VARIATION OF DISSOLVED ORGANIC MATTER COMPOSITION IN EFFLUENT FROM SWINE WASTE-RETENTION BASINS: A CASE STUDY OF YUJIANG COUNTY, CHINA

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ABSTRACT

The main purpose of this study was to elucidate the variation of dissolved organic matter (DOM) composition in effluent from swine waste-retention basins of different concentrated swine feeding operations (CSFOs) located in Yujiang County of Jiangxi Province, China. Three fluorescence components were identified using three-dimensional excitation-emission matrix and parallel factor analysis, including tryptophan-like C1, tyrosine-like C2, and humic-like C3. For all samples examined, percentage of C1 varied from 0.49 to 0.61, and the range of C2 and C3 was 0.05–0.31 and 0.09–0.45, respectively. Moreover, C1 linked positively with C2 ($R^2 = 0.285$, $p < 0.05$), while negatively related to C3 ($R^2 = 0.577$, $p < 0.001$). Humification degree of DOM was adverse to biological activities ($R^2 = 0.946$, $p < 0.001$), and humic substances were predominantly derived from terrestrially DOM. In the present study, dissolved organic carbon concentration and C/N ratio significantly affected DOM composition in swine effluent.

KEYWORDS:
Concentrated swine feeding operations; dissolved organic matter; three-dimensional excitation-emission matrix; parallel factor analysis; redundancy analysis

INTRODUCTION

In 2008, concentrated swine feeding operations (CSFOs) accounted for 56 percent of pig farms in China, and the ratio has approached to 65% by the year 2015. With the increase of CSFOs, more and more public concerns have been focused on their potential environmental effects [1]. At present, industrial-scales increased the risks of water contamination owing to large amounts of swine effluent containing high organic loading generated at a central place [2-7].

In swine effluent, dissolved organic matter (DOM) accounted for the most fractions of nutrients [6]. As one kind of organic polymer, DOM is generally composed of a complex mixture, ranging from low molecular weight (MW) substances, such as amino acids and carbohydrates, to high MW components, such as humic substances [8, 9]. Meanwhile, DOM properties differed with pig farms owing to the different farming scales, and feed formulas [4]. Furthermore, microorganism also played a more important role in the degradation of organic matter in wastewater [10, 11]. So, it is often difficult to characterize DOM component with traditional methods. Since the works of Stedmon and Bro (2008), fluorescent spectroscopy has been used effectively to characterize DOM components with parallel factor analysis (PARAFAC) [12-15]. Two major fractions of fluorescence DOM found in swine slurry were humic-like and protein-like components [16-18]. To date, although piles of studies focused on DOM have been carried out, knowledge about the variation of DOM composition between different CSFOs is still limited.

In the present study, a case study was carried out to elucidate the variation of DOM composition in swine effluent from waste-retention basins of different CSFOs, which encompassed a scale gradient ranged from 2000 to 24000 head, located in Yujiang County. The main purpose of this study was to characterize the DOM components in swine effluent using the combined technique of three-dimensional excitation-emission matrix (3DEEM) and PARAFAC, and made certain of the main environmental parameters that affected the variation of DOM composition.

MATERIAL AND METHODS

Study area and sample collection. Yujiang County (116°41′–117°09′ E, 28°04′–28°37′ N), located in the northeast of Jiangxi Province, China, is characterized by a subtropical humid monsoon climate with annual average temperature, rainfall and sunshine hours of 17.6°C, 1766 mm, and 1809 h, respectively [19]. In 2010, the percentage of pig farms with ≤ 2000, 2000–10000, and > 10000 heads accounted for 78.6%, 19.9%, and 1.2% of the total CSFOs, respectively, with the number of pigs being 41.4%, 43.4%, and 12.3%, respectively [20].

