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RESEARCH ARTICLE

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Effects of maize (*Zea mays* L.) growth and photosynthesis on $\delta^{13}C$ in soil respiration

Abstract As a safe, stable and practical labeling method, the natural abundance of ^{13}C has been widely used in a carbon cycle in the soil-plant system. In order to understand the effects of maize growth and photosynthesis on the value of $\delta^{13}C$ in soil respiration, the value of $\delta^{13}C$ in soil respiration was determined by mass spectrum after being trapped in a NaOH solution under a closed static chamber and then turned into barium carbonate in a pot experiment. The results showed that maize growth and photosynthesis significantly affected the value of $\delta^{13}C$ in the soil respiration. In maize-planted soil, the value of $\delta^{13}C$ in soil respiration had a clear seasonal variation. It changed with maize growth in the range of −14.57‰ to −12.3‰ and decreased during the period of trumpeting > ripening > flowering stages. The difference of $\delta^{13}C$ in soil respiration during various maize growth stages added up to about 2.3‰. However, in bare soil, $\delta^{13}C$ in soil respiration ranged from −19.34‰ to −19.13‰ and did not change significantly over time. The $\delta^{13}C$ in soil respiration in the maize-planted soil was the lowest at flowering stage. This was mainly due to the decline of the input in assimilates into soil and the decrease in root activity. However, the $\delta^{13}C$ increased at ripening stage, due to the decomposition and ingestion of senescent and died roots by soil microorganisms. In the planted soil, $\delta^{13}C$ in soil respiration was significantly higher during daytime than at nighttime at flowering and ripening stages. The difference of $\delta^{13}C$ in soil respiration between day and night periods added up to about 1.4‰ and 2.1‰ at flowering and ripening stages, respectively. Shading maize plants at the trumpeting stage decreased the value of $\delta^{13}C$ in soil respiration significantly. The difference of $\delta^{13}C$ in soil respiration between the treatment of non-shading and shading plants added up to 2.85‰. It was concluded that $\delta^{13}C$ in soil respiration was remarkably controlled by the maize growth and photosynthesis in planted soil. Soil respiration was mainly derived from the recent assimilates during maize growth.

Keywords maize growth, photosynthesis, soil respiration, $\delta^{13}C$

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1 Introduction

As an important composition in the global carbon cycle, soil respiration is the primary path by which CO_2 fixed by land plants returns to the atmosphere and is estimated at approximately 75 Pg C/a (Schlesinger and Andrews, 2000). There are three sources of the CO_2 release by a system of living roots and soil: (1) root respiration; (2) microbial respiration utilizing root-derived materials; and (3) microbial respiration using original soil C (Cheng et al., 1994; Cheng, 1996; Domanski et al., 2001). Total rhizosphere respiration is defined as the sum of root respiration and rhizo-microbial respiration (Cheng, 1996). The isotope ratios are expressed as $\delta^{13}C$ value where

$$\delta^{13}C = \frac{\left(^{13}C/^{12}C\right)_{sample} - \left(^{13}C/^{12}C\right)_{standard}}{\left(^{13}C/^{12}C\right)_{standard}} \times 1000$$

(Andrews et al., 2000; Kristiansen et al., 2004). The $\delta^{13}C$ value in atmosphere is around −7‰ (Balesdent et al., 1987). Because the assimilation of ^{13}C demands more energy than the assimilation of ^{12}C, the photosynthesis of plants discriminates ^{13}C. Plants with different photosynthesis paths are different in the discrimination of ^{13}C. With more discrimination of ^{13}C in C_3 plants than in C_4 plants, the $\delta^{13}C$ value in C_4 plants was higher than in C_3 plants, with a difference about 14‰ (Nyberg et al., 2000). The $\delta^{13}C$ value averaged at −12‰ in C_4 plants and at −27‰ in C_3 plants (Balesdent et al., 1987;

