

Article

Forest Soil Profile Inversion and Mixing Change the Vertical Stratification of Soil CO₂ Concentration without Altering Soil Surface CO₂ Flux

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Received: 28 December 2018; Accepted: 19 February 2019; Published: 21 February 2019



Abstract: In order to gain more detailed knowledge of the CO₂ concentration gradient in forest soil profiles and to better understand the factors that control CO₂ concentration along forest soil profiles, we examined the soil surface CO₂ flux, soil properties and soil profile CO₂ concentration in upright (CK), inverted and mixed soil columns with a depth of 60 cm in two subtropical forests in China from May 2008 to December 2009. The results showed that: (1) The SOC (soil organic carbon), TN (total N) and microbial biomass were higher in the deeper layers in the inverted soil column, which was consistent with an increase in CO₂ concentration in the deeper soil layer. Furthermore, the biogeochemical properties were homogenous among soil layers in the mixed soil column. (2) CO₂ concentration in the soil profile increased with depth in CK while soil column inversion significantly intensified this vertical stratification as the most active layer (surface soil) was now at the bottom. The stratification of CO₂ concentration along the soil profile in the mixed soil column was similar to that in CK but it was not intensified after soil was mixed. (3) The soil surface CO₂ flux did not significantly change after the soil column was inverted. The surface CO₂ flux rate of the mixed soil column was higher compared to that of the inverted soil column but was not significantly different from CK. Our results indicated that the profile soil CO₂ production was jointly controlled by soil properties related to CO₂ production (e.g., SOC content and soil microbial biomass) and those related to gas diffusion (e.g., soil bulk density and gas molecular weight), but the soil surface CO₂ flux was mainly determined by soil surface temperature and may be affected by the intensity of soil disturbance.

Keywords: CO₂ production and diffusion; soil properties; CO₂ emission; surface soil layer

1. Introduction

An increase in atmospheric CO₂ concentration is considered to be one of the main causes for global warming [1,2]. As the largest terrestrial source and potential sink for CO₂, soil is particularly

important in the global carbon cycle [3–7]. All CO₂ produced in the soil would be emitted through soil surface efflux on a long-term basis [8]. The soil profile CO₂ concentration was reported to drive and accelerate this surface emission process [9] and therefore, this would influence the carbon balance of the forest. Some models estimated the soil CO₂ effluxes from the soil CO₂ concentration profiles [10–12]. Thus, we need to gain more detailed knowledge of the soil profile CO₂ concentration in order to better assess its contribution to soil surface CO₂ emission and global warming.

The majority of the forest soil CO₂ is produced in the surface layer since the majority of SOM (soil organic matter) and roots are distributed in the surface soil. However, the soil profile CO₂ concentration is high in the deep soil layer and low in the surface soil, which is the opposite to the production source layer [13]. Microbial biomass acts as both a source and sink for nutrients and participates in C, N and P transformation. Although it contributes less than 5% to SOM, it plays an active role in the soil C cycle [14]. The soil profile CO₂ concentration depends on both the processes of CO₂ production and diffusion. Studies have shown that soil CO₂ production and diffusion often has a strong and remarkable dependence on temperature and moisture [15–17]. It was also affected by soil properties, such as soil organic matter, total N (TN) and bulk density, root dynamics and microbial biomass [18,19]. However, there were limited studies focused on the relationship between the variation of soil properties and soil CO₂ concentration.

Agricultural practice, such as tillage, plays an important role in the storage and release of C within the terrestrial C cycle. The conventional intensive tillage was found to increase the emission of CO₂ by 16.0% in a subtropical rice farm [20]. Significantly greater CO₂ fluxes were also observed in subtropical paddy ecosystems after tillage operations [21]. Tillage disturbance does not occur as frequently as croplands in forests but during the process of restoring damaged ecosystems, tree planting and occasionally tillage are usual practices. Consequently, soil mixing is inevitable during ploughing.

In the present study, we manipulated a soil column experiment with upright, inverted and mixed soil columns in order to investigate the soil surface CO₂ flux, the distribution of CO₂ concentration in soil profiles and their influencing factors. The field site was a forest restoration ecosystem. We mixed the soil in “mixed” columns to identify the influence of “tillage” disturbance on soil CO₂. The purpose of our study was to examine the dependence of soil profile CO₂ concentration on soil properties in order to better understand the mechanism of the vertical stratification of soil profile CO₂ concentration and the relationship of soil profile CO₂ with soil surface CO₂ flux.

2. Materials and Methods

2.1. Study Area

The study was conducted over a 20-month period (from May 2008 to December 2009) in two subtropical plantation forests at the Heshan Hilly Land Interdisciplinary Experimental Station (112°50' E, 22°34' N), Guangdong Province, China. These selected forests included a coniferous forest (CF) mixed by *Pinus massoniana* Lamb, *Cunninghamia lanceolata* (Lamb) Hook and a broad-leaved forest (BF) dominated by *Schima wallichii* Choisy. The soil of the field site was an Orthic Acrisol [22] and the surface soil pH was about 4.0. The soil SOC (soil organic carbon) was 13.08 and 19.26 g kg⁻¹ while the TN was 0.99 and 1.11 g kg⁻¹ in CF and BF, respectively. The trees were about 25 years old when the current experiment started in 2008.

2.2. Experimental Design and Sample Analysis

A randomized block design with six replicates for each treatment was used in the soil column manipulation experiment. The soil column treatments were: (1) Upright soil column (CK); (2) inverted soil column (Inverted); and (3) mixed soil column (Mixed). The soil pillar was carefully dug and sheathed in the PVC (polyvinyl chloride) pipe to make a soil column cylinder. Each soil column had a diameter of 40 cm and a depth of 60 cm. In the upright and inverted soil columns, the soil pillar was undisturbed but in the mixed soil column, the topsoil and subsoil were thoroughly mixed. All soil

columns were sealed at the bottom and placed back into the original location where the soil column was manipulated. Each soil column was equipped with gas tubes and three-way stopcocks at depths of 20, 40 and 60 cm to sample soil CO₂ while a water tube was added at the bottom to sample the dissolved soil organic carbon and to avoid waterlog (Figure A1). Several holes with a diameter of 2 cm were made onto the wall of the PVC pipe to allow for the free exchange of soil air. All vegetation and litter fall were removed carefully from the soil surface of each soil column and were not present during the experiment period, which was achieved without disturbing the soil. All these manipulations were completed in May 2008.