Eight CSFOs were selected randomly for study, and the abbreviation and annual pig marketing rate were embodied in the parenthesis, respectively: Guan Zhucheng (GZC, 2000 head), Wu Yanggao (WYG, 2000 head), Yong Sheng (YS, 4000 head), Cheng Lin (CL, 5000 head), Pan Luping (PLP, 5000 head), Zhong Tong (ZT, 12000 head), Wan Gu (WG, 20000 head), and Zheng Bang (ZB, 24000 head). More detail information about the geographical locations of these CSFOs is shown in the Supplemental file Fig.S1. In general, a swine feed formula, which consisted of 63–70% corn flour, 20–30% soybean pulp, and 5–20% wheat bran, was applied to these CSFOs. A mean of 2 kg solid manure, 3.3 kg urine and 8.0 kg wastewater (plus rinsed water) was generated by a fattening swine every day [20].

Swine effluent samples were collected in 500-mL acid-cleaned polyethylene bottles from the surface of waste-retention basins in triplicate at each CSFOs in December 2013. Sampling points were close to the center of basins at 5-meter intervals in a north-south line, and all samples were stored in an ice box and delivered back to laboratory within 24 hours. Prior to analyze DOM, all samples were filtered through pre-combusted 0.7 μm GF/F filters (Whatman, UK), and the filtrate was stored in pre-combusted (550 °C for 6 h) glass bottles and preserved in refrigerator (4 °C) for a further analysis. All measurements were completed within 7 days.

UV-Visible absorbance and fluorescence analysis. The absorption features of DOM between 200 and 600 nm were measured using an ultraviolet and visible spectrophotometer (Eppendorf), with a 1 cm quartz cuvette and MilliQ water as a reference. In the present study, the ultraviolet absorbance at 254 nm (UV_{254}) was applied to indicate the aromatic character of the DOM in swine effluent [21].

Prior to the fluorescence analysis, all samples were diluted and adjusted them comparable to each other with a dissolved organic carbon (DOC) concentration in 7 mg/L [16]. Fluorescence analysis was completed using a Hitachi F-7000 fluorescence spectrometer, and the excitation/emission scanning ranges were 200–450 nm and 250–600 nm, respectively; the readings were collected at 5-nm intervals for excitation and at 1-nm intervals for emission with a scanning speed of 2400 nm/min, and the band-passes of excitation and emission were both set at 5 nm [22]. A MilliQ water (18.0 Ω) blank was subtracted from fluorescence excitation-emission matrices (EEMs) to eliminate the Raman scatter peaks.

In order to remove the inner-filter effect thoroughly, the EEMs were revised for absorbance by multiplying every reading in the EEMs with a revision modulus, based on the premise that the average path length of absorption of the emission and excitation light is 1/2 of the cuvette length [23]. The revision was expressed as follows:

$$F_{Re} = F_{Obs} \times 10^{(A_{Ex} + A_{Em})/2}$$

(1)

where, F_{Re} and F_{Obs} are revised and unrevised fluorescence intensities, and A_{Em} and A_{Ex} are the absorbance values at the current emission and excitation wavelengths, respectively.

Fluorescence intensity was normalized as quinine sulfate units (QSU), where 1 QSU is defined as the maximum fluorescence intensity of 0.01 mg/L of quinine in 1 N H_2SO_4 at the excitation/emission wavelength of 350 nm/450 nm [24]. Moreover, the Rayleigh scatter effects were eliminated from the data matrices by excluding any emission measurements in the two triangle regions (emission wavelength ≤ excitation wavelength + 5 nm, and ≥ excitation wavelength + 300 nm), and the missing values of the two triangle regions were replaced with zeroes [22].

PARAFAC modeling. Without any assumptions on the spectral shape or the number of fluorescent components, the complex mixture of DOM has been decomposed into individual fluorescent components using PARAFAC [22]. Compared with bilinear solutions (e.g. PCA), PARAFAC decodes the data matrix into a set of trilinear terms and a residual array, and the modeling has been completed by the processes of minimizing the sum of square residuals [25]:

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{i,jk}$$

(2)

where, x_{ijk} represents the fluorescence intensity of the ith sample at emission wavelength j and excitation wavelength k, and F is the number of components. The a_{if} is directly proportional to the fth component concentration in the ith sample; both b_{jf} and c_{kf} are linearly linked to the emission and excitation spectra at wavelengths j and k

respectively for the fth component, and ε_{ijk} is the variability not accounted for by the model [26].

