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# Enhanced biological nitrogen fixation in pea-canola intercrops quantified using $^{15}N$ isotopic analysis

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ABSTRACT

Legume and non-legume intercrops are a promising tool to increase productivity and reduce reliance on nitrogen (N) fertilizers through $N_2$ fixation. However, limited research quantifying N contributions in pea-based intercrops warrants further exploration. Across Saskatchewan, pea-oat (PO) and pea-canola (PC) intercrops, along with their monocrops, were grown under three N fertilizer rates (0, ¼, and ½ of N applied to non-legume monocrops), while canola and oat monocrops received their full recommendation rates. Using the $^{15}N$ dilution method, the percentage of N derived from the atmosphere (%Ndfa) was higher in intercropped peas than in pea monocrops. On a per pea plant basis, PC increased N fixation by 22 % over pea monocrop, but PO reduced N fixation by 15 %. Soil was the major source of N for intercrops (61–73 %), followed by atmosphere (20–38 %), and then fertilizer (1–7 %). Comparing intercrops, PO recovered 22 % more N fertilizer than PC; however, neither intercrop recovered more fertilizer N than non-legume monocrops. Increasing N fertilizer supply to intercrops did not affect N uptake or N fertilizer recovery but reduced %Ndfa by up to 14 % and N fixation by up to 36 %, indicating that no N fertilizer is needed for intercrops. The intercrops had similar biomass N to monocrops, but PC's greater N fixation led to more efficient N use than PO and monocrops on a per land area basis. The results indicated that pea-based intercrops significantly increased %Ndfa and altered N sources compared to pea monocrop, providing an alternative pathway for sustainable N management.

## 1. Introduction

Nitrogen (N) fertilizers are the reason our arable land can support more than double the population it did 100 years ago and are; therefore, essential for food production (Erisman et al., 2008); however, they have come with unforeseen consequences. Excessive fertilization can pollute our water and atmosphere (Fustec et al., 2010). Therefore, there is a need for improved management practices that reduce fertilizer rates without sacrificing yield. Legume-based intercropping provides a promising strategy for N management, because the growth of multiple crops together can utilize resources more efficiently and reduce the amount of N fertilizer required for optimal yield production (Cowden et al., 2020).

Legume and non-legume intercrops are commonly used because of the legume's ability to fix atmospheric N, their enhanced resource use efficiency, and the land equivalent ratio advantages over monocrops (Fletcher et al., 2016; Unay et al., 2021; Bremer et al., 2024). Grown in an intercrop, legumes can derive a higher percentage of their N from the atmosphere because the non-legume crop competes for soil N and forces the legume to rely more on biological N fixation (BNF) (Hauggaard-Nielsen et al., 2009; Demie et al., 2025). There has also been evidence that nodule numbers can increase when pea is grown in intercrops rather than monocrops (Chapagain and Riseman, 2014), leading to pea-barley intercrops deriving 73 % of N from the atmosphere compared to 66 % in pea monocrops (Hauggaard-Nielsen et al., 2009). This intercrop combination also utilizes 18–20 % more soil N (including fertilizer) than their respective monocrops, leading to a 30–40 % increase in N use efficiency (NUE) compared to monocropping systems (Hauggaard-Nielsen et al., 2009).

Additionally, evidence demonstrates that intercropped legumes can transfer N to their companion crop, although results vary on the quantity of N transfer between crops (Chapagain and Riseman, 2014; Gungaabayar et al., 2023; Reid et al., 2024). Because of their ability to utilize resources more efficiently, these intercrops have shown yield increases of up to 75 % compared to their monocrops, but these advantages decrease with increasing N fertilizer rates (Bremer et al., 2024). Thus,

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intercrops have the potential to reduce fertilizer applications and utilize N more efficiently through increased BNF, N transfer, and recovery of more fertilizer/soil N while increasing productivity (Hauggaard-Nielsen et al., 2009; Chapagain and Riseman, 2014; Gungaabayar et al., 2023).

Intercrops have demonstrated success in other regions, but limited research has been conducted across western Canada. Pea-cereal intercrops are common because of cereals' competitive ability to reduce pest issues and lodging in pea (Ghaley et al., 2005), while pea-oilseed intercrops can provide the same advantages (Waterer et al., 1994) and show great promise in Saskatchewan (Reid, 2022). In intercrops, non-legume crops can directly benefit from the legume's fixed N through N transfer. This is particularly true for crops that can form arbuscular mycorrhizal fungi (AMF) associations (Chapagain and Riseman, 2014). Pea-cereal intercrops can also recover more N fertilizer than pea monocrops, but additional research is needed to compare the differences in N fertilizer recovery between different types of intercrops (Cowden et al., 2020).

Many studies have used the $^{15}$N isotope to trace N and determine practices that maximize N use efficiency (Cowden et al., 2020; Reid et al., 2024). The $^{15}$N dilution method enables researchers to determine the percentage of plant N derived from the atmosphere (%Ndfa), fertilizer (%Ndff), and soil (%Ndfs) (International Atomic Energy Agency, 1990) and has been used in previous studies involving intercrops (Chapagain and Riseman, 2014; Cowden et al., 2020; Reid et al., 2024). Many studies have found that intercrops can increase %Ndfa compared to monocrops (Chapagain and Riseman, 2014; Bremer et al., 2024). Cowden et al. (2020) determined that intercropped pea derived 112 % more N from soil than pea monocrops. Gaining a better understanding of how intercrops source their N will allow for accurate N fertilizer recommendations in intercropping systems and enable producers to manage N more efficiently. Additionally, increased NUE could incentivize growers to incorporate intercrops if they can reduce fertilizer applications while maintaining productivity to increase their profits (Peoples and Craswell, 1992). Further research is needed to understand N dynamics and how intercrops source their N (atmosphere, fertilizer, and soil) differently than monocropping systems, along with differences between intercrop types, so that productivity can be maximized without oversupplying crops with N fertilizer.

The objectives of this paper were to determine: 1) how intercrops vs. monocrops, 2) intercrop type, and 3) N fertilizer rate affected %Ndfa, N fixation, %Ndff, %Ndfs, sources of plant N uptake, and N fertilizer recovery using the $^{15}$N dilution approach. We hypothesized that N would be used most efficiently in the intercrops compared to the monocrops, PO would have greater N fixation than PC because oat is a mycorrhizal associated crop, whereas canola is non-mycorrhizal, and the unfertilized treatment in intercrops would maximize the overall benefits of BNF.

## 2. Material and methods

### 2.1. Site description

This was a two-year (2021 and 2022) field study conducted at Agriculture and Agri-Food Canada's Swift Current Research and Development Centre (50°16' N, 107°46' W) and Melfort Research Farm (52°49' N, 104°35' W). Swift Current soil is a silty loam (Swinton Orthic Brown Chernozem) and Melfort is a silty clay loam (Orthic Black Chernozem). The precipitation and mean daily temperature for each site are reported below (Environment Canada, 2025) (Table 1). Plots were 4 m wide and 7 m long in Melfort and 10 m long in Swift Current.

### 2.2. Experimental design

Pea-oat and PC intercrops were grown along with pea (PMono), oat (OMono), and canola (CMono) monocrops. Canola and oat monocrops received their full recommendation of N fertilizer, while intercrops received three rates: 0, ¼, and ½ the amount of N applied to the recommended non-legume monocrops. Monocrop fertilizer rates were determined based on targeted yields and estimated N removals, and PMono received no N fertilizer. Therefore, the treatments included PMono, OMono, CMono, PO 0 N, PO ¼ N, PO ½ N, PC 0 N, PC ¼ N, and PC ½ N. These nine treatments were established in a randomized complete block design with four replicates for a total of 36 plots per site.

