

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Available online at www.sciencedirect.com

## **ScienceDirect**



www.jesc.ac.cn

www.elsevier.com/locate/jes

# Pronounced temporal changes in soil microbial community and nitrogen transformation caused by benzalkonium chloride

## Rui Yang<sup>1,2</sup>, Shaohong Zhou<sup>1,2</sup>, Lilan Zhang<sup>1,2,\*</sup>, Cunli Qin<sup>1,2</sup>

<sup>1</sup> State Key Laboratory of Coal Mine Disaster Dynamics and Control, Chongqing University, Chongqing 400044, China

<sup>2</sup> Key Laboratory of Three Gorges Reservoir Region's Eco-Environment, Ministry of Education, Chongqing University, Chongqing 400045, China

#### ARTICLE INFO

Article history: Received 23 November 2021 Revised 2 April 2022 Accepted 5 April 2022 Available online 15 April 2022

Keywords: Benzalkonium chloride Soil Degradation kinetics Microbial community structure Nitrogen cycling

#### ABSTRACT

As one typical cationic disinfectant, quaternary ammonium compounds (QACs) were approved for surface disinfection in the coronavirus disease 2019 pandemic and then unintentionally or intentionally released into the surrounding environment. Concerningly, it is still unclear how the soil microbial community succession happens and the nitrogen (N) cycling processes alter when exposed to QACs. In this study, one common QAC (benzalkonium chloride (BAC) was selected as the target contaminant, and its effects on the temporal changes in soil microbial community structure and nitrogen transformation processes were determined by qPCR and 16S rRNA sequencing-based methods. The results showed that the aerobic microbial degradation of BAC in the two different soils followed first-order kinetics with a half-life (4.92 *vs.* 17.33 days) highly dependent on the properties of the soil. BAC activated the abundance of N fixation gene (*nifH*) and nitrification genes (AOA and AOB) in the soil and inhibited that of denitrification gene (*narG*). BAC exposure resulted in the decrease of the alpha diversity of soil microbial community and the enrichment of Crenarchaeota and Proteobacteria. This study demonstrates that BAC degradation is accompanied by changes in soil microbial community structure and N transformation capacity.

© 2022 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

## Introduction

Quaternary ammonium compounds (QACs) are a group of cationic surfactants widely used in medicine, industry, environmental protection, and agriculture for their excellent surface activity and bactericidal property (Kahrilas et al., 2015). Recently, the U.S. Environmental Protection Agency has approved some surface disinfectants in response to the coronavirus disease 2019 outbreak. QACs are the most common of these recommended disinfectants, (Chen et al., 2021). Global sales of surface disinfectants totaled \$4.5 billion in 2020, representing a dramatic increase (>30%) over the previous year (Lewis, 2021). Most QACs (>75%) cannot be removed from wastewater and then discharged into the environment due to the limitations of wastewater treatment technology

\* Corresponding author. E-mail: lilanzhang@cqu.edu.cn (L. Zhang).

https://doi.org/10.1016/j.jes.2022.04.004

1001-0742/© 2022 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

(Dai et al., 2018; Kim et al., 2020; Zhang et al., 2015). The potential environmental risk of the tremendous increase in QACs entering the environment should be paid special attention.

There is now ample evidence suggesting that QACs can enter and accumulate in the soil through sludge return, wastewater irrigation, and pesticide spraying (Pateiro-Moure et al., 2013; Xiang et al., 2016). Some researchers predicted that the concentration of QACs entering the soil through the application of organic fertilizers was about 3.5 mg/kg (Mulder et al., 2018). Moreover, up to 28.5 mg/kg of benzalkonium chlorides (BACs) were still detected in soils in one region of South Korea for 6 months after the application of disinfectants containing BACs (Kang and Shin, 2016). Besides, QACs were detected in some farmland soils at concentrations as high as 0.82 mg/kg due to irrigation with QACs-tainted wastewater (Jardak et al., 2016). Soil health is closely related to healthy food production. Thus, it is crucial to know the fate and toxic effects of QACs in soil.

