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Urbanization gradient of selected pharmaceuticals in surface water at a watershed scale

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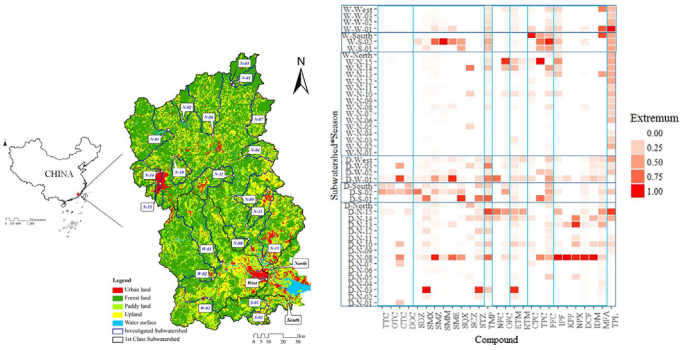
HIGHLIGHTS

- Instream pharmaceutical loads along a subwatershed-based urbanization gradient.
- 27 out of 33 selected compounds in 3 categories were quantified in surface water.
- Urban land use in subwatersheds was highly correlated to instream pharmaceuticals.
- Evident seasonality of level and number of instream pharmaceuticals was observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Ubiquitous detection of pharmaceuticals in the aquatic environment around the world raises a great public concern. Aquatic residuals of pharmaceuticals have been assumed to relate to land use patterns and various human activities within a catchment or watershed. This study generated a gradient of human activity in the Jiulong River watershed, southeastern China by urban land use percentage in 20 research subwatersheds. Thirty-three compounds from three-category pharmaceuticals [26 compounds of 5 antibiotic groups, 6 compounds of non-steroidal anti-inflammatory drugs (NSAIDs), and 1 compound of respiratory system drugs (RSDs)] were quantified in stream water before the research subwatershed confluences with two sampling events in dry and wet seasons. In total, 27 out of the 33 pharmaceutical compounds of interest were found in stream waters. Seasonality of instream pharmaceuticals was observed, with less compounds and lower concentrations in the wet season sampling event than in the dry season one. Urban land use in the research subwatershed was identified as the main factor influencing in stream pharmaceutical concentrations and composition regardless of season. Rural land uses

Abbreviations: Tetracyclines (TCs), Tetracycline (TTC), Oxytetracycline (OTC), Chlortetracycline (CTC), Doxycycline (DOC); Sulfonamides (SAs), Sulfadiazine (SDZ), Sulfamethoxazole (SMX), Sulfamethazine (SMZ), Sulfamerazine (SMR), Sulfamonomethoxine (SMM), Sulfaquinoxaline (SQX), Sulfadimethoxine (SDM), Sulfameter (SME), Sulfaclozine (SCZ), Sulfathiazole (STZ), Synergist: Trimethoprim (TMP); Fluoroquinolones (FQs), Norfloxacin (NFC), Ofloxacin (OFC), Ciprofloxacin (CFC), Enrofloxacin (EFC), Difloxacin (DFC); Macrolides (MLs), Erythromycin-H2O (ETM), Roxithromycin (RTM), Tylosin (TLS); Chloramphenicols (CPs), Chloramphenicol (CPC), Thiamphenicol (TPC), Florfenicol (FFC); Non-steroidal anti-inflammatory drugs (NSAIDs), Naproxen (NPX), Ketoprofen (KPF), Diclofenac (DCF), Ibuprofen (IPF), Indomethacin (IDM), Mefenamic acid (MFA); Respiratory system drug (RSD), Theophylline (TPL); Standards, D_4 sulfamethazine (D_4-SMZ), D_8 ofloxacin (D_8-OFC), Erythromycin (*N* dimethyl ^{13}C) (^{13}C-ETM), D_5 chloramphenicol (D_5-CPC), D_3 mecoprop (D_3-MCP), ^{13}C phenacetin (^{13}C-PNC), D_5 Atrazine (D_5-ATZ), methyl D_3 benzeneamide D_4 (D_7-DEET), D_6 gemfibrozil (D_6-GFZ); SPE, Solid phase extraction; DEM, Digital elevation map; QA/QC, Quality assurance and quality control; LOD, Limit of detection; S/N, Signal-to-noise; LOQ, Limit of quantification; One-way ANOVA, One-way analysis of variance; NMDS, Non-metric multidimensional scaling; WWTP, Wastewater Treatment Plant.

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Land use pattern
Jiulong River

contributed a mixture of human and veterinary pharmaceuticals possibly from agricultural application of manure and sewage sludge and aquaculture in the research subwatersheds. Erythromycin in both sampling events showed medium to high risks to aquatic organisms. Results of this study suggest that urban pharmaceutical management, such as a strict prescription regulations and high-efficient removal of pharmaceuticals in wastewater treatment, is critical in reducing aquatic pharmaceutical loads.

1. Introduction

Large amounts of pharmaceutical compounds have been widely used as human and veterinary medicines and growth promoters in bee-keeping, livestock production, and aquaculture (Kümmerer, 2009) over decades. China produced approximately two million tons of active pharmaceutical ingredients in 2011, which doubled the production of 2003, accounting for over 20% of worldwide production (Liu and Wong, 2013). It was estimated that China consumed over 162,000 tons of antibiotics in 2013 of which approximately 48% was human medication (Zhang et al., 2015). An estimate indicated that Chinese daily dosage per thousand persons of antibiotics was approximately 6 times greater than residents in UK, USA, Canada, and European countries in 2013 (Zhang et al., 2015).

Given a huge variation of pharmaceutical uptake efficiency among various organisms, a considerable amount of pharmaceutical residuals has been assumably discharged into the aquatic environment (Kümmerer, 2009). Pharmaceutical compounds have been ubiquitously detected in natural ecosystems (Chen et al., 2013; Lapworth et al., 2012; Li et al., 2016; Luo et al., 2011; Peng et al., 2014; Tran et al., 2014; Wu et al., 2014; Xu et al., 2007) besides wastewaters from households, hospitals, livestock farms, aquacultural ponds, and pharmaceutical manufactures (Jiang et al., 2013; Luo et al., 2011; Sim et al., 2011; Xi et al., 2015; Zhu et al., 2013), In total, 94 pharmaceutical compounds, consisting of over 75% antibiotics, were reported at least once in surface waters and sediments in China mostly at a level of ng L^{-1} or ng g^{-1}, comparable to the rest world (Bu et al., 2013). Environmental residuals of pharmaceutical compounds are defined as a group of emerging micro-pollutants (Boxall et al., 2012; Daughton, 2004; Daughton and Ternes, 1999; Field et al., 2006) according to their ecosystem impacts on antibiotic resistance, endocrine disruption, and microbial community structure and function (Oaks et al., 2004; Peng et al., 2008; Pruden et al., 2006; Xi et al., 2015; Su et al., 2017; Zhang et al., 2015; Zhu et al., 2017).

Although most pharmaceuticals, such as antibiotics, are related to natural products (Li and Vederas, 2009), their escalating concentrations in the aquatic environment are anthropogenically caused. Pharmaceutical residues in the aquatic environment have been assumed in relation to the catchment land use patterns (Davis et al., 2006; Kemper, 2008; Li et al., 2016). A number of anthropogenic sources (or endmembers) of pharmaceutical residues in the aquatic environment has been identified, including above-mentioned wastewater discharges and the effluents from wastewater treatment plants (Arlos et al., 2014; Fairbairn et al., 2016; Hanamoto et al., 2018). However, few studies have linked the pharmaceutical residues in stream water to specific anthropogenic activities, such as urbanization or animal farming and agricultural production except implications (Yu et al., 2013; Fairbairn et al., 2016). Hanamoto et al. (2018) found that instream loadings of 12 WWTPs-derived pharmaceuticals could be predicted by human population in the catchments. However, Veach and Bernot (2011) found comparable concentrations of 12 pharmaceutical compounds in stream water between agricultural and urban influenced sites in an Indiana watershed, USA. The source-sink relationship between pharmaceutical residues in surface water and anthropogenic activity in the catchment needs further clarifications, which will be helpful to understand bio-physico-chemical behaviors and ecological effects of pharmaceutical residuals in the aquatic environment.