### 2.3. Field management

The previous season, fields were seeded to wheat to maintain consistency across sites. Pea (*Pisum sativum* cv. CDC Inca), oat (*Avena sativa* cv. CDC Arborg), and canola (*Brassica napus* cv. Clearfield PV 200) monocrops were seeded, along with PO and PC intercrops, using a no-till plot seeder. The trial was seeded in early- to mid-May (depending on weather conditions) and monocrops were seeded at a rate of 125 (PMono), 300 (OMono), and 200 seeds m$^{-2}$ (CMono). These rates are consistent with local recommendations (Canola Council of Canada, 2025; Prairie Oat Growers Association, 2020; Saskatchewan Pulse Growers, 2025). The PO and PC intercrops were grown in mixed row arrangements, where pea was seeded at 2/3 its regular rate (85 seeds m$^{-2}$) and oat and canola were seeded at ½ their regular rate (150 and 100 seeds m$^{-2}$, respectively). Studies found that intercrops performed better when legumes were seeded above 50 % of their monocrop seeding rate (Hauggaard-Nielsen et al., 2009; Gungaabayar et al., 2023). Accordingly, the pea seeding rate was set at 2/3 of the monocrop seeding rate to give it an advantage to compete against the non-legume crop and provide higher BNF that could benefit both crops in the intercrop. Row spacing was 25 cm in Swift Current and 30 cm in Melfort.

Nitrogen fertilizer application rates were determined based on crop grain N removal for the monocrop, with ¼ and ½ of the monocrop N rate

**Table 1**
Precipitation and mean daily temperature at Swift Current and Melfort Environment Canada weather stations: 2021, 2022, and long-term norms.

| | Precipitation (mm) | | | Mean daily temperature (°C) | | |
|---|---|---|---|---|---|---|
| | 2021 | 2022 | Long-term norms (1991–2020) | 2021 | 2022 | Long-term norms (1991–2020) |
| ***Swift Current*** | | | | | | |
| May | 35.9 | 51.2 | 45.3 | 9.5 | 10.9 | 10.7 |
| June | 29.6 | 37.7 | 91.7 | 18.4 | 15.9 | 15.2 |
| July | 38.9 | 90.4 | 46.2 | 21.7 | 19.8 | 18.3 |
| August | 55.7 | 7.5 | 48.1 | 18.0 | 20.9 | 18.0 |
| Total | 160.1 | 186.8 | 231.3 | | | |
| ***Melfort*** | | | | | | |
| May | 31.4 | 90.8 | 33.0 | 9.6 | 9.9 | 10.1 |
| June | 37.6 | 78.1 | 77.5 | 18.2 | 15.2 | 15.2 |
| July | 0.2 | 34.9 | 79.3 | 20.1 | 18.2 | 17.5 |
| August | 69.3 | 36.5 | 52.3 | 16.9 | 18.7 | 16.6 |
| Total | 138.5 | 240.3 | 242.1 | | | |

applied in the intercrop N treatments as defined by the specific treatments. Urea (46−0−0) was side-banded at both sites, where OMono received 50 kg N ha$^{-1}$ and CMono received 81 kg N ha$^{-1}$, except Melfort 2021, where OMono received 56 kg N ha$^{-1}$ and CMono received 112 kg N ha$^{-1}$. Along with urea, monoammonium phosphate (11−52−0) was seed placed at 40 kg ha$^{-1}$ across all plots at both sites. At Swift Current, 3.7 kg ha$^{-1}$ of TagTeam granular inoculant (*Rhizobium leguminosarum* + *Penicillium bilaiae*) (NexusBioAg) was applied to treatments containing pea and at Melfort, pea seed was treated with a liquid inoculant (2.78 mL kg$^{-1}$) Cell-Tech® (*Rhizobium leguminosarum*) (NexusBioAg).

Weeds were controlled using a pre-seeding application of glyphosate (900 g a.i. ha$^{-1}$) and then during the season, herbicides were applied when necessary. Solo (20 g a.i. ha$^{-1}$) and Assure II (47 g a.i. ha$^{-1}$) were used in PMono, CMono, and PC intercrops, and MCPA (198 g a.i. ha$^{-1}$) in PO intercrops. Buctril M (560 g a.i. ha$^{-1}$) was applied to OMono when necessary.

### 2.4. Soil sampling and analysis

Before seeding, soil characteristics were determined for each site at 0–15 cm (Table 2). The $NH_4^+$ and $NO_3^-$ were determined using 2 M KCl (Maynard et al., 2007) and $PO_4^-$ and K were determined using 0.5 M $NaHCO_3$. Inorganic C was removed using 6 M HCl before determining organic %C using dry combustion. Total %N was also determined using dry combustion. The McKeague (1978) saturated paste method was used to determine pH and EC.

After harvest, soil samples were taken from 0 to 15 cm and 15–30 cm depths for mineral N analysis ($NO_3^-$ and $NH_4^+$). Inorganic N was extracted from 10 g of air-dried soil with 50 mL of 2 M KCl. The samples were shaken for 15 min before being filtered through Whatman No. 40 filter paper and analyzed by a SEAL AutoAnalyzer 3 Continuous Segmented Flow Analyzer (Kitchener, Ontario, CA).

### 2.5. Plant sampling and analysis

After the crops were seeded, two 1 m x 1 m micro-plots were established in the centre of plots with 2 m spacing between them. The micro-plots enabled us to trace N through the system using the $^{15}$N dilution method (McAuliffe et al., 1958). One week after emergence, 0.3701 g m$^{-2}$ of 50 atom% $^{15}$N-enriched urea (equivalent to 5 kg ha$^{-1}$ of N at 18 atom% $^{15}$N urea) was applied to one micro-plot ($^{15}$N micro-plot), while non-enriched urea (0.3701 g m$^{-2}$) was applied to another (control). A steel frame was inserted into the soil, delineating the micro-plot area and the fertilizers were dissolved in 2 L of water before evenly applying them across the micro-plots. Depending on soil moisture, 2–4 L of additional water was added to the micro-plots to leach the applied fertilizer into the rooting zone.

**Table 2**
Soil physical and chemical characteristics of 0–15 cm depth soils at Swift Current and Melfort before seeding in the spring of 2021 and 2022.

| | 2021 | | 2022 | |
|---|---|---|---|---|
| | Swift Current | Melfort | Swift Current | Melfort |
| $NH_4^+$ (mg N kg$^{-1}$) | 3.3 | 11.8 | 3.2 | 6.6 |
| $NO_3^-$ (mg N kg$^{-1}$) | 4.1 | 11.2 | 2.7 | 6.8 |
| $PO_4^{-3}$ (mg P kg$^{-1}$) | 16.2 | 6.5 | 10.4 | 5.2 |
| K (mg K kg$^{-1}$) | 266.3 | 223.0 | 299.5 | 277.9 |
| Total N (g kg$^{-1}$) | 1.4 | 4.4 | 1.5 | 3.9 |
| Organic C (g kg$^{-1}$) | 14.0 | 49.6 | 15.5 | 47.2 |
| pH | 7.1 | 6.7 | 7.3 | 6.0 |
| EC (mS cm$^{-1}$) | 0.9 | 0.6 | 1.2 | 0.7 |

Once crops reached physiological maturity, biomass samples were hand-harvested from the micro-plots. Afterwards, the central five rows in each plot were harvested using a plot-combine, avoiding the micro-plot areas. Grain harvested from the intercrops was cleaned and separated based on crop type to determine crop yield (Liu et al., 2025). Biomass samples were separated by crop type and dried at 45ºC then threshed to determine grain and straw yields of both crops in the intercrops. Dried straw was ground using a Wiley® Mill (Thomas Scientific, Swedesboro, NJ, United States) and seeds were ground using a laboratory mill (Perten Instruments, Shelton, CT, United States) and then both samples were finely ground using a ball mill (<0.5 mm) (Mixer Mill MM 500 Vario, Retsch USA Verder Scientific Inc., Newtown, PA, United States). Subsamples (4 mg +/- 0.01) of the ground seed and straw were weighed using a Mettler Toledo Balance XSR105 (Switzerland) into 8 × 5 mm tin capsules (Isomass Scientific Inc., Calgary, Canada), where they were compacted airtight and placed into 96-well trays (Thermo Fischer Scientific, Voltaweg 22, 2627 BC Delft, The Netherlands). These samples were analyzed for total %N and atom% $^{15}$N using a Flash 2000 Elemental Analyzer (Thermo Fisher Scientific, Voltaweg 22, 2627 BC Delft, The Netherlands), coupled with a Finnigan Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Electron, Bremen, Germany).

