Frequency of field pea in rotations impacts biological nitrogen fixation

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Knight, J. D. 2012. **Frequency of field pea in rotations impacts biological nitrogen fixation**. Can. J. Plant Sci. **92**: 1005–1011. Economic, environmental and energy concerns about the use of nitrogen (N) fertilizers in crop production have prompted the examination of increasing the frequency of pulses in crop rotations to capitalize on biological nitrogen fixation (BNF). Plots from a field experiment established in 1998 at the Agriculture and Agri-Food Canada Research Farm at Scott, SK, were sampled in 2008, 2009 and 2010. Rotations that included pea every year (continuous pea), every second year (pea-wheat), every third year (pea-canola-wheat) and every fourth year (canola-wheat-pea-wheat) were evaluated for BNF using the enriched ^{15}N isotope dilution technique. Nitrogen from BNF in the seed and straw, total above-ground N, seed and straw yield and soil available N and P were evaluated. In 2 of 3 yr, the highest BNF occurred in the two most diverse rotations. Continuous cropping of pea resulted in drastically low BNF in 2008 and 2009. Nitrogen derived from atmosphere in the continuous pea was 15% compared with an average of approximately 55% across all other rotations in these 2 yr. The reduction in BNF was not due to lower productivity in the continuous pea rotation, nor from higher initial soil inorganic N levels inhibiting BNF. In the third year of the study (2010), the more than double the normal precipitation received during the growing season stimulated BNF in pea in the continuous pea rotation. Determining whether the rotation effects on BNF are due to N mineralization of the previous years' crop residues requires further investigation.

Key words: Continuous pea, crop rotation, *Pisum sativum* L., ^{15}N enrichment

Knight, J. D. 2012. **La fréquence du pois dans les assolements influe sur la fixation biologique de l'azote**. Can. J. Plant Sci. **92**: 1005–1011. Les préoccupations concernant l'emploi d'engrais azotés (N) en agriculture liées à l'économie, à l'environnement et à l'énergie ont fait en sorte qu'on s'intéresse à un recours plus fréquent aux légumineuses dans les assolements, cela afin de profiter au maximum de la fixation biologique de l'azote (FBA). En 2008, 2009 et 2010, les auteurs ont prélevé des échantillons des parcelles sur un terrain aménagé en 1998 à la ferme expérimentale d'Agriculture et Agroalimentaire Canada de Scott, en Saskatchewan. Ils ont évalué la FBA d'assolements comprenant le pois chaque année (monoculture), tous les deux ans (pois-blé), tous les trois ans (pois-canola-blé) et tous les quatre ans (canola-blé-pois-blé) par la technique de dilution enrichie à l'isotope ^{15}N. Il a ainsi été possible d'évaluer la présence d'azote issu de la FBA dans la graine et la paille, la concentration de N total dans les organes aériens, le rendement grainier et le rendement en paille ainsi que la concentration de N et de P disponibles dans le sol. Deux années sur trois, la plus forte FBA est survenue dans les deux assolements les plus variés. La monoculture du pois a débouché sur une FBA dramatiquement faible en 2008 et 2009. En effet, la proportion d'azote venant de l'atmosphère n'était que de 15 % dans la monoculture du pois, comparativement à une moyenne d'environ 55 % pour les autres assolements durant ces deux années. La diminution de la FBA ne dérive pas de la productivité inférieure de la monoculture, ni d'une concentration initiale élevée de N inorganique dans le sol, qui aurait pu nuire à la FBA. La troisième année de l'étude (2010), des précipitations supérieures au double de la normale pendant la période végétative ont stimulé la FBA par le pois dans la monoculture. Il faudrait entreprendre des recherches plus poussées pour établir si les effets de l'assolement sur la FBA résultent de la minéralisation du N présent dans les résidus agricoles de l'année antérieure.