Microbial degradation is considered to be the primary pathway for the QACs dissipation in the soil environment. It has been well established that under aerobic conditions, QACs can be used as carbon sources by some bacteria such as Aeromonas, Xanthomonas, and Pseudomona (Bergero and Lucchesi, 2015; Patrauchan and Oriel, 2003; Tezel et al., 2012). With the participation of molecular oxygen and NADPH, the hydroxylation reaction catalyzed by monooxygenase marks the initiation step of QACs biodegradation (Khan et al., 2015; Patrauchan and Oriel, 2003; Tezel et al., 2012). Whereas, as one antibacterial agent, QACs inevitably expose adverse effects on other organisms. For example, in aerobic wastewater treatment systems, QACs could inhibit the microbial degradation of organic pollutants and cause damage to key enzymes (such as ammonium monooxygenase and nitrite oxidoreductase) (Zhang et al., 2018). QACs induced the decline in total microbial abundance and diversity in the lake and the enrichment of Rheinheimera, Pseudomonas, and Vogesella (Yang and Wang, 2018). In membrane bioreactors with QACs, some microorganisms such as Pseudomonas and Xanthomonadaceae gradually evolved into dominant populations (Lai et al., 2017). Even QACs at 5 mg/L completely inhibited the nitrification in the biological denitrification reactor (Hajaya and Pavlostathis, 2012). Our previous study also proved that benzalkonium chlorides (BAC) could inhibit photosynthesis and cause oxidative stress response of cyanobacteria Microcystis aeruginosa at EC50 concentration (Qian et al., 2022). Despite recent advances, the dissipation of QACs in the soil environment and their impact on soil health remains poorly addressed

Soil microorganisms are regarded as an essential indicator of soil health, and their diversity and stability are fundamental to the stability of the soil ecosystem. It is widely established that exogenous substances in the soil can alter the main microbial functional groups (Wang et al., 2020). Furthermore, as the driver of the nutrient cycle, changes in soil microbes might affect the nitrogen (N) cycling capacity (Ma et al., 2018; van der Heijden, 2010). It was found that QACs could inhibit soil microbial ammonia oxidation activity (Frühling et al., 2001) and stimulate dehydrogenase activity and potential nitrification at low concentrations, whereas QACs at high concentrations caused the opposite effect (Sarkar et al., 2010). Exploring microbial response is key to uncovering the effects of QACs exposure on soil health, which need to be fully explored.

To address the above-mentioned scientific issues, BAC, a typical QACs, was chosen as the target contaminant to study its effects on microbial community structure and the N conversion capacity. The present study aimed to investigate (1) the degradation kinetics of BAC in two different soils; (2) the temporal changes of microbial community structure during BAC degradation; (3) the variation of the abundance of N transformation-related genes.

## 1. Materials and methods

## 1.1. Soil and chemicals

Two surface soils with different physicochemical properties were selected as the subject for the present study: acid forest soil (XS) and alkaline cultivated soil (HN), and their physicochemical properties are given in Appendix A Table S1. After sieving through a 2 mm sieve to remove large pieces of vegetation and debris, the soils were stored in a refrigerator at 4°C until use. The soil samples were incubated at 20% moisture content for a week at room temperature to recover the microbial activity before the spiking experiment.

BAC (CAS Nos. 139-07-1, purity 99%) from Shanghai Mokai Biotechnology Co., Ltd. (Shanghai, China). All the other reagents were obtained from Bio-Rad Laboratories Co., Ltd. (California, USA) and the Chongqing Huanghui Chemical Dangerous Goods Sales Co., Ltd. (Chongqing, China). The used primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). BAC (0.1 g) were accurately weighed and made into stock solutions (5 mg/mL) with 20 mL methanol. The stock solution was diluted in ultrapure water to the desired concentration at the time of use.

#### 1.2. Experimental design

#### 1.2.1. Soil preparation

Abiotic soil controls were sterilized through three cycles of 30 min autoclaving at 0.1 Mpa and 121°C with intermittent incubation at  $(22 \pm 2)^{\circ}$ C for 3 days between each autoclaving cycle. An appropriate amount of BAC stock was added to the soil sample, then stirred continuously in a fume hood for more than 3 hr to ensure methanol volatilized completely. Soil samples with the final concentration of BAC of 10 mg/kg were obtained. To investigate the dynamic degradation process and ecotoxicity of BAC in soil, the following three groups were set up: (1) the experimental group with the addition of 10 mg/kg (dw) BAC only; (2) control group with the addition of sterile water to the unspiked soil; (3) abiotic soil control group with the addition of 10 mg/kg BAC and 0.4 mg/g NaN<sub>3</sub>. Incubation experiments were performed in a 125 mL screw-capped brown bottle containing 15 g of soil, and all treatments were run in triplicate. All samples were then placed in a constant temperature incubator at 20°C, and destructive samplings were taken for analysis on days 0, 1, 3, 7, 15, 30, and 60. During the incubation period, soil moisture was maintained at 20% of the moisture content by weight the loss. Gas chromatography (SC-3000B-020T, Chongqing Chuanyi Analytical Instrument

#### 1.2.2. Extraction and analysis of BAC

The soil (0.5 g) was mixed homogeneously with a 10% methanolic hydrochloric acid (15 mL, V/V). The samples were then sonicated at 62°C for 1 hr, followed by centrifugation at 7000 r/min for 10 min to obtain the supernatants. The extraction procedure was repeated three times, and all supernatants were combined and filtered with a 0.45 µm filter membrane. Samples were stored at -20°C until analysis. BAC was determined using LC-MS with a Shim-pack GISS 1.9 µm C18 column (2.1 mm  $\times$  50 mm) by injecting 10 µL. Eluents were 0.1% formic acid in water (A) and methanol (B). The details of the elution process are provided in Appendix A Table S2. The column temperature was set at 25°C and the detection wavelength at 210 nm. The recovery rate of BAC in soil was 92.4%  $\pm$  2.6%, and the background of BAC in HN and XS soil was 0.01 and 0.45 mg/kg, respectively. In parallel, BAC in the air of the bottle was detected by SPE columns (C18 300 mg, DIONEX, USA), and the results indicated that the BAC concentrations were below the detection limit (see the Appendix A Text S2 for additional details).