The PARAFAC analysis was performed using MATLAB (R2009a, USA) with the DOMFluor toolbox according to the works carried out by Stedmon and Bro (2008) [12]. A total of 24 EEMs of effluent samples from these CSFOs were applied for PARAFAC analysis, and the fluorescent spectra of components were drawn using MATLAB

Fluorescence indices. Humification index (HIX) was introduced by Zsolnay et al. (1999) and applied to determine the maturation degree of soil DOM initially [27]. It was defined as the ratio of two spectral region areas (300 nm and 345 nm, 435 nm and 480 nm) from the emission spectrum scanned for excitation both at 254 nm [28]. In the present study, to decrease the inner filtration effects, HIX was defined as the ratio of the fluorescence intensity from 435 to 480 nm divided by the sum of fluorescence intensity from 300 to 345 nm and from 435 to 480 nm, both excited at 255 nm and the reading of excitation determined at 5-nm intervals mentioned earlier [22, 29]. Moreover, HIX values are in the range of 0–1, increasing with the degree of aromaticity of DOM.

Biological index (BIX) is often used to determine the presence of the β fluorophore, characteristic of autochthonous biological activity in water samples, and defined as the ratio of the fluorescence intensity at an emission wavelength of 380 nm divided by the maximum fluorescence intensity within 420–435 nm in this study, both excited at 310 nm [28]. High BIX value (> 1) indicated more autochthonous origin of DOM, whereas a low value (0.6–0.7) indicated lower autochthonous DOM production in natural waters [22].

To distinguish sources of isolated aquatic fulvic acids, McKnight et al. (2001) has introduced a fluorescence index (FI_{370}): the ratio of fluorescence intensity at emission wavelength of 450 nm divided by fluorescence intensity at emission wavelength of 500 nm, both excited at 370 nm [23]. The index value of microbial-derived fulvic acids is ≥ 1.9, and the value of terrestrial-derived fulvic acids is ≤ 1.4 [22].

Chemical measurement and statistic analysis. Electrical conductivity (EC) and pH were measured using a FE30 conductivity meter (Mettler Toledo, Switzerland) and a pH meter (HANAN, Italy), respectively. Chemical oxygen demand (COD) was analyzed according to the standard method [30]. DOC was measured from the difference between TC and IC using a Multi N/C 3100 analyzer (analytikjena, Germany), and total dissolved nitrogen (TDN) was determined by UV digestion method using flow analysis and spectrometric detection (Skalar San++, Dutch). In the study, the C/N ratio is defined as the ratio of DOC divided by TDN [31].

Statistical analyses, including the mean value, coefficient of variation, and analysis of variance, were completed with SPSS 20.0 software (Statistical Program for Social Sciences). All linear regression analyses were completed using OriginPro 9.1 (USA), and the redundancy analysis (RDA) was performed using R 3.0.3 statistic software (New Zealand) with a vegan package. All statistically significant differences were at the level of $p < 0.05$ unless stated otherwise.

RESULTS AND DISCUSSION

Environmental parameters in swine effluent. The variation of environmental parameters in swine effluent is summarized in Table 1.

