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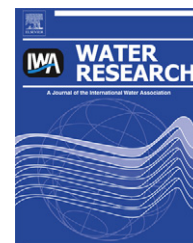
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Temporal flux and spatial dynamics of nutrients, fecal indicators, and zoonotic pathogens in anaerobic swine manure lagoon water[☆]

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ABSTRACT

Confined animal feeding operations (CAFOs) often use anaerobic lagoons for manure treatment. In the USA, swine CAFO lagoon water is used for crop irrigation that is regulated by farm-specific nutrient management plans (NMPs). Implementation of stricter US environmental regulations in 2013 will set soil P limits; impacting land applications of manure and requiring revision of NMPs. Precise knowledge of lagoon water quality is needed for formulating NMPs, for understanding losses of N and C in ammonia and greenhouse gas emissions, and for understanding risks of environmental contamination by fecal bacteria, including zoonotic pathogens. In this study we determined year-round levels of nutrients and bacteria from swine CAFO lagoon water. Statistical analysis of data for pH, electrical conductivity (EC), inorganic and organic C, total N, water-soluble and total minerals (Ca, Cu, Fe, K, Mg, Mn, P, and Zn) and bacteria (*Escherichia coli*, enterococci, *Clostridium perfringens*, *Campylobacter* spp., *Listeria* spp., *Salmonella* spp., and staphylococci) showed that all differed significantly by dates of collection. During the irrigation season, levels of total N decreased by half and the N:P ratio changed from 9.7 to 2.8. Some seasonal differences were correlated with temperature. Total N and inorganic C increased below 19 °C, and decreased above 19 °C, consistent with summer increases in ammonia and greenhouse gas emissions. Water-soluble Cu, Fe, and Zn increased with higher summer temperatures while enterococci and zoonotic pathogens (*Campylobacter*, *Listeria*, and *Salmonella*) decreased. Although their populations changed seasonally, the zoonotic pathogens were present year-round. Increasing levels of *E. coli* were statistically correlated with increasing pH. Differences between depths were also found. Organic C, total nutrients (C, Ca, Cu, Fe, Mg, Mn, N, P, and Zn) and *C. perfringens* were higher in deeper samples, indicating stratification of these parameters. No statistical interactions were found between collection dates and depths.

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1. Introduction

Open anaerobic lagoons provide primary manure treatment for confined animal feeding operations (CAFOs) in the south-eastern USA. Lagoon water is continuously pumped to flush barn pits and is seasonally pumped to supply spray irrigation systems with water and fertilizer nutrients for summer crops (Sistani et al., 2008). Water for irrigating crops may be land-applied only from spring through fall in compliance with site-specific nutrient management plans under EPA – (US Environmental Protection Agency) regulated state permits. In 2013 the US will implement a revised standard for nutrient management plans (Sharpley et al., 2011). The new standard will give greater emphasis to soil P levels and require state-by-state and farm-by-farm revision of current plans. In Europe, a reduced land base available for manure spreading also requires careful manure nutrient management (Webb et al., 2005; Oenema et al., 2007).

Nutrient management plans typically concern only crop fertilization requirements and N and P levels in irrigated soils, and do not address microbial levels in the soils or in the wastewater used for irrigation. The US-EPA regulates standards for microbial levels in drinking water, but not for animal agricultural sources of irrigation water applied to pasture and hay crops (Bradford et al., 2008; USEPA and USAID, 2004). Regulatory standards and recommendations for reuse of agricultural wastewater are based on fecal coliform levels, and vary widely (Gerba and Smith, 2005). Recommendations to the World Health Organization (WHO) (Blumenthal et al., 2000) suggest acceptable fecal coliform counts up to $\leq 1 \times 10^5$ 100 mL⁻¹ for spray irrigation of pasture and fodder crops. The WHO guidelines (WHO, 2006) suggest crop-based targets for reduction of fecal bacteria counts in wastewater used for irrigation. In the US, individual state standards for coliforms are as low as 2.2 fecal coliforms 100 mL⁻¹ for wastewater reused on nonfood crop land in Hawaii (USEPA and USAID, 2004). Coliforms and other fecal bacteria are good indicators of fecal contamination, but poor indicators of contamination by zoonotic bacteria. A 2009 report of anaerobic swine manure lagoons in the southeastern US (McLaughlin et al., 2009) found that levels of thermotolerant coliforms and enterococci were poorly correlated with levels of *Campylobacter* spp. and *Salmonella* spp., and were not correlated with levels of *Listeria* spp., despite the high incidence of these zoonotic bacteria across 37 lagoons tested (100, 89, and 81 percent, respectively). That report concluded that these indicator bacteria should not be used to estimate zoonotic bacterial levels and that direct testing was required for detection of zoonotic bacteria in swine lagoon water.

In a recent review Davies (2011) highlighted the need for quantitative information on enteric zoonotic bacteria in the pork production environment in order to accurately assess risks and improve food safety. Because many earlier studies of swine anaerobic lagoon water were conducted during the summer crop irrigation season (Sistani et al., 2008) and did not include microbial data, these studies provide only limited water quality data. More recent studies of temporal changes in swine anaerobic lagoons reported significant seasonal variability in the nutrient and microbial content of lagoon water

(Lovanh et al., 2009; Cook et al., 2010; Westerman et al., 2010). Lovanh et al. (2009) and Cook et al. (2010) used molecular analyses to estimate total bacterial levels, but except for *Clostridium* spp., did not report levels of zoonotic bacteria. In an earlier report from North Carolina, Hill and Sobsey (2003) measured year-round levels of fecal bacteria, including *Clostridium perfringens* and *Salmonella* spp., in swine waste lagoons. These few reports and their limited coverage of zoonotic pathogens point toward the need for a more comprehensive temporal analysis of bacterial populations, especially zoonotic pathogens, in anaerobic swine manure lagoon water. Additional data were needed for a more complete temporal picture of nutrients, fecal bacteria (including zoonotic species), and coincident environmental factors that may interact with nutrients and bacteria to impact lagoon water quality, nutrient management plans, and risk assessments associated with use of anaerobic treatment lagoons.

The present study was conducted to obtain data on temporal and spatial changes in nutrient and bacterial levels of swine anaerobic lagoon water and to test the null hypothesis that respective nutrient and bacterial levels in lagoon water would not differ by time of year or depth in the lagoon water column. Our objectives were to measure and compare levels of nutrients and bacteria (including fecal indicators and zoonotic species) in swine anaerobic lagoon water collected in different seasons and at different depths and to assess potential correlations of environmental variables with any observed changes in these levels. Nutrient and bacterial levels in a 3.4-ha anaerobic swine CAFO sow farm lagoon were determined at 5–10-wk intervals and at two depths over a 14-month period and analyzed to document temporal and spatial differences. Correlations of nutrient and bacterial levels with air and lagoon water temperature, and lagoon water pH, are described. Implications for lagoon management and NMPs are presented.