We measured the soil surface CO₂ flux for each column once per month with the static chamber-gas chromatograph (GC) technique [23] from May 2008 to December 2009. PVC chamber with a diameter of 20 cm and a height of 20 cm was gently inserted 2 cm below the soil. Gas samples were collected four times at 10 min intervals from each soil column with 60 mL polypropylene syringes. Measurements were always made between 09:00 and 11:00 as suggested by Xu and Qi to represent the diurnal averages [19,24,25]. Soil CO₂ concentrations at depths of 20, 40 and 60 cm were sampled after the surface measurements and were determined using GC within 24 h. The soil temperature at depths of 5, 20, 40 and 60 cm was recorded every 0.5 h with an iButton DS1923 digital thermometer equipped in the soil column.

Gas flux was calculated based on the soil surface gas concentration change within the chamber over the measurement period, which was estimated as the slope of linear regression between concentration and time. It was expressed in the following equation [26]:

$$F = \frac{\Delta m}{\Delta t} \cdot D \frac{V}{A} = hD \frac{\Delta m}{\Delta t} \quad (1)$$

where F is the gas flux ($\text{mg m}^{-2} \text{h}^{-1}$); h is the height of the chamber (m); D is the gas density in the chamber ($D = n/v = P/RT$, in mg m^{-3} where P is the air pressure; T is the temperature inside of the chamber and R is the air constant; $\Delta m/\Delta t$ denotes the linear slope of concentration changing with time over the measurement period.

The soil along the profiles was sampled in May and November both in 2008 and 2009. All soil samples were sieved with a 2 mm sieve before analysis. Soil water content (SWC) was measured by oven-drying for 48 h at 105 °C; SOC was determined by the dichromate oxidation method; soil TN was estimated by Kjeldahl digestion with UV spectrophotometric analysis [27]; and soil bulk density was determined by the intact soil core method. The soil microbial biomass and community structure was analyzed using the phospholipid fatty acids (PLFAs) method as described by Bossio and Scow [28]. Different PLFAs were considered to represent different groups of soil microorganisms. The abundance of individual fatty acids was calculated based on the 19:0 internal standard concentrations. Bacteria were identified by 10 PLFAs (i15:0, a15:0, 15:0, i16:0, 16:1 ω 7, i17:0, a17:0, 17:0, cy17:0 and cy19:0) while 18:2 ω 6c and 18:1 ω 9c were used as the indicators of fungal biomass [29]. The ratio of fungal PLFAs to bacterial PLFAs was used to estimate the ratio of fungal to bacterial biomass (F/B) in soil [30]. The results of soil properties were the average of four measurements.

2.3. Statistical Analysis

A repeated measures analysis of variance (RM ANOVA) was performed to examine the monthly changes in CO₂ concentration and the soil surface CO₂ flux. Two-way ANOVA and LSD (least significant difference) tests were performed to compare the physicochemical and microbial traits among forest types and soil treatments. All statistics were performed using IBM SPSS Statistics 21 (IBM Corporation, New York, NY, USA) and SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Precipitation and Air Temperature

The annual precipitation of the site was 1319.6 mm and 1525.6 mm while the average temperature was 21.60 °C and 22.49 °C in 2008 and 2009, respectively. The precipitation from May to September (high temperature period, monthly mean temperature >25 °C) in 2008 was 922.60 mm, which was significantly less than that in the same period in 2009 (1148.20 mm). The mean air temperature was 26.95 °C in this period in 2008, which was lower by 0.62 °C compared to 2009 (Figure A2). The mean soil temperature at a depth of 5 cm was 24.73 °C in CF in the study period, which was higher by 1.29 °C compared to BF.

3.2. Variation of Soil Profile CO₂ Concentration

Large variations of CO₂ concentration in soil profiles were observed both throughout time and with different depths in all treatments (Figure 1a–f). In general, the CO₂ concentration increased with depth, with the highest concentrations observed at a depth of 60 cm. CO₂ concentrations in soil profiles were quite different between the two years, with the peak accumulation having occurred in the high temperature period of the second year. The CO₂ concentration in the BF soil was higher than that in the CF soil.

