Characteristics of Nitrogen Uptake, Use and Transfer in a Wheat-Maize-Soybean Relay Intercropping System

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Abstract: Intercropping and relay intercropping systems, which significantly improve land use efficiency, are used worldwide to increase crops yield. The wheat-maize-soybean relay intercropping system has been widely employed by famers in Southwestern China for years, but the detailed mechanisms through which the nitrogen fertilizer use efficiency reach the high level in this system remain unclear. In the present study, two separate pot experiments were performed by 15N isotope dilution (ID) labeling and direct 15N foliar feeding (FF) assays, and a solid barrier was employed to prevent the roots intergrowth and N movement among crops in the first experiment, using no barrier as the control. The results showed that, under the no-barrier condition, the grain yields, 15N uptake and 15N recovery efficiency of wheat and maize were significantly increased, but those measures in soybean were decreased compared to the solid barrier condition. Furthermore, bi-directional N transfer was detected during the co-growing stage of crops, the amount (Ntransfer) and percentage (%NT) of 15N transferred varied significantly with the fertilizer-N rate, and the maximum reached at 150 – 300 kg N ha⁻¹ level. The Ntransfer from maize to wheat was 16.1% – 163.0% higher than that from wheat to maize; the Ntransfer from soybean to maize was 1.7 – 6.0 times higher than those from maize to soybean, while the %NT from soybean to maize were 6.7 – 22.2 times higher than those from maize to soybean. Conclusively, this study revealed that the interaction of the roots among crops significantly increased the uptake efficiency and recovery efficiency, and further, the positive N competition and bi-directional N transfer of each crops were the main contributors to improve the N use efficiency in the wheat-maize-soybean relay intercropping system.

Key words: 15N, N uptake, N transfer, Relay intercropping, Interspecific competition.

The global human population has been projected to reach 10 billion by the year 2050, and therefore, the demand for food will continue to increase for another 40 years to meet the needs of food for the growing population growth (Tilman et al., 2002; Godfray et al., 2010). In China, the annual crop production should be approximately 580 Mt by the year 2030 with a 2% annual increase to feed the growing population (Fan et al., 2012). Despite having the largest population in the world, China has lower per capita arable land area and urbanization exacerbates problems related to the limited availability of arable land (Tan et al., 2005). Consequently, increasing food production on currently available farmland is becoming a major challenge in China. Furthermore, implementing efficient agronomic practices with the goal of increasing the multiple crop index of land is of dire importance (Yan et al., 2010). Diverse studies demonstrated that relay intercropping and intercropping are two practical and desirable multi-cropping systems, which markedly enhance crop yield, increase farmers’ income and improve land-use efficiency (Li et al., 2006; Zhang et al., 2011; Gao et al., 2010; Li et al., 2001; Zhang et al., 2003; Xiang et al., 2012). Consequently, these systems are widespread in the North China Plain, the Yellow and Huai River Valleys, and the Southern China, including the wheat-maize relay intercropping system (Knörzer et al., 2011; Li et al., 2001), wheat-faba bean intercropping system (Xiao et al., 2004), wheat-soybean strip intercropping system (Li et al., 2001), rice-peanut intercropping system (Shen and Chu., 2004), maize-soybean strip intercropping system (Yang et al., 2014) and alfalfa-maize intercropping system (Zhang et al., 2011). Importantly, the optimal combination
of relay intercropping and intercropping may reduce the fertilizer inputs, and thus, minimize environmental pollution (Exner et al., 1999).

Complementary N use (Chalk, 1998) and interspecific competitiveness (Li et al., 2001; Zhang et al., 2011) allow legume-cereal intercropping to result in increased N uptake and yield in both cereals and legumes (Li et al., 2001; Eaglesham et al., 1983). Different crops possess distinct resource requirements. For example, legumes use N₂ while cereals use NO₃⁻-N and NH₄⁺-N; this alleviates the competition for resources allowing both crops to meet their N requirements (Vandermeer, 1992). Therefore, growing crops in an intercropping system enhances N use efficiency by complementation rather than competition (Xiao et al., 2004). In addition, N transfer from legumes to cereal also increases the N use efficiency (Stern et al., 1993).

The increasing economic and environmental pressures of the excess N inputs have led researchers to focus on N transfer between crops (Elgersma et al., 2000; Chu et al., 1993). Previous studies revealed that N use efficiency was improved in faba bean-maize and wheat-faba bean intercropping systems through interspecific N transfer from legume to the companion cereal crops (Li et al., 2003; Xiao et al., 2004). In detail, the intercropped legume could transfer 40 kg of N ha⁻¹ to the associated maize crop (Willey et al., 1979). The N-fixing root nodules of cowpea transferred 24.9% of N to maize in the maize-cowpea intercropping system (Eaglesham et al., 1983). ¹⁵N dilution and ¹⁵N-leaf-labeling are the universal techniques used to estimate N transfer in the intercropping system (Xiao et al., 2004; Sierra et al., 2007; Høgh-Jensen, 2006).