Mots clés: Monoculture du pois, assolement, *Pisum sativum* L., enrichissement au ^{15}N

Continuous cropping of non-legume annual crops is heavily dependent on the use of N fertilizers. Rising oil prices combined with an increased demand for N fertilizers has resulted in dramatic increases in N fertilizer prices over the past decade (Canadian Fertilizer Institute 2012). Moreover, production and application of N fertilizers accounts for 57 to 65% of total CO_2 equivalent (CO_2e) emissions from Canadian prairie agricultural systems (Gan et al. 2011) making it a major agricultural contributor to climate change.

Crop rotations that include pulse crops offer an alternative to the traditional cereal-oilseed intensive rotations, which are reliant on inorganic N fertilizers. Pulse crops are not dependent on N fertilizers because of their ability to form symbiotic associations with N_2-fixing bacteria (i.e., *Rhizobium*) (Crews and Peoples 2004). Optimizing biological nitrogen fixation (BNF) in crop rotations should provide an economic benefit to producers, as well as an environmental benefit to society as a whole. However, reports in the literature of

Abbreviations: BNF, biological nitrogen fixation; **%Ndfa**, percentage of nitrogen derived from atmosphere; **Cont-P**, continuous pea; **P**, pea; **W**, wheat; **C**, canola

percentages of N derived from atmosphere (%Ndfa) by different pulse crops are extremely variable (Walley et al. 2007; Evans et al. 2001), making it difficult to assess the N fixation benefits conferred by the pulse. Inferior strains of *Rhizobium*, low soil temperatures, drought, excess water conferring anaerobic conditions, among other environmental constraints have all been reported to adversely affect BNF. Van Kessel and Hartley (2000) suggest that in addition to using improved *Rhizobium* strains, any practice that increases the N demand by the host plant should increase BNF in pulse crops, including improving the overall growing conditions for the pulse by using best management practices for pest management, soil structure, and overall soil fertility while maintaining low levels of available N. If we are to optimize the role of pulses in crop rotations, thereby capitalizing on reduced fertilizer N inputs, it is necessary to understand how pulses function in these rotations in terms of constraints on BNF.

Crop rotations implemented by a producer depend on a variety of factors including, but not limited to, available moisture and nutrients, diseases and weed levels, herbicide history, equipment availability, commodity prices and risk adversity of the individual producer. The "best" rotation can vary from field to field on the same farm and from year to year for the same field. Traditionally, partly because of commodity prices, "typical" rotations in Saskatchewan tended to focus on cereal production and, more recently, canola (*Brassica napus* L.) production in wetter areas of the province, with pulse crops included to diversify the rotation and break disease and weed cycles. Pulse crops, such as field pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.), are included infrequently, normally ranging from once every 3 to 5 yr. The current recommendation for Saskatchewan is 3 consecutive years between pulse cops to minimize plant disease problems (Saskatchewan Pulse Growers 2008). However, with increased focus on the economics of N fertilizer application and improving the environmental sustainability of agricultural production, focus is turning to reevaluating the role of pulse crops in rotations, and the possibility of increasing the frequency with which pulses are included in rotation. The objectives of the study were to determine how frequency of field pea in rotation affected BNF, N uptake and yield in the pea phase of four distinct rotations that have been in place since 1998. Pea was included once every year, every 2 yr, every 3 yr or every 4 yr in rotation with spring wheat (*Triticum aestivum* L.) or spring wheat and canola.

MATERIALS AND METHODS

The study made use of an existing field experiment located at the Agriculture and Agri-Food Canada (AAFC) Research Farm at Scott, SK, in the 2008, 2009 and 2010 cropping years. Select plots from a larger on-going experiment were used in this study. The field experiment was established in 1998 on a loam Dark Brown Chernozem (Typic Boroll). Prior to establishing the experiment, the site had been continuously cropped with cereals, except in 1997 when canola was grown. The overall plot design was a split-plot with four replicates, 12 crop rotations (main plots) and two fungicide treatments (treated and untreated) as sub-plots. Main-plot dimensions were 21 m × 100 m; subplots were split across the width of the main plot with a 10-m-wide pathway resulting in subplot dimensions of 21 m × 45 m. All phases of each crop sequence were present every year. For the current study only pea plots were sampled. Malhi et al. (2011) provide a detailed description of the larger experiment and details of fertilizer and herbicide applications. Pea plots received 15 kg ha^{-1} as triple superphosphate. Pea yield estimates were obtained from a 12-m × 45-m strip down the centre of the subplot in 2008, and a 5-m × 45-m strip down the centre of the subplot in 2009 and 2010. Growing season precipitation was about normal in 2008, below normal in 2009 and very wet in 2010 (Table 1).