Objectives of this study were to explore responses of instream pharmaceutical concentrations to land use patterns in subwatersheds along a gradient of urban land use composition during dry and wet seasons. Based on existing research, it was hypothesized that concentration and composition of instream pharmaceuticals would be associated with the land use patterns in subwatersheds relating to human activities, such as human medication uses in urban areas and animal farming uses and agricultural application of pharmaceutical contaminated sludge/composts in rural areas. Seasonality, dry and wet seasons, might also differentiate instream pharmaceutical interactions with human activity in subwatershed due to dilution effects of seasonal precipitation (Fairbairn et al., 2016). Our previous study found 330 compounds of 9 pharmaceutical categories were detected in surface sediments from the coastal tidal section of the Jiulong River (Chen et al., 2013). Other studies indicated approximately 1.8 million pigs were farmed in the Jiulong River watershed (Zhang et al., 2012), and the swine wastewater discharges elevated instream antibiotic concentrations (Jiang et al., 2013; Zhang et al., 2011). This study selected 20 research subwatersheds among the 3 Class I subwatersheds (the main tributaries draining through) in the Jiulong River watershed in southeastern China (Fig. 1). The 20 research subwatersheds formed a gradient of urban land use composition in the subwatershed. Two seasonal sampling events of stream water were conducted above the confluences of the 23 subwatersheds (20 research subwatersheds and 3 Class I subwatersheds) to the main river during the dry season (November 2014) and the wet season (June 2015). The pharmaceutical compounds of interest in this study included 3 categories, i.e. 26 compounds of five-group antibiotics, 6 compounds of non-steroidal anti-inflammatory drugs (NSAIDs), and 1 compound of respiratory system drugs.

2. Materials and methods

2.1. Study watershed and sampling

The 258-km long Jiulong River drains the 2nd largest watershed in Fujian Province, China (24°13′-25°51′ N, 116°47′-118°02′ E). There are three Class I tributary confluences meeting in its estuary, namely the North Stream, the West Stream, and the South Stream. The watershed covers 14,741 km^2 and hosts two regional cities, Longyan and Zhangzhou, with a population over 10.5 million (Fig. 1). The monsoon climate brings annual precipitation of 1716 mm (2014) and 2114 mm (2015) in the upper catchment (Longyan) and 1578 mm (2014) and 1824 mm (2015) in the lower catchment (Zhangzhou) (Fig. S1 in the Supplementary information).

The Jiulong River watershed is divided into 197 minimal subwatersheds according to the digital elevation map (DEM) using an ArcGIS (Ver. 10 for Windows). Five land use types in the watershed, i.e. forest land, upland, paddy land, urban land, and water surface, were identified using LandSat ETM+ images (August 2014) using the ArcGIS with field validations (Fig. 1). Briefly, the forest land is coved by mature and full canopy forests; upland is a rain-fed and irrigated cultivated field with crops, vegetables, orchards, nursery plants, and grasses; paddy land referred to irrigated rice paddy land and other aquatic plants; urban land was covered by impervious surfaces; and water surface included streams, lakes and ponds, and reservoirs. The minimal subwatersheds were grouped by a cluster analysis with parameters of land use composition (%) and 20 research subwatersheds were

selected according to a gradient of urban land use (Fig. 1 and Table S1). In this study, a simplified land use interpretation has been implemented as urban land use (human/pet medication and pharmaceutical manufacture), rural land use (consisting of upland and paddy land with animal farming and pharmaceutical contaminated sludge/compost application), water surface (aquaculture), and natural land use (forest land) to represent an intensive gradient of human impacts on pharmaceutical discharges (Table S1). Three Class I subwatersheds are shown in Fig. 1. The North Stream subwatershed has 15 research subwatersheds (labeled as N-01 to N-15) with a urban land use percentage ranging from 0.1 to 26.3% (Fig. 1 and Table S1). The West Stream has 3 research subwatersheds (labeled as W-01 to W-03) and the South Stream has 2 research subwatersheds (labeled as S-01 and S-02) (Fig. 1 and Table S1).

Two surface water sampling events were carried out above the confluence of each research subwatershed to the main river in dry season (November 2014) and wet season (June 2015) (Fig. S1). The water sample was collected at a 30-cm depth below the water surface using a 1-L amber glass bottle. The bottles were rinsed three times with stream water prior to collection. The collected stream water was acidified to pH 3 with 4 mol L^{-1} H_2SO_4. Microbial activity was inhibited by adding methanol (5%, v/v) to the sample. Three parallel water samples were taken along the crossing transect of each confluence in 2 h. The samples were transported to the laboratory on ice and stored in a dark refrigerator at 4 °C prior to processing within 24 h.

2.2. Extraction of pharmaceutical compounds in stream water

The stream water samples were filtered through glass fiber filters (GF/F 0.7 μm, Whatman, Maidstone, UK). A portion of 0.2 g $Na_2EDTA \cdot 2H_2O$ was added to the filtrate that was then spiked with 100 ng of internal standards (D_4 sulfamethazine (D_4-SMZ), D_8 ofloxacin (D_8-OFC), Erythromycin (*N* dimethyl ^{13}C) (^{13}C-ETM), D_5 chloramphenicol (D_5-CPC), and D_3 mecoprop (D_3-MCP); Dr. Ehrenstorfer GmbH, Augsburg, Germany) and surrogate standards (^{13}C phenacetin (^{13}C-

