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Fate of photosynthetically-fixed carbon in soybean crops measured using ^{13}C labelling after long-term fertilization of *Phaeozem* soils in Northeast China

Yunfa QIAO¹, Lucas C. R. SILVA², Xiaozeng HAN¹, Shujie MIAO¹,
Chunjuan YAN³, William R. HORWATH²

¹Northeast Institute of Geography and Agro-ecology, Chinese Academy of Sciences
No. 138 Haping road, 150081 Harbin, Heilongjiang province, China
E-mail: miaoshujie@126.com

²Biogeochemistry and Nutrient Cycling Laboratory, Department of Land, Air and Water Resources,
University of California
Davis, USA

³Crop Institute, Liaoning Academy of Agricultural Science
Shenyang 110161, Liaoning, China

Abstract

A good understanding of the fate of photosynthetically-fixed carbon (C) in the plant-soil system following fertilization is crucial to manage agricultural soils. The objective of this study was to analyse the allocation of C fixed by soybean and its response to long-term fertilization. We conducted a ^{13}C pulse labelling experiment, using soybean grown in *Phaeozem* (PH) soils (FAO) under five fertilization treatments: inorganic fertilization (NPK, NP, NK), inorganic and organic fertilization (NPKM) and no fertilization (CK) as control. The ^{13}C pulse labelling was conducted at full pod (R_4) stage. Results showed that fertilization always increased C accumulation in the biomass of soybean plants, with the highest C content measured in the NPKM treatment (35%) and lower values observed in NP, NK and CK than in the NPK treatment. The net C fixed after labelling varied between 8.6 and 21.4 mg ^{13}C per plant with maximal values observed in the NPKM treatment. More than 4/5 of the fixed ^{13}C was found in shoots at day 0. The label was exported from shoots to roots with 53.3% remaining in shoots and 7.5% measured in roots at day 7 after labelling. The percent of net fixed C incorporated into soils was also affected by fertilization. Compared to CK, the incorporation of ^{13}C into soils was 24.4, 14.8, 18.2 and 50.8 % in NP, NK, NPK and NPKM treatments, respectively. The net accumulation of labelled C in soils changed with fertilization from 160 to 564 kg ha⁻¹ C season⁻¹. This indicates that the accumulation of soybean-derived C following NPKM addition would significantly increase C levels in Northeast Chinese *Phaeozem* soils.

Key words: ^{13}C , soil carbon, fertilization, *Phaeozem*, soybean.

Introduction

The carbon (C) fixed by plants through photosynthesis is the primary source of C in agricultural ecosystems (Warembourg, Paul, 1977). Predicting the long-term allocation of the assimilated C in plant-soil system is useful to better understand the terrestrial C and nutrient cycling (Silva et al., 2010; 2011 a; 2011 b), optimally manage soil organic matter (Jin et al., 2011), and adequately assess regional and national contributions of agriculture to the global C budget (Prince et al., 2001; Bolinder et al., 2007).

After photosynthetic fixation, reallocation of C fixed to the plant-soil system occurs through rapid transport from shoots to roots and release into soil (Ostle et al., 2003). The proportion of below-ground C allocation varies among plant species. Cereals (wheat and barley) transfer 20–30% of total assimilated C into soil, and in pastures this value ranges between 30% and

50% (Kuzyakov, Domanski, 2000). On average, 7–13% of the total assimilated C is used for root growth, while 1–2% is allocated as exudates, secretes, root hairs and fine roots; and root respiration accounts for 7–14% of the total assimilated (Kuzyakov et al., 2001). However, fertilization may change the amount of C allocated below-ground as well as the amount of C transferred to soil pools (Kuzyakov, Domanski, 2000). The portion of assimilated C allocated below-ground by wheat (Liljeroth et al., 1990), maize (Merckx et al., 1987) and lettuce (Kuzyakov, Domanski, 2000) has been shown to decrease with nitrogen (N) fertilization. While the application of N generally favours the turnover rate of recently fixed C, it may also delay the decomposition of other, more complex and recalcitrant, organic compounds (Thirukkumaran, Parkinson, 2000; Neff et al., 2002; Hagedorn et al., 2003). Therefore, even though it is well established that biomass

accumulation responds positively to fertilization, it is difficult to predict the net effect of fertilization on plant-derived C accumulation in soil pools.