The crop sequences examined in the present study included: field pea grown every year (Cont-P), field pea-wheat (P-W), field pea-canola-wheat (P-C-W) and a field pea-wheat-canola-wheat sequence (P-W-C-W). Only the pea phase of the sequences was sampled and all samples were obtained from the fungicide-treated subplots. In the fall of 2008, 2009 and 2010, soil samples were obtained from each plot by extracting two 3.8-cm-diameter cores to a 60-cm depth using a hydraulic punch. The cores were split into 0 to 15 cm, 15 to 30 cm, and 30 to 60 cm. In 2010 only, bulk density was estimated on the same samples by determining soil dry weight per core volume from these same cores. Because the entire soil core length to 60 cm was extracted, compaction was negligible. Soil samples were air-dried and ground to pass a 2-mm sieve. Nitrate-N was extracted with 2 M KCl in a 1:5 (wt:vol) soil:extractant ratio. Concentration was determined with a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY). Phosphorus in soil was extracted using Mehlich extract, and the concentration determined using a Technicon Autoanalyzer II. Analyses for the two cores per plot were averaged for a per plot estimate.

Biological nitrogen fixation was estimated using the ^{15}N enriched isotope dilution method (Hardarson and Danso 1990). After the pea crop was seeded, two 1-m × 1-m microplots were established adjacent to one another

Table 1. Monthly precipitation (mm) during the growing season (GS) in 2008, 2009 and 2010

Year	May	June	July	August	GS total
2008	12.7	87.0	85.4	20.8	205.9
2009	19.0	0.4	74.6	57.6	151.6
2010	128.1	145.6	122.4	61.8	457.9
LTA[z]	38.1	64.1	68.3	45.5	216.0

[z]LTA = long-term average for Scott, SK, for the period 1981 to 2010 (Environment Canada).

within each of the pea plots. In one of the microplots, five rows of wheat were hand-seeded into the area with 22.5-cm row spacing for use as a non-fixing reference crop. The second microplot was marked but left unmanaged. Approximately 1 wk after emergence of the pea crop, pea plants that emerged within the wheat microplot were removed by hand. A 10 atom%[15]N-urea solution was applied at an equivalent rate of 5 kg N ha^{-1} to both the wheat and pea microplots. Approximately 5 L of solution containing the total amount of urea for the plot was evenly applied to the soil surface within the microplot area. The urea application was followed by an equal volume of water to wash any ^{15}N-urea on the plant shoots into the rooting zone.

Within 5 d of final harvest of the plot, the middle three rows of plants in each of the microplots were hand-harvested for mass spectrometry analysis. The outer rows were hand-harvested and discarded. Plant samples were oven-dried (65°C), and the seed and straw ground separately with a Wiley mill (Thomas Scientific, Swedesboro, NJ) and then reground in a rotating ball-mill. Subsamples of wheat and pea seed and straw were analysed for atom%[15]N using a Costech Elemental Combustion System coupled to a Delta V Advantage Mass spectrometer (Isomass Scientific Inc. Calgary, AB). Percentage of N derived from atmosphere (%Ndfa) was calculated according to Hardarson and Danso (1990) as:

$$\%Ndfa = \left[1 - \left(\frac{atom\%^{15}Nexcess_{fixing}}{atom\%^{15}Nexcess_{non-fixing}} \right) \right] \times 100 \quad (1)$$

Where atom%[15]Nexcess is the ^{15}N content of the sample minus the ^{15}N content of atmospheric N_2 (i.e., 0.36637) in the fixing and non-fixing crops. %Ndfa was calculated for the seed and straw separately. Amount of N fixed was calculated according to Hardarson and Danso (1990) by:

$$Nfixed = \frac{\%Ndfa \times totalN_{fixing}}{100} \quad (2)$$

Nitrogen harvest index (NHI) was calculated as the amount of N in the seed relative to the total amount of N in the above-ground tissue. Total N acquired from the soil was calculated as the difference between total N in the plant minus N acquired through BNF.

