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Effects of moisture and carbonate additions on CO_2 emission from calcareous soil during closed-jar incubation

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Abstract: Calcareous soil contains organic and inorganic carbon (C) pools, which both contribute to CO_2 emission during closed-jar incubation. The mineralization of organic C and dissolution of inorganic C are both related to soil moisture, but the exact effect of water content on CO_2 emission from calcareous soil is unclear. The objective of this experiment was to determine the effect of soil water content (air-dried, 30%, 70%, and 100% water-holding capacity (WHC)), carbonate type ($CaCO_3$ or $MgCO_3$), and carbonate amount (0.0, 1.0%, and 2.0%) on CO_2 emission from calcareous soil during closed-jar incubation. Soil CO_2 emission increased significantly as the water content increased to 70% WHC, regardless of whether or not the soil was amended with carbonates. Soil CO_2 emission remained the same or increased slowly as the soil water content increased from 70% WHC to 100% WHC. When the water content was ≤30% WHC, soil CO_2 emission from soil amended with 1.0% inorganic C was greater than that from unamended soil. When the soil water content was 70% or 100% WHC, CO_2 emission from $CaCO_3$ amended soil was greater than that from the control. Furthermore, CO_2 emission from soil amended with 2.0% $CaCO_3$ was greater than that from soil amended with 1.0% $CaCO_3$. Soil CO_2 emission was higher in the $MgCO_3$ amended soil than from the unamended soil. Soil CO_2 emission decreased as the $MgCO_3$ content increased. Cumulative CO_2 emission was 3–6 times higher from $MgCO_3$ amended soil than from $CaCO_3$ amended soil. There was significant interaction effect between soil moisture and carbonates on CO_2 emission. Soil moisture plays an important role in CO_2 emission from calcareous soil because it affects both biotic and abiotic processes during the closed-jar incubation.

Keywords: calcareous soil; soil moisture; organic carbon; CO_2 emission

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Soil is both a large carbon (C) sink and a large C source in the terrestrial ecosystem (Schlesinger and Andrews, 2000). Soil respiration releases 68–80 Pg C/a, the second largest C flux between the terrestrial ecosystem and the atmosphere (Raich et al., 2002). Even a small change in soil respiration could significantly intensify or mitigate current atmospheric increases of CO_2, with potential feedbacks to climate change (Freer-Smith et al., 2008).

Most CO_2 released by soil respiration is from the mineralization of soil organic C. Mineralization is primarily a biotic process, but it is also influenced by environmental factors, especially soil moisture (Leirós et al., 1999). Closed-jar incubation is a common method for estimating soil respiration (Alef, 1995). The closed-jar method is widely used to quantify soil microbial activity and to investigate the effects of precipitation change on CO_2 emission from different soil types

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(Borken et al., 2003; Rey et al., 2005; Inglima et al., 2009; Merbold et al., 2009; Muhr et al., 2010). Most studies have focused on CO_2 emission from acidic or neutral soils. Few researchers have studied the effect of soil moisture on CO_2 emission from calcareous soil.

Calcareous soil, which covers more than 30% of the Earth's land surface (Chen and Barak, 1982), contains two pools of C, i.e. organic C and inorganic C. The inorganic C content of calcareous soil is typically 2–5 times greater than the organic C content. In some arid regions, inorganic C content can be ten times greater than the organic C content (Schlesinger, 1982). Some researchers have suggested that carbonates could dissolve and release CO_2 during incubation, leading to an overestimation of organic C mineralization in soil (Alef, 1995; Bertrand et al., 2007). The dissolution of $CaCO_3$ is a chemical process that is closely related to soil water content (Birkeland, 1984). Thus, the biotic and abiotic processes that contribute to soil CO_2 emission are both influenced by soil moisture. The exact effect of water content on CO_2 emission from calcareous soil is unclear.

There are two types of carbonates in calcareous soil: $CaCO_3$ and $MgCO_3$ (Guo, 1992). Due to its higher dissolubility, $MgCO_3$ is easily dissolved in the soil and leached. The content of $MgCO_3$ is less than 10% in calcareous soil. In contrast, low dissolubility contributes to the easy accumulation and conservation of $CaCO_3$ in the soil, making it the main composition of soil carbonate. Despite its low content in calcareous soil, $MgCO_3$ is more active than $CaCO_3$. So when we discuss the stabilization of soil carbonates, the influence of $MgCO_3$ should not be ignored.