The wheat-maize-soybean relay intercropping system was widely employed by farmers for years, especially in Southwestern China. However, there are few studies that focused on the fertilizer utilization efficiency in this system (Xiang et al., 2012), and the mechanism of efficient use of N has not been revealed. In particular, it is unclear whether or not N recovery or crop N accumulation would be increased, whether competition for applied nutrients would have positive or negative effects on each crop species, and whether N transfer could be possible or impossible in the soil from one species to another. We designed root partitioning and ¹⁵N-isotope foliar feeding experiments to solve these questions. Our study should provide new insights into the movement of N as well as N use efficiency in a three crop intercropping system. Consequently, these results will help us precisely design relay intercropping system to maximize crop yield and meet the growing demand for food crops.

Materials and Methods

1. Experimental sites and plant materials

The experiments were performed from the year 2009 through 2011 in pots maintained in a glasshouse at the Research Farm of Sichuan Agricultural University (29°50' N, 105°00' E and 576 m a.s.l.), which is located in the Southwestern China. The subtropical humid climate of this region features a mean annual temperature of 16.2°C (data from 1971 to 2000) and a mean annual rainfall of 1200 mm. The frost-free period lasts approximately 300 days, and solar radiation during this period was 3750 MJ m⁻² y⁻¹.

Soil used in the experiments was collected from a fallow field on the experimental farm and was sieved with a 2 mm mesh. The typical purplish soil type had an organic matter content of 8.96 g kg⁻¹, total N content of 1.21 g kg⁻¹, nitrate N content of 62.35 mg kg⁻¹, total P content of 0.61 g kg⁻¹, Olsen-P content of 25.34 mg kg⁻¹, total K content of 11.44 g kg⁻¹, available K content of 63.7 mg kg⁻¹, and a pH in water of 6.55 (the ratio of soil to water is 1:2).

The winter wheat cultivar *Triticum aestivum* L. Chuannong 18, maize cultivar *Zea mays* L. Chuandan 418 and soybean cultivar *Glycine max* L. Gongxuan 1 were planted in the relay intercropping system. Wheat, maize and soybean materials were provided by the Agronomy College, the Maize Research Institute of Sichuan Agricultural University and the Zigong Institute of Agricultural Sciences, respectively.

2. Experimental design and crop management

(1) Experiment 1

This experiment was performed from 2009 to 2011, and a 2 × 2 factorial completely randomized with three replicates was employed, including root barrier patterns and varied fertilizer-N application rates. The fertilizer-N application rates were no N application (N0) and 4.8 g ¹⁵N-urea pot⁻¹ (N1, ¹⁵N atom excess of 10.24%), which was equivalent to 300 kg N ha⁻¹. ¹⁵N-urea was provided by the Chemical Industry and Research Institute of Shanghai. The root barrier patterns were designed according to the protocol described elsewhere with no barrier or solid barrier treatments (Xiao et al., 2004). Each pot was separated into two halves with a plastic sheet to prevent roots of different crop species from intergrowing. One compartment of each pot was planted with wheat and subsequently with soybean after harvest of wheat, while the other compartment was planted with maize.

The experiment was performed in 36 plastic pots with 15 kg air-dried soil capacity (34 cm in diameter and 55 cm in height). Among them, 12 N0 test pots were used to measure the abundance of naturally occurring ¹⁵N, and 24 pots were used with ¹⁵N application for measuring ¹⁵N abundance of labeled crops. 12 of 24 pots were fertilized...
with $^{15}$N-urea ($^{15}$N W.M) for the wheat and maize, soybean was fertilized with normal urea, and the other 12 pots were fertilized with $^{15}$N-urea for the soybean, wheat and maize with normal urea. The amount of N fertilizer applied for each crop was the same whether labeled by $^{15}$N-urea or not. For wheat, maize and soybean, the amount of urea was 1.37 g pot$^{-1}$, 2.74 g pot$^{-1}$ and 0.69 g pot$^{-1}$, respectively. The crops were labeled with $^{15}$N twice, once at the sowing date and again at flowering. To investigate N use efficiency, CO ($^{15}$NH$_2$) was dissolved in water, and then injected into the soil around the roots at the depth of 10 cm with a 25 mL syringe. All $^{15}$N labeling processes were strictly controlled to ensure that there was no cross contamination. In addition, N, P and K were also applied as follows: 0.6 g P$_2$O$_5$ pot$^{-1}$ and 0.8 g K$_2$O pot$^{-1}$ for wheat, 0.9 g P$_2$O$_5$, pot$^{-1}$ and 1.0 g K$_2$O pot$^{-1}$ for maize, 0.6 g P$_2$O$_5$, pot$^{-1}$ and 0.5 g K$_2$O pot$^{-1}$ for soybean, respectively.

Wheat was sown on 5 November 2009, maize on 25 March 2010, and soybean was sown on 11 June 2010. Dates of harvesting for wheat, maize and soybean were 12 May 2010, 3 August 2010 and 25 October 2010, respectively. Post-emergence densities were limited to six wheat, two maize, and two soybean plants per pot. In 2011, the crops were planted by rotation. Wheat and soybean were sown where maize had been sown previously, and maize was sown where soybean had been sown previously. The sowing dates of each crop in 2011 were the same as those in 2010, and the harvest dates of wheat, maize and soybean were 15 May, 5 August and 22 October, respectively.