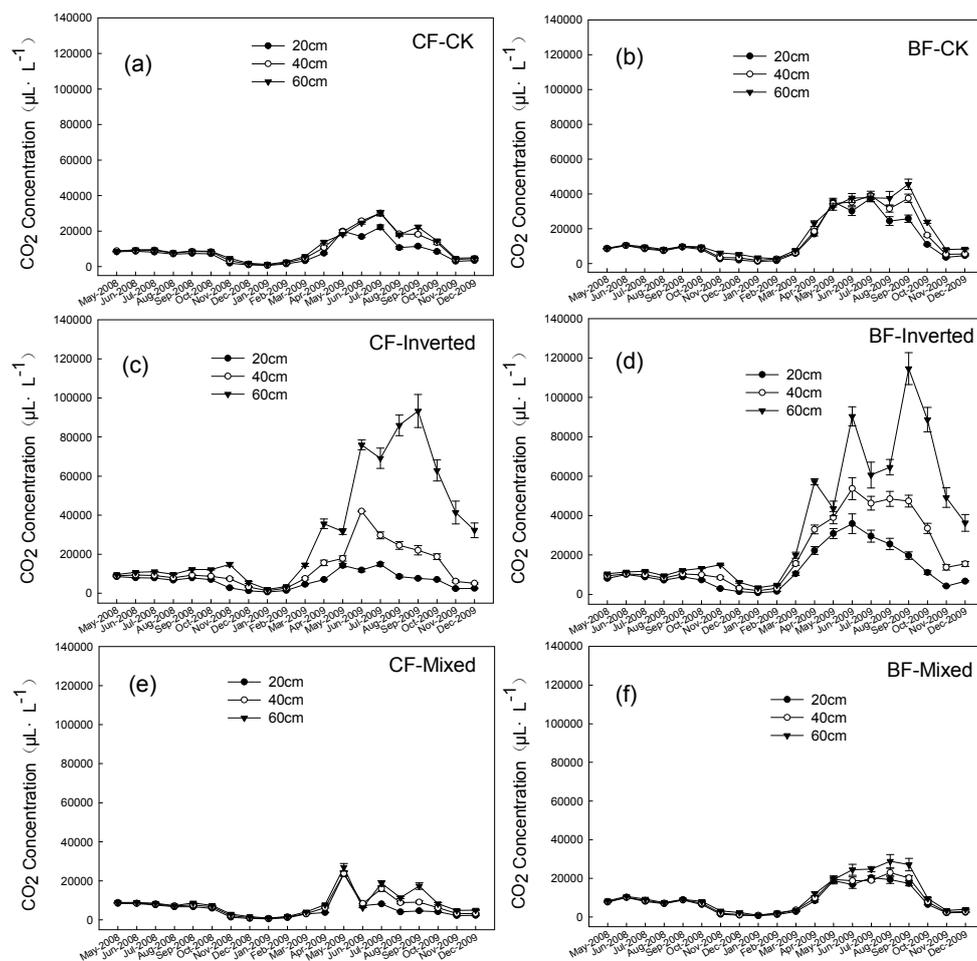


Figure 1. Seasonal variations of CO₂ concentration along soil profiles in a coniferous forest (CF) and a broad-leaved forest (BF). Different soil column treatments were: (a) CF-CK (upright soil column); (b) BF-CK; (c) CF-Inverted; (d) BF-Inverted; (e) CF-Mixed; and (f) BF-Mixed. Data are shown as means ± SE, $n = 6$.

The average CO₂ concentration in CF at a depth of 60 cm was $1.1 \times 10^4 \mu\text{L}\cdot\text{L}^{-1}$, which was 39% higher than that in 20 cm. The CO₂ concentration in the inverted soil column at a depth of 60 cm reached $3.2 \times 10^4 \mu\text{L}\cdot\text{L}^{-1}$, which was about 4.8 times that of the 20 cm. In other words, the vertical stratification of CO₂ concentration in the soil profile was intensified in the inverted soil column compared to CK. In the mixed soil columns, the CO₂ concentration in each layer was lower than CK. Similar patterns were observed in BF, which showed that the inverted soil column intensified the stratification of CO₂ concentrations in soil profiles, while CO₂ concentrations in the “Mixed” soil column were lower than CK despite still maintaining its stratification.

3.3. Seasonal Variation of Soil Surface CO₂ Flux

Soil surface CO₂ flux rates varied significantly during the study period, with higher CO₂ flux rates observed during the summer both in BF and CF (Figure 2a,b). The repeated measures analyses of variance indicated a significant interaction between months and treatments ($p < 0.001$). The average soil surface CO₂ flux rates in CF were 185.69, 155.70 and 201.81 mg m⁻² h⁻¹ for the CK, inverted and mixed soil columns, respectively. In BF, the rates were 183.42, 159.95 and 197.70 mg m⁻² h⁻¹, respectively. The soil surface CO₂ flux rates of the inverted soil column were 13%–16% lower than CK while that of the “mixed” soil column were somewhat higher although these differences were not significant. However, soil surface CO₂ flux rates of the “mixed” soil column was significantly higher than that in the inverted soil column in BF ($p < 0.05$). Soil surface CO₂ flux rates from CF did not significantly differ from the rates measured in BF.

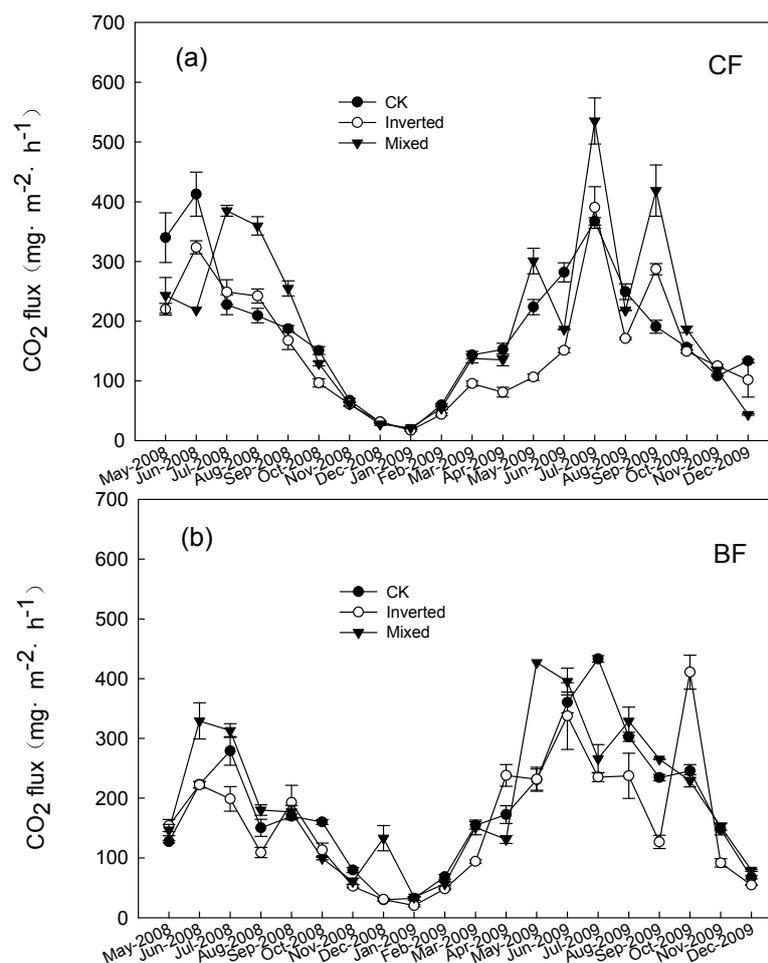


Figure 2. Fluxes of CO₂ in a coniferous forest (CF) (a) and a broad-leaved forest (BF) (b) in different soil column treatments: CK, inverted and mixed. Error bars represent standard errors of the mean ($n = 6$).

