Received: 19 January 2023 | Accepted: 19 June 2023 | Published online: 23 August 2023

DOI: 10.1002/agj2.21419

Agronomy Journal

ORIGINAL ARTICLE

Agronomy, Soils, and Environmental Quality

Nitrogen fixation among pea and lentil varieties in the Northern Great Plains

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Assigned to Associate Editor Trang Nguyen

Funding information
United States Department of Agriculture—Agricultural Research Service—Pulse Crop Health Initiative, Grant/Award Number: 58-3060-8-025; Montana Fertilizer Advisory Committee

Abstract

Pulse crops, including lentil (*Lens culinaris* Medik.) and pea (*Pisum sativum* L.), can improve the sustainability of Northern Great Plains cropping systems, largely through biological N fixation. Greater N fixation amounts can help producers to increase yield while decreasing N fertilizer inputs for the following crop. There may be potential to breed greater N-fixing pulse varieties, yet little is known about varietal differences in N fixation. Nitrogen fixation of pea and lentil varieties was quantified at two sites in Montana from 2019 to 2021 using an N difference approach and the ^{15}N natural abundance method. Riveland and CDC Richlea were frequently high N-fixing lentil varieties, both fixing ca. 130 kg N ha^{-1} in the site-year with the most favorable growing conditions. No pea variety had consistently greater N fixation than others, despite N fixation ranging from 88 to 135 kg N ha^{-1} in one site-year among varieties. Nitrogen fixation by lentil had an inverse relationship with days to flowering but was not correlated with days to maturity. Nitrogen fixation by pea was positively correlated with days to maturity but was not correlated with days to flowering. Breeding lentil and pea for high N fixation by selecting high N-fixing varieties is likely difficult, as varieties performed differently under variable environmental conditions. Breeding efforts based on traits, such as days to flowering, could be more successful. There were more positive correlations between N fixation parameters and seed yield for pea than for lentil, suggesting that breeding for greater yields could increase N fixation for pea but not lentil.

1 | INTRODUCTION

Nitrogen is widely accepted as the most limiting nutrient for plant growth. Consequently, humans globally apply over 108 Tg of N fertilizer to crops each year at a cost of over 28 billion USD (FAO, 2018). Reliance upon synthetic N inputs is especially common in agroecosystems dominated by monocultures, such as the Northern Great Plains (NGP) where cereal crops predominate. Adding to economic costs, applied N fertilizers are prone to environmental losses via denitrification, volatilization, and leaching (Galloway et al., 2003; Pan et al., 2016). Repeated use of N fertilizer can cause soil acidification, due to release of H$^+$ ions during nitrification of ammonium-based fertilizers (Brown et al., 2008). Through biological N fixation, pulse crops such as pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) rotated with cereals

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Abbreviations: fNdfa, fraction of N derived from atmosphere; NA, ^{15}N natural abundance; NARC, Northern Agricultural Research Center; ND, N difference; NGP, Northern Great Plains; PF, Arthur H. Post Agronomy Research Farm.

can decrease N input needs while serving as a profitable commodity for producers.

Pulse crops, annual legumes harvested for grain, are well adapted to NGP semiarid growing conditions and have increased in popularity in recent years, with the area planted with lentil or pea increasing from approximately 19,000 ha in 2000 to 419,000 ha in 2022 in Montana (National Agriculture Statistics Service, 2020). Pulse crops are often grown to replace summer fallow (Long et al., 2014), a practice known to decrease soil organic matter (Romero et al., 2019) and increase susceptibility to soil erosion (Carlyle, 1997) and nitrate leaching (John et al., 2017). Several studies have found increases in cereal crop yield (Arcand et al., 2014; Beckie & Brandt, 1997; Bremer et al., 2011; Miller et al., 2003; Stevenson and van Kessel, 1996) and protein concentration (Bremer et al., 2011; Miller et al., 2003) when grown after pulse crops, likely due to N supplied by pulse crop residues (Arcand et al., 2014; Beckie & Brandt, 1997;). While a majority of fixed N is removed from the system during grain harvest (Hossain et al., 2017), pulse crop residues left in the field are more readily mineralized than cereal residues because of their lower C/N ratio (Lupwayi et al., 2004), resulting in more N availability for subsequent crops. By increasing N fixation by pulse crops, producers may be able to further decrease N fertilizer rates for the subsequent crop. Additionally, growing a pulse crop in place of a cereal or oilseed results in substantial fertilizer N savings because pulse crops require little to no N inputs. In Saskatchewan and Alberta, Canada, adding pulses to crop rotations decreased fertilizer N requirements by 38% for subsequent crops (Khakbazan et al., 2022). Pulses can directly benefit from higher N fixation, which has been associated with higher legume seed yield and protein (Hossain et al., 2017; Zimmer et al., 2016).

One possible strategy to increase N fixation is through breeding. Pulse crop breeding has historically focused on increasing yield and other beneficial agronomic traits, with little attention on boosting N fixation despite its recognized potential to improve the sustainability of cropping systems. Grain legume varieties vary in the fraction of N derived from the atmosphere (fNdfa; Abi-Ghanem et al., 2011; Hossain et al., 2017; Zimmer et al., 2016), number of root nodules (Abi-Ghanem et al., 2011), total plant N (Abi-Ghanem et al., 2013), and the amounts of N fixation (Hossain et al., 2017). The presence of genetic variation in N fixation and its associated parameters within a given species indicates that selection through breeding may be possible.

Despite the promise of focusing on N fixation as a measurable trait for lentil and pea breeding programs, only one study evaluating N fixation by different pulse varieties has been identified in the NGP (Hossain et al., 2017). Therefore, we initiated a study to fill this research void. The primary objective of this study was to evaluate differences in N fixation, biomass N accumulation, and fNdfa among regionally grown lentil and pea varieties. A secondary objective was to investigate the relationship between N fixation and days to flowering, days to maturity, and seed yield, to assist with future pulse breeding efforts.