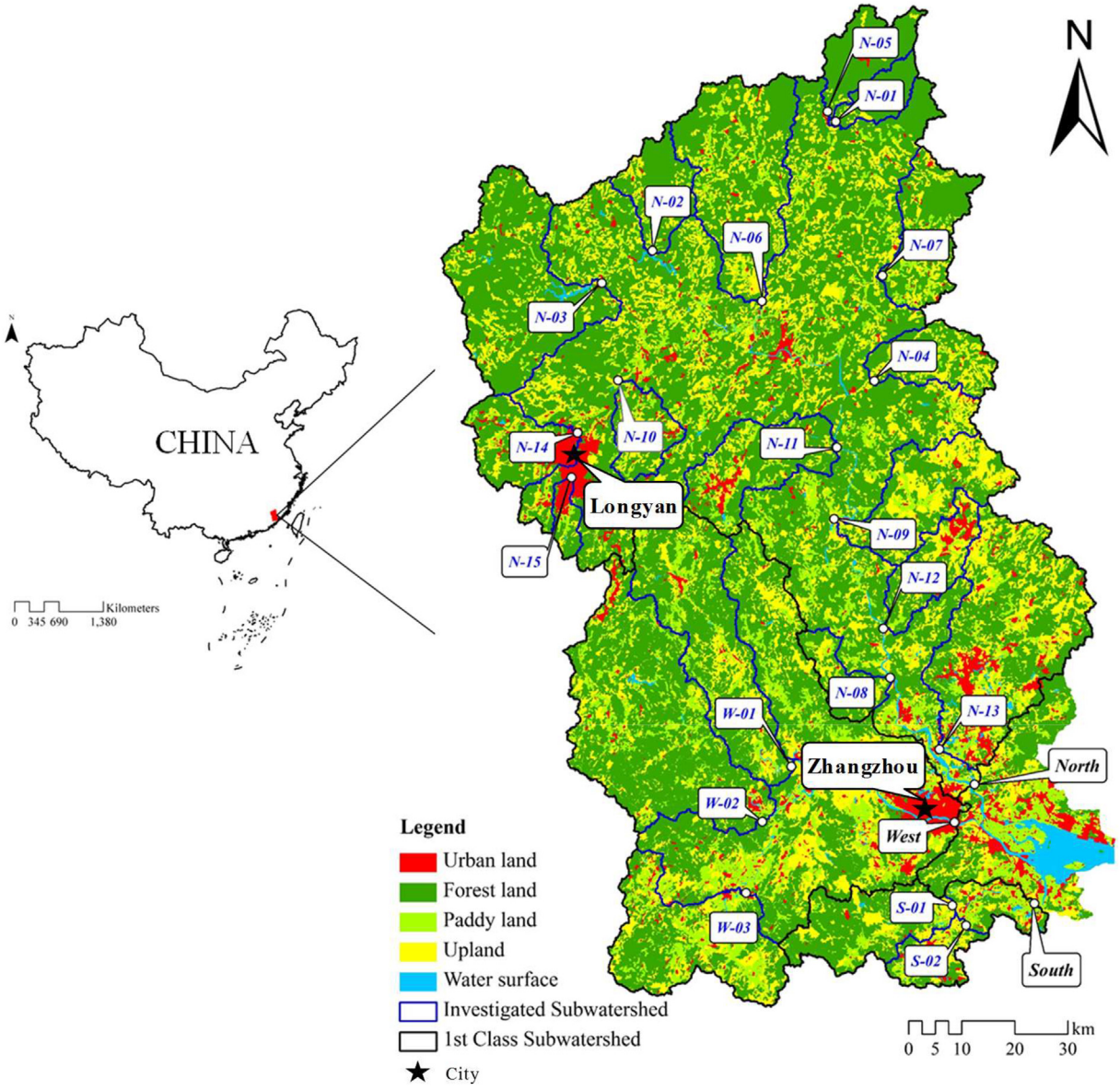

Fig. 1. Locations of the Jiulong River watershed in southeastern China with 3 Class I subwatersheds with land use patterns. The 15 research subwatersheds in the North Stream (labeled as "North", the Class I subwatershed) were labeled as "N-01" to "N-15" with an increasing urban land use percentage in the subwatershed by 2014, ranging from 0.1% to 26.3% as Table S1 in Supplementary Information. There were 3 research subwatersheds (W-01, W-02, and W-03) in the West Stream (labeled as "West", the Class I subwatershed) and 2 research subwatersheds (S-01 and S-02) sampled in the South Stream (labeled as "South", the Class I subwatershed). Land use composition of each research subwatershed and Class I subwatershed referred to Table S1.

PNC), D_5-Atrazine (D_5-ATZ), methyl D_3 benzeneamide D_4 (D_7-DEET), and D_6 gemfibrozil (D_6-GFZ); Toronto Research Chemicals Inc., Toronto, Canada).

Solid phase extraction (SPE) cartridges (Oasis HLB cartridge (500 mg and 6 mL), Waters, Milford, MA, USA) were rinsed with 10 mL of ultrapure water (water purification system, Thermo Scientific, Iowa, USA) and preconditioned with 10 mL of methanol (HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA). Then, the standard-spiked filtrates were passed through the SPE cartridges at a flow rate of 5–10 mL min^{-1} maintained by a vacuum. The filtrate bottle was rinsed twice using 50 mL of 5% (v/v) methanol in ultrapure water and the rinsed waters were passed through the SPE cartridge of each sample. Then the loaded cartridge was rinsed with 10 mL of ultrapure water and vacuumed until dry for 2 h to remove excess water. The pharmaceutical compounds were eluted from the loaded cartridge using 12 mL of methanol. The eluates were evaporated to near dryness with a gentle stream of pure nitrogen gas and re-dissolved in 1 mL of methanol-ultrapure water (v/v = 1:1). The re-dissolved sample was passed through a syringe filter (PTFE 0.2 μm, Millipore, Massachusetts, USA) and stored in a freezer at −18 °C prior to the HPLC-MS/MS analysis.

2.3. Chemical analysis

Standards of the 33 selected pharmaceutical compounds were purchased from Sigma-Aldrich Shanghai Trading Co. Ltd. (Shanghai, China) and Dr. Ehrenstorfer GmbH (Augsburg, Germany). The 33 pharmaceuticals included 26 antibiotics (4 tetracyclines (TCs): tetracycline (TTC), oxytetracycline (OTC), chlortetracycline (CTC), and doxycycline (DOC); 10 sulfonamides (SAs): sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfamerazine (SMR), sulfamonomethoxine (SMM), sulfaquinoxaline (SQX), sulfadimethoxine (SDM), sulfameter (SME), sulfaclozine (SCZ), and sulfathiazole (STZ) and synergist (trimethoprim (TMP)); 5 fluoroquinolones (FQs): norfloxacin (NFC), ofloxacin (OFC), ciprofloxacin (CFC), enrofloxacin (EFC), and difloxacin (DFC); 3 macrolides (MLs): erythromycin-H_2O (ETM), roxithromycin (RTM), and tylosin (TLS); and 3 chloramphenicols (CPs): chloramphenicol (CPC), thiamphenicol (TPC), and florfenicol (FFC)), 6 non-steroidal anti-inflammatory drugs (NSAIDs) (naproxen (NPX), ketoprofen (KPF), diclofenac (DCF), ibuprofen (IPF), indomethacin (IDM), and mefenamic acid (MFA)), and 1 respiratory system drug (RSD) (theophylline (TPL)). Basic physicochemical properties of the selected 33 compounds are listed in Table S2. Stock solutions of all standards were dissolved in methanol and stored in a freezer at −18 °C. Working standard solutions were prepared by diluting the stock solution just before the analysis.

A Shimadzu liquid chromatography (LC) coupled with an ABI 3200 triple quadruple tandem mass spectrometry (MS/MS) (AB SCIEX, USA) were used to analyze the selected 33 pharmaceutical compounds in the SPE samples. Chromatographic separation was performed using a Kromasil 100–5 C18 column (150 mm × 4.6 mm × 2.6 μm, Akzo Nobel, USA) and a binary gradient with a flow rate of 0.65 mL min^{-1} for the negative electrospray ionization (ESI) mode and 0.5 mL min^{-1} for the positive ESI mode, respectively. The mobile phase was acetonitrile (HPLC grade, TEDIA, Fairfield, OH, USA; B solution) with 2 mmol L^{-1} ammonia acetate (CNW Technologies GmbH, Düsseldorf, Germany) in ultrapure water (A solution) for the negative ESI mode and methanol (B solution) with 0.1% formic acid in ultrapure water (A solution) for the positive ESI mode. The gradient elution programs in both positive and negative ESI modes were shown in Table S3. All analytes were identified and quantified using multiple reaction monitoring (MRM) mode. The declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) were optimized. Two precursor ion/product ion transition pairs were used for all analytes. Detailed analytical parameters of the selected compounds were shown in Table S4.

2.4. Quality control and quality assurance

Strict quality assurance and quality control (QA/QC) were conducted during the analytical procedures. Recovery rates of the 33 pharmaceutical compounds were obtained by spiking the mixed standards into stream water at 100 ng L^{-1} in triplicate. Limits of detection (LOD) of selected pharmaceutical compounds were determined as the lowest concentration resulting in a signal-to-noise (S/N) ratio of 3. The limit of quantification (LOQ) was calculated with an S/N ratio of 10. The recovery rate, LOD, and LOQ of each pharmaceutical compound of interest are listed in Table S5. Concentrations below the LOQ were defined as zero during statistical analyses. A solvent blank, a procedural blank, and standards were run successively for each batch to validate background and instrument performance. The selected pharmaceutical compounds of interest were not detected in the blanks.

