

## The influence of legume cropping sequences on above and below ground carbon and nitrogen inputs

K. S. Slater<sup>1</sup>, J. Knight<sup>2</sup>, R. Farrell<sup>3</sup> & R. Lemke<sup>4</sup>

Dept. of Soil Science, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5A8

Phone: 306- 966-4963 Fax: 306-966-6881, Email: kms163@mail.usask.ca

### Abstract

Pulse crops included in cereal and oilseed crop rotations can provide environmental and economic benefits such as reduced greenhouse gas (GHG) emissions and a reduced dependence on synthetic nitrogen (N) fertilizers. Pulse crop rotations have shown positive impacts on soil nutrient cycling and crop yields due to contributions of bioavailable carbon (C) and N to soils. Pulse crops in symbiosis with rhizobia can input N to soils from atmospheric N fixation. Soil C inputs are derived from aboveground crop residue inputs and below ground root exudates.

Stable isotope (<sup>13</sup>C and <sup>15</sup>N) methodologies are being utilized to quantify above and belowground contributions (roots and rhizodeposits) of both C and N inputs from chickpea, pea and lentil residues to soil. <sup>15</sup>N is introduced into the stem of plants using a stem feeding technique (stem wick method). <sup>13</sup>C is introduced using atmospheric pulse <sup>13</sup>CO<sub>2</sub> labeling. The more precise nutrient budgets can be used to determine the best pulse crop rotations to improve GHG budgets, reduce farmer dependence on N fertilizers and therefore improve environmental and economic sustainability of the system. Preliminary results will be discussed.

### Introduction

Carbon and nitrogen are the building blocks of soil organic matter (SOM). The majority of C in agricultural systems is derived from photosynthesis by plants. Plants either respire C or use it in the production of organic compounds. The C containing compounds that are not harvested with the plants are added to soils either as above ground crop residues (CR) or belowground inputs. Soil microbes respire a portion of the C inputs and the remainder is humified, to produce SOM. Accumulation of SOM occurs when the amount of C, added via CR, is greater than the rate of SOM decomposition (Janzen, 2006). The ability for agricultural soils to act as a C sink or source is a growing area of research, due to the recent focus on improving GHG budgets (Thomsen et al., 2008; Gershenson and Consulting, 2012; Stockmann et al., 2013).

Pulse crops have the potential to improve GHG budgets of agricultural systems due to their unique ability to fix dinitrogen gas (N<sub>2</sub>) from the atmosphere, and perhaps also via improved soil C storage, relative to non pulse crops (van Kessel and Hartley, 2000; Lemke et al., 2007). These processes can reduce farmer dependence upon N fertilizers, reduce GHG emissions and improve soil nutrient status for use by succeeding crops (Mayer et al., 2003; Gan et al., 2011).

A significant proportion of the soil C and N credits produced by pulse crops is due to rhizodeposition (belowground deposits) and root material (Wichern et al., 2008). The C:N ratio of above ground inputs, roots and root exudates will affect the distribution, the storage and the phytoavailability of C and N in the various SOM pools (Knicker, 2011; Gårdenäs et al., 2011).

Vitousek et al. (1997) highlights the increased need for research into the interactions of the C and N cycles since N inputs to soils from synthetic fertilizers and atmospheric N gases have surpassed soil N inputs derived from microbial N fixation. Consideration of the above and

belowground inputs of C and N to soils from pulse crops is essential to providing an accurate nutrient budget of pulse crop rotations (Wichern et al., 2007; Wichern et al., 2008).

## Materials and Methods

### Study I: Partitioning of Above and Belowground Nitrogen and Carbon in Continuous Chickpea, Lentil and Pea Soil Cores

This experiment took place in the greenhouse. Chickpea, lentil and pea plants were planted in intact soil cores from the Semi-Arid Prairie Agricultural Research Centre (SPARC), in Swift Current. The cores are 20 cm in diameter and 30 cm in length and have been cropped with chickpea, lentil or pea in the previous 2 rotations (Table 1).

Table 1. Rotations to be tested in Study I (Winter/Spring 2013)

Crop grown in SPARC field trial			Proposed crop grown in Greenhouse study
2009	2010	2011	Winter/Spring 2013
Wheat	Pea	Pea	Pea
Wheat	Chickpea	Chickpea	Chickpea
Wheat	Lentil	Lentil	Lentil

number of cores = 3 treatments x 4 reps (enrichment) + 3 treatments x 4 reps (natural abundance) = **24 cores**

The enriched soil cores were organized in four blocks of three, in a random complete block design. Each block of four cores were planted with chickpea, lentil or pea. Each core received eight seeds inoculated with *Rhizobium leguminosarum*. After germination the plants were culled to four plants per pot, based on seeding rates recommended by Saskatchewan Pulse Growers (2011).

Labeling the plants with  $^{15}\text{N}$ -urea (99 atom%) was accomplished using the cotton stem wick method (Russell and Fillery, 1996). The method involves drilling a 0.5 mm hole in the stem of the plants, 3 cm above the surface of the soil. A cotton wick was fed through the drilled hole and each end is placed in a solution of  $^{15}\text{N}$ -urea (99 atom%). The solution was delivered in repeat pulses in order to achieve homogenous labeling of the plant with  $^{15}\text{N}$ -urea. Labeling began three to four weeks after planting. Untreated control soil cores and plants were kept in a separate greenhouse to account for natural background levels of  $^{15}\text{N}$  (Wichern et al., 2007).

