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RESEARCH ARTICLE

Estimates on nitrogen uptake in the subsequent wheat by above-ground and root residue and rhizodeposition of using peanut labeled with ^{15}N isotope on the North China Plain

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Abstract

Leguminous crops play a vital role in enhancing crop yield and improving soil fertility. Therefore, it can be used as an organic N source for improving soil fertility. The purpose of this study was to (i) quantify the amounts of N derived from rhizodeposition, root and above-ground biomass of peanut residue in comparison with wheat and (ii) estimate the effect of the residual N on the wheat-growing season in the subsequent year. The plants of peanut and wheat were stem fed with ^{15}N urea using the cotton-wick method at the Wuqiao Station of China Agricultural University in 2014. The experiment consisted of four residue-returning strategies in a randomized complete-block design: (i) no return of crop residue (CR0); (ii) return of above-ground biomass of peanut crop (CR1); (iii) return of peanut root biomass (CR2); and (iv) return of all residue of the whole peanut plant (CR3). The 31.5 and 21% of the labeled ^{15}N isotope were accumulated in the above-ground tissues (leaves and stems) of peanuts and wheat, respectively. N rhizodeposition of peanuts and wheat accounted for 14.91 and 3.61% of the BG^{15}N, respectively. The ^{15}N from the below-ground ^{15}N-labeled of peanuts were supplied 11.3, 5.9, 13.5, and 6.1% of in the CR0, CR1, CR2, and CR3 treatments, respectively. Peanut straw contributes a significant proportion of N to the soil through the decomposition of plant residues and N rhizodeposition. With the current production level on the NCP, it is estimated that peanut straw can potentially replace 104 500 tons of synthetic N fertilizer per year. The inclusion of peanut in rotation with cereal can significantly reduce the use of N fertilizer and enhance the system sustainability.

Keywords: cropping system, crop residues, rhizodeposition, ^{15}N labeling

1. Introduction

In modern agriculture, nitrogen (N) fertilizers are widely used to increase the yield in non-legume crops (Peña and Pueyo 2012; Zhang *et al.* 2013). Large amounts of N fertilizer input in crop production has detrimental consequences to the environment; nitrate leaching may pollute underground

water; N_2O emissions contribute to climate change (Yang *et al*. 2014). Biologically fixed N can act as a sustainable source of N and can complement or replace N fertilizer inputs (Garg and Geetanjali 2007). Legume roots produce free nitrogen atoms from their N_2 form through two continuous processes, N_2 fixation and N rhizodeposition. The released N from legume roots could be used by an adjacent crop in intercropping systems (Hu *et al*. 2017), or by a subsequent crop grown in rotation (Gan *et al*. 2014). Therefore, the inclusion of legumes into rotations or intercropping systems is regarded as an alternative approach to reduce N fertilizer input in agroecosystems (Fustec *et al*. 2010; Gan *et al*. 2015; Tang *et al*. 2018). Numerous studies have shown that diversifying crop rotation with the inclusion of legumes could increase crop productivity, improve soil fertility (Yuan and Xu 2012; Qiu *et al*. 2014; Yang *et al*. 2014), and enhance environmental sustainability (Gan *et al*. 2014). The peanut nodules play a vital role for enhancing N_2 fixation and transferring N from the rhizosphere to the soil (Zgadzaj *et al*. 2016).

The North China Plain (NCP) is one of the major grain production areas in China, where farmers usually apply more than 300 kg N ha^{-1} to their cereal crops annually. Use of large amounts of N fertilizer has led to the loss of soil fertility (Zhang *et al*. 2011) and the degradation of soil quality (Zhang *et al*. 2013; Su *et al*. 2014; Shi *et al*. 2016). A key strategy in the sustainable agricultural development is to minimize the input of synthetic fertilizers and improve nutrient use efficiency in cropping systems. Rotating leguminous crops with cereals, coupled with the use of appropriate tillage practices, have been found to be an effective strategy (Zhang *et al*. 2008; Fustec *et al*. 2010; Wang *et al*. 2016). One of the most commonly used rotation models on the NCP is peanut (*Arachis hypogaea* L.) in rotation with winter wheat (*T. aestivum* L.).

Peanut is an important economic crop in China, and the NCP is one of the largest peanut producing areas. Peanut straw can be used for feed processing (Zhao *et al*. 2017), and it can be properly managed on farm level to increase the renewable N resource on the farming systems (Fischer *et al*. 2002; Van den Putte *et al*. 2010; Preissel *et al*. 2015).