## 1.3. Determination of soil urease activity

The urease activity was measured according to the methodology described by Yang et al. (2021a). In short, mix 5 g of soil with 10 mL citrate buffer, 5 mL urea solution (10%), and 1 mL toluene in a 50 mL volumetric flask. The absorbance was measured after the samples were incubated in a constant temperature incubator at  $37^{\circ}$ C for 24 h.

#### 1.4. Gene quantification and high-throughput sequencing

The soil DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Extracted DNA was then stored at  $-80^{\circ}$ C until tested. N transformation-related gene abundance was quantified through real-time qPCR (Bio-Rad, Foster, USA). The PCR reaction system had a total volume of 20 µL and contained primer (1.2 µL), template DNA (0.8 µL), sterile water (8 µL), and 10 µL 2 × IQ SYBR Green Supermix (Bio-Rad, USA). For additional details on qPCR procedures, see Appendix A Text S3. High-throughput sequencing was carried out by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) with the Illumine novaseq 6000 platform. The analysis process and quality control are described in detail in the Appendix A Text S4.

## 1.5. Statistical analysis

In this study, first-order degradation kinetics model was used to describe the kinetics of BAC biodegradation. First-order degradation kinetic has the following form:

$$\frac{d(C_t - C_s)}{dt} = -k \times (C_0 - C_s) \tag{1}$$

which was also equal to the following style:

$$C_t - (C_0 - C_s) \exp(-k \cdot t) + C_s$$
 (2)

$$T_{50} = \frac{\ln 2}{k} \tag{3}$$

where, k is the biodegradation rate constant; t (day) is the incubation time;  $C_t$  (mg/kg) is the soil concentration of BAC;  $C_0$ (mg/kg) is the initial BAC concentration;  $C_s$  (mg/kg) is BAC adsorbed to the surface of the soil;  $T_{50}$  (day) is the half-life of BAC.

Significance differences were examined using the twosample t-test to compare two groups and one-way ANOVA tests to compare multiple groups (IBM SPSS Statistics 26.0, USA). Pearson's correlation between the abundance of the microbial community and the concentration of BAC was considered statistically robust if Pearson's correlation coefficient (|r|) was >0.8 and the *p*-value was <0.05. The experimental data were processed and mapped using OriginPro 2020b (OriginLab Corporation, Northampton, Massachusetts, USA). Co-occurrence network analysis was performed in the R environment via the igraph package, and the network was visualized via the interaction platform Gephi.

## 2. Results and discussion

## 2.1. Biodegradation kinetics of BAC in two different soils

To reveal the effects of soil properties on the environmental fate of BAC, two different soils were selected. XS from forest areas was sorted as acidic silt loam soil, and HN collected from agricultural region was alkaline loam soil. The detailed properties of the two soils are listed in Appendix A Table S1. The aerobic biodegradation process of BAC in the two different soils is presented in Fig. 1. The degradation process of BAC followed well first-order kinetic in both HN and XS soils, and the kinetic equations and fitting parameters are provided in Appendix A Table S5, revealing that the half-life of in XS soil was  $(17.33 \pm 0.34)$  days, 3.5 times higher than in HN soil  $(4.92 \pm 0.15)$ days (Eqs. (1)–(3)). This was accompanied by a nearly constant BAC content in the sterile control group for 15 days. The assay results of BAC in the air inside the bottle showed that the mass of BAC volatilized into the air was less than 0.02% of the BAC initially added to the soil. The evidence above suggests that microbial degradation is the predominant pathway of BAC dissipation in the soil.

It could be clearly seen that the degradation rates of BAC in the two soils were totally different. The degradation rate of BAC in HN soil was significantly higher than that of BAC in XS soil (p < 0.05). The two different soils used in this experiment showed notable differences in organic matter, pH, and other physicochemical properties, especially the organic matter content in the XS was about 3-fold higher than that in the HN (Appendix A Table S1). This might be an essential contributor to the apparent difference in the degradation rates of BAC



Fig. 1 – Aerobic biodegradation kinetics of benzalkonium chloride (BAC) in two different soils. The solid line in the figure indicates the degradation kinetics of BAC, while the dotted line indicates the degradation rate of BAC. XS from forest areas was acidic silt loam soil, and HN from agricultural region was alkaline loam soil.

in those two soils. Previous studies established that microorganisms can utilize BAC as a carbon source, leading to the degradation of BAC. However, this process could be postponed by the more easily utilizable carbon sources (Zhang et al., 2011). Moreover, the high organic matter content could lead to irreversible adsorption of surfactants to the soil (Atay et al., 2002; Matsuda et al., 2009). Additionally, in HN and XS soil, 95.4% and 83.9% of BAC were calculated to be degraded on the day 203 of the incubation period, respectively (data not shown), indicating BAC could pose persistent stress on the soil ecosystem.

