 \cdot \text{Mg} + 2.8 \cdot \text{Fe} + 54 \cdot \text{Mn} \\ & - 226 \cdot \text{Sand} - 415 \cdot \text{Silt} - 235 \cdot \text{Ln} - \text{green colony} - \text{forming bacteria}\end{aligned}$$

4 | CONCLUSIONS

Shrimp production across farming cycles typically does not significantly alter most soil properties or the quantity of microorganisms; however, the level of easily decomposable organic matter (EDOM) does increase throughout the farming period. Compared with other soil parameters, EDOM has a significant impact on several critical aspects of shrimp farming. It influences pond soil and water quality, affects pathogen levels, and plays a significant role in shrimp growth and disease incidence. Nevertheless, effective cleaning of the pond bottom post-harvest can substantially reduce the residual EDOM. This reduction is critical for maintaining the pond's long-term usability, preventing degradation, and reducing the need for extended pond drying periods that were traditionally required to decompose residual organic matter.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The data supporting this study are available upon request from the authors.

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