(2) Experiment 2

The experiments were performed in 2010 and 2011. Fertilizer-N was applied at 2.4, 4.8, and 7.2 g urea pot$^{-1}$, which were equivalent to 150, 300, and 450 kg N ha$^{-1}$, respectively. Fertilizer-N was applied twice: one half was mixed evenly into the soil immediately prior to sowing, the other half was applied at the flowering stage as a top-dressing. All treatments were in a completely randomized design with three replicates. The pot size and planting model for experiment 2 were similar to the experiment 1.

The pots in Experiment 2 were divided into two groups. One group of pots was used to investigate the direct transfer of N among wheat, maize and soybean in a relay intercropping system. $^{15}$N-urea was labeled by foliar feeding (FF) for each crop, and then the abundance of $^{15}$N in crops was detected (Shen and Chu., 2004). The foliar application of $^{15}$N-urea with 10.24 atom % was given to wheat at the flowering stage, maize at jointing (co-growing with wheat) and silking (co-growing with soybean) stages, and soybean at the branching stage (co-growing with maize). Briefly, a 1% (w/w) solution of $^{15}$N-labelled urea with 10.24 atom % $^{15}$N enrichment was spread on the surface of the plant leaves. The labeling process was controlled strictly to avoid cross-contamination to other plants by enclosing the plants in PVC cylinders (25 by 50 cm) open at both ends, and the plant leaves were immediately covered with sealed polyethylene bags after spraying. The plants received a 14 mL $^{15}$N solution once a day for three days continuously, and the total volume was 42 mL pot$^{-1}$ (0.42 g $^{15}$N pot$^{-1}$). As $^{15}$N donor of wheat, the N transfer from wheat to the associated maize was calculated by detecting the amount of $^{15}$N in maize. Another group of pots with the same amount of urea without $^{15}$N labeling were used as a control to calculate the amount of background of $^{15}$N present under these experimental conditions.

3. Sampling and $^{15}$N analysis

Plant samples were collected from each pot after crops were harvested in the two experiments. All samples were divided into roots, stems and grains, and then were dried in a forced-draft oven at 80ºC to a constant weight. Total N content of plant samples were investigated by the micro-Kjeldahl analysis method, and the atomic % $^{15}$N value was determined by a ZHT-03 isotope mass spectrometer (Beijing Analytical Instrument Factory, Beijing, China). Control samples were also collected and analyzed in the same manner to provide background $^{15}$N concentrations.

4. Calculations

(1) Nutrient competitive ratio

The nutrient competitive ratio (NCR) reflects the competitive ability of different species (Willey and Rao, 1980), and has been used as an index of the advantage in nutrient uptake by one species in intercropping over the other; it can be calculated as follows (Morris et al., 1993; Li et al., 2001):

$$NCR_a = \frac{NU_a / (NU_a \times F_a)}{NU_a / (NU_a + NU_b)}$$

where $NU_a$ and $NU_b$ denote N uptake by species $C_a$ and $C_b$ in the sole cropping (solid barrier); $NU_a$ and $NU_b$ denote N uptake by species $C_a$ and $C_b$ in the sole cropping (solid barrier); $F_a$ and $F_b$ denote the proportions of the area occupied by crops ‘a’ and ‘b’ in the relay strip intercropping; the value of $F_a / F_b$ was 1.

(2) N transfer indices

The atomic % $^{15}$N was corrected for the atomic % $^{15}$N background. It was estimated based on the measurements made on control plants grown in separate pots not supplied with $^{15}$N and placed among the other plant containers. The percentage of N transfer (%NT) was defined as the recovery of the donor N in the receiver plant, and was calculated as described by Johansen and Jensen (1996), and by Shen and Chu (2004).
Table 1. Biomass of wheat, maize and soybean with different root barrier and fertilizer-N application rate treatments (Experiment 1).

| N treatments | Wheat grain | Wheat straw | Maize grain | Maize straw | Soybean grain | Soybean straw |
|--------------|-------------|-------------|-------------|-------------|---------------|---------------|
|              | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) |
| 2010 year    |             |             |            |             |             |            |             |             |            |             |             |            |             |             |            |
| N0\(^a\)    | 22.53 a     | 24.21 a     | 23.57 b    | 51.66 a     | 56.88 a     | 54.27 b     | 62.95 a     | 63.93 a     | 63.14 b     | 22.84 a     | 20.69 a     | 21.51 b     | 74.04 a     | 40.52 b     | 57.28 a     |
| N1          | 31.80 b     | 37.20 a     | 34.30 a    | 75.98 b     | 70.63 a     | 64.30 a     | 67.80 b     | 84.13 a     | 80.52 a     | 26.22 a     | 22.65 b     | 24.43 a     | 27.53 a     | 29.32 a     | 28.42 a     |
| Mean        | 27.16 b     | 30.70 a     | 29.71 a    | 61.74 a     | 65.43 a     | 64.69 a     | 69.63 a     | 74.03 a     | 69.45 a     | 24.28 a     | 21.67 b     | 24.01 a     | 27.59 a     | 29.32 a     | 28.42 a     |

