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Original article

Allocation of photosynthetic carbon to nodules of soybean in three geographically different Mollisols

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ABSTRACT

The development of legume nodules and N_2-fixation demand fair proportion of photosynthetic carbon (C). Soil as a determinant of nodulation may influence C allocation to nodule and subsequent underground C fates. However, there is little information on the photosynthetic C allocation to the nodule of soybean grown in Mollisols in Northeast China. Such research is crucial for optimizing the strategies of C/N management to improve nodulation and productivity in soybean farming systems. With a pot experiment, soybean plants were grown in three Mollisols sampled from low- to high-latitude in Northeast China and labeled with $^{13}CO_2$ at the R4 (full pod) and R5 (initial pod filling) stages. The nodule characteristics and underground ^{13}C distribution were investigated. The nodule number and nodule density were in the order of Mid- > High- > Low-latitude Mollisol, resulting in 980, 578 and 252 nodules per plant, and 11.1, 8.2 and 2.7 nodules per m of root length, respectively. The $^{13}CO_2$ pulse-chase labeling showed that higher proportion of ^{13}C was recovered in Mid-latitude Mollisol at R4, and in both Mid- and High-latitude at R5. Moreover, a 53.8% of increase on C-growth rate of nodule was also found in the two soils compared to Low-latitude. This suggests that nodules in high-latitude Mollisols have stronger C-sink activity than low-latitude ones, which attribute to nodule number and C-growth rate of nodule. The nodules accounted for 15.7%, 28.0% and 11.5% of net underground ^{13}C in Low-, Mid- and High-latitude Mollisols, respectively. Therefore, the extent of nodule-driving underground C allocation varied with Mollisols from geographically different regions.

1. Introduction

Nodulation and N_2 fixation are closely related to carbohydrate supply by photosynthesis [1,2]. When nodule develops, it gradually becomes a strong sink of photosynthate for the energy source. Ito et al. [3] reported that the carbon (C) allocation to the inoculated roots did not increase during the early stages of nodule formation, while it markedly increased after emergence of the nodules. Therefore, the great demand of C in nodule may occur when nodule enters linear growth stages.

Soybean as a legume species is the major crop in Northeast China with the sown acreage accounting for 33% of the nation's total [4–6]. Mollisols is the main farming soil type there and amounts to more than 4 million hectares [4]. However, the chemical and biological properties of Mollisols such as organic C, pH and microbial activity, are various with geographic locations due to biotic (plants) and abiotic factors (temperature and rainfall) [4]. These discrepancies on soil properties may influence the nodulation. Lindström et al. [7] indicates that the nodulation varies when legumes grown in different soils. Studies found that the diversity of *rhizobium* species isolated from bean nodules in Austrian, Mexican and French soils were different, which affected the infection effectiveness and nodule characteristics [8,9]. Soils from different localizations where the temperature and rainfall were different, had different communities of rhizobium [10,11]. Therefore, soil types or soils from separated locations may affect biomass and number of nodules and N_2 fixation. Consequently, the demand of C for the development and function of nodules may change with soils, especially when nodules are at fast growing stages. To our knowledge, the information on the soybean photosynthetic C allocation to nodule in geographically different Mollisols is poorly understood, which is essential for how to optimize N_2 fixation by soybean to save fossil N in the perspective of sustainable agriculture in Northeast China.

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The $^{13}CO_2$ labeling techniques include continuous labeling and pulse labeling are widely used to trace photosynthetic C [12–19]. With the labeling techniques, most studies focused on the underground fate of photosynthates in agricultural cereals and pasture species [20], while much less is known about N_2-fixed soybean plants grown in Mollisols. Since pulse-labeling can be implemented to determine the seasonal dynamics of assimilate partitioning [21,22], and the residence time in various carbon pools [23,24], the $^{13}CO_2$ pulse-labeling was used in this experiment to quantitatively analyze the photosynthetic ^{13}C distribution to nodule. The aims of this study are (1) to determine the nodule characteristics in three Mollisols from low- to high-latitude of the soil distributed region, and (2) subsequently quantify the differentiation of C allocation to the nodules in soils.

2. Materials and methods

2.1. Soil and plant materials

Three soils used in this study were taken from low- to high-latitude regions of Mollisols (named as Low-, Mid- and High-latitude Mollisol, respectively) in Northeast China. The soil from each location was air-dried, sieved through a 4 mm sieve, and mixed. The general chemistry characteristics of soil samples were showed in Table 1.

A soybean cultivar, i.e. Suinong 14 was used in this experiment. It has been widely grown over 2 million ha and produced more than 937 million kg in China [25,26].

