Co-effects of salinity and moisture on CO₂ and N₂O emissions of laboratory-incubated salt-affected soils from different vegetation types

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ABSTRACT

The temporal variation of precipitation and relevant salinity fluctuation can significantly affect greenhouse gas (GHG) emissions of salt-affected soils in the Yellow River Delta (YRD) of China. The current study aims to investigate the effects of salinity and moisture on CO₂ and N₂O emissions of saline soils. Soils collected from different vegetation communities were incubated in glass Mason jars under different levels of salinity and moisture. Gas samples were collected from the headspace of jars and analyzed using gas chromatography during the incubation period. Soil CO₂ and N₂O emission rates decreased steadily over time, and then were relatively stable during the final incubation. Cumulative CO₂ and N₂O emissions increased steadily across the incubation period in all treatments. However, cumulative N₂O emissions in bare land with no vegetation cover increased steadily. In general, production rate and cumulative emission of CO₂ were highest in herbaceous communities, were intermediate in woody community, and were lowest in bare land under all treatments. The negative relationship between cumulative GHG emission and soil salinity was more significant in soils that contained low levels of salt, than that in other soils. The significant positive correlation between cumulative GHG emissions and soil moisture was found in all soils. The effects of salinity on GHG emission were stronger in soils with low levels of salt. Compared with soils collected from bare land with no vegetation cover, soils from different vegetation communities emitted more CO₂ and N₂O. Perhaps more attention, therefore, should be paid to pulse emissions of GHG as a result of destruction of vegetation in the course of exploitation and utilization of saline soil resources.

1. Introduction

Soil salinization is considered to be one of the most common land degradation processes. There are about 831 million ha (> 6%) of salt-affected agricultural land worldwide (Amini et al., 2016), with 397 million ha of saline soils and 434 million ha of sodic soils (FAO, 2015). Soils that contain excess salts not only interfere with the normal soil processes, but also affect the nutrient and water uptake by plant, which impair plant growth (Nelson and Ham, 2000).

Excess salts affect the microbial activity, apart from plant growth inhibition, and interfere with microbe-mediated soil processes (Liang et al., 2005; Tejada et al., 2006). Soil carbon (C) and nitrogen (N) mineralization increases or decreases following varied microbial respiration, which was affected by high concentration of salt (Pathak and Rao, 1998; Wichern et al., 2006). As a stress to soil microorganisms, increasing salinity inhibits organic matter decomposition and causes a decline of N mineralization (Rietz and Haynes, 2003). However, Khoi et al. (Khoi et al., 2006) found that N mineralization rate was inhibited temporarily and recovered at later stages. Many factors, such as soil types and incubation conditions, could be responsible for the differences.

Carbon dioxide (CO₂) and nitrous oxide (N₂O), two major radioactively active greenhouse gases (GHG) contributing to global warming, were driven by microbial activities, such as denitrification and metabolism, and may be significantly affected by salt and moisture conditions (Houska et al., 2017; Maucieri et al., 2017; Setia et al., 2011b; Shi...
et al., 2015). In general, severe drying and excess salt limit microbial activity by osmotic stress (Smith et al., 2003; Stark and Firestone, 1995; Yemadje et al., 2016), and soil aeration can be limited with increasing levels of water (Mentges et al., 2016; Yuste et al., 2017). Zhang et al. (2016) and Oren (1999) reported that considerable amounts of N₂O emitted from salt-affected soils result from prevailed denitrification. Similarly, C mineralization has also been reported to increase with increasing salinity (Marton et al., 2012). However, Kontopoulou et al. (2015) found that salinity has no significant effect on CO₂ and N₂O productions. Many studies (Kessavalou et al., 1998; Qian et al., 1997; Schaufler et al., 2010; Sehy et al., 2003) showed that emission of soil N₂O increase significantly along a soil moisture gradient, but CO₂ production is highest at an intermediate soil moisture. Salinity is usually determined by changed moisture of soil resulting from rainfall, evaporation, irrigation, and drainage (Ghosh et al., 2017; Rabie et al., 2018). Therefore, GHG production of salt-affected soil could be affected by the interactive effect of salinity and moisture.