Global warming is predicted to increase the number of abnormal rainfall events (IPCC, 2001). Therefore, it is important to understand the effect of soil moisture on CO_2 emission from calcareous soil. The aims of this study are (1) to evaluate the effect of soil moisture on CO_2 emission from calcareous soil during closed-jar incubation, and (2) to estimate the effect of soil carbonate type and amount on CO_2 emission during incubation.

1 Materials and methods

1.1 Study area

Soil samples were collected from the Experiment Station at Northwest A&F University, Yangling, Shaanxi province, China (34º17'56"N, 108º04'07"E). The elevation at the site is 523 m, the annual average temperature is 13ºC, and the average annual precipitation is 600 to 650 mm. The soil type is Lou soil, which is classified as an Eum-Orthric Anthrosol (equivalent to a Udic Haplustalf in the USDA system). Lou soil has been cultivated for more than two thousand years. The inorganic C content in the 0–200 cm soil profile ranged between 0.7 and 22.8 g/kg.

1.2 Soil samples

The soil samples in this study were from the eluvial horizon (60–80 cm depth) of the profile. We chose this depth in order to minimize the effects of preexisting carbonates on CO_2 emission during incubation. The inorganic C content of the eluvial horizon was 0.74 g/kg. The soil was air-dried, passed through a 2-mm sieve, and then stored.

The physical and chemical properties of the soil were measured using standard methods. Soil organic C was measured with the Walkley-Black acid digestion method, inorganic C with the aerometric method, total N with the Kjeldahl method, and soil pH with a pH meter (soil:water=1:5). The soil organic C content was 5.81 g/kg, the total nitrogen (N) content was 0.24 g/kg, and the soil pH was 7.67.

1.3 Incubation method

To investigate the effects of soil moisture and carbonate addition on CO_2 emission during closed-jar incubation, we designed an experiment that included four soil moisture contents (air-dried, 30%, 70% and 100% of field water holding capacity (WHC)) and three carbonate concentrations (0, 1.0% and 2.0% inorganic C added as either $CaCO_3$ or $MgCO_3$). Twenty grams of air-dried soil were placed in 50-mL plastic bottles. Deionized water was added to adjust the water content and then $CaCO_3$ or $MgCO_3$ was added to the soil. The samples were put into 500-mL jars along with bottles containing 10-mL 0.1 mol/L NaOH. The jars were sealed and then incubated at 25°C. Soil moisture was kept constant throughout the incubation by weighing the bottles every three days and adding deionized water when necessary. The NaOH solutions were changed on day 1, 2, 4, 8, 12, 16, 23, 30, 40, 51, 61,

67 and 83. The incubation time was chosen because cumulative CO_2 emission would remain unchanged after a 83-d incubation. And the jars were sealed during the incubation except adding water every three days. The amount of CO_2 trapped in the NaOH was measured with the HCl-$BaCl_2$ titration method (Alvarez et al., 1995). Each treatment was replicated three times.

1.4 Statistic analysis

CO_2 emission during the interval i (C_i) was calculated as Eq. 1 (Chen et al., 2009):

$$C_i = \frac{(V_s - V_a) \times n \times 12 \times 1000}{20 \times 2}. \quad (1)$$

Where V is the volume of HCl used to neutralize the NaOH solution, s stands for the soil samples, a stands for the air in the jar, and n stands for the molar concentration of HCl solution.

The cumulative CO_2 emission (C) during the incubation was calculated as Eq. 2:

$$C = \sum C_i. \quad (2)$$

Cumulative CO_2 emission was calculated for each treatment combination using mean CO_2 mineralization rates (n=3). Soil CO_2 emission rates were analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS (SAS Institute Inc., 2000). When the effects of soil moisture or C content were significant (P<0.05), paired t-tests were used to compare CO_2-C emission between the treatments. The interaction effect between soil moisture and inorganic C content on CO_2 emission was analyzed by ANOVA. The relationship between soil moisture and CO_2 emission was determined using nonlinear regression analysis (Sigmaplot 12.0, 2012). The values used in the regression analyses were the mean values of each treatment.

2 Results

2.1 Effect of moisture on soil CO_2 emission

Cumulative CO_2 emission increased significantly as the soil water content increased from air-dried to 70% WHC (P<0.01). There was generally no significant difference in cumulative CO_2 emission between the 70% WHC treatment and the 100% WHC treatment (Fig. 1). The emission of CO_2 was 63% and 30% higher in the 30% and 70% WHC treatments respectively than in the air-dried treatment (P<0.01). Cumulative CO_2 emission was 6.51% less in the 70% WHC treatment than in the 100% WHC treatment.