2.2. Plant culture

A pot experiment was conducted at Northeast Institute of Geography and Agroecology (45°41.8′ N, 126°38.1′ E), Chinese Academy of Sciences, Harbin, China. The experiment was completely randomized block design with three Mollisols, and each received six pots for labeling and another six as control. Twelve kilograms of a soil/sand mixture (1:1 w/w) was filled into each container (29.0 cm in height and 25.5 cm in diameter). The reason for mixing soil with sand in this study was to facilitate root washing at harvest. Basal nutrients were applied at the following rates (mg kg^{-1}): 217 urea, 219 KH_2PO_4, 167 $CaCl_2.2H_2O$, 43 $MgSO_4.7H_2O$, 9 Fe-EDTA, 6 $ZnSO_4$ and 5 $CuSO_4$, 0.7H_3BO_3, 6.7 $MnSO_4.H_2O$, 10 $ZnSO_4.7H_2O$, 2 $CuSO_4.5H_2O$, 0.3 $CoSO_4.7H_2O$, 0.2 $Na_2MoO_4.2H_2O$ [27]. Nutrients were thoroughly mixed with the soil and sand. Nine seeds were sown to each pot on May 10, 2009, and the plants were thinned to three per pot on the 14th day after sowing. The plants were grown in a glasshouse with night temperature of 16–20 °C and day temperature of 24–28 °C during the experiment. Water content in soil was well controlled at 80 ± 5% of field water capacity.

2.3. Labeling process and measurements

Soybean plants were labeled with $^{13}CO_2$ on the R4 (full pod) stage when nodules rapidly grow, and the R5 (initial pod filling) stage when nodules reach maximum biomass. On labeling dates, plants were moved into an airtight glass chamber (area 130 × 80 cm^2, height 130 cm). Before labeling, CO_2-free air was pumped through the chamber for 30 min to eliminate $^{12}CO_2$ inside. After that, five hundred ml of pure $^{13}CO_2$ (Chemical purity ≥ 99.9%) was injected with a syringe through a rubber gasket into the chamber [28]. The average $^{13}CO_2$ concentration maintained about 350–400 ppm. Plants were labeled for 6 h in the chamber where a fan was installed to homogenize the atmosphere inside. After labeling, plants in three pots were harvested at intervals of 0 and 12 days, respectively. Plants without ^{13}C labeling were harvested as control at the same time. The entire root system was carefully removed from the pot after shoots were cut off at the soil surface. The roots were washed with tap water to remove soil and sand adhering roots. Nodules on root system were picked off, counted and weighed. The measurement of root length was performed with Win-RHIZO version 2004a (Regent Instruments, Quebec, Canada). All plant samples were dried at 70 °C for 72 h and then ground before further analysis. Soil samples were took at the same time, air-dried and ground.

The C concentrations and stable ^{13}C isotope ratios of all samples were determined with an isotope ratio mass spectrometer (Delta^{plus}, Finnigan MAT GmbH, Bremen, Germany). The natural abundance of ^{13}C isotopes was presented as ratio per thousands relative to the international standard VPBD using delta units (δ).

2.4. Calculation

Atomic $^{13}C\%$ was expressed as:

$$\text{Atomic } {}^{13}C\% = [[(\delta^{13}C + 1000) \times \text{RVPBD}]/[(\delta^{13}C + 1000)] \times R_{\text{VPBD}} + 1] \times 100$$

where $R = {}^{13}C/{}^{12}C$ and $R_{\text{VPBD}} = 0.0111802$ [29].

The net photosynthetic ^{13}C in roots, nodules and soils was the difference between ^{13}C contents of the labeled samples and those of the nonlabeled samples as follows:

$$C_{\text{sample}} = \left[(\text{atomic } {}^{13}C\%)_{\text{l}} - (\text{atomic } {}^{13}C\%)_{\text{nl}}\right]_{\text{sample}} \times TC_{\text{sample}} \times 100$$

where "l" and "nl" indicate labeling and nonlabeling; "TC" is the total C content of sample.

By immediate investigation after labeling (0 day), the sum of the ^{13}C amounts in shoots, roots, nodules and soils was assumed as the total ^{13}C photosynthetic by plants during 6-h ^{13}C labeling [30].

The percentage distribution of ^{13}C at each harvesting time was estimated as [30]:

$$\text{Distribution}\% = {}^{13}C_{\text{sample}}/\text{net}\,{}^{13}C \text{ assimilation}$$

The underground ^{13}C allocation was sum of $^{13}C_{\text{root}}$, $^{13}C_{\text{nodule}}$, and $^{13}C_{\text{soil}}$.

The C-growth rates of root and nodule were calculated according to the C accumulation of those during the interval of the two-harvest at each growth stage which was divided by 12 days.

Table 1
General chemical characteristics of three geographically different Mollisols in Northeast China.

Mollisols	Sites	Locations	C_{org} (%)	pH (1:5 v/v)	Total (g kg^{-1})			Available (mg kg^{-1})		
					N	P	K	N	P	K
Low-latitude	Yushu, Jilin	43°20′ N, 124°30′E	1.04	7.1	0.80	0.31	16	75	24.0	100
Mid-latitude	Hailun, Heilongjiang	47°32′ N, 126°57′ E	2.90	7.1	1.72	0.78	16	154	37.1	160
High- latitude	Beian, Heilongjiang	48°17′ N, 127°15′ E	5.05	6.1	3.65	0.92	12	266	32.5	130