Considering Belowground Nitrogen of Crops Grown in Prairie Agroecosystems

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Abstract

Grain legumes can improve the yield of succeeding cereal crops through nitrogen and non-nitrogen benefits. Included among these is the input of symbiotically-fixed N from the remaining legume residues following grain harvest. However, the contribution of fixed-N to the soil system can be underestimated due to inadequate physical recovery of roots and unaccounted N released from living legume roots (rhizodeposition) during crop growth. This paper reports on N partitioning in pea and canola plants using ^{15}N stable isotope methods to track N from the plant into the soil. Results illustrate the importance of accounting for belowground N, particularly rhizodeposit N, as it accounted for more of the total N that remained in the residues compared to the aboveground residues including straw and chaff. Preliminary results also indicate different allocation of plant N between canola and pea with potential implications for N cycling between these two crops.

Introduction

Diversification of crop rotations has improved the sustainability of prairie agricultural systems. In particular, including oilseed and pulse crops in traditional cereal-based crop rotations breaks disease cycles, improves energy use efficiency, and can reduce greenhouse gas emissions (Gan et al., 2011). Nitrogen management in these systems is a fine balance between ensuring an adequate N supply to match crop requirements while reducing losses of N to water and the atmosphere. The residues that remain in the soil following crop harvest differ in quality as well as the quantity among the variety of crop species. Pulse crops, which rely on N fixation in addition to soil N to meet their total N requirements may incur a net input of fixed N to soil, but only where fixed N in the residues that remain in the field are greater than the amount of soil derived N that is exported in the grain at harvest (Walley et al., 2007). Adequate budgets of N inputs are necessary in order to determine whether N may be accrued in soils following pulse crops or if N is depleted. While it is straightforward to get an account of the aboveground residue N that remains on the soil, assessing the belowground contributions of N in roots and rhizodeposits is difficult.

The harvest of grain from pulse crops can result in the removal of two thirds of the total plant N (Mayer et al., 2003). Therefore, any input of N to soil from legumes grown for grain is derived from N released by roots during crop growth and decomposition of root and stubble residues.

Despite this, N balance studies in pulses often do not account for root N and therefore N contribution from N_2 fixation of grain legumes is likely underestimated (Walley et al., 2007). In fact, N release from roots through the process of rhizodeposition, can represent a significant proportion of total plant N (Mayer et al., 2003). N fixation as a proportion of total plant N may be underestimated by 10% when N lost from roots is not accounted (Sawatsky and Soper, 1991).

Stable isotope methods can be used to provide an improved estimate of the input of N from roots and rhizodeposits. These methods allow for the determination of N inputs from roots during crop growth, as a result of rhizodeposition processes, and can account for any root-derived N that may be released to the soil through decomposition of roots that have died before the plant is harvested. This paper reports on results from stable isotope experiments using pea and canola to assess the total plant N partitioning aboveground (grain, chaff, straw) and belowground (roots and rhizodeposits). In the first experiment, pea was labelled with ^{15}N enriched urea and harvested at vegetative growth stage, at early flowering, and at physiological maturity in order to assess the temporal change in plant N partitioning above and belowground. In the second experiment, both pea and canola were labelled with ^{15}N enriched urea and harvested at physiological maturity in order to compare the inputs of N from a pulse crop with that of another broadleaf crop commonly grown in the Canadian prairies.

Materials and Methods

^{15}N Labelling Method

In experiment I and experiment II, pea and pea and canola, respectively, were grown and labelled with ^{15}N enriched urea in order to track the partitioning of plant-derived N between aboveground and belowground components, particularly into soil as rhizodeposits. The method for introducing the ^{15}N enriched urea followed the cotton wick method described by Russell and Fillery (1996). The method was applied similarly in both experiments. Briefly, a 0.5 mm hole was drilled into the stem of the plant, approximately 5 cm from the soil surface. A cotton thread was fed through the hole in the stem using a thin needle and both ends of the cotton wick were protected within silicone tubing (4 cm length). The silicone tubing was adhered to the stem of the plant using plasticine. The ends of the cotton thread were fed through the cap of a 2 mL vial and immersed in a ^{15}N enriched urea solution (0.4% (w/v), 99.2 atom % ^{15}N). To prevent solution loss, the cap of the vial consisted of a septum with a hole small enough to allow for the silicone tubing and the thread to feed through. A needle and syringe were used to replenish the ^{15}N urea solution over the course of the experiment in small increments (0.10-0.50 mL) through the septum of the vial cap. Following the last incremental addition of ^{15}N urea solution, 0.40 mL of deionized water was added to maximize ^{15}N urea solution uptake from the string into the plant stem.