2.5. Risk assessment of quantified pharmaceutical compounds in stream water

Hazard quotient (HQ) of each quantified pharmaceutical compound in stream water was calculated by ratios of the measured environmental concentration (MEC) to predicted no-effect concentration (PNEC) according to the Technical Guidance Document on Risk Assessment (European Commission, 2003). The PNEC value for each quantified pharmaceutical compound were obtained from literatures (Dalla Bona et al., 2014; Komori et al., 2013; Lin et al., 2008; Orias and Perrodin, 2013; Sui et al., 2012; Zhou et al., 2016). The classification of HQ risk follows the Technical Guidance Document on Risk Assessment (European Commission, 2003), i.e. "no risk" with Log_{10} HQ < −2, "low risk" with −2 < Log_{10} HQ < −1, "medium risk" with −1 < Log_{10} HQ < 0, and "high risk" with Log_{10} HQ > 0.

2.6. Data processing and statistical analysis

One-way analysis of variance (One-way ANOVA) was conducted for residual levels of individual, grouped, and summed pharmaceutical compounds to identify differences among 3 Class I subwatersheds and 20 research subwatersheds. Two-sample paired *t*-test was performed to determine differences of pharmaceutical residual levels and composition between the dry and wet seasons. Pearson correlation analysis was used to explore correlations between pharmaceutical compounds during the dry and wet seasons and between pharmaceutical compounds and subwatershed land use composition (%). Relationships between land uses composition (subwatershed percentages of urban and rural land, and water surface) and pharmaceutical residues at each confluence were explored using a Canonical correlation analysis coupled with a Redundancy analysis. Patterns of pharmaceutical compound concentrations as a function of land use percent composition were explored by non-metric multidimensional scaling (NMDS) using the R (version 3.3.2). Multiple linear regressions were employed to determine effects of land use and rainfall on pharmaceutical residues. All statistical analyses, except NMDS, were conducted using the SAS® Studio (Release 3.6, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Occurrence

Twenty-seven out of 33 selected pharmaceutical compounds were found in surface waters of the subwatersheds sampled in the Jiulong River watershed. Of the 27 quantified compounds, two tetracyclines' compounds (TTC and DOC), one sulfonamides' compound (STZ), one fluoroquinolones' compound (NFC), and three NSAIDs' compounds (NPX, KPF, and IDM) were observed in the dry season samples but were below detection limits in the wet season samples (Table S5).

ETM was the only compound detected in all subwatersheds during both dry and wet season samples. TMP, RTM, DCF, and MFA were detected in all subwatersheds only in the dry season samples (Table S5). The residual compounds with a detection frequency > 74% included SMX, SMZ, SMM, FFC, and SDZ in the dry season samples and SMX, SMZ, SMM, TMP, FFC, and TPL in the wet season samples (Table S5).

Higher pharmaceutical residuals were observed in the dry season samples, ranging from 29.5 to 15,099 ng L^{-1} with a median of 990 ng L^{-1}. Wet season sample residuals ranged from 12.5 to 5862 ng L^{-1} with a median of 291 ng L^{-1} (Fig. 2). Antibiotic compounds contributed the highest instream pharmaceutical residuals in both dry and wet season samples. Residuals of NSAIDs ranged from 7.09 to 1687 ng L^{-1} with a median of 25.7 ng L^{-1} in the dry season samples and from LOQ to 273 ng L^{-1} with a median of 55.8 ng L^{-1} in the wet season samples (Fig. 2). The one respiratory system drug (TPL) instream residuals ranged from LOQ to 14.7 ng L^{-1} in the dry season samples and from LOQ to 16.1 ng L^{-1} in the wet season samples (Fig. 2). Few extremes were located in the South Stream, i.e. OTC (8417 ng L^{-1} at S-01, 2993 ng L^{-1} at S-02, and 1651 ng L^{-1} at South), TTC (2120 ng L^{-1} at S-01), DOC (2030 ng L^{-1} at S-01), and SDZ (4092 ng L^{-1} at S-02) in the dry season and SMM (3551 ng L^{-1} at S-02) in the wet season (Fig. 2).

Antibiotic compounds contributed 58–100% of the pharmaceutical residuals in the dry season and 52–100% in the wet season. The remaining fractions were dominated by NSAID compounds. TPL, the respiratory system drug, was observed only at three sampling points (N-04, N-09, and N-12) in the wet season, and contributed up to 21% of the total residual concentrations (Fig. 3). Antibiotic residuals compounds of SAs and MLs were predominant (Fig. 3). IPF and DCF, two NSAID compounds, were present regardless of the seasons; KPF was only observed in the dry season. No NSAID compounds in the wet season samples were detected in one third of the research subwatersheds in the North Stream, i.e. N-01, N-07, and N-09 (less 10% of urban land use in the subwatersheds) and N-11 and N-12 (considerable urban land use in the subwatersheds) (Table S1 and Fig. 3).

3.2. Spatiotemporal pattern

Spatially, concentrations of pharmaceutical residuals were significantly greater in the subwatersheds of N-03, N-08, N-15, S-01, S-02, W-01 and the South Stream in the dry season ($p < 0.05$, Fig. 3). However, in the wet season, only the S-02 subwatershed had a comparable concentration of pharmaceuticals as in the dry season. The other subwatersheds had lower concentrations, which were significantly lower in N-03, N-08, N-15, S-01, and W-01 ($p < 0.05$, Fig. 3).

After extremum normalization transformation, concentrations of the 27 pharmaceutical compounds exhibited clear temporal and spatial patterns (Fig. 4). The dry season samples had higher residuals than the wet season with some exceptions, such as TPL, which was higher in the wet season in the entire Jiulong River Watershed (Fig. 4). Spatially, the lower sections of the watershed, especially the South Stream and West Stream Class I subwatersheds, had higher concentrations of instream pharmaceuticals than the North Stream Class I subwatershed. This result was regardless of season, except for several compounds in the dry season, when the N-03 and N-08 subwatersheds had higher concentrations of SAs and MLs, and NSAIDs and SAs, respectively (Fig. 4). A clear spatiotemporal separation was observed for instream pharmaceuticals between the dry and wet seasons and between the upper and lower subwatersheds using non-metric multidimensional scaling (NMDS) analysis (Fig. 5). The spatial separation of the upper and lower subwatersheds showed a relatively similar pattern in both dry and wet seasons by subwatershed urban land percentage, while the two seasons resulted in a clear temporal separation (Fig. 5).

3.3. Relationship between in stream pharmaceuticals and land use composition in subwatersheds

Pharmaceutical concentrations were negatively correlated with the percentage of forest land use in the investigated subwatersheds, regardless of season (Table 1). Instream concentrations of FQs and MLs were exclusively and positively correlated with urban land use percentage for both seasons ($p < 0.01$, Table 1). The respiratory system drug, TPL,