3.4. Soil Biogeochemical Properties in Different Columns

The SOC (at a depth of 0–20 cm) was significantly higher in the BF soil than in the CF soil ($p < 0.05$, Table 1). SWC, TN and bulk density did not differ between the two forests. In the upright soil column, the SOC and TN in the topsoil were significantly higher than in the subsoil ($p < 0.05$) while the soil bulk density was higher in the subsoil. Naturally, the opposite pattern was observed in the inverted soil column, which showed that SOC and TN were higher in “new subsoil” but soil bulk density was higher in the “new topsoil”. In the mixed soil column, all measured soil properties did not differ significantly among the three soil layers.

Table 1. Soil physical and chemical properties by depth and soil columns manipulation, including soil water content (SWC), total organic carbon (TOC), total nitrogen (TN) and bulk density. Error bars represent the standard errors of the mean ($n = 4$). Different letters represent significant differences (LSD test, $p < 0.05$).

Forest Type	Treatments	Profile	SWC (%)	SOC ($\text{g}\cdot\text{kg}^{-1}$)	TN ($\text{mg}\cdot\text{L}^{-1}$)	Bulk Density ($\text{g}\cdot\text{cm}^{-3}$)
CF	CK	0–20 cm	19.66 \pm 4.00a	13.09 \pm 2.74a	1.17 \pm 0.14a	1.40 \pm 0.05a
		20–40 cm	18.95 \pm 2.54a	7.62 \pm 2.21b	0.83 \pm 0.18b	1.48 \pm 0.06a
		40–60 cm	19.93 \pm 2.97a	5.75 \pm 1.45b	0.70 \pm 0.10b	1.48 \pm 0.08a
	Inverted	0–20 cm	19.08 \pm 3.21a	5.98 \pm 1.00b	0.66 \pm 0.15b	1.49 \pm 0.02a
		20–40 cm	20.04 \pm 1.86a	6.17 \pm 1.76b	0.71 \pm 0.20b	1.46 \pm 0.11ab
		40–60 cm	21.36 \pm 2.58a	10.12 \pm 2.98a	0.99 \pm 0.20a	1.35 \pm 0.09b
	Mixed	0–20 cm	20.19 \pm 2.63a	10.89 \pm 1.82a	0.97 \pm 0.18a	1.31 \pm 0.03a
		20–40 cm	21.04 \pm 2.67a	9.25 \pm 1.35a	0.94 \pm 0.16a	1.30 \pm 0.05a
		40–60 cm	21.80 \pm 1.53a	9.42 \pm 0.69a	0.90 \pm 0.16a	1.28 \pm 0.09a
BF	CK	0–20 cm	23.15 \pm 1.82a	17.25 \pm 1.53a	1.42 \pm 0.41a	1.39 \pm 0.02b
		20–40 cm	21.07 \pm 2.15a	6.97 \pm 1.94b	0.81 \pm 0.29b	1.55 \pm 0.05a
		40–60 cm	21.44 \pm 1.60a	4.76 \pm 1.54b	0.68 \pm 0.33b	1.48 \pm 0.08a
	Inverted	0–20 cm	20.15 \pm 1.82a	5.51 \pm 1.44b	0.75 \pm 0.41a	1.39 \pm 0.09a
		20–40 cm	20.62 \pm 2.51a	5.31 \pm 1.49b	0.76 \pm 0.31a	1.48 \pm 0.10a
		40–60 cm	23.29 \pm 1.83a	12.79 \pm 5.54a	1.15 \pm 0.29a	1.39 \pm 0.02a
	Mixed	0–20 cm	21.80 \pm 2.31a	9.43 \pm 2.16a	0.97 \pm 0.33a	1.32 \pm 0.08a
		20–40 cm	22.22 \pm 1.57a	9.18 \pm 3.01a	0.92 \pm 0.21a	1.35 \pm 0.03a
		40–60 cm	22.50 \pm 3.93a	8.33 \pm 2.22a	0.96 \pm 0.40a	1.32 \pm 0.04a

In the upright soil columns, the mean total soil microbial biomass (PLFA), fungal biomass, bacterial biomass and F/B ratio all decreased with depth both in CF and BF soil (Table 2). In the inverted soil column, bacterial biomass was higher in the “new subsoil” while fungal biomass was higher in the “new topsoil”. In the mixed soil column, the biogeochemical properties were homogenous among different soil layers.

Table 2. Soil microbial community characters in each soil layer over all manipulated soil columns. PLFA, total PLFA; Fun, fungi; Bac, Bacteria; F/B, the ratio of fungal to bacterial biomass. Error bars represent the standard errors of the mean ($n = 4$). Different letters represent significant differences (LSD test, $p < 0.05$).

Forest Type	Treatments	Profile	PLFA ($\text{nmol}\cdot\text{g}^{-1}$)	Fun (mol%)	Bac (mol%)	F/B%
CF	CK	0–20 cm	6.34 \pm 3.52a	4.14 \pm 1.34a	28.64 \pm 5.76a	14.69 \pm 4.23a
		20–40 cm	4.07 \pm 2.24a	1.99 \pm 0.98ab	21.33 \pm 8.61b	10.75 \pm 6.12a
		40–60 cm	4.15 \pm 2.30a	1.53 \pm 1.12b	17.86 \pm 4.93b	8.89 \pm 5.83a
	Inverted	0–20 cm	5.45 \pm 2.66a	3.52 \pm 3.39a	17.39 \pm 3.31b	19.64 \pm 18.31a
		20–40 cm	4.72 \pm 2.25a	1.18 \pm 0.72a	17.06 \pm 3.36b	7.34 \pm 4.67b
		40–60 cm	5.10 \pm 1.90a	2.94 \pm 0.51a	27.51 \pm 6.05a	11.18 \pm 4.54a
	Mixed	0–20 cm	7.31 \pm 4.60a	5.01 \pm 2.60a	27.25 \pm 3.00a	17.43 \pm 8.86a
		20–40 cm	5.57 \pm 2.73a	3.21 \pm 0.89a	25.28 \pm 3.84a	12.09 \pm 3.37a
		40–60 cm	5.45 \pm 1.77a	2.76 \pm 0.56a	25.83 \pm 2.10a	10.19 \pm 2.06a

Table 2. Cont.