The %N in biomass samples was averaged between the two micro-plots, then multiplied by biomass to determine above-ground biomass (biomass$_a$) N uptake. According to Eq. 1 (Mead and Willey, 1980), N land equivalent ratio (NLER) was calculated, where values above 1 indicate an intercrop N advantage and values below 1 indicate an intercrop N disadvantage. Oat was replaced with canola when calculating NLER for the PC intercrop.

$$NLER = NLERp + NLERo \quad (1)$$

*where* $NLER_p$= N $Uptake_{PeaPO}$/N $Uptake_{PMono}$ *and* $NLER_o$= N $Uptake_{OatPO}$/N $Uptake_{OMono}$

The atom% $^{15}$N in plant samples enabled us to calculate the %Ndfa in pea (Eq. 2) and the amount of N fixed by pea (Eq. 3) (Chalk and Craswell, 2018). As described above, NLER values > 1 indicate an intercrop advantage on a per-land-area basis. To assess NLER on a per pea plant basis, a reference value of 0.67 (rather than 1) was used since intercrop pea was seeded at 2/3 the rate of PMono. Therefore, $NLER_{pBNF}$ assesses N fixed on a per-pea-plant basis to determine if individual plant N fixation efficiency was improved in intercrops (Eq. 4). A similar method was used in Hauggaard-Nielsen et al. (2009). Eq. 4 was used for both PO and PC intercrops.

$$\%Ndfa = \frac{^{15}N\ atom\%\ excess_{OMono} - {}^{15}N\ atom\%\ excess_{Pea}}{^{15}N\ atom\%\ excess_{OMono}} \times 100\% \quad (2)$$

$$N\ Fixation(kg\ N\ ha^{-1}) = (\%Ndfa_{Grain} \times N\ Uptake_{Grain}) + (\%Ndfa_{Straw} \times N\ Uptake_{Straw}) \quad (3)$$

$$NLER_{pBNF} = N\ Fixation_{Pea\text{-}in\ intercrop}/N\ Fixation_{PMono} \quad (4)$$

Additionally, the percentage of total biomass$_a$ N in the intercrop derived from BNF (%NdfBNF) was determined using Eq. 5. This differs from %Ndfa, where we only observed N within pea that was derived from the atmosphere. This equation is specific to intercrops, where more than one crop is grown, to observe the percentage of biomass$_a$ N derived from the atmosphere.

Furthermore, the percentage of N derived from N fertilizer (%Ndff) (Eq. 6) and from soil (%Ndfs) (Eq. 7) were calculated to determine where crops sourced their N from (International Atomic Energy Agency, 1990).

$$\%NdfBNF = \frac{N\ Fixation}{Biomass_a\ N_{Pea} + Biomass_a\ N_{Oat/Canola}} \times 100\% \quad (5)$$

*where* $Biomass_a$= above-ground biomass

$$\%Ndff = \frac{^{15}N\ atom\ \%\ excess\ in\ plant}{^{15}N\ atom\ \%\ excess\ in\ fertilizer} \times 100\% \quad (6)$$

$$\%Ndfs = 100 - \%NdfBNF - \%Ndff \quad (7)$$

*where* atom % $^{15}N$ excess is the difference between $^{15}N$ abundance in enriched plants and non-enriched plants from the micro-plots. The OMono was used as a reference crop.

Using %Ndff, N fertilizer recovery efficiency (%NREf) was determined using Eq. 8.

$$\%NREf = \frac{\%Ndff \times Biomass_a\ N}{N\ Fertilizer} \times 100\% \quad (8)$$

### 2.6. Statistical analysis

Data were analyzed using R Studio (Wickham, 2016; R Core Team, 2021; Wickham and Girlich, 2022). A mixed linear effect model (Kuznetsova et al., 2017) was used with treatment as a fixed factor and site, year, and replicate as random factors. Three separate analyses were conducted: 1) PO intercrops compared to their monocrops, 2) PC intercrops compared to their monocrops, and 3) factorial analysis with intercrop type and N fertilizer rate to compare the intercrops, fertilizer rates, and their interaction. Since the preliminary results showed significant effects of N rates on most N response variables, individual intercrops (at each N rate) were compared with monocrops instead of pooling intercrops across three N rates for the intercrop versus monocrop comparison. Sites were combined for the analyses to create a robust dataset. Differences of least squares means (Kuznetsova et al., 2017) were used to determine treatment differences.

*P-values* $\leq 0.05$ were considered significant. Data points outside three standard deviations from the mean were considered outliers (Lehmann, 2013) and removed from the dataset. The normality of the residuals was tested using the Shapiro-Wilk statistic and the homogeneity of variance was tested visually using fitted vs. residual plots. Log transformations were conducted where necessary and back-transformed least squares means were reported.

## 3. Results

### 3.1. Land equivalent ratio of plant nitrogen uptake

Among effects of intercrop type, N fertilizer rate, and their interactions on NLER of grain, straw, and $biomass_a$, only intercrop type affected NLER of grain N ($p < 0.001$) and $biomass_a$ N ($p = 0.003$). The PC intercrops provided straw N and $biomass_a$ N uptake advantages over monocrops and PO intercrops increased straw N uptake over monocrops (Fig. 1). Straw N in the intercrops was 23 % (PO) and 20 % (PC) higher than monocrops and no differences were measured between the two types of intercrops (Fig. 1B). In contrast, both intercrops had reduced grain N compared to their monocrops, but PC produced an 8 % higher grain NLER than PO (Fig. 1A). Similarly, PC's $biomass_a$ NLER was larger than PO and showed a 3 % NLER advantage over its monocrops, whereas PO produced 4 % less $biomass_a$ N than its monocrops (Fig. 1C). Oat was the dominant crop in PO ($biomass_a$ $NLER_o$=0.60; $biomass_a$ $NLER_p$=0.36), compared to PC where pea was dominant ($biomass_a$ $NLER_p$=0.70; $biomass_a$ $NLER_c$=0.33). Pea in PC produced over double the biomass as in PO (Choo-Foo, 2024), which translated to pea N uptake being almost double in PC compared to PO (Fig. 1). Additional information on crop biomass and N uptake of individual crops can be found in Choo-Foo (2024), along with NUE indices (%NHI, NUtE, $NUE_{crop}$).

### 3.2. Biological nitrogen fixation

Pea-based intercrops significantly increased %Ndfa compared to PMono, while their effects on the amount of N fixed varied (Table 3). For PO intercrops, grain %Ndfa was 34–59 % higher and $biomass_a$ %Ndfa was 37–49 % higher in PO intercrops than in PMono. However, grain N fixation was 48–63 % lower and $biomass_a$ N fixation was 38–51 % lower in PO intercrops than in PMono. There was no difference in either %Ndfa or N fixation among PO intercrops, although increasing N rate generally reduced %Ndfa and N fixation (Table 3). Similarly, PC 0 N and PC ¼ N increased grain %Ndfa by 28 and 31 % and $biomass_a$ %Ndfa by 25 and 20 % compared to PMono, respectively (Table 3). Increasing N rates significantly reduced N fixation in PC intercrops, but at the lower N rates (e.g., 0 and ¼ N rate), N fixation in PC intercrops was similar to that in PMono (Table 3).