Soybean (*Glycine max* L. Merr.) is one of the most important crops in China with the yield of 120 million Magnesium (Mg) in 2011 (<http://www.askci.com>) and, thus, any changes in the fate of C fixed in soybean crops is of major interest in predicting shifts in the Chinese C budget. The largest production areas in China are located in the Northeast region, which accounts for 33% of the nation's total yields (Liu, Herber, 2002). In this region, the productivity of soybean in *Phaeozems* is relatively high, compared with other soils (Liu et al., 2005; Jin et al., 2010). However, intensive cropping with no return of crop residues and other organic inputs results in the loss of soil organic C and fertility across all soil types including humus-rich *Phaeozems*. Due to the concern about soil fertility and permanent loss of organic matter, a number of long-term experiments were set up in the agricultural regions of Northeast China to test the effects of application of inorganic and organic fertilizers (Zhang et al., 2002; Dang et al., 2003). These experiments offer a unique opportunity to quantify allocation of fixed C to soils using long-term fertilization trials. So far, there is little information on photosynthates partition in plant-soil system as affected by long-term fertilization in *Phaeozems*. Therefore, the objective of the present study is to quantify the fate of C fixed by soybean in these soils, following different (inorganic and organic) fertilization treatments.

Materials and methods

Soil preparation and plant culture. We prepared a pot experiment using soils from the tillage layer collected from long-term fertilization experiment field plots, located at the State Key Agro-ecological Experiment Station (Hailun county, Heilongjiang province, 47°26' N, 126°38' E) in 2009. Basic conditions of the long-term experiment were described by Han et al. (2005) and the field fertilization was conducted for 19 years (since 1990). The soils in the research plots are classified as *Phaeozem* (PH), according to FAO and contained 27.9 g kg⁻¹ of total organic carbon (C), 2.2 g kg⁻¹ of total nitrogen (N), 0.7 g kg⁻¹ of total phosphorus (P) and 25.2 g kg⁻¹ of total potassium (K). Soil pH was about 7 (1:2 V/V). Representing the main cropping system in this region, soybean-wheat-corn was the planting rotation pattern established in the long-term fertilization trials. The soils studied here represent experimental plots comprised of five fertilization treatments with four replicates: 1) no fertilizer or control (CK), 2) addition of nitrogen and phosphorus (NP) fertilizer, 3) addition of nitrogen and potassium (NK) fertilizer, 4) addition of nitrogen, phosphorus and potassium (NPK) fertilizer, 5) addition of nitrogen, phosphorus and potassium plus organic manure (NPKM). The inorganic fertilizers were applied at the following rates: for the corn and wheat crops, 112.5 kg ha⁻¹ N as urea, 19.6 kg ha⁻¹ P as superphosphate, and 49.8 kg ha⁻¹ K as K₂SO₄; and for the soybean crop, 13.5 kg ha⁻¹ N, 15.1 kg ha⁻¹ P, and 49.8 kg ha⁻¹ K. In the organic treatment, the equivalent of 15,000 kg ha⁻¹ of pig manure was incorporated during the soybean and wheat stages and 30,000 kg ha⁻¹ during the corn stage.

Three soil samples were taken from the soil surface (0–20 cm) using a shovel to make a composite sample for each fertilizer treatment. Soil samples were air-dried at room temperature (25°C) and passed through a 5-mm sieve to favour growth of soybean plants. We

filled pots using 6.5 kg of sieved soil according to Yang and Cai (2006), and compacted to the bulk density (1.24 g cm⁻³), which is a value identical to that measured in the field. Applications of 0.023 g kg⁻¹ nitrogen (N), 0.057 g kg⁻¹ diphosphorus pentoxide (P₂O₅), and 0.034 g kg⁻¹ dipotassium monoxide (K₂O) were used as the basal fertilization needed to grow soybean plants for one season. A soybean cultivar 'Heinong 35' (maturity group 0, days to maturity 120) was used in this experiment. Eight uniform seeds were sown to each pot on 5 May, 2009. Two weeks later, the plants were thinned to two seedlings per pot. The plants were grown in natural sunlight conditions with a day temperature range of 22–28°C and night temperature of 16–20°C. Soil moisture of all pots was maintained at about 60% water-holding capacity (WHC) over the entire experimental period.