Forest Type	Treatments	Profile	PLFA (nmol·g ⁻¹)	Fun (mol%)	Bac (mol%)	F/B%
BF	CK	0–20 cm	10.55 ± 1.92a	3.35 ± 1.90a	24.24 ± 6.24a	13.04 ± 5.24a
		20–40 cm	7.75 ± 1.94a	2.05 ± 0.74a	20.83 ± 3.85a	9.79 ± 4.14a
		40–60 cm	6.23 ± 1.05b	0.98 ± 0.60a	14.37 ± 3.15b	7.32 ± 4.10a
	Inverted	0–20 cm	6.54 ± 1.55a	3.71 ± 4.90a	16.79 ± 3.93a	8.46 ± 4.25a
		20–40 cm	6.83 ± 2.49a	1.39 ± 0.41a	18.38 ± 3.64a	8.81 ± 3.39a
		40–60 cm	7.11 ± 0.96a	1.74 ± 0.48a	21.24 ± 1.06a	8.93 ± 1.67a
	Mixed	0–20 cm	7.05 ± 2.27a	2.21 ± 2.04a	20.96 ± 4.47a	9.88 ± 8.07a
		20–40 cm	8.49 ± 2.37a	1.44 ± 1.30a	16.15 ± 6.37a	8.19 ± 5.49a
		40–60 cm	9.10 ± 3.08a	1.32 ± 1.11a	16.32 ± 4.66a	7.93 ± 4.95a

3.5. Correlations of Soil CO₂ Concentration and Temperature

The dependence of soil CO₂ concentration on temperature was strong and consistent in two forests in the CK and mixed soil columns (Figure 3a–f). The relationship was well fit by an exponential growth regression model ($p < 0.001$). The temperature explained 26%–76% of the variation in soil CO₂ concentration in the upright and mixed soil columns. In the inverted soil columns, this dependence was significantly weaker.

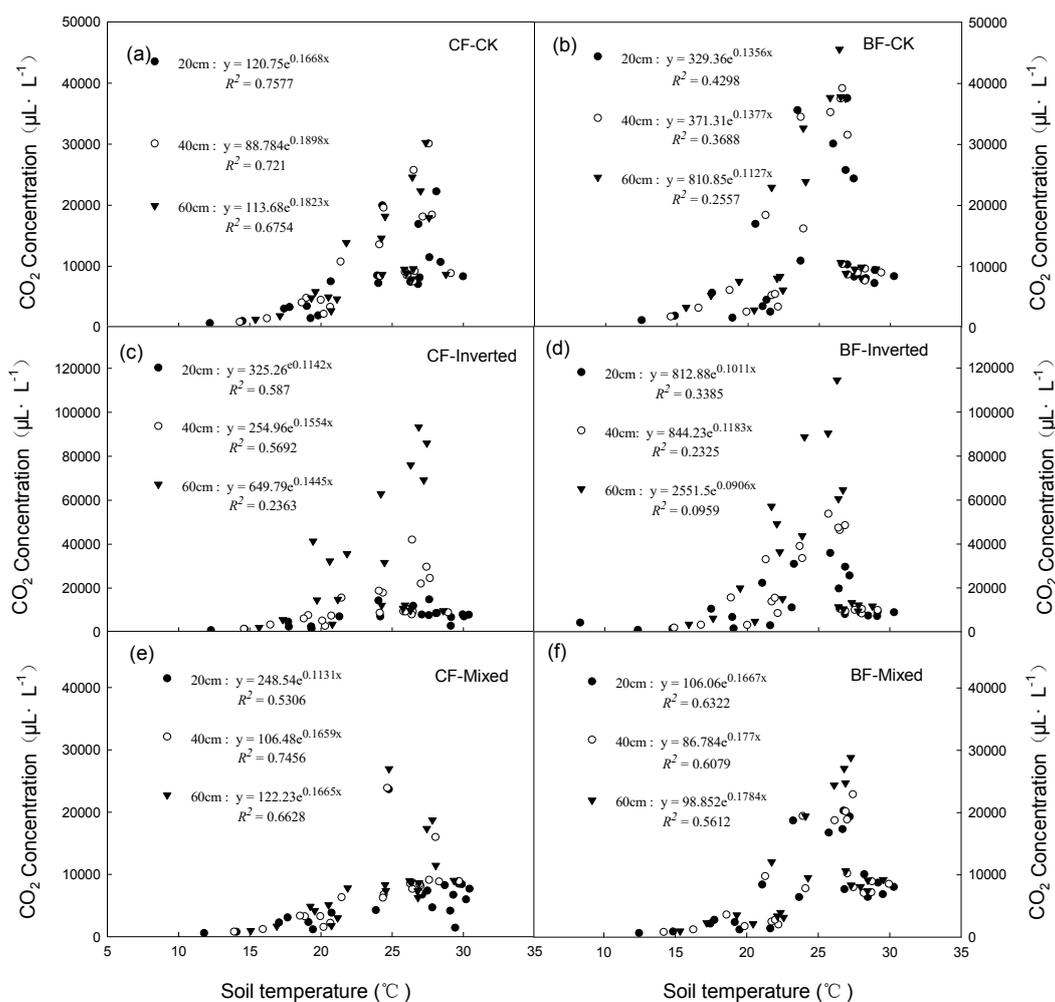


Figure 3. Relationship between soil profile CO₂ concentration and soil temperature. Different soil column treatments were: (a) CF-CK; (b) BF-CK; (c) CF-Inverted; (d) BF-Inverted; (e) CF-Mixed; and (f) BF-Mixed.