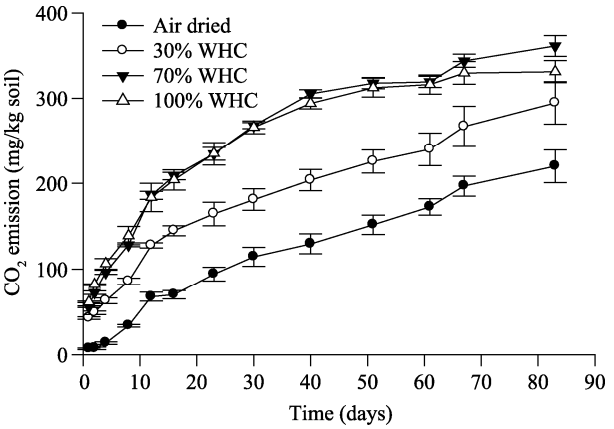

Fig. 1 Cumulative CO_2 emission from soil without carbonate amendment. Error bars represent the standard error of the mean. WHC, water holding capacity. n=3.

2.2 Effects of moisture and $CaCO_3$ addition on soil CO_2 emission

Cumulative CO_2 emission increased significantly as soil moisture increased in the $CaCO_3$ amended soil (P<0.01) (Fig. 2). In the 1.0% $CaCO_3$ treatment, CO_2 emissions from the wetted soils (i.e. 30%, 70%, and 100% WHC) were 53% to 78% higher than those from the air-dried soil. In the 2.0% $CaCO_3$ treatment, CO_2 emissions from the wetted soils were 90% to 334% higher than those from the air-dried soil. There was a significant quadratic relationship between cumulative CO_2 emission (y) and soil moisture (x) (y= $-0.0208x^2+4.0689x+0.0071$, R^2=0.999).

The effect of $CaCO_3$ addition on CO_2 emission varied depending on soil water content (Fig. 2). The interaction effect between soil moisture and $CaCO_3$ concentration on CO_2 emission was significant (P<0.01). When soil moisture was ≤70% WHC, CO_2 emission was less in the $CaCO_3$ amended soil than in the unamended soil. Furthermore, CO_2 production decreased as the amount of $CaCO_3$ added to the soil increased. However, when soil moisture was 100% WHC, CO_2 emission was greater in the $CaCO_3$ amended soil than in the unamended soil. Furthermore, CO_2 production increased as the $CaCO_3$ content increased.

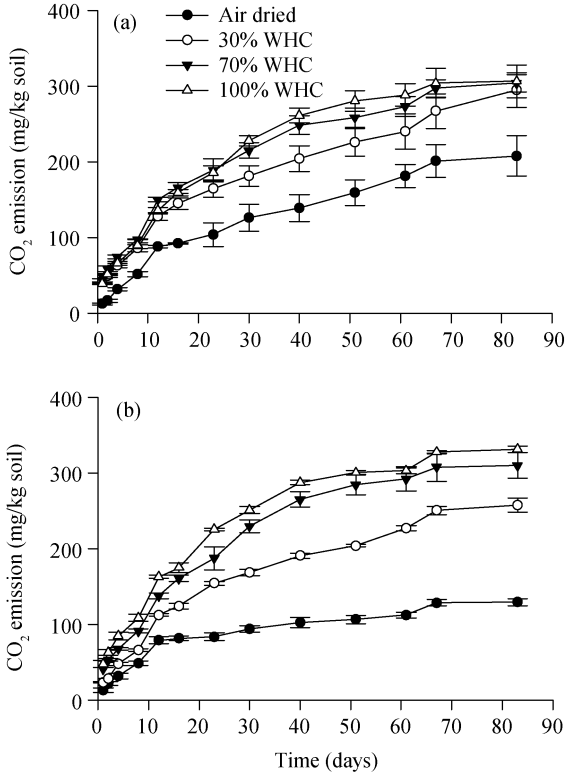

Fig. 2 CO_2 emission from soil amended with 1.0% $CaCO_3$ (a) and 2.0% $CaCO_3$ (b). Error bars represent the standard error of the mean. WHC, water holding capacity. n=3.