#### 2.2. Effect of BAC on soil N transformation

As demonstrated in the preceding studies, the soil N transformation capacity could be revealed by the N transformation functional enzymes and the genes encoding these enzymes (Song et al., 2019; Zhang et al., 2021). To further quantify the change in soil N conversion ability, urease activity (Appendix A Text S6), and the abundance of N transformation-related functional genes (*nifH*, AOA, AOB, *narG*) were assayed in HN and XS soil.

## 2.2.1. Effect of BAC on nifH

The gene of nifH encodes nitrogenase ferritin, and its abundance is associated with soil N fixation activity (Bossolani et al., 2020). The changes in the abundance of nifH were plotted in Fig. 2a. The abundances of nifH in XS and HN soils were  $1.81 \times 10^6 - 4.55 \times 10^6$  copies/g and  $5.95 \times 10^5 1.35 \times 10^6$  copies/g, respectively. The background abundance of nifH in XS soil was significantly higher than that in HN soil (p<0.05). This may be related to the higher organic matter content in XS soil. The available experimental data suggested a positive correlation between the abundance of N-fixing bacteria and organic carbon content (Manzoni et al., 2012; Yi et al., 2022). As a whole, the abundance of nifH increased in the two soils with the degradation of BAC. On day 60, the relative abundance of nifH in XS soil increased by 91% and 79% in HN soil. It could be hypothesized that some N-fixing bacteria could utilize BAC as carbon sources.



Fig. 2 - Abundance variations of nifH (a), AOA (b), AOB (c), and narG (d) genes in both soils.



Fig. 3 - The temporal variation of relative abundances of bacterial phylum (a) and genus (b) in the XS soil spiked with BAC.

## 2.2.2. Effect of BAC on AOA and AOB

AOA and AOB refer to the ammonia monooxygenase gene (amoA), and their abundances can reflect the ammonia oxidation capacities of the soil archaea and bacteria, respectively (Philippot et al., 2002). Fig. 2b and c illustrate the dynamic changes of AOA and AOB abundances during the degradation of BAC. Similar patterns can be observed for the response of AOA and AOB to the BAC degradation. After days 7, 15, and 60 of incubation, the abundances of AOA and AOB were significantly higher in both soils than on day 0 (p<0.05). The maximum abundances of AOA and AOB in XS and HN soils were documented at day 60. For AOA, the maximum activation rates were 160% and 195% in XS and HN soils, respectively, compared to day 0. For AOB, the maximum activation rates were 55% and 368% in XS and HN soils, respectively, compared to day 0. It suggested a possible increase of the soil nitrification rate following the degradation of BAC.

Consistent with our results, the activation of AOA and AOB genes by pollutants was also observed in previous studies (Du et al., 2018; Zhang et al., 2021). Evidence suggested that ammonia monooxygenase produced by ammonia-oxidizing bacteria could participate in the co-metabolism of organic matter when they are stressed by organic pollutants, thereby increasing the abundance of AOB (Tran et al., 2013).

## 2.2.3. Effect of BAC on narG

The gene of *narG* is responsible for encoding the gamma subunit of nitrate reductase, which is ubiquitous in prokaryotes and can therefore mark the function of soil nitrate reduction (Fierer et al., 2005). The changes in the abundance of *narG* are shown in Fig. 2d. It is apparent from the plot that the abundance changes of the *narG* gene under the single treatment of BAC were distinctly different from that of the other three N transformation-related genes. Throughout the incubation,



Fig. 4 – Network co-occurrence analysis of microbial community in the XS soil spiked with BAC. A connection stands for a strong (Spearman's r > 0.8) and significant (p value < 0.01) correlation. The size of each node is proportional to the number of connections (degree). Red edges indicate positive interactions between two bacterial nodes, while green edges indicate negative interactions. Each node is colored based on the bacterial phyla. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

the narG abundance in XS soil increased significantly only on the days 3 and 7, with the maximum abundance on the day 7 which increased by 184.03% compared to day 0. For HN soils, the abundance of narG increased significantly only on days 7 and 15, reaching a maximum on day 15 with an increase of 15.45% compared to day 0. On the day 60, the abundance of narG in HN and XS soils decreased by 97% and 17%, respectively. This inhibition was also found in the effect of herbicides on denitrification-related genes (Zhang et al., 2018). A decrease in the abundance of denitrification-related genes implies a drop in the soil denitrification capabilities, which is conducive to the uptake of N by crops and may help reduce the greenhouse gas N<sub>2</sub>O (Gu et al., 2017; Zhang et al., 2018).