2. Materials and methods

2.1. Lagoon site

Water samples were collected from a single-stage 3.4-ha anaerobic swine manure lagoon in east central Mississippi. The lagoon was 2.4 m deep and had a capacity of 7.6×10^4 m³. The lagoon had been in continuous operation for 17 years and had an appreciable bottom sludge layer of unknown depth. The lagoon received fresh manure slurry directly from 13 confinement barns, on a sow farm (Fig. 1). The lagoon water retained a permanent red-purple color, which is characteristic of mature phototrophic (purple sulfur bacteria) lagoons (Chen et al., 2003). Each barn typically housed 220–1000 gilts for breeding and gestation, or 384 sows and 3500 piglets during farrowing. Lagoon water was pumped from the lagoon for irrigating and fertilizing warm season grass hay crops on the farm from April through September; land application was not permitted from October through March. Thus, the annual lagoon cycle was one of reducing the lagoon water volume during the drier warm season to accommodate increased rainfall water volume during the cooler season. Nutrient

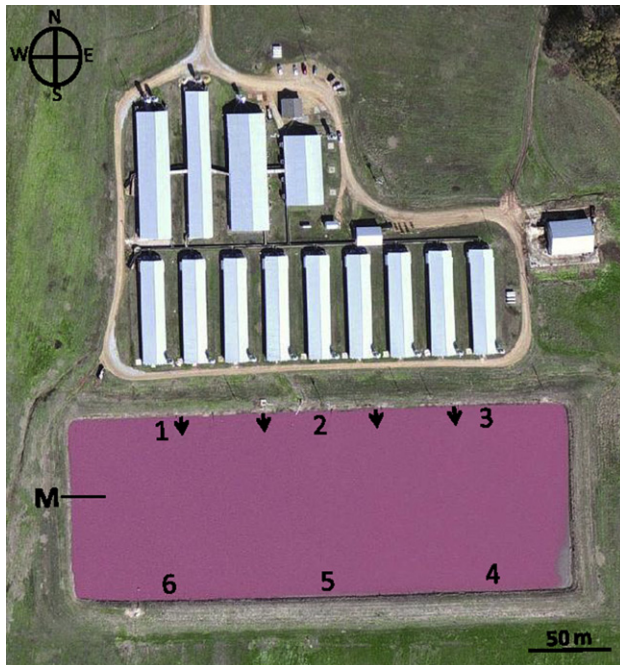


Fig. 1 – Aerial image (courtesy of the U.S. Geological Survey) of the anaerobic lagoon and swine CAFO used in the present study. Lagoon labels show the direction of manure slurry influent flow (arrows), the locations of six water sample collection sites (1–6), and the weather and lagoon temperature monitoring station (M) with lagoon temperature probe (bar).

levels and some bacterial levels in water from the lagoon had been periodically analyzed and reported in other studies (Adeli and Varco, 2001; McLaughlin and Brooks, 2009; McLaughlin et al., 2009) but temporal levels and changes over the annual cycle had not been studied.

2.2. Environmental monitoring

Environmental conditions at the lagoon site were monitored continuously using appropriate sensors and data logged electronically using a weather station (Hobo Micro Station, Onset Computer Corp., Bourne, MA) located on the lagoon bank (Fig. 1). Conditions that were monitored included lagoon temperature (via a probe deployed 0.5 m beneath a floating PVC platform tethered 10 m from the bank), ambient air temperature, relative humidity, rainfall, ambient solar radiation, and wind speed and direction. Mean hourly and daily values for logged data parameters were determined using HOBOWare Pro v.3.2.0 (Onset Computer Corp.) and exported to Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA) for additional analyses.

2.3. Water sample collection

Water samples were collected on 26 Jan, 9 Mar, 27 Apr, 15 Jun, 17 Aug, 5 Oct, and 16 Dec in 2009 and 22 Feb 2010. On the first two sampling dates, collections were made at three depths

(0.04, 0.47, and 1.0 m), but analyses showed no differences in bacterial levels between 0.04 and 0.47 m samples (data not shown), so subsequent collections used only 0.04 and 1.0 m depths and only those results are reported here. Six samples were collected for each depth and date. All samples were collected at the same equidistantly spaced locations along two opposite sides of the lagoon (Fig. 1). Three locations were proximal to the barns and influent flow and three locations were on the opposite side of the lagoon, distal to the barns and influent flow. To avoid collection of sludge and possible plugging of collection lines by sludge, and to allow 1-m deep samples to be drawn from just above the sludge interface, water samples at each location were collected 6–7 m out from the edge of the lagoon. Samples were drawn through sterile silicon tubing using a remote-controlled 12-V DC vacuum pump mounted on a tethered floating collection platform. Samples were drawn into sterile 250-ml polypropylene bottles through luer-lock fittings in custom installed top ports in their sealed caps. For each location, a separate set of collection tubing, cut to length for different sample depths and taped to a PVC pipe (12 mm diameter, 1.5 m long), was lowered from the collection platform by remote control. Separate sets of bottles, tubing, and PVC pipe were used at each sample collection point. Vacuum source lines and the pump were protected by a 0.22- μ m inline filter and liquid traps. Samples were placed in a cooler on ice immediately after collection, returned to the laboratory, held at 5 °C, and processed within 3–4 h of collection.

2.4. Water sample analyses

All water samples were analyzed individually. Chemical properties (pH and electrical conductivity) and nutrient levels (mg L⁻¹ inorganic and organic C, total N, and total and water-soluble (ws) P, K, Ca, Cu, Fe, Mg, Mn, and Zn) were determined as previously published (McLaughlin et al., 2009).

Ammonia–nitrogen (NH₃–N) flux estimates were calculated using the observational model (Eq. (1)) of Aneja et al. (2000)

$$\text{Log}_{10}(\text{NH}_3\text{--N flux}) = 0.048 T_L + 2.1 \quad (1)$$

with NH₃–N flux expressed as (μ g N m⁻² min⁻¹) and T_L measured as lagoon water temperature (°C). Log₁₀ NH₃–N flux estimates (Eq. (1)) were back transformed to NH₃–N flux (μ g N m⁻² min⁻¹) and plotted over time to estimate seasonal temperature effects.

Bacterial levels (cfu 100 ml⁻¹ for enterococci, staphylococci, *C. perfringens*, and *Escherichia coli*; and MPN 100 ml⁻¹ for *Campylobacter* spp., *Listeria* spp., and *Salmonella* spp.) were determined using cultural and molecular methods as previously published (McLaughlin et al., 2009, 2010).

