

## Microbial phototrophic fixation of atmospheric CO<sub>2</sub> in China subtropical upland and paddy soils

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### Abstract

Autotrophic microorganisms, which can fix atmospheric CO<sub>2</sub> to synthesize organic carbon, are numerous and widespread in soils. However, the extent and the mechanism of CO<sub>2</sub> fixation in soils remain poorly understood. We incubated five upland and five paddy soils from subtropical China in an enclosed, continuously <sup>14</sup>CO<sub>2</sub>-labeled, atmosphere and measured <sup>14</sup>CO<sub>2</sub> incorporated into soil organic matter (SOC<sup>14</sup>) and microbial biomass (MBC<sup>14</sup>) after 110 days. The five upland soils supported dominant crops (maize, wheat, sweet potato, and rapeseed) in the region, while all paddy soils were cultivated in a regime consisting of permanently-flooded double-cropping rice cultivation. The upland and paddy soils represented typical soil types (fluvisols and ultisols) and three landforms (upland, hill, and low mountain), ranging in total carbon from low (<10 g kg<sup>-1</sup> soil organic carbon) to medium (10–20 g kg<sup>-1</sup>) to high (>20 g kg<sup>-1</sup>). Substantial amounts of <sup>14</sup>CO<sub>2</sub> were fixed into SOC<sup>14</sup> (mean 20.1 ± 7.1 mg C kg<sup>-1</sup> in upland soil, 121.1 ± 6.4 mg C kg<sup>-1</sup> in paddy soil) in illuminated soils (12 h light/12 h dark), whereas no <sup>14</sup>C was fixed in soils incubated in continuous darkness. We concluded that the microbial CO<sub>2</sub> fixation was almost entirely phototrophic rather than chemotrophic. The rate of SOC<sup>14</sup> synthesis was significantly higher in paddy soils than in upland soils. The SOC<sup>14</sup> comprised means of 0.15 ± 0.01% (upland) and 0.65 ± 0.03% (paddy) of SOC. The extent of <sup>14</sup>C immobilized as MBC<sup>14</sup> and that present as dissolved organic C (DOC<sup>14</sup>) differed between soil types, accounting for 15.69–38.76% and 5.54–18.37% in upland soils and 15.57–40.03% and 3.67–7.17% of SOC<sup>14</sup> in paddy soils, respectively. The MBC<sup>14</sup>/MBC and DOC<sup>14</sup>/DOC were 1.76–5.70% and 1.69–5.17% in the upland soils and 4.23–28.73% and 5.65–14.30% in the paddy soils, respectively. Thus, the newly-incorporated C stimulated the dynamics of DOC and MBC more than the dynamics of SOC. The SOC<sup>14</sup> and MBC<sup>14</sup> concentrations were highly significantly correlated ( $r = 0.946$ ;  $P < 0.0001$ ). We conclude that CO<sub>2</sub> uptake by phototrophic soil microorganisms can contribute significantly to carbon assimilation in soil, and so warrants further future study.

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### 1. INTRODUCTION

Increasing concentrations of atmospheric CO<sub>2</sub>, primarily due to the large consumption of fossil fuels since the beginning of the industrial revolution (IPCC, 2007), are believed to be closely associated with global warming. Both terrestrial and marine ecosystems are major sinks of this

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CO<sub>2</sub>, leading to much interest in carbon (C) cycling in terrestrial ecosystems and its potential to mitigate atmospheric CO<sub>2</sub> concentrations through C sequestration (Lal, 2004; Hill et al., 2006).

The global C cycle in terrestrial ecosystems involves two primary processes: the photosynthetic fixation of atmospheric CO<sub>2</sub> and the mineralization of biosynthesized organic materials through plant and microbe respiration in soil and wetland ecosystems. Taking the estimation of the global C budget by IPCC Fourth Assessment Report, the total C emitted into the atmosphere through fossil fuel combustion, deforestation, land-use changes and soil cultivation was at an annual approximate rate of 8.0 Pg C (IPCC, 2007). Of this, 27.5% was fixed by the ocean, 40% remained in the atmosphere, and the remaining 32.5% (about 2.6 Pg C yr<sup>-1</sup>) was trapped by terrestrial ecosystems (Grace, 2004; Lal, 2008). However, globally, the annual C fixation rate by plant photosynthesis (120 Pg C yr<sup>-1</sup>, annual gross (terrestrial) primary production) is almost equivalent to biosphere respiration (IPCC, 2007; Lal, 2008). This leaves open the question of how terrestrial ecosystems can trap the 40% of the global total CO<sub>2</sub> emitted into the atmosphere.

Autotrophic microorganisms contribute a substantial part to the photosynthesis of atmospheric CO<sub>2</sub> in aquatic ecosystems such as oceans and wetlands (Stanley et al., 2003; Koblížek et al., 2006; Savage et al., 2010). For example, Cannon et al. (2001) showed that oceanic cyanobacteria and chemoautotrophs were responsible for about 40% of annual CO<sub>2</sub> fixation. Stanley et al. (2003) estimated that algae accounted for 4–10% of total C fixation in wetlands. Autotrophic bacteria, green and blue algae with the capability to assimilate CO<sub>2</sub> via the Calvin cycle, are widespread in terrestrial ecosystems. Nanba et al. (2004) even discovered facultative autotrophic microorganisms, including aerobic CO<sub>2</sub>-oxidizing bacteria such as *Bradyrhizobium japonicum*, *Sinorhizobium meliloti*, and colorless and purple sulfide-oxidizing microbe such as *Chromatium vinosum*, in volcanic soils. Similarly, large populations of previously unidentified autotrophic microorganisms (e.g. *Oligotropha carboxidovorans*, *B. japonicum*, *Burkholderia xenovorans*, and *Nitrospira* sp.) were also identified in agricultural and forest soils (Slesi et al., 2005; Tolli and King, 2005). The *cbbL* gene, which encodes the large subunit of form I RubisCO, has been used as a functional marker for molecular ecological research into organisms which assimilate CO<sub>2</sub> in ecosystems (Paul et al., 2000). Our recent study showed that the dominant *cbbL*-containing bacteria were *Azospirillum lipoferum*, *Rhodospseudomonas palustris*, *B. japonicum*, *Ralstonia eutropha*, and *cbbL*-containing chromophytic algae of the genera *Xanthophyta* and *Bacillariophyta* in paddy and upland soils (Yuan et al., 2012). However, whether the autotrophic microorganisms in soil can contribute significantly to terrestrial CO<sub>2</sub> fixation is not yet known.