#### 2.3. Effect of BAC on soil microbial community structure

The changes in soil N transformation-related functional genes may have been accompanied or induced by a pronounced alter in the microbial community structure. Given that the following two reasons, we monitored the microbial community succession process in XS soil with the degradation of BAC: (1) The N transformation-related functional genes in both soils had similar response patterns to the degradation of BAC; (2) BAC had a longer half-life in XS soil, which might have a more profound impact.

The changes in alpha diversity of microbial community in XS soil are recorded in Appendix A Table S7 during BAC degradation, indicating the alpha diversity of the microbial community gradually decreased with the increase of exposure time. The composition of the microbial community at the phylum and genus levels are plotted in Fig. 3. In all microbial taxa, 3 phyla were identified as the dominant group, namely Actinobacteriota (22.35%-36.46%), Chloroflexi (13.49%-29.96%), and Proteobacteria (15.40%-34.40%) (Fig. 3a). The relative abundance of Proteobacteria increased during the first seven days after BAC administration and then decreased. This is consistent with the rapid BAC degradation in the initial period of incubation, implying that certain species in Proteobacteria could contribute to the BAC degradation (Das et al., 2021; Kidd et al., 2021; Mohapatra and Phale, 2021). The relative abundance of Crenarchaeota was significantly higher than that of day 0 except for day 7, and its abundance was positively correlated with the concentration of BAC (Appendix A Fig. S2). There are some evidences suggest that Crenarchaeota is one of the main taxa of AOA (Liu et al., 2021). Considering the results of the above-mentioned AOA gene together, it may be prudent to consider that BAC may facilitate the nitrification process of the soil.

The community heatmap of the top 50 genera is shown in Fig. 3b. During the degradation of BAC, the top 4 dominant genera were AD3 (12.29%-19.75%), Acidothermus (5.24%-10.72%), HSB\_OF53-F07 (1.79%-13.89%), and Gaiellales (4.87%-8.63%). And, the abundance changes of Xanthobacteraceae and Nitrososphaeraceae were positively correlated with the variation of BAC concentration, implying that the two genera might be potential BAC degrading bacteria (Appendix A Fig. S2). Previously, Xanthobacteraceae was reported to degrade organic pollutants (Martirani-Von Abercron et al., 2017), and also to oxidize ammonia and fix N (Lee et al., 2005; Muñoz-Palazon et al., 2019; Obermeier et al., 2020). Nitrososphaeraceae, known ammonia oxidizing archaea, could oxidize ammonia to hydroxylamine, which serves as the first step in the nitrification process (Yang et al., 2021b). Thus, the increase in the abundance of N conversion-related genes could be attributed to the enrichment of these two bacteria. This further proves that BAC in the soil can perturb the N transform functions by changing the microbial community structure. Intrasporangiaceae and Nocardioides, as functional microbiota in the soil, are responsible for the decomposition of cellulose and sugar as well as the mineralization of multiple organic compounds, respectively (Harada et al., 2006; Schellenberger et al., 2010; Takagi et al., 2009). In the present study, the two genera abundances firstly decreased and then increased during the degradation of BAC. This suggests that BAC might interrupt the mineralization processes of organic compounds in the soil.

## 2.4. Analysis on soil microbiomes co-occurrence network

The co-occurrence network analysis was used to characterize the effect of BAC on the connections between soil microorganisms (Fig. 4). As shown in Fig. 4, the positive edges (64.69%) percentage was dramatically higher than that of the negative edges (35.31%). This showed that possible widespread positive interactions exist with bacteria in response to adverse environmental disturbances (Yi et al., 2022). Proteobacteria is a critical functional microbiota in the soil, which plays a crucial role in the soil carbon, N, and sulfur cycles, and can participate in the degradation of carbon-containing compounds (Spain et al., 2009). We found that Proteobacteria is highly correlated with other microorganisms during the degradation of BAC. It could be concluded that Proteobacteria is the core microbial phylum of XS soil and plays a crucial role in maintaining the stability of microbial community structure. Within the Proteobacteria clusters, the *Xanthobacteraceae* was positively correlated with *Bradyrhizobium* and *Methyloligellaceae*. *Xanthobacteraceae* was capable of ammonia oxidation and N fixation, while *Bradyrhizobium* has a significant positive correlation with organic carbon and available phosphorus (Obermeier et al., 2020; Yan et al., 2014). Therefore, functional microbes in soil that can tolerate BAC are the key drivers of changes in microbial community structure during the degradation of BAC.