Core Ideas

- Variation in environmental conditions strongly influenced N fixation by lentil and pea varieties.
- Differences in N fixation among varieties were up to 21 kg N ha^{-1} for lentil and 47 kg N ha^{-1} for pea.
- Two lentil varieties often fixed more N than others, while no pea varieties performed consistently well.
- Breeding lentil for shorter days to flower could increase N fixation, suggested by an inverse relationship.
- Breeding pea for greater yield or longer days to maturity could increase N fixation, suggested by positive relationships.

2 | MATERIALS AND METHODS

2.1 | Site selection and experimental design

Field experiments were conducted at Montana State University's Arthur H. Post Agronomy Research Farm (PF) near Bozeman, MT (45°40′48″ N; 111°9′00″ W) and Northern Agricultural Research Center (NARC) near Havre, MT (48°29′24″ N; 109°47′60″ W) from 2019 to 2021. Historically, these two sites approximately span the precipitation gradient of Montana's dryland agricultural areas, with average annual precipitation amounts of 306 and 417 mm at NARC and PF, respectively. The soil type at PF was Amsterdam silt loam (fine-silty, mixed, superactive, frigid Typic Haplustoll) in all years. Soil types at NARC were Telstad-Joplin loam (fine-loamy, mixed, superactive, frigid Aridic Argiustoll) in 2019 and 2020 and Kenilworth-Fort Benton fine sandy loam (fine-loamy, mixed, superactive, frigid Aridic Argiustoll) in 2021. Site selection was based on soil nitrate-N (0.12 M KCl, Cd reduction), exchangeable K (1 M ammonium acetate, ICP), sulfate-S (0.12 M KCl, turbidimetric), Olsen P (0.5 M $NaHCO_3$, colorimetric), and soil organic matter (loss on ignition) in soil samples collected the previous fall to identify locations within suitable fields that had relatively similar fertility levels and relatively low nitrate-N levels. Soil at each selected field site was sampled (two locations per block) in the spring of each growing season and analyzed for pH (1:1 soil:water), organic matter, Olsen P, exchangeable K, sulfate-S, and nitrate-N. Both fall and spring analyses were

TABLE 1 General soil properties for Arthur H. Post Agronomy Research Farm (Post Farm) and Northern Agricultural Research Center N fixation study sites, 2019–2021.

Soil property	2019	2020	2021
	Post Farm		
Pea/lentil history (year)[a]	2017	None	None
Soil texture[b]	Silt loam	Silt loam	Silt loam
Soil pH	6.7	7.7	7.4
Organic matter (g kg^{-1})	29	23	28
Olsen P (mg kg^{-1})	14	17	15
Exchangeable K (mg kg^{-1})	371	324	350
Sulfate-S (mg kg^{-1})	4.8	4.0	1.4
Nitrate-N, 0–90 cm (kg ha^{-1})	20	33	74
	Northern Agricultural Research Center		
Pea lentil/history (year)	2016	2016	None
Soil texture	Loam	Loam	Sandy loam/loam
Soil pH	8.3	6.8	7.7
Organic matter (g kg^{-1})	20	15	15
Olsen P (mg kg^{-1})	9	21	23
Exchangeable K (mg kg^{-1})	313	325	341
Sulfate-S (mg kg^{-1})	4.5	16.8	1.0
Nitrate-N, 0–90 cm (kg ha^{-1})	46	95	19

Note. Average of 16 soil samples (8 samples each from lentil and pea), 0–15 cm unless otherwise noted.

[a] 10 previous years.

[b] *Source*: USDA-NRCS Web Soil Survey.

performed by an independent laboratory (AgVise, Northwood, ND, USA). Soil characteristics and pulse crop histories are summarized in Table 1. Ten regionally grown varieties of lentil and pea were each grown in a randomized complete block design with four replicates (Table 2).

2.2 | Plot management

Pulses were sown into no-till managed fields that contained standing cereal stubble from the previous crop year at a target rate of 150 pure live lentil plants m^{-2} and 85 pure live pea plants m^{-2} in 23-cm rows at PF and 30-cm rows at NARC. Plot sizes were 6 by 1.5 m at PF and 6.7 by 2 m at NARC. Seeding depth was 5.1 cm for both lentil and pea at PF, and was 3.2 cm for lentil and 3.8 cm for pea at NARC. Dates for planting and other field activities are shown in Table 3. Seeds were inoculated with *Rhizobium leguminosarum* biovar. *viciae* and treated with fungicide (mefenoxam and fludioxonil) according to manufacturer labels. In 2019, one row of flax was hand sown as a non-N fixing reference crop. Due to poor results using one row, flax was mechanically seeded adjacent to pulse crops in 2020 and 2021. Triple super phosphate (0–46–0) was applied in furrow at a rate of 12.2 kg P ha^{-1} in all years. In 2021, pelletized gypsum ($CaSO_4 \bullet 2H_2O$) was applied in furrow to provide 8 kg S ha^{-1} to prevent S deficiencies due to low soil sulfate-S levels (1–2 mg kg^{-1}) at both sites. Pre-plant (glyphosate, pendimethalin, and saflufenacil) and in-crop (quizalofop p-ethyl) herbicides were applied to all plots.

