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Applicability of soil column incubation experiments to measure CO_2 efflux**

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Abstract. Accurate measurements of CO_2 efflux from soils are essential to understand dynamic changes in soil carbon storage. Column incubation experiments are commonly used to study soil water and solute transport; however, the use of column incubation experiments to study soil CO_2 efflux has seldom been reported. In this study, a 150-day greenhouse experiment with two treatments (no-tillage and tillage soils) was conducted to evaluate the applicability of soil column incubation experiments to study CO_2 efflux. Both the chamber measurement and the gradient method were used, and results from the two methods were consistent: tillage increased soil cumulative CO_2 efflux during the incubation period. Compared with fieldwork, incubation experiments can create or precisely control experimental conditions and thus have advantages for investigating the influence of climate factors or human activities on CO_2 efflux. They are superior to bottle incubation because soil column experiments maintain a soil structure that is almost the same as that in the field, and thus can facilitate analyses on CO_2 behaviour in the soil profile and more accurate evaluations of CO_2 efflux. Although some improvements are still required for column incubation experiments, wider application of this method to study soil CO_2 behaviour is expected.

Keywords: column incubation experiment, CO_2 efflux, tillage, chamber method, gradient method

INTRODUCTION

Global warming caused by an increase in atmospheric greenhouse gas (GHG) concentrations, such as CO_2, N_2O, and CH_4 threatens sustainable development. Soils can function as either a source or a sink for atmospheric GHG depending on land use and soil management. Appropriate agricultural management enables increasing carbon storage and decreasing CO_2 emissions (Lal, 2010; Luo *et al.*, 2010). Accurate quantitation of CO_2 efflux from soils is essential to assess dynamic changes in soil carbon storage.

Field monitoring using the chamber method is often employed to evaluate CO_2 efflux from soils (Ahmad *et al.*, 2009; Morell *et al.*, 2011). However, field monitoring is laborious and time-consuming. Field monitoring is easily affected by environmental conditions, and the required experimental conditions are difficult to create or control. Therefore, laboratory incubation experiments have been carried out to evaluate soil CO_2 production under different agricultural conditions. Plante and McGill (2002) incubated soils with differing simulated tillage frequencies in the laboratory to study the dynamics of soil organic matter. Dong *et al.* (2014) controlled water content and carbonate concentration in the soil to investigate the effects of moisture and carbonate addition on CO_2 emission during closed-jar incubation.

Most of these experiments incubate a small amount of disturbed soil in closed bottles. The structure of the incubated soil is altered and different from that in the field; however, many previous studies have suggested that soil structure has a considerable effect on soil carbon dynamics (Conant *et al.*, 2007; Six and Paustian, 2014). Column incubation experiments, using undisturbed soil sampled from the field, can maintain soil structure and evaluate the effects of changes in soil structure on soil CO_2 efflux.

Additionally, bottle incubation experiments neglect the contributions of gas diffusion, which is the main mechanism of CO_2 transport in the soil, to CO_2 efflux. Assuming

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that molecular diffusion is predominantly responsible for gas transport in the soil, the gradient method based on Fick's law can be used to estimate CO_2 efflux from the soil. Although the chamber method is commonly used for measuring CO_2 efflux from the soil surface, there are several difficulties with this method. Precise measurement of CO_2 efflux is difficult with the chamber method during rainfall or windy days because the environments are different within and outside the chamber (Maier and Schack-Kirchner, 2014; Wolf *et al.*, 2011). Disturbance of the concentration gradient between soil and the atmosphere caused by covering the chamber may also increase experimental error in this method (Kutzbach *et al.*, 2007). Thus, the gradient method has been used as an alternative or supplemental method to estimate soil CO_2 efflux. Jassal *et al.* (2005) and Tang *et al.* (2003) estimated the CO_2 efflux with Fick law and suggested the estimated data agreed well with the measured ones. Considering the advantages of monitoring soil properties in the profile, estimation of CO_2 efflux with the gradient method can be carried out with column incubation experiments. However, existing studies commonly used soil column incubation to solve problems of water and solute transport in soils, and only a few studies evaluated CO_2 efflux using soil column incubation. For example, Rottmann and Joergensen (2011) compared four methods of measuring CO_2 production using the column incubation method, considering that this method could accurately regulate environmental conditions. Additionally, Camarda *et al.* (2009) used this method to investigate the effects of soil gas permeability and recirculation flux on soil CO_2 efflux, considering that this experiment system could maintain soil structure and was available commercially. Volcanic ash soil is an important resource for agriculture in various regions of the world because of its unique chemical and physical properties, especially its high organic carbon content (Prado *et al.*, 2007; Shoji *et al.*, 1993). Because volcanic ash soil is an important carbon pool, it possesses a major challenge to mitigating emission of CO_2 in the soil during agricultural activities. Optimum agricultural activities are considered to have the potential to restrict CO_2 emissions from soil by changing soil properties (Gregorich *et al.*, 2005; Tenesaca and Al-Kaisi, 2015). No-tillage farming has been known to improve soil structure, reduce soil erosion, and conserve soil moisture (Gupta and Sayre, 2007; Zuber *et al.*, 2015). Recently, no-tillage farming has received much attention because many studies have shown that it contributes to the reduction of CO_2 efflux from agricultural fields (Alvaro-Fuentes *et al.*, 2007; Jabro *et al.*, 2008). Finding ways to decrease soil CO_2 efflux from agricultural soil requires accurate measurements of CO_2 efflux.