Studies have shown that the rhizodeposition of leguminous crop could have a significant impact on soil fertility throughout the world (Fustec *et al*. 2010; Høgh-Jensen 2012). Therefore, exploration the rhizodeposition of peanut on the effect of soil fertility would be necessary in China. In the present study, we designed both field and micro-plot experiments in which ^{15}N-labeling technique was used to explore the regulation of nitrogen translocation in a legume-cereal rotation system. The purposes of this study were (i) to quantify rhizodeposition N, and the uptake and distribution of nitrogen between the plant and soil; and (ii)

to investigate the effect of N derived from rhizodeposition (NdfR) of peanut residues on the peanut plant growth and productivity and the performance of the subsequent wheat crop following peanut in the rotation.

2. Materials and methods

2.1. Study sites

The NCP is located between 34°46′–40°25′N and 112°30′–119°30′E, and this region includes parts of five locations, Beijing, Tianjin, Hebei, Shandong, and Henan, and has an area of 139 200 km² and a population of 133 million people (Liang *et al*. 2009; Zhang *et al*. 2013; Pei *et al*. 2015). The study was conducted at the Wuqiao Station of China Agricultural University, in Wuqiao County (37°37′N, 116°26′E, altitude 18–21 m), Hebei Province, a representative area of the agricultural production and climate conditions in the central NCP (Fig. 1). The field experiments were conducted from 2014 to 2015. The soil is sandy clay loam, with pH of 8.2, contained 10.09 g kg^{-1} of organic matter, 0.78 g kg^{-1} of total N, 34.5 mg kg^{-1} of Olsen phosphorus, and 87.49 mg kg^{-1} of available potassium.

2.2. Weather conditions

The experimental area has a warm, temperate, and semi-humid monsoon climate with an average annual temperature of 12.9°C and a mean precipitation of 562 mm (56% during July and August). The annual accumulated temperature (≥0°C) to 4 826, 201 days of the frost-free period, and the annual number of sunshine hours was 2 724. Meteorological data for the period 2011–2016 were provided by Wuqiao County Meteorological Bureau. During the experimental period 2014–2015, there was 475.2 mm of precipitation, and the average temperature was 14.4°C. The average annual rainfall from 2011 to 2016 was 558 mm. During the growing seasons, we irrigated with 66.7 mm water twice per year, in mid-March and mid-October.

2.3. Experimental design and crop management

The study was conducted in a peanut and winter wheat rotation system from 2014 to 2015. The experiment consisted of four residue-returning strategies in a randomized complete-block design: (i) no return of crop residue (CR0); (ii) return of above-ground biomass of peanut crop (CR1); (iii) return of peanut root biomass (CR2); and (iv) return of all residue of the whole peanut plant (CR3); each with four replicates. The main treatments were implemented for wheat, and the micro-plot included the ^{15}N-labeled crop residue from the previous peanut. The peanut and winter
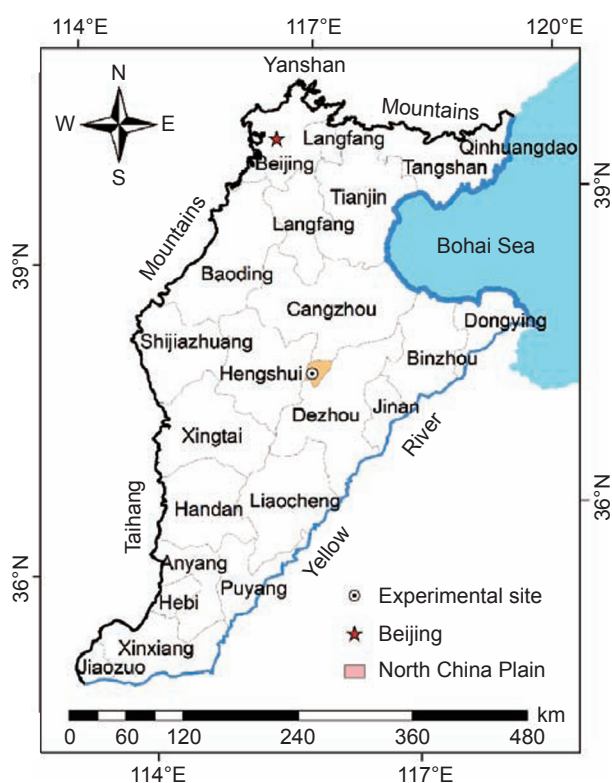
Fig. 1 Location of the North China Plain.

wheat varieties used in this experiment were Jihua 4 and Jimai 22, respectively.