2.3 | Data collection

A representative area, consisting of three 1-m rows at PF (0.69 m^2) or two 1-m rows at NARC (0.61 m^2), from each pulse plot was sampled for aboveground biomass (hereafter termed "biomass") at early to late pod fill by cutting plants at the soil surface. In 2019 and 2020, one corresponding flax biomass sample (one 1-m row) was harvested for every three pulse plots. To better account for high and variable soil nitrate-N observed in spring sampling of 2021 PF pea plots, and issues encountered when there was variable soil nitrate-N in 2020 at NARC, a flax sample adjacent to each pea plot was harvested at PF in 2021. Biomass samples were oven dried (50˚C, 72 h), weighed, and ground (<0.5 mm). Subsamples were analyzed for N concentration via combustion with a LECO TruSpecCN, and approximately 10% of samples were analyzed in duplicate to assess precision (2.5% mean CV). Isotope ^{15}N was analyzed at the UC Davis Stable Isotope Facility (Davis, CA) using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (2.4% mean CV; Sercon Ltd.).

TABLE 2 Lentil and pea varieties grown at Arthur H. Post Agronomy Research Farm (Post Farm, Bozeman, MT) and Northern Agricultural Research Center (Havre, MT), 2019–2021.

Lentil variety	Type	Registration article
CDC[a] Impala	Extra small red	–
CDC Maxim	Small red	–
CDC Redberry	Small red	Vandenberg et al. (2006)
CDC Richlea	Medium green	–
CDC Robin	Small red	Vandenberg et al. (2002)
CDC Rosetown	Extra small red	–
CDC Viceroy	Small green	–
ND Eagle[b]	Small green	–
Pennell	Large green	Muehlbauer and McPhee (2004)
Riveland	Large green	McPhee and Muehlbauer (2009)
Pea variety		
Aragorn[c]	Green	–
Arvika	Forage	–
Carousel[c]	Yellow	–
CDC Mozart	Yellow	Vandenberg and Slinkard (2002)
Cruiser[c]	Green	–
Delta	Yellow	–
DS Admiral	Yellow	Andersen et al. (2002)
Hampton[d]	Green	–
Lifter	Green	McPhee and Muehlbauer (2002)
Majoret	Green	–

[a] Crop Development Centre.

[b] North Dakota Crop Improvement and Seed Association.

[c] ProGene Plant Research.

[d] USDA-ARS.

Plots were harvested with a small plot combine and pulse seeds were cleaned and weighed. Seed yield (kg ha^{-1}) was determined on a dry matter basis. Shortly after pulses were harvested, soil was sampled to a depth of 90 cm (3.0-cm diameter core) in each pulse plot, and at each location where flax was sampled, and split into three 30-cm segments. Soils were dried (50˚C, 72 h) and weighed, from which bulk density was calculated. Samples were ground (<2 mm), extracted using 1 M KCl, and analyzed for nitrate-N with a flow injection colorimetric analyzer (Lachat). Nitrate-N pool (kg N ha^{-1}) for each 90-cm sample was calculated from each depth's nitrate concentration and bulk density, and then summed.

Pea data at NARC in 2019 were not collected due to heavy deer grazing, and lentil data at PF in 2020 were not collected due to substantial residual herbicide injury. Seed yield was not measured for lentil or pea at PF in 2021 because of poor emergence at the plot scale resulting from high cereal straw residue.

2.4 | Measuring N fixation

Aboveground N fixation was estimated using two methods: an N difference approach (ND) and the ^{15}N natural abundance (NA) method. The ND method was used only in 2020 and 2021 because of the lack of confidence in flax biomass N based on only one seed row in 2019. The ND method used the following equation as reported by Unkovich et al. (2008) with all units in kg N ha^{-1}.

$$\text{N fixation} = \left(\text{biomass N}_{\text{legume}} - \text{biomass N}_{\text{reference}}\right) + \left(\text{soil N}_{\text{legume}} - \text{soil N}_{\text{reference}}\right) \tag{1}$$

Biomass N is the product of dry aboveground biomass and its N concentration. Soil N is the nitrate-N in the upper 90 cm from the soil sample taken shortly after pulse crop harvest.

A lower bound of 0 for N fixation was used because N fixation cannot be negative. An upper bound for N fixation was set as each plot's biomass N_{legume} as defined in Equation (1). Amounts of N fixation outside the set bounds (12.5% of ND observations) were omitted from analysis.

For the NA method, the (fNdfa was calculated using the following equation, first published by Shearer and Kohl (1986):

$$\text{fNdfa} = \frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference}} - B} \tag{2}$$

Where flax is the reference crop, and δ^{15}N is:

$$\delta^{15}\text{N}(‰) = \left(\frac{\text{atom\%N}_{\text{sample}} - \text{atom\%N}_{\text{atmosphere}}}{\text{atom\%N}_{\text{atmosphere}}}\right) 1000 \tag{3}$$

The B in Equation (2) refers to the δ^{15}N of a pulse crop grown in the absence of soil N, meaning it reflects conditions in which all plant N is fixed. Variety specific B-values were obtained in a greenhouse experiment (Baber, 2022). Nitrogen fixation for the NA method was calculated using Equation (4).

$$\text{N fixation} = \text{biomass N}_{\text{legume}} * \text{fNdfa} \tag{4}$$

2.5 | Statistical analyses

All statistical procedures were conducted using R (R Core Team, 2022). Linear mixed models for response variables (N fixation using NA and ND, biomass N, fNdfa, and yield) were fit using variety as a fixed effect and block as a random

TABLE 3 Field activities and dates for Arthur H. Post Agronomy Research Farm (Post Farm) and Northern Agricultural Research Center (NARC), 2019–2021 N fixation study sites.