The Yellow River Delta (YRD), one of the three biggest deltas in China, is the fastest growing delta and the most active land–ocean interaction regions among the large river deltas in the world (Wang et al., 2012). Due to its great exploitation potential, the YRD is called as the “Golden Triangle” and gets more and more attention. However, rainfall in this area is scarce and irregular, with about 70% of precipitation occurring between June and August, and excessive salt exists in underground water. These conditions cause soil salinization and alkali-desalinization, leaving only a few tolerant plant species, thus reducing plant diversity. *Tamarix chinensis*, *Suaeda salsa* and *Phragmites australis* is three dominant plant species adapt the saline-alkaline habitat in this region. Since vegetation plays an important role in regulating the temporal and spatial variations of soil respiration by controlling a variety of environmental variables (Barba et al., 2013; Han et al., 2014; Jenkins and Adams, 2010), Zhang et al. (2013, 2015) and Song et al. (2013) investigated GHG production of saline soils in above-mentioned three vegetation communities and in bare land with no vegetation cover in situ. They found that temporal variations of GHG emissions were related to the interactions of abiotic factors, such as soil water content and electrical conductivity, while spatial variations were mainly affected by the vegetation composition at spatial scale. Exploring the complex interaction among different environmental factors on GHG emission is necessary for better management of soil and environment. Measurement of soil gas production under laboratory-controlled conditions offer an opportunity to understand the effects of specific factors on CO₂ and N₂O emissions (Ghosh et al., 2017).

To our knowledge, even though variations in soil salinity and moisture are considered as the main driver of GHG emission very few studies have been conducted to investigate the effects of salinity and moisture on CO₂ and N₂O emissions of saline soils under different vegetation types. In this laboratory incubation study, therefore, we sought to examine the effects of salinity, moisture, and their interaction on CO₂ and N₂O emissions of salt-affected soils collected in bare land (BL) and three adjacent vegetation communities, *Tamarix chinensis* (TC), *Suaeda salsa* (SS) and *Phragmites australis* (PA). The objectives of the current study were to assess the effects of soil salinity and moisture on CO₂ and N₂O emissions, and compare the difference of CO₂ and N₂O emissions among soils collected from different vegetation communities.

### Table 1

| Soil types | pH       | EC (mS cm⁻¹) | WHC (g g⁻¹) | NH₄⁺-N (mg kg⁻¹) | NO₃⁻-N (mg kg⁻¹) | TOC (g kg⁻¹) | TN (g kg⁻¹) |
|------------|----------|-------------|-------------|------------------|------------------|-------------|-------------|
| BL         | 7.78 ± 0.05 | 14.84 ± 1.21 | 0.36 ± 0.01 | 4.50 ± 0.35 | 11.87 ± 0.83 | 7.52 ± 0.81 | 0.35 ± 0.02 |
| TC         | 7.62 ± 0.03 | 10.46 ± 1.02 | 0.41 ± 0.01 | 4.09 ± 0.38 | 10.24 ± 0.78 | 8.83 ± 0.77 | 0.46 ± 0.01 |
| SS         | 7.41 ± 0.03 | 5.18 ± 0.37  | 0.50 ± 0.01 | 4.30 ± 0.29 | 7.71 ± 0.49  | 9.33 ± 0.79 | 0.83 ± 0.02 |
| PA         | 7.36 ± 0.02 | 2.47 ± 0.22  | 0.53 ± 0.01 | 5.09 ± 0.41 | 3.90 ± 0.27  | 11.78 ± 0.86| 1.05 ± 0.08 |

Bias, bare land; TC, *Tamarix chinensis*; SS, *Suaeda salsa*; PA, *Phragmites australis*.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from saline-alkaline soil with no vegetation cover (i.e. bare land BL), with *T. chinensis* community (TC), with *S. salsa* community (SS) and with *P. australis* community (PA), which are located in the Nature Reserve of the Yellow River Delta (37°35′–38°12′N, 118°33′–119°20′E) in Dongying City, Shandong Province, China. Samples from four areas were collected from 0 to 10 cm depth, air-dried at room temperature and passed through a 2-mm stainless steel sieve. Soil characteristics are shown in Table 1.