The present study attempted to use column incubation experiments to investigate the CO_2 efflux from an Andisol under two treatments (no-tillage and tillage) and to test the applicability of column incubation experiments. Measurements of the soil CO_2 efflux with both the chamber method and the gradient method were conducted during the column incubation experiments.

MATERIALS AND METHODS

The soil was sampled at the experimental farm of the University of Tokyo located in Nishitokyo (139°54′E, 35°73′N), Tokyo, Japan. The area has a subtropical monsoon climate with an average annual air temperature of 16.5°C, average rainfall of 1 500 mm, daily solar radiation of 10 MJ m^{-2}, and relative humidity of 60%. The soil is an Andisol derived from volcanic ash. Some of the soil physical properties are shown in Table 1. The sampling site used as an upland field was fallow for two years and covered by approximately 10 cm tall weeds. Prior to sampling, the weeds were removed, and 0.74 kg m^{-2} of leaf compost (with a total carbon content of 340.3 g kg^{-1}) that is similar to the usual application rate of organic carbon in this region was applied to the soil.

Table 1. Soil characteristics of the sampling site

Depth (cm)	Bulk density (Mg m^{-3})	Saturated hydraulic conductivity (m s^{-1})	Particle size distribution* (%)	Particle density* (Mg m^{-3})	Total carbon (g kg^{-1})
0-5	0.90	3.5 10^{-7}	sand 40.5		55.9
5-10	0.86	1.0 10^{-6}	silt 37.9	2.57	54.5
10-15	0.90	2.0 10^{-6}	clay 21.6		55.7
15-25	0.82	1.1 10^{-5}	sand 44.9		55.5
25-35	0.80	1.2 10^{-5}	silt 39.4	2.55	54.6
			clay 15.7		

*Soil particle size and density were measured at depths of 2.5 and 17.5 cm.

The experiments consisted of two treatments: no-tillage (NT) and tillage (T). For the T treatment, surface soil was tilled with a hoe to a depth of about 15 cm in the plot after compost application, and then slightly compacted. The tillage operation was conducted in one day. Undisturbed soil columns (15 cm in diameter; 40 cm high) were acquired by driving 50 cm long polyvinyl chloride (PVC) cylinders (inner diameter, 15 cm; sidewall thickness, 3 mm) into the soil and carefully extracting the soil. A thin metal ring with a cutting edge was attached to the bottom of the PVC cylinder to prevent damage to the soil column during insertion. Because a 40 cm soil column was sampled, 10 cm of headspace was left in the PVC cylinder.

During the incubation period, copper-constantan thermocouples (Fig. 1A) were inserted into the soil 3 cm from the sidewall to monitor the soil temperature. Soil water pressure was monitored using porous cups (Fig. 1B) that were connected with pressure transducers. Thermocouples and porous cups were installed horizontally at depths of 2.5, 12.5, and 30.0 cm of the soil column. A data logger stored temperature and water pressure data at 10 min intervals (CR10X, Campbell Scientific Inc., Logan, UT). A gas sampling port (Fig. 1C) used for CO_2 sampling was inserted at a depth of 2.5 cm. The gas sampling port was a 7 cm-long porous resin tube that was permeable to gas but not soil and water.