4. Discussion

4.1. Effects of Environment Variables on CO₂ Concentration in Soil Profiles and Soil Surface CO₂ Flux

In general, the soil CO₂ concentration was greater in the deeper soil layer regardless of the way that the soil columns were manipulated in the present study. We considered that this was mainly due to the difficulty of CO₂ diffusion within the soil profile to the atmosphere [15]. Studies have demonstrated that the molecular diffusion trait is the most important factor affecting CO₂ transportation within the soil profile. Furthermore, this was strongly dependent on soil porosity, which was closely related to soil moisture [9]. Rainfall could lead to an increase in soil moisture and a decrease in CO₂ diffusivity, which would subsequently result in CO₂ accumulation in soil and increase the CO₂ concentration in deep soil [16]. Our data also showed that CO₂ concentration in soil profiles increased with the seasonal temperature rise, which was probably due to an increase in soil microbial respiration with increasing temperature. This suggests that temperature is also a major factor that influences CO₂ concentration along the soil profile. The summer of 2009 was relatively hotter and wetter than the summer of 2008 during the study period, which resulted in a significantly higher CO₂ concentration in the summer of 2009 thus, verifying that temperature and moisture are major factors determining soil CO₂ concentration.

4.2. Soil Properties and Soil CO₂ Concentration Stratification

The inverted soil column was found to intensify the stratification of CO₂ concentration due to the replacement of the subsoil with the original topsoil. In this case, the SOC, TN and microbial biomass were higher in the deeper layer in the inverted soil column, which was consistent with an increase in CO₂ concentration in the deeper soil layer. SOC and TN provide energy and nutrients for microbial growth and thus, the CO₂ in the soil profiles mainly resulted from microbial activity. In addition, the higher soil bulk density in the “new topsoil” in the inverted soil column would have a negative effect on CO₂ emission from the soil since CO₂ diffusion within the soil profile depends on soil porosity, which is tied closely with soil bulk density.

As we observed, soil organic carbon, total nitrogen and microbial biomass were often higher in original topsoil and they coincided with higher CO₂ production. However, the soil CO₂ concentration was generally higher in the deeper soil layer. Furthermore, soil properties in the mixed soil columns did not differ significantly among soil layers but the CO₂ concentration along the soil profile showed a clear stratification. These results indicated that although CO₂ concentration was highly influenced by soil properties, it was the gravity that determined the vertical distribution of soil CO₂ concentration since the molecular weight of CO₂ is greater than air.

4.3. CO₂ Profile Concentration and its Relationship to Soil Surface CO₂ Flux

The surface CO₂ flux rates of the mixed soil column were 8%–9% higher than those in CK although these were not statistically significantly. This was much less than those in the croplands under conventional tillage [20,21]. Soil profile CO₂ is transported into the atmosphere primarily by diffusion and air turbulence at the forest soil surface, which could significantly impact the carbon balance of the forest ecosystem [31,32]. These important processes often occurred near the soil surface but had little effect on the subsoil CO₂ storage. Wiaux et al. [10] observed that approximately 90%–95% of the surface CO₂ fluxes originated from the top 10 cm of the soil profile. On one hand, the upward movement of CO₂ is a slow process limited by soil surface texture and turbulence. On the other hand, CO₂ has the tendency to sink down along the soil profile as the molecular weight of CO₂ is heavier than the average molecular weight of air [13].

In the present study, it is important to note that soil column inversion significantly intensified the vertical stratification of soil profile CO₂ concentration. However, this did not intensify soil surface CO₂ flux rate, which was even lower compared to CK. In contrast, soil column mixing increased the soil surface CO₂ flux to some extent (although this was not statistically significant). C content in BF

in the topsoil was 32% more than that in CF, while the soil surface CO₂ flux rates from BF were not significantly different from those in CF. These results suggested that CO₂ production was stimulated by the increased CO₂ production sources (SOC, TN and microbial communities) while surface soil CO₂ exchange could be altered by changing the soil texture (i.e., soil bulk density) and soil surface temperature (mean soil temperature at a depth of 5 cm was 1.29 °C higher in CF than in BF).

5. Conclusions

The soil profile CO₂ concentration appeared to be strongly affected by environmental factors (temperature and precipitation) and soil properties (SOC, TN, soil bulk density and microbial communities) in the current study. The surface CO₂ fluxes rates remained relatively stable when the CO₂ concentration in soil profile was increased to a significant extent. These results increased our understanding of the factors influencing CO₂ concentration in forest soil profile and the relationship of soil profile CO₂ with soil surface CO₂ flux. We concluded that the interaction of soil properties and environmental factors controlled the CO₂ production in the soil profile, but the soil surface CO₂ emission could be affected by the intensity of the disturbance or soil temperature variation. Although all CO₂ produced in the soil would be eventually emitted to the atmosphere through soil surface efflux on a long-term basis, CO₂ stored in the subsoil may be relatively stable in the deeper soil layers.

Author Contributions: Data curation, X.W. and Q.T.; formal analysis, X.W.; funding acquisition, J.L. and H.X.; investigation, X.W., Y.L. and Q.T.; project administration, S.F. and L.Z.; supervision, S.F.; writing—original draft, X.W.; writing—review and editing, X.Z. and W.Z.

Funding: This research was funded by the National Natural Science Foundation of China, grant numbers 41501268 and 30771704; and GDAS' Project of Science and Technology Development, grant number 2019GDASYL-0103060 and 2018GDASCX-0107.