Intercrop type affected %Ndfa and N fixation (Table 4). Compared to PC intercrops, PO intercrops increased grain %Ndfa by 19 % and $biomass_a$ %Ndfa by 20 %, but reduced N fixation by 47 % in grain and 40 % in $biomass_a$ (Table 4). Nitrogen fertilizer rate did not affect $biomass_a$ %Ndfa but affected grain %Ndfa and both grain and $biomass_a$ N fixation (Table 4). Increasing N rates in intercrops showed a declining trend in %Ndfa and N fixation. Applying N at the ½ N rate reduced grain %Ndfa by 11–14 %, grain N fixation by 32–36 %, and $biomass_a$ N fixation by

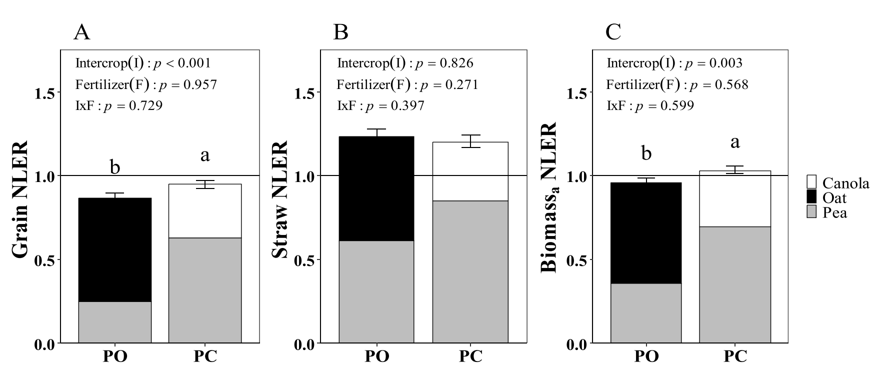


**Fig. 1.** Grain (A), straw (B), and above-ground biomass (C) N land equivalent ratios (NLER) in pea-oat (PO) and pea-canola (PC) intercrops. Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops); monocrops received their full N recommendation, except pea which received no N fertilizer. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022. Bars are means with standard errors ($n = 48$) and $p \leq 0.05$ were considered significant. NLER values $\geq 1$ indicate an intercrop advantage, whereas values $\leq 1$ indicate an intercrop disadvantage.

**Table 3**
Nitrogen derived from the atmosphere (%Ndfa) and N fixation by pea in pea-oat (PO) intercrops, pea-canola (PC) intercrops, and pea monocrop (PMono). Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops) and PMono received no N fertilizer. Results are the combined analysis Swift Current and Melfort sites in 2021 and 2022.

| | %Ndfa | | N fixation (kg N ha$^{-1}$) | |
|---|---|---|---|---|
| | Grain | Biomass$_a$[1] | Grain | Biomass$_a$ |
| ***Pea-Oat*** | | | | |
| PO 0 N | 60.3 a[2] | 55.6 a | 16.7 b | 24.0 b |
| PO ¼ N | 62.7 a | 55.1 a | 16.3 b | 24.2 b |
| PO ½ N | 53.0 a | 50.9 a | 11.8 b | 19.0 b |
| PMono | 39.5 b | 37.2 b | 31.9 a | 38.9 a |
| | ***p*<0.001** | ***p*<0.001** | ***p*<0.001** | ***p*<0.001** |
| ***Pea-Canola*** | | | | |
| PC 0 N | 50.6 a | 46.6 a | 33.4 a | 43.3 a |
| PC ¼ N | 51.9 a | 44.8 a | 30.8 a | 37.7 a |
| PC ½ N | 44.9 ab | 42.9 ab | 20.6 b | 29.3 b |
| PMono | 39.5 b | 37.2 b | 31.9 a | 38.9 a |
| | ***p*=0.011** | ***p*=0.032** | ***p*=0.001** | ***p*=0.004** |

[1] Biomass$_a$= above-ground biomass.
[2] Means ($n$ = 16) followed by the same letter are not significantly different ($p$ > 0.05).

23–29 % compared to the 0 and ¼ N rates (Table 4). The interaction between intercrop type and N fertilizer rate had no effect on %Ndfa or N fixation, with $p$-values ranging from 0.099 to 0.967 (Table 4).

Nitrogen fixation was positively correlated with pea biomass$_a$ (Fig. 2). Regression analysis indicated that approximately 0.01 kg of N was fixed per kg of pea biomass$_a$. As pea grain N fixation increased, so did overall grain N uptake in the intercrop ($r^2$= 0.80; $p$ < 0.001) and a similar trend occurred with biomass$_a$ N fixation and N uptake ($r^2$= 0.71; $p$ < 0.001). Based on NLER$_{pBNF}$, the large N fixation of PC was evident, providing 37 % higher N fixation than PO (Table 4). However, neither intercrop provided an N fixation advantage over PMono (i.e., NLER$_p$<1) (Table 4). That being said, intercropped pea was seeded at 2/3 the rate of PMono; thus, NLER$_{pBNF}$ was compared to 0.67 (instead of 1) to determine if intercrops provided an N fixation advantage over PMono on a per pea plant basis. Based on this, PC increased N fixation by 22 % over PMono (Table 4). Nevertheless, PO showed a 15 % disadvantage compared to PMono (Table 4).

### 3.3. *Percentage of plant nitrogen derived from atmosphere, fertilizer, and soil*

In PO intercrops, increasing N rates significantly decreased the proportion of plant N derived from BNF, with 26 %, 25 %, and 20 % derived from BNF when supplied with 0 N, ¼ N, and ½ N, respectively (Fig. 3A). However, all PO intercrops had 30–45 % lower %NdfBNF compared to PMono (Fig. 3A). Opposite to %NdfBNF, %Ndff increased with increasing fertilizer rate, where OMono had a higher %Ndff than all three intercrops, and the three intercrops were higher than PMono (Fig. 3A). Although PO 0 N and PMono had no N fertilizer applications, they did receive small amounts of N from monoammonium phosphate and $^{15}$N fertilizer applications; therefore, %Ndff could still be calculated for these treatments. Observing %Ndfs, OMono was most reliant on soil N and derived an average of 22 % more of its N from the soil than PO intercrops (Fig. 3A). Overall, soil was the largest source of N for the crops, followed by BNF, and fertilizer contributed the least to biomass$_a$ N.

In the PC intercrops, N application had no effect on the proportion of plant N derived from BNF (Fig. 3B). The PC 0 N, PC ¼ N, and PC ½ N treatments derived 38 %, 34 %, and 32 % of their N from BNF, respectively, comparable to PMono, which derived 37 % from BNF (Fig. 3B). The %Ndff in PC experienced a similar pattern to those in PO, where it increased with increasing fertilizer rates (Fig. 3B). The %Ndff and %Ndfs in CMono were higher than in PC intercrops (Fig. 3B). Similar to PO intercrops, soil was the largest N source for PC intercrops, followed by BNF, and then fertilizer.