The model for statistical analysis was a randomized complete block design with crop rotation and year as the main factors and four replicates. Rotation and replicate were considered as fixed factors and year as a random factor. F-statistics and their associated probability were calculated using the IBM SPSS Statistics ver. 19 software package. Effects were considered significant at $P < 0.05$. Tukey's HSD was calculated for expressing significant rotation effects.

RESULTS

Despite different crops grown in sequence with field pea, crop sequence did not affect soil inorganic N or P levels or bulk density (Table 2). Crop year affected levels of inorganic N in the 30- to 60-cm depth of the profile (Table 2), but values were always low, ranging from 8 to 33 kg ha^{-1} depending on depth. Inorganic N levels in the top 15 cm of soil were 28 kg N ha^{-1} in 2010, compared with 15.1 kg N ha^{-1} in 2008 and 18.7 kg ha^{-1} in 2009. Throughout the 60-cm profile values ranged from 46 to 58 kg ha^{-1} in the 3 yr, and were not statistically different ($P > 0.05$). Neither crop year nor rotation affected inorganic P level (Table 2). The highest levels of P in the 0- to 15-cm soil depth occurred in 2009 (98 kg ha^{-1}) with the lowest levels in these surface soils occurring in 2008 (63 kg ha^{-1}). Subsequent to soil sampling the plots were fertilized with 15 kg P ha^{-1}.

Rotation affected many of the N uptake and productivity parameters measured for field pea and affected N uptake and productivity differently depending on the year (Table 3). In 2008, pea in the P-W-C-W rotation had higher total shoot N contents than P grown in the rotations with pea included more frequently (Fig. 1). There was no difference among the three less diverse rotations. Pea grown in all of the rotations had approximately the same distribution of N between the seed and straw. NHI ranged from 0.73 in the Cont-P rotation to 0.77 in the P-W-C-W rotation. The differences in tissue N contents among rotations were less pronounced in 2009 and 2010 (Fig. 1). In 2009, the second most diverse rotation (P-W-C) had the highest total N uptake and in 2010 the rotations all had approximately the same amount of N in the pea tissues. However, in both years NHI was more variable ranging from 0.59 in Cont-P to 0.74 in P-W-C in 2009 and from 0.57 (P-W-C) to 0.64 (P-W-C-W) in 2010. In 2 of the

Table 2. Summary of *P* values from ANOVA for select soil characteristics in the spring prior to pea being grown in four crop rotations with different frequencies of pea

	Inorganic N at the depth (cm) of				Inorganic P at the depth (cm) of				Bulk density at the depth (cm) of		
	0–15	15–30	30–60	Sum	0–15	15–30	30–60	Sum	0–15	15–30	30–60
Rotation (R)	0.642	0.539	0.205	0.405	0.421	0.781	0.529	0.436	0.462	0.274	0.220
Year (Y)	0.118	0.118	**0.032**	0.344	0.111	0.508	0.105	0.170	—[z]	—	—
R × Y	0.131	0.391	0.250	0.122	0.349	0.295	0.198	0.246	—	—	—

[z]Bulk density was only measured in 2010.

Table 3. Summary of *P* values from ANOVA for nitrogen acquisition (kg ha^{-1})^{z} and productivity parameters (kg ha^{-1}) for pea grown in four crop rotations with different frequencies of pea

	Seed N	Straw N	Total N in shoots	NHI^{z}	Fixed N in seed	Fixed N in straw	Total fixed N	Total N from soil	Seed yield	Straw yield	Total above-ground biomass
Rotation (R)	0.498	0.447	0.556	0.306	0.130	**0.008**	0.082	**0.028**	0.531	0.316	0.417
Year (Y)	**0.030**	0.477	0.068	**0.009**	0.402	**0.009**	0.697	**0.007**	**0.019**	**0.042**	**0.028**
R × Y	**0.015**	0.525	0.054	**0.036**	**0.011**	0.691	**0.023**	0.458	**0.003**	0.094	**0.016**

^{z}Nitrogen harvest index (unitless) = seed N/total N in above-ground biomas.