Cheng, 1996). The input of plant assimilates into soil was the source of soil organic matter (Saggar et al., 1997; Kuzyakov, 2002), so the $\delta^{13}C$ value in soil long-term growing C_4 plants was near to the $\delta^{13}C$ value in C_4 plants, which could be called as C_4 soil, and the $\delta^{13}C$ value in the soil long-term growing C_3 plants was near to the $\delta^{13}C$ value in C_3 plants, which could be called as C_3 soil. Planting C_3 plants in C_4 soil or planting C_4 plants in C_3 soil was equivalent to the ^{13}C isotope labeling in plants and soil at one time. The effects of plant growth and photosynthesis on soil respiration would be reflected by the determination of $\delta^{13}C$ value during plant growth. In order to provide the evidence of understanding the effects of plant growth on a soil carbon cycle and applying the natural ^{13}C abundance in the studying of a carbon cycle, the effects of plant growth and photosynthesis on soil respiration were investigated by measuring the $\delta^{13}C$ value of soil respiration at various growth stages of maize and after shading maize plants.

2 Materials and methods

2.1 Materials

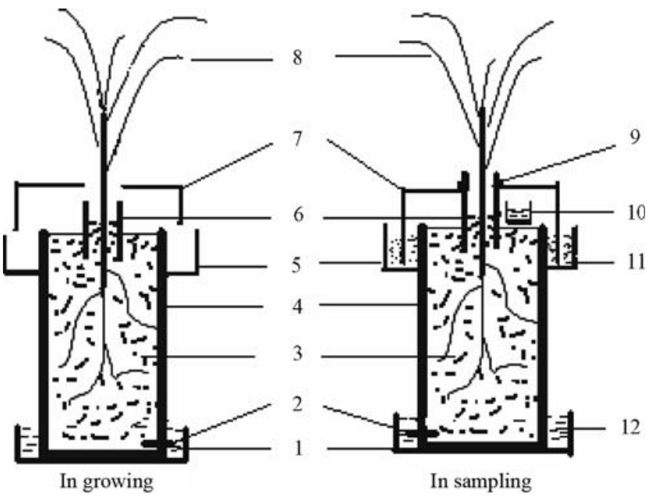
The plant used in this experiment was maize (*Zea mays* L.) and the variety was Yedan 14.

The soil used in this experiment was Udic luvisols, which was derived from the matrix of quaternary loss. Soil organic carbon and nitrogen content was 4.9 g/kg and 0.6 g/kg and soil pH was 5.80 (H_2O, 2.5 : 1). The content of sand (20–200 μm), silt (2–20 μm) and clay (< 2 μm) was 325, 462 and 213 g/kg, respectively, and the soil texture was heavy loam.

2.2 Methods

2.2.1 Pot experiment

Pots were in the shape of column, made of polyvinyl chloride (PVC) with 20 cm in height and 15 cm in diameter. At the edge of pot mouth, there was a trough with 5 cm in height, 25 cm in width and 25 cm in length. For the convenience of sampling, a PVC tube with 2.5 cm in inner diameter and 6 cm in length was tightly positioned at the center of the pot (2.5 cm above the pot mouth and 3.5 cm inserted into soil) for insulating plants from soil during sampling, in which maize seeds were sowed in about 2 cm of soil depth. This made the sample of soil respiration included only belowground respiration. Plants were cultivated in a net-house. Nine pots were used in this experiment and 4.0 kg of air-dried soil sieved through a 5-mm sieve was filled into each pot. Basal fertilizer was 150 mg N/kg, 75 mg K/kg and 150 mg P/kg, provided in the form of $(NH_4)_2SO_4$, $K_2H_2PO_4$ and K_2SO_4. Three unplanted pots were used as control and the other six pots were used to sow two maize seeds on April 30, 2003. Seedlings were emerged on May 5. After emergence of the maize seeds, one maize plant exhibiting consistent growth characteristics was retained in each pot and the other was discarded. In order to maintain the same water content in each pot, after being opened a hole with 2 cm in diameter at the edge of pot bottom and crammed with sponge into it, each pot was placed in a pottery basin with 19 cm in internal diameter and 5 cm in height. During the period of experiment, the basin was full of water, and this made the water content in each pot maintain the same level because of the capillary force. The device of pot and the sampling of soil respiration are showed in Fig. 1 in details. The pots were placed on a movable experimental bench on the rail in order to facilitate movement between the netted house on fine days and into glass house on rainy days. The air, temperature and light for plant growth were controlled naturally.