TABLE 1
Variations of environmental parameters in swine effluent

CSFOs	pH	EC (mS/cm)	COD (O₂, mg/L)	UV₂₅₄ (cm⁻¹)	DOC (mg/L)	TDN (mg/L)	TP (mg/L)	C/N ratio
GZC	7.62±0.01 e	2.27±0.02 f	898.0±129.5 b	2.07±0.13 cd	136.6±13.5 de	185.7±3.9 d	47.8±1.1 a	0.74±0.08 b
WYG	8.44±0.05 a	6.01±0.07 a	1628.3±20.7 a	6.37±0.16 a	574.8±33.6 a	518.9±2.8 a	14.9±2.9 de	1.11±0.06 a
YS	7.93±0.01 d	3.30±0.01 e	168.6±44.9 de	1.99±0.12 cd	213.1±39.0 bc	315.7±3.0 c	24.0±1.1 c	0.67±0.12 b
CL	8.34±0.01 b	5.28±0.07 c	1003.0±194.1 b	4.21±0.65 b	238.5±34.6 b	476.8±2.4 b	16.6±0.5 d	0.50±0.07 c
PLP	8.09±0.01 c	5.42±0.03 b	272.3±0.0 de	2.35±0.08 c	164.7±70.3 cd	478.6±12.4 b	31.4±0.7 b	0.34±0.14 d
ZT	8.06±0.10 c	0.88±0.01 h	138.9±25.2 e	0.64±0.12 e	12.1±1.1 f	54.9±7.9 e	13.1±1.1 e	0.22±0.03 de
WG	7.98±0.02 d	2.17±0.01 g	730.3±115.5 c	1.75±0.24 d	88.2±3.4 e	178.7±3.7 d	24.8±0.3 c	0.49±0.01 c
ZB	7.92±0.02 d	3.41±0.00 d	340.0±36.2 d	1.69±0.12 d	26.8±13.2 f	312.9±2.0 c	25.1±0.2 c	0.09±0.04 e
CV (%)	3.1	48.5	77.0	66.0	97.3	50.9	43.4	60.5

CSFOs, concentrated swine feeding operations; EC, electrical conductivity; COD, chemical oxygen demand; UV₂₅₄, ultraviolet absorbance at 254 nm; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; TP, total phosphorus; C/N, the ratio of DOC divided by TDN; CV, coefficient of variation. The error was measured using three replicates; different lowercases within the same column indicate the significant at the level of $p < 0.05$.

For all samples examined, pH and EC ranged from 7.62 to 8.44 with a coefficient of variation (CV) of 3.1% and 0.88 mS/cm to 6.01 mS/cm (CV = 48.5%), respectively, indicating a low alkalinity and high salinity. The concentration of COD varied from 138.9 mg/L to 1628.3 mg/L (CV = 77.0%), suggesting a high organic loading in swine effluent. The range of UV_{254} and DOC was 0.64–6.37 cm^{-1} (CV = 66.0%) and 12.1–574.8 mg/L (CV = 97.3%), respectively. TDN and TP varied from 54.9 mg/L to 518.9 mg/L (CV = 50.9%) and from 13.1 mg/L to 47.8 mg/L (CV = 43.4%), respectively. The range of C/N ratio was 0.12–1.06 (CV = 60.5%). In all environmental parameters, DOC had the largest coefficient of variation followed by COD, indicating a huge variation of organic loading between these CSFOs.

The high free ammonia concentration accounted for the slightly basic pH for all effluent samples examined [32]. EC was linked with TDN significantly (R^2 = 0.991, p < 0.001, data not shown), and it had been applied to predicate the nutrients value in swine slurry as positive relationships were noticed between EC and NH_3-N [33]. A positive relationship was observed between UV_{254} and DOC (R^2 = 0.895, p < 0.001, data not shown), and both can be used as surrogate indices for the DOM in wastewater [21]. Moreover, the TDN varied as same as DOC (R^2 = 0.418, p < 0.01, data not shown), suggesting a common source or the same variation [34]. The range of TP concentration was consistent with the previous study. In Yujiang, the range of phosphorus was 4.1-66.6 mg kg^{-1} in the wastewater discharged from biogas system of the CSFOs [20]. In swine effluent, a high concentration of TDN was apt to bias a low C/N ratio. According to the previous studies, a low C/N ratio would restrict the removal efficiency of organic matter from swine effluent [35, 36].