3 yr the lowest NHI was calculated for pea in the Cont-P rotation and in the third year this rotation had the second lowest NHI.

The origin of N in the tissues, either from BNF or from the soil, varied widely with rotation and also interacted with year (Table 3). In 2008 and 2009, very little of the N in pea in the Cont-P rotation was obtained from BNF (Fig. 2); 18% in 2008 and 14% in 2009. In contrast, pea grown in the other three rotations obtained between 50 and 59% of their N from BNF. In both years, pea grown in the two most diverse rotations obtained a larger percentage of their N from BNF than the pea grown in the less diverse rotations. In 2010, pea grown in the Cont-P rotation obtained substantially more of its N from BNF (50%) than in previous years. Despite this improvement in BNF, this rotation still had the lowest N uptake through BNF of all the rotation sequences.

Despite the low BNF occurring in the Cont-P rotation in 2008 and 2009 (Fig. 2), the N that was fixed biologically was preferentially found in the seed (Fig. 3). In the 2010 growing season, when BNF contributed 50% of the N to the pea shoot in the Cont-P rotation, 80% of the biologically fixed N was found in the seed compared with 64 to 70% for the other rotations.

The poor BNF observed in the Cont-P rotation was not due to poor growth of the pea in this rotation in any year (Fig. 4). With the exception of the P-W-C-W rotation in 2008 and the P-W-C rotation in 2009, all of the other rotations had similar yields. Furthermore,

in 2010, when differences among all of the rotations were less pronounced for N uptake parameters, all of the rotations yielded comparable amounts of straw and seed.

DISCUSSION

Varying the frequency of field pea in rotation with wheat and canola affected both the amount of N and the origin of the N in the above-ground biomass of the pea phase of the rotation. In 2 of 3 yr, pea grown in more diverse rotations that included canola (P-W-C-W in 2008 and P-W-C in 2009) had the highest tissue N contents and the amount of tissue N fixed through BNF was highest in these rotations.

The main influence that living plants have on microbial populations in the soil is through root exudation. Some of the substances released into the rhizosphere serve as carbon sources for bacteria and fungi and support the metabolic activities of diverse groups of microorganisms (Bais et al. 2006). The composition of root exudates is influenced by plant species. For example, exudates from pea roots had a lower percentage of carboxylic acids and higher percentage of sugars compared with maize roots (Gransee and Wittenmayer 2000). Considering that bacteria isolated from different plants (Lemanceau et al. 1995) and even bacteria isolated from different cultivars of the same plant species (Misko and Germida 2002) have different carbon utilization profiles, it follows that the organic compounds released
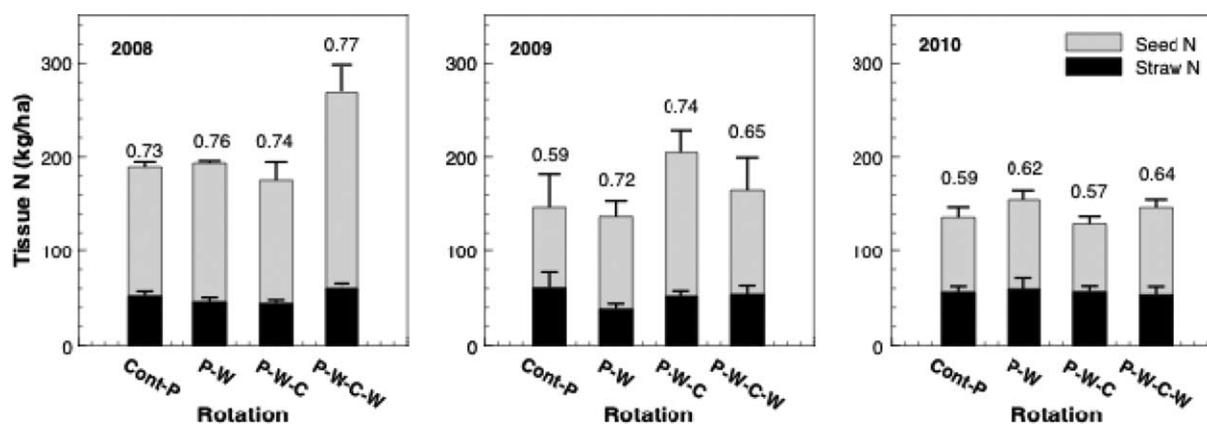