The micro-plot (with an area size of 0.2 m×0.2 m; and a depth of 50 cm) was established using plastic film in the summer of 2014. The surface soil (0–30 cm) from the field was collected, air-dried, and passed through a 2-mm sieve to remove gravel and plant residue. Approximately 18 kg of dry soil was placed in each micro-plot and watered to maintain a similar water content as in the field. The micro-plots were treated the same as the field plot, but the peanut plants were labeled with the cotton-wick method, which an isotope solution was taken up by the whole plant *via* a cotton wick that passes through the plant stem (Russell and Fillery 1996). This method has been used for the ^{15}N labeling isotope of leguminous plants by other researchers (Arcand *et al*. 2014; Chalk *et al*. 2014). A ^{15}N urea solution (99 atom% ^{15}N) was taken up from a reservoir by a wick, which was passed through a hole into the stem. The labeling solution was 0.3% (w/v) ^{15}N-enriched (99 atom%) urea. The solutions were prepared separately using sterile deionized water under aseptic conditions with twice the target concentration and were mixed together in the injection bottle when the labeling system was set (Yang *et al*. 2012; Zang *et al*. 2015). All the materials used in the system were sterilized for 20 min at 121°C.

Peanut plants were stem fed with ^{15}N urea using the cotton-wick method (Mahieu *et al*. 2009). The ^{15}N urea (99 atom% ^{15}N) solution was taken up from a reservoir by a wick, which was passed through a hole into the stem. The hole was made in the middle of the internode located 3 to 5 cm from the soil surface, and the wick was passed through the hole. Labeled urea was supplied continuously, and the reservoir was supplied with 1 mL of 0.3% ^{15}N urea solution once a week (total four times) at the flowering and pegging stages for peanut and at the jointing stage for wheat. After absorption, the reservoir was washed with 1 mL of deionized water, which was absorbed by the plant.

2.4. Method of sampling and determination

During sampling, the micro-plots were first removed from the field, and the entire above-ground portion of each plant was cut from the soil surface (Fig. 2). Peanuts and winter wheat were separated into grains/nuts, leaves, stems, shell/glume, and peg. The soil and roots were also sampled at the same time. Subsequently, the soil was passed through a 2-mm sieve, and all visible roots were collected manually (Chen *et al*. 2011). The sieved soil was thoroughly mixed, and a subsample of the soil was obtained for analysis. After being dried at 75°C for at least 72 h or to a constant weight, the soil sample was weighed and the ^{15}N content was determined. The samples were then ground to a fine powder using a centrifuge mill and a ball mill, and then the total N content and ^{15}N enrichment in the plant and soil samples were determined using an isotope ratio mass spectrometer (Vario EL, Elementar, Germany) coupled with a Vario PYRO Cube Elemental Analyzer (Elementar, Germany). Plant samples were dried at 75°C to a constant weight, and the dry matter weight was determined.

2.5. Calculation formula

Excess values of atom% ^{15}N in soil and roots were determined by subtracting the atom% ^{15}N values from the soil and roots of non-labeled natural abundance control plants from the atom% ^{15}N values of the ^{15}N-labeled plants (Arcand *et al*. 2014).

The percentage of soil N derived from rhizodeposition (%NdfR) was calculated according to the following equation (Janzena and Bruinsmaa 1989; Laberge *et al*. 2011; Li *et al*. 2015):

$$NdfR\ (\%)=A/B\times100 \tag{1}$$

Where, A is the excess atom% ^{15}N of the soil and B is the excess atom% ^{15}N of the roots.