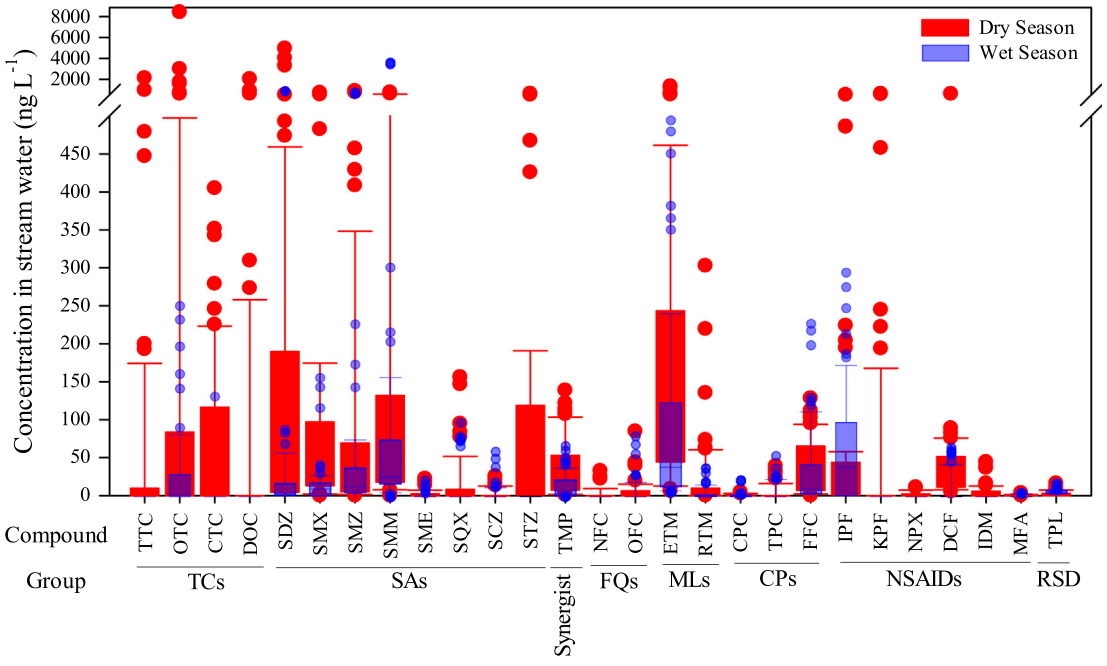

Fig. 2. Concentrations of twenty-seven quantified pharmaceutical compounds in stream water from 3 Class I subwatersheds and 20 research subwatersheds of the Jiulong River watershed, southeast China during dry (November 2014 in red) and wet (June 2015 in blue) seasons. Each subwatershed had three parallel samples along the transect before its confluence. The boxplot showed the median, 10th, 25th, 75th, and 90th percentiles as a vertical box and error bar and dots are outliers. Abbreviations of compounds were referred to Table S2.

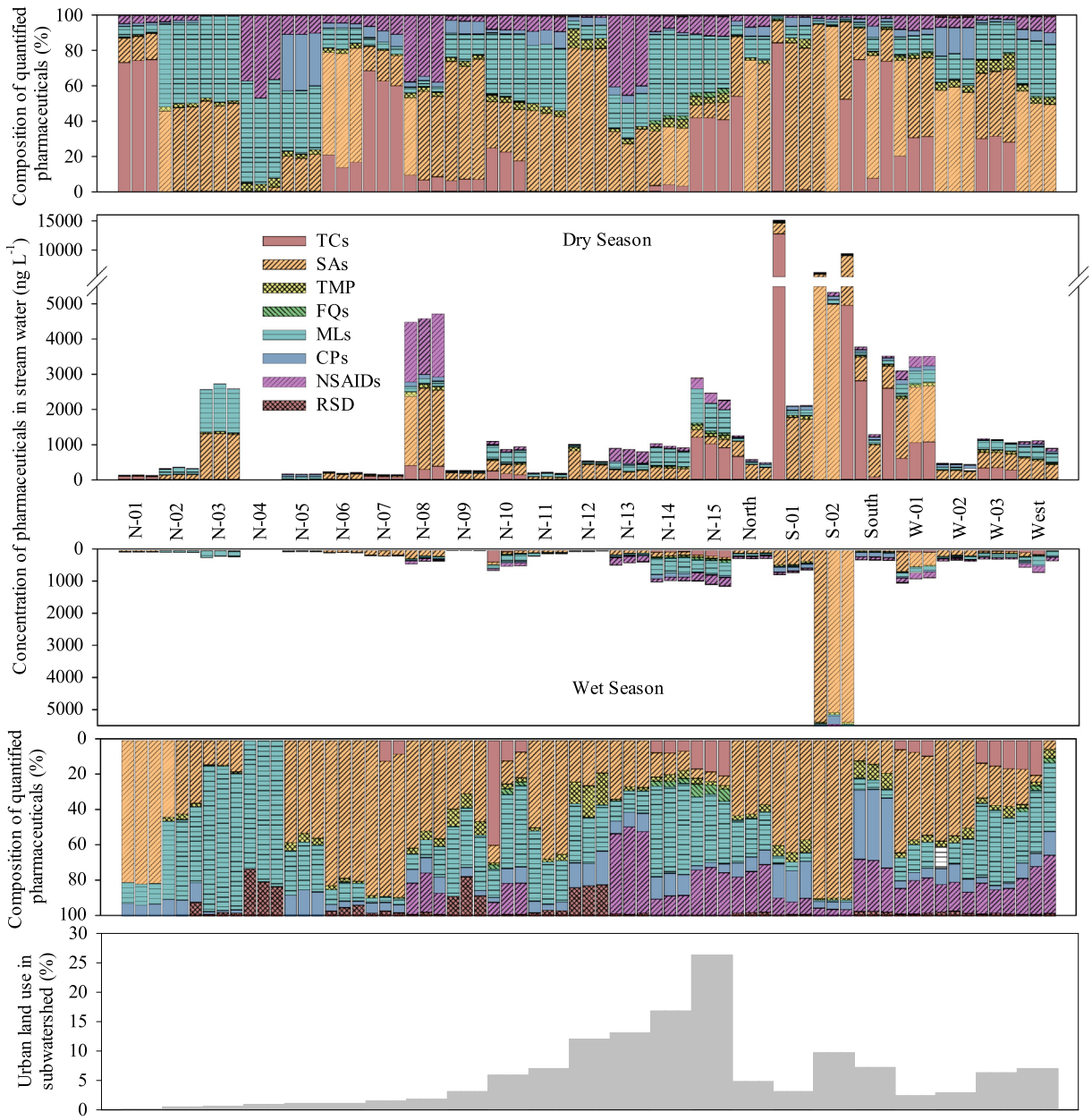

Fig. 3. Composition changes of pharmaceutical compounds in stream water from 3 Class I subwatersheds and 20 research subwatersheds of the Jiulong River watershed, southeast China during the dry (November 2014) and wet (June 2015) seasons. Each subwatershed had three parallel samples along the transect before its confluence. Percentages of urban land use in each research subwatershed were illustrated in gray and the land composition of each investigated subwatershed referred to Table S1 in Supplementary Information. Labels of subwatersheds referred to Fig. 1. Abbreviations of compounds were referred to Table S2.

showed a positive significant correlation with urban land use percentage in the dry season, but with both rural and urban land use percentages in the wet season ($p < 0.01$, Table 1). Rural land use percentage had positive and significant correlations with the compounds of SAs, CPs, NSAIDs, and TPL in the subwatersheds ($p < 0.05$, Table 1). Water surface percentage in the subwatersheds had broad positive significant correlations with the instream concentrations of compounds of TCs, SAs, MLs, CPs in the dry season, and SAs, CPs, NSAIDs, and RSD in the wet season ($p < 0.05$, Table 1).

Canonical correlation analysis of instream pharmaceuticals and subwatershed land use composition (excluding forest land due to the significantly negative correlations with the compounds) produced an $R^2 = 0.803$ for both seasons ($p < 0.001$, Table 2). Urban land percentage was highly correlated to the pharmaceuticals in the dry and wet seasons (squared coefficients: 0.442 and 0.645, respectively), which were greater than correlations to rural land percentage in both seasons (Table 2). Water surface percentage only exhibited a correlation (0.212) to pharmaceuticals in the dry season (Table 2). Pharmaceuticals had a higher correlation to land use composition in the wet season (0.243) than in the dry season (0.117). Among the instream pharmaceutical categories, RSD had the highest correlation with land use composition, followed by the antibiotics of FQs and CPs in the dry season. In the wet season, the highest correlations with the land use composition switched to the antibiotics of FQs, MLs, and TCs, followed by NSAIDs (Table 2).