Between intercrops, there was no difference in %Ndff; however, %NdfBNF was 48 % higher in PC than PO, while %Ndfs was 16 % lower in PC than PO (Fig. 4A). Nitrogen fertilizer rates had no effects on %Ndfs but affected %NdfBNF and %Ndff in the intercops (Fig. 4B). Increasing N rate reduced %NdfBNF. Compared to the 0 N treatment, ½ N reduced %NdfBNF by 20 % and by 13 % compared to ¼ N. In contrast, increasing N rates increased %Ndff. Compared to the 0 N treatment, ¼ N increased %Ndff by 236 % and ½ N increased %Ndff by 469 % (Fig. 4B). That being said, the intercrops had minimal %Ndff, with an average of only 4 %. The interaction between intercrop type and N rate had no effect on %

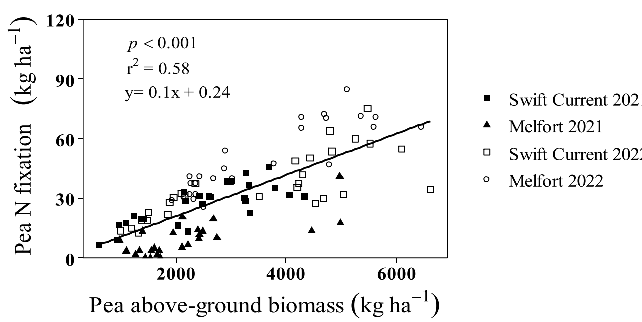


**Fig. 2.** Nitrogen fixed by pea in relation to above-ground pea biomass ($n$ = 112).

**Table 4**
Percentage of nitrogen derived from atmosphere (%Ndfa) and biological N fixation in pea-oat (PO) and pea-canola (PC) intercrops receiving three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops). Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022.

| | | %Ndfa | | N fixation (kg N ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | | Grain | Biomass$_a$[1] | Grain | Biomass$_a$ | NLER$_{pBNF}$[2] |
| **Intercrop** | PO | 58.5 a[3] | 53.8 a | 14.9 b | 22.3 b | 0.52 b |
| ($n$ = 48) | PC | 49.1 b | 44.8 b | 28.3 a | 36.9 a | 0.89 a |
| **Fertilizer rate** | 0 N | 55.3 a | 51.0 | 25.3 a | 34.0 a | 0.80 a |
| ($n$ = 32) | ¼ N | 57.1 a | 50.1 | 23.8 a | 31.2 a | 0.75 a |
| | ½ N | 49.0 b | 46.9 | 16.2 b | 24.0 b | 0.58 b |
| ***P*-values** | | | | | | |
| Intercrop (I) | | **0.001** | **0.001** | **< 0.001** | **< 0.001** | **< 0.001** |
| Fertilizer rate (F) | | **0.044** | 0.285 | **< 0.001** | **0.001** | **0.001** |
| I x F | | 0.959 | 0.967 | 0.099 | 0.128 | 0.323 |

[1] Biomass$_a$= above-ground biomass.
[2] Partial N land equivalent ratio for pea biological N fixation (NLERpBNF); values ≥ 0.67 indicate an intercrop advantage, whereas, values ≤ 0.67 indicate an intercrop disadvantage.
[3] Means followed by the same letter in a given column are not significantly different ($p$ > 0.05).

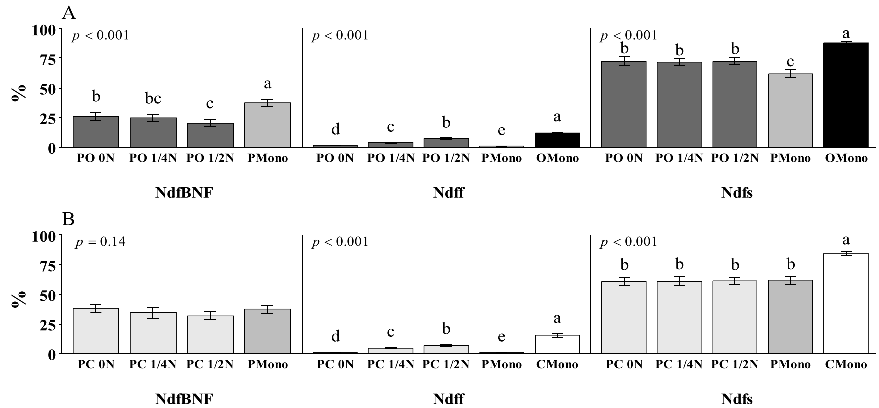


**Fig. 3.** Percentage of above-ground biomass N derived from BNF (NdfBNF), fertilizer (Ndff), or soil (Ndfs) in pea-oat (PO) (A) and pea-canola (PC) (B) intercrops compared to their respective monocrops (PMono, OMono, and CMono). Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops); monocrops received their full N recommendation, except pea, which received no N fertilizer. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022. Bars are means with standard errors ($n = 16$). Bars marked with different letters in each panel show significant differences among treatments for each N source at $p \leq 0.05$ level.

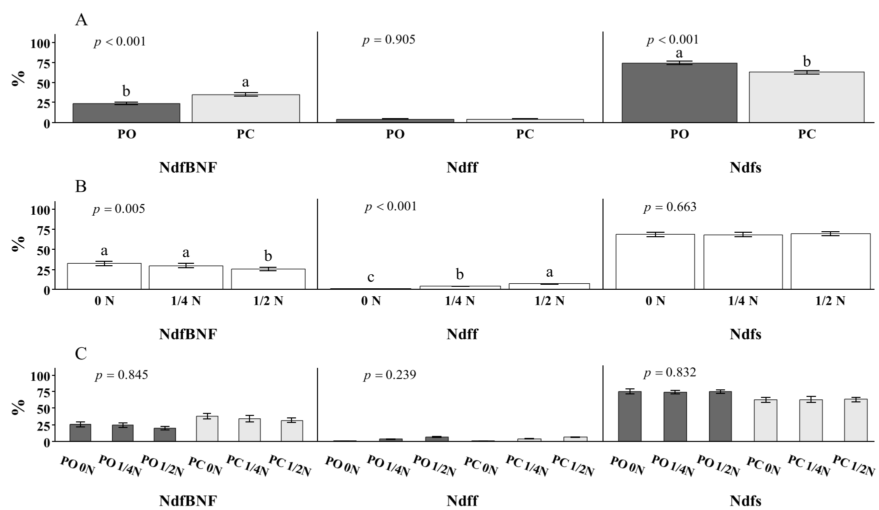


**Fig. 4.** Comparison of intercrop type (PO vs PC intercrops) (A), N fertilizer rates (B), and their interaction (C) for the percentage of above-ground N uptake that was derived from BNF (NdfBNF), fertilizer (Ndff), and soil (Ndfs). Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops). Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022. Bars marked with different letters show significant differences among treatments for each N source at $p \leq 0.05$ level. Bars are means with standard errors (Fig. 4A $n = 48$, Fig. 4B $n = 32$, Fig. 4C $n = 16$).

NdfBNF, %Ndff, or %Ndfs (Fig. 4C).

### 3.4. Nitrogen recovery efficiency of fertilizer N

The PO intercrops recovered 18–26 % more fertilizer N than PMono but showed no difference in %NREf compared to OMono (Fig. 5A). The PC intercrops showed no difference in %NREf compared to their monocrops (Fig. 5B), with their NREf ranging from 13 % to 15 %.