$$NdfR=N_{total}\ (mg/plant)\times NdfR\% \tag{2}$$

Where, N_{total} is the total N in the bulk and rhizosphere soil fractions. These calculations do not consider N
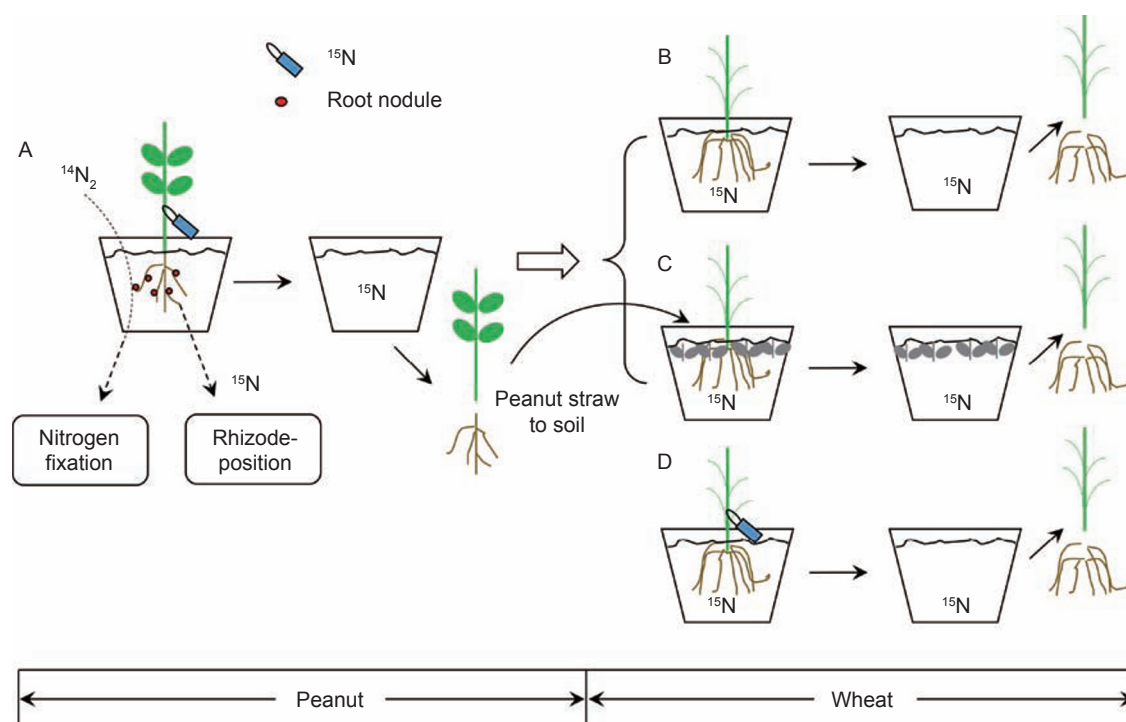
Fig. 2 Use of nitrogen (N) fixed by peanuts explains how the peanut rhizodeposition of N in traditional legume-cereal rotation systems can be used without increasing N loss to the environment and without decreasing crop yield. A, root exudate (peanut roots secrete substances into the soil), and peanuts were marked using the ^{15}N isotope. B, no crop residue isotope was transferred to the different plant parts of crops from N derived from rhizodeposition (NdfR). C, peanut residues decomposed in the soil, and crop rotation absorbed root exudates from the soil by the previous season's peanut crop. D, wheat was marked using the ^{15}N isotope.

reabsorption; thus, they represent net N rhizodeposition.

$$NdfR \ transfer \ (\%)=(C-D)\times 100 \tag{3}$$

Where, NdfR transfer (%) is the value of excess atom% ^{15}N of the subsequent wheat crop, C is the atom% ^{15}N in wheat grown on ^{15}N-labeled peanut below-ground residues (BGR), and D is the atom% ^{15}N in wheat grown on non-labeled peanut BGR. The excess atom% ^{15}N of peanut BGR was assumed to equal the excess atom% ^{15}N in the roots (Mayer and Heß 2003).

$$RN_{total} \ (kg \ ha^{-1})=Soil \ total \ N \ (g \ kg^{-1})\times Soil \ depth \ increment \ (cm)\times Soil \ bulk \ density \ (g \ cm^{-3})\times Ndff_{soil}/10^{-6} \tag{4}$$

Where, RN_{total} is the N added from rhizodeposition present in the soil.

The excess values of atom% ^{15}N of peanut and wheat BGR were assumed to equal the excess atom% ^{15}N in the roots.

2.6. Data analysis

Data were processed using Excel (version 2013, Microsoft Inc., USA) and one-way analysis of variance was performed using SPSS (version 13.0, Systat Software Inc., USA). In the analysis, crop traits were considered a fixed effect and block (replicate) as a random effect. Comparisons of mean effects were performed using Tukey's honestly significant difference test. Origin (Vision 8.6, Systat Software Inc., USA) was used for graphics rendering.