Note: 1) pottery pot; 2) the hole and the sponge; 3) soil; 4) PVC pot; 5) trough; 6) PVC tube for isolating plant and soil; 7) sampling chamber; 8) maize plant; 9) silicone gel to seal the joint; 10) glass utensil and the solution of NaOH; 11) water for sealing; 12) water supplying for soil and plants

Fig. 1 Device of experimental pot and sampling

At the trumpeting stage of maize growth (47 days after emergence, from May 5 to Jun 21, 2003), three pots with maize plants were randomly taken into one group as replicates for stopping photosynthesis for three days by shading the plants with black cloth bags. To commence the shading of the plants, the aerial parts of each plant were placed into an iron wire cylinder covered with a double-layered, black cloth bag which was suspended above the plant. The bottom of the bag was tightly fastened at the base of plant stem with a plastic rope for light-proofing. This shading device eliminated almost 100% of available light. As soon as the shading was over, soil respiration samples in shading and non-shading maize plants were trapped in a NaOH solution under a closed static chamber during one whole day for measuring their $\delta^{13}C$ values. After sampling of soil respiration, the shaded maize plants were harvested in aerial parts and roots, respectively.

At flowering (59 days, from May 5 to July 3, 2003) and ripening (80 days, from May 5 to July 24, 2003) stages, soil respiration samples during one whole day were trapped two daytimes and two nights in NaOH solutions for measuring their $\delta^{13}C$ values.

2.2.2 Sampling preparation

Each pot was fitted up an open bottom PVC chamber with 20.5 cm in length, 20.5 cm in width and 6 cm in height which had a hole on the center of chamber's cover to fit the PVC tube exactly. The chamber was crossed on the trough during un-sampling. When sampling, as soon as the glass utensil with 30 mL in volume was place on the soil surface in pot, in which 15 mL of 1 mol/L NaOH solution was poured, the chamber was moved downward to exactly fit in the trough with 200 mL water and PVC tube so that the water prevented air from exchanging between inside and outside the chamber. Silicon gel was used to seal the connecting joints between the chamber and tube making it leakproof. After being trapped for a certain time, the chamber was removed up and the glass utensil was covered and taken back to laboratory. The solution in utensil was transferred into centrifugal tube to centrifuge after the solution of $BaCl_2$ was poured into the utensil. The solution was discarded and the precipitate was washed with distilled water to centrifuge and replicated this process for three to four times. Then the centrifugal tube together with precipitate was placed into an oven at 50°C–60°C for drying. The precipitate was milled into powder with a glass rod and transferred into a glass bottle to the oven at 105°C for 6 h. Subsequently, the bottle was tightly covered to store up in a desiccator for analyses after being cooled in it.

2.2.3 Sampling analysis

The concentrations of C and N in soil were determined by a PIE 2400-II CHNS/O analyzer (Perkin Elmer Corporation, USA). Soil texture was measured by LS-230 laser diffraction particle size analyzer and soil pH was determined by an exact acidimeter.

Barium carbonate derived from soil respiration sample was turned into CO_2 by H_3PO_4 which was purified in the cooling trap system of liquid nitrogen and the $\delta^{13}C$ value of CO_2 released from barium carbonate was determined by the isotope mass spectrometer (Delta Plus MAT253, USA).