Pulse labelling and isotopic measurements. The $^{13}\text{CO}_2$ labelling was performed at the full pod (R₄), which has the largest growth rate of the entire growth period of soybean plants. The detailed method of $^{13}\text{CO}_2$ labelling can be found in Jin et al. (2011). Briefly, six pots of each fertilization treatment were transferred into an airtight glass chamber. Ten hundred ml of pure $^{13}\text{CO}_2$ (chemical purity $\geq 99.9\%$) was injected with a syringe through a rubber gasket into the chamber during 6 h of a sunny day. The $^{13}\text{CO}_2$ concentration was maintained at 350–400 ppm. Photosynthetically active photon flux density was $\sim 1000 \mu\text{E m}^{-2} \text{ s}^{-1}$. Temperature in chamber was kept at 26–28°C, and relative humidity was 70–90%. Plants in three pots were harvested immediately after labelling (Day 0). The other harvest time was 7 days later (Day 7), unlabelled plants were used as controls following each harvest. Shoots were cut off at the soil surface, and roots were carefully separated from soil by gentle washing. Plant samples were dried at 80°C until stable dry mass was achieved and ground for analysis. Soil samples collected from each pot were also mixed thoroughly, air-dried, and milled for analysis. The C contents in the aerial parts and roots of the soybean plant as well as in the soil were determined by an elemental analyzer "Vario EL CHN" ("Heraeus Elementar", Germany). The stable ^{13}C isotope ratios of all samples were analysed with an isotope ratio mass spectrometer "Delta plus" ("Finnigan MAT GmbH", Germany).

Calculation. We calculated the net photosynthetically fixed ^{13}C in the total biomass, the percent distribution of ^{13}C in roots and shoots at harvest time, C-growth rates and contribution of the fixed C to soils. The calculations used here are the same as reported by Jin et al. (2011). The percentage distribution of ^{13}C at harvest time was estimated as:

$$\text{Distribution \%} = \frac{{}^{13}\text{C}_{\text{sample}}}{\text{total fixed } {}^{13}\text{C}}$$

The C-growth rates of plant were calculated according to the C accumulation during the interval of 0 and 7 day. The contribution of the fixed C at the growth stages to soil organic carbon (SOC) was estimated as:

$$\text{Contribution (mg C plant}^{-1} \text{ day}^{-1}) = \frac{({}^{13}\text{C}_{\text{soil}})_{\text{end}}}{(\text{total fixed } {}^{13}\text{C})_{\text{day0}}} \times \text{C-growth rate,}$$

where $({}^{13}\text{C}_{\text{soil}})_{\text{end}}$ represents the C amount in soil at the harvest time and $(\text{total fixed } {}^{13}\text{C})_{\text{day0}}$ is the amount of fixed ^{13}C immediately after the pulse labelling event.

Statistics. The effects of fertilization on C content in plants and soils and the contribution of applied ^{13}C to the total biomass and soil organic matter between treatments were analyzed using analysis of variance, followed by a Tukey's mean comparison test ($P = 0.05$). We also used a correlation analysis to describe the relationship between ^{13}C fixed by plants and accumulated

in soils. All statistical analysis was performed using SAS 9.1.3 statistical software (SAS Institute Inc., USA). The least significant difference (LSD at $P < 0.05$) test was adopted to assess the differences among the means of three replicates of factors.

Results

Carbon accumulation in soybean plants. There was a significant ($P < 0.05$) difference in C accumulation in soybean plants among the fertilization treatments (Table 1). The largest change was observed in the NPKM treatment, where C content in soybean shoots increased by 53.2% in relation to the CK treatment and 35.0% in relation to the NPK treatment. In relation to the NPK treatment, shoot C content was 28.2, 12.6 and 27.2 % lower in CK, NP and NK, respectively. The same pattern was observed in roots, where 23.1% higher C content was observed in the NPKM treatment and 53.8, 38.5 and 53.8 % lower C content in CK, NP and NK in relation to the NPK treatment, respectively. As a result, total C content in soybean plants was 28.2, 12.6 and 17.5 % lower in CK, NP and NK, and 35.0% higher in NPKM compared to NPK (Table 1). When comparing plants within treatments, the C accumulation in shoots was always larger than in roots (Table 1).