Solar radiation is the most important energy source to ecosystems on Earth. Algae, microphytes, macrophytes, and anoxygenic photosynthetic bacteria all use energy from sunlight (Harada et al., 2005). However, the effects of phototrophic microorganisms on soil microbial assimilation of

atmospheric CO<sub>2</sub> in agricultural soils have never been studied, as far as we know. Paddy soil, which is different from upland soil because it is regularly flooded and intermittently irrigated, makes up the largest anthropogenic wetlands on Earth (Kögel-Knabner et al., 2010). China is the world's largest rice producer, accounting for 30% of total world production (<http://beta.irri.org/statistics>). Rice paddy soils, which represent 80% of the rice fields in China, have been recognized as an important C sink (Lal, 2002).

We designed a microcosm experiment to test the capacity of soil microorganisms in Chinese paddy and upland soils to assimilate atmospheric CO<sub>2</sub>. We incubated five paddy soils and five upland soils in a continuously <sup>14</sup>CO<sub>2</sub>-enriched atmosphere under either continuous darkness or a 12 h:12 h light:dark cycle for 110 days. We had three aims: (1) to determine how much <sup>14</sup>CO<sub>2</sub> was incorporated into soil organic matter (SOC<sup>14</sup>) and what proportions were in active pools as microbial biomass carbon (MBC<sup>14</sup>) and dissolved organic carbon (DOC<sup>14</sup>); (2) to investigate whether there were significant differences between paddy and upland soils in microbial CO<sub>2</sub> fixation rates; and (3) to determine whether the autotrophs in paddy and upland soils were primarily phototrophs or chemotrophs.

## 2. METHODS

### 2.1. Soil preparation

Five upland soils (U1 to U5) and five rice paddy soils (P1 to P5) were sampled in the Hunan Province, in the subtropical region of China (28°08'–29°56'N 111°03'–113°31'E) (Table 1). The five upland soils represented the dominant crop soils (maize, wheat, sweet potato, and rapeseed) in the region, while all paddy soils were under permanently-flooded double-cropping rice cultivation. The upland and paddy soils represented typical soil types (fluvisols and ultisols) and three landforms (plain, hill, and low mountain), and they ranged in total carbon from low (<10 g kg<sup>-1</sup> soil organic carbon) to medium (10–20 g kg<sup>-1</sup>) to high (>20 g kg<sup>-1</sup>). All soils were sampled in November, 2010, (the dry season) after the final crop harvest, to minimize the effects of direct fertilization. The soil sampling sites each had a mean annual temperature of approximately 17 °C and an annual rainfall of about 1400 mm. A bulk sample of each soil was collected from the Ap horizon (0–20 cm depth) to include both photosynthetic (primarily surface) organisms as well as chemotrophs. Soils were hand-sorted to remove visible plant residues, then sieved (<5 mm mesh). Prior to use, all upland soils were uniformly adjusted to about 45% water-holding capacity (WHC) (Priha and Smolander, 1999), and the paddy soils were flooded with distilled water. All soils were equilibrated for 2 weeks prior to analysis to permit microbial activity to stabilize following the disturbance (Butterly et al., 2011).

### 2.2. Experimental design

Two sets of microcosms of the 10 soils, each with four replicates, were prepared by weighing 1.0 kg (on an oven-dried basis) of fresh soil into plastic tubes (10 cm diame-

Table 1  
Characteristics of the upland and paddy soils.