2.2. Experimental design and set-up

We used a 4 × 3 factorial design with the following main factors: 1) salinity as the main factor (control or 1 mg/g, 3 mg/g and 5 mg/g, represented by S1, S2, S3 and S4, respectively); 2) moisture as a secondary factor (40%, 70% and 130% water-holding capacity (WHC), represented by W1, W2 and W3, respectively). Therefore, there were 12 treatment combinations in the present experiment, each with three replicates. At the beginning of experiment, 80 g of air-dried soil was put into a 1-L glass Mason jar. Soil salinity was adjusted using deionized and sea water to ensure the salt types were similar with those in field soil. The deionized or salinized water was used to adjust soil moisture. The incubation began when water content of all soils reached to required levels. The jars were kept at 25 ± 1 °C during the entire incubation period, and were weighed daily to correct the soil moisture by adding deionized water onto the soil surface.

2.3. Greenhouse gas measurements

Gas samples were collected and measured after 1, 2, 4, 7, 10, 15, 20, 27, 37 and 52 days of incubation according to a procedure similar to that described by McDaniel et al. (2014) and Sun et al. (2014). Jars were thoroughly flushed with fresh air using an air compressor for 5 min to ventilate air in all jars, they were sealed with gas tight lids equipped with three-way valve to allow collection of CO₂ and N₂O samples from the headspace. Gas samples were immediately collected via syringe and injected into 20-ml pre-evacuated dark cool packs. Soils were subsequently incubated for 24 h before a second gas sample was collected. After that, the jars were opened until the next sampling date. Packs were analyzed for greenhouse gases content within 24 h of gas sampling using gas chromatography (Agilent 7890A) equipped with FID and ECD. Soil GHG production rate was calculated as the difference in CO₂ and N₂O concentrations between the two sampling time points (McDaniel et al., 2014). Production rates of CO₂ and N₂O were measured more frequently at the beginning of the incubation and less frequently toward the end of the experiment during the study.

The emission rate (F) of CO₂ (mg CO₂ kg⁻¹ d⁻¹) or N₂O (µg N₂O kg⁻¹ soil⁻¹ d⁻¹) was calculated by the following equation (Sun et al., 2014):

\[
F = \rho \times \frac{V}{M} \times \frac{dc}{dt} \times 273 \times \frac{1}{T}
\]

where \( \rho \) is the density of CO₂ or N₂O in standard temperature and
pressure (1.98 g/L and 1.97 g/L), V is the volume of the glass jar (L), M is the mass of soil (g), \( \frac{dc}{dt} \) is the slope of the linear regression for gas concentration gradient through time and \( T \) is the incubation temperature (K). By using trapezoidal rule, the cumulative CO2 or N2O emission was calculated as the sum of the area bounded by the rate.

Soil EC was potentiometrically measured in the supernatant suspension of a 1:5 soil:water mixture after 1 h end-over-end shaking at 25 °C (Setia et al., 2011b) at the end of incubation.

2.4. Data analysis and statistics

Production of GHGs was calculated assuming constant rates of
production, and the “area-under-the-curve” approach was used to calculate cumulative CO₂ and N₂O productions of each jar (Maucieri et al., 2017). Cumulative production of CO₂ and N₂O and properties of incubated soil were analyzed with two-way ANOVA and significant effects of different soils with varying moisture levels, salinity levels, and their interaction were checked through Turkey’s test. Normality test and equal variance test of the original data were checked prior to analysis of variance (ANOVA). Regression analysis between GHG production and soil EC were conducted. IBM SPSS statistics 23 and SigmaPlot 12.0 were used to perform statistical analysis of data. The data were presented as means of the replications, with standard deviation (SD).