2.5. Statistical analyses

Bacterial count data (cfu or MPN 100 mL⁻¹) were adjusted by addition of 1.0 (to convert zeros to positive numbers) and Log₁₀-transformed to stabilize the variance for statistical analyses. Transformed bacterial data and raw data for chemical and nutrient analyses were subjected to analysis of

variance using SAS (v9.2 for Windows XP-Pro, SAS Institute, Inc., Cary, NC) MIXED model analysis of sampling dates (eight) using a repeated measures design with sample location (six) and depth (two) as repeated variables and a heterogeneous autoregressive covariance structure. Data from one sampling date each for *Campylobacter* (9 Mar 2009), enterococci (16 Dec 2009), and wsZn (16 Dec 2009) did not fit the heterogeneous autoregressive covariance structure, so these variables were analyzed using data for seven dates each and missing data were estimated by linear models for graphical comparisons. Data from six 1-m-depth samples (two from each of three sampling dates) that contained significant amounts of sludge were excluded in the analysis. Main effects in the MIXED model were compared by *F* tests and differences between means were compared at the $P \leq 0.05$ level. Correlations and graphical analyses were done using Microsoft Office Excel 2007 (Microsoft Corporation) and differences were compared at the $P \leq 0.05$ level, unless stated otherwise.

3. Results and discussion

3.1. Environmental factors

Local environmental conditions and temporal changes at the lagoon site are shown in Fig. 2. Annual rainfall (Fig. 2a) in the area averages 1400 mm, with higher amounts from late fall through winter to early spring and generally less rainfall in the warm season from mid-April through September, which encompasses the 6-month irrigation season when lagoon water is applied to surrounding crop land. Relative humidity at the lagoon site (Fig. 2b) varied widely, but generally was

higher from summer through winter than in spring. Ambient air temperatures (Fig. 2c) displayed typical seasonal fluctuations with highs in mid-summer and lows in mid-winter corresponding with seasonal changes in day length (Fig. 2d). Lagoon water temperature changes paralleled air temperature changes, but averaged about 3 °C higher (Fig. 2c), an observation also reported by Lohan et al. (2009) for an anaerobic lagoon in Kentucky. Although seasonal temperature ranges may differ from those reported for lagoons in other regions of the USA, the seasonal pattern reported in the present study is similar to those reported for swine lagoons in Iowa (Merrill and Halverson, 2002), Kansas (DeSutter and Ham, 2005), Kentucky (Cook et al., 2010), and North Carolina (Vanotti and Szogi, 2008). Correlations between air and lagoon temperatures and emissions of ammonia, greenhouse gasses, and malodorants have been widely studied, and it is generally agreed that lagoon water temperature is a significant factor in volatilization processes (Harper et al., 2000; DeSutter and Ham, 2005; Aneja et al., 2008; Blunden and Aneja, 2008; Blanes-Vidal et al., 2010; Loughrin et al., 2011). Correlations observed in the present study are described in 3.2.2., 3.2.3., 3.2.5., and 3.3.1.

3.2. Chemical properties

Chemical properties and total nutrient levels of the lagoon water samples are reported in Tables 1 and 2. Tests of sampling date were significant for all variables. Tests of sampling depth were significant for some variables. Property and nutrient means averaged over sampling depth and compared across sampling dates are shown in Table 1. Significant seasonal changes in nutrients with volatile forms

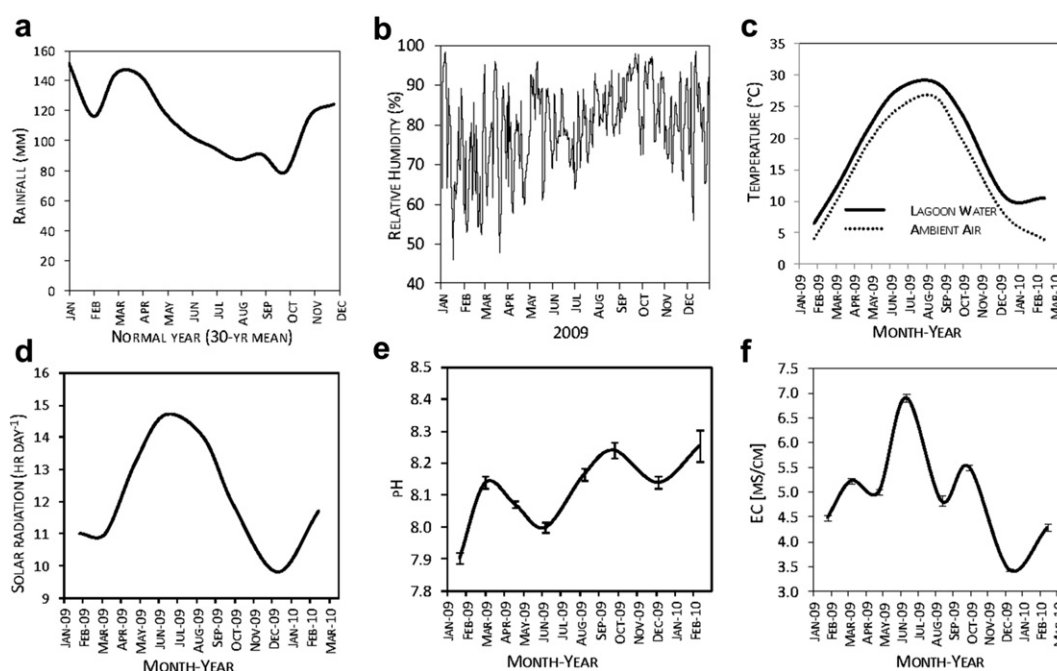


Fig. 2 – Lagoon environmental conditions: (a) normal (30-yr mean) rainfall; (b) mean daily percent relative humidity; (c) mean hourly temperatures for 10-day periods before each lagoon water sample collection; (d) mean daily hours of solar radiation; (e) lagoon water pH; and (f) lagoon water EC. The pH and EC are combined means (\pm SE bars) for 4-cm and 1-m depths at each collection date ($n = 12$).

Table 1 – Collection date means for chemical properties of lagoon water samples from an anaerobic manure lagoon on a commercial swine farrowing (sow farm) CAFO in Mississippi.