<sup>a</sup> Soils	Location	Landform	Soil type	Crop	pH	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	CEC (cmol kg <sup>-1</sup> )	Clay content (%)	WHC (%)
U1	E113°03'; N28°12'	Low mountain	Fluvisol	Maize	5.95 ± 0.04	11.1 ± 0.28	1.6 ± 0.01	12.1 ± 0.41	20.9 ± 0.56	18 ± 1
U2	E112°41'; N28°14'	Low mountain	Fluvisol	Wheat	7.15 ± 0	8.7 ± 0.13	1.2 ± 0.00	20.5 ± 0.13	36.3 ± 0.99	17 ± 1
U3	E113°05'; N28°12'	Hill	Ultisol	Wheat	4.40 ± 0.03	6.2 ± 0.04	1.4 ± 0.00	11.1 ± 0.10	31.4 ± 0.55	39 ± 2
U4	E111°31'; N29°13'	Plain	Ultisol	Sweet potato	7.38 ± 0	20.6 ± 0.65	2.2 ± 0.01	13.6 ± 0.10	17.4 ± 0.18	26 ± 1
U5	E112°28'; N29°19'	Plain	Ultisol	Rapeseed	6.60 ± 0.01	18.7 ± 0.10	2.6 ± 0.02	13.7 ± 0.10	26.1 ± 1.84	43 ± 3
P1	E113°16'; N28°08'	Hill	Fluvisol	Double-crop rice	5.09 ± 0	17.0 ± 0.16	2.5 ± 0.03	5.6 ± 0.05	22.1 ± 0.55	69 ± 1
P2	E113°11'; N28°08'	Plain	Ultisol	Double-crop rice	6.67 ± 0.01	17.4 ± 0.40	2.2 ± 0.01	15.8 ± 0.24	24.5 ± 0.39	30 ± 2
P3	E111°31'; N29°13'	Plain	Ultisol	Double-crop rice	5.66 ± 0.01	20.9 ± 0.72	2.8 ± 0.00	13.2 ± 0.23	33.2 ± 0.43	64 ± 2
P4	E111°31'; N29°13'	Hill	Fluvisol	Double-crop rice	6.09 ± 0	14.7 ± 0.35	2.5 ± 0.03	11.5 ± 1.17	44.9 ± 0.26	60 ± 3
P5	E112°11'; N29°56'	Low mountain	Fluvisol	Double-crop rice	6.15 ± 0.01	21.9 ± 1.03	2.6 ± 0.02	11.8 ± 0.32	39.8 ± 1.40	66 ± 2

Values are mean ± SD. SOC and CEC indicate soil organic carbon and cation exchange capacity, respectively.

<sup>a</sup> U1, U2, U3, U4, and U5 were upland soils; P1, P2, P3, P4, and P5 were paddy soils.

ter × 22 cm height). One set, the control, was covered with 0.7 cm of dark, porous, low-density polymeric foams (MF-ST-48i, Jiangsu, China; pore size: 200 μm, gas diffusivity: 1.05 × 10<sup>4</sup> g d<sup>-1</sup> m<sup>-2</sup>, density: 1.8 kg m<sup>-3</sup>) to stop light but permit gas exchange. The other set was not covered, to permit illumination. Both sets of microcosms were then placed in a growth chamber (80 × 250 × 120 cm high). During the incubation period, the air temperature in the chamber was maintained at 22 °C between 8:00 pm and 8:00 am, and at 28–32 °C between 8:00 am and 8:00 pm, while the relative humidity was maintained at 80–90% throughout. Plant growth lamps (CSS-ZWD-600, Hangzhou, China) acted as artificial light (about 500 mmol photons m<sup>-2</sup> s<sup>-1</sup> PAR) between 8:00 am and 8:00 pm every day. The <sup>14</sup>CO<sub>2</sub> was generated from Na<sub>2</sub><sup>14</sup>CO<sub>3</sub> (1.0 M, specific activity 1.68 × 10<sup>4</sup> Bq μg<sup>-1</sup> C) and HCl (2 M) and maintained at approximately 350 μl l<sup>-1</sup> during the incubation by adjusting with Na<sub>2</sub><sup>14</sup>CO<sub>3</sub> weekly. We used an air release valve (including tubing and a set of 4 × 250 ml flasks, each containing 100 ml of 2 M NaOH) to remove <sup>14</sup>CO<sub>2</sub> from the growth chamber when the CO<sub>2</sub> concentration exceeded 350 μl l<sup>-1</sup>. The concentration of CO<sub>2</sub> in the growth chamber was monitored by infrared CO<sub>2</sub> sensor (GasCard NG, 6132A, Guangzhou, China). Full details are given by Ge et al. (2012). The paddy soils were permanently-flooded, while the upland soils were maintained at 45% WHC by the addition of sterile distilled water as required.

Soils were incubated for 110 days to simulate post-harvest fallow fields, in which plant-derived carbon inputs cease for approximately three months each year. Upon completion of the 110 days incubation, the surface water

was removed from the paddy soils. Each soil was then mixed thoroughly then divided into two portions. One portion was oven-dried at 70 °C to a constant weight to determine the amount of SOC<sup>14</sup> fixed from <sup>14</sup>CO<sub>2</sub>, and the other was stored at 4 °C to determine MBC<sup>14</sup> and DOC<sup>14</sup>. Soil moisture was also determined at harvest and was nearly identical to the soil water content at the beginning of the experiment (data not shown).

### 2.3. Analytical methods

Total SOC and total N both before and after incubation were determined by dry combustion using an elemental analyzer (Elementar Vario MAX C/N, Hanau, Germany). Soil pH was determined in 1:2.5 (w/v) soil:H<sub>2</sub>O extracts. Soil clay content was determined using the pipette method (Müller and Höper, 2004).

The SOC<sup>14</sup> was measured according to Wu and O'Donnell (1997). Briefly, 1.50 g of air-dried soil (<150 μm) was added to 20 ml 0.2 M potassium dichromate and 30 ml of a mixture of concentrated H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> (5:1 v:v) in a double-necked round-bottom flask. This mixture was digested at 165 °C for 8 min under pure O<sub>2</sub> that was continually replenished during the digestion and for 10 min thereafter. The evolved CO<sub>2</sub> was trapped in 40 ml 0.4 M NaOH. The NaOH (1 ml) was then mixed with 9 ml of RIA cocktail (Beckman Coulter, Brea, CA, USA) and <sup>14</sup>C was measured in an automated liquid scintillation counter (LS-6500, Beckman) for 5 min. The amount of SOC<sup>14</sup> (mg C kg<sup>-1</sup> soil) fixed by each soil was calculated following published procedures (Xiao et al., 2007; Ge et al., 2012):

$$\text{SOC}^{14} = F_1 R_s / R_p W \quad (1)$$

where  $F_1$  represents the factor to convert the counting volume (1 ml from 40 ml plus soil water volume in ml),  $R_s$  and  $R_p$ , radioactivity ( $\text{Bq l}^{-1}$ ; blank counts omitted) for a trap solution and  $\text{Na}_2^{14}\text{CO}_3$  ( $\text{Bq mg}^{-1} \text{C l}^{-1}$ ) used to produce  $^{14}\text{C}$ - $\text{CO}_2$  in the growth chamber,  $W$ , the weight (kg) of soil on an oven-dry basis, respectively.