Acknowledgments: We thank Dima Chen for advice on the content and Xingquan Rao and Heshan Station for providing meteorological data. We thank Yanmei Xiong for improving the English of this manuscript. We are grateful to the editors and the three anonymous reviewers for helpful comments on the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

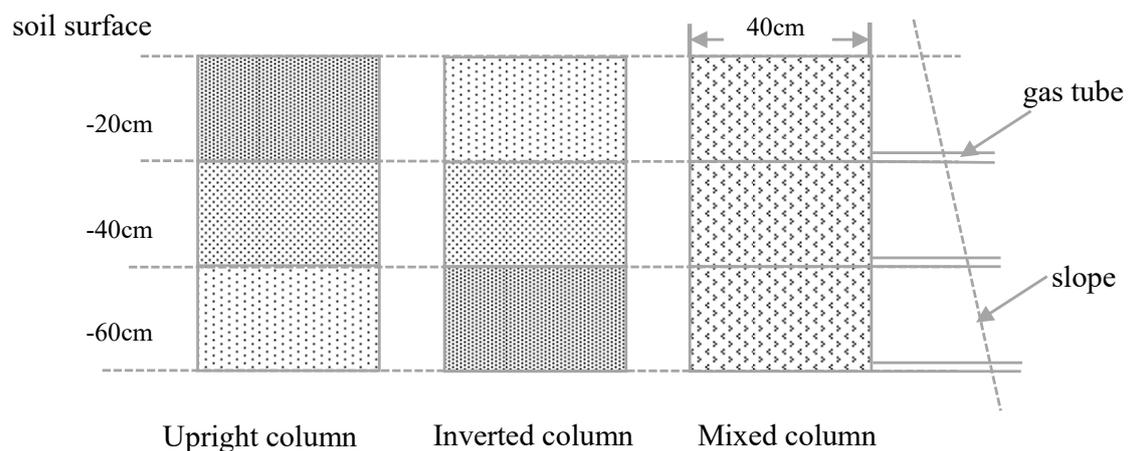


Figure A1. Diagram depicting soil column manipulation for field measurements of CO₂.

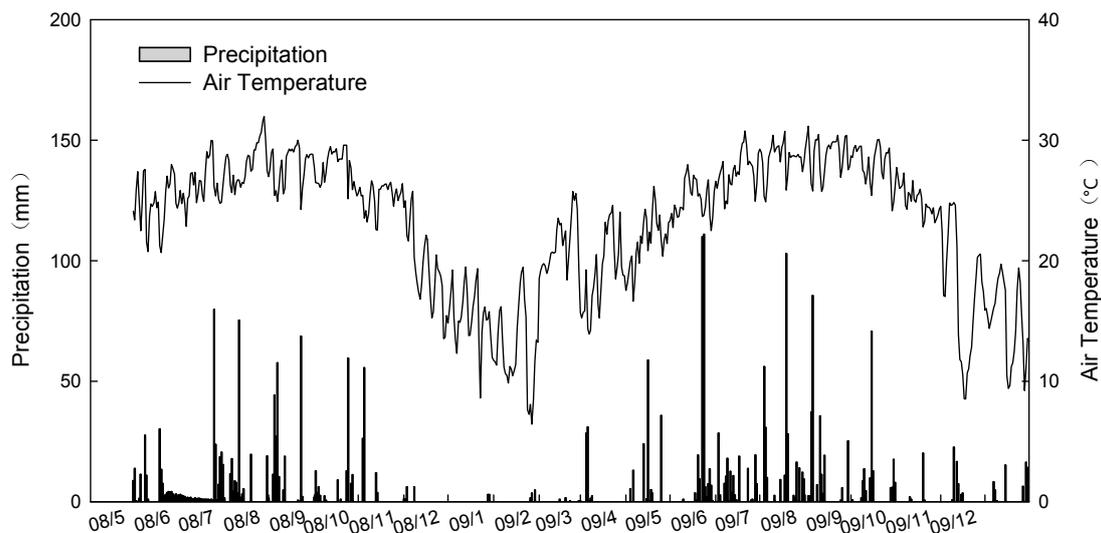


Figure A2. Precipitation and air temperature in Heshan station during the study period.

References

- Houghton, J.T.; Meira-Filho, L.G.; Callander, B.A.; Harris, N. *Climate Change 1995: The Science of Climate Change*; Kathenberg, N., Maskell, K., Eds.; Cambridge University Press: New York, NY, USA, 1996.
- Stocker, T.F.; Qin, D.; Plattner, G.K.; Tignor, M.; Allen, S.K.; Boschung, J.; Nauels, A.; Xia, Y. *Climate Change: The Physical Science Basis*; Bex, V., Midgley, P.M., Eds.; Cambridge University Press: New York, NY, USA, 2013.
- Grüning, M.M.; Germeshausen, F.; Thies, C.; L.-M.-Arnold, A. Increased forest soil CO₂ and N₂O emissions during insect infestation. *Forests* **2018**, *9*, 612. [[CrossRef](#)]
- Lee, S.; Yim, J.; Son, Y.; Son, Y.; Kim, R. Estimation of forest carbon stocks for national greenhouse gas inventory reporting in south Korea. *Forests* **2018**, *9*, 625. [[CrossRef](#)]
- Schlesinger, W.H.; Andrews, J.A. Soil respiration and the global carbon cycle. *Biogeochemistry* **2000**, *48*, 7–20. [[CrossRef](#)]
- Valentini, R.; Matteucci, G.; Dolman, A.J.; Schulze, E.D.; Rebmann, C.; Moors, E.J.; Granier, A.; Gross, P.; Jensen, N.O.; Pilegaard, K.; et al. Respiration as the main determinant of carbon balance in European forests. *Nature* **2000**, *404*, 861–865. [[CrossRef](#)] [[PubMed](#)]
- Raich, J.W.; Schlesinger, W.H. The global carbon-dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus B* **1992**, *44*, 81–99. [[CrossRef](#)]
- Maier, M.; Schack-Kirchner, H.; Hildebrand, E.E.; Schindler, D. Soil CO₂ efflux vs. soil respiration: Implications for flux models. *Agric. For. Meteorol.* **2011**, *151*, 1723–1730. [[CrossRef](#)]
- Pihlatie, M.; Pumpanen, J.; Rinne, J.; Ilvesniemi, H.; Simojoki, A.; Hari, P.; Vesala, T. Gas concentration driven fluxes of nitrous oxide and carbon dioxide in boreal forest soil. *Tellus B* **2007**, *59*, 458–469. [[CrossRef](#)]
- Wiaux, F.; Vanclooster, M.; Van-Oost, K. Vertical partitioning and controlling factors of gradient-based soil carbon dioxide fluxes in two contrasted soil profiles along a loamy hillslope. *Biogeosciences* **2015**, *12*, 4637–4649. [[CrossRef](#)]
- Tang, J.W.; Baldocchi, D.D.; Qi, Y.; Xu, L.K. Assessing soil CO₂ efflux using continuous measurements of CO₂ profiles in soils with small solid-state sensors. *Agric. For. Meteorol.* **2003**, *118*, 207–220. [[CrossRef](#)]
- Maier, M.; Schack-Kirchner, H. Reply to comment on “Using the gradient method to determine soil gas flux: A review”. *Agric. For. Meteorol.* **2014**, *197*, 256–257. [[CrossRef](#)]
- Luo, Y.Q.; Zhou, X.H. Processes of CO₂ transport from soil to the atmosphere. In *Soil Respiration and the Environment*; Elsevier Inc.: Amsterdam, The Netherlands, 2006; pp. 61–76.
- Hopkins, D.W.; Sparrow, A.D.; Shillam, L.L.; English, L.C.; Dennis, P.G.; Novis, P. Enzymatic activities and microbial communities in an antarctic dry valley soil: Responses to c and n supplementation. *Soil Biol. Biochem.* **2008**, *40*, 2130–2136. [[CrossRef](#)]
- Risk, D.; Kellman, L.; Beltrami, H. Carbon dioxide in soil profiles: Production and temperature dependence. *Geophys. Res. Lett.* **2002**, *29*, 111–114. [[CrossRef](#)]