2.3 Effects of moisture and $MgCO_3$ addition on soil CO_2 emission

Cumulative CO_2 emission increased significantly as soil moisture increased in the $MgCO_3$ amended soil (P<0.05) (Fig. 3). In the 1.0% $MgCO_3$ treatment, CO_2 emissions from the wetted soils (i.e. 30%, 70%, and 100% WHC) were 96% to 155% higher than those from the air-dried soil. A quadratic relationship existed between cumulative CO_2 emission and soil moisture (y=–0.1011x^2+18.675x+12.959, R^2=0.995). In the 2.0% $MgCO_3$ treatment, CO_2 emissions from the wetted soils were 106% to 183% higher than those from the air-dried soil. In soil amended with 2.0% $MgCO_3$, CO_2 emissions were 183% higher in the 100% WHC treatment, 164% higher in the 70% WHC treatment, and 106% higher in the 30% WHC treatment than in the air-dried soil. There was also a significant quadratic relationship between the cumulative CO_2 emission and soil moisture (y= –0.1353x^2+23.48x+13.865, R^2=0.996).

Cumulative emissions were highest in the 2.0% $MgCO_3$ treatment and lowest in the non $MgCO_3$ treatment (Fig. 3). The trend was different for the $CaCO_3$ amended soil. There was an exponential relationship between cumulative CO_2 emission and the amount of $MgCO_3$ added to the soil at different soil moisture contents. The interaction effect between soil moisture and $MgCO_3$ on CO_2 emission was also significant (P<0.01).

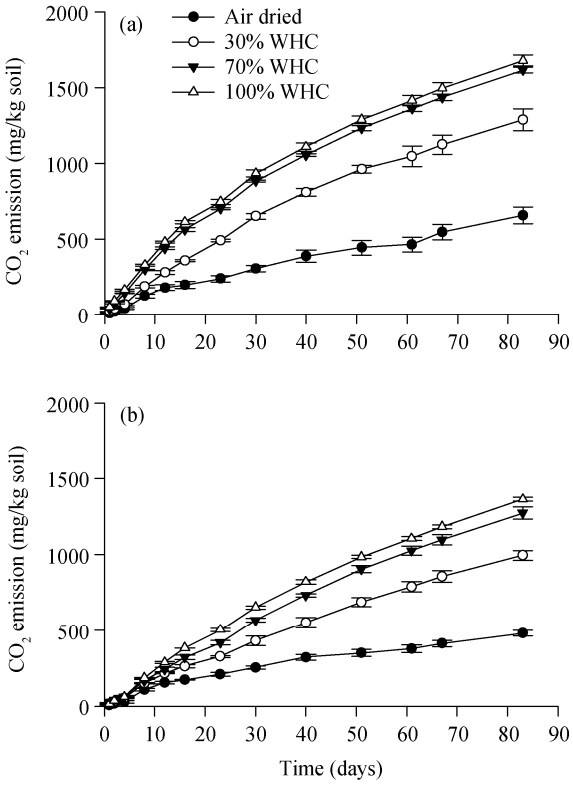

Fig. 3 CO_2 emission from soil amended with 1.0% $MgCO_3$ (a) and 2.0% $MgCO_3$ (b). Error bars represent the standard error of the mean. WHC, water holding capacity. n=3.

3 Discussion

3.1 Soil moisture and CO_2 emission

CO_2 emitted from soil during this incubation experiment originated either from organic matter or from inorganic carbon. The mineralization of organic matter is mainly controlled by the biological activities in soil and is affected by environmental factors, such as soil temperature and moisture (Rey et al., 2005; Jia et al., 2006; Wang et al., 2010). The release of CO_2 from inorganic sources is a chemical process and is mostly

affected by environmental factors (Ryskov et al., 2008). This study showed a significant relationship between CO_2 emission and soil moisture, regardless of whether or not the soil was amended with carbonates. This indicates that soil water content affects both the biotic and abiotic processes that result in CO_2 emission.

Soil CO_2 emission increased when the soil water content was ≤70% WHC, regardless of whether or not the soil was amended with carbonates. When the soil moisture content was >70% WHC, the CO_2 emission remained the same or increased slowly as the water content increased. Other studies showed that CO_2 emission increased as the water content increased in peat soil (Wang et al., 2010), paddy soil (Zhang et al., 2007) and forest soil (Bowden et al., 1998). However, CO_2 emission decreased when soil water content exceeded 60% or 70% WHC. The significant interaction effect between soil water content and CO_2 emission reflects the fact that soil water content affects both the biotic and abiotic processes which result in CO_2 emission. When soil moisture is low, microbial activities are inhibited and the dissolution of inorganic C is reduced. Both of these factors contribute to a reduction in CO_2 emission. Microbial activities increase as soil moisture increases. Soil CO_2 emission reached a peak when soil water content was between 50% and 80% WHC (Raich and Potter, 1995). The dissolution of inorganic C also accelerated as the soil water content increased, leading to an additional increase in soil CO_2 emission. The concentration of O_2 in the soil declines as the soil water content increases to above 80% WHC. Near anaerobic conditions could have inhibited microbial activities and reduced CO_2 emission (Raich and Potter, 1995). Therefore, when evaluating the effect of soil moisture on CO_2 emission from calcareous soil, both biotic and abiotic processes should be considered.