To determine  $\text{MBC}^{14}$ , moist soil portions (20 g) were fumigated according to Wu et al. (1990), followed by extraction with 80 ml 0.5 M  $\text{K}_2\text{SO}_4$ . The  $^{14}\text{C}$ -radioactivity in the extractant (1 ml), together with that (1 ml) extracted from equivalent unfumigated portions, were determined as above. The soil  $\text{DOC}^{14}$  concentrations were determined in the non-fumigated soils (Ge et al., 2012).

The amounts of  $\text{DOC}^{14}$  ( $\text{mg C kg}^{-1}$  soil) and  $\text{MBC}^{14}$  ( $\text{mg C g}^{-1}$  soil) were calculated from:

$$\text{DOC}^{14} = F_2 R_{uf} / R_p W \quad (2)$$

$$\text{MBC}^{14} = F_3 (R_f - R_{uf}) / R_p W k_c \quad (3)$$

where  $F_2$  and  $F_3$  represent the factor to convert the counting volume (from 1 ml to the volume of 80 ml plus soil water volume in ml);  $R_f$  and  $R_{uf}$ , radioactivity ( $\text{Bq l}^{-1}$ ; blank counts subtracted for the extractants of the fumigated soil and unfumigated soil, respectively);  $R_p$ , radioactivity of  $\text{Na}_2^{14}\text{CO}_3$  ( $\text{Bq mg}^{-1} \text{C ml}^{-1}$ ) used to produce  $^{14}\text{C}$ - $\text{CO}_2$  in the growth chamber;  $W$ , the weight (kg) of soil on an oven-dry basis, and  $k_c$ , the factor (0.45) converting measured  $^{14}\text{C}$  into biomass  $^{14}\text{C}$  (Wu et al., 1990).

#### 2.4. Statistics

Data were processed using Excel 2000 (Microsoft, Redmond, WA, USA) to calculate the means and the standard errors. Multiple comparisons of significant differences were made using a one-way ANOVA followed by a Tukey test ( $P < 0.05$ ). Correlation analyses were done using the Pearson correlation method with significance defined at  $P < 0.05$ , unless otherwise stated. Statistical analyses were performed using SPSS 13.0 software for Windows XP (IBM, Armonk, NY, USA).

### 3. RESULTS

#### 3.1. Soil organic C derived from $^{14}\text{CO}_2$

After 110 days of incubation,  $^{14}\text{C}$  in the soils incubated in darkness was undetectable. In the soils incubated under a light/dark regime, the  $\text{SOC}^{14}$  ranged from 10.63–47.94  $\text{mg kg}^{-1}$  in upland soils and 104.95–133.81  $\text{mg kg}^{-1}$  in paddy soils (Fig. 1A), accounting for 0.09–0.25% and 0.57–0.82% of total soil organic C, respectively (Table 2). The largest concentration of  $\text{SOC}^{14}$  was measured in P5 (133.81  $\text{mg kg}^{-1}$ ) and the smallest in U1 (10.63  $\text{mg kg}^{-1}$ ). The mean concentrations of  $\text{SOC}^{14}$  in the five upland soils ( $20.08 \pm 15.83 \text{ mg kg}^{-1}$ ,  $\text{CV} = 79\%$ ) were significantly ( $P < 0.01$ ) lower than in the paddy soils ( $121.11 \pm 14.31 \text{ mg kg}^{-1}$ ,  $\text{CV} = 12\%$ ).

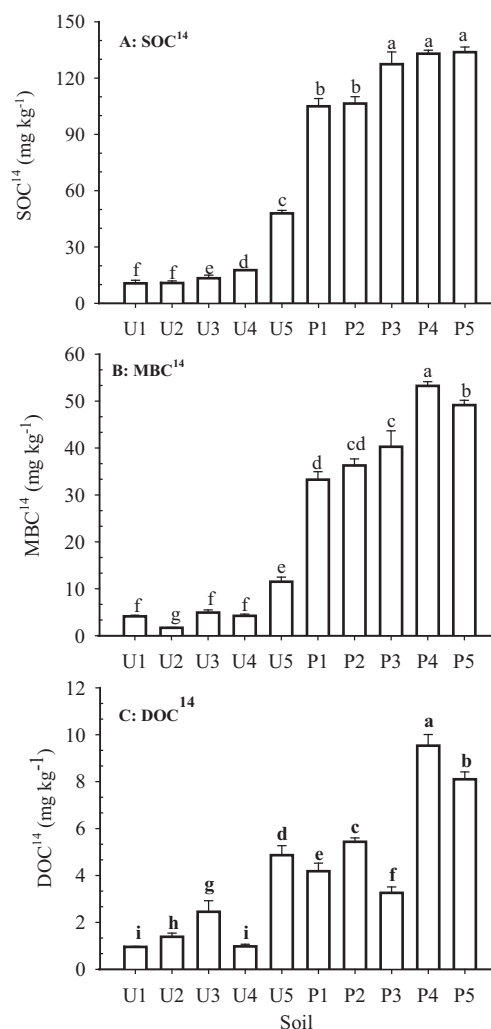


Fig. 1. The concentrations of  $\text{SOC}^{14}$  (A),  $\text{MBC}^{14}$  (B), and  $\text{DOC}^{14}$  (C) in soils incubated in a growth chamber with  $^{14}\text{CO}_2$  for 110 days. Error bars indicate the standard error of the mean ( $n = 4$ ) and means with the same letter are not significantly different ( $P > 0.05$ ).