Date	Chemical properties ^a														
	pH	EC mS cm ⁻¹	iC	oC	tC	N	P	K	Ca	Fe	Mg	Na	Cu	Mn	Zn
26 Jan 09	7.90 ± 0.017	4.49 ± 0.06	464 ± 4	368 ± 10	832 ± 15	403 ± 3	51 ± 6	403 ± 6	59 ± 9	1.8 ± 0.6	21 ± 1	213 ± 3	107 ± 24	256 ± 104	678 ± 203
09 Mar 09	8.14 ± 0.016	5.23 ± 0.04	517 ± 3	477 ± 11	994 ± 11	505 ± 2	52 ± 2	431 ± 4	60 ± 2	2.4 ± 0.2	24 ± 0.4	226 ± 2	129 ± 10	253 ± 27	1144 ± 136
27 Apr 09	8.07 ± 0.010	5.08 ± 0.02	485 ± 4	461 ± 4	947 ± 1	496 ± 1	59 ± 1	424 ± 4	58 ± 1	2.2 ± 0.1	29 ± 1	218 ± 1	112 ± 9	170 ± 14	1318 ± 154
15 Jun 09	8.00 ± 0.015	6.91 ± 0.07	399 ± 5	450 ± 5	848 ± 4	404 ± 1	32 ± 1	220 ± 3	18 ± 0	0.6 ± 0.0	15 ± 1	112 ± 1	40 ± 3	60 ± 6	266 ± 44
17 Aug 09	8.18 ± 0.013	4.82 ± 0.13	338 ± 2	586 ± 4	923 ± 6	322 ± 2	97 ± 7	480 ± 8	52 ± 8	4.1 ± 1.1	41 ± 3	255 ± 4	234 ± 78	405 ± 119	1603 ± 501
05 Oct 09	8.24 ± 0.027	5.51 ± 0.05	283 ± 3	459 ± 8	742 ± 10	261 ± 2	94 ± 11	355 ± 3	56 ± 14	5.7 ± 2.2	39 ± 4	190 ± 2	326 ± 124	556 ± 221	1999 ± 692
16 Dec 09	8.14 ± 0.021	3.43 ± 0.03	330 ± 3	403 ± 26	733 ± 26	313 ± 5	84 ± 23	416 ± 4	61 ± 13	3.3 ± 2.2	32 ± 12	223 ± 2	158 ± 121	372 ± 261	1832 ± 830
22 Feb 10	8.21 ± 0.018	4.33 ± 0.02	401 ± 5	351 ± 4	750 ± 8	391 ± 2	65 ± 5	415 ± 5	63 ± 5	3.0 ± 0.6	22 ± 2	213 ± 2	147 ± 31	356 ± 96	1289 ± 212
a Values are least squares means (±SE) of combined data for 12 samples from six locations and two collection depths (4 cm and 1 m) per date. Samples were individually tested for pH, electrical conductivity (EC), inorganic carbon (iC), organic carbon (oC), total carbon (tC), and total nutrients (as listed above). Means followed by the same letter within columns were not significantly different (P = 0.05) in pairwise t-tests.															

^a Values are least squares means (±SE) of combined data for 12 samples from six locations and two collection depths (4 cm and 1 m) per date. Samples were individually tested for pH, electrical conductivity (EC), inorganic carbon (iC), organic carbon (oC), total carbon (tC), and total nutrients (as listed above). Means followed by the same letter within columns were not significantly different ($P = 0.05$) in pairwise *t*-tests.

(tN and iC) and in water-soluble metal levels (wsCu, wsFe, wsZn) were observed and are described later (3.2.2. and 3.2.5., respectively). Chemical property and nutrient means averaged over sampling dates and compared between sampling depths are shown in Table 2.

3.2.1. pH and EC

The pH of lagoon water samples (Fig. 2e) varied between collection dates (Table 1), but remained within a relatively narrow range (7.9–8.2) that was slightly higher than the range (7.3–7.9) reported for sow farm lagoons in the region (McLaughlin et al., 2009). Mean pH was statistically higher in shallow (8.13) than deep (8.09) samples (Table 2), but this difference was not correlated with observed changes in nutrients or bacteria and did not appear to be chemically or biologically significant. Although pH tended to increase slightly through the study period, values remained within the optimal range reported for purple sulfur bacteria (Chen et al., 2003). The pH flux observed in the present study showed significant correlation only with *E. coli* levels, as described in 3.3.1.

Electrical conductivity (Fig. 2f) also varied between collection dates (Table 1) with a peak summer mean EC value (6.91 ± 0.071 mS cm⁻¹ on 15 Jun 2009) nearly double the lowest winter mean EC value (3.43 ± 0.021 mS cm⁻¹ on 16 Dec 2009) and slightly above the range (2.7–6.0 mS cm⁻¹) reported for sow farms in the region (McLaughlin et al., 2009). The influence of increased summer temperatures on increased solubility of some metals as noted above, and thereby on EC values, are described in 3.2.5.

3.2.2. Nitrogen, phosphorus and potassium

Levels of the principal fertilizer nutrients N, P, and K varied in lagoon water with dates of collection (Fig. 3), but only N and P differed between lagoon depths (Table 2). Annual mean levels of lagoon water N (393 mg L⁻¹), P (64 mg L⁻¹), and K (480 mg L⁻¹) in the present study were similar to those reported earlier (N = 412 mg L⁻¹; P = 61 mg L⁻¹; K = 401 mg L⁻¹) from a 1994–1996 study of irrigation water from this lagoon (Adeli and Varco, 2001). This earlier study, however, did not report levels and changes during these years or irrigation seasons. Furthermore, at the time of the earlier study, the farm was a complete farrow to finish operation, consequently animal numbers and age ratios, feed inputs, and management were different than during the present study and likely produced different lagoon water quality than the present operation.

Annual flux in total N content of lagoon water in the present study was estimated by a third order polynomial equation ($R^2 = 0.98$) as:

$$y = 6.0 \times 10^{-5}x^3 - 0.034x^2 + 4.7236x + 309.42 \quad (2)$$

where *y* is the concentration of total N (mg L⁻¹) and *x* is the day (from one to 366) of the calendar year. To test this simple model (Eq. (2)), data were used from an earlier study (McLaughlin et al., 2009), in which the mean concentration of total N from six samples of shallow lagoon water had been determined following collection on 5 Sep 2007 (day 249 of the calendar year) from the same lagoon used in the present study. Fitting $x = 249$ in Eq. (2) estimated $y = 304$ mg N L⁻¹ for the 5 Sep 2007 samples, which, based on the earlier analysis

Table 2 – Chemical property means of lagoon water samples at two depths in the water column of an anaerobic manure lagoon on a commercial swine farrowing (sow farm) CAFO in Mississippi.

Depth	Chemical properties ^a														
	pH	EC	iC	oC	tC	N	P	K	Ca	Fe	Mg	Na	Cu	Mn	Zn
		mS cm ⁻¹													
4 cm	8.13 ^b ± 0.008	4.95 ± 0.03	401 ± 2	434 ± 6	835 ± 6	383 ± 1	56 ± 5	394 ± 3	43 ± 5	1.5 ± 0.7	24 ± 2	207 ± 1	78 ± 43	137 ± 77	771 ± 232
1 m	8.09 ± 0.008	5.01 ± 0.03	403 ± 2	455 ± 6	858 ± 6	390 ± 1	77 ± 5	392 ± 3	64 ± 5	4.3 ± 0.7	32 ± 3	206 ± 2	236 ± 44	470 ± 80	1761 ± 240
P > F	<0.01	ns ^c	ns	0.02	0.02	0.01	0.02	ns	0.02	0.02	0.05	ns	0.02	0.01	<0.01

^a The lagoon was sampled eight times over 14 months from Jan 2009 to Feb 2010. Samples were collected at six locations such that 1-m-depth samples were from the bottom of the water column just above the sludge interface. Samples were tested for pH, electrical conductivity (EC), inorganic carbon (iC), organic carbon (oC), total carbon (tC), and total nutrients (as listed above). Data from six 1-m-depth samples (two from each of three dates) that contained significant amounts of sludge were excluded in the means analysis.

^b Least squares means (±SE); 4-cm, n = 48; 1-m, n = 42.