Among the main and interaction effects of intercrop type and N rate, only intercrop type affected %NREf (Fig. 6). The PC had 20 % lower %

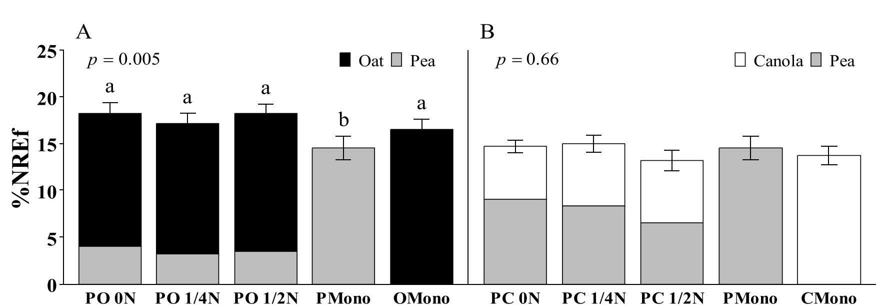


**Fig. 5.** Nitrogen recovery efficiency of N fertilizer (%NREf) by pea-oat (PO) (A) and pea-canola (PC) (B) intercrops compared to their respective monocrops (PMono, OMono, and CMono). Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops); monocrops received their full N recommendation, except pea, which received no N fertilizer. Different letters show significant differences between treatments. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022. $P \leq 0.05$ were considered significant and bars are means with standard errors ($n = 16$).

NREf than PO. In the PO intercrops, oat recovered, on average, 14 % of the fertilizer applied and pea recovered 4 %. In the PC intercrop, pea recovered the more fertilizer N with an average of 8 % and canola recovered an average of 6 %. This followed a similar pattern to biomass$_a$ N, where oat was PO's dominant crop and pea was PC's dominant crop.

### *3.5. Post-harvest soil residual mineral nitrogen*

Post-harvest soil mineral N did not differ between PC intercrops and their respective monocrops; however, post-harvest soil mineral N was lower in PO intercrops than in their respective monocrops (Table 5). Compared to PMono, soil mineral N following PO intercrops was 12–20 % lower.

Nitrogen fertilizer rate did not affect post-harvest soil mineral N, but intercrop type and the interaction between intercrop type and N fertilizer rate did (Table 6). Post-harvest soil mineral N was 10 % higher in PC intercrops than in PO intercrops, with the most mineral N present in PC ½ N and the lowest in PO 0 N.

## 4. Discussion

### *4.1. Pea productivity determined nitrogen land equivalent ratio in pea-based intercrops*

Previous studies have reported NLER advantages of 10–75 % (Hauggaard-Nielsen et al., 2001, 2009; Malhi, 2012; Monti et al., 2016; Bremer et al., 2024), whereas our intercrops demonstrated no grain NLER advantages. Swift Current received 69 % (2021) and 81 % (2022) of long-term rainfall and Melfort received 57 % (2021) and 99 % (2022) of long-term rainfall during the growing seasons (Table 1). The drought reduced plant growth (Liu et al., 2025) and thus affected N uptake (Thomas et al., 2004), limiting the NLER advantages we witnessed.

Under variable conditions, plant stand can become of major importance to the production of intercrops (Bremer et al., 2024). In PO intercrops, pea represented 35 % of the plant stand but it represented 57 % of plants in PC intercrops (Choo-Foo, 2024). In intercrops, there is a positive correlation between pea plants per m$^2$ and pea productivity (Monti et al., 2016). Therefore, the poor pea plant stand in PO enabled oat to become dominant, reducing pea biomass$_a$, whereas the strong pea establishment in PC enabled pea to produce over double the pea biomass$_a$ of PO. A reduction in the legume component of an intercrop negatively affects LER (Bremer et al., 2024; Bahia et al., 2025), explaining the low NLER observed in this study. This further explains why PO had lower grain and biomass$_a$ NLER than PC, reflecting larger

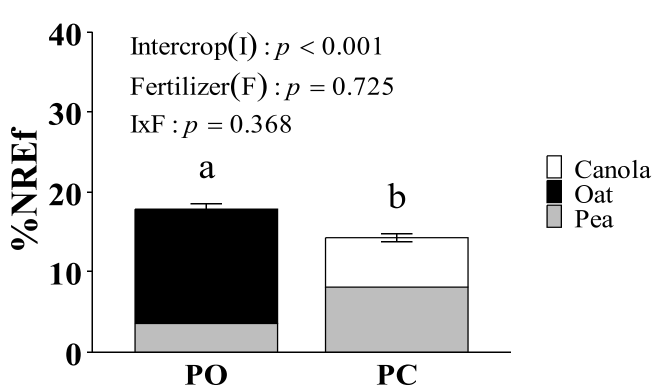


**Fig. 6.** Comparison of N recovery efficiency of N fertilizer (%NREf) between pea-oat (PO) and pea-canola (PC) intercrops. Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops). Different letters show significant differences between treatments. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022. $P \leq 0.05$ were considered significant and bars are means with standard errors ($n = 48$).

**Table 5**
Post-harvest soil mineral N content among pea-oat (PO), pea-canola (PC) intercrops and their respective monocrops (PMono, OMono, and CMono). Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops); monocrops received their full N recommendation, except pea, which received no N fertilizer. Soil was collected from the 0–30 cm depth. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022.

| | Soil mineral N |
|---|---|
| | — kg N ha$^{-1}$ — |
| ***Pea-Oat*** | |
| PO 0 N | 21.8c[1] |
| PO ¼ N | 24.2 bc |
| PO ½ N | 22.1c |
| PMono | 27.4 a |
| OMono | 25.5 ab |
| | ***p*< 0.001** |
| ***Pea-Canola*** | |
| PC 0 N | 24.2 |
| PC ¼ N | 24.1 |
| PC ½ N | 26.5 |
| PMono | 27.4 |
| CMono | 24.4 |
| | *p*=0.055 |

[1] Means ($n = 16$) followed by the same letter are not significantly different ($p > 0.05$).

**Table 6**
Post-harvest soil mineral N as affected by intercrop type (PO and PC intercrops), N fertilizer application rates, and their interaction. Intercrops received three N fertilizer rates (0, ¼, and ½ of recommended N rates for non-legume monocrops). Soil was collected from the 0–30 cm depth. Results are the combined analysis of Swift Current and Melfort sites in 2021 and 2022.

| | | Soil mineral N |
|---|---|---|
| | | — kg N ha$^{-1}$ — |
| **Intercrop** | PO | 22.7 b[1] |
| ($n = 48$) | PC | 24.9 a |
| **Fertilizer rate** | 0 N | 23.1 |
| ($n = 32$) | ¼ N | 24.2 |
| | ½ N | 24.2 |
| **Intercrop x Fertilizer rate** | PO 0 N | 21.8c |
| ($n = 16$) | PO ¼ N | 24.2 b |
| | PO ½ N | 22.1 c |
| | PC 0 N | 24.2 ab |
| | PC ¼ N | 24.1 b |
| | PC ½ N | 26.5 a |
| ***P*-values** | | |
| Intercrop (I) | | **< 0.001** |
| Fertilizer rate (F) | | 0.295 |
| I x F | | **0.014** |

[1] Means followed by the same letter are not significantly different ($p > 0.05$).

pea plant stand and more pea biomass$_a$ production in PC. Previous studies have also determined that oat is dominant in PO intercrops and that pea is dominant in PC intercrops (Chalmers, 2016; Monti et al., 2016).

Trends demonstrated that pea productivity decreased and companion crop productivity increased as intercrops were supplied with more N (Choo-Foo, 2024); however, fertilizer rate had no impact on NLER. Based on other findings, a decrease in pea productivity reduced NLER (Andersen et al., 2005). Nonetheless, grain NLER$_p$ declined with increasing N rate, from 0 N (0.51), ¼ N (0.45), and ½ (0.35), yet this reduction in pea productivity was smaller than the pronounced difference between PO (0.25) and PC (0.63). Therefore, a decrease in NLER is likely related to the reduced pea productivity, while the different N fertilizer rates did not sufficiently affect pea N to influence NLER.

Bremer et al. (2024) found similar results where changing plant densities in intercrops only changed the proportion each crop contributed to LER but did not influence the overall LER.