2011 year

| N0\(^a\)    | 18.00 a     | 15.19 a     | 16.59 b    | 16.40 a     | 15.41 a     | 15.90 b     | 6.36 a      | 3.33 a      | 2.85 a      | 3.29 b      | 4.03 b      | 3.62 b      | 15.62 a     | 15.34 a     | 15.47 a     |
| N1          | 24.35 b     | 28.68 a     | 28.51 a    | 50.83 b     | 64.01 a     | 61.92 a     | 67.30 a     | 69.20 a     | 68.25 a     | 26.21 a     | 16.59 b     | 19.09 a     | 24.68 a     | 28.27 a     | 26.69 a     |
| Mean        | 21.17 b     | 23.93 a     | 22.67 b    | 33.99 a     | 33.76 a     | 32.47 a     | 50.10 b     | 54.77 a     | 53.63 a     | 18.61 a     | 15.96 a     | 17.06 a     | 27.99 a     | 30.02 a     |

\(^a\) N0 = No N fertilizer applied; N1 = 4.8 g urea pot\(^{-1}\).

\(^b\) Values are means of three replicate treatments. Different letters within the same row excluding the mean column indicate a significant difference at the \(P < 0.05\) level, and different letters within the mean column indicate a significant difference at the \(P < 0.05\) level.

Table 2. N uptake of intercropped wheat, maize and soybean with different root barrier and fertilizer-N application rate treatments (Experiment 1).

| N treatments | Wheat grain | Wheat straw | Maize grain | Maize straw | Soybean grain | Soybean straw |
|--------------|-------------|-------------|-------------|-------------|---------------|---------------|
|              | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) | Solid barrier | No barrier | Mean\(^b\) |
| 2010 year    |             |             |            |             |             |            |             |             |            |             |             |            |             |             |            |
| N0\(^a\)    | 0.26 a      | 0.29 a      | 0.27 b     | 0.14 a      | 0.16 a      | 0.15 b     | 0.34 b      | 0.50 a      | 0.42 b     | 0.46 b      | 0.61 a      | 0.54 b     | 1.61 a      | 1.50 a      | 1.55 b     |
| N1          | 0.47 b      | 0.65 a      | 0.56 a     | 0.49 a      | 0.51 a      | 0.49 a     | 0.63 b      | 0.92 a      | 0.77 a     | 0.63 b      | 0.72 a      | 0.67 a     | 1.91 a      | 1.59 b      | 1.75 a     |
| Mean        | 0.36 b      | 0.47 a      | 0.31 a     | 0.33 a      | 0.48 b      | 0.71 a     | 0.54 a      | 0.60 a      | 0.67 a     | 1.76 a      | 1.54 b      | 0.67 a     |

2011 year

| N0\(^a\)    | 0.26 a      | 0.19 b      | 0.22 b     | 0.07 a      | 0.08 a      | 0.07 b     | 0.07 a      | 0.05 a      | 0.05 b     | 0.16 a      | 0.15 a      | 0.15 b     | 0.82 a      | 0.78 a      | 0.80 b     |
| N1          | 0.36 b      | 0.61 a      | 0.48 a     | 0.16 a      | 0.22 a      | 0.19 a     | 0.41 b      | 0.59 a      | 0.49 a     | 0.38 b      | 0.45 a      | 0.41 a     | 1.72 a      | 1.32 b      | 1.51 a     |
| Mean        | 0.30 b      | 0.40 a      | 0.11 b     | 0.14 a      | 0.24 b      | 0.31 a     | 0.27 a      | 0.29 a      | 0.27 a     | 1.27 a      | 1.04 a      | 0.40 a     |

\(^a\) N0 = No N fertilizer applied; N1 = 4.8 g urea pot\(^{-1}\).

\(^b\) Values are means of three replicate treatments. Different letters within the same row excluding the mean column indicate a significant difference at the \(P < 0.05\) level, and different letters within the mean column indicate a significant difference at the \(P < 0.05\) level.

\[ %NT = \frac{15N \text{content}_{\text{receiver}} \times 100}{15N \text{content}_{\text{source}} + 15N \text{content}_{\text{donor}}} \]  

\[ \text{atom } %15N \text{Nexcess}_{\text{plant}} = \frac{\text{atom } %15N \text{Nexcess}_{\text{plant}} \times \text{total } N_{\text{plant}}}{\text{atom } %15N \text{Nexcess}_{\text{labelled } N}} \]  

\[ \text{atom } %15N \text{Nexcess}_{\text{plant}} = \frac{\text{atom } %15N \text{Nexcess}_{\text{labelled crop}} - \text{atom } %15N \text{Nexcess}_{\text{unlabelled crop}}}{\text{atom } %15N \text{Nexcess}_{\text{labelled crop}}} \]  

In Equation (5), the total amount of N (mg pot\(^{-1}\)) transferred from the donor, \(N_{\text{transfer}}\), was calculated as:

\[ N_{\text{transfer}} = \frac{%N_{\text{transfer}} \times \text{total } N_{\text{donor}}}{100 - %N_{\text{transfer}}} \]  