3. Results

3.1. Biomass and N uptake of cotton-labeled peanut and wheat

Compared with peanut, wheat plants had lower biomass in each plant component, but root biomass was 1.22-fold (Fig. 3-A). The root to above-ground biomass ratios were 0.06 for peanut and 0.45 for wheat. Total plant N uptake was greater for peanuts than for wheat due to high N accumulation in the above-ground parts of peanut with the greatest differences in N uptake being in the biomass of individual plants (Fig. 3-B).
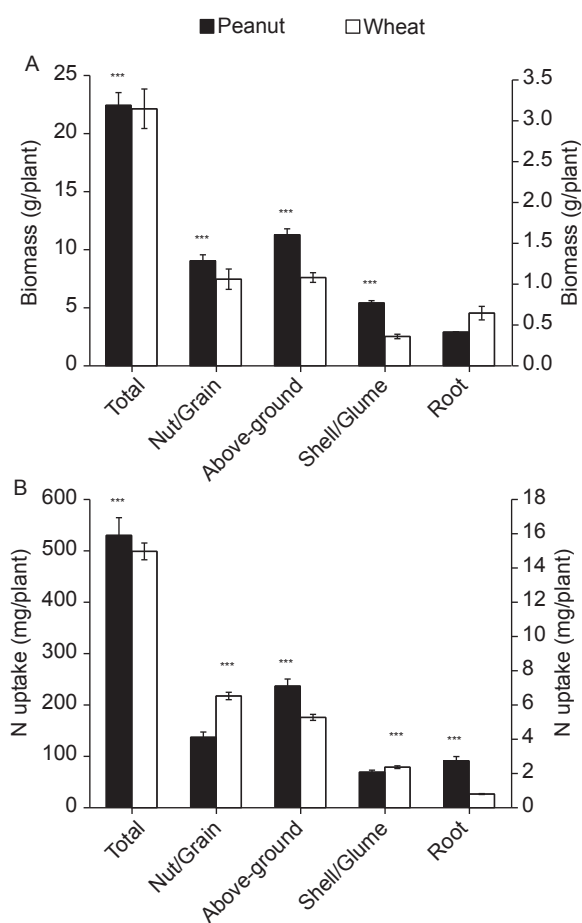
This study investigated ^{15}N isotope recovery and distribution in crop plant (Table 1), where the excess atom% ^{15}N was the highest in the stems of peanut, at 8.23%, and the other parts of peanut were between 0.69 and 8.23%. In comparison, wheat plant excess atom% ^{15}N was higher than that of peanuts with the grain and stem excess atom% ^{15}N being 5.69 and 6.44%, respectively. The general

trend of excess atom% ^{15}N for different plant parts was stems>grain/nuts>shell/glume>underground plant N. The total ^{15}N recovery of peanut plants was 52.07%, which was lower than that in wheat, at 68.19%. Peanut and wheat stems contained approximately 20% of the marked isotopes. In peanuts, approximately 31.5% of the recycled marked isotopes were distributed in the above-ground residue (leaves and stems), and the percentage in wheat was approximately 21%. In peanut, rhizosphere nitrogen deposition accounted for 14.91% of the below-ground ^{15}N (BG^{15}N), whereas in wheat, the value was 3.61%. These findings suggested that peanut plants are involved in the secretion of nitrogen-containing organic matter to the soil in a more profound manner than wheat.

3.2. ^{15}N distribution ratios within the plants

Relative to the total amount of ^{15}N in the crop residues remaining in the soil system following seed harvest, the

above-ground plant parts contributed the highest proportion of recovered ^{15}N for peanuts (Fig. 4-A).

The largest proportion of marked isotope was in the grains (wheat) and nuts (peanuts). Wheat grains had the highest percentage at 66.12%, significantly greater than that in peanuts at 43.34% in the nuts (Fig. 4-B). The quantity of the BG^{15}N was 4.96 mg/micro-plot for peanuts and 2.41 mg/micro-plot for wheat. In total, the CR0, CR1, CR2, and CR3 treatments supplied 11.3, 5.9, 13.5, and 6.1% of the ^{15}N from the BG^{15}N of peanuts, respectively, to the subsequent wheat crop in rotation. There was no difference in ^{15}N recovery in wheat roots grown on the two BG^{15}N, but a higher percentage of ^{15}N from the BG^{15}N of peanuts was supplied to wheat grains and shoots.