#### 3.2. Contribution of soil microbial C fixation to the microbial biomass and dissolved organic C dynamics

The concentrations of  $\text{MBC}^{14}$  in soils ranged from 1.70 (U2) to 11.51 (U5)  $\text{mg kg}^{-1}$  in upland soils and from 33.26 (P1) to 53.24 (P4)  $\text{mg kg}^{-1}$  in paddy soils, and  $\text{DOC}^{14}$  varied from 0.96 (U1) to 4.87 (U5)  $\text{mg kg}^{-1}$  in the upland soils and from 3.26 (P3) to 9.53 (P4)  $\text{mg kg}^{-1}$  in paddy soils (Fig. 1B and C). Both  $\text{MBC}^{14}$  and  $\text{DOC}^{14}$  concentrations differed significantly between soils. The  $\text{MBC}^{14}$  and  $\text{DOC}^{14}$  concentrations in paddy soils ( $42.4 \pm 8.5$  and  $6.1 \pm 2.6 \text{ mg kg}^{-1}$ , respectively) were significantly higher ( $P < 0.05$ ) than in upland soils ( $5.3 \pm 3.7$  and  $2.1 \pm 1.6 \text{ mg kg}^{-1}$ , respectively).

The  $\text{MBC}^{14}$  as a proportion of  $\text{SOC}^{14}$  ( $\text{MBC}^{14}/\text{SOC}^{14}$ ) ranged from 15.69% (U2) to 36.90% (U3) in upland soils and from 15.57% (P2) to 40.03% (P4) in paddy soils after 110 days (Table 2). The proportion of  $\text{MBC}^{14}/\text{SOC}^{14}$  in paddy soils (mean =  $30.45 \pm 1.63\%$ ) was significantly greater

Table 2

Distribution of  $^{14}\text{C}$  in SOC pools from ten contrasting cropping soils during 110 days of continuous  $^{14}\text{CO}_2$  labeling and incubation of soils under 12 h day/12 h night.

Soils	$^{14}\text{C}$ -SOC/SOC (%)	$^{14}\text{C}$ -MBC/ $^{14}\text{C}$ -SOC (%)	$^{14}\text{C}$ -DOC/ $^{14}\text{C}$ -SOC (%)	$^{14}\text{C}$ -MBC/MBC (%)	$^{14}\text{C}$ -DOC/DOC (%)
U1	0.12 ± 0.01 <sup>b</sup>	38.76 ± 1.16 <sup>a</sup>	8.99 ± 0.21 <sup>c</sup>	5.70 ± 0.44 <sup>a</sup>	5.07 ± 0.08 <sup>a</sup>
U2	0.09 ± 0.01 <sup>c</sup>	15.69 ± 0.05 <sup>d</sup>	12.85 ± 0.84 <sup>b</sup>	2.51 ± 0.15 <sup>d</sup>	5.10 ± 0.37 <sup>a</sup>
U3	0.21 ± 0.02 <sup>a</sup>	36.90 ± 2.34 <sup>b</sup>	18.37 ± 1.27 <sup>a</sup>	4.42 ± 0.14 <sup>b</sup>	2.92 ± 0.66 <sup>b</sup>
U4	0.09 ± 0.0 <sup>c</sup>	23.97 ± 1.50 <sup>c</sup>	5.54 ± 0.33 <sup>d</sup>	1.76 ± 0.20 <sup>e</sup>	1.69 ± 0.24 <sup>c</sup>
U5	0.25 ± 0.01 <sup>a</sup>	24.01 ± 0.63 <sup>c</sup>	10.15 ± 0.86 <sup>c</sup>	3.58 ± 0.39 <sup>c</sup>	5.17 ± 0.78 <sup>a</sup>
Means	0.15 ± 0.03	27.87 ± 2.07	11.18 ± 1.30	3.59 ± 0.59	3.99 ± 0.82
P1	0.58 ± 0.02 <sup>c</sup>	31.69 ± 1.41 <sup>c</sup>	3.98 ± 0.18 <sup>c</sup>	5.39 ± 0.45 <sup>d</sup>	7.70 ± 0.64 <sup>b</sup>
P2	0.57 ± 0.02 <sup>c</sup>	15.57 ± 0.38 <sup>d</sup>	5.11 ± 0.25 <sup>b</sup>	28.73 ± 1.17 <sup>a</sup>	13.43 ± 0.36 <sup>a</sup>
P3	0.64 ± 0.01 <sup>b</sup>	28.24 ± 2.54 <sup>c</sup>	3.67 ± 0.18 <sup>c</sup>	4.23 ± 0.57 <sup>d</sup>	5.65 ± 0.45 <sup>c</sup>
P4	0.82 ± 0.04 <sup>a</sup>	40.03 ± 0.47 <sup>a</sup>	7.17 ± 0.61 <sup>a</sup>	16.94 ± 0.41 <sup>b</sup>	13.38 ± 0.77 <sup>a</sup>
P5	0.64 ± 0.02 <sup>b</sup>	36.74 ± 3.37 <sup>b</sup>	6.05 ± 0.39 <sup>b</sup>	7.98 ± 0.22 <sup>c</sup>	14.30 ± 0.68 <sup>a</sup>
Means	0.65 ± 0.08	30.45 ± 2.63	5.20 ± 0.42	12.65 ± 4.67	10.89 ± 1.84

Values are means ± SD. Different lower case letters indicate significant differences among upland soils and among paddy soils at the 0.05 level.

