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Insight into a direct carbon dioxide effect on denitrification and denitrifying bacterial communities in estuarine sediment

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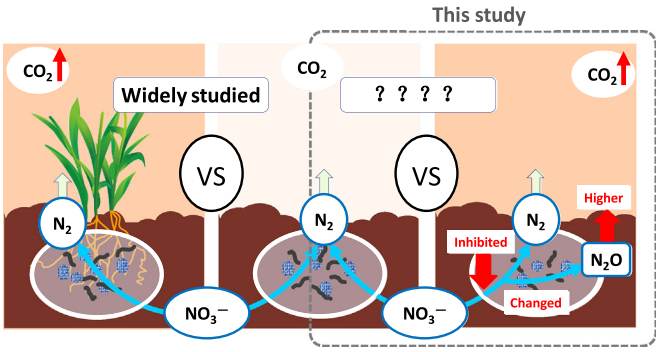
HIGHLIGHTS

- The short-term and long-term exposure to CO_2 directly inhibited sediment denitrification.
- Long-term exposure to 30,000 ppm CO_2 caused >276-fold N_2O emissions than the control.
- CO_2 directly decreased the abundance of denitrifying bacteria.
- CO_2 decreased the abundance of fermenting bacteria and inhibited the decomposition process.

GRAPHICAL ABSTRACT



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ABSTRACT

With the elevation of atmospheric CO_2 content, the potential effects of CO_2 on organisms and various environmental processes have gained increasing concern. Most previous studies on denitrification have been conducted on ecosystems comprising plants, soils and microbes, but they have ignored the direct effect of CO_2 on denitrification and denitrifying bacterial communities. Here, by excluding the effects of plants, we found that both short- and long-term exposure to CO_2 directly inhibited the denitrification process, and caused the total nitrogen removal efficiency to decrease by up to 37%. Compared with the control, long-term exposure to CO_2 (30,000 ppm) also caused >276-fold increase in N_2O emissions, and significantly inhibited the decomposition process. Enzymatic and qPCR assays showed that CO_2 decreased the denitrifying enzymes activity (DEA) and the copy numbers of denitrifying genes, which directly resulted in the inhibitory effect of CO_2 on denitrification process. Further study indicated that adverse effect of CO_2 on DEA and denitrifying genes were caused by reducing the relative abundance of denitrifying bacteria. Moreover, the relative abundance of fermenting bacteria also decreased as CO_2 concentration increased, which might result in insufficient liable carbon for the activity of denitrifying bacteria, and ultimately exacerbate the negative denitrification performance. Overall, this study suggests that, in the absence of plants, CO_2 could directly affect the denitrifying and fermenting bacterial community, and inhibit denitrification and decomposition processes, which is detrimental to sediment nitrogen and carbon cycles.

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1. Introduction

The cumulative anthropogenic CO_2 emissions to the atmosphere between 1870 and 2016 comprised 2075 ± 205 $GtCO_2$ (Quéré et al., 2016). Approximately 40% of these emissions remained in the atmosphere, which led to an increase in the atmospheric CO_2 concentration from 280 ppm before Industrial Revolution to 400 ppm in 2016 (Collins et al., 2013; Spady et al., 2018). According to multi-model predictions, the average atmospheric CO_2 concentration in 2100 might reach 985 ± 97 ppm (Collins et al., 2013). In contrast, due to the activity of microbes and plants, soil CO_2 levels are >10 times higher than the atmospheric concentration (Sotomayor and Rice, 1999). Geologic CO_2 sequestration is commonly considered to be a feasible approach to alleviate the increase in atmospheric CO_2 content, but leakage and accidental emissions from sequestration sites could result in an observed CO_2 concentration of up to 40,000 ppmv (McAlexander et al., 2011). Therefore, the effect of CO_2 on organisms and various environmental processes has been widely studied. In the ocean, increasing atmospheric CO_2 changes the property of seawater. For instance, it may decrease the seawater pH and change the calcium carbonate saturation state, thus affecting aquatic organisms. In the literature, variations in the chemical characteristics of the seawater have been reported to threaten the future of coral reefs by reducing the concentration of carbonate ions (Mollica et al., 2018), and affect the coastal biodiversity and structural complexity of marine biogenic habitats (Sunday et al., 2017). In addition, a great number of evidences had suggested that increased CO_2 stimulated the productivity and re-productivity of plants. The effects could further propagate to heterotrophic soil microorganisms (Chung et al., 2007; Jablonski et al., 2002), which ultimately disturb underground bio-processes.

As a key process in the nitrogen cycle, denitrification is driven by diverse heterotrophic denitrifying bacteria (Altabet et al., 2002; Wan et al., 2018). Specifically, by utilizing carbon substrates as electron donors, NO_3^- is stepwise reduced to NO_2^-, NO, N_2O, and N_2, with the catalysis of nitrate reductase (NAR), nitrite reductase (NIR), nitric oxide reductase (NOR), and nitrous oxide reductase (N2OR), respectively (Wan et al., 2016). Therefore, denitrification performance is largely dependent on bacterial populations and microbial enzymatic activity, both of which are correlated to a variety of physical and chemical conditions, such as temperature, carbon substrate availability and types (e.g., acetate, methanol, even industrial or agricultural waste), as well as toxic compounds (e.g., nanoparticles, heavy metals) (Wang and Chen, 2016; Warneke et al., 2011). For instance, via decreasing the microbial enzymatic activity and changing the microbial community, the long-term exposure to ZnO nanoparticles has been reported to impact denitrification profile (Wang et al., 2016). As mediators of the denitrification process, denitrifying bacteria can be quantified by functional genes that encode key denitrifying enzymes (Wan et al., 2016). It was reported that Al_2O_3 nanoparticles have affected denitrification by decreasing the denitrifying functional genes (such as *nirK*, *nirS*) (Chen et al., 2012).