16. Jassal, R.; Black, A.; Novak, M.; Morgenstern, K.; Nestic, Z.; Gaumont-Guay, D. Relationship between soil CO₂ concentrations and forest-floor CO₂ effluxes. *Agric. For. Meteorol.* **2005**, *130*, 176–192. [[CrossRef](#)]
17. Albanito, F.; Saunders, M.; Jones, M.B. Automated diffusion chambers to monitor diurnal and seasonal dynamics of the soil CO₂ concentration profile. *Eur. J. Soil Sci.* **2009**, *60*, 507–514. [[CrossRef](#)]
18. Billings, S.A.; Richter, D.D.; Yarie, J. Soil carbon dioxide fluxes and profile concentrations in two boreal forests. *Can. J. For. Res.* **1998**, *28*, 1773–1783. [[CrossRef](#)]
19. Xu, M.; Qi, Y. Soil-surface CO₂ efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. *Glob. Chang. Biol.* **2001**, *7*, 667–677. [[CrossRef](#)]
20. Zhang, Z.S.; Cao, C.G.; Guo, L.J.; Li, C.F. Emissions of CH₄ and CO₂ from paddy fields as affected by tillage practices and crop residues in central china. *Paddy Water Environ.* **2016**, *14*, 85–92. [[CrossRef](#)]
21. Li, C.F.; Kou, Z.K.; Yang, J.H.; Cai, M.L.; Wang, J.P.; Cao, C.G. Soil CO₂ fluxes from direct seeding rice fields under two tillage practices in central China. *Atmos. Environ.* **2010**, *44*, 2696–2704. [[CrossRef](#)]
22. FAO. *World Reference Base for Soil Resources 2006*; World Soil Resources Report; FAO: Rome, Italy, 2006; p. 103.
23. Wang, Y.S.; Wang, Y.H. Quick measurement of CH₄, CO₂ and N₂O emissions from a short-plant ecosystem. *Adv. Atmos. Sci.* **2003**, *20*, 842–844.
24. Larionova, A.A.; Rozonova, L.N.; Samoylov, T.I. Dynamics of gas exchange in the profile of a gray forest soil. *Sov. Soil Sci.* **1989**, *24*, 1359–1372.
25. Jian, J.S.; Steele, M.K.; Day, S.D.; Quinn, T.R.; Hodges, S.C. Measurement strategies to account for soil respiration temporal heterogeneity across diverse regions. *Soil Biol. Biochem.* **2018**, *125*, 167–177. [[CrossRef](#)]
26. Zhou, C.Y.; Zhou, G.Y.; Zhang, D.Q.; Wang, Y.H.; Liu, S.Z. CO₂ efflux from different forest soils and impact factors in Dinghu Mountain, China. *Sci. China Ser. D* **2005**, *48*, 198–206.
27. Liu, G. *Analysis of Soil Physical and Chemical Properties and Description of Soil Profiles*; China Standard: Beijing, China, 1996.
28. Bossio, D.A.; Scow, K.M. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. *Microb. Ecol.* **1998**, *35*, 265–278. [[CrossRef](#)] [[PubMed](#)]
29. Frostegard, A.; Baath, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fert. Soils* **1996**, *22*, 59–65. [[CrossRef](#)]
30. Baath, E.; Anderson, T.H. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biol. Biochem.* **2003**, *35*, 955–963. [[CrossRef](#)]
31. Massman, W.J.; Sommerfeld, R.A.; Mosier, A.R.; Zeller, K.F.; Hehn, T.J.; Rochelle, S.G. A model investigation of turbulence-driven pressure-pumping effects on the rate of diffusion of CO₂, N₂O and CH₄ through layered snow packs. *J. Geophys. Res.-Atmos.* **1997**, *102*, 18851–18863. [[CrossRef](#)]
32. Bowling, D.R.; Massman, W.J. Persistent wind-induced enhancement of diffusive CO₂ transport in a mountain forest snowpack. *J. Geophys. Res.-Biogeo.* **2011**, *116*, 352–370. [[CrossRef](#)]