Field activity	2019	2020	2021
	Post Farm		
Spring soil sampling	25 April	10 April	7 May
Planting	7 May	21 April	15 April
Lentil biomass sampling	7 August	–[a]	13 July
Pea biomass sampling	31 July	28 July	13 July
Lentil harvest	3 September	–	–[b]
Pea harvest	20 August	19 August	–
Fall soil sampling	4 September	21 August	16 August
	Northern Agricultural Research Center		
Spring soil sampling	15 April	20 April	24 March
Planting	22 April	23 April	8 April
Lentil biomass sampling	25 July	17 July	25 June
Pea biomass sampling	–[c]	17 July	25 June
Lentil harvest	1 August	1 August	7 August
Pea harvest	–	1 August	20 July
Fall soil sampling	25 September	11 August	11 August

[a] Lentil at Post Farm in 2020 not sampled due to herbicide injury.

[b] Seed yield not measured at Post Farm in 2021 because of poor stand.

[c] Pea at NARC in 2019 not sampled or harvested due to extensive deer grazing.

TABLE 4 Precipitation and air temperature data for Arthur H. Post Agronomy Research Farm (Post Farm, Bozeman, MT) and Northern Agricultural Research Center (Havre, MT), 2019–2021 study sites.

	Precipitation (mm)				Mean temperature (˚C)			
	2019	2020	2021	LTA[a]	2019	2020	2021	LTA
	Post Farm							
September–March[b]	150	210	120	161	−0.7	1.7	1.9	1.5
April	84	22	28	44	6.1	5.6	5.9	6.3
May	44	20	79	69	9.5	11.5	10.1	10.8
June	52	93	20	71	15.0	15.3	18.6	14.8
July	68	17	21	35	18.2	18.3	22.5	18.8
Growing season[c]	248	151	148	220	12.2	12.7	14.3	12.7
	Northern Agricultural Research Center							
September–March[b]	107	125	119	110	−2.3	0.0	0.9	0.7
April	23	14	13	25	7.1	3.5	5.5	7.4
May	39	40	77	55	9.6	11.9	9.9	12.8
June	82	68	6	66	16.3	17.3	19.4	17.3
July	14	18	8	43	19.7	20.0	23.8	21.0
Growing season[c]	158	140	104	189	13.2	13.2	14.7	14.6

[a] Long-term average, 1981–2010 from the Western Regional Climate Center, Desert Research Institute, Reno, NV.

[b] Over-winter preceding the growing season.

[c] April–July.

TABLE 5 Means and summary of analysis of variance for nitrogen (N) fixation by lentil varieties as determined by the ^{15}N natural abundance (NA) method by year and site.

	N fixation (kg N ha^{-1})			
	Post Farm[a]		Northern Agricultural Research Center[b]	
Lentil variety	2019	2021	2019	2021
CDC Impala	109	29 b	32 abc	18 c
CDC Maxim	138	42 ab	43 ab	21 bc
CDC Redberry	116	35 ab	35 abc	21 bc
CDC Richlea	128	51 a	45 a	26 ab
CDC Robin	122	36 ab	24 c	18 c
CDC Rosetown	118	36 ab	39 abc	24 abc
CDC Viceroy	128	49 ab	42 abc	21 bc
ND Eagle	154	42 ab	26 bc	26 ab
Pennell	118	39 ab	36 abc	25 ab
Riveland	134	48 a	41 abc	30 a
	p-values			
	0.13	**0.04**	**0.03**	**<0.01**

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$). Bolded values indicate $p < 0.1$.

[a] No 2020 data at Arthur H. Post Agronomy Research Farm (Post Farm) due to herbicide damage.

[b] No 2020 data at Northern Agricultural Research Center due to high/variable nitrate.

TABLE 6 Means and summary of analysis of variance for nitrogen (N) fixation by pea varieties as determined by the ^{15}N natural abundance (NA) method by year and site.

	N fixation (kg N ha^{-1})			
	Post Farm		Northern Agricultural Research Center[a]	
Pea variety	2019	2020	2021	2021
Aragorn	91 bcd	109 ab	53	47
Arvika	110 a	112 ab	65	43
Carousel	77 d	104 ab	57	40
CDC Mozart	96 abc	129 ab	53	43
Cruiser	87 bcd	88 b	63	39
Delta	95 bc	135 a	43	42
DS Admiral	89 bcd	110 ab	55	40
Hampton	99 ab	118 ab	63	47
Lifter	98 ab	113 ab	70	48
Majoret	82 cd	91 ab	56	46
	p-values			
	0.07	**0.10**	0.13	0.74

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$). Bolded values indicate $p < 0.1$.

[a] At Northern Agriculture Research Center, no 2019 data due to deer grazing and no 2020 data due to highly variable soil nitrate that affected ^{15}N and N fixation.

effect in the "lme4" package (Bates et al., 2015). A combined model was attempted, but site-year variance dominated such that it may have obscured treatment differences in response variables. Therefore, site-years were analyzed independently for variety effects using analysis of variance (ANOVA). Differences among varieties were further evaluated using Tukey–Kramer HSD (honestly significant difference) familywise comparisons of means (alpha = 0.1) with the "multcomp" package (Hothorn et al., 2008). Correlation analyses were conducted to analyze relationships (Pearson correlation coefficients) between N fixation (NA method only because of more site-years of data) and days to flowering and maturity. To combine site-years, relative N fixation and days to flowering and maturity were calculated by dividing each value by the highest value in each site-year. Correlations were also determined between N fixation parameters (N fixation using both methods, fNdfa, and biomass N) and grain yield.

3 | RESULTS AND DISCUSSION

3.1 | Weather context

Yearly weather varied substantially at both sites over the course of the study (Table 4).

The 2019 growing season was more favorable than 2020 and 2021 at both sites, with cumulative growing season precipitation being 113% and 84% of the long-term average for PF and NARC, respectively. Both 2020 and 2021 at PF had below average growing season precipitation, with 2021 exhibiting more drought stress at both sites due to much lower overwinter precipitation at Post Farm, lower growing season precipitation at NARC, and hotter air temperatures in June–July at both sites. Drought was particularly severe at NARC in 2021, where precipitation for June, a pivotal month for pulse growth in the NGP, was 9% of the long-term average.