^c ns, no significant difference (P > 0.05) between depths.

had total N = 294 mg L⁻¹. The error of the estimate was three percent. This relationship provides a promising practical tool for nutrient management of this particular lagoon within the relatively stable cyclical input parameters that comprise this specific farm operation. With more extensive testing of additional lagoons over multiple years this simple tool might find wider application to other lagoons and locations.

In the present study, levels of N and P were higher in 1-m than in 4-cm samples (Table 2). No interactions of date and depth were observed. Because P is often bound to sediment suspended in lagoon water, it was not surprising that deeper samples had higher total P content. In fact, six 1-m samples containing appreciable amounts of sediment and noted at the times of collection as darker in color and high in sludge (likely collected in the sludge interface rather than in lagoon water alone), were excluded from subsequent comparisons of lagoon water nutrient levels (2.5) because of their very high levels of some nutrients, especially P, which were five to ten times higher than other 1-m lagoon water samples collected at the same times from above the sludge interface. Chen et al. (2003) also reported nutrient stratification in sow farm lagoons and Lovanh et al. (2009) showed much higher nutrient levels in the sludge layer beneath the water column than in water samples above.

3.2.3. Nitrogen and carbon flux

Nitrogen levels in lagoon water increased during the cool season and decreased steadily during the warm season (Fig. 3a). Westerman et al. (2010) observed a similar seasonal trend weakly correlated with annual rainfall in North Carolina lagoons and suggested that N losses in summer could be due to increased ammonia emissions. Estimated NH₃-N flux in the present study showed such a seasonal effect (Fig. 3b), suggesting a temperature effect. Although gaseous emissions from the lagoon were not measured directly in the present study, the pattern of N loss observed during summer and a similar pattern of inorganic C loss (Fig. 3c) together suggest that gaseous emissions during the warm season played a significant role in reducing the levels of these nutrients in lagoon water. In addition to N loss from increased NH₃ emissions, other important greenhouse gas emissions affected by seasonal temperature changes are carbon dioxide, methane, and nitrous oxide (Kayranli et al., 2010).

Lagoon temperature effects on total N and inorganic C levels in lagoon water were further examined by correlation analysis. Changes in total N and inorganic C levels were calculated by comparing their respective levels at each collection date with levels at the previous collection date. This calculation assumed annual cyclical changes in both nutrients such that the estimated changes in levels for the first (late winter) collection date (26 Jan 2009) were calculated based on the respective levels for (early winter) samples collected on 16 Dec 2009. Changes in temperature were calculated using hourly mean temperatures for 10-day periods immediately preceding each respective collection date and determining their respective deviations from an empirically derived threshold of 19 °C. At the threshold temperature, N and inorganic C inputs to lagoon water from conversion of manure nutrients in the lagoon should approximate N and inorganic C losses (to gaseous emissions) from the lagoon (no net change).

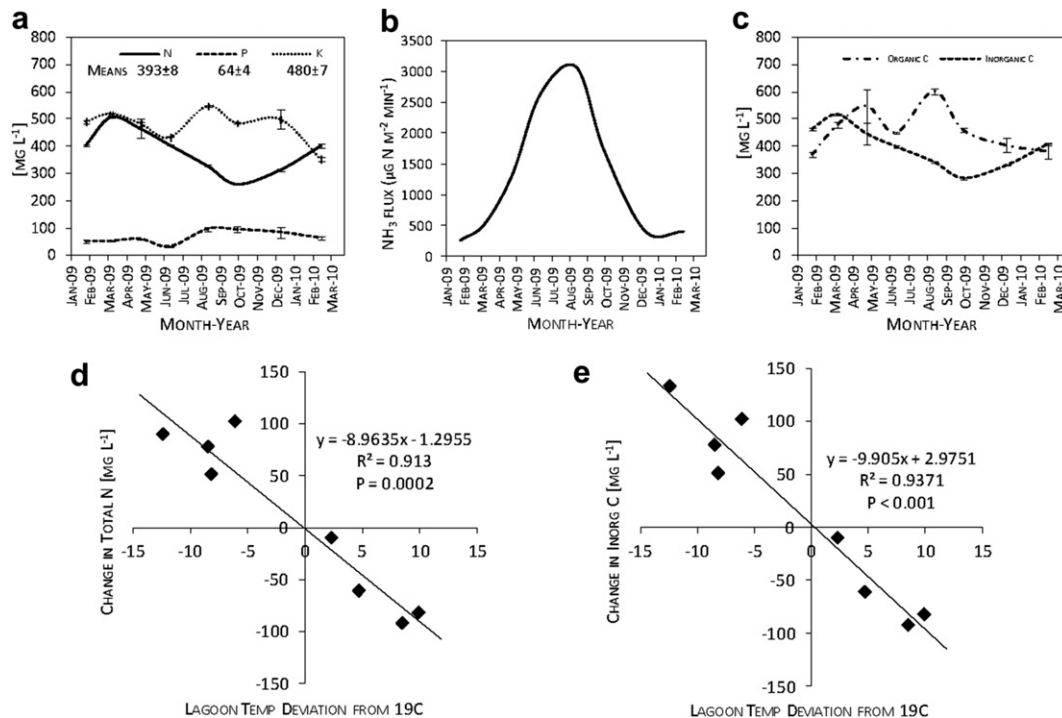


Fig. 3 – Temporal distribution of (a) N, P, and K; (b) estimated temporal NH₃–N flux from gaseous emissions; and (c) organic and inorganic C in lagoon water from 26 Jan 2009 through 22 Feb 2010. Models of changes in (d) total N in lagoon water; and (e) inorganic C in lagoon water based on changes in lagoon water temperature (deviation from 19 °C in 10-day hourly mean temperatures before each collection date). Nutrient data are the combined means for 4-cm and 1-m samples (±SE bars for each collection date, $n = 12$). NPK means shown in the legend in (a) are annual lagoon cycle means (±SE) for combined depths ($n = 90$; excludes six 1.0-m samples which contained significant amounts of sludge; two each on 27 Apr and 17 Aug 2009 and 22 Feb 2010).

At temperatures below this threshold, N and inorganic C accumulated in lagoon water. At temperatures above this threshold, N and inorganic C losses exceeded inputs. Analyses of changes in levels of N (Fig. 3d) and inorganic C (Fig. 3e) showed significant correlations with deviations from the threshold temperature. This simple temperature correlation of total N and inorganic C levels in lagoon water was consistent with observations of earlier studies (Ibusuki and Aneja, 1984; Harper et al., 2000) and with more recent and comprehensive models (Blanes-Vidal et al., 2010).