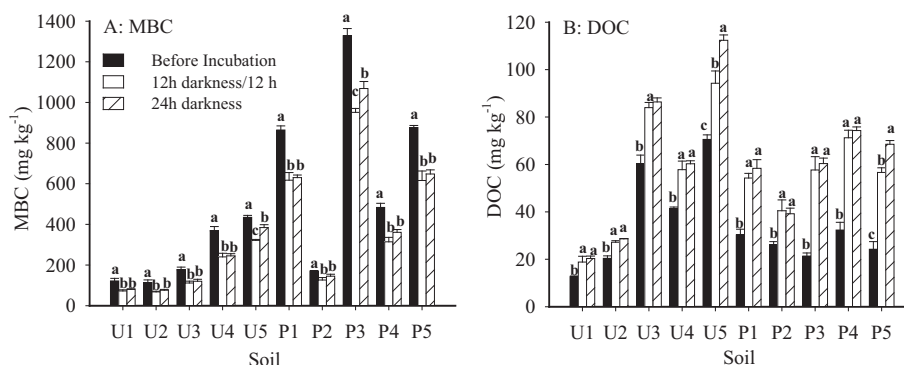


Fig. 2. The amounts of MBC (A) and DOC (B) in upland and paddy soils before (■) and after incubation with  $^{14}\text{CO}_2$  for 110 days in light (12 h:12 h) (□) and 24 h darkness (▨). Different lower case letters indicate significant differences among different treatments of the same soil type at the 0.05 level.

than in upland soils (mean = 27.87 ± 1.13%). Similarly, the  $\text{DOC}^{14}$  as a proportion of  $\text{SOC}^{14}$  ( $\text{DOC}^{14}/\text{SOC}^{14}$ ) ranged from 5.54% (U4) to 18.37% (U3) in upland soils and from 3.67% (P3) to 7.17% (P4) in paddy soils. The proportion of  $\text{DOC}^{14}/\text{SOC}^{14}$  in paddy soils (mean = 5.20 ± 0.32%) was significantly lower ( $P < 0.05$ ) than in upland soils (mean = 11.18 ± 0.70%). In addition,  $\text{MBC}^{14}$  as a percentage of total MBC ( $\text{MBC}^{14}/\text{MBC}$ ) ranged from 1.76% (U4) to 5.70% (U1) in the upland soils and from 4.23% (P3) to 28.73% (P2) in the paddy soils. The  $\text{DOC}^{14}$  as a percentage of total DOC ( $\text{DOC}^{14}/\text{DOC}$ ) ranged from 1.69% (U4) to 5.17% (U5) (mean 3.99%) in the upland soils and from 5.65% (P3) to 14.30% (P5) (mean 10.89%) in paddy soils. Furthermore, the values for  $\text{MBC}^{14}/\text{MBC}$  and  $\text{DOC}^{14}/\text{DOC}$  in the upland soils were much lower ( $P < 0.05$ ) than those in the paddy soils.

### 3.3. Changes in microbial biomass and dissolved organic C during incubation

The mean MBC in upland (162.54 mg kg<sup>-1</sup>) and paddy soils (525.07 mg kg<sup>-1</sup>) during the 110-day incubation period with illumination decreased to 66.9% and 70.6%, respectively, compared to that at the start of the experiment (average 242.86 mg kg<sup>-1</sup> in upland soil, 744.11 mg kg<sup>-1</sup> in paddy soil) and to 25.4% (181.19 mg kg<sup>-1</sup>) and 23.3% (570.64 mg kg<sup>-1</sup>), respectively in darkness (Fig. 2A). In contrast, DOC increased in both illuminated and dark soils (Fig. 2B). The DOC in illuminated soils was 1.37 (upland soils) and 2.08 (paddy soils) times higher after the 110-day incubation than at the start of the experiment and in the dark soils was 1.50 (upland soils) and 2.24 (paddy soils) times higher.



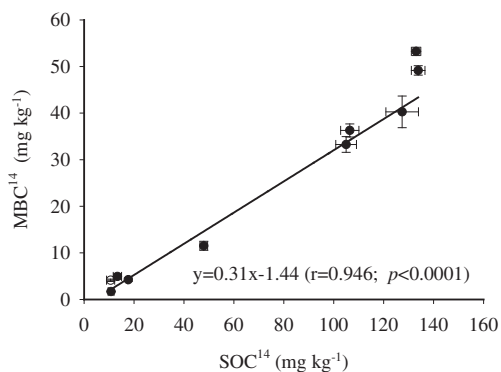


Fig. 3. Relationship between SOC<sup>14</sup> and MBC<sup>14</sup> from 5 upland and 5 paddy soils during 110 days <sup>14</sup>CO<sub>2</sub> continuous labeling incubation of soils for 110 days with 12 h darkness/12 h light. Points represent individual tested soil means. Bars represent the standard errors. For data on SOC<sup>14</sup>, MBC<sup>14</sup>, see Fig. 1.

### 3.4. Relationships between soil organic C<sup>14</sup> and microbial biomass C<sup>14</sup>

There was a significant positive linear relationship between the concentrations of SOC<sup>14</sup> and MBC<sup>14</sup> ( $r = 0.99$ ,  $P < 0.0001$ ) (Fig. 3). This relationship was independent of the differences in the soil management and other factors, such as soil organic C and total N concentrations and pH (data not shown).