Soil columns were incubated in the glass greenhouse built on the top floor of the Life Science Research Building at the University of Tokyo. The incubation duration was 150 days (August 18 – January 14). Sunshine was the major source of light, and lamps were placed in the greenhouse for supplementary lighting on cloudy days. The temperature in the room was set to 30°C during the day (06:00-18:00) and 25°C during the night (18:00-06:00). During incubation, the soil surface was irrigated with 13.8 mm of water every 5 d to maintain soil moisture. This was equal to the amount of water evaporation in the greenhouse, and thus, soil water content was controlled to fluctuate within a certain range.

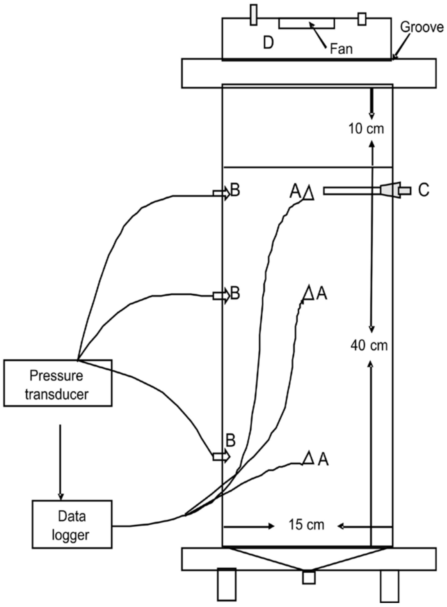

Fig. 1. Schematic of the experimental setup: A – thermocouples, B – porous cups, C – gas sampling port, and D – the chamber.

During the incubation period, CO_2 efflux and concentration were measured at 7 day intervals. An acrylic cylindrical chamber (inner diameter 16.3 cm; height 5.5 cm) was fixed to the groove on the top of the PVC cylinder (Fig. 1D) to measure the CO_2 efflux. The perimeter of the chamber was sealed with water to prevent gas leakage during measurement, and a circulating fan in the top of the chamber was used to mix the headspace air homogeneously. The gas inside the chamber was sampled using a 5 ml plastic syringe at 0, 10, 20, and 30 min after the chamber was closed. Gas samples were stored in 5 ml vacuum glass vials and then brought back to the laboratory for quantification with a gas chromatograph (GC 2014, Shimadzu Inc., Tokyo). The CO_2 efflux was calculated using the following equation:

$$F = \rho\left(\frac{273}{T}\right)\left(\frac{V}{A}\right)\left(\frac{\Delta C}{\Delta t}\right), \tag{1}$$

where: F is the CO_2 efflux (g CO_2-C m^{-2} d^{-1}), ρ is the gas density (g m^{-3}), T is the temperature (K), V is the volume of the chamber (m^3), A is the area of the soil column surface (m^2), ΔC is the concentration increment (m^3 m^{-3}), and Δt is the time interval (in days).

After CO_2 efflux measurement, the soil CO_2 concentration at a depth of 2.5 cm was determined by sampling the soil gas through the gas sampling port and quantifying it by gas chromatography. The CO_2 concentration in the atmosphere was determined by sampling the air near the soil surface with a syringe and quantifying it by gas chromatography.

After incubation, the soil columns were split, and the soil properties of each layer were measured. Water retention curves were determined by the hanging water method (-10 cm > soil water potential > -100 cm) and the pressure plate method (-100 cm > soil water potential > -1 000 cm) (Klute, 1986). Soil volumetric water content was calculated from the water retention curves and soil water potentials. Dry bulk density was determined gravimetrically by using 100 cm^3 undisturbed soil cores (Blake and Hartge, 1986a). Soil particle density was determined by the pycnometer method (Blake and Hartge, 1986b). Soil total porosity was computed from the data on dry bulk density and particle density. The difference between the total porosity and volumetric water content was assumed to be the air-filled porosity of the soil.