The  $^{15}\text{N}$ -urea was administered on a supply and demand need, to obtain as evenly distributed label as possible. At the same time of the  $^{15}\text{N}$ -urea labeling, the enriched chickpea, lentil and pea treatments (Table 1) were atmospherically labeled with  $^{13}\text{CO}_2$  every 3 to 5 days. A labeling event involved placing four blocks of three of the soil cores containing stem-wick plants under poly (methyl methacrylate) chambers measuring 45cm x 55cm x 60cm An infrared gas analyser (IRGA) was connected to each chamber to provide estimates of the overall  $\text{CO}_2$  levels and the  $^{13}\text{CO}_2$  concentrations within the chambers.

The atmosphere was enriched with approximately 150 ppm  $^{13}\text{CO}_2$  by injecting 4 mol  $\text{L}^{-1}$  hydrochloric acid (HCl) into the chamber, through a fitted port with a septum. The HCl was

injected beneath the port where a  $\text{NAH}^{13}\text{CO}_2$  (99 atom %) solution was contained. The solution was added through out the labeling period to maintain a  $\text{CO}_2$  concentration of 360 to 430  $\mu\text{mol mol}^{-1}$  (Sangster et al., 2010). The atmospheric labeling took place every week until the plants reached maturity.

The chickpea, lentil and pea plants were harvested at maturity and divided into shoot, leaf, and grain and pod components. Their biomass yields were recorded after drying for 24 hours at 60°C (Gan et al., 2009; Sangster et al., 2010). The plant parts were ground to a very fine texture.

Roots were extracted from the soil cores by hand and the soil was sieved (<2mm). Due to time constraints and the large volumes of soil being handled, fine roots were included in the bulk soil. A subsample of fresh soil from each core was taken and soils were separated into heavy, light and very light fractions as well as the water extractable organic matter fraction.

In the Stable Isotope Laboratory, each soil fraction will be analyzed for DOC, total C and the  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  isotope ratios using an elemental combustion system and mass spectrometry (Mayer et al., 2003). The plants and roots will be analyzed for their total N and C content and their  $^{15}\text{N}/^{14}\text{N}$  and  $^{13}\text{C}/^{12}\text{C}$  isotope ratios.

Inorganic N and MB pools were also extracted with KCl (Maynard et al., 1993) and chloroform fumigation (Brookes et al., 1985; Voroney et al., 2008), respectively. These extracts will be analyzed for  $^{15}\text{N}/^{14}\text{N}$  isotope ratios as well as the  $^{13}\text{C}/^{12}\text{C}$  isotope ratio in the MB (Carter and Gregorich, 2008).

## Study II: Partitioning of Belowground Nitrogen and Carbon in Mustard and Wheat intercropped with Chickpea, Lentil and Pea Soil Cores

This experiment will follow the exact same procedures as those described in Study I, but will involve the use of cores that have been cropped with mustard or wheat in the previous rotation (Table 2).

Table 3. Rotations to be tested in Study II (Summer 2013)

Crop grown in SPARC field trial			Proposed crop grown in Greenhouse study
2009	2010	2011*	Winter/Spring 2013
Wheat	Pea	Mustard	Pea
Wheat	Pea	Mustard	Chickpea
Wheat	Pea	Wheat	Pea
Wheat	Pea	Wheat	Chickpea
Wheat	Chickpea	Wheat	Chickpea
Wheat	Lentil	Wheat	Lentil

\*soil cores will be taken at the end of this rotation phase

total number of cores = 3 treatments x 4 reps (enrichment) + 2 treatments x 4 reps (natural abundance) + 3 treatments x 3 reps (enrichment) + 4 treatments x 3 reps (natural abundance) = **41 cores**

## Preliminary Results

**Table 1. C and N derived from rhizodeposition in the bulk soil, the heavy fraction, the light fraction and the very light fraction organic matter.**

Rotation	NdfR (mg)			
	Bulk	Heavy fraction	Light fraction	Very light fraction
Chickpea	116.78 ±36.83	0.05 ±0.02	0.006 ±0.0005	0.04 ±0.01
Lentil	128.49 ±43.47	0.04 ±0.02	0.008 ±0.002	0.03 ±0.01
Pea	115.48 ±35.69	0.05 ±0.02	0.006 ±0.001	0.04 ±0.01

  

Rotation	CdfR (mg)			
	Bulk	Heavy fraction	Light fraction	Very light fraction
Chickpea	1032.16 ±231.64	0.67 ±0.34	0.09 ±0.01	1.42 ±0.004
Lentil	784.18 ±43.37	-0.21 ±0.24	0.13 ±0.06	0.78 ±0.003
Pea	973.71 ±35.69	0.87 ±0.59	0.06 ±0.02	0.63 ±0.01

Study 1 has shown that there are no significant differences in the amount of C and N derived from rhizodeposition between the chickpea, lentil and pea rotations. The majority of the C derived from rhizodeposition appears to be present in the very light fraction organic matter, which is consistent with much of the previous literature.

### Future Expected Results and Discussion:

It is expected that the variability in the response of pulse crop C and N partitioning to the previous crop will be better understood, as well as the effects of crop sequence on the aboveground and belowground interactions of the two nutrients. Distribution of both the <sup>15</sup>N and the <sup>13</sup>C labels will allow conclusions to be drawn on the aboveground and belowground interactions of the two nutrients, in both the continuous pulse crop (study I) and non continuous pulse crop rotations (study II).

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