## 4. DISCUSSION

### 4.1. Contributions of soil microbes to CO<sub>2</sub> fixation and dissolved organic C and microbial biomass C dynamics

The flooding water was removed before soil sampling and the DOC<sup>14</sup> and MBC<sup>14</sup> were not determined. Although previous studies on the aquatic ecosystems or wetland soils showed that cyanobacteria or algae were responsible (4–40%) for CO<sub>2</sub> fixation (Cannon et al., 2001; Stanley et al., 2003), in the present study, the water layer (sterile water) over the paddy soil was very thin (1–2 cm), oligotrophic, and unfavorable for the growth of cyanobacteria or algae. Indeed, none were observed. Therefore, the contribution of CO<sub>2</sub> fixation from flooding water microorganisms was considered to be negligible.

We measured labeled SOC as a percentage of total SOC (SOC<sup>14</sup>/SOC) to estimate how much <sup>14</sup>CO<sub>2</sub> was incorporated into soil organic matter. Percentages averaged 0.15% in upland soils and 0.65% in paddy soils. Ge et al. (2012) estimated values ranging from 0.14% to 0.38% in unplanted soils after 80 days of continuous labeling incubation; these values likely differed from ours due to differences in soil characteristics, labeling techniques, and experiment durations. Our range (0.15–0.65%) was much lower than the percentages measured in planted soils. In rice-soil and maize-soil systems, the proportions of plant-derived C during the course of a growing season varied from 0.73–1.99% and from 0–12.3% of total SOC, respectively (Liang et al., 2002; Ge et al., 2012). The maximum

proportion reported was around 20%, of which 64–86% was rapidly respired by soil microorganisms and only 2–5% was incorporated into SOC (Lynch and Whipps, 1990; Zagal, 1994; Hütsch et al., 2002). In wheat-soil and maize-soil systems, new SOC was 4.0% and 3.3%, respectively, of net assimilated C (Merckx et al., 1987; Kuzyakov and Schneckenberger, 2004), lower than the 0.15–0.65% measured in our study. This difference in soil C inputs among plant species is primarily attributable to the distinct characteristics of each plant as well as to different soil environments that control organic C decomposition.

Our new finding of C transformations that contribute significantly to labile soil C components, especially MBC and DOC, are closely related to CO<sub>2</sub> and CH<sub>4</sub> emissions (Liang et al., 2002; Amiotte-Suchet et al., 2007). About 8.13% of assimilated <sup>14</sup>C was recovered as MBC after 110 days (Table 2). The amounts of MBC<sup>14</sup> in our upland and paddy soils ranged from 1.70–11.51 mg kg<sup>-1</sup> and from 33.26–53.24 mg kg<sup>-1</sup> and accounted for 1.76–5.70% (mean 3.59%) and 4.23–28.73% (mean 12.65%) of the total MBC, respectively. This finding suggested that indigenous microbes assimilated C inputs in soil that affected MBC dynamics, as supported by previous studies (Lu et al., 2002; Ge et al., 2012). Rapid incorporation of <sup>14</sup>C into MBC proved that ‘new’ C derived from recent assimilates was readily used by microorganisms in paddy and upland soils.

The MBC<sup>14</sup> may have had three fates: a fraction released as CO<sub>2</sub> through respiration, another transformed to structural components of microorganisms, and a third entering the SOC as microbial metabolites because of microbial turnover. The ratios of MBC<sup>14</sup>/MBC and DOC<sup>14</sup>/DOC were much larger than those of SOC<sup>14</sup>/SOC in both paddy and upland soils. These values indicated that the newly-incorporated C stimulated the dynamics of DOC and MBC more than the dynamics of SOC. However, we have little information about the accumulation of the ‘new’ SOC derived from assimilation of CO<sub>2</sub> at the soil interface.

The MBC in both illuminated and dark soils decreased after 110 days of continuous labeling, compared with day 0. Ge et al. (2012) also found that MBC decreased in unplanted paddy soils. The decrease in MBC during the incubation suggested that anaerobic (flooded) conditions cause the death of soil microorganisms, presumably resulting in changes in the microbial community structure after soil flooding. However, in upland soil, the reasons for the decrease in MBC was more probably due to the lack of fresh input.

In contrast, DOC increased in both illuminated and dark soils ( $P < 0.05$ ). Because no organic fertilizer was applied to the soils in this experiment, the increase in DOC must have resulted from the release of soluble organic substrates and residues and their decomposition products. Also, the DOC<sup>14</sup> dynamics were largely consistent with the SOC<sup>14</sup> concentrations (the amounts of SOC<sup>14</sup> and DOC<sup>14</sup> incorporated were highly correlated ( $r = 0.819$ ;  $P < 0.05$ )), suggesting that the DOC was released from organic materials originally assimilated by microbes from atmospheric CO<sub>2</sub>. The contribution of microbial C to DOC<sup>14</sup> averaged 8.19% after 110 days. Previous

experiments showed that the contribution of the assimilated  $^{14}\text{C}$  to  $\text{CH}_4$  emitted into the atmosphere was 3–6% in a rice-soil (Dannenberg and Conrad, 1999), 0.1–0.5% in natural wetland soils (King and Reeburgh, 2002), and 1.76–7.68% in paddy soils (Ge et al., 2012). The contribution of assimilated C to DOC in our experiment appeared to be relatively high, indicating that DOC served as a reservoir for microbial cells and a supply of C for  $\text{CH}_4$  production in paddy soils. This discrepancy between our results and previous studies may be attributable to differences in experimental conditions, such as soil type and labeling periods (Saggar et al., 1997).