Soil gas diffusivity was measured using 100 cm^3 soil cores at five water potentials (-20, -63, -200, -500, and -1 000 cm). Prior to measurement, the water potentials of the soil cores were adjusted to a certain value using the pressure-plate chamber. The diffusion chamber, with O_2 as the tracer gas, was used for the measurement of soil gas diffusivity (Hamamoto *et al.*, 2011). The upper end of the soil core was exposed to the atmosphere, and the bottom was connected with the diffusion chamber. The diffusion chamber was flushed using pure N_2 gas before measurement. The O_2 inside the diffusion chamber was measured by a galvanic electrode (Oxygen Sensor Ke12, GS Yuasa Co., Kyoto) connected to a data logger. The soil gas diffusivity was calculated by assessing the changes in the O_2 concentration in the chamber using the following equation (Currie, 1960):

$$\frac{C(L_s,t)-C_i}{C_0-C_i}=\frac{2h\exp(-D_p a_1^{2} t/\varepsilon)}{L_s(a_1^{2}+h^{2})+h}, \qquad (2)$$

where: D_p is the gas diffusion coefficient in the soil ($m^2\ s^{-1}$), $C(L_s,\ t)$ is the O_2 concentration in the diffusion chamber ($g\ m^{-3}$), C_i is the O_2 concentration in the atmosphere ($g\ m^{-3}$), C_0 is the O_2 concentration at $t = 0$ ($g\ m^{-3}$), ε is the air-filled porosity ($m^3\ m^{-3}$), L_s is the height of the soil core (m), a_1 is the first positive square root of $hL_s = aL_s\tan(aL_s)$, and $h = \varepsilon/L_a$ (L_a is the height of the diffusion chamber, m). Three replicates were conducted in this experiment.

The least significant difference method (LSD) was used to determine any significant differences between treatments. Non-linear regression analysis was used to model the soil gas diffusivity as a function of air-filled porosity. The relationship between the estimated and measured CO_2 efflux was analysed using Pearson correlation analysis. All statistical analyses were performed using SPSS software procedures (IBM SPSS statistics 20.0).

RESULTS AND DISCUSSION

The column incubation experiments maintained a soil structure comparable to that in the field experiments, and CO_2 effluxes under the NT and T treatments were investigated with the chamber method. The difference in soil CO_2 efflux between the NT (2.39 g $m^{-2}\ d^{-1}$) and T (4.48 g $m^{-2}\ d^{-1}$) treatments on the first day (August 18) was notable ($p<0.05$, n=3). In this experiment, the tillage operation was conducted 1 day before the first measurement of the CO_2 efflux. Tillage disturbed the soil and accelerated the gas exchange between the soil air phase and the atmosphere. The CO_2 stored in the air-filled pores of the soil was quickly released into the atmosphere over the first several days. As a consequence, the CO_2 efflux under the T treatment was obviously higher than that under the NT treatment on the first day. Kessavalou *et al.* (1998) and Reicosky and Archer (2007) reported results similar to those of the present study. No noticeable increase or decrease in the CO_2 efflux was observed after the first day of the incubation period (August 19 – January 14) (Fig. 2). The soil CO_2 efflux under the T treatment fluctuated in the range of 0.2-1.1 g $m^{-2}\ d^{-1}$, which was similar to that in the NT treatment (0.0-1.0 g $m^{-2}\ d^{-1}$). Soil cumulative CO_2 efflux under the T treatment (95.67 g m^{-2}) for the 150 days of incubation was higher than that under the NT treatment (84.97 g m^{-2}) ($p<0.05$, n=3).

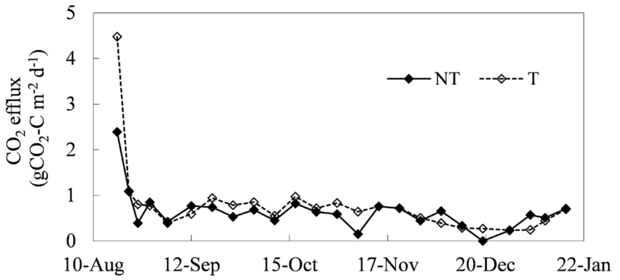

Fig. 2. Soil CO_2 effluxes under the no-tillage (NT) and tillage (T) treatments during the incubation period.