3.2 | Evaluation of N fixation methods

Both the ND and NA methods of estimating N fixation require certain assumptions to be met. The ND method is invalidated when the pulse and reference crops have access to different pools of soil nitrate-N (Unkovich et al., 2008). At NARC in 2020 and PF in 2021, post-harvest soil nitrate-N was highly variable, with average standard errors of the mean (SEM) for each variety ranging from 11 to 27 kg N ha^{-1} and nitrate-N differences between each pulse plot and its paired flax value were inconsistent among replicates. This indicates the pulse crop and reference crop likely accessed different amounts of soil nitrate-N over the growing season, violating

TABLE 7 Means and summary of analysis of variance for lentil biomass N and fraction of N derived from atmosphere (fNdfa; from ^{15}N natural abundance method) by year and site.

	Biomass N (kg ha^{-1})			fNdfa	
Lentil variety	**2019**	**2020**	**2021**	**2019**	**2021**
	Post Farm				
CDC Impala	145 b	–[a]	54 b	0.57	0.53 b
CDC Maxim	171 ab	–	62 ab	0.47	0.68 a
CDC Redberry	152 b	–	58 ab	0.55	0.61 ab
CDC Richlea	168 ab	–	81 a	0.53	0.63 ab
CDC Robin	148 b	–	61 ab	0.45	0.60 ab
CDC Rosetown	165 ab	–	59 ab	0.61	0.61 ab
CDC Viceroy	167 ab	–	69 ab	0.61	0.70 a
ND Eagle	193 a	–	64 ab	0.49	0.66 a
Pennell	147 b	–	59 ab	0.63	0.67 a
Riveland	166 ab	–	75 ab	0.49	0.64 ab
	p-values				
	0.01	–	**0.06**	0.25	**0.03**
	Northern Agricultural Research Center[b]				
CDC Impala	67	116	27 d	0.52	0.65
CDC Maxim	66	107	31 bcd	0.65	0.68
CDC Redberry	63	103	30 cd	0.55	0.69
CDC Richlea	76	98	36 abc	0.58	0.70
CDC Robin	55	108	27 d	0.43	0.67
CDC Rosetown	67	102	35 abcd	0.57	0.69
CDC Viceroy	68	113	32 abcd	0.61	0.67
ND Eagle	60	120	39 ab	0.43	0.68
Pennell	70	102	34 abcd	0.52	0.75
Riveland	76	99	39 a	0.54	0.75
	p-values				
	0.28	0.64	**<0.01**	0.12	0.11

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$). Bolded values indicate $p < 0.1$.

[a] No 2020 data at Arthur H. Agronomy Research Farm (Post Farm) due to herbicide damage.

[b] No 2020 fNdfa data at Northern Agricultural Research Center due to variable nitrate.

FIGURE 1 Large green lentil variety Riveland (left) and small red variety CDC Robin (right), June 7, 2021 at Northern Agricultural Research Center.

TABLE 8 Means and summary of analysis of variance for pea biomass N and fraction of N derived from atmosphere (fNdfa; from ^{15}N natural abundance method) by year and site.

	Biomass N (kg ha^{-1})			fNdfa		
Pea variety	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
	Post Farm					
Aragorn	132	130	74 ab	0.74 ab	0.75 abc	0.71
Arvika	148	143	97 ab	0.79 a	0.82 a	0.67
Carousel	116	144	89 ab	0.68 b	0.71 bc	0.65
CDC Mozart	137	163	82 ab	0.73 ab	0.74 abc	0.65
Cruiser	131	117	85 ab	0.73 ab	0.65 c	0.75
Delta	131	177	64 b	0.75 ab	0.72 abc	0.68
DS Admiral	127	143	86 ab	0.75 ab	0.70 bc	0.66
Hampton	136	142	96 ab	0.77 a	0.77 ab	0.65
Lifter	133	134	102 a	0.76 a	0.79 ab	0.69
Majoret	117	126	85 ab	0.74 ab	0.69 bc	0.66
	p-values					
	0.51	0.24	**0.08**	**0.03**	**<0.01**	0.22
	Northern Agricultural Research Center					
Aragorn	–[a]	86 b	61	–	–[b]	0.78
Arvika	–	147 a	58	–	–	0.74
Carousel	–	102 ab	54	–	–	0.73
CDC Mozart	–	107 ab	56	–	–	0.77
Cruiser	–	109 ab	52	–	–	0.75
Delta	–	108 ab	58	–	–	0.73
DS Admiral	–	107 ab	51	–	–	0.78
Hampton	–	133 a	56	–	–	0.84
Lifter	–	118 ab	60	–	–	0.81
Majoret	–	102 ab	59	–	–	0.79
	p-values					
	–	**0.04**	0.92	–	–	0.43

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$). Bolded values indicate $p < 0.1$.

[a] Pea data not collected at Northern Agricultural Research Center in 2019 due to deer grazing.

[b] fNdfa not reported due to high/variable nitrate.

an assumption of the ND method. There was little confidence in results using the ND method for these two site-years, and they were omitted from analysis. The other site-years had SEMs for lentil and pea studies ranging from 1.3 to 5.5 kg ha^{-1}, which was deemed small enough to have confidence in the ND results. The NA method is sensitive to variation in soil ^{15}N natural abundance. Natural abundance of ^{15}N in soil is known to have inherent spatial variation (Hauggard-Nielson et al., 2010; Walley et al., 2001), and this is likely worsened when nitrate-N levels are highly variable. Based on observed nitrate-N variability between pulse and flax plots in 2020 at NARC, confidence in results was compromised and NA results are not reported here for either crop for NARC in 2020.