The percentage of N in the receiver derived from transfer (%NDFT) was calculated as formula (6).

\[ %\text{NDFT} = \frac{N_{\text{transfer}} \times 100}{\text{total } N_{\text{receiver}}} \]  

5. Statistical analysis

Analysis of variance (ANOVA) allowed analysis of the statistical significance of differences between treatments, and mean values were compared by least significance difference (LSD) using multiple comparisons with the General Linear Models Procedure of SPSS (version 15, SPSS, Chicago, IL, USA).
Results

1. Dry matter yields and N uptake

The results showed that both treatments, root barrier between wheat and maize and exogenous N application, significantly affected wheat growth and N uptake (Table 1, Table 2). There were no significant differences in dry matter and N uptake of straw and grain between solid barrier and no-barrier pots without N application. However, the dry matter and N uptake in the pots without barrier were significantly higher than those in the pots with a solid barrier with N application. Furthermore, in the treatments with N application, the yield and N uptake of wheat grain in the pots with no barrier were 17% and 38.3% higher in 2010, and 34.2% and 69.4% higher in 2011, respectively, compared to the pots with a solid barrier.

Maize growth was also influenced significantly by both root barrier and N application treatments (Table 1 and 2). Without exogenous N application, the dry matter and N uptake of maize grain were greater in the pots with no barrier than in the those with a solid barrier in 2010. This was reversed in 2011 (Tables 1 and 2). This difference can be attributed to intensive competition between maize and wheat. With exogenous N application, the dry matter and N uptake in maize plants in the pots with no barrier were significantly larger ($p < 0.05$), than in the pots with a solid barrier. The increase rate in maize grain yield and N uptake were 21.8% and 46.0%, respectively, in 2010, 7.0% and 43.9%, respectively, in 2011.

In contrast to wheat and maize, yield and N uptake of soybean grain were slightly lower in pots with no barrier than in pots with a solid barrier irrespective of N application (Tables 1 and 2). Without N application, the dry matter and N uptake of soybean grain and straw in the pots with a solid barrier were not significantly different from those in the pots with no barrier. With N application, however, there was a 13.6% and 16.8% decrease in soybean grain yield and N uptake, respectively, in 2010, and 23.2% and 23.3% decrease in 2011, respectively.

2. $^{15}$N uptake and recovery efficiency

$^{15}$N uptake in the straw, grain and root of wheat plants was significantly influenced by the root barrier treatment (Table 3). In the pots with no barrier, the $^{15}$N uptake in wheat grain and straw increased by 67.2% and 9.0%, respectively, compared with that in the pots with a solid barrier. The recovery efficiency of $^{15}$N showed a trend similar to that of $^{15}$N uptake, with a 38.1% increase in wheat plants in the pots with no barrier as compared to those in pots with a solid barrier.

$^{15}$N uptake and recovery efficiency of crop straw, grain and root of maize grown in the pots with no barrier were higher than in those of the plants in the pots with a solid barrier when the soil covered with wheat and maize, was fortified with labeled $^{15}$N ($^{15}$NW.M). The $^{15}$N uptake and recovery efficiency of maize grain in the pots with no barrier increased by 37.9% and 37.8%, respectively, compared to pots with a solid barrier. Furthermore, when soybean was labeled by $^{15}$N ($^{15}$NS), the amount of $^{15}$N in straw, grain and root of maize increased tremendously in the pots with no barrier, and the total $^{15}$N accumulated was 188.02 mg · pot$^{-1}$, which was 13.2% of the total N uptake by maize; the $^{15}$N recovery efficiency was 27.45%.

$^{15}$N uptake and recovery efficiency in soybean grain and straw in the pots with no barrier decreased significantly, compared to the pots with a solid barrier, when soybean was labeled with $^{15}$N ($^{15}$NS). Furthermore, $^{15}$N% abundance was detected in soybean plant in 2010, when soybean was planted in the stubble soil of wheat ($^{14}$NW.M);

### Table 3. The $^{15}$N uptake and recovery efficiency of crops with solid root barrier and no barrier treatments (Experiment 1, 2010).

| Crops | Model of $^{15}$N labeled | Root barrier | $^{15}$N uptake (mg pot$^{-1}$) | $^{15}$N recovery efficiency (%) |
|-------|---------------------------|--------------|---------------------------------|---------------------------------|
|       |                           |              | Straw  | Grain  | Root  | Total  | Straw  | Grain  | Root  | Total  |
| Wheat | $^{15}$NW.M               | Solid barrier| 189.12 | 208.60 | 8.58  | 406.29 | 13.80  | 15.23  | 0.67  | 29.66  |
|       |                           | No barrier   | 206.22 | 348.77 | 5.99  | 500.98 | 15.05  | 25.46  | 0.44  | 40.95  |
| Maize | $^{15}$NW.M               | Solid barrier| 186.55 | 362.83 | 11.79 | 681.92 | 6.81   | 13.24  | 0.43  | 24.89  |
|       |                           | No barrier   | 201.74 | 500.16 | 12.46 | 853.48 | 7.36   | 18.25  | 0.46  | 31.15  |
| Soybean| $^{15}$NS                 | Solid barrier|   –   |   –   |   –   |   –   |   –    |   –    |   –    |   –    |
|       |                           | No barrier   | 63.69  | 90.49  | 0.65  | 188.02 | 9.29   | 13.21  | 0.09  | 27.45  |