Selected results of the temporal changes in soil temperature and volumetric water content that could be controlled by column incubation are shown in Fig. 3. Soil temperatures at depths of 2.5, 12.5, and 30 cm under the two treatments fluctuated with the greenhouse temperature and ranged between 25 and 30°C (Fig. 3a, 3b). Moreover, the range of fluctuation of soil temperature under the NT treatment was slightly larger than that under the T treatment. Soil disturbance caused by tillage leads to a greater air phase and fewer particles, which in turn depress soil thermal conductivity. Changes in soil thermal conductivity contribute to the differences in soil temperatures between the two treatments. After irrigation, volumetric water contents of the surface soils (2.5 cm) under the two treatments rapidly increased and then decreased gradually as a result of evaporation and redistribution (Fig. 3c, 3d). Upon irrigation, water contents at depths of 12.5 cm and 30 cm also increased but to a lesser extent and then decreased. Soil water content fluctuated within a certain range because of supplemental irrigation during incubation, and similar soil water content was observed at a depth of 30 cm in both treatments. However, the T treatment resulted in smaller volumetric water content at depths of 2.5 and 12.5 cm as compared with the NT treatment. Tillage loosened the soil of the plough layer, and this led to a reduction in bulk density and a rise in total porosity of the plough layer (Table 2). Since tillage increased soil unsaturated hydraulic conductivity (Kargas and Londra, 2015; Miller *et al.*, 1998), lower soil volumetric water content was observed under the T treatment. Corresponding to lower volumetric water content of shallow soils, tillage increased the soil air-filled porosity at depths of 2.5 cm and 12.5 cm (Fig. 3e, 3f). Therefore, during the incubation period, soil aeration resulting from the air-filled porosity would be higher under the T treatment than that under the NT treatment. High aeration because of tillage increased the O_2 level in the soil, which accelerated microbial respiration (Gomez and Garland, 2012; Silva *et al.*, 2011), and thus might contribute to the higher cumulative CO_2 efflux from the tilled soil.

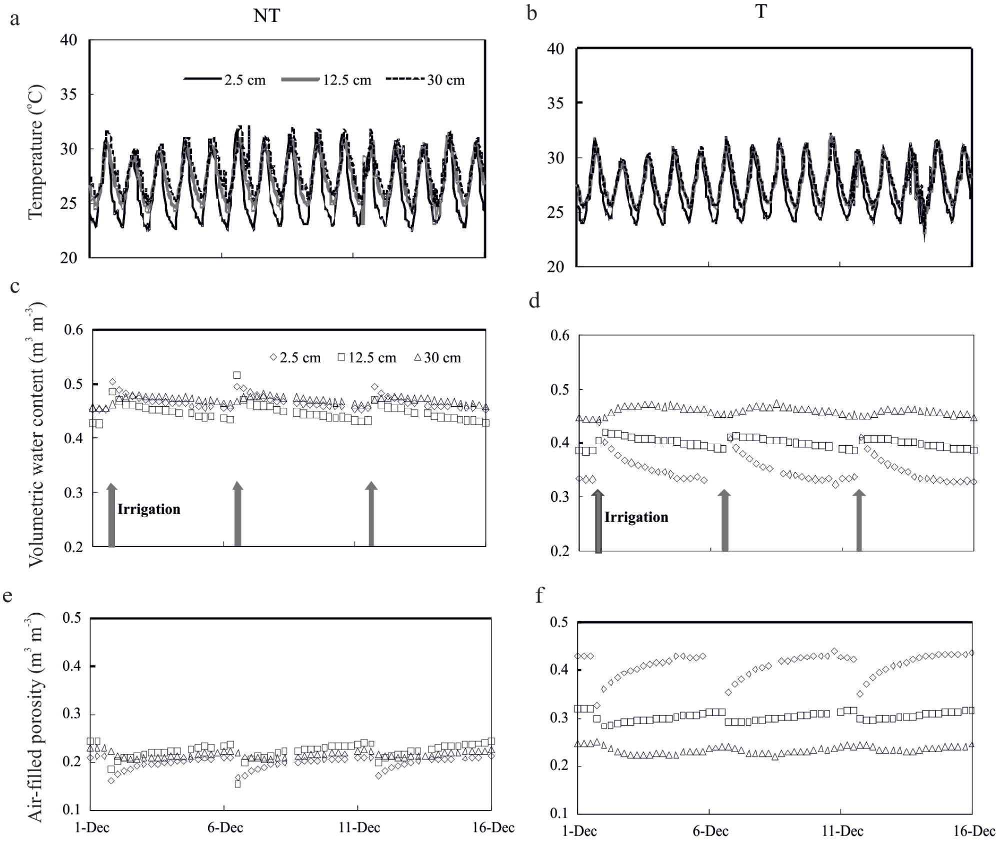

Fig. 3. Soil temperatures, volumetric water contents, and air-filled porosities at depths of 2.5, 12.5, and 30 cm under no-tillage (NT) and tillage (T) treatments (15 day); temperatures in: a – NT, b – T; volumetric water contents in: c – NT, d – T; air-filled porosities in: e – NT, f – T.