3.4. Risk assessment

Given no PNEC value in literature available for SME, only 26 quantified pharmaceutical compounds had been calculated for hazard quotient (HQ). A majority of the quantified instream pharmaceutical compounds had Log_{10} HQ < −2, suggesting no risk to aquatic organisms,

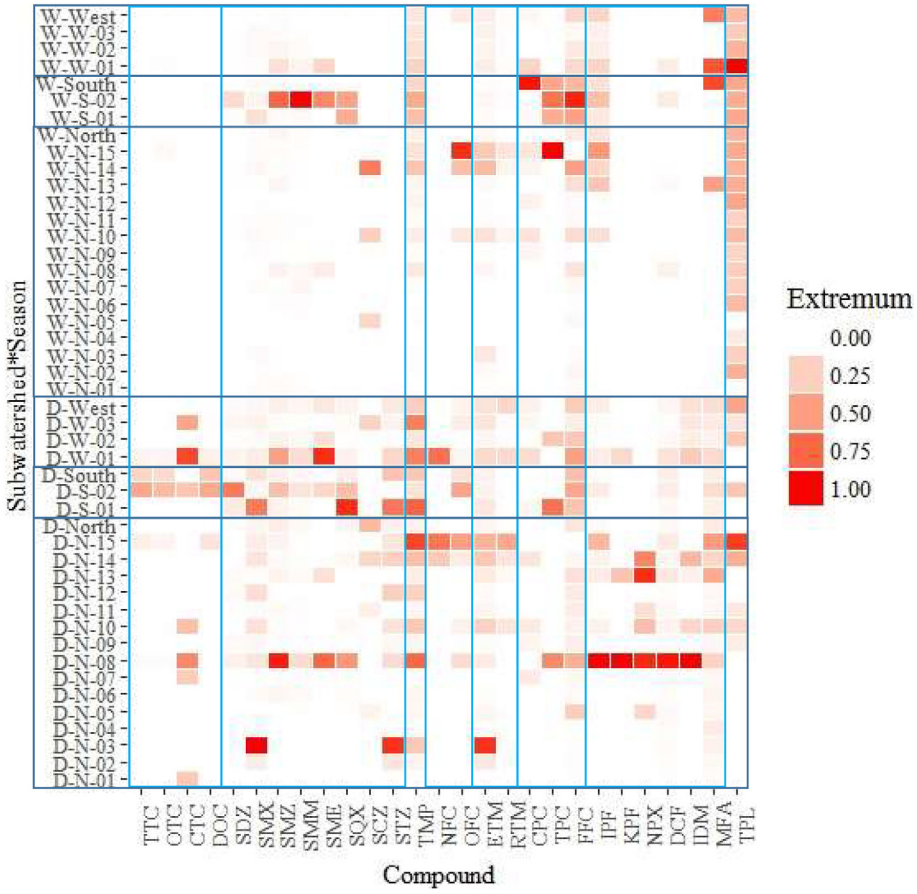

Fig. 4. Instream intensity of twenty-seven quantified pharmaceutical compounds in 3 Class I subwatersheds and 20 research subwatersheds of the Jiulong River watershed, southeast China during dry (with the capital D before the subwatershed logs along the Y axis and sampled in November 2014) and wet (with the capital W and sampled in June 2015) seasons. Concentrations of the quantified compounds in the stream water of both seasons were standardized using an extremum normalization transformation approach. The heat map was generated using R. Abbreviations of compounds were referred to Table S2.

such as algae, daphniid, and fish according to the Technique Guidance Document on Risk Assessment (European Commission, 2003) regardless of the dry and wet season sampling events (Fig. 6). In comparison with NSAIDs and RSD compounds, antibiotic compounds had considerable great HQs except outliers (Fig. 6). ETM had the highest HQs up to 33.3 in both dry and wet season sampling events, posing at least medium risk to aquatic organisms in the Jiulong River (Fig. 6). SMM ranked the second with the maximum HQs up to 4.13 and 20.2 in the dry and wet season sampling events, respectively (Fig. 6).

4. Discussion

Catchment land use patterns have been assumed to influence instream concentrations of pharmaceuticals. In this study, urban land use percentage in the research subwatersheds showed the highest correlation with the presence of instream pharmaceuticals for both dry and wet season sampling events (Table 2). The WWTPs-derived pharmaceuticals were considered as the main urban source in watersheds (Arlos et al., 2014; Fairbairn et al., 2016). Hanamoto et al. (2018) was able to predict the instream loadings of 12 WWTPs-derived pharmaceuticals by human population in a Japanese watershed. It suggests that human medications served as the major contributor of the instream pharmaceuticals.

Given direct contributions from domestic animal farming, aquaculture and secondary contributions from cropping using pharmaceutical enriched manures from the animal farms, rural land use in the catchment is expecting to have a good correlation to instream pharmaceuticals. Interestingly, the rural land use percentage in this study, however, had the lowest correlations with instream pharmaceuticals in the dry season and slightly higher in the wet season (Table 2). Hanamoto et al. (2018) observed that instream loading of two veterinary drugs (sulfamonomethoxine (SMM) and lincomycin) were positively correlated with swine population in the watershed. The research watershed, Jiulong River watershed, had large livestock production in the past (Zhang et al., 2011, 2012). Especially, the upstream city, Longyan, was one of the largest livestock farming region of the province and had been found as the major source of veterinary antibiotics (mostly TCs) in stream water (Table S6, Jiang et al., 2013). In this study, the rural land percentage (sum of paddy land and upland) in the subwatershed had positively significant correlations with veterinary only (SQX and FFC), human only (KPF, IPF, IDM, MFA, and TPL), and both human and veterinary pharmaceuticals (SDZ, SMZ, SMM, SME, TMP, and DCF) ($p < 0.05$, Table 2 and S7). This might be related to the transport of pharmaceutical compounds via surface runoff from agricultural fields (Davis et al., 2006; Li et al., 2016) with animal manure and sewage sludge applications (Jaffrézic et al., 2017; Tang et al., 2015; Zhu et al., 2013) in the subwatersheds. It implicates that the rural land contributes a mixed source of instream pharmaceuticals in the subwatersheds, which resulted in the weak impact of rural land percentage on instream pharmaceuticals.

Aquaculture might contribute instream pharmaceutical residuals. The water surface percentage showed a comparable correlation as the urban land percentage in the dry season sampling event (Table 2) and positively and significantly related to aquacultural pharmaceuticals (DOC, SDZ, SMX, SMM, FFC, and TPC) (Table 2 and S7). Li et al. (2016) found antibiotics discharges from aquacultural ponds via flooding or drainage to the Tiaoxi watershed, China during the wet season as well as in the Hai River watershed, northern China (Luo et al., 2011).