Table 1. Carbon (C) content of shoot, root and total biomass of soybean plants at full pod (R_4) stage in *Phaeozem* soils under five fertilization treatments

Treatments	Shoot g plant ⁻¹ C	Root g plant ⁻¹ C	Total C g plant ⁻¹ C
CK	7.4 ± 0.61	0.6 ± 0.13	8.0 ± 0.48
NP	9.0 ± 0.48	0.8 ± 0.21	9.8 ± 0.69
NK	7.5 ± 0.67	0.6 ± 0.06	8.1 ± 0.66
NPK	10.3 ± 0.39	1.3 ± 0.03	11.7 ± 0.39
NPKM	13.9 ± 0.50	1.6 ± 0.02	15.5 ± 0.49
LSD ($P < 0.05$)	3.24	0.25	1.83

Notes. Inorganic fertilizer treatment were NP, NK and NPK; inorganic and organic fertilizer treatment was NPKM; no fertilizer was CK. LSD means least significant deviation.

Table 2. Net ¹³C assimilation after a 6 h labelling event in soybean tissues at full pod (R_4) stage grown under five fertilization treatments in *Phaeozem* soils

Treatments	Fixed ¹³ C mg plant ⁻¹ ¹³ C	Shoot	Root	Soil
		%	%	%
day 0 after labelling				
CK	8.6 ± 0.76	92.4 ± 2.42	5.0 ± 0.17	2.6 ± 0.13
NP	14.6 ± 0.37	95.6 ± 4.81	4.6 ± 0.72	0.2 ± 0.06
NK	10.7 ± 0.71	88.7 ± 1.63	6.3 ± 0.71	4.9 ± 0.41
NPK	15.2 ± 1.41	91.9 ± 3.22	5.6 ± 0.49	2.5 ± 0.24
NPKM	21.4 ± 1.61	94.3 ± 4.67	3.8 ± 0.27	1.9 ± 0.19
LSD ($P < 0.05$)	2.04	9.63	1.02	0.48
day 7 after labelling				
CK	–	59.6 ± 2.46	7.9 ± 1.06	7.1 ± 0.66
NP	–	53.3 ± 3.21	6.5 ± 0.72	8.6 ± 0.41
NK	–	55.0 ± 3.69	8.8 ± 0.97	7.1 ± 0.34
NPK	–	53.2 ± 2.26	8.0 ± 0.76	8.7 ± 0.97
NPKM	–	45.4 ± 3.64	6.5 ± 0.46	9.9 ± 1.01
LSD ($P < 0.05$)	–	4.17	0.83	1.04

Notes. Inorganic fertilizer treatments were NP, NK and NPK; inorganic and organic fertilizer treatment was NPKM; no fertilizer was CK. LSD means least significant deviation. Changes in the relative distribution of the fixed ¹³C in the plant-soil system represent reallocations occurred within 7 days following the labelling event.