3.2.4. N:P

Total N and total P data were also examined as temporal changes in the ratio of N to P in lagoon water. This ratio is useful in nutrient management planning for applications of lagoon water in irrigation and fertilization of crops. Total P levels showed an overall increase during the summer irrigation season, in contrast to the coincident decrease in total N (Fig. 3a). On 9 Mar 2009 before the start of the irrigation season the N:P of lagoon water was 9.7. On 5 Oct 2009 at the close of the irrigation season the N:P was 2.8. The irrigation season mean N:P was 6.7 (four sampling dates); and the annual mean N:P was 6.8 (eight sampling dates). These results were consistent with the 3-yr irrigation season mean lagoon water N:P of 6.7 reported in an earlier study of this lagoon (Adeli and Varco, 2001). Consequently, N-based lagoon water

applications require informed adjustments during the irrigation season to prevent nutrient insufficiency or excess. Failure to increase application rates as the summer growing season progresses and lagoon water N levels decline could result in N insufficiency. Conversely, increasing lagoon water application rates in summer to increase N rates could result in P excesses. In the case of summer hay crops, there are many different species and varieties of forage crops available, which offer a range of N:P requirements to help growers and nutrient management planners optimize individual nutrient management plans (Sistani et al., 2008). Managing the N:P status of land-applied manures will become even more critical in the future, especially in the USA, following the scheduled 2013 implementation of a revised state-by-state national nutrient management standard establishing upper limits for soil P levels (Sharpley et al., 2011).

3.2.5. Other nutrients

Levels of other nutrients also changed significantly across sampling dates Table 1. While water-soluble and total levels of the highly soluble nutrients, Ca and Mg, could be expressed on the same respective scales, less soluble nutrients, Fe, Cu, and Zn, showed 10-fold differences between their respective total and water soluble levels (data not shown). Assuming relatively stable feed inputs of these nutrients year round, increased levels of water-soluble metals, wsFe, wsCu, and

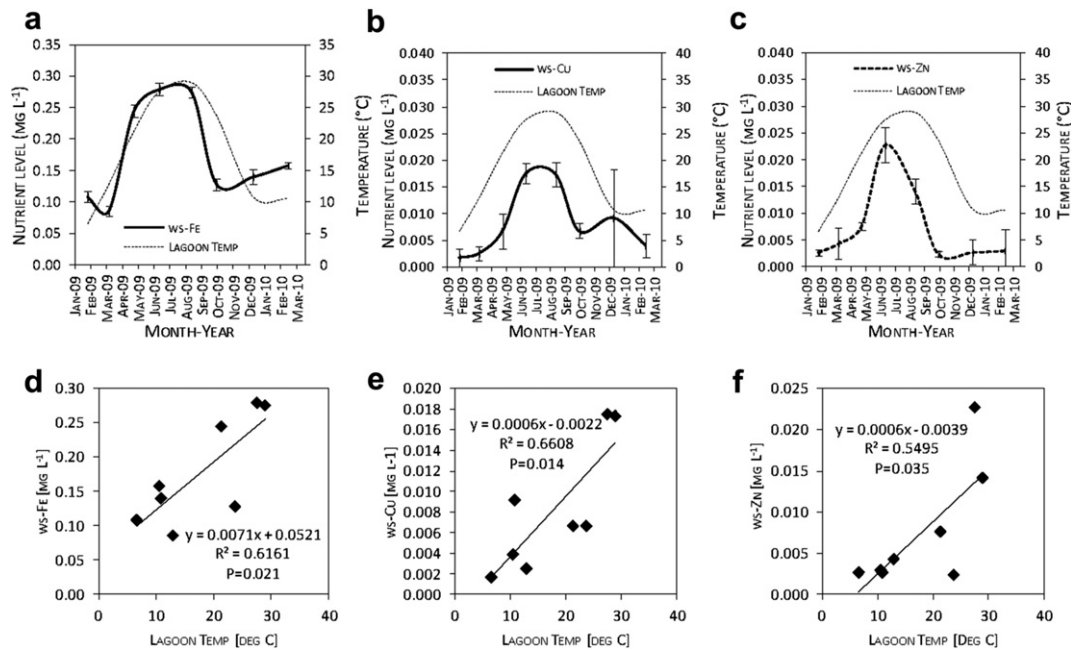


Fig. 4 – Temporal changes in lagoon water temperature and levels of water soluble (ws-) (a) Fe; (b) Cu; and (c) Zn. Combined means (4 cm and 1 m) and standard error bars of nutrient levels are shown ($n = 12$; excludes six 1.0-m-depth samples which contained significant amounts of sludge, two each on 27 Apr and 17 Aug 2009 and 22 Feb 2010; ws-Zn levels for 16 Dec 2009 were estimated by linear model using 5 Oct 2009 and 22 Feb 2010 data). Linear correlations of nutrient level and lagoon water temperature for (d) Fe, (e) Cu, and (f) Zn.

wsZn, in summer suggested a temperature effect (Fig. 4a–c). To examine these data for such an effect, water-soluble levels of these three metals were plotted with hourly mean lagoon water temperatures calculated for 10-day periods immediately before each collection date. Temperatures at the time of collection were also examined (data not shown), but appeared more variable and were believed to be more coincidental and less effectual than 10-day means for the periods before sample collections. Linear correlation analyses of wsFe, wsCu, and wsZn levels against lagoon water temperatures showed significant positive correlations and possible temperature effects (Fig. 4d–f).

Levels of other total nutrients, except for the highly mobile inorganic C, K, and Na also differed between depths (Table 2). Levels of organic and total C, and total levels of Ca, Cu, Fe, Mg, Mn, N, P, and Zn were all higher in 1-m water samples than in 4-cm water samples. Such stratification was not unexpected as similar observations have been reported for top and bottom samples from a farrowing farm lagoon in Kentucky (Lovanh et al., 2009) and for water samples from different depths in farrowing farm lagoons in Nebraska (Chen et al., 2003).

3.3. Bacteria

Combined bacterial population means Log₁₀ (cfu or MPN 100 mL⁻¹, $n = 90$) across all sampling dates and both depths were, from highest to lowest: staphylococci (6.4 ± 0.08); *E. coli* (6.0 ± 0.05); *C. perfringens* (6.0 ± 0.05); *Campylobacter* spp. (4.4 ± 0.06); enterococci (4.3 ± 0.18); *Listeria* spp. (2.3 ± 0.11); and *Salmonella* spp. (1.4 ± 0.11). The relative order and levels of these respective population means were similar to those

reported for sow farms in the region (McLaughlin et al., 2009). Reports of the presence and persistence of these bacterial indicators and pathogens in swine manure lagoons in other states and Canada revealed many single event collections of these bacteria, but few longer term temporal studies (Hill and Sobsey, 2003; Guan and Holley, 2003; McLaughlin and Brooks, 2009). The present study is the first to document temporal changes in zoonotic bacterial pathogen populations in anaerobic swine lagoon water in the Mid-South USA.