PARAFAC modeling of DOM components. In the present study, although two protein-like components and one humic-like component were identified by PARAFAC analysis based on a total of 24 EEMs of effluent samples, it did not mean that only three fluorophores exist in all the samples or all the three fluorophores were present in each sample examined. The EEMs spectra of these fluorescence components are shown in Fig. 1.
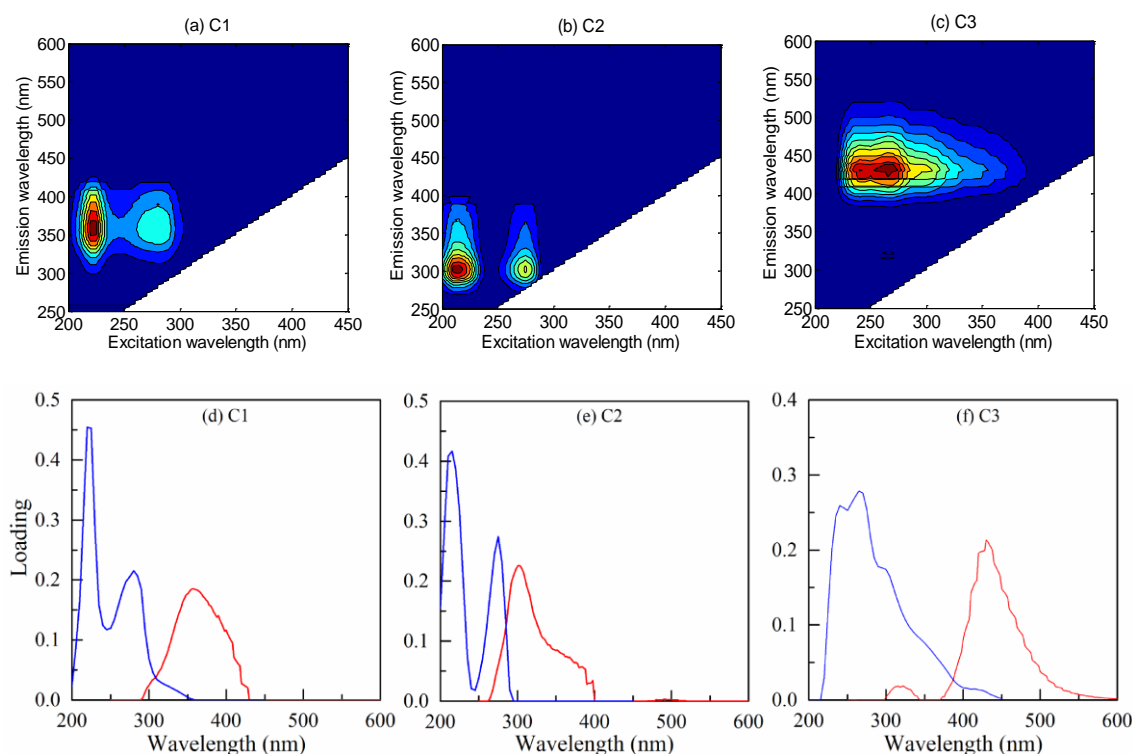

FIGURE 1
PARAFAC model output showing fluorescence signatures of three fluorescence components (a-c), split-half validation results of the three components, excitation (left, blue) and emission (right, red) spectra were estimated from two independent halves of the dataset; C1-tryptophan-like component, C2-tyrosine-like component, C3-humic-like component.

C1 exhibited two fluorescence peaks, with the major and minor peaks at 225/350 and 275/350 nm, respectively (Fig.1a, d), similar to the tryptophan-like fluorophores observed in seawater or lakes and was named as peak T generally [22, 37, 38]. The documented sources of tryptophan-like fluorescence were both autochthonous products and bio-available substrates [39, 40]. C2 also presented two excitation maxima (at 200 nm and 350 nm) at the corresponding maximum emission wavelength of 300 nm (Fig.1b, e). These fluorescence characteristics were identical to the previously reported tyrosine-like fluorescence and named as peak B generally [37], which were derived from autochthonous processes [22, 40]. In swine effluent, the protein-like components would come from the swine forage containing a higher percentage of crude protein. C3 displayed a primary fluorescence peak at an excitation/emission wavelength of 265 nm/425 nm (Fig.1c, f), similar to the UVA humic-like components whose main constituent was fulvic-like acid and noted in many aquatic systems [16, 25, 37, 40]. Moreover, the blue shift of C3 with respect to peak A, is observed, which may be attributed to the high concentration of artificial materials (e.g. N-containing compounds) in swine effluent [40]. Compared with humic-like component, tryptophan-like component was the predominant fluorophore in all examined samples, mostly in GZC and WYG.