## 3. Conclusions

The present paper investigated the temporal evolution of soil microbial communities and N transformation functions during BAC degradation. Aerobic microbial degradation was the main pathway of BAC dissipation in natural soils, and its degradation process followed a first-order kinetic model, and its half-life (4.92 vs. 17.33 days) may depend on the organic matter content of the two soils. A decrease in the alpha diversity of the soil microbial community was found during BAC degradation, which was accompanied by a significant change in the abundance of some functional microorganisms. For example, Xanthobacteraceae, involved in ammonia oxidation and N fixation, and Nitrososphaeraceae, responsible for nitrification, were found to be significantly correlated with BAC concentrations. This implies that the soil N cycle function is inevitably disturbed during the degradation of BAC. This can be further demonstrated by the up-regulation of nitrogen fixation and nitrification genes in combination with the down-regulation of denitrification genes. These findings suggest that while paying attention to the ecotoxicity of BAC in soil, it is important to focus on changes in the soil microbial structure and nutrition cycle.

## **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 42177363); the National Key R&D Program of China (No. 2019YFC1805502); the Open Project of National and Local Joint Engineering Research Center of Shale Gas Exploration and Development (No. YYQKTKFGJDFLHGCYJZX-201904); the Innovation Support Program for Chongqing Overseas Returnees (2017).

## Appendix A Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jes.2022.04.004.

## REFERENCES

- Atay, N.Z., Yenigun, O., Asutay, M., 2002. Sorption of anionic surfactants SDS, AOT and cationic surfactant hyamine 1622 on natural soils. Water Air Soil Pollut. 136, 55–67.
- Bergero, M.F., Lucchesi, G.I., 2015. Immobilization of Pseudomonas putida a (ATCC 12633) cells: a promising tool for effective degradation of quaternary ammonium compounds in industrial effluents. Int. Biodeterior. Biodegrad. 100, 38–43. Bossolani, J.W., Crusciol, C.A.C., Merloti, L.F., Moretti, L.G.,
- Costa, N.R., Tsai, S.M., et al., 2020. Long-term lime and gypsum amendment increase nitrogen fixation and decrease nitrification and denitrification gene abundances in the rhizosphere and soil in a tropical no-till intercropping system. Geoderma 375, 114476.
- Chen, B., Han, J., Dai, H., Jia, P., 2021. Biocide-tolerance and antibiotic-resistance in community environments and risk of direct transfers to humans: Unintended consequences of community-wide surface disinfecting during COVID-19? Environ. Pollut. 283, 117074.
- Dai, X., Wang, C., Lam, J.C.W., Yamashita, N., Yamazaki, E., Horii, Y., et al., 2018. Accumulation of quaternary ammonium compounds as emerging contaminants in sediments collected from the pearl river estuary, China and Tokyo Bay. Jpn. Mar. Pollut. Bull. 136, 276–281.
- Das, N., Kotoky, R., Maurya, A.P., Bhuyan, B., Pandey, P., 2021. Paradigm shift in antibiotic-resistome of petroleum hydrocarbon contaminated soil. Sci. Total Environ. 757, 143777.
- Du, P., Wu, X., Xu, J., Dong, F., Liu, X., Zhang, Y., et al., 2018. Clomazone influence soil microbial community and soil nitrogen cycling. Sci. Total Environ. 644, 475–485.
- Fierer, N., Jackson Jason, A., Vilgalys, R., Jackson Robert, B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120.
- Frühling, W., Rönnpagel, K., Ahlf, W., 2001. Effect of zinc and benzalkonium chloride on Nitrosomonas communis and potential nitrification in soil. Environ. Toxicol. 16, 439–443.
- Gu, J., Yuan, M., Liu, J., Hao, Y., Zhou, Y., Qu, D., et al., 2017. Trade-off between soil organic carbon sequestration and nitrous oxide emissions from winter wheat-summer maize rotations: Implications of a 25-year fertilization experiment in Northwestern China. Sci. Total Environ. 595, 371–379.
- Hajaya, M.G., Pavlostathis, S.G., 2012. Fate and effect of benzalkonium chlorides in a continuous-flow biological nitrogen removal system treating poultry processing wastewater. Bioresour. Technol. 118, 73–81.
- Harada, N., Takagi, K., Harazono, A., Fujii, K., Iwasaki, A., 2006. Isolation and characterization of microorganisms capable of hydrolysing the herbicide mefenacet. Soil Biol. Biochem. 38, 173–179.
- Jardak, K., Drogui, P., Daghrir, R., 2016. Surfactants in aquatic and terrestrial environment: occurrence, behavior, and treatment processes. Environ. Sci. Pollut. Res. 23, 3195–3216.
- Kahrilas, G.A., Blotevogel, J., Stewart, P.S., Borch, T., 2015. Biocides in hydraulic fracturing fluids: a critical review of their usage, mobility, degradation, and toxicity. Environ. Sci. Technol. 49, 16–32.