Fig. 1. Distribution of total above-ground nitrogen in seed and straw from pea grown in four different cropping sequences in 3 crop years. Numbers above the bars are nitrogen harvest indices (NHI). Error bars are standard errors of the mean.
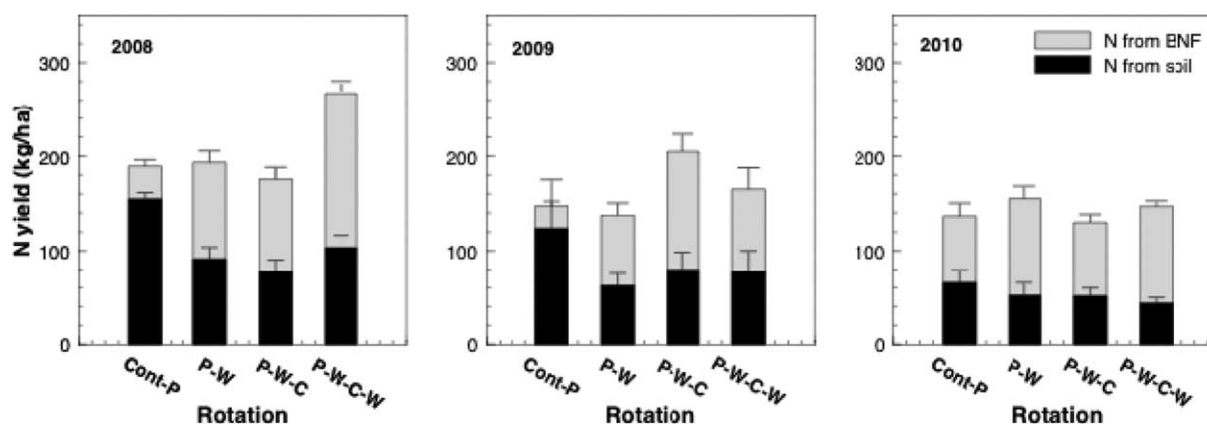
Fig. 2. Nitrogen from biological nitrogen fixation (BNF) and soil uptake in pea grown in four different cropping sequences in 3 crop years. Error bars are standard errors of the mean.

from plant roots are at least partially responsible for the diversity of microorganisms associated with different plant root systems. Certainly, soils that grow a diversity of plant species support more diverse microbial populations (Lupwayi et al. 1998; Wieland et al. 2001). Including canola with pea and wheat in rotation may increase the microbial biodiversity of the rotation making it more flexible and able to respond to environmental and/or biotic fluctuations that affect BNF.

The continuous pea rotation supported the lowest BNF in all years of the study and the lowest proportion of above-ground N acquired through BNF. Differences in BNF were not due to the concentration of inorganic N in the continuous pea rotation inhibiting BNF since rotation had no effect on inorganic N levels in the pea phase of the study. Nayyar et al. (2009) reported generally smaller microbial biomass and higher root rot disease ratings under an 11-yr continuous pea rotation compared with a pea-wheat rotation at Indian Head, SK, that accounted for differences in dry matter production between pea in the two rotations. Although diseases were

not assessed in the current study at Scott, if there were higher incidences of disease in the continuous rotation compared with the more diverse rotations, they were not severe enough to affect N uptake, nor productivity of continuous pea to any greater extent than the other rotations. Total N yield and residue and seed yields were not consistently lower in the continuous pea rotation at Scott. Malhi et al. (2011) reported cumulative seed yields for 1998 through 2005 for all of the rotations at the same study site in Scott, SK. Reductions in seed yield for continuous pea were modest, ranging from only 1% compared with the P-W-C rotation, to 13% compared with the P-W rotation. In contrast, Lafond et al. (2011) reported a 25% reduction in yield of continuous pea compared with a P-W rotation at Indian Head SK, but no yield reduction when pea in the P-W rotation was compared with W-W-P. Kutcher and Brandt (2008) similarly reported no yield differences for pea grown every 2, 3 or 4 yr.