Fluorescence components percentage. The relative concentration of each component was defined as the ratio of the maximum fluorescent intensity (F_{max}) of each component divided by the sum of F_{max} of all identified components. Compared with tyrosine-like C2 and humic-like C3, tryptophan-like C1 had a higher F_{max} generally in all samples examined. The percentage of C1 was in the range of 0.49–0.61 (CV = 7.7%). C2 and C3 varied from 0.05 to 0.31 (CV = 55.4%) and from 0.09 to 0.45 (CV = 53.6%), respectively. Moreover, with the increase in the farming scale, the percentage of protein-like components decreased roughly, while humic-like component increased accordingly. More detail information is shown in the Supplemental file Table S1.

In swine effluent, high concentrations of protein-like components were attributed to autochthonous biological degradation of plentiful terrestrial DOM [25, 40], and its presence indicated a low humification degree and a high level of bio-available organic matter in swine effluent [41]. In general, the tryptophan-like peak was more pronounced than the tyrosine-like peak at the same molar concentration, due to the higher quantum yield of former [42, 43]. Meanwhile, compared with humic-like components, the protein-like components were apt to be used by the heterotrophic bacteria as its ephemeral nature in aquatic system [39-41]. Through linear regression, a positive relationship was observed between C1 and C2 ($p < 0.05$, Fig.2a), and a negative relationship existed between C1 and C3 ($p < 0.001$, Fig.2b). It had been reported that both of the fluorescence intensities of protein-like components were significantly correlated with the concentration of amino acids [39], suggesting a common source. Moreover, as one possible mechanism, amino acids and proteins can be incorporated into humic substances for avoiding the microbial degradation [39]. Thus, the concentration of humic-like component would be strengthened as the microbial degradation of protein-like components [41].

Variation of fluorescence indices. For all samples examined, the HIX ranged from 0.92 to 0.99 (CV = 3.1%), and the BIX and FI_{370} were varied within 0.74–2.42 (CV = 46.2%) and 1.14–1.69 (CV = 12.3%), respectively. In general, with the increase in the farming scale, HIX increased gradually, while BIX decreased accordingly. Moreover, the values of FI_{370} indicated the primary source of humic substances was terrestrially-derived DOM in swine effluent, except for WG, which was a mixture of terrestrially- and microbially-derived DOM. More detail information is shown in Supplemental file Table S1.
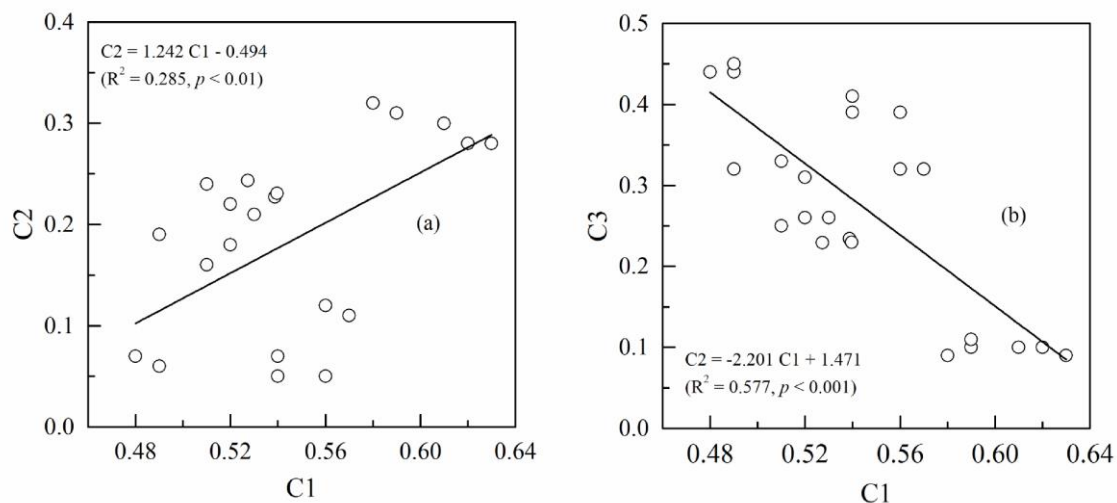
FIGURE 2
Linear relationships between C1 and C2 (a), C1 and C3 (b); C1-tryptophan-like component, C2-tyrosine-like component, C3-humic-like component.