Kang, H.I., Shin, H.S., 2016. Rapid and sensitive determination of benzalkonium chloride biocide residues in soil using liquid

chromatography–tandem mass spectrometry after ultrasonically assisted extraction. Bull. Korean Chem. Soc. 37, 1219–1227.

- Khan, A.H., Topp, E., Scott, A., Sumarah, M., Macfie, S.M., Ray, M.B., 2015. Biodegradation of benzalkonium chlorides singly and in mixtures by a *Pseudomonas* sp. isolated from returned activated sludge. J. Hazard. Mater. 299, 595–602.
- Kidd, P.S., Álvarez, A., Álvarez-López, V., Cerdeira-Pérez, A., Rodríguez-Garrido, B., Prieto-Fernández, Á., et al., 2021.
  Beneficial traits of root endophytes and rhizobacteria associated with plants growing in phytomanaged soils with mixed trace metal-polycyclic aromatic hydrocarbon contamination. Chemosphere 277, 130272.
- Kim, S., Ji, K., Shin, H., Park, S., Kho, Y., Park, K., et al., 2020. Occurrences of benzalkonium chloride in streams near a pharmaceutical manufacturing complex in Korea and associated ecological risk. Chemosphere 256, 127084.
- Lai, Y.S., Ontiveros-Valencia, A., Ilhan, Z.E., Zhou, Y., Miranda, E., Maldonado, J., et al., 2017. Enhancing biodegradation of C16-alkyl quaternary ammonium compounds using an oxygen-based membrane biofilm reactor. Water Res. 123, 825–833.
- Lee, K.B., Liu, C.T., Anzai, Y., Kim, H., Aono, T., Oyaizu, H., 2005. The hierarchical system of the 'Alphaproteobacteria': Description of Hyphomonadaceae fam. nov., Xanthobacteraceae fam. nov. and Erythrobacteraceae fam. nov. Int. J. Syst. Evol. Microbiol. 55, 1907–1919.
- Lewis, D., 2021. COVID-19 rarely spreads through surfaces. So why are we still deep cleaning? Nature 590, 26–28.
- Liu, J., Liu, W., Zhang, Y., Chen, C., Wu, W., Zhang, T.C., 2021. Microbial communities in rare earth mining soil after in-situ leaching mining. Sci. Total Environ. 755, 142521.
- Ma, B., Zhao, K., Lv, X., Su, W., Dai, Z., Gilbert, J.A., et al., 2018. Genetic correlation network prediction of forest soil microbial functional organization. ISME J. 12, 2492–2505.
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91.
- Martirani-Von Abercron, S.M., Marín, P., Solsona-Ferraz, M., Castañeda-Cataña, M.A., Marqués, S., 2017. Naphthalene biodegradation under oxygen-limiting conditions: Community dynamics and the relevance of biofilm-forming capacity. Microb. Biotechnol. 10, 1781–1796.
- Matsuda, M., Kaminaga, A., Hayakawa, K., Takisawa, N., Miyajima, T., 2009. Surfactant binding by humic acids in the presence of divalent metal salts. Colloids Surf. Physicochem. Eng. Asp. 347, 45–49.
- Mohapatra, B., Phale, P.S., 2021. Microbial degradation of naphthalene and substituted naphthalenes: Metabolic diversity and genomic insight for bioremediation. Front. Bioeng. Biotech. 9, 602445.
- Mulder, I., Siemens, J., Sentek, V., Amelung, W., Smalla, K., Jechalke, S., 2018. Quaternary ammonium compounds in soil: implications for antibiotic resistance development. Rev. Environ. Sci. Bio. 17, 159–185.
- Muñoz-Palazon, B., Rodriguez-Sanchez, A., Hurtado-Martinez, M., de Castro, I.M., Juarez-Jimenez, B., Gonzalez-Martinez, A., et al., 2019. Performance and microbial community structure of an aerobic granular sludge system at different phenolic acid concentrations. J. Hazard. Mater. 376, 58–67.
- Obermeier, M.M., Minarsch, E.M.L., Durai Raj, A.C., Rineau, F., Schröder, P., 2020. Changes of soil-rhizosphere microbiota after organic amendment application in a hordeum vulgare L. short-term greenhouse experiment. Plant Soil 455, 489–506.
- Pateiro-Moure, M., Arias-Estévez, M., Simal-Gándara, J., 2013. Critical review on the environmental fate of quaternary ammonium herbicides in soils devoted to vineyards. Environ. Sci. Technol. 47, 4984–4998.

Patrauchan, M.A., Oriel, P.J., 2003. Degradation of benzyldimethylalkylammonium chloride by Aeromonas hydrophila sp. K. J. Appl. Microbiol. 94, 266–272.

Philippot, L., Piutti, S., Martin-Laurent, F., Hallet, S., Germon Jean, C., 2002. Molecular analysis of the nitrate-reducing community from unplanted and maize-planted soils. Appl. Environ. Microbiol. 68, 6121–6128.