We also observed that there was a decreasing trend of CO_2 emission from unamended soil when soil moisture increased from 70% to 100% WHC, whereas CO_2 emission from soil amended with either $CaCO_3$ or $MgCO_3$ increased. The most likely reason for this is that although biological activities were reduced at 100% WHC, the dissolution of inorganic C was promoted when the content of carbonates in soil was high.

3.2 Forms of carbonates and CO_2 emission

This study indicated that the effect of additional inorganic C on total CO_2 emission during closed-jar incubation varied with soil moisture. When the soil water content was <30%, soil CO_2 emissions were higher in the 1.0% $CaCO_3$ treatment than in the control treatment. In contrast, CO_2 emissions were lower in the 2.0% $CaCO_3$ amendment than in the control treatment. However, when the water content reached 70% or 100% WHC, CO_2 emission in the $CaCO_3$ amended soil was higher than in the control treatment. Soil CO_2 emission in the soil increased with the increase of $CaCO_3$ content.

Soil CO_2 emission was greater in the $MgCO_3$ amended soil than in the control treatment. Furthermore, CO_2 emission decreased as the carbonate content of the soil increased. Soil CO_2 emissions were 3 to 6 times greater in $MgCO_3$ amended soil than in the $CaCO_3$ amended soil. These results could be explained by the dissolution and formation of carbonates in the following equations (Birkeland, 1984):

$$\text{Organic C} \rightarrow CO_2, \quad (3)$$

$$CaCO_3(s)+CO_2(g)+H_2O(l) \leftrightarrow Ca^{2+}(aq)+HCO_3^-(aq), \quad (4)$$

$$MgCO_3(s)+CO_2(g)+H_2O(l) \leftrightarrow Mg^{2+}(aq)+HCO_3^-(aq). \quad (5)$$

The partial pressure of CO_2 and the soil water content are two factors affecting the dissolution of carbonates. When the soil water content is <30% WHC, soil microbial activities are low and the amount of CO_2 derived from soil organic C is relatively small. Under this condition, carbonates in the 1.0% $CaCO_3$ treatment would dissolve quickly and reach an equilibrium. However, it would take longer for the system to equilibrate in the 2.0% $CaCO_3$ treatment. Microbial activities are higher at soil moisture contents of 70% and 100% WHC than at 30% WHC. Under these conditions, microbial activities would produce enough CO_2 to complete the balance of Eq. 4. The $CaCO_3$ could dissolve and release CO_2 at these two levels of soil moisture. Soil CO_2 emission increased as the amount of $CaCO_3$ added to the soil increased whereas CO_2 emission decreased as the amount of $MgCO_3$

added to the soil increased. One explanation is that $MgCO_3$ dissolves more readily than $CaCO_3$ (Patnaik, 2003). At low soil moisture, $MgCO_3$ can complete the balance of Eq 5.

In this experiment, we studied the effect of soil water content on CO_2 emission from calcareous soil during closed-jar incubation. It must be emphasized that the incubation conditions in the laboratory are different from those in the field. The dissolution and movement of carbonates in soil are more complex under field conditions. During the rainy season, carbonates in soil are leached, accumulating in the lower depths of the soil profile. Furthermore, Ca^{2+} in the soil can react with HCO_3^- produced by plants to form heavy calcium carbonates (Guo, 1992). Therefore, seasonal variation of precipitation should be taken into consideration when evaluating the effect of soil water content on CO_2 emission in the field.

4 Conclusions

In summary, soil moisture had a significant effect on CO_2 emission from calcareous soil during closed-jar incubation. Furthermore soil moisture and carbonate content had significant interaction effects on CO_2 emission. Therefore, when determining CO_2 emission from calcareous soil, the contribution of CO_2 from carbonates needs to be considered, especially when soil moisture is not constant. In this study, we could not distinguish between CO_2 released by biotic processes and that released by abiotic processes. The $\delta^{13}C$ method can be used to solve this problem in future studies.

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