Through linear regression, the BIX correlated significantly with both of HIX and FI_{370} ($p < 0.001$, Fig.3a-b), respectively, and a significant positive relationship was observed between HIX and FI_{370} ($p < 0.001$, Fig.3c). As the aforementioned, a high concentration of protein-like components indicated a low humification degree and a high level of bio-available organic matter in swine effluent [41], which could favor a high degree of biological activities [44-46]. The humification degree of DOM would be strengthened as the microbial degradation of organic matter during the process of anaerobic or aerobic digestion [18, 32, 47]. In general, DOM containing a higher aromaticity was difficult to be utilized by microorganisms and induced a lower microbial activity subsequently [48, 49]. Thus, a high humification degree was adverse to biological activities in aquatic systems. Meanwhile, compared with previous studies, the conclusions about relationship between FI_{370} and BIX were controversial. Guo et al. (2012) studied that the FI_{370} increased with the production of microbially-derived organic matter and linked negatively with the BIX [16]. However, a significantly positive relationship had also been observed between BIX and FI_{370} in lakes of Yungui Plateau [22]. In general, FI_{370} increased with the formation of microbially DOM consisted of a low aromaticity, and decreased with the production of terrestrially DOM commonly contained a higher aromaticity [23]. Thus, the possible reasons for the inconsistent were that the rapid metabolism and self-degradation of a high level of microorganisms contributed to the increase of microbially-derived DOM in eutrophic systems (e.g. swine slurry), whereas more terrestrially-derived DOM were utilized for the maintenance of microbial metabolism in oligotrophic streams [49]. Moreover, the FI_{370} increased with the HIX owing to the decreased biological activities as the aforementioned.

Relationships between environmental parameters and DOM components. Fig.4 had visualized the potential relationships between environmental parameters and DOM components across all effluent samples examined through the ordination of redundancy analysis (RDA), and pH, DOC, TP, and C/N were remained as the main constrained factors. All the four constrained factors had explained a total of 51.6% of the variance of DOM composition between different CSFOs, and only RDA1 was the significant and explained 48.6% of the total variance ($p < 0.01$). The relative significance of each factor was elucidated in the ordination diagram by the length of their corresponding centrifugal lines, and two significant factors were screened out, namely DOC ($R^2 = 0.41$, $p < 0.001$) and C/N ($R^2 = 0.47$, $p < 0.001$), respectively. The variance explained by the two ordination axes was a bit low, which was attributed to the absent of biological factors.
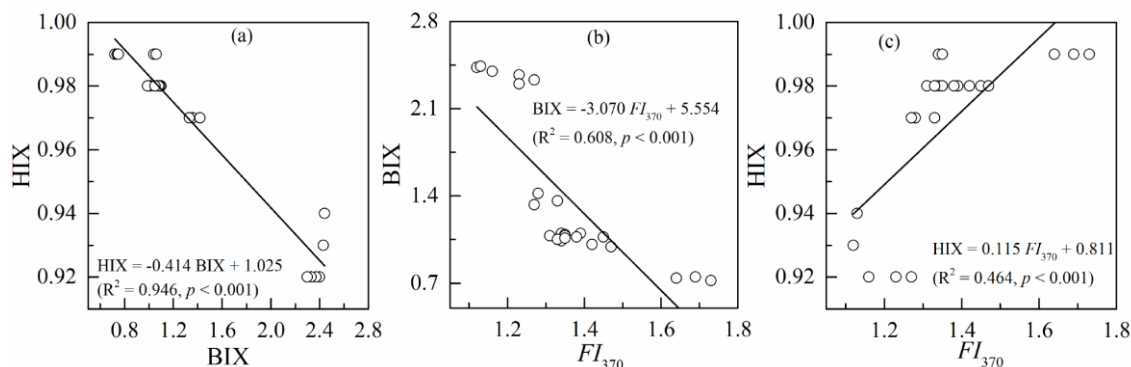
FIGURE 3