Table 2. Soil bulk density and total porosity under the no-tillage (NT) and tillage (T) treatments

Depth (cm)	Bulk density (g cm^{-3})		Total porosity (m^3 m^{-3})	
	NT	T	NT	T
2.5	0.85a	0.64b	0.67a	0.75b
7.5	0.87a	0.62b	0.66a	0.76b
12.5	0.83a	0.76b	0.68a	0.71b
20	0.81a	0.78a	0.68a	0.69a
30	0.81a	0.79a	0.68a	0.69a

Significant differences between treatments are indicated with different letters (p<0.05, n=3).

Gas diffusion is the main mechanism of gas transport within and between soils and the atmosphere, and hence the gradient method based on Fick law (Eq. (3)) has been proposed to estimate soil CO_2 efflux from the soil surface. Accordingly, based on the gas diffusivity and soil CO_2 concentration gradient (0-2.5 cm) data obtained from the column incubation experiment, we used Eq. (3) to estimate CO_2 efflux:

$$F = -D_P \frac{\Delta C}{\Delta z}, \tag{3}$$

where: F is the gas flux from the soil (g CO_2-C m^{-2} d^{-1}), D_p is the gas diffusion coefficient in the soil (m^2 d^{-1}), ΔC is the soil CO_2 concentration gradient (g m^{-3}), and Δz is the thickness of the soil layer (m).

Because continuous measurement of gas diffusivities is difficult, predictive models developed by fitting curves were used to estimate soil gas diffusivities (Moldrup *et al.,* 2000; Deepagoda *et al.,* 2011). As shown in Fig. 4, an exponential relationship was observed between soil air-filled porosity and gas diffusivity; hence, soil gas diffusivity could be described as a function of the air-filled porosity. However, the range of air-filled porosities at water potentials of -20 to -1 000 cm H_2O under the T treatment was lower than that under the NT treatment; this difference may be attributed to changes in soil structure and, thus, water retention curves due to tillage. Based on the measured gas diffusivities at five specific water potentials (-20, -63, -200, -500, and -1 000 cm H_2O), fitting curves were developed for both NT ($Y = 2.37X^{2.78}$) and T ($Y = 3.18X^{3.65}$) treatments. Because water potentials in soils during the incubation period were monitored accurately in the column experiment,

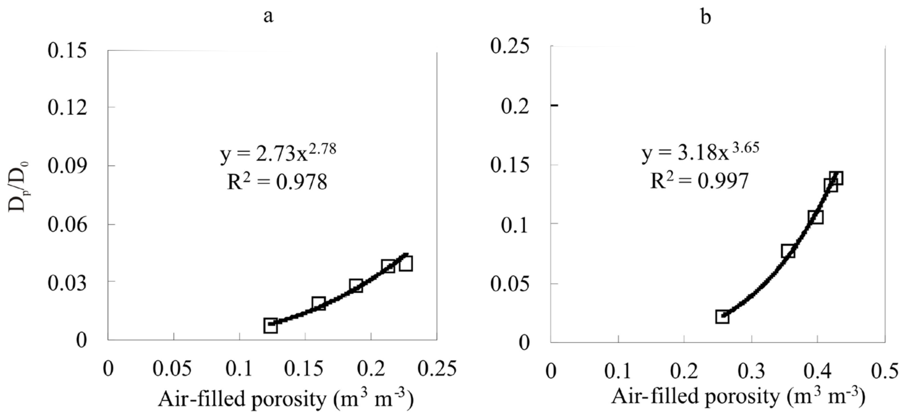

Fig. 4. Exponential relationships between soil gas diffusivities (D_p/D_0) and air-filled porosities for: a – no-tillage (NT) and b – tillage (T) treatments. D_p and D_0 are the gas diffusion coefficient in the: soil and air (m^2 s^{-1}), respectively.

temporal changes in soil air-filled porosity in the two treatments were available. Therefore, soil gas diffusivities at depths of 0-5 cm could be predicted (Fig. 5a) with values of air-filled porosity and fitting curves.