Nitrogen fixation between methods in the same site-year were generally similar (Tables S1 and S2). Using both methods increases confidence in N fixation amounts (McCauley et al., 2012). The NA method detected N fixation differences among lentil varieties in three site-years and among pea varieties in two site-years, whereas there were no pea or lentil varietal effect on N fixation using the ND method. This indicates that for this study that the NA method is more sensitive in detecting differences than the ND method. Based on this finding, we only report on the NA N fixation results below, which we refer to as "N fixation" in the text.

3.3 | N fixation, aboveground biomass N, and fNdfa

Climate and site influenced N fixation of lentil and pea (Tables 5 and 6).

TABLE 9 Pearson correlation coefficients (*r* values) between N fixation amounts and number of days to flowering and maturity at Arthur H. Post Agronomy Research Farm (PF) and Northern Agricultural Research Center (NARC).

Site-year	Days to flowering	Days to maturity
	Lentil	
PF 2019	**−0.65**	0.28
PF 2021	−0.38	−0.15
NARC 2021	−0.35	–[b]
Combined[a]	**−0.41**	−0.07
	Pea	
PF 2019	**0.66**	**0.59**
PF 2020	0.03	**0.55**
PF 2021	**0.60**	0.45
NARC 2021	0.10	–[b]
Combined	0.26	**0.32**

Note. Bold indicates $p < 0.1$.

[a] All available site-years using relative N fixation amounts and flowering/maturity dates to account for differences among site-years.

[b] Days to maturity not recorded at NARC in 2021.

Overall, N fixation by lentil and pea varieties trended to be higher at PF than NARC, which corresponds to precipitation differences (Table 4). The 2019 growing season was favorable at both sites and resulted in high N fixation by lentil at PF, with a mean of 127 kg N ha^{-1} (Table 5). In 2021, a drought year, the mean lentil N fixation at PF was 41 kg N ha^{-1}. The difference between years was less drastic at NARC, with a 2019 N fixation mean of 36 kg N ha^{-1} and a 2021 mean of 23 kg N ha^{-1}. The smaller range at NARC was likely due to a smaller growing season precipitation range among years (ca. 50 vs. 100 mm for NARC and PF, respectively).

At PF, N fixation by pea trended higher in 2019 and 2020 than in 2021 (Table 6). While 2020 and 2021 had similar cumulative precipitation over the growing season, 2020 likely had greater N fixation because of more overwinter precipitation, timely rainfall in June, and a cooler June and July (Table 4) which would have conserved water. Under water stress, N fixation decreases before other plant physiological processes (King and Purcell, 2001; McCauley et al., 2012), likely in part due to less N demand from less biomass growth. Studies in Western Canada found that N fixation increased when pulses experienced favorable growing conditions (Clayton et al., 2004; Hossain et al., 2017). Nodules require large amounts of C from the plant for N fixation (Liu et al., 2018). Under water stress, pulses likely prioritize C allocation to other tissues over nodules to complete their growth cycle since N is less limiting when biomass growth, and hence N demand, is reduced by drought stress.

Lentil variety had a significant effect ($p < 0.1$) on N fixation in three of the four site-years (Table 5). In 2019 at NARC, CDC Richlea fixed an average of 20 kg ha^{-1} more N than CDC Robin and ND Eagle, and CDC Maxim fixed 19 kg ha^{-1} more N than CDC Robin. In 2021, CDC Richlea and Riveland were among the top N-fixing varieties at both sites. CDC Richlea and Riveland fixed 12 and 8 kg ha^{-1} more N, respectively, than CDC Impala at NARC in 2021, and 18 and 22 kg ha^{-1} more N than CDC Impala at PF in the same year. Differences in N fixation are not surprising given that different varieties respond differently to variable weather (McPhee & Muehlbauer, 2001).

The N fixation by pea varieties varied in two (PF 2019 and 2020) of the four site-years (Table 6). In 2019 at PF, Arvika, the only forage pea tested, fixed an average of 25 kg N ha^{-1} more than the five lowest varieties (Carousel, Cruiser, Delta, DS Admiral, and Majoret). Arvika flowers later in the season and likely benefitted from consistent precipitation later in the 2019 growing season and accumulated more biomass N. In 2020 at PF, N fixation varied among pea varieties. In this drier growing season, Arvika was not among the high or low N-fixing varieties, while Delta fixed much more N (47 kg ha^{-1}) than Cruiser. Cruiser was among the earlier maturing varieties tested. Under water stress, pea does not fix significantly more N after flowering (McCauley et al., 2012). Cruiser may have fixed less N because its earlier flowering decreased the time for actively fixing N compared to other varieties.

Aboveground biomass N amounts (hereafter referred to biomass N) differed among lentil varieties in three of the five site-years (Table 7). Riveland and CDC Richlea, two of the top N-fixing varieties, were consistently in the top statistical groupings for biomass N, while CDC Impala, which generally fixed less N, was frequently in the bottom grouping. CDC Richlea and Riveland were two of the larger-seeded varieties tested, which may explain their robust growth (Figure 1) and hence higher biomass N in 60% of site-years. Lentil variety fNdfa values only varied at PF in 2021. The fNdfa value for CDC Impala was 0.53, which was on average 0.15 lower than CDC Maxim, CDC Viceroy, ND Eagle, and Pennell. For this site-year, varieties with high fNdfa values generally had greater N fixation.