3 Results

3.1 Effects of growth stage on $\delta^{13}C$ value of soil respiration

Fig. 2 indicated that the $\delta^{13}C$ value in maize-planted soil was significantly higher than that in the bare soil. In the maize-planted soil, the $\delta^{13}C$ value varied with the maize growth periods in the range of −14.57‰ to −12.30‰, but in the bare soil, it slightly varied with crop growing in the range of −19.34‰ to −19.13‰. The $\delta^{13}C$ value of soil respiration was different in various growing stages and the difference was as high as 3.3‰, and decreased in the order of trumpeting > ripening > flowering stages in the maize planted soil, while it had no significant difference in the bare soil.

3.2 Effects of photosynthesis on $\delta^{13}C$ value of soil respiration

To understand the effects of photosynthesis on the $\delta^{13}C$ value of soil respiration, soil respiration was sampled after shading maize plants for three days at trumpeting stage, and the soil respiration during daytime (7:00–19:00) and nighttime (19:00–7:00) was respectively sampled at flowering and ripening stages.

Fig. 3 showed that three days of plant shading decreased the $\delta^{13}C$ value of soil respiration significantly and the difference of $\delta^{13}C$ value of soil respiration between shading and non-shading treatments reached 2.85‰.

Fig. 4 implied that at both flowering and ripening stages, the $\delta^{13}C$ values of soil respiration in maize-planted soil were remarkably higher in daytime than in nighttime. The difference of $\delta^{13}C$ values between day and night was 1.36‰ at flowering stage and as high as 3.11‰ at ripening stage. The $\delta^{13}C$ value of soil respiration during daytime was significantly higher at ripening stage than at flowering stage, but its difference during nighttime between ripening and flowering stages was not significant.