Under the increasing CO_2 scenario, the effect of CO_2 on denitrification has also been investigated in various ecosystems, such as grasslands, forests, and meadows (Barnard et al., 2004b; Lee et al., 2012; Regan et al., 2011; Zheng et al., 2008), for which different results have been observed. It has been reported that potential denitrifying activity increased by 3 to 24 times with increasing CO_2, which was stimulated by the exudation and accumulation of root nonstructural carbohydrate from upland vegetation (Smart et al., 1997). However, an insignificant effect of CO_2 on denitrification was observed in mesocosms where two perennial C3 grass monocultures were planted (Barnard et al., 2004b). On the other hand, significant decreases in denitrification activities were reported in grassland monoliths in France and forest soils beneath *Pinus sylvestriformis* in China upon exposure to high CO_2 concentration (Barnard et al., 2004a; Zheng et al., 2008). Changes in the denitrifying bacterial community were also observed with changing CO_2 concentration in wetland ecosystems (Lee et al., 2012). Indeed, variations in soil environmental conditions (such as labile C availability, soil water content, soil oxygen concentration, and microbial communities) caused by CO_2 increase were identified as the main reason for affecting denitrification performance in previous studies. However, these factors were also affected by the vegetation in their respective ecosystems due to the complexity of interactions between CO_2, plants, soil, and soil microorganisms (Barnard et al., 2004b; Chung et al., 2007; Jablonski et al., 2002). As such, the actual effect of CO_2 on microbial denitrification might be occluded by the responses of vegetation. Our previous studies have indicated that an increase in the environmental CO_2 concentration could directly inhibit the carbon metabolism, electron transport and consumption of denitrifiers, which resulted in a negative effect on denitrification (Wan et al., 2018; Wan et al., 2016). To date, the direct effect of CO_2 on in situ denitrification and the denitrifying bacterial communities in the absence of plants has seldom been documented.

As an important nitrogen sink for both terrestrial and marine ecosystems, estuarine sediment plays a vital role in alleviating coastal eutrophication and modifying nitrogen loads in marine systems, therefore estuaries are excellent ecosystems for the study of the diversity and functional ecology of denitrifying bacterial communities (Brin et al., 2017; Nogales et al., 2002). Herein, the aim of this paper is to investigate the direct influences of CO_2 on denitrification in the estuarine sediment. The direct short-term effects of CO_2 on nitrate reduction, N_2O emission, and denitrifying enzyme activity (DEA) were first studied using fresh sediments. To investigate the long-term effect of CO_2, sediments were cultured under different concentrations of CO_2 for 60 days. Batch tests were then performed to investigate the effects of CO_2 on the nitrate reduction, N_2O emission, and the DEA of the cultured sediments. Finally, the variations in sediment compositions, functional denitrifying genes (*nirS* and *nirK*) and bacterial community were further analyzed to disclose the underlying mechanisms of the long-term denitrifying performance. This paper provides evidence for understanding the direct response of microbes to CO_2 concentration.

2. Materials and methods

2.1. Environmental sampling

Estuarine sediment samples were collected from the intertidal zone of the Huangpu River estuary (N31°23′, E121°30′) in Shanghai, China in November 2015. At low tide, surface sediment (0–5 cm) was randomly collected, carefully stored in sterile plastic bags, and transported to the laboratory in <2 h. In the laboratory, after discarding roots and stones, sediments were sieved through a two-millimeter plastic mesh, homogenized with a Teflon spoon until no textural difference could be observed, and then stored at 4 °C for <12 h. In addition, near the sediment sampling sites (within 20 m), 15 L of estuarine water was collected in sterile polyethylene bottles at the high tide and stored at 4 °C for <5 days. The characteristics of the fresh sediment and estuarine water samples are provided in the supplementary materials (Tables S2 and S3).

2.2. Short-term effect of CO_2 on denitrification in sediment

To investigate the short-term effect of CO_2 on sediment denitrification, the fresh sediments were exposed to two CO_2 concentrations (0 ppm, 3000 ± 210 ppm and 30,000 ± 315 ppm CO_2), which were composed of CO_2 and N_2, and purchased from a gas cylinder supplier (ChunYu Special Gases Co., Ltd. Shanghai, China). The denitrifying medium was detailed in our previous publication with a minor modification (Wan et al., 2016), which contained (per liter): 0.15 g NH_4NO_3, 0.18 g KNO_3, 0.59 g sodium acetate, 0.1 g $MgSO_4 \cdot 7H_2O$, 2.44 g KH_2PO_4, 4.65 g Na_2HPO_4, and 1 mL trace elements. The trace elements solution contained (per liter): 7.30 g Na_2-EDTA, 2.50 g $FeSO_4 \cdot 7H_2O$, 0.02 g $MnCl_2 \cdot 4H_2O$, 0.242 g $Na_2MoO_4 \cdot 2H_2O$, 0.135 g $CuCl_2 \cdot 2H_2O$, and