Data represent means of three replications and different letters in the same column show significant differences at $P<0.05$. $^{15}$NW.M denotes that soil planted with wheat and maize is labeled $^{15}$N. $^{15}$NS denotes that soil planted with soybean is labeled $^{15}$N. **"** denotes that $^{15}$N was not detected for the crop.
the $^{15}$N uptake and recovery efficiencies in soybean grain and straw in the pots with no barrier were higher than those in the pots with a solid barrier. Thus, our data suggest that N could also be transferred from maize to soybean in an indirect manner.

Based on the observed $^{15}$N residues, the total $^{15}$N uptake and recovery efficiency of the cropping system in pots with no barrier were 1727.55 mg pot$^{-1}$ and 36.0%, respectively, and which were 34.4% and 34.3% higher than those in the pots with a solid barrier.

### 3. The Interspecific Competition Regarding the N Uptake

The results of N uptake analysis revealed the existence of interspecific competition of N and facilitation of N absorption in a wheat-maize-soybean relay strip intercropping system. To analyze the difference of the ability of crops to uptake N, we calculated an index of N competition in the form of a ratio (NCRab, where a and b denote the crops). When NCRab was greater than 1, the competitive ability in N uptake of crop “a” was greater than that of crop “b” in the relay strip intercropping system, and vice versa. Based on our calculations, in 2010, NCRwm (w, wheat; m, maize) was 0.91, and the NCRms (s, soybean) was 1.48. This indicated that the ability of maize to take N was greater than that of wheat and soybean during the co-growing stage of wheat, maize and soybean. In the pots without a barrier, the biomass and N uptake of wheat were greater than that in the pots with a solid barrier, which suggested that wheat absorbed N from co-growing maize. In 2011, the NCRwm reached 1.21, which further indicated the strong N uptake competitive ability of wheat. The NCRms value reached 1.44, which indicated that the N uptake competitiveness of maize was much higher than that of soybean.

### 4. N Transfer

To further evaluate the nitrogen transfer among symbiotic crops in the relay intercropping system, we determined the $^{15}$N transfer directly by foliar $^{15}$N labeling. The foliar application of $^{15}$N-urea to wheat, maize and soybean caused the donor crops to absorb more $^{15}$N than normal (Table 4), which was transferred to other co-growing crops (Table 5). The amounts of $^{15}$N uptake and transfer by plants were significantly varied among different N application rates ($P < 0.05$). When wheat was the donor, $^{15}$N was transferred to the associated maize, and the amount and percentage of $^{15}$N transfer decreased significantly with an N application rate-dependent manner. The maximum N transfer occurred at the N application rate of 150 kg ha$^{-1}$ level, with an increase of 72.4% and 42.5% in the amount of N transfer and %NT respectively from wheat to maize, compared to the effect of N 450 kg ha$^{-1}$ level. In addition, the %NDFT showed the same trends.

N transfer occurred both from maize to wheat and from maize to soybean when maize acted as the $^{15}$N donor. With the increasing of N application rates, the amount of N transfer initially increased and decreased thereafter. Moreover, the amount of N transfer, %NT and %NDFT were highest at N 300 kg ha$^{-1}$ level. Meanwhile, the amount of N transfer was varied in different receiver crops. The amount of N transfer, %NT and %NDFT from maize to wheat was higher than that from maize to soybean when the three N treatments were given. The total amount of N transfer from maize to wheat was 16.1% – 163.0% higher than from wheat to maize, especially for the 300 and 450 kg ha$^{-1}$ N treatments. Moreover, $^{15}$N uptake was lowest in maize in the 300 kg ha$^{-1}$ N treatment (Table 4), suggesting that much of the foliar $^{15}$N absorbed by maize was transferred to wheat. Therefore, the N application rate of 300 kg ha$^{-1}$ favored N transfer from maize to wheat in the wheat-maize-soybean relay strip intercropping system.

When soybean acted as the $^{15}$N donor, the amount of $^{15}$N transferred increased with an increase in the N application rate, but the %NDFT decreased. Although, the

| $^{15}$N labeling crop | Fertilizer-N application rates (kg N ha$^{-1}$) | $^{15}$N uptake of donor crop (mg pot$^{-1}$) |
|-----------------------|-----------------------------------------------|---------------------------------------------|
|                       | Grain  | Straw  | Root  | Total |
| Wheat                 | 150    | 62.71 a | 40.26 b | 0.33 a | 103.30 b |
|                       | 300    | 63.24 a | 51.30 a | 0.27 ab | 114.81 a |
|                       | 450    | 45.50 b | 42.71 b | 0.20 b  | 88.40 c  |
| Maize                 | 150    | 66.80 b | 49.66 a | 3.47 a  | 119.93 b |
|                       | 300    | 67.56 b | 27.04 c | 1.81 c  | 96.40 c  |
|                       | 450    | 81.24 a | 40.42 b | 2.48 b  | 124.15 a |
| Soybean               | 150    | 26.84 b | 10.15 b | 0.54 b  | 37.53 b  |
|                       | 300    | 38.05 a | 12.47 a | 1.19 a  | 51.70 a  |
|                       | 450    | 20.17 c | 7.53 c  | 0.78 ab | 28.48 c  |

Data represent means of three replications and different letters in the same column show significant differences at $P < 0.05$.  