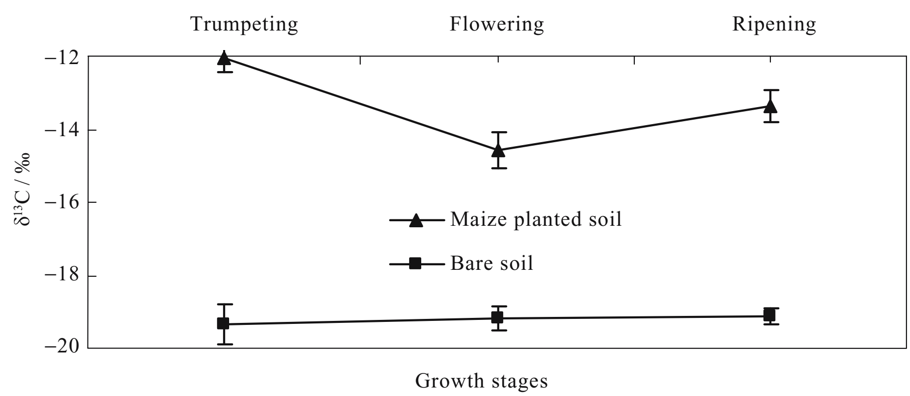


Fig. 2 Effects of maize growth periods on $\delta^{13}C$ in soil respiration

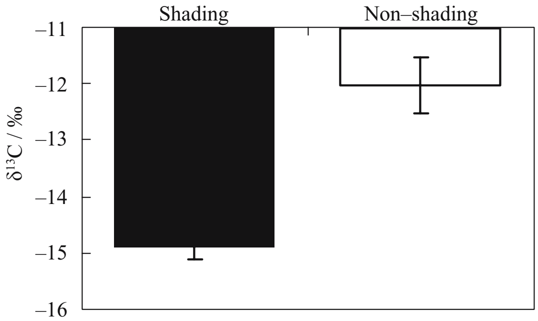


Fig. 3 Effects of shading plants on the $\delta^{13}C$ in soil respiration

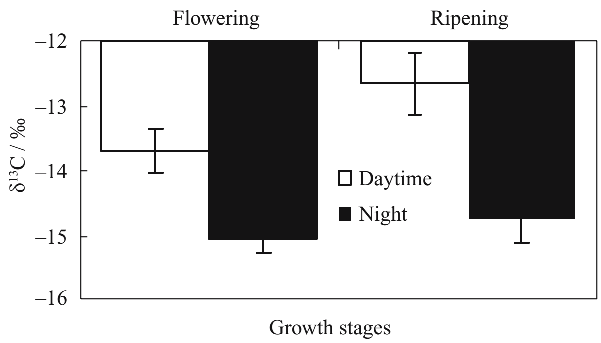


Fig. 4 $\delta^{13}C$ in soil respiration during daytime and nighttime

4 Discussion

Natural abundance of $\delta^{13}C$ is corresponded to a natural and homogeneous isotope labeling method, and is used more and more widely in recent years because of its free radioactivity, long term labeling and usage in soil organic composition derived from plants, the dynamic of soil organic matter in the whole and in different size of particle or in various densities, and the influence of agricultural managements and environmental factors on the dynamic of soil organic matters (Dou and Zhang, 2001). In this experiment, the variation of $\delta^{13}C$ value of soil respiration during maize growth was measured to study the effects of maize growth and photosynthesis on soil respiration.

4.1 Effects of plant growth on $\delta^{13}C$ value of soil respiration

That the $\delta^{13}C$ value of soil respiration in the maize-planted soil was significantly higher than that in the bare soil suggested that maize growth influenced soil respiration significantly. In the planted soil, soil respiration was controlled by plant growth. In the system of soil plants, soil respiration was made up the respiration of assimilates transformed belowground and soil original organic matters, so the change of the $\delta^{13}C$ value of soil respiration originated from the respiration of assimilates input into soil by plants. The different $\delta^{13}C$ values of soil respiration at the trumpeting, flowering and ripening stages exhibited that there was a seasonal variation of $\delta^{13}C$ in soil respiration during maize growth which coincided with the results conducted in pot (Fu et al., 2002) and in field (Rochette and Flanagan, 1997; Rochette et al., 1999). These meant that it was feasible to use natural abundance of the $\delta^{13}C$ in studying the dynamic of soil respiration during plant growth. The trumpeting stage was the period of maze growing most flourishingly in vegetative growth, during which the root respiration was strong in order to get more water and nutrients for supporting the flourishing growth. The energy in root absorption of nutrients and water was provided by the root respiration of assimilates. Between 10% and 15% of belowground allocated C is respired by roots to generate metabolic energy which is used for growth, maintenance and transport process (Kuzyakov et al., 2002). At flowering stage between maize vegetative growing and reproductive growing stages, as the turning point, one reason for the decrease in $\delta^{13}C$ value of soil respiration was the decrease in input of photosynthate into soil leading to the decrease in rhizosphere respiration. Generally speaking, the input of assimilates into soil at the vegetative growing stage was higher than that at the reproductive growing stage. Gregory and Atwell (1991) showed that the assimilate input into soil was between 33% and 35% of the net assimilates at wheat and barley tillering stage, but it was lower than 10% at the seeding swelling stage. In labeling *Lolium* using ^{14}C in laboratory and field, Meharg and Killham (1990) found that in laboratory, the portion of the assimilated C transformed below ground was between 20% and 28% at the time 23–51 days after *Lolium* was sowed and only 8.1% at 65 days; but in field, it was 67.1%, 37.7% and 13.8% at the time four, six and twelve weeks after *Lolium* was sowed. Another reason for the decrease in the $\delta^{13}C$ value of soil respiration at the flowering stage was the decrease in maize root activity leading to the decrease in root respiration. Roots growth presented a growth cycle, undergoing the period of growth stagnancy, logarithm growth, linear growth and senescence (Pan, 2004). During the growing period of black spruce, the contribution of root to soil respiration increased with the growth at first, but it went down when it reached the highest, and reached the lowest at the end of its growth (Bond-Lambery et al., 2004), which implied that soil respiration was the reflection of roots activity. Maize roots grew faster than that of aerial parts and finished their development on the whole at elongating stage (Wang, 1998). It was reported that maize roots began to senescence in about 50 days after emergence (Chen and Zhang, 1994). In this experiment, the soil respiration sample at the flowering stage lasted 59 days after emergence which was just the period of decrease in roots activity. Roots respiration contributed considerably to soil respiration (Kelting et al., 1998), but varied largely with ecosystems, measuring methods and growth periods. It ranged from 50% to 93% in the tundra ecosystem, from 40% to 50% in the temperate forest ecosystem and from 17% to 70% in the grass ecosystem (Zhang et al., 2005). It was between 20% and 80% measured by the method of component synthesis and between 40% and 70% by roots exclusion (Cheng and Zhang, 2003), and between 75% and 90% in agroecosystem by the method of isotope labeling (Swinnen et al., 1994). It was estimated that the contribution of root respiration to soil respiration in the growing season in a cool-temperate deciduous forest ranged from 27% to 71% (Lee et al., 2003). Thus the decrease in soil respiration resulting from the decrease in roots activity led to the decrease in $\delta^{13}C$ value of soil respiration. However, the increase in $\delta^{13}C$ value of soil respiration at the ripening stage was due to the decomposition of senescent and dead roots. The soil respiration sample at ripening stage was conducted in 80 days after the emergence, and it was certain that the senescent and dead roots were decomposed in this period, for example, the increase in soil N_2O emission during the soybean ripening stage resulted from the decomposition of roots (Yang and Cai, 2005). CO_2 released from the decomposition of senescent and dead roots increases the contribution of rhizosphere respiration to soil respiration, so as to increase the $\delta^{13}C$ value of soil respiration at the ripening stage.