### 3.3. ^{15}N uptake on subsequent wheat derived from ^{15}N-labeled peanut

The uptake of nitrogen derived from ^{15}N-labeled peanut residues is shown in Fig. 5-A. The contents of ^{15}N isotopes in the CR1 and CR3 of the peanut season were higher than those of the other two treatments. During the wheat growing season the following year, the accumulation of ^{15}N isotopes in wheat plants was in the order of CR3>CR1>CR2>CR0.

Nitrogen derived from ^{15}N-labeled peanut residues and rhizodeposition with different treatments on wheat (Fig. 5-B). The N content of wheat from NdfR was 1.9 kg ha^{-1} when grown on peanut BG^{15}N. The rhizodeposition-N of peanut

Fig. 3 Biomass (A) and N uptake (B) of mature peanut and wheat grown in field micro-plots. Black histogram refer to the left *y*-axis, and white histogram refer to the right *y*-axis. Error bars represent standard error of the mean (*n*=3). *** indicates a significant difference between peanuts and wheat at *P*≤0.001.

Table 1 Enrichment with ^{15}N, recovery and distribution of the ^{15}N label, and distribution of plant N in peanuts and wheat

Item[1]	Atom% ^{15}N excess	Recovery of ^{15}N	Distribution of recovery ^{15}N	Distribution of total N
Peanut (%)				
Nuts	3.673±1.370	24.82±3.21	43.34±5.61	40.62±9.48
Shells	2.535±0.796	3.95±0.18	6.90±0.32	8.98±2.97
Pegs	2.014±0.368	0.59±0.19	1.02±0.33	1.63±0.74
Stems	8.226±1.661	11.24±1.32	19.64±2.30	7.48±1.03
Leaves	1.330±0.448	5.18±0.99	9.05±1.73	21.84±4.33
BG^{15}N	0.022±0.014	9.79±0.92	17.10±1.60	–
Roots	0.694±0.104	0.23±0.10	0.41±0.18	1.76±0.49
NdfR	–	1.46±0.01	2.54±0.02	17.68±4.78
Total	–	52.07±5.17	100	100
Wheat (%)				
Grains	5.690±0.318	45.09±2.46	66.12±3.60	58.36±5.50
Glumes	2.803±0.017	2.73±0.32	4.01±0.47	7.18±0.52
Stems	6.441±1.047	12.19±0.21	17.88±0.31	13.95±0.34
Leaves	2.215±0.105	2.12±0.13	3.11±0.19	7.05±1.13
BG^{15}N	0.011±0.002	5.27±0.57	7.73±0.84	–
Roots	1.006±0.059	0.60±0.19	0.87±0.28	4.35±0.91
NdfR	–	0.19±0.04	0.19±0.06	9.13±1.10
Total	–	68.19±3.42	100	100

[1] BG^{15}N, below-ground ^{15}N; NdfR, N derived from rhizodeposition. Values are mean±SE (*n*=4). –, missing value.
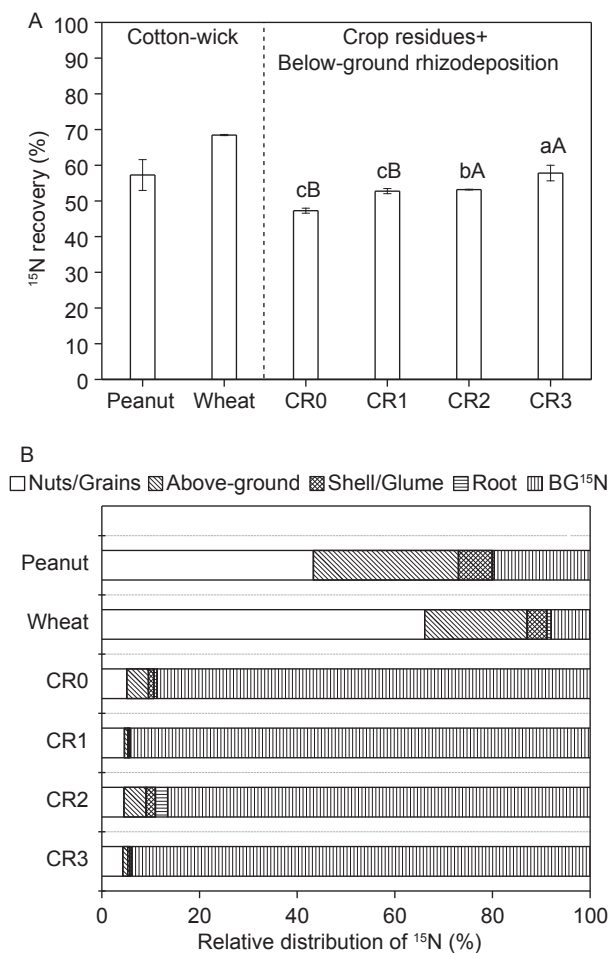
Fig. 4 Recovery of below-ground ^{15}N (BG^{15}N) of the previous peanut crop in subsequently mature wheat grown in the micro-plot. Different letters indicate significant differences between the treatments CR0, CR1, CR2, and CR3 ($P \leq 0.05$ and $P \leq 0.01$). CR0, no return of crop residue; CR1, return of above-ground biomass of peanut crop; CR2, return of peanut root biomass; CR3, return of all residue of the whole peanut plant. Error bars represent the standard error of the mean (n=3). Distribution of ^{15}N (A) and relative ^{15}N (B) in crop residues, including above-ground, root, and N derived from rhizodeposition and supplied with ^{15}N-urea using the cotton-wick labeling method.