The CO_2 concentrations in the surface air and at a depth of 2.5 cm under the two treatments are shown in Fig. 5b, c. No clear difference in the CO_2 concentration in the surface air was observed between the NT and T treatments. The soil CO_2 concentration under the NT treatment at a depth of 2.5 cm was higher than that under the T treatment. Greater air-filled porosity and gas diffusivity of the tilled soil could be a cause of the lower soil CO_2 concentration.

Similar to the measured results, distinct differences in the estimated CO_2 efflux between the NT (1.90 g m^{-2} d^{-1}) and T (4.91 g m^{-2} d^{-1}) treatments were observed on the first day of the incubation period. Except for the first day, no noticeable increase or decrease in the CO_2 efflux was observed, as shown in Fig. 5d. The estimated cumulative CO_2 efflux under the T treatment (79.14 g m^{-2} d^{-1}) during the 150-day incubation period was higher than that under the NT treatment (65.02 g m^{-2} d^{-1}). A linear relationship ($p<0.05$) was observed between the measured and estimated soil CO_2 effluxes (Fig. 6). However, the estimated data showed a tendency to underestimate the soil CO_2 effluxes, similar to results reported by Fierer *et al.* (2005) and Kusa *et al.* (2008). A source of a bias for the gradient method may come from an assumption that the CO_2 flux at 0-2.5 cm in depth represents the CO_2 efflux from the soil surface to the atmosphere. This assumption neglects soil CO_2 production in the 0-2.5 cm – depth layer. However, the shallow soil layer could be active in CO_2 production because the soil O_2 level might be high due to quick gas exchange with the atmosphere. With compensation by using measured CO_2 efflux, the gradient method used in the column incubation experiment could provide an acceptable estimation of CO_2 efflux.

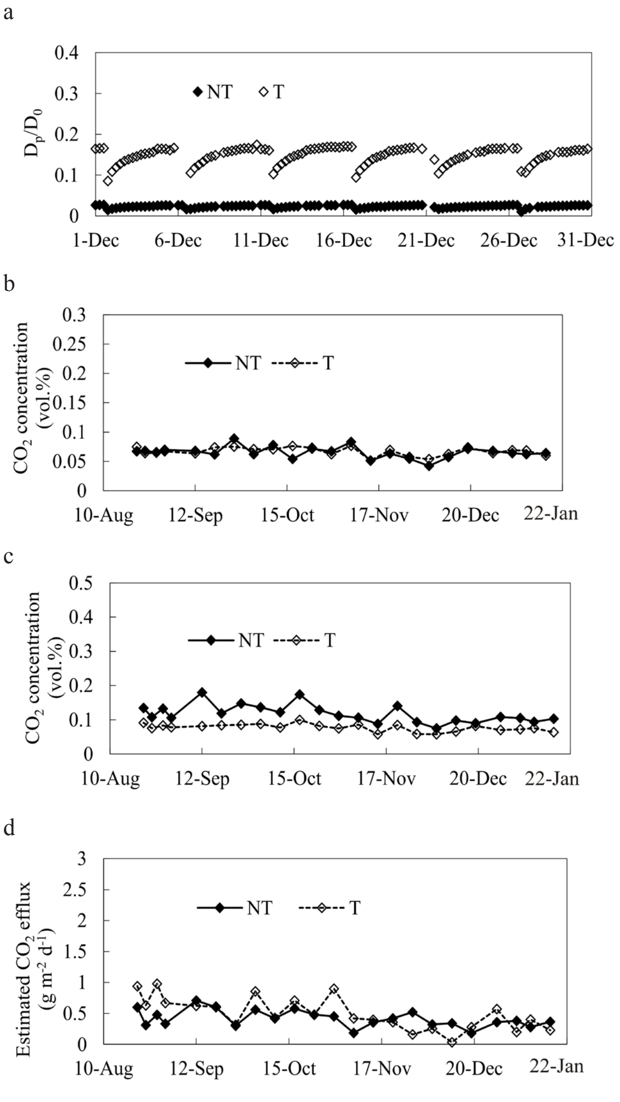

Fig. 5. Soil gas diffusivities at depths of 0-5 cm (a) (selected results of soil gas diffusivities are shown), soil CO_2 concentrations in the surface air (b), CO_2 concentrations at a depth of 2.5 cm (c), estimated CO_2 effluxes (d) for the no-tillage (NT) and tillage (T) treatments (August 19 – January 14).