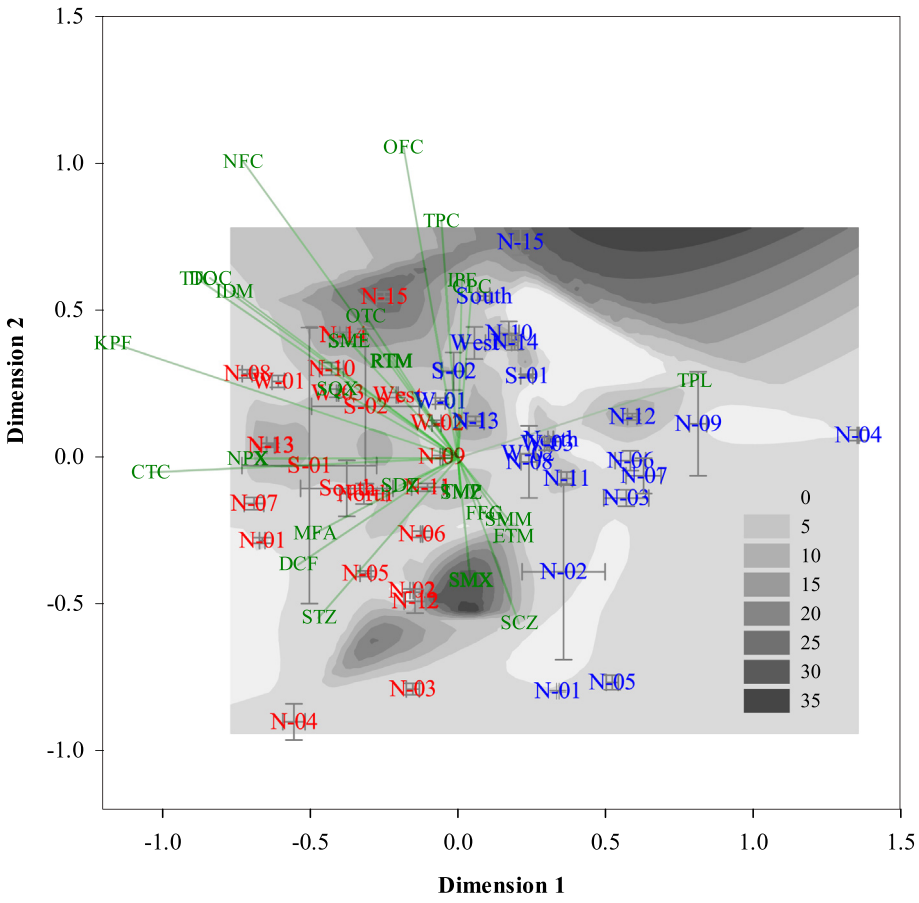

Fig. 5. Patterns of concentrations of twenty-seven quantified pharmaceutical compounds in stream water at the confluences as a function of land use composition (percentage) of 3 Class I subwatersheds and 20 research subwatersheds in the Jiulong River watershed, southeast China, explored by non-metric multidimensional scaling (NMDS) analysis using R. The urban land use (percentage) contour was in gray scale with a scale of 0–35% of total area of each subwatershed. Stream water samples were taken before the confluence of each subwatershed in dry (November 2014 in red) and wet (June 2015 in blue) seasons with three parallel samples along the transect. Twenty-seven quantified compounds were from three pharmaceutical categories. Land use composition of each investigated subwatershed referred to Table S1 in Supplementary Information. Labels of the investigated subwatersheds referred to Fig. 1. Abbreviations of compounds were referred to Table S2.

Therefore, the intensity gradient of human activity impacts as the simplified land use interpretation (i.e. in the order of urban land use > rural land use ≥ water surface (aquaculture) ≫ forest land use, Table S1) in this study clearly differentiated their influences on instream pharmaceuticals in both concentration and composition, of which were changed between the dry and wet seasons (Table S5 and Fig. 3). This finding is in agreement to the hypothesis, i.e. land use pattern in the subwatersheds influenced the instream pharmaceuticals in concentration and composition.

On the other hand, a dilution effect was hypothesized that seasonal precipitation might result in less compounds and lower concentration of instream pharmaceuticals quantified and weaken the land use impacts during the wet season. The dilution effect of seasonal precipitation on seasonality of instream pharmaceuticals was observed by many watershed studies, such as in the Zumbro River watershed, Minnesota, USA (Fairbairn et al., 2016), in the highly impacted Grand River watershed in southern Ontario, Canada (Arlos et al., 2014), in the Huangpu River watershed, Shanghai (Jiang et al., 2011) and the Haihe River watershed (Luo et al., 2011) in China. The reduced instream flows during the dry season also led to greater pharmaceutical loadings in wastewater (Yu et al., 2013), reclaimed wastewater, and even drinking water (Loraine and Pettigrove, 2006). In this study, an evident dilution effect of instream pharmaceuticals was also observed, including less number and lower concentration of compounds quantified in the wet season sampling event than those in the dry season sampling event (Table S5, Figs. 2 and 3) with relative homogeneity (Table S8). Coefficients of multiple linear regression models between instream concentrations of the quantified pharmaceutical groups (except CPs) and land use composition of the subwatersheds were improved by weighting the rainfall in the 15-day period before the samplings (Table S9), confirming the seasonal dilution effect. However, the canonical redundancy analysis integrating both concentration and composition of the instream pharmaceuticals revealed that the land use impacts were greater in the wet season sampling event than in the dry season sampling event (Table 2). It suggests that both composition and concentration of the instream pharmaceuticals might be equally responded to the land use pattern in the subwatershed or catchment although the less quantified number of instream pharmaceuticals in the wet season or high-flow season might be because their concentrations were below the quantification limit of the used protocol with the dilution effect.

Besides the seasonal dilution effect by rainfall, the environmental loading of pharmaceuticals might be related to seasonality of diseases, such as the infectious diseases (Fisman, 2007). The seasonality of infectious diseases (e.g. influenza) determined the amount and type of anti-infection medicine usages, resulting in a seasonality of concentration and composition of specific pharmaceutical compounds in the environment. Theophylline (TPL), an asthma drug and exclusive human medication (Table S7), in this study was significantly correlated with the urban land use ($p < 0.001$, $n = 69$, Table 1). It acted as the top indicator in response to land use composition in the subwatersheds during the dry season sampling event (November 2014) when asthma usually breaks out. Meanwhile, it also had significant correlations with compositions of rural, urban, and water surface ($p < 0.01$, n = 69, Table 1) in the wet season (June 2015) due to homogenization of rainfall or popular

Table 1
Correlations between instream concentrations of quantified pharmaceuticals and land use composition (%) in the subwatersheds in dry (November 2014) and wet (June 2015) seasons in the Jiulong River watershed, southeast China, explored by Pearson Correlation Analysis ($N = 69$).

Compound[a]		Dry season				Wet season			
		Land use composition (%)				Land use composition (%)			
		Forest	Rural	Urban	Water surface	Forest	Rural	Urban	Water surface
TCs	Group				*			***	
	TTC[b]				*				
	OTC				*			***	
	CTC								
	DOC[b]				*				
SAs	Group	-**	**		***	-*			**
	SDZ	-**	*		***	-*	*		***
	SMX				**				*
	SMZ	-*	**			-*	*		**
	SMM	-**	***						**
	SME		**			-*	*		*
	SQX		*						***
	SCZ						-*	**	
	STZ[b]				**				
Synergist	TMP	-***	**	***		-***	**	***	***
FQs	Group	-*		***				***	
	NFC[b]			***					
	OFC	-*		***				***	
MLs	Group			**				***	
	ETM		-*		*			***	
	RTM			***				***	
CPs	Group	-**	***		**	-**	*	**	***
	CPC								***
	TPC					-**		***	***
	FFC	-**	***		***	-**	*		***
NSAIDs	Group		*			-***	**	***	***
	IPF	-*				-***	*	***	***
	KPF[b]		*						
	NPX[b]								
	DCF		*			-**	**		*
	IDM[b]		*						
	MFA	-***		***		-**	**		***
RSD	TPL	-*		***		-***	***	**	**

*, **, and *** represented significance levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, and "-" represented "negative correlation".
[a] Abbreviations of compounds were referred to Table S2.
[b] The compound was not quantified in stream water in the wet season (Table S5).

tea (*Camellia sinensis*) drinking in the region. On the other hand, ibuprofen (IPF), a NSAID's medication for human only to treat pain, fever, and inflammation (Table S7), was the only pharmaceutical compound of interest with a significantly elevation in the wet season sampling event in comparison to that in the dry season sampling event ($p < 0.01$, $n = 138$, Fig. 2). Furthermore, the IPF concentration was only significantly correlated to land use composition of the subwatersheds during the wet season ($p < 0.05$, n = 69, Table 1), when influenza and fever likely break out more frequently due to the weather transformation. Compounds from sulfonamides (SAs) and chloramphenicols (CPs), most uses for veterinary and aquaculture (Table S7), had greater significant correlations with rural land use percentage of the subwatersheds in

Table 2
Explanations between anthropogenic land use patterns and grouped pharmaceutical levels in stream water of the dry and wet seasons from 3 Class I subwatersheds and 20 research subwatersheds of the Jiulong River watershed, revealed by Canonical Redundancy Analysis (Proc CANCORR).