was higher than that of wheat. The CR1 and CR3 involved the return of peanut straw to the soil, which affected the amount of N absorbed by the subsequent wheat crop, and the N content of the CR3 treatment was the highest, with residue input levels of 4.3 and 2.4 kg ha^{-1}. However, the CR2 and CR3 returned 1.7 and 0.9 kg ha^{-1} of peanut straw to the soil, respectively. Whereas the biomass of the subsequent wheat crop increased with increasing N uptake in wheat when grown on peanut BG^{15}N, there was no significant relationship between the N content of wheat and wheat biomass when grown on the BG^{15}N of peanuts. Nevertheless, these estimates were based on only small amounts of residual N with absorption by the following crops.

By using ^{15}N-marked peanut crop residues in a micro-plot experiment, these experiments have shown that the peanut provided approximately 2.1–25.0% of the straw-N and rhizodeposition-N to the subsequent wheat.

4. Discussion

4.1. N allocation in the crop plant and soil system

The cotton-wick method did not affect the N and biomass partitioning. Several studies have demonstrated N allocation in peanut plants and in the soil (Wichern *et al*. 2008). The N content of the wheat from NdfR was 1.9 kg ha^{-1} when grown on the BG^{15}N of peanuts. The importance of NdfR is undisputed, but reliable quantitative data are sparse, as no methodology is available to clearly distinguish rhizodeposition due to the death and decay of below-ground tissues from rhizodeposition due to the exudation of soluble compounds (Peña and Pueyo 2012). The allocation of root ^{15}N to rhizodeposition occurs through the phloem and the roots. With different isotopic labeling methods, ^{15}N enrichment is generally greater in the above-ground parts than in the roots (Chalk *et al*. 2002). The N rhizodeposition in this study was calculated from the relationship between the ^{15}N enrichment in the roots and soil (Li *et al*. 2015). According to the 2015 experiment, approximately 35.7 kg ha^{-1} of the N rhizodeposition was transferred to the soil by peanuts. This finding indicates that peanuts contribute substantially to soil fertility.

4.2. N transfer from peanut to subsequent wheat under different residue-returning strategies

The ^{15}N of the different treatments from the BG^{15}N in peanut (CR0, CR1, CR2, and CR3) were 11.3, 5.9, 13.5, and 6.1%, respectively; this N was available to the subsequent wheat crop grown in the rotation. Compared with CR0, approximately 1.7, 0.9, and 2.4 kg ha^{-1} of the N in the subsequent wheat was derived from the residues of preceding peanut plants in CR1, CR2, and CR3, respectively. These values represent, respectively, about 0.64, 0.40, and 0.88% of the total N in the subsequent wheat plants. Our findings indicated that despite of possible nitrogen losses in legume-cereal rotation systems (Chalk *et al*. 2014), peanut residues returning back to the soil were immediately immobilized by soil microbes, and the released N from the legume residue decomposition was readily available to the growing cereal plants.