3.3.1. Bacterial temporal flux

Levels of all bacterial groups enumerated in the present study varied significantly across dates of sample collection (Table 3). The temporal distributions of several of the bacterial groups in the present study, especially *Campylobacter* spp. and *Listeria* spp. showed decreases from spring to summer followed by increases in fall, which suggested potential temperature effects. Inactivation effects from increased ultraviolet light exposures might also increase with longer day length, however, the extreme turbidity of the lagoon water likely protected the target bacterial populations from UV light, even at 4-cm depths. Therefore, the respective bacterial groups were analyzed to investigate potential correlations with lagoon temperature only. Significant ($P < 0.05$) correlations with lagoon temperature were found for *Campylobacter* spp. (Fig. 5a), enterococci (Fig. 5b), *Listeria* spp. (Fig. 5c), and *Salmonella* spp. ($P < 0.1$) (Fig. 5d). Bui et al. (2011) and others (Placha et al., 2001; Arrus et al., 2006) have reported declining survival of *Campylobacter coli* and *Salmonella*, respectively, with increasing temperatures in swine waste environments.

Table 3 – Collection date means of bacterial populations from anaerobic swine manure lagoon water samples.

Sample collection date	Bacterial population (Log ₁₀ cfu or MPN 100 mL ⁻¹) ^a						
	Campylobacter MPN	C. perfringens cfu	E. coli cfu	Enterococcus cfu	Listeria MPN	Salmonella MPN	Staphylococcus cfu
26 Jan 09	4.92 a ± 0.06	6.00 b ± 0.06	5.46 d ± 0.18	5.17 a ± 0.04	2.92 a ± 0.09	2.83 a ± 0.13	6.71 ab ± 0.14
09 Mar 09	md ^b	6.19 b ± 0.06	6.06 c ± 0.04	5.04 b ± 0.06	3.27 a ± 0.15	1.00 c ± 0.26	6.76 a ± 0.04
27 Apr 09	4.15 c ± 0.11	5.62 d ± 0.07	6.01 c ± 0.06	4.51 c ± 0.15	2.34 b ± 0.13	1.36 b ± 0.29	6.33 c ± 0.03
15 Jun 09	3.59 d ± 0.13	5.79 c ± 0.06	5.93 c ± 0.07	4.70 c ± 0.11	1.91 b ± 0.29	1.04 c ± 0.30	6.52 bc ± 0.04
17 Aug 09	3.78 d ± 0.11	5.99 bc ± 0.13	5.89 cd ± 0.14	4.79 c ± 0.03	0.13 c ± 0.21	0.88 c ± 0.31	6.54 bc ± 0.07
05 Oct 09	4.45 b ± 0.07	6.31 ab ± 0.16	6.16 bc ± 0.04	4.97 b ± 0.03	1.95 b ± 0.04	0.87 c ± 0.21	6.29 c ± 0.07
16 Dec 09	4.56 b ± 0.09	5.47 d ± 0.11	6.34 ab ± 0.07	md	2.56 b ± 0.08	1.27 bc ± 0.32	5.45 c ± 0.51
22 Feb 10	4.62 ab ± 0.14	6.53 a ± 0.03	6.35 a ± 0.01	5.03 b ± 0.01	2.94 a ± 0.09	1.89 b ± 0.07	6.57 bc ± 0.04

a Least squares means (±SE) of bacterial populations measured as colony forming units (cfu) or most probable number (MPN), combined across 4-cm and 1-m sampling depths for each collection date. Means followed by the same letter within a bacterial population column were not significantly different ($P > 0.05$).

b Missing data (md).

As enteric pathogens, well adapted to animal gut temperatures of 37–39 °C, *Salmonella* spp. are well adapted to the relatively higher temperatures recorded in the present study from lagoon water in summer (30 °C). Therefore, temperature alone is likely not the direct cause of the observed decline in *Salmonella* counts. Increased levels of water-soluble metals could be a factor, for although Cu, Fe, Mn, and Zn, are important in oxidation–reduction reactions and trace amounts are required for microbial enzymatic processes (Kayranli et al., 2010; Burgin et al., 2011; Glass and Orphan, 2012), higher concentrations of these metals, especially Cu and Zn, have known microbial toxicity (Özbelge et al., 2005). Both Cu and Zn are added to swine diets to improve performance and as antibacterial agents to reduce enteric pathogens and prevent disease (Wells et al., 2010). Other potential factors in the seasonal *Salmonella* decline include increased solar radiation (Fig. 2d) and increased competition and predation from other bacteria and microorganisms, including bacteriophages (Callaway et al., 2011). *Salmonella*-specific bacteriophages have been isolated from water samples collected from this and similar lagoons in the area (McLaughlin et al., 2006), although their temporal distribution and concentrations are unknown. Furthermore, the dynamics of *Salmonella* population flux in lagoon water may not be fully explained by lagoon factors alone. The cyclical nature and continually overlapping litters of the CAFO sow farm farrowing operation, combined with the integrated system of moving replacement gilts and feeder pigs within and between farms, and the constant recycling of lagoon water to flush barn pits, carries the inherent potential for continuous exposure of naïve animals in new litters to *Salmonella* and subsequent resupply of freshly shed *Salmonella* in variable but continuous inputs back to the lagoon (Door et al., 2009; Davies, 2011).

Potential correlations of bacterial levels with nutrient levels, pH, and EC were also examined graphically and by correlation analysis. Plotting *E. coli* and pH over time showed a potential correlation of *E. coli* and pH (Fig. 6a), which was confirmed by correlation analysis (Fig. 6b). No other effects on bacterial levels were found from further analyses of nutrient levels, pH or EC.

3.3.2. Bacterial spatial flux

In the present study, only *C. perfringens* counts differed ($P < 0.05$) between water sample depths, with means of 6.085 (±0.063) Log₁₀ cfu 100 mL⁻¹ in 1-m samples ($n = 42$) and 5.89 (±0.06) Log₁₀ cfu 100 mL⁻¹ in 4-cm samples ($n = 48$). Cook et al. (2010), using quantitative polymerase chain (qPCR) reaction methods to compare samples from different depths in a sow farm lagoon in Kentucky over a 12-month period, concluded that species of *Clostridiales* were widespread in slurry, and reported much higher cell counts than those of the present study. Although statistical analysis of the Cook et al. (2010) data were not reported, estimated cell counts ranged from <5 logs mL⁻¹ to >7 logs mL⁻¹ with lower counts in some bottom (>198 cm deep) samples, little difference between spatial counts (top, 51 cm, and 152 cm), and temporal variations within a range of about 1.5–2 logs. By comparison, results from the present study showed a range from about 5.5 to 6.5 Log₁₀ cfu 100 mL⁻¹, but higher counts in deeper samples, as noted above. In addition, the present study found even