Qian, Y., He, Y., Li, H., Yi, M., Zhang, L., Zhang, L., et al., 2022. Benzalkonium chlorides (C12) inhibits growth but motivates microcystins release of Microcystis aeruginosa revealed by morphological, physiological, and iTRAQ investigation. Environ. Pollut. 292, 118305.

Sarkar, B., Megharaj, M., Xi, Y., Krishnamurti, G.S.R., Naidu, R., 2010. Sorption of quaternary ammonium compounds in soils: Implications to the soil microbial activities. J. Hazard. Mater. 184, 448–456.

Schellenberger, S., Kolb, S., Drake, H.L., 2010. Metabolic responses of novel cellulolytic and saccharolytic agricultural soil *Bacteria* to oxygen. Environ. Microbiol. 12, 845–861.

Song, Z., Wang, J., Liu, G., Zhang, C., 2019. Changes in nitrogen functional genes in soil profiles of grassland under long-term grazing prohibition in a semiarid area. Sci. Total Environ. 673, 92–101.

Spain, A.M., Krumholz, L.R., Elshahed, M.S., 2009. Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J. 3, 992–1000.

Takagi, K., Iwasaki, A., Kamei, I., Satsuma, K., Yoshioka, Y., Harada, N., 2009. Aerobic mineralization of hexachlorobenzene by newly isolated pentachloronitrobenzene-degrading Nocardioides sp. strain PD653. Appl. Environ. Microbiol. 75, 4452–4458.

Tezel, U., Tandukar, M., Martinez, R.J., Sobecky, P.A., Pavlostathis, S.G., 2012. Aerobic biotransformation of n-tetradecylbenzyldimethylammonium chloride by an enriched Pseudomonas spp. community. Environ. Sci. Technol. 46, 8714–8722.

Tran, N.H., Urase, T., Ngo, H.H., Hu, J., Ong, S.L., 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Bioresour. Technol. 146, 721–731.

Van der Heijden, M.G.A., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91, 1163–1171.

Wang, Y., Du, L., Liu, H., Long, D., Huang, M., Wang, Y., et al., 2020. Halosulfuron methyl did not have a significant effect on diversity and community of sugarcane rhizosphere microflora. J. Hazard. Mater. 399, 123040.

- Xiang, L., Sun, T.F., Zheng, M.J., Li, Y.W., Li, H., Wong, M.H., et al., 2016. Sorption of dodecyltrimethylammonium chloride (DTAC) to agricultural soils. Sci. Total Environ. 560-561, 197–203.
- Yan, J., Han, X.Z., Ji, Z.J., Li, Y., Wang, E.T., Xie, Z.H., et al., 2014. Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. Appl. Environ. Microbiol. 80, 5394–5402.

Yang, R., Wang, J., Zhu, L., Wang, J., Yang, L., Mao, S., et al., 2021a. Effects of interaction between enrofloxacin and copper on soil enzyme activity and evaluation of comprehensive toxicity. Chemosphere 268, 129208.

Yang, Y., Herbold, C.W., Jung, M.Y., Qin, W., Cai, M., Du, H., et al., 2021b. Survival strategies of ammonia-oxidizing archaea (AOA) in a full-scale WWTP treating mixed landfill leachate containing copper ions and operating at low-intensity of aeration. Water Res. 191, 116798.

Yang, Y., Wang, W., 2018. Benzyldimethyldodecyl ammonium chloride shifts the proliferation of functional genes and microbial community in natural water from eutrophic lake. Environ. Pollut. 236, 355–365.

Yi, M., Zhang, L., Qin, C., Lu, P., Bai, H., Han, X., et al., 2022. Temporal changes of microbial community structure and nitrogen cycling processes during the aerobic degradation of phenanthrene. Chemosphere 286, 131709.

Zhang, C., Cui, F., Zeng, G., Jiang, M., Yang, Z., Yu, Z., et al., 2015. Quaternary ammonium compounds (QACs): A review on occurrence, fate and toxicity in the environment. Sci. Total Environ. 518-519, 352–362.

Zhang, C., Tezel, U., Li, K., Liu, D., Ren, R., Du, J., et al., 2011. Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge. Water Res. 45, 1238–1246.

Zhang, M., Wang, W., Tang, L., Heenan, M., Xu, Z., 2018. Effects of nitrification inhibitor and herbicides on nitrification, nitrite and nitrate consumptions and nitrous oxide emission in an Australian sugarcane soil. Biol. Fertil. Soils 54, 697–706.

Zhang, Y., Zhang, J., Shi, B., Li, B., Du, Z., Wang, J., et al., 2021. Effects of cloransulam-methyl and diclosulam on soil nitrogen and carbon cycle-related microorganisms. J. Hazard. Mater. 418, 126395.