Linear relationship between HIX and BIX (a), BIX and FI_{370} (b), HIX and FI_{370} (c), HIX-humification index, BIX-biological index, FI_{370}-the ratio of fluorescence intensity at an emission wavelength of 450 nm divided by fluorescence intensity at an emission wavelength of 500 nm, both excited at 370 nm.
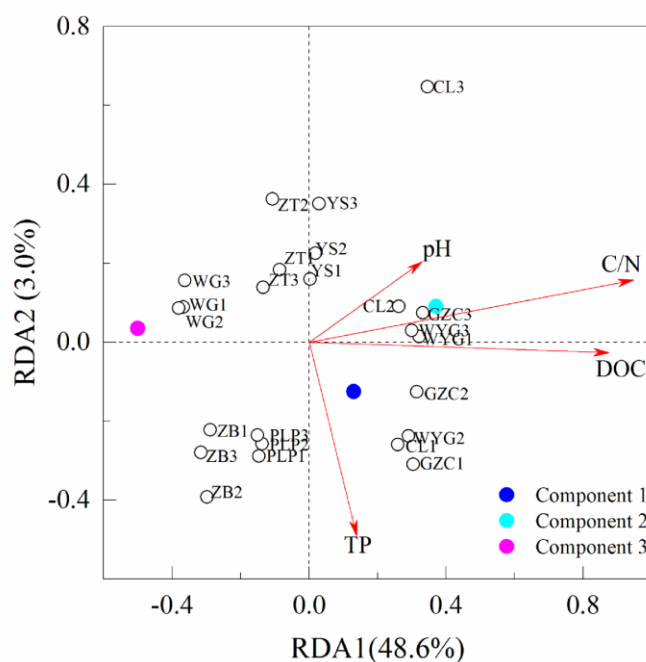

FIGURE 4

RDA plot showing the relationships between environmental parameters and fluorescence components across all effluent samples. The RDA1 explained 48.6%, and RDA2 explained 3.0% of the total variances of DOM composition. Red lines represent the environmental parameters; Component1-tryptophan-like, Component2-tyrosine-like, Component3-humic-like; GZC, WYG, YS, CL, PLP, ZT, WG, and ZB-the abbreviations of concentrated swine feeding operations.

At present, DOC was widely used to represent the organic loading in wastewater discharged from the biogas system of CSFOs [20, 50], and a significant and positive relationship was observed between DOC concentration and protein-like fluorescence intensity [51]. Moreover, fluorescence intensity of protein-like components would be strengthened as the addition of fresh swine slurry [16], and protein-like peak was significantly correlated with the concentration of total organic acids [17]. In general, compared with small-scale CSFOs (GZC and WYG), sufficient

treatments (e.g. anaerobic or aerobic digestion) have often been compulsively applied to process the swine effluent at large-scales (WG and ZB). Anaerobic or aerobic digestion has been proved to be effective methods to remove chemical oxygen demand and organic pollutants from wastewater [52]. Thus, high DOC concentration favored a high level of protein-like components and biological activities. In general, the rate of carbon consumption was 25–30 times faster than that of nitrogen during the course of anaerobic digestion [53]. Compared with large-scale CSFOs, the C/N ratio of small-scale CSFOs was higher owing to a higher content of undigested or fresh swine slurry. Moreover, it was well established that an optimal anaerobic or aerobic microbial metabolism could achieve with a higher C/N ratio [54]. Thus, the high concentration of DOC and C/N ratio accounted for the variation of DOM composition in swine effluents.

CONCLUSIONS

In swine effluent, there existed discrepancies in DOM properties between different CSFOs. In the present study, three fluorescence components (tryptophan-like, tyrosine-like, and humic-like) were identified using the combined technique of 3DEEMs and PARAFAC. Compared with humic-like component, protein-like components were the predominant fluorophores generally in swine effluent, mostly in small-scale CSFOs. Meanwhile, the biological activities linked negatively with humification degree of DOM, and the primary source of humic substances was a mixture of terrestrially-derived DOM in all samples examined. Through redundancy analysis, both the DOC concentration and C/N ratio significantly affected the DOM composition. In sum, the combined technique of 3DEEM and PARAFAC could provide a feasible and convenient method for analyzing the variances of DOM composition in swine effluent.

ACKNOWLEDGEMENTS

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