Fig. 2. Temporal variations of N₂O production rates of soils, collected from bare land (BL), T. chinensis community (TC), S. salsa community (SS) and P. australis community (PA), at the four different moisture levels or at the three different salinity levels. The bars indicate the standard deviations of means (± SD).
3. Results

3.1. Temporal variations of CO₂ and N₂O emissions

Similarly temporal dynamics of production rates and cumulative emissions for soil CO₂ and N₂O were observed under treatment with salinity and moisture (Figs. 1 to 4). The two GHG emission rates decreased steadily over time, and then were relatively stable after 15 days of incubation in TC, SS and PA, except for N₂O emission rates in SS and PA under W3 treatment, which were highest on the 20th day of incubation (Figs. 1 and 2). Although production rates of CO₂ and N₂O in TC, SS and PA showed exponential declines, the two greenhouse gases
in BL were highly variable showing no distinct pattern. Cumulative CO$_2$
and N$_2$O emissions increased steadily across the incubation period in all
treatments (Figs. 3 and 4). However, cumulative N$_2$O emissions in BL
decreased steadily across the incubation period.

In general, production rates and cumulative emissions of CO$_2$
were highest in SS and PA, were intermediate in TC, and were lowest in BL
under all treatments (Figs. 1 and 3). However, N$_2$O emission rates and
cumulative productions increased significantly in the order TC > PA > SS > BL (Figs. 2 and 4).

Fig. 4. Cumulative N$_2$O emissions of soils, collected from bare land (BL), T. chinensis community (TC), S. salsa community (SS) and P. australis community (PA), at the four different moisture levels or at the three different salinity levels. The bars indicate the standard deviations of means (± SD).
3.2. Effects of salinity and moisture on cumulative CO₂ and N₂O emissions

Salinity showed significant and identical effects on CO₂ and N₂O emissions (Table 2). Cumulative CO₂ and N₂O emissions were lower than control with increasing salinity. Higher cumulative N₂O emissions under high salinity were only found in TC and PA under W3 treatment.

Soil moisture also had a significant effect on CO₂ and N₂O productions (Table 2). The elevated soil water content significantly increased CO₂ emissions in all soils (Fig. 3). Cumulative N₂O emissions in BL, SS and PA increased with increasing moisture. However, TC had the highest N₂O production under W2 treatment (Fig. 4).

CO₂ and N₂O emissions of all soils, by the end of incubation, were significantly influenced by interaction between salinity and moisture (Fig. 5, Table 2). Cumulative CO₂ emissions of different soils were highest under S1 treatment along different soil moisture gradient. While N₂O emissions did not differ among salinity treatments when % WHC was 40%, when %WHC was increased to 70% and 130%, the least saline treatment emitted significantly more N₂O than treatments S2 to S4. At 130% WHC, only N₂O emissions in TC and PA under S4 treatment were significantly higher than control.

3.3. Soil salinity and moisture and correlation with CO₂ and N₂O emissions

Regardless of soil types, soil EC increased with increasing salinity and moisture, and was significantly influenced by salinity, moisture and their interaction (Table 3). Soil CO₂ and N₂O emissions were significantly negatively correlated with soil EC in SS. However, no significant relationship was found in other soils (Fig. 6). The relationship between cumulative GHG emissions and soil salinity was more significant in SS and PA, which contained low levels of salt, than that in BL and TC. The significant positive correlation between cumulative GHG emissions and soil moisture was found in most of soils (Fig. 6).

4. Discussion

The availability of labile C could be responsible for the decrease of soil CO₂ emissions during incubation period (Maucieri et al., 2017). Heterotrophic consumption of relatively abundant labile C of the initial incubation period are likely lead to rapid rates of CO₂ emission, and exhaustion of labile C likely result in slower rates of emission in the final stages (Cheng et al., 2008; Zimmerman et al., 2011). On the other hand, in the present study, soil CO₂ production rates were highest in SS and PA, were intermediate in TC, and were lowest in BL, which were consistent with TOC content of different soils (Table 1). These results further confirmed that soil CO₂ emissions could be significantly affected by availability of soil organic carbon. Similarly, N₂O emitted fast initially, and slowly emitted from approximately 10-15th day of incubation. Two different processes, ammonia oxidation and linked nitrifier denitrification or denitriﬁcation pathway, could be responsible for N₂O emission dynamics (Huang et al., 2014; Sánchez-García et al., 2014).