4.2 Effects of photosynthesis on $\delta^{13}C$ value of soil respiration

In the maize-planted soil, higher $\delta^{13}C$ in soil respiration during daytime than that during nighttime and the sharp decrease in $\delta^{13}C$ by crop shading testified that there was a direct connection between photosynthesis and soil respiration. No difference of $\delta^{13}C$ of soil respiration in nighttime and the significant difference of $\delta^{13}C$ of soil respiration in daytime between flowering and ripening stages indicated that the difference in photosynthesis intensity in daytime was the reason for the difference of $\delta^{13}C$ in soil respiration among the various growth periods and the soil respiration was controlled by the recent assimilates. A study of pulse labeling ryegrass with ^{14}C showed that about 80% of the root-derived CO_2 efflux originated from the C assimilated after defoliation (Kuzyakov et al., 2002). Craine et al. (1999) found two days of shading grassland decreased 40% of soil CO_2 efflux. Kuzyakov and Cheng (2004) prolonged the nighttime to 36 and 84 h leading to 39% and 68% of decrease in soil respiration and the contribution of rhizoshpere respiration to soil respiration was 82%, 68% and 56% in 12, 36 and 84 h of nighttime, respectively. Johansson (1993) indicated that soil respiration went down rapidly after stopping photosynthesis. Warembourg and Esterlrich (2001) confirmed that the recent assimilates in plant could be rapidly utilized by root and soil organism. Illeris et al. (2003) reported that a large part of belowground respiration derived from the recent assimilates. Ekblad and Högberg (2001) calculated that the recent assimilates contributed at least 65% to total soil respiration. Soil $^{14}CO_2$ flux can be detected 1 h after labeling ryegrass with ^{14}C (Kuzyakov et al., 1999). It was found that soil $^{14}CO_2$ began to emit only after 30 min of labeling winter wheat and ryegrass with ^{14}C (Cheng et al., 1993) and the notable mount of ^{14}C was found in all carbon pool 4 h after labeling tudra plant with ^{14}C (Olsrud and Christensen, 2004). All these results testified that the process of CO_2 assimilated by plants and the transportation of assimilates belowground were very rapid and the speed of assimilates transformed down to the roots was about 100 cm/h (Kuzyakov et al., 2001).

In sum, maize growth altered the $\delta^{13}C$ value of soil respiration. In the maize-planted soil, the $\delta^{13}C$ value of soil respiration varied with maize growth periods. The significant effects of photosynthesis on the $\delta^{13}C$ value of soil respiration reflected that the rhizosphere respiration derived principally from the recent assimilates in plants.

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