	Standardized Variance of the **Land uses** Explained by				Standardized First Variance of the **Pharmaceuticals** Explained by			
	Their own Canonical variables	R^2	*p*	The opposite Canonical variables	Their own Canonical variables	R^2	*P*	The opposite Canonical variables
	Proportion			Proportion	Proportion			Proportion
Dry season	0.273	0.803	<0.001	0.219	0.146	0.803	<0.001	0.117
Wet season	0.287	0.803	<0.001	0.230	0.303	0.803	<0.001	0.243
Squared Multiple Correlations between the **Land uses** and the First M Canonical Variables of the **Pharmaceuticals**					Squared Multiple Correlations between the **Pharmaceuticals** and the First M Canonical Variables of the **Land uses**			
Land use	M1				Pharmaceutical group	M1		
	Dry season			Wet season		Dry season		Wet season
Urban	0.442			0.645	RSD[a]	0.377		0.005
Water surface	0.212			0.011	FQs	0.210		0.776
Rural	0.003			0.035	CPs	0.128		0.012
					TMP	0.099		0.037
					SAs	0.079		0.001
					MLs	0.019		0.555
					TCs	0.015		0.307
					NSAIDs	0.009		0.253

[a] Abbreviations of compounds were referred to Table S2.

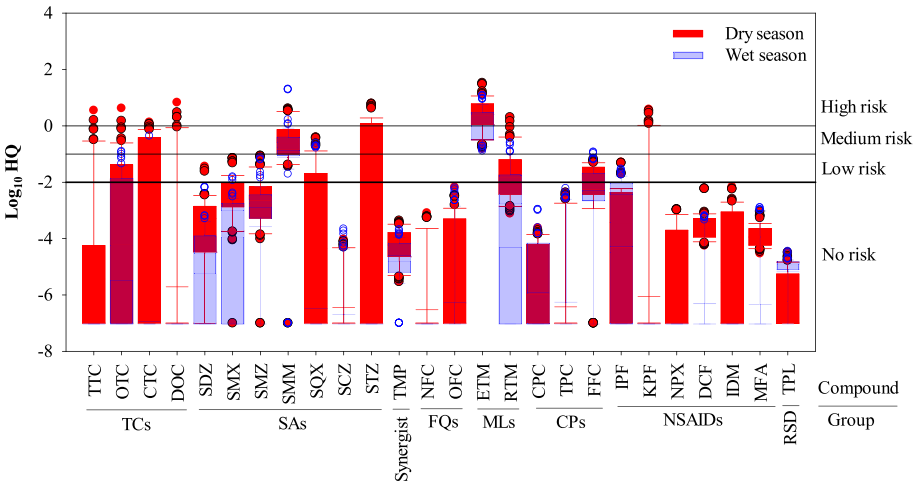

Fig. 6. Hazard quotients of 26 quantified pharmaceutical compounds in stream water at confluences of 3 Class I subwatersheds and 20 research subwatersheds in the Jiulong River watershed, southeast China during dry (November 2014 in red) and wet (June 2015 in blue) seasons. The boxplot shows the median, 10th, 25th, 75th, and 90th percentiles as a vertical box and error bars and dots are outliers. Abbreviations of compounds were referred to Table S2.

the dry season but with water surface in the wet season when fish diseases mostly take place. These evidences might suggest that the seasonality of instream concentration of pharmaceuticals is influenced by the seasonal diseases. The seasonally increased uses had been used to explain the seasonal elevations of instream concentration and loading of prescription pharmaceuticals in wastewater of WWTPs (Yu et al., 2013) and in downstream water of a WWTP (Fairbairn et al., 2016). On the other hand, instream attenuation of some pharmaceutical compounds had also been observed in Yodo River watershed, Japan (Hanamoto et al., 2018), such as complete photolysis of OTC in sea surface water over 21 days (Lunestad et al., 1995) and NSAIDs in surface water with a half-life of 8–32 days via photolysis and biodegradation (Tixier et al., 2003). The attenuation might explain some instream NSAIDs with a low quantified frequency and concentration in this study (Table S5, Figs. 2 and 3). The attenuation of instream pharmaceuticals might be related to their chemical and biological properties (Table S2).

In terms of concentration of instream pharmaceutical compounds of interest, the quantified ranges of this study were comparable to other watersheds in China and other countries (Ashton et al., 2004; Bu et al., 2013; Kolpin et al., 2002; Luo et al., 2011; Murata et al., 2011). TCs' compounds were widely quantified in stream water around the world. For example, an extreme instream OTC concentration (8417 ng L^{-1} in the dry season sampling event, the greatest concentration of TCs' compounds) was quantified in this study, which was lower than the instream concentration in the Tiaoxi River watershed (19,810 ng L^{-1}, Li et al., 2016) but much greater than those in the Haihe River watershed (450 ng L^{-1}, Luo et al., 2011) in China and the Cache La Poudre River watershed, USA (1210 ng L^{-1}, Kim and Carlson, 2007). On the other hand, an extreme concentration of ETM was also quantified (1259 ng L^{-1}) in the dry season sampling event of this study, which was lower than the concentration in the Victoria Harbor, Hong Kong, China (1900 ng L^{-1}, Minh et al., 2009) but nearly doubled that in the Pearl River watershed, China (636 ng L^{-1}, Xu et al., 2007) and tripled that in the Cache La Poudre River watershed, USA (450 ng L^{-1}, Kim and Carlson, 2007). However, ETM had a greater HQ than any compounds of TCs in this study regardless of seasonality (Fig. 6), suggesting greater impacts on aquatic organisms. Xi et al. (2015) identified that RTM, another ML compound, significantly responded to the microbial community structure shift in fishpond sediments. Although a great risk of DCF (HQ > 1) was reported in seawater of North Portugal (Lolić et al., 2015), most risks were derived from antibiotics rather than NSAIDs and RSD in stream water of this study (Fig. 6).

5. Conclusion

Along the gradient of urban land use percentage in the 20 research subwatersheds and the 3 Class I subwatersheds of the Jiulong River watershed, southeastern China, the interactions between the land use pattern in the subwatersheds and instream pharmaceuticals (3 categories and 33 compounds) at their confluences were studied via two sampling events during the dry and wet seasons. This study revealed that land use pattern in the watershed or catchment embarks human activity information as well as anthropogenic contaminants. The interactions between land use pattern in the watershed or catchment and instream pharmaceuticals at the confluence were explored by both concentration and composition instead of concentration alone using a multivariate analysis combining canonical correlation and redundancy analysis. Urban land use in the subwatersheds predominately drove instream human-use pharmaceuticals in concentration and composition while rural land use contributed a mixed human and veterinary source. Seasonality of instream pharmaceuticals was related to the dilution effect of seasonal precipitation but the impact of land use pattern in the subwatersheds on instream pharmaceuticals was enhanced. Among the quantified compounds, antibiotics had greater HQs than NSAIDs and RSD in stream water. But, only ETM showed medium to high risks to aquatic organisms regardless of seasons. The results from this study also suggest that evaluating eco-environmental risk of pharmaceuticals should consider both concentration and composition of instream pharmaceuticals instead of concentration of individual compound. Integrating socioeconomic development and epidemic survey with land use pattern in a catchment or watershed might be able to improve our understandings of interactions between human activity and pharmaceutical pollution and their possible ecological and health risks.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.392.

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