Salinity is one of the most important factors in affecting gas production. The present study showed that production rate and cumulative emission of CO₂ decreased with increasing salinity (Figs. 1, 3, and 5). The negative effect of salinity on CO₂ emission of salt amended soils has been found in many previous studies in laboratory incubation experiments (Maucieri et al., 2017; Reddy and Crohn, 2014; Setia et al., 2010; Walpola and Arunakumara, 2010). The adverse effects of salinity, such as ion toxicity (Na⁺ specifically) (Rath et al., 2016), osmotic stress (Setia et al., 2011a; Setia et al., 2011b), or their cooperation (Maucieri et al., 2017), could inhibit the growth and activity of heterotrophic soil microorganisms, and thus reduce CO₂ emission. Chandra et al. (2002) and Wong et al. (2009), however, found that carbon mineralization increased with increasing salinity. The discrepancy was likely due to varied type of salts used for developing salinity in different studies (Setia et al., 2011b), because different salts may have different impact on carbon mineralization (McClung and Frankenberger, 1987). In the
current study, therefore, soil salinity was developed using sea water to simulate the effect of salt type in the field.

Many previous studies (Maucieri et al., 2017; Reddy and Crohn, 2014; Zhang et al., 2016) showed that N$_2$O production increased with increasing salinity. The following reasons could be responsible for the above result. Firstly, N$_2$O reductase may be depressed under saline conditions, leading to N$_2$O accumulation from denitrification (Menyailo et al., 1997). Secondly, an increase of the ionic concentration in the soil solution can reduce N$_2$O solubility and favor its emission (Cayuela et al., 2013; Heincke and Kaupenjohann, 1999). Furthermore, the accumulation of soil NO$_2$ results from incomplete nitrification under salt inhibition, leading to an increase in N$_2$O production (Zhang et al., 2016). In the present study, however, soil salinity only enhanced cumulative N$_2$O emissions in TC and PA under 130%WHC treatment.

Soil mineral N, including NH$_4$$^+$-N and NO$_3$-N, and organic matter are important factors influencing N$_2$O emission (Huang et al., 2017;
Salinity affects N transformations in soil by retarding several biological or microbial processes responsible for mineralization and nitrification (Lodhi et al., 2009). Our results, however, showed that soil NH₄⁺-N concentration was higher in soil of PA that contained low levels of salt, and NO₃⁻-N concentration decreased with decreasing salinity (Table 1). The results indicated that nitrogen mineralization was inhibited by salt, but nitrification increased under higher salinity condition. Our results were inconsistent with previous studies (Irshad et al., 2005; Kumar et al., 2007), which suggested that salinity can negatively affect soil microbes responsible for nitrification, and reduce the conversion of NH₄⁺-N to NO₃⁻-N. The soil NO₃⁻-N is the electron-acceptor for denitrifiers responsible for N₂O emission and reduced N₂O emission is generally associated with lower soil NO₃⁻-N concentration (Gillam et al., 2008). Although NO₃⁻-N concentration was highest in soil of BL, it emitted lowest cumulative N₂O. On the other hand, the higher NO₃⁻-N concentration increased cumulative N₂O emission in different vegetation communities. It can be assumed that, therefore, denitrification could be the main process behind N₂O emissions in BL with no vegetation cover, but nitrification could be responsible for N₂O production in vegetation covered soil. In general, soil organic matter (SOM) increases N₂O production because it provides a substrate for nitrifiers/denitrifiers (Huang et al., 2004; Huang et al., 2017). Since higher C:N ratio of residue competes with soil microorganisms for available N, however, the negative effect or no significant effect of SOM on N₂O emissions was also found (Ambus et al., 2001; Malhi et al., 2006). In the present study, soil of BL, which contained lowest TOC, emitted less N₂O than vegetation covered soil. On the other hand, cumulative N₂O emission of soil in TC was highest, but its TOC content was lower than soils in SS and PA. These indicated that SOM may accelerate N₂O production. Nevertheless, the interaction between SOM and other soil characteristics could complicate the situation of N₂O emission.

Although soil salinity negatively affected CO₂ and N₂O emissions, the negative correlations between EC and GHG were more significant only in SS and PA (Fig. 6). This was likely due to the difference of EC in different soils. Compared to the more tolerance of soil microorganisms in BL and TC, which contained higher levels of salt, the salt tolerance of microorganisms in low-salinated SS and TA were more vulnerable to the sudden increase in EC after salt addition because they do not have time to adapt to the increased osmotic stress (Setia et al., 2011b). Soil CO₂ and N₂O emissions, therefore, were more significantly inhibited in SS and PA than those in BL and TC.