Despite our low NLER, other studies have found similar patterns, where straw NLER advantages were larger than grain NLER (Neugschwandtner and Kaul, 2015; Monti et al., 2019). The large straw NLER could be due to "haying off", where more straw is produced than grain under limited moisture conditions (Table 1). Cereals' large water uptake in intercrops can cause stress to legume crops (Demie et al., 2025) and this could exacerbate "haying off" in the intercrops to produce straw NLER advantages over monocrops. The large production of straw, suppressing grain production, could further explain the low grain NLER. Nonetheless, under ideal conditions, intercrops could provide larger benefits since grain NLER of 1.16 (PO) and 1.12 (PC) were determined in Melfort 2022 and straw NLER of 1.58 (PO) and 1.24 (PC) (Choo-Foo, 2024). Bahia et al. (2025) also found that intercrops provided advantages under high rainfall compared to disadvantages under dry conditions.

Overall, $biomass_a$ NLER of PC demonstrated a 3 % advantage over monocrops and 7 % over PO, indicating that PC used N more efficiently than monocrops and PO. Within intercrops, the lateral root growth of crops can increase compared to monocrops, facilitating greater acquisition of soil nutrients (Hauggaard-Nielsen et al., 2001). Additionally, N resources can be used more efficiently in intercrops since the companion crops have different rooting depths and legumes can access atmospheric N, allowing them to access different N pools (Gan et al., 2009; Hauggaard-Nielsen et al., 2009), thus leading to these NLER advantages. However, between companion crops, cereals are more competitive for soil N because of their ability to quickly grow fibrous roots at the beginning of the growing season (Andersen et al., 2005). This competition between crops in intercrops for soil and fertilizer N can largely influence intercrop advantages and early competition from cereals can have lasting negative effects on legume crops (Bremer et al., 2024; Demie et al., 2025). Therefore, the PC likely had better niche complementarity than PO, which allowed it to utilize N resources most efficiently (Bahia et al., 2025). Pea-canola intercrops have previously been found to provide larger LER advantages than pea-barley intercrops because of canola's ability to acquire N for an extended period of time over the growing season, thereby using N resources more efficiently than pea-cereal intercrops (Andersen et al., 2005).

### *4.2. Pea-based intercrops increased %Ndfa compared to pea monocrop*

The %Ndfa results in our study were comparable to those reported by Gungaabayar et al. (2023), who conducted their study at similar sites to ours, but lower than those observed in other studies (Hauggaard-Nielsen et al., 2009; Chapagain and Riseman, 2014). Saskatchewan has reported some of the lowest N fixation across Canada because of its arid climate (Yang et al., 2010). Both of our intercrops showed increased %Ndfa compared to PMono, consistent with previous reports (Andersen et al., 2005; Hauggaard-Nielsen et al., 2009; Chapagain and Riseman, 2014; Gungaabayar et al., 2023). These increases are due to complementary competition, where the companion crop competitively takes up soil N, forcing pea to acquire more of its N from the atmosphere (Hauggaard-Nielsen et al., 2009; Bremer et al., 2024). This limits crops' niche overlap and enables intercrops to utilize resources more efficiently than monocrops (Lithourgidis et al., 2011). Additionally, Chapagain and Riseman (2014) reported that intercrops increased nodule production, which may partially explain intercrops' increased %Ndfa over PMono, although nodulation was not assessed in this study.

Due to the extreme competitiveness of oat (Lauk and Lauk, 2008), it likely outcompeted pea for soil N and forced PO to produce the highest %Ndfa of all treatments. The greater %Ndfa in PO may also be associated with the mycorrhizal nature of oat, as mycorrhizal hyphae may facilitate N transfer from pea to oat during growing season, thereby enhancing %Ndfa. However, a higher %Ndfa does not necessarily imply a greater N fixation, which is also determined by pea $biomass_a$ production factor. In intercrops, cereals can increase root growth compared to monocrops and this vigorous growth can limit legume development (Demie et al., 2025). Since biomass production is the primary factor affecting BNF (Hauggaard-Nielsen et al., 2001, 2009; Yang et al., 2010; Gungaabayar et al., 2023), the limited pea biomass in PO constrained its capacity for N fixation relative to PMono. Previous studies found similar results (Gungaabayar et al., 2023; Reid et al., 2024). In the current study, PC fixed comparable amounts of N to PMono, despite being seeded at a reduced pea rate, likely due to larger pea $biomass_a$ (compared to PO) and higher %Ndfa (compared to PMono). The PC intercrop produced over double the pea $biomass_a$ that was produced in PO (Choo-Foo, 2024). The positive relationship between $biomass_a$ and nodule biomass may explain its increased BNF (Bargaz et al., 2021). To confirm this, a further study examining pea roots and nodules in intercrops is needed.

Taking into account the reduced seeding rate of intercropped pea, PC fixed 22 % more N than PMono on a per plant basis ($NLER_{pBNF}$). Andersen et al. (2005) found similar results, where PC intercrops fixed more N (on a per pea plant basis) than pea-barley intercrops and PMono. In their study, pea-barley and PMono fixed comparable amounts of N, but our PO intercrop decreased $NLER_{pBNF}$ by 15 %. They suggested that complementary competition can be lost if the companion crop is too competitive (Andersen et al., 2005). Because oat was the dominant crop in PO and pea $biomass_a$ was reduced, this was likely no longer complementary competition and oat impeded pea's ability to fix N. This is reflected in the 4 % $biomass_a$ N disadvantage PO had compared to its monocrops. In contrast, the increased N fixed by PC explains its 3 % $biomass_a$ NLER advantage over its monocrops since BNF was positively correlated with N uptake.

Nitrogen fertilizer application decreased %Ndfa, which is in line with other studies (Voisin et al., 2002; Andersen et al., 2005; Gungaabayar et al., 2023). Biological N fixation can be reduced by up to 50 % when N fertilizer is applied because of a reduction in nodulation (Voisin et al., 2003). As previously mentioned (4.1), $NLER_p$ decreased with increasing N fertilizer rates and this reduction in pea $biomass_a$ directly limited BNF (Hauggaard-Nielsen et al., 2001, 2009; Gungaabayar et al., 2023). High soil mineral N also decreases %Ndfa since peas preferentially utilize available soil N rather than fixing atmospheric $N_2$ (Voisin et al., 2002; Hardarson and Atkins, 2003; Ghaley et al., 2005). This mechanism further explains why intercrops receiving the ½ N rate had the lowest %Ndfa and BNF.

### *4.3. Impacts of intercropping on nitrogen sourced from the atmosphere, fertilizer and soil*

The majority of N taken up by crops was derived from soil (61–73 %), then BNF (20–38 %), and fertilizer (1–7 %). Cowden et al. (2020) found that, in a pea-barley intercrop, %Ndfs (36 %) and %NdfBNF (35 %) were similar but higher than %Ndff (29 %). Our %NdfBNF was comparable to that of Cowden et al. (2020); however, the higher %Ndfs observed in our study was likely caused by low %Ndff, as plants absorb N fertilizer primarily via mass flow. The dry conditions (Table 1) created a challenging environment for crops to access N fertilizer, ultimately lowering %Ndff. Another study determined that 81 % of N uptake in faba bean-wheat intercrops was from soil N (Demie et al., 2025), consistent with our findings that soil N was the main source of N for the intercrops. Future studies are needed to quantify %Ndff and %Ndfs of each crop species in intercrops to deepen our understanding of crop competition and N acquisition.