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amount of 15N acquired by soybean was highest at N 300 kg ha⁻¹ (Table 4), the %NT transferred from soybean to maize was lowest (Table 5) because NT% was significantly affected by the N uptake of receiver maize. The total amount of N transferred from soybean to maize was 1.7 – 6.0 times larger than that from maize to soybean, and the NT% from soybean to maize was 6.7 – 22.2 times higher than that from maize to soybean.

**Discussion**

1. **Yield advantage in a wheat-maize-soybean relay strip intercropping system**

   As human populations increase globally, the demand for food will soar and efforts will need to be focused on overcoming environmental challenges and increasing land use efficiency to improve land productivity (Tilman et al., 2002; Rathmann et al., 2010; Spiertz, 2012). The use of relay cropping and intercropping in developing countries helps to improve grain production by increasing the multiple crop index of land (Li et al., 2006; Gao et al., 2010; Zhang et al., 2011). The wheat-maize-soybean relay strip intercropping system, which efficiently uses resources, such as light, temperature and water, is widely practiced by farmers in the southwestern part of China (Yang et al., 2014). The present study was conducted to show that three way intercropping has a distinct advantage over double intercropping when there is sufficient availability of soil nutrients and the environmental factors are favorable. A solid barrier assay was used to mimic single cropping practices while intercropping was simulated by the absence of a barrier in the experimental pots. Our results demonstrated that the yield of wheat and maize in pots with no barrier increased by an average of 25.6% and 14.4%, respectively, when compared to plants with solid barrier. However, soybean yield with no barrier pots was reduced by 18.4% as compared to plants in the pots with a solid barrier (Table 1). The total grain yield of the relay intercropping system was significantly higher than that of monoculture system.

   Previous studies revealed that the yield advantage of relay intercropping system may have resulted from the complementary use of diverse growth resources, such as light, land and nutrition (Li et al., 2007; Gao et al., 2010). Crops can use the available resources more efficiently during intercropping, resulting in higher yields than the corresponding monocultures (Vandermeer, 1990; Zhang, et al., 2011). Intercropped species do not compete for exactly the same growth resource at any given time and tend to use the available resources in a complementary way resulting in higher yield (Hauggaard-Nielsen et al., 2001).

2. **The relationship between ecological niches and N uptake and use**

   The burst usage of global nitrogen fertilizer in the past decades has not only disrupted the global N cycles, but also caused serious hazards to the environment and human health (Vitousek et al., 1997; Foley et al., 2011). These environmental problems reduce the ability of cropland ecosystems to provide goods and services at both country and regional levels (Tilman et al., 2002; Pretty, 2008; Ju et al., 2009). Therefore, construct a low-input and resource-efficient agricultural system based on ecological principles, is essential to sustainable agriculture development (Altieri, 1999; Spiertz, 2012). Intercropping exploits the ability of diverse crops to increase N uptake as well as N use efficiency as a result of “complementary N use” (Chalk,
different ecological niches. The present study demonstrated uptake by maize and the inferred N transfer characteristics (Table 3), which demonstrated that an increase of N absorbed from soil and fertilizer and the by wheat (Li, et al., 2011). The amounts of wheat N were in succession, which was beneficial for the N uptake during the seeding stage. Therefore, their peaks of nutrient demand increased, while maize decreased (Xiao, et al., 2004). When wheat and maize were grown together, wheat had a high N requirement in the flowering and seed filling stages, while maize had a higher N requirement in the seeding stage. Therefore, their peaks of nutrient demand were in succession, which was beneficial for the N uptake by wheat (Li, et al., 2011). The amounts of wheat N absorbed from soil and fertilizer and the \( ^{15}N \) recovery efficiency increased in no barrier treatment. In the cereal-legume intercropping system, the ecological niches of the two species partially overlap. Finally, cereals possess the faster rate of root growth with high N requirement, and therefore, compete for inorganic nitrogen uptake more than legumes (Lithourgidis et al., 2011; Hauggaard-Nielsen et al., 2001, 2003).

In our study, the amounts of \( ^{15}N \) uptake and N uptake of the soybean in the pots without barrier decreased by 46.44% and 9.75%, compared to the solid barrier treatment (Table 3), suggesting that soybean was a self-abnegation crop in the wheat-maize-soybean relay intercropping system. When maize and soybean grew together, maize was more competitive for N than soybean because of its faster growth and higher demand for N. Our results revealed that NCRMs was greater than 1.0, indicating that maize was the dominant species, and could absorb more N from soil and fertilizer than soybean. Maize increased its absorption of N and reduced the residual N in the soil. Furthermore, the strong competition for N uptake by maize could decrease the available N in the rhizosphere soil of soybean, which would force soybean to fix more N from the air; then, the fixed N was transferred from soybean to maize (Li et al., 2006). The \( ^{15}N \) recovery efficiency of maize increased when no barrier was used (Table 3), which demonstrated that an increase of N uptake by maize and the inferred N transfer characteristics existed in the maize-soybean relay intercropping system.