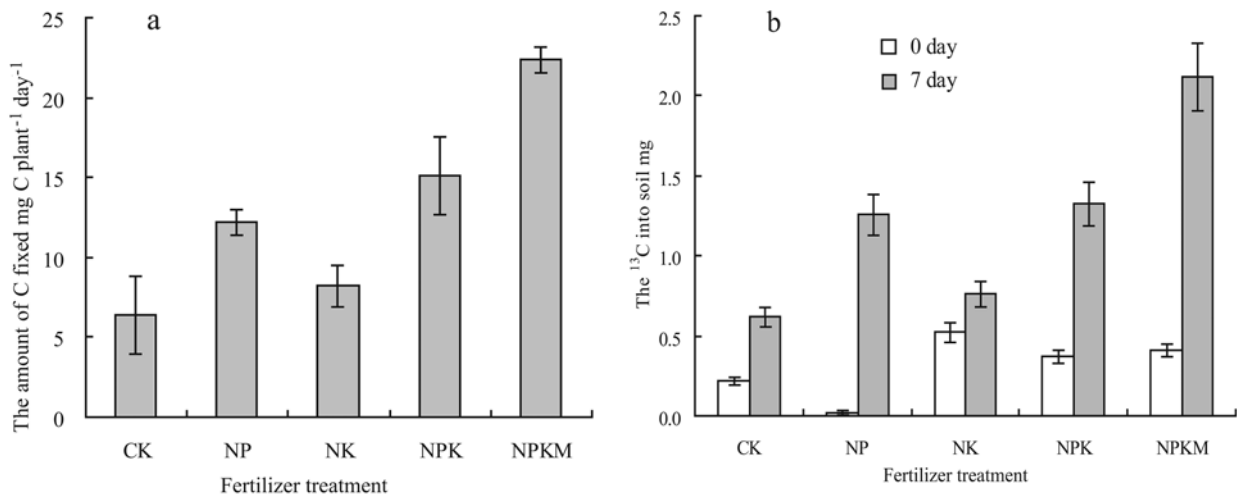
Fixation and distribution of C after labelling.

After 6-h labelling at R_4 stage, the net fixed ¹³C varied from 8.6 to 21.4 mg ¹³C per plant, with maximum values observed in the NPKM treatment, on average, 6.2 mg plant⁻¹ higher than measured in the NPK treatment (Table 2). The net fixed ¹³C in soybean plants in CK, NP and NK treatments was 6.6, 0.6 and 4.5 mg plant⁻¹ lower than measured in the NPK treatment, respectively. Compared to CK, the corresponding values were 6.0, 2.1, 6.6 and 12.8 mg plant⁻¹ lower in NP, NK, NPK and NPKM, respectively (Table 2). Most of the fixed ¹³C (88.7–95.6%) was recovered in the above-ground biomass (shoots) at day 0 across all five fertilization treatments. No significant differences were found among treatments (Table 2). On average, at day 0 the ¹³C recovered in roots accounted only for 5.1% of the total assimilated ¹³C and the recovery of ¹³C in soils was close to zero.

Compared to day 0, the proportions of fixed ¹³C in shoots decreased significantly by day 7, while the recovery of ¹³C in roots and soil significantly increased (Table 2). On average, 53.3% of the ¹³C assimilated during the pulse labelling was retained in shoots and 7.5% in roots 7 days after the labelling event. In contrast, the recovery of assimilated ¹³C in soils increased significantly ($P < 0.05$), representing over 7% of the assimilated label across all treatments. The highest value of ¹³C transferred from roots to soils was 8.7% in the NPK treatment, and 9.9% in the NPKM treatment (Table 2).

Accumulation of assimilated ¹³C in soils.

The transfer of photosynthetically fixed ¹³C from plant to soil was calculated by the difference between the isotopic signatures of soils at day 0 and day 7 following the labelling event. The percentage of ¹³C accumulated in soils in relation to the net fixed ¹³C was affected by fertilization. The amount of fixed ¹³C by plant was affected significantly by fertilization ($P < 0.05$) (Fig. 1 a). Compared to NPKM treatment, the amount of ¹³C fixed by plant per day of CK, NP, NK and NPK treatment was lower 72.7, 45.5, 63.6 and 31.8 %. Even though, the ¹³C transferred to soil was very not affected significantly by fertilization at the labelling day. After one week, the ¹³C into soil was affected by fertilization in an order of NPKM > NPK > NP > NK > CK (Fig. 1 b). In comparison with NPKM, the ¹³C transferred to soil in CK, NP, NK and NPK treatment occupied by 29.2, 59.4, 35.8 and 62.7 % of that in NPKM.



Notes. Vertical bars represent standard error of means ($n = 3$). Inorganic fertilizer treatment were NP, NK and NPK; inorganic and organic fertilizer treatment was NPKM; no fertilizer was CK.