Wheat recovery of ^{15}N from the ^{15}N-labeled peanuts (52.07%) and wheat (68.19%) were not particularly high. But it was similar to the ^{15}N recoveries reported from crop residues in previous studies (Yang *et al*. 2012; Zang
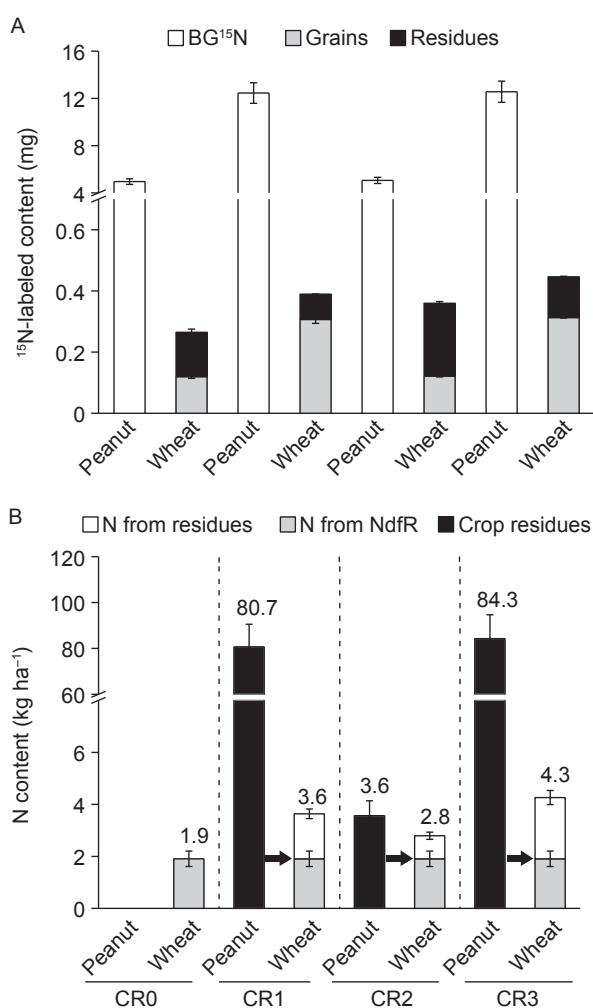
Fig. 5 ^{15}N-labeled content (A) and N content (B) uptake of nitrogen derived from ^{15}N-labeled peanut residues and rhizodeposition with different treatments on wheat. CR0, no return of crop residue; CR1, return of above-ground biomass of peanut crop; CR2, return of peanut root biomass; CR3, return of all residue of the whole peanut plant. BG[15]N, below-ground ^{15}N; NdfR, N derived from rhizodeposition. Error bars represent the standard error of the mean (*n*=3).

et al. 2015). For example, their researches had 52.1 to 67.3% of ^{15}N of the above-ground and root components of ^{15}N-labeled mung bean and oat were recovered in cropped or intercropped systems. When rhizodeposition, roots, and above-ground residues of the different legume crops were included, ^{15}N recoveries can range from 8 to 20% in the subsequent cereal crops (Mayer *et al.* 2003; Arcand *et al.* 2014).

The magnitude of N release from crop residues depends on many factors, such as bacteria in the soil, root herbivory, mycorrhizal fungi and their ability in straw decomposition (Mayer and Heß 2003; Esther *et al.* 2014; Li *et al.* 2015). In the present study, we found a large amount of N derived from ^{15}N-labeled peanut residues and the amount of N

transferred into the soil by legumes accounted for 3.1 to 7.1% of the total N by the subsequent cereal crops. Similar results have been reported by other researchers for different legumes such as lentil (*Lens culinaris*) and cowpea (*Vigna unguiculata*) (Laberge *et al.* 2011; Arcand *et al.* 2014).

5. Conclusion

Using the ^{15}N-labelling technique, we found that the N content of wheat from NdfR was 1.9 kg ha^{−1} when grown on peanut BG[15]N, and 0.9 to 2.4 kg ha^{−1} from peanut straw-N. Meanwhile, the total nitrogen content of peanuts was 1.4 times more than wheat in the legume-cereal rotation system on the NCP. The rhizodeposition-N from peanut roots was averaged 35.7 kg ha^{−1}, significantly greater compared with wheat (13.5 kg ha^{−1}). Therefore, we consider that the legume-cereal rotation in combination with peanut straw returning back to the soil is an effective approach to enhance N use in crop production. For the long term, the legume-cereal rotation may help improve soil quality because of the large amounts of root rhizodeposition. More detailed research is needed to enhance our understanding of the role of the N immobilization associated with the decomposition of plant residues and N rhizodeposition in the rotation systems. Enhanced use of soil residual N will aid in the sustainable development of agricultural systems on the NCP.

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