Experimental I soils, planting and harvest

Soil was collected from the Agriculture and Agri-food Canada (AAFC) research station at Scott, SK, which was an Orthic Dark Brown Chernozem. The soil was air dried, sieved (4 mm) to remove any rocks, and mixed with silica sand in a 1:1 ratio by weight to facilitate easy recovery of roots during the experiment. Soils were packed to a bulk density of 1.3 g cm^{-3} in pots (12 cm

dia., 30 cm deep) constructed of polycarbonate tubing. Five pea seeds, inoculated with *Rhizobium leguminosarum*, were sown into each pot and thinned to one plant per pot. Plants were watered regularly with deionized water to maintain 80% field capacity. The pots were arranged on a greenhouse bench as a completely randomized design with nine replicates for each harvest period (vegetative, flowering, maturity) for the ^{15}N labelled treatments and with four replicates for the ^{15}N natural abundance control plants. Labelling commenced at 18 days after planting (DAP) and continued until 25, 41, and 72 DAP for plants harvested at vegetative growth (32 DAP), early flowering (55 DAP), and physiological maturity (96 DAP), respectively.

Experimental II soils, planting and harvest

Soil was collected from the Agriculture and Agri-food Canada (AAFC) research station at Swift Current, SK, which was an Orthic Brown Chernozem. The soil was air dried, sieved (4 mm) to remove any rocks, and mixed with silica sand in a 1:1 ratio by weight. Soils were packed to a bulk density of 1.4 g cm^{-3} in commercial planting pots (20 cm dia., 20 cm deep). Five pea seeds or five canola seeds were planted per pot and thinned to one plant per pot. Plants were watered with deionized water to 80% field capacity. The pots were arranged on a greenhouse bench as a randomized complete block design with eight replicate pots per crop species for both the ^{15}N labelled plants and the natural abundance control plants. Both pea and canola plants were supplied with ^{15}N urea from 21 to 56 DAP. Pea was harvested at 100 DAP and canola at 130 DAP.

Soil and plant sample preparation and analysis

At harvest in both experiments, the aboveground plant components were separated into leaves, stems, pods, and grain, dried at 60°C. The pots containing soil and the intact roots were stored at 2°C until roots could be removed from the soil. Roots were carefully removed from the soil using a 2 mm sieve and tweezers; soil adhering to the roots (rhizosphere soil) was retained and collected upon root washing. Roots were washed on a 0.5 mm sieve with deionized water and were dried at 60°C. The soil-water slurry from root washing was collected and dried in an oven at 75°C; the remaining soil was considered rhizosphere soil. Soil (bulk and rhizosphere) and plant samples were finely ground in a ball mall and were weighed and analyzed for N content (%) and $^{15}N/^{14}N$ isotope ratios using isotope ratio mass spectrometry (Delta V Advantage, Thermo Fisher Scientific Inc.).

Calculating N rhizodeposition

The proportion of soil N derived from roots (pNdfR) was calculated based on the equation of Janzen and Bruinsma (1989):

$$\text{pNdfR} = \frac{(\text{atom } \%^{15}N \text{ excess soil})}{(\text{atom}\%^{15}N \text{ excess roots})}$$

where natural abundance ^{15}N of the atmosphere (0.3663 atom % ^{15}N) was used to calculate the excess ^{15}N in soil and roots. In order to calculate the total amount of N in rhizodeposits, the total amount of N in the soil (bulk or rhizosphere) was multiplied by pNdfR.