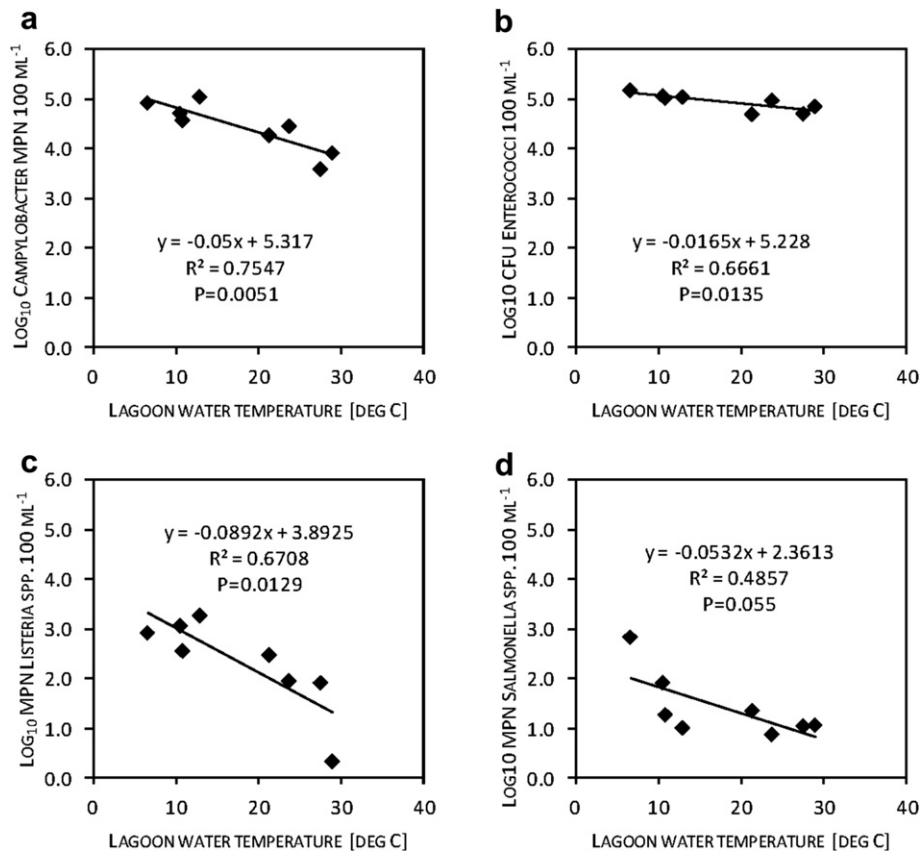


Fig. 5 – Correlations of lagoon water temperature and bacterial counts were significant ($P < 0.05$) for (a) *Campylobacter* spp., (b) enterococci, (c) *Listeria* spp., and (d) ($P < 0.1$) *Salmonella* spp.

higher counts from six 1-m samples (two samples each from 27 Apr and 17 Aug 2009, and 22 Feb 2010) that were excluded in statistical comparisons because they contained appreciable amounts of sludge (3.2.2). The *C. perfringens* counts from these six sludge-containing samples ranged from 6.7 to 8.3 Log₁₀ cfu 100 mL⁻¹ with a mean of 7.6 (± 0.5) Log₁₀ cfu 100 mL⁻¹.

Apparent differences in spatial distribution and magnitude of counts between the study by Cook et al. (2010) and the present study may be due to inherent differences between the

respective lagoons and to differences in methodology. Higher overall counts typically result from qPCR, which measures DNA from live and dead cells. Cultural assay in the present study specifically targeted spores by eliminating live somatic cells with a heat shock treatment (McLaughlin et al., 2009). Spore counts and qPCR inhibitors would be expected to be higher in sediment (Mueller-Spitz et al., 2010) and spores would be expected to be more resistant than somatic cells to DNA extraction. Additionally, Cook et al. (2010) reported total cell numbers from qPCR using *Clostridia-Eubacteria* group primers

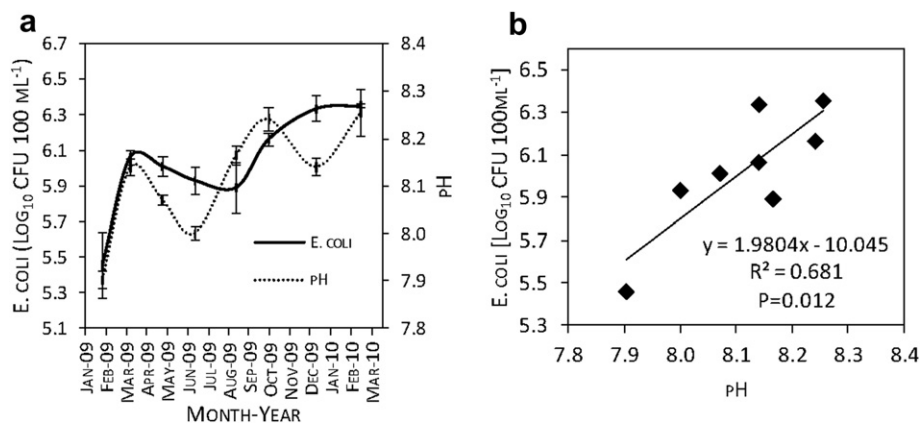


Fig. 6 – Correlation between *E. coli* and pH in lagoon water. (a) Combined means (4-cm and 1-m samples, $n = 12$) and standard error bars for collection dates, and (b) linear model of the means.

that detected multiple genera and species, while cultural methods in the present study detected only *C. perfringens*.

4. Conclusions

This study characterized the water quality of an anaerobic swine manure lagoon over a 14-month period. Temporal and spatial differences in levels of nutrients and bacteria, including zoonotic species, were described and analyzed. These results and the conclusions noted below will enable better informed, safer, and more efficient management and use of anaerobic manure lagoons and other impounded water resources.

- Stratification (higher levels in deeper samples) of most nutrients and *C. perfringens*, suggested that water samples should be collected at multiple depths to accurately characterize these components of lagoon water quality; however, lack of stratification of most bacterial groups, including *E. coli*, enterococci, *Campylobacter* spp., *Listeria* spp., *Salmonella* spp., and staphylococci confirmed that relatively shallow surface samples are adequate for their respective characterizations.
- Temporal changes observed in nutrient and bacterial levels suggests that water samples should be collected at least seasonally for accurate characterization of lagoon water quality.
- An empirical formula (Eq. (2)) developed to estimate annual flux of total N in anaerobic lagoon water may be a useful and practical tool for managing N levels in other lagoons.
- Levels of total N and inorganic C were highly correlated with water temperature; both accumulated below 19 °C and declined above 19 °C with direct implications for increased summertime emissions of ammonia and greenhouse gasses.
- Temporal changes in levels of heavy metals and some bacteria were correlated with other parameters, including: increasing *E. coli* levels with increasing pH; decreasing populations of *Campylobacter* spp., enterococci, *Listeria* spp., and *Salmonella* spp. with increasing temperature; and higher levels of the water-soluble heavy metals Cu, Fe, and Zn, with increasing temperature.
- Levels of the respective bacteria in lagoon water varied significantly during the year, but all species, including zoonotics, were consistently present year-round; thus, the zoonotic species pose ever present, but seasonally changing risks that make risk assessments more complex.

A declining ratio of N:P in lagoon water observed during the summer crop irrigation season, suggests the need for testing of nutrient levels during the season, coincident adjustment of irrigation rates, and periodic review and adjustment of nutrient management plans to prevent excess P accumulations in soils fertilized to meet crop N demand.

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