Differences in BNF may be related to differences in microbial populations among the different rotations.
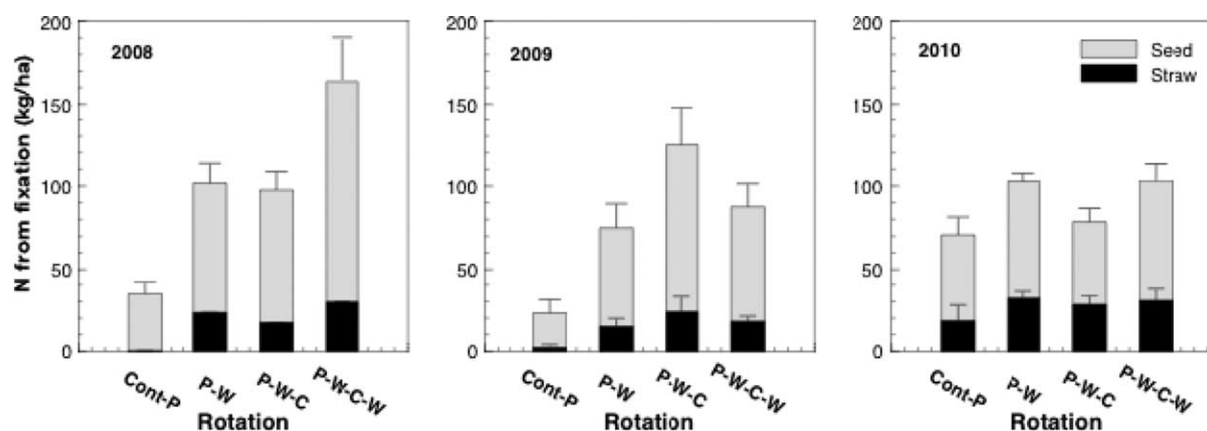

Fig. 3. Distribution of nitrogen from biological nitrogen fixation in seed and straw from pea grown in four different cropping sequences in 3 crop years. Error bars are standard errors of the mean.
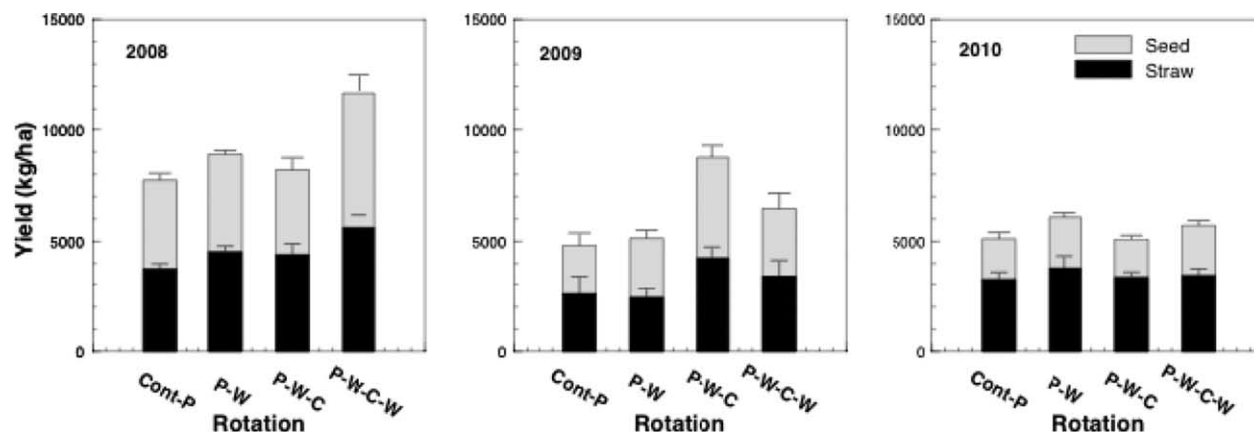
Fig. 4. Seed and straw yield from pea grown in four different cropping sequences in 3 crop years. Error bars are standard errors of the mean.