Figure 1. The carbon (C) fixed by soybean with five fertilization treatments at the end of growing season (a), and transferred to soil (b)

Discussion

The present study shows that long-term fertilization leads to substantial changes in carbon cycling and accumulation in soybean culture. The long-lasting effects of the combined organic and chemical fertilization remarkably increased plant productivity and also lead to proportionally greater transference of assimilated C into soils. Chemical fertilization alone also increased both plant productivity and soil C accumulation, but this effect was much lower than observed when organic fertilization was applied. According to our calculations (extrapolated from the results of the pot labelling experiment), 564 kg ha⁻¹ C season⁻¹ of photosynthetically assimilated C would be accumulated under field conditions, following fertilization with manure treatment and NPK. Between 200 and 400 kg ha⁻¹ C season⁻¹ would be accumulated following various combinations of chemical fertilizers and, under no fertilization, only 160 kg ha⁻¹ C season⁻¹ would be transferred from soybean plants to soil. Considering the common density of soybean in farming system, an extrapolation from the pot experiment to field conditions derived ~210 kg C input per ha over the whole growing season (Jin et al., 2011). However, the C transference into soil that calculated represents the amount of accumulated ^{13}C as a function of photosynthetic rate (De Bruin et al., 2010) and does not account for losses such as mineralization and microbial respiration. Other factors, such as plant growth rates (Jin et al., 2011), respiration (Swinnen et al., 1994) and redistribution of fixed C to either shoots or roots can also affect net C accumulation in soils. However, here we unambiguously show that fertilization type controls the amount of C fixed by plants and transferred to soils. Despite much greater C loss by respiration in plants growing under NPKM soils (these plants had the highest leaf area and above ground biomass; data not shown), we observed the highest plant to soil C transference in that same treatment. We therefore conclude that the increase in C-growth rate of plants, and the large amount of fixed-C were the main mechanisms behind the increase in NPKM soil C content. A regression analysis showed a highly significant positive

relationship between C-growth rate and the amount of fixed C to soil ($r = 0.9803^{**}$) (Fig. 2).

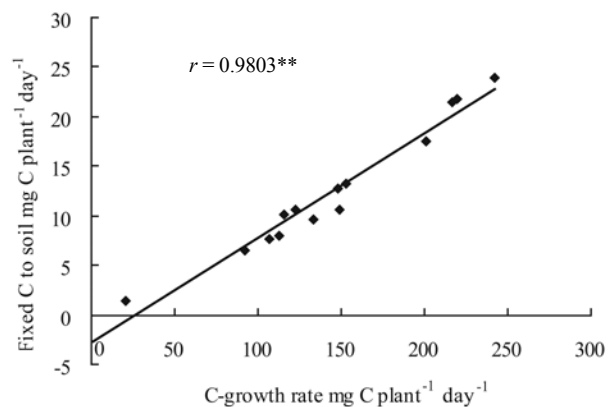


Figure 2. The relationship between the amount of fixed carbon (C) to soil and C-growth rate of soybean across all five fertilization treatments at the end of growing season

Our results thus show that the amount of C allocated to soil depends on the amount of total photosynthesized C, a relationship that holds despite differential losses and distinct distribution patterns that may occur in response to different fertilization treatments (Lu et al., 2002). In soybean plants, about half of below-ground translocated C is used for root growth, one third is used by roots and rhizosphere microorganism respiration, and the rest remains in soil (Lu et al., 2002). Previous reports showed that the allocation of C in low N treatments is higher than that in high N treatments (Paterson, Sim, 1999; Kuzyakov, Domanski, 2000; Kuzyakov, 2002). Here, the percentage of fixed C translocated to soil in the NP treatment was similar to that found in the NPK treatment, but higher than that observed in the NK treatment (Table 2). This shows that not only N levels, but also addition of P and organic manure, promote plant-derived C accumulation in soils (Table 2). These findings

are in agreement with the results found by Wani et al. (2003), who concluded that P application in soybean culture increased C sequestration by 7.4 t ha⁻¹ in whole growth season. In our experiment, we demonstrated that the percentages of distribution of the fixed C to soil in NPK, NK, NP and CK treatments were lower than those measured in the NPKM treatment (Table 2). This further supports the notion that even a balanced application of chemical fertilizers of N, P, K is not an ideal practice from the point of view of C sequestration in soils (Cai, Qin, 2006). However, when mixed with organic fertilizer NPK fertilization increase both plant productivity and C sequestration in typical agricultural soils of the Huang-Huai-Hai region in China.