Soil moisture was one of the most important regulating variables on soil CO₂ emission rate (Maucieri et al., 2017). Many studies have confirmed that soil CO₂ emissions increase with increasing soil water content, but excessive soil moisture depresses soil respiration by limiting the transport of CO₂ in the soil profile (Gaumont-Guay et al., 2006; Yan et al., 2014). In this study, production rates of CO₂ of the initial incubation period were higher under 40% WHC to 70% WHC than those under 130% WHC in all soils. However, both production rates and cumulative emissions of CO₂ in all soils increased with increasing WHC after 4 days of incubation. The result of elevated CO₂ emission with increasing moisture was consistent with other studies (Borken et al., 2003; Maucieri et al., 2017; Wang et al., 2015; Yu et al., 2017). Water-blocked soil pores and reduced diffusivity could be responsible for increasing CO₂ emissions under excessive soil moisture stress, and result in the accumulation of CO₂ in the soil profile (Gaumont-Guay et al., 2006; Pumpen et al., 2008). Since at least 70% of annual precipitation occurs in between June and August, this finding suggests that more intense wetting events in summer will increase pulse additions of CO₂ to the atmosphere in the YRD.

Soil N₂O emission can be significantly affected by small changes of soil moisture (Smith et al., 2003). In general, rewetting of dry soil lead to a pulse of N₂O emission (Borken and Matzner, 2009; Xiang et al., 2008). The synthesis of N₂O originate from complex and multiple routes, of which nitrification-related pathways, including ammonia oxidation and nitrifier denitrification, and heterotrophic denitrification are dominant sources of N₂O under dry and wet soil conditions, respectively (Hu et al., 2015). The relationship between soil moisture and N₂O emission was significantly positive in our study (Fig. 6). This was consistent with results of many previous studies (Cardoso et al., 2017; Kiese and Butterbach-Bahl, 2002; Wang and Cai, 2008; Zhang et al., 2016). We speculate that denitrification facilitated N₂O emissions under moisture treatment in the present experiment.

### Table 3

| Treatments | BL | TC | SS | PA |
|------------|----|----|----|----|
| **Salinity** |    |    |    |    |
| S1         | 15.18 ± 0.53 b | 10.73 ± 0.03 d | 4.92 ± 0.23 d | 2.53 ± 0.21 d |
| S2         | 15.34 ± 0.58 b | 11.91 ± 0.19 c | 6.46 ± 0.23 c | 3.64 ± 0.12 c |
| S3         | 16.07 ± 0.05 a | 12.60 ± 0.05 b | 7.80 ± 0.07 b | 7.64 ± 0.07 b |
| S4         | 16.21 ± 0.29 a | 13.99 ± 0.24 a | 8.37 ± 0.06 a | 7.94 ± 0.18 a |
| **Water**  |    |    |    |    |
| W1         | 15.17 ± 0.35 b | 11.99 ± 0.13 b | 7.17 ± 0.09 a | 5.14 ± 0.16 b |
| W2         | 16.02 ± 0.42 a | 12.41 ± 0.12 a | 6.61 ± 0.21 c | 5.34 ± 0.11 a |
| W3         | 15.64 ± 0.31 ab| 12.53 ± 0.13 a | 6.88 ± 0.13 b | 5.23 ± 0.11 ab|
| **ANOVA**  |    |    |    |    |
| Salinity   | 0.001 | < 0.001 | < 0.001 | < 0.001 |
| Water      | 0.004 | < 0.001 | < 0.001 | 0.019 |
| Salinity × Water | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

BL, bare land; TC, Tamarix chinensis; SS, Suaeda salsa; PA, Phragmites australis.
production of \( N_2O \) with increasing moisture treatment. Compared with bare land, soils collected from different vegetation communities produced more \( CO_2 \) and \( N_2O \) emissions, this finding suggests that the destruction of vegetation in the course of exploitation and utilization of saline soil resources will likely increase pulse additions of GHG to the atmosphere.

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