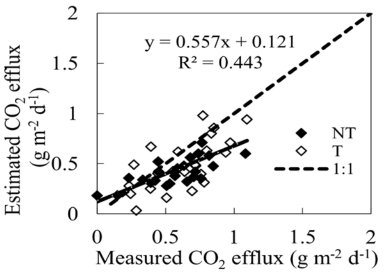

Fig. 6. Relationship between the measured and estimated soil CO_2 effluxes.

The incubation experiment has advantages in observing the experimental process and saving labours. More importantly, compared with fieldwork, simulation or accurate control of environmental factors can be done in column incubation experiments. In the present study, moisture and temperature, important factors affecting soil respiration, were controlled within a relatively stable range, and thus the effects of tillage on soil CO_2 efflux could be accurately estimated. Rather than temperature and moisture conditions, other experimental conditions (*eg* climate factors, agricultural managements, soil gas state) can be created or precisely controlled in column incubation experiments (Case *et al.*, 2014; De Graaff *et al.*, 2008). In the investigation of the influence of climate factors or human activity on CO_2 release, incubation experiments are preferred if special or precise experimental conditions are required.

In the present study, changes in soil properties, particularly water content and air-filled porosity, were accurately observed during the incubation period. The observed results could help to understand higher CO_2 production from tilled soil, which would be due to the increase in the air-filled porosity and gas diffusivity at the 0-15 cm depth caused by tillage. Additionally, the column incubation method can be used to monitor the gas concentration and thus to examine gas transport in soil. Based on the theory of gas diffusion, the gradient method could be conducted with column incubation experiments to estimate temporal changes in the soil CO_2 efflux by using soil CO_2 concentration and gas diffusivity. Unlike the chamber method, measurements of soil CO_2 concentration and moisture conditions are not affected by climatic conditions. Therefore, this method can compensate for the shortcomings of chamber measurements and serve as an alternative or supplemental method for CO_2 efflux evaluation. This type of analysis of CO_2 production and transport in the soil profile cannot be achieved through bottle incubation.

Moreover, the structure of the undisturbed soil column used for this experiment was almost the same as that in the field, and thus the results were more accurate than those obtained in bottle incubation. Chu *et al.* (2007) reported values of 0.427-0.524 g CO_2-C m^{-2} d^{-1} CO_2 efflux from an Andisol upland field in Japan under different types of fertilizer management using a closed chamber method that was the same as that used in the present study. Similarly, Nakadai *et al.* (2002) conducted a field experiment using a chamber method and reported that the average soil CO_2 efflux from an Andisol under the tillage treatment was in the range of 0.73-1.39 g CO_2-C m^{-2} d^{-1} according to the season. However, the values of CO_2 efflux from Andisol determined from bottle incubation were significantly overestimated (Dumale *et al.*, 2009; Nakadai *et al.,* 2002), because the gas status and soil structure in bottle incubation were significantly different from those in the field experiment. The results of CO_2 efflux from soils measured with the column incubation experiment were superior to those from bottle incubation experiments.

However, compared with the bottle incubation, preparing the soil column is complicated and costly. During the experiment, controlling the environmental conditions often requires special techniques or instruments. That may be the main reason that limited studies have been carried out with column incubation. Because column incubation has obvious advantages for analysis of CO_2 behaviour in soils, further improvements in this method are expected.

CONCLUSIONS

1. To evaluate the applicability of soil column incubation experiments in studying CO_2 effluxes, a 150-day column incubation experiment was conducted to measure CO_2 efflux from no-tilled and tilled soils. Both the chamber measurement and the gradient method were used, and results from the two methods were consistent: tillage increased the cumulative CO_2 efflux from the soil during the incubation period.

2. Compared with the fieldwork, incubation experiments allow creating or precisely controlling experimental conditions and thus have advantages in investigating the influence of climate factors or human activity on CO_2 efflux. Superior to bottle incubation, soil column experiments can focus on analysis of the mechanism of CO_2 behaviour in soil profiles and yield more accurate results. Considering the advantages of column incubation experiments, we expect them to be more widely employed in the analysis of CO_2 behaviour in soils.

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