At PF in 2019 and 2020, where differences in N among pea varieties were observed using NA, fNdfa values were higher for varieties with greater N fixation (Table 8). Generally, pea varieties with higher fNdfa values fixed more N. Arvika, Hampton, and Lifter on average had fNdfa values 0.09 (12%) higher than Carousel at PF in 2019. In 2020 at PF, Arvika, Hampton, and Lifter again had higher fNdfa values than Carousel, as well as Cruiser, DS Admiral, and Majoret. Biomass N varied among pea varieties in two of the five site-years, yet this did not translate to differences in N fixation using either method.

We only identified one other study that evaluated N fixation parameters of any of the varieties tested herein. Specifically, Abi-Ghanem et al. (2011) measured fNdfa in two lentil varieties (Pennell and Riveland) and two pea varieties (Delta and

TABLE 10 Means and summary of analysis of variance for seed yield of lentil and pea varieties by year and site.

	Yield (kg ha^{-1})			
	Post Farm[a]	**Northern Agricultural Research Center**		
Lentil variety	**2019**	**2019**	**2020**	**2021**
CDC Impala	2661 ab	948 d	1896 cd	578 ab
CDC Maxim	2874 a	1211 abcd	2118 bcd	473 d
CDC Redberry	2682 ab	1215 abcd	2042 bcd	506 cd
CDC Richlea	2669 ab	1393 a	2228 ab	549 bc
CDC Robin	2574 ab	992 cd	2023 bcd	385 e
CDC Rosetown	2786 a	1045 bcd	1814 e	626 a
CDC Viceroy	2593 ab	1283 ab	2423 a	457 d
ND Eagle	2835 a	1262 abc	2163 abc	567 abc
Pennell	2763 a	1134 abcd	1765 d	301 f
Riveland	2387 b	1209 abcd	1953 bcd	378 e
	p-values			
	0.01	**<0.01**	**<0.01**	**<0.01**
Pea variety	**Post Farm[b]**		**Northern Agricultural Research Center[c]**	
	2019	**2020**	**2020**	**2021**
Aragorn	2045 bc	2840 bc	2285 abc	761 cd
Arvika	2283 ab	2879 bc	2006 cde	545 e
Carousel	2326 ab	3255 abc	2464 a	838 bcd
CDC Mozart	2374 ab	3577 a	1961 cde	1002 a
Cruiser	2696 a	2887 cd	2265 abcd	629 e
Delta	2040 bc	3490 ab	2148 bcde	851 bc
DS Admiral	2152 abc	3358 ab	2117 bcde	739 d
Hampton	1938 bc	3127 abc	2159 abcde	981 a
Lifter	1534 c	3481 ab	1885 e	936 ab
Majoret	2038 bc	2000 d	2392 ab	619 e
	p-values			
	<0.01	**<0.01**	**<0.01**	**<0.01**

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$). Bolded values indicate $p < 0.1$.

[a] No Arthur H. Post Agronomy Research Farm (Post Farm) lentil yield data in 2020 due to herbicide damage or in 2021 due to poor emergence.

[b] No Post Farm pea yield data in 2021 due to poor emergence.

[c] No Northern Agricultural Research Center pea yield data in 2019 due to deer grazing.

Lifter) that were evaluated in our study. Values for fNdfa were up to 30% lower in our study, likely because Abi-Ghanem et al. (2011) used a greenhouse environment with a low-N media while we used a field study and crops accessed more soil nitrate-N. Comparing varieties grown in both studies, there were no differences in fNdfa between the two lentil varieties and the two pea varieties in any year for either study.

N fixation can vary among varieties within legume crop species (Hossain et al., 2017; Zimmer et al., 2016), although in a temperate environment where fNdfa < 0.50, N fixation of organic pea varieties did not vary (Gollner et al., 2019). The N fixation calculation includes fNdfa and biomass N. A strong positive correlation ($R^2 = 0.65$) between biomass production and N fixation has been established for legume crops (Unkovich et al., 2010). McCauley et al. (2012) reported that lentil and pea had greater N fixation (by both NA and ND methods) when the crops accumulated more biomass N. In our study, lentil varieties with greater biomass N accumulation had greater N fixation in 50% of site-years. Pea N fixation appeared to be influenced more by fNdfa than biomass N, as varieties tended to fix more N when they had higher fNdfa. In a Western Canada field study, trends in fNdfa often matched trends in N fixation (Hossain et al., 2017). A greenhouse study revealed that fNdfa of lentil varieties was positively correlated ($r = 0.25$, $p < 0.001$) with nodule numbers, suggesting that breeding for higher rates of rhizobia infection may boost fNdfa (Abi-Ghanem et al., 2011).

TABLE 11 Pearson correlation coefficients (*r* values) between N fixation parameters and seed yield at Arthur H. Post Agronomy Research Farm (PF) and Northern Agricultural Research Center (NARC).

			Yield	
Year	**Site**	**Parameter**	**Lentil**	**Pea**
2019	PF	N fixation[a]	−0.17	0.12
		fNdfa[b]	−0.19	**0.54**
		Biomass N	0.05	**0.34**
	NARC	N fixation	**0.31**	–[c]
		fNdfa	0.04	–
		Biomass N	**0.62**	–
2020	PF	N fixation	–	**0.58**
		fNdfa	–	**0.54**
		Biomass N	–	**0.65**
	NARC	Biomass N	0.14	0.24
2021	NARC	N fixation	−0.05	0.19
		fNdfa	−0.19	0.19
		Biomass N	0.06	0.11

Note. Bold indicates significance ($p < 0.1$).

[a] N fixation using ^{15}N natural abundance method.

[b] Fraction of N derived from atmosphere (^{15}N natural abundance method).

[c] No data.