#### 4.2. Differences in microbial $\text{CO}_2$ fixation rates between paddy and upland soils

The need for a better understanding of the global C cycle has focused considerable attention on the microbial transformation of plant-derived carbon and on the role of the microbiota in the fixation and long-term sequestration of C. Although the importance of plant input chemistry to the long-term stability of soil organic matter has been questioned (Schmidt et al., 2011), it is generally accepted that microbial interactions with the soil physicochemical environment have a key role in determining structure/function relationships in soil (O'Donnell et al., 2007), including the long-term sequestration of soil carbon (Liang et al., 2002). However, the mechanisms by which microbial carbon, or microbially modified plant carbon, are incorporated into stabilized soil carbon remain poorly understood. Despite these unknowns there is a growing expectation that C sequestration in agricultural systems can be enhanced by management practices designed to conserve and build soil carbon. In the present study, the (newly synthesized)  $\text{SOC}^{14}$  and  $\text{MBC}^{14}$  concentrations were generally greater in paddy soils than in upland soils (Fig. 1). The statistically significant linear correlation between  $\text{MBC}^{14}$  and total  $\text{CO}_2$  fixation (measured as  $\text{SOC}^{14}$ ) indicated that the C utilized was derived from microbial fixation. More microbially-fixed C was measured in paddy soils, as indicated by  $\text{MBC}^{14}$  ( $42.4 \pm 8.5 \text{ mg kg}^{-1}$ ,  $\text{CV} = 20.0\%$ ,  $n = 5$ ), than in upland soils ( $5.3 \pm 3.7 \text{ mg kg}^{-1}$ ,  $\text{CV} = 69.8\%$ ,  $n = 5$ ). Furthermore, the anaerobic soil conditions in flooded paddy soils may substantially slow the decomposition of fresh (new) organic carbon and the mineralization rates of native SOC compared with aerobic conditions in upland soils (Guo and Lin, 2001).

#### 4.3. Phototrophic versus chemotrophic fixation of $\text{CO}_2$

Essentially no C fixation occurred in soils incubated in the dark, but phototrophic microbial  $\text{CO}_2$  fixation accounted for 0.09–0.82% ( $10.63\text{--}133.81 \text{ mg SOC}^{14} \text{ kg}^{-1}$ ) of the total SOC in the illuminated soils. In the illuminated soils, 15.57–40.03% (mean 29.16%) of the total  $\text{SOC}^{14}$  was present as  $\text{MBC}^{14}$ , indicating microbial fixation of  $^{14}\text{CO}_2$ . We therefore concluded that phototrophic microorganisms were the main contributors to soil microbial  $\text{CO}_2$  fixation. Miltner et al. (2005) discovered that non-photosynthetic soil microbes fixed amounts of  $\text{CO}_2$  corresponding to

0.05% of the total organic carbon after six weeks of incubation in a  $^{14}\text{C}$ -labeled  $\text{CO}_2$  atmosphere. They suggested that this non-phototrophic  $\text{CO}_2$  fixation was mainly driven by aerobic heterotrophic microorganisms. The differences between this study and that of Miltner et al. (2005) may be due to the extremely high ( $319 \text{ mg g}^{-1}$ ) SOC content in their soils and their addition of readily available substrates (such as acetic acid), both of which stimulated the growth of aerobic heterotrophic microorganisms. In our previous experiments, the activity of  $\text{CO}_2$ -assimilating microbes (including photo- and chemo-autotrophic microbes) was suppressed, and the microbial population and community structure declined substantially in the dark (Yuan et al., 2012). We did not detect any chemotrophically-generated  $^{14}\text{C}$  in any of our soils. Thus, from our results, indigenous phototrophs play an important role in  $\text{CO}_2$  fixation in soils while non-phototrophic  $\text{CO}_2$  fixation makes only a minor contribution.

In natural soils, phototrophic processes are restricted to the top few millimeters of the soil profile, although microbially-assimilated C may move from the soil surface to the subsoil. Our soils were separately mixed before the chemical analyses, so the newly-formed  $\text{SOC}^{14}$  at the surface would have been much diluted with bulk soil. Further work is clearly required to investigate which components of SOC are most affected by  $\text{CO}_2$  assimilation in soils. We are currently studying the spatial distribution and dynamics of atmospheric  $\text{CO}_2$  fixation by different soils as a function of the distance from the soil surface.

### 5. CONCLUSIONS AND IMPLICATIONS

We demonstrated that  $\text{CO}_2$  in upland and paddy soils was fixed almost exclusively by phototrophic microbes and that fixation rates were significantly higher in paddy soils. Clearly, an important limitation of our study, a bioassay, was that the measurements were made under ideal experimental conditions, which are seldom, or never, reached in the field. For example, the soils were incubated at 45% WHC soils and never allowed to dry, and the temperature and humidity regimes may not have represented actual field conditions, because they were carefully controlled in the laboratory to control for these effects. Further work is required to determine these measurements under natural field conditions.

Additionally, large variations in microbial  $\text{CO}_2$  assimilation rates were also observed across the tested soils in the present study, although the mechanism was not clear. Soil types, management practices, and environmental conditions could influence the activities and populations of soil microorganisms involved in C cycling process. Further studies on the abundance and composition of C-fixing microorganisms, their adaptations to soil habits and responses to external interference, and the functional ecological implications of their C fixation are clearly warranted.

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