The %Ndff was largely influenced by N fertilizer rates, following similar patterns to the N inputs they received (Ghaley et al., 2005). This led OMono and CMono to have the highest %Ndff as they received the most N fertilizer. However, N fertilizer contributed minimally to the crop N uptake (average of 6 %). Similar trends were observed by Cowden et al. (2020), with the cereal monocrop exhibiting the highest %

Ndff, followed by the intercrop, and PMono showing the lowest %Ndff. Their intercrop had 29 % Ndff compared to ours which ranged from 1 to 7 %. This difference may be attributed to differences in N fertilizer management, as we applied fertilizer at seeding, whereas in Cowden's study, fertilizer was applied twice throughout the growing season, which likely increased N uptake from fertilizer.

As intercrops received small amounts of N from fertilizer, %NdfBNF exert a strong influence on %Ndfs. The %NdfBNF (24 %) of PO intercrops were smaller than the 35 % NdfBNF reported in a pea-barley intercrop (Cowden et al., 2020), likely due to the drought conditions in this study (Table 1). The ability of intercrops to fix N reduced their reliance on soil N compared to non-legume monocrops, confirming previous findings (Ghaley et al., 2005; Demie et al., 2025). Because PC fixed large amounts of N relative to PO, %Ndfs in PC was reduced but remained comparable to PMono, whereas PO derived more N from soil than PMono. Similarly, pea-barley intercrops have also increased %Ndfs compared to PMono (Cowden et al., 2020). Higher %Ndfs in PO intercrops are likely due to the cereal's ability to quickly grow roots and search for nutrients at the beginning of the season (Andersen et al., 2005). As described in Section 4.2, this early competition by the cereal can create lasting impacts throughout the season and greatly reduced N fixation. Therefore, since PO obtained limited N from fertilizer and atmosphere, it was most reliant on soil N.

Similar to the impact fertilizer rate had on N fixation, %NdfBNF decreased while %Ndff increased with increasing N fertilizer rates. These counterbalanced one another, leading to fertilizer rate having no effect on %Ndfs. Similar patterns were observed in pea-wheat intercrops (Ghaley et al., 2005). Overall, N fertilizer rates had minimal impact on N uptake but changed the relative sources of N: lower N rate treatments relied more on atmospheric N and higher N rate treatments relied more on N fertilizer.

### *4.4. Nitrogen fertilizer recovery and post-harvest soil N in pea-based intercrops*

Crops only recovered 13–18 % of applied N fertilizer, lower than the 43 % reported in a meta-analysis (Smith and Chalk, 2018). Fertilizer recovery has a positive relationship with precipitation (Gardner and Drinkwater, 2009), and the drought conditions (Table 1) likely explained the reduced %NREf and lower %Ndff in our study. Nonetheless, when applying 40 kg N ha$^{-1}$, Ghaley et al. (2005) found PMono recovered only 15 %, similar to our findings. The PMono had lower %NREf than PO intercrops because pea roots are shallower and less competitive than cereals for N fertilizer uptake (Andersen et al., 2005; Gan et al., 2009). Oat recovered the majority of N fertilizer in the intercrops, explaining the higher %NREf in PO than PMono. Cowden et al. (2020) found similar results, with the barley monocrop showing the highest recovery, followed by intercrops, and then PMono. In contrast, no differences between PC and its monocrops indicated that canola did not have higher N recovery efficiency from N fertilizer. Canola relies on lateral root growth for nutrient uptake but can be negatively impacted by heat stress at flowering, reducing N accumulation and yield production (Wu et al., 2020). Given June and July temperatures were higher than normal in this study (Table 1), we speculate that heat stress contributed to canola's reduced %NREf, comparable to PMono.

The PO intercrop recovered 22 % more fertilizer N than PC because of cereals' greater competitive ability to grow roots at the beginning of the season and to increase root biomass in water-limited conditions (Hauggaard-Nielsen et al., 2001; Andersen et al., 2005; Bargaz et al., 2021). No difference in %Ndff was observed between the intercrops, indicating PO did not benefit from the additional fertilizer N recovery. Thus, the higher %NREf observed in PO likely resulted from its lower rate of N fertilizer, which increased the percentage recovered but not the absolute amount of fertilizer applied. Furthermore, canola retains a large portion of N in the root system (Arcand et al., 2013). Since root biomass and N content were not measured in this study, a substantial fraction of fertilizer N recovered by canola may have been overlooked, potentially leading to an underestimated %NREf by canola. Incorporating root analysis in future work would provide a more holistic picture of fertilizer N recovery.

The lack of fertilizer rate effect on %NREf contradicts previous findings showing larger recovery at lower fertilizer application rates (Andersen et al., 2005). In the present study, treatments receiving higher N fertilizer rates left more N in the soil because %NREf is a proportion rather than an absolute amount. Thus, intercrops receiving lower N rates likely left less residual fertilizer N in the soil compared to those receiving higher fertilizer rates. These findings are similar to the results reported by Ghaley et al. (2005), who found that fertilizer rate had no impact on fertilizer recovery in intercropping systems.

The lower rates of N fertilizer applied to PO, high %NREf, low N fixation, and high %Ndfs reduced post-harvest soil mineral N by 9 % compared to PC. Additionally, increased biomass$_a$ N is positively correlated with rhizodeposition (Mahieu, 2008); therefore, the greater pea biomass$_a$ in PC, compared to PO, likely increased N deposited by PC, contributing to higher soil mineral N content. Furthermore, the PC ½ N treatment received the highest amount of fertilizer N of all intercrops, which likely contributed to the highest mineral N. Although PC 0 N fixed the most atmospheric $N_2$, much of the fixed $N_2$ was removed in the grain, resulting in only a relatively small contribution to soil N (Peoples and Craswell, 1992). Therefore, the high N fertilizer input and N-fixing capacity in the PC ½ N treatment explained the highest post-harvest soil mineral N.

No differences in post-harvest soil mineral N were observed between PC intercrops and their monocrops, indicating that even when PC received 0 N it acquired its required N from the atmosphere without depleting soil N reserves. A lower soil N drawdown can be attributed to intercrops' abilities to fix and transfer N (Reid et al., 2024). In contrast, the minimal N fixed by pea in PO intercrops and the lower N fertilizer rates applied likely caused PO intercrops to have lower post-harvest soil mineral N than their monocrops. Although OMono had the highest %Ndfs, its higher N fertilizer rate compared with PO likely contributed to the higher post-harvest soil mineral N than PO. Previous studies found increased soil residual nitrate following intercrops because of increased NUE but they noted that this effect can be limited under dry conditions (Bahia et al., 2025), which may explain the absence of post-harvest mineral N advantages in pea-based intercrops relative to non-legume monocrops.

## 5. Conclusion

This study showed that pea-based intercrops sourced their N primarily from soils, followed by atmosphere $N_2$ through BNF, and to a lesser extent, from N fertilizer. Both PO and PC intercrops had significantly higher %Ndfa compared to PMono. Between the intercrops, PC fixed substantially more atmospheric $N_2$ than PO on a per pea plant basis, even though %Ndfa was much lower in PC than in PO. Higher N fixation in PC was mainly attributed to greater above-ground pea biomass production. Although PO showed higher N fertilizer recovery efficiency than PC, overall N use efficiency may have been underestimated, particularly for canola, without accounting for belowground N pools, including root N, nodule-associated N, and rhizodeposited N. Quantifying these belowground N inputs and in-season N transfer from pea to non-legume crops will improve understanding of N cycling in intercrops. Overall, this study advanced our knowledge of N fixation and sourcing in pea-based intercrops and provided insight for developing low N input pea-based intercropping systems that enhance ecological benefits through improved N cycling.

## CRediT authorship contribution statement

**Kui Liu:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Kennedy Choo-Foo:**

Writing – original draft, Formal analysis, Data curation, Conceptualization. **J. Diane Knight:** Writing – review & editing, Supervision, Methodology, Conceptualization.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

Data will be made available on request.

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