Results and Discussion

Distribution of Recovered ^{15}N

The aboveground components of the plants were preferentially enriched with ^{15}N relative to the roots and rhizodeposits in both experiments (Tables 1 and 2). The ^{15}N urea was applied directly to the stem of the plant and was transferred into the plant via the transpiration stream where it was metabolized and transferred among the various plant parts. It is not surprising that the aboveground components are preferentially enriched given that the ^{15}N is artificially introduced directly into the stem using unnatural means of N incorporation. However, by supplying the ^{15}N label in small increments (referred to as pulse labelling) over the duration of plant growth, the label can be more homogenously distributed throughout aboveground and belowground parts relative to one time ^{15}N label application (Mahieu et al., 2009). Even label distribution is particularly important in follow up studies that might examine the fate of root-derived N in soil (Wichern et al., 2008).

Table 1. Distribution (%) of recovered ^{15}N in plant parts and bulk and rhizosphere soil rhizodeposits of pea at various growth stages in Experiment I

Harvest stage	Grain	Leaves	Roots	Rhizodeposits	
				Rhizosphere	Bulk
Vegetative	-----------	83.0 (0.53)	2.8 (0.24)	1.8 (0.08)	12.5 (0.45)
Flowering	-----------	87.3 (0.57)	4.1 (0.61)	1.1 (0.13)	7.5 (0.45)
Maturity	79.7 (1.13)	11.2 (1.13)	2.4 (0.12)	0.1 (0.01)	6.6 (0.14)

Values are means (n=9) and standard errors are in brackets

The relative distribution of ^{15}N in pea at various stages of maturity remained relatively constant among above and belowground components of pea (Table 1). However, as the plants reached physiological maturity, a greater proportion of the ^{15}N label ended up in the grain and leaves relative to the rhizodeposits, although the distribution of total ^{15}N in roots remained relatively stable. The relative distribution of ^{15}N in pea plants harvested at maturity in experiment I and II are in close agreement (Table 1 and 2), with grain comprising 80 and 79% of the recovered ^{15}N, respectively. However, in experiment II the N rhizodeposits in bulk soil were not yet analyzed and therefore the percent distribution in all components is slightly overestimated. Interestingly, distribution of the recovered ^{15}N differed between canola and pea, with more ^{15}N being allocated to belowground components (roots, in particular) of canola relative to pea (Table 2). Pea grain comprised 79% of the recovered ^{15}N, while only 53% of recovered ^{15}N was found in canola. The labelling period between both plants were identical, therefore the ^{15}N label had equal amount of time to distribute within the tissues of the two crop species.

Enrichment levels of bulk and rhizosphere soil, expressed as atom % ^{15}N excess, increased with plant age in pea in bulk soils, but decreased in rhizosphere soils. This suggests that N released into the soil from pea roots at early stages of plant growth eventually gets transferred out of the rhizosphere into the bulk soil over time. In addition, as the plant ages a higher proportion of root will have already started to die and decompose. Therefore, the recovered ^{15}N in the rhizosphere

soil is more representative of newly released root derived N rather than N associated with root decomposition.

Table 2. Distribution (%) of recovered ^{15}N in plant parts and rhizosphere soil rhizodeposits of pea and canola harvested at maturity in Experiment II

Crop	Grain	Pods	Leaves	Senesced leaves	Roots	Rhizodeposits in rhizosphere soil
Pea	78.6 (1.51)	3.5 (1.24)	15.7 (1.52)	-----------	2.1 (0.66)	0.2 (0.09)
Canola	52.8 (6.1)	8.4 (0.54)	21.0 (6.70)	6.5 (1.06)	10.4 (0.74)	0.9 (0.13)

Values are means (n=8) and standard errors are in brackets

Total N and distribution of plant N in soil

Distribution of N within the plant changed over time as found in experiment I (Table 3). Belowground N comprised 45% of total plant N during vegetative stage of pea. As the plant aged, the proportion of total plant N that was allocated belowground diminished since more N was needed for grain development. By the time the pea plant reaches maturity root N comprises 3.6% of total plant N and rhizodeposits comprise 9.9% of total plant N. Therefore, if rhizodeposited N had not been considered the majority of belowground N contributions to soil would not have been accounted. In a review of N rhizodeposition, Wichern et al. (2008) report that the mean belowground N distribution for field pea was 32% of total plant N, but that the range was 14-74%, reflecting the differing methodologies used to assess N rhizodeposition. Because this experiment was conducted in the greenhouse using pots, root growth was likely restricted compared to field conditions and contributions of belowground N under field conditions are likely higher (Mayer et al., 2003). In fact, using physical recovery of roots in a field study, Gan et al. (2010) found that roots comprised 14% of total plant N; however this study did not attempt to quantify rhizodeposits. Considering that 80% of total plant N was removed during harvest of pea seed in this study, it is clear that adequate accounting of belowground inputs of N, including rhizodeposits, is vital to getting an accurate N balance in cropping systems that include pea.