Xiao et al. (2004) observed a similar model of N uptake and transfer in a wheat-faba bean intercropping system. N transfer to maize resulted in decreased \( ^{15}N \) availability in soybean. This suggests that maize promoted N fixation in soybean, and the fixed N compensated for the N transferred to maize. Thus, interspecific competition and nutrition use existed in the wheat-maize-soybean relay strip intercropping system, which highlight the merits of relay strip intercropping.

### 3. The effect of N transfer among crops on N uptake and use

N transfer was detected from legumes to cereals (Xiao et al., 2004; Ofosu-Budu et al., 1993; Johansen et al., 1996), e. g. from white clover to grass (Rasmussen et al., 2013), from legume tree to the associated grass (Sierra, et al, 2007) in some intercropping systems. In addition, there was bi-directional N transfer in rice-peanut intercropping system, the N transferred from peanut to rice reached 22.6 mg N plant\(^{-1}\), accounting for 10.9% of the total N accumulated in rice plants, which improved the yield of rice; the amount of transferred N from rice to peanut was much less than that from peanut to rice (Shen and Chu, 2004). In this study, we found that the amount of N transfer between wheat and maize decreased with increasing N fertilizer application rate. The maximum transfer amount was 26.65 mg pot\(^{-1}\) from maize to wheat at the N fertilizer application rate of 300 kg ha\(^{-1}\), accounting for 1.76% of the total N accumulated in wheat plants, and the transfer amount from wheat to maize was 10.48 mg pot\(^{-1}\). Thus, the final transfer direction was from maize to wheat during the inter growth stage of both crops. Furthermore, bidirectional N transfer occurred between maize and soybean. The maximum of \%NDFT from soybean to maize was 2.17, which was lower than 8% of the same data that from alfalfa to maize (Jordan et al., 1993). The causal factors may be that less N secreted by soybean in soil because of the short co-growing time of maize and soybean (Table 2). The amount of N transferred from soybean to maize increased in N application level-dependent manner, because the N uptake and competition of soybean decreased, which lead to the significant increment of maize N uptake by N competing, and reduce of soybean \( ^{15}N \) uptake (Table 3). The amount of N transferred from soybean to maize was much larger than that from maize to soybean at all three N application rates; Therefore, the final N transfer direction was from soybean to maize.

In the wheat-maize-soybean relay intercropping system, soybean acts as a disadvantage when competing for N because wheat and maize dominated N uptake. N use characteristics of the three crops were consistent with the characteristics of N transfer in relay intercropping system. N uptake and recovery efficiency of wheat and maize
increased while soybean decreased. Interspecific N transfer disrupted the original N balance among crops, which stimulated N uptake and promoted the N use efficiency of wheat and maize. N uptake of maize tended to increase in N application-dependent manner, while N uptake of stems and leaves in soybean decreased (Table 2). Moreover, in maize, $^{15}$N recovery efficiency in pots with no barrier was higher than in the pots with a solid barrier; while soybean showed the opposite trend. Subsequently, it was suggested that the NH$_4^+$ and NO$_3^-$ released by soybean roots were taken up by maize, which was characterized by N transfer, resulting in improvement the N use efficiency in maize. It decreased the amount of available nitrogen for soybean thereby stimulating it to fix atmospheric N (Xiao et al., 2004; Shen and Chu, 2004).

The mechanism of N transfer in intercropping systems is complex and often ambiguous. Previous reports proposed feasible mechanisms such as root-to-root contact, the involvement of mycorrhizal fungi, and the release of N in exudates and turnover in soil, as well as root and nodule turnover (Høgh-Jensen, 2006). A study on the spatial and temporal variation of N transfer in grass-white clover mixtures showed that N transfer in both grass and white clover cannot be solely explained by competition for soil inorganic N, and may involve N transfer in organic N forms (Rasmussen et al., 2013). In a wheat-maize-soybean relay intercropping system, the requirement of N amount as well as specific types of N, such as NO$_3^-$, NH$_4^+$, N and N$_2$, varies with the symbiotic crops. Aside from the possible mechanism mentioned by former researchers, the variation in N content and types in the rhizosphere may be one of the important reasons for promoting a bi-directional N transfer between wheat and maize, and between maize and soybean; however, the exact mechanism remains to be elucidated.

Conclusions

Our present study demonstrated that N recovery and total N accumulation were increased in the wheat-maize-soybean relay intercropping system, compared to the monoculture system. Competition for N favored wheat and maize rather than soybean, and therefore wheat and maize were the dominant species in the relay intercropping system. N applied to leaves could be transferred to other species through N uptake from the soil. During the co-growing stage of wheat and maize, net N transfer occurred from maize to wheat; during the co-growing stage of maize and soybean, a net N transfer occurred from soybean to maize. Positive N competition and bidirectional N transfer were main contributors to efficient N use in the wheat-maize-soybean relay intercropping system.

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