Changes in microbial communities in response to crop rotation have been documented (O'Donnell et al. 2001; Yin et al. 2010) although the mechanisms of how soil microbial communities are affected are poorly understood and appear to be highly complex (Yin et al. 2010). Nayyar et al. (2009) reported significantly lower percentages of arbuscular mycorrhizal fungi (AMF) colonizing the roots of pea in continuous rotation compared with pea in a P-W rotation that could affect the tripartite relationship between pea roots, rhizobia and AMF (Chalk et al. 2006). However, despite the statistically significant difference between 31% colonization in the continuous pea rotation and 34% colonization in the P-W rotation (Nayyar et al. 2009) it is unlikely that these differences in AMF colonization have biological significance.

The most dramatic difference in N acquired through BNF occurred between study years. In 2008 and 2009, the inhibition of N fixation was extreme in the Cont-P rotation, with less than 20% of N acquired through N fixation in these 2 yr, whereas in 2010, 50% of the total N was acquired through BNF. The enhanced fixation in 2010 may be due to an effect similar to a fertilizer "starter-N" effect. Nitrogen levels in the 0- to 15-cm depth were more than 10 kg ha^{-1} higher in 2010 than levels in 2008 and 2009 and may have stimulated early root growth, leading to enhanced nodulation and N fixation (Voisin et al. 2002). Alternatively, the extreme amounts of precipitation received during the 2010 growing season probably had a direct effect on microbial populations in the soil. While very wet conditions typically contribute to anaerobic conditions and hence decreased BNF, the fairly even distribution of the rainfall throughout the growing season months appears to have provided enough time for drainage to occur providing adequate conditions for BNF.

Biologically fixed N was found preferentially in the seed of pea in all of the rotations. Van Kessel (1994) reported that at maturity % Ndfa in lentil pods and seed was as high as 91%, whereas significantly less (ca. 80%) N in the leaves and stems was derived from BNF. In the continuous pea rotation, while only a small amount of N was obtained from BNF in 2008 and 2009, all of the fixed N was found in the seed. This implies that active BNF in this rotation was delayed until seed set. In general, early N uptake from the soil is used for vegetative growth and the development of photosynthetic tissues and once the soil N source is depleted BNF becomes the dominant mechanism for N capture. Considering that initial soil N levels were not different among the rotations, some other mechanism must have been at play to delay BNF in this rotation. The C:N ratios in pulse residues are narrower than those in cereals and oilseeds making pulse residues more susceptible to N-mineralization. Pea grown on pea stubble in the continuous rotation would be exposed to inorganic N from mineralization as soon as microbial populations were actively mineralizing the pea residue. In contrast, the wide C:N ratio in the rotations with wheat and canola would initially cause N to be immobilized thereby depleting the inorganic N levels in the soil, providing ideal conditions for BNF to occur. It is not until later in the season when the easily mineralized pea residue in the continuous pea rotation is depleted that conditions favorable for N fixation arise. In the very wet growing season in 2010, the contribution of BNF to the straw in the continuous pea rotation indicates that conditions suitable for BNF occur earlier than the previous years. This could be due to the high rainfall leaching mineralized N out of the rooting zone, or possibly due to more rapid mineralization of the pea residue.

In summary, under near-normal growing season precipitation the rotations that included pea in sequence with wheat and canola every 3 or 4 yr, supported the highest BNF in the pea phase of the rotation. In these years with near-normal precipitation, increasing the frequency of pea to every second year and even every year resulted in comparable N uptake and seed yields to

all but the highest producing rotation in a particular year. However, increasing the frequency of field pea to every year drastically reduced BNF. Determining whether or not the inhibition of BNF was due to the inhibitory effects of inorganic N from mineralization of the previous year's pea residue requires further investigation.

ACKNOWLEDGMENTS

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