Total N on a per plant basis was greater overall for pea than it was for canola in experiment II. Despite this, the total amount of belowground N was greater for canola (19.3 mg) than for pea (6.0 mg). A very high proportion of total plant N was allocated for grain development in pea (79%). The high allocation of N to grain development in pea resulted in only 5% of total plant N remaining belowground in pea, while 20% of total plant N remained in canola. The complete N budget for this experiment will be complete once samples are analyzed for the bulk soil (root-free). Although this data shows that there is a great input of residue N from canola compared to pea in this experiment, data from other experiments indicate that the quality of residues from these two plant species differ (Sangster et al., 2010), which will have implications for residue decomposition and nutrient turnover.

Table 3. Total plant N (mg) and distribution of plant N (%) in plant parts and bulk and rhizosphere soil rhizodeposits of pea at various growth stages in Experiment I

Harvest stage	Grain	Leaves	Roots	Rhizodeposits	
				Rhizosphere	Bulk
			-----Total N (mg)-----		
Vegetative	-----------	36.2 (2.22)	4.7 (0.45)	3.1 (0.22)	21.1 (1.05)
Flowering	-----------	178.4 (10.84)	20.3 (3.89)	5.4 (0.76)	35.5 (2.05)
Maturity	178.4 (11.52)	25.1 (1.33)	14.4 (0.80)	0.6 (0.06)	39.5 (1.62)
			-----N distribution (%)-----		
Vegetative	-----------	55.4 (1.27)	7.24 (0.57)	4.7 (0.17)	32.6 (1.17)
Flowering	-----------	74.5 (0.76)	8.2 (1.05)	2.2 (0.27)	15.1 (0.92)
Maturity	80.2 (0.49)	6.3 (0.35)	3.4 (0.17)	0.1 (0.01)	9.8 (0.31)

Values are means (n=9) and standard errors are in brackets

Table 4. Total plant N (mg) and distribution of plant N (%) in plant parts and rhizosphere soil rhizodeposits of pea and canola harvested at maturity in Experiment II

Crop	Grain	Pods	Leaves	Senesced leaves	Roots	Rhizodeposits in rhizosphere soil
			-----Total N (mg)-----			
Pea	94.0 (9.09)	2.9 (0.47)	16.3 (1.44)	-----------	5.6 (0.33)	0.4 (0.14)
Canola	44.2 (3.97)	6.6 (0.44)	22.1 (3.70)	8.6 (0.70)	17.8 (1.13)	1.5 (0.22)
			-----N distribution (%)-----			
Pea	78.6 (2.02)	3.3 (0.67)	12.8 (1.25)	-----------	4.9 (0.49)	0.4 (0.08)
Canola	48.2 (4.14)	7.8 (0.31)	16.0 (5.08)	8.4 (1.15)	18.2 (1.03)	1.5 (0.24)

Values are means (n=8) and standard errors are in brackets

Conclusion

Nitrogen rhizodeposition comprises an important part of the total N budget of growing crops. In two controlled environment studies using the cotton-wick method for ^{15}N labelling, N allocation belowground was significant in pea. The results stress the importance of using ^{15}N isotope techniques to track N transfer from the plant to the soil, particularly as N inputs are quantified to calculate the net input or export of symbiotically fixed N in pulse crops. Preliminary results comparing the N distribution between pea and canola highlight that canola allocated more of its N resources belowground than pea, indicating that at least on a per plant basis, N inputs are higher. However, when considering the overall sustainability of the farm, one must consider that a significant proportion of total plant N in canola would have been derived from fertilizer-N, while a pulse crop would have accessed ~60% of its total N through biological N$_2$ fixation.

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