Conclusion

Long-term (19-years) addition of chemical fertilizers in combination with organic manure improved the C-growth rate of soybean plants and, consequently, the amount of plant-derived C accumulated in soils of Northeast China. The combination of inorganic and organic fertilizers had a much larger effect on plant productivity and C sequestration than any combination of inorganic application of N, P and K, alone. The application of P and organic manure played a key role in promoting the transfer and accumulation of photosynthetically assimilated C in soils. Over one growing season, the equivalent of ~564 kg ha⁻¹ photosynthetically assimilated C was accumulated in NPKM fertilized soils, ~380 kg ha⁻¹ C in NPK fertilized soils and only ~160 kg ha⁻¹ C in the control treatment. We also found a highly significant positive relationship between plant C-growth rate and the amount of C transferred to soils across all treatments. Our results show NPKM application would significantly contribute to C sequestration in typical agricultural soils of Northeast China.

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Fotosintetiškai fiksuotos anglies kiekis sojų pasėlyje, pamatuotas naudojant ^{13}C , po juosvažemio ilgalaikio tręšimo Šiaurės Rytų Kinijoje

Y. Qiao¹, L. C. R. Silva², X. Han¹, S. Miao¹, C. Yan³, W. R. Horwath²

¹Kinijos mokslų akademijos Šiaurės Rytų geografijos ir agroekologijos institutas

²Kalifornijos universiteto Žemės, oro ir vandens išteklių katedra, JAV

³Liaoning žemės ūkio mokslų akademijos Augininkystės institutas, Kinija

Santrauka

Fotosintetiškai fiksuotos anglies (C) pasiskirstymo sistemoje augalas–dirvožemis po tręšimo supratimas yra labai svarbus siekiant tinkamai ūkininkauti žemės ūkio paskirties žemėje. Tyrimo tikslas – išanalizuoti sojos augalų fiksuotos C pasiskirstymą ir nustatyti jo atsaką į ilgalaikį tręšimą. Atliktas pupinių augalų žymėjimo ^{13}C tyrimas, panaudojus sojas, augintas juosvažemyje (*Phaeozem*, PH), ir penkis tręšimo variantus: neorganinis tręšimas (NPK, NP, NK), neorganinis bei organinis tręšimas (NPKM) ir netręšta (kontrolinis variantas, CK). ^{13}C žymėjimas atliktas pilnos ankštaros tarpsniu (R_d). Tyrimo rezultatai parodė, kad tręšimas visuomet didino anglies kaupimąsi sojos augalų biomasėje, o didžiausias kiekis (35%) anglies nustatytas NPKM variante; šio rodiklio mažesnės vertės buvo nustatytos NP, NK ir CK variantuose nei NPK variante. Žymėtos suminės fiksuotos anglies kiekis augale svyravo nuo 8,6 iki 21,4 mg ^{13}C , o didžiausios vertės nustatytos NPKM variante. Daugiau nei 4/5 fiksuotos ^{13}C ūgliuose buvo rasta iškart po žymėjimo. Žymėta anglis transportuota iš ūglių į šaknis, 53,3 % jos liko ūgliuose, o 7,5 % aptikta šaknyse 7 dieną po žymėjimo. Suminės fiksuotos anglies, įterptos į dirvožemį, kiekiui taip pat turėjo įtakos tręšimas. Palyginus su CK, ^{13}C įterpimas į dirvožemį buvo 24,4, 14,8, 18,2 ir 50,8 % atitinkamai NP, NK, NPK ir NPKM variantuose. Priklausomai nuo tręšimo, suminės anglies kiekis dirvožemyje keitėsi nuo 160 iki 564 kg ha⁻¹ C per sezoną. Tai rodo, kad iš sojos augalų gautos anglies susikaupimas po tręšimo NPKM smarkiai padidintų anglies lygį Šiaurės Rytų Kinijos juosvažemyje.

Reikšminiai žodžiai: ^{13}C , dirvožemio anglis, tręšimas, juosvažemis, soja.