3.4 | Nitrogen fixation relationship with days to flower and maturity

Correlation coefficients for N fixation and days to flower and maturity were generally negative for lentil and positive for pea (Table 9). When lentil site-years were combined, the correlation coefficient for N fixation and days to flowering was weakly negative ($r = -0.41$, $p < 0.1$), with days to flowering explaining 16% of variation in N fixation, while the correlation coefficient for N fixation and days to maturity was not significant. When pea site-years were combined, the correlation coefficient for N fixation and days to maturity was weakly positive ($r = 0.32$, $p < 0.1$), and days to maturity explained just 9% of variation in N fixation. For both crops, the strength of correlations for combined site-years was likely diluted by weak correlations in some site-years. For example, pea had moderately strong correlation coefficients between days to flowering and N fixation at PF in 2019 and 2020, but correlation coefficients were not significant at PF in 2020 and at NARC in 2021.

The differences in relationships observed between lentil and pea could be explained by growth habit. The pea and lentil varieties used in this study were all indeterminate, but pea appears to be determinate when water is depleted and growth consequently stops. McCauley et al. (2012) found that pea fixed no further N after flowering in a dry year and only 22% of the total N fixation occurred after flowering in a wet year. Conversely, 53% and 72% of lentil's total N fixation occurred between after flowering in a dry and wet year, respectively (McCauley et al., 2012). This aligns with our findings, where in two site-years N fixation by pea increased with longer vegetative growth periods (i.e., pre-flowering), whereas N fixation by lentil never increased with pre-flowering time. Pea may cease fixing N as it approaches maturity in order to translocate more carbon toward pods instead of nodules, while lentil, absent of drought stress, would benefit from more N fixation during reproductive growth to produce more seed. Plant size could also explain the difference between species, as lentil plants are small when flowering begins and may be able to predominantly use soil N up to that point. Lentil may then rely more heavily upon N fixation as plants increase in size, whereas pea plants are much larger by flower and likely already depend heavily on N fixation.

3.5 | Seed yield

Seed yield varied ($p < 0.1$) for all four site-years among lentil and pea varieties (Table 10). Average yields were somewhat inconsistent among site-years, likely reflecting notable variation in weather among years and between sites. In 2019 at NARC, CDC Richlea yielded greater than the three lowest varieties (CDC Impala, CDC Robin, and CDC Rosetown). In 2021 at the same site, CDC Impala, CDC Rosetown, and ND Eagle were the three highest yielding varieties, demonstrating how site-year growing conditions can greatly affect yield ranking among varieties (McPhee & Muehlbauer, 2001; Tullu et al., 2001). The site-year with the greatest lentil yields overall was PF in 2019, highlighting the unusually favorable growing conditions experienced that year.

For pea, CDC Mozart consistently was among the highest yielding varieties, while Majoret was among the lowest yielding varieties in all three site-years (Table 10). Other varieties showed less consistency. Cruiser, for example, was in the top statistical grouping at PF in 2019 but was in the bottom statistical grouping at PF in 2020 and at NARC in 2021. The inconsistency in Cruiser's yield is not explained by maturity date, as it is often one of the earlier maturing varieties. We would expect an early-maturing variety to have a yield advantage in dry, warm years, but Cruiser had greater yield relative to more than one variety or more only at PF in 2019, the wettest and coolest site-year, and NARC in 2020. The pea variety Arvika may have had its seed yield most affected by low rainfall, yielding over 2000 kg ha^{-1} at PF in 2019 and 2020 but just 545 kg ha^{-1} at NARC in 2021. Arvika is a late-flowering forage variety, so drought in 2021 likely stressed Arvika during flowering and consequently decreased seed yield. Hossain et al. (2017) reported an interaction of year and cultivar in pea and chickpea yield response when growing season precipitation ranged from 74% to 124% of the long-term average over a 3-year study, but cultivar characteristics that may have explained the interaction were not addressed.

3.6 | N fixation and yield relationships

Overall, correlation coefficients of N fixation parameters (biomass N, fNdfa, and N fixation) and seed yield were inconsistent in direction and strength, ranging from −0.19 to 0.65 (Table 11); however, when the correlations were significant, they were always positive, consistent with previous work on grain legumes (Hossain et al., 2017, Zimmer et al., 2016).

More non-significant correlations than significant suggests that factors other than N fixation also influenced seed yield, like precipitation amounts and timing. However, at PF in 2019, favorable precipitation allowed for robust growth which should have caused N to be more limiting than water, yet there were no correlations between N fixation and yield for pea or lentil. Correlation analysis was likely affected by sampling area, as seed yield was measured at the plot scale (~16 m^2) and N fixation parameters were obtained from biomass subsamples (~0.6 m^2) based on a subjective representative area. Of the 20 correlations (10 for each species), 7 were significant ($p < 0.1$). A significant association with yield was more likely when there was variation in N fixation parameters. The 2021 growing season at NARC had N fixation parameters least correlated with yield. This was likely because yields were primarily limited by severe drought. Excluding 2021, 71% of pea N fixation parameters had positive relationships with yield, while just 29% of lentil N fixation parameters were positively related with yield. The positive relationships with pea yield and N fixation parameters were most notable at PF in 2019 and 2020 when there were excellent growing conditions due to above average growing season precipitation and below average temperature (2019) or above average overwinter precipitation and timely high June precipitation (2020). This finding reinforces the premise that yield and N fixation are more tightly aligned when moisture is less limiting, thereby increasing the probability that N will limit yield.

Legume crop breeding efforts have historically focused on increasing yields and improving agronomic factors such as disease tolerance. Herridge and Rose (2000) proposed that selecting for higher yielding legume varieties will in turn increase N fixation due to higher seed N demand. However, intensive breeding and domestication may have diminished legume crops' capacity for N fixation, as breeding programs often utilize soils with high mineral N and inadvertently select lower N-fixing lines that yield higher without the C sink of N fixation (Liu et al., 2020). Our results suggest that modern pea varieties in the NGP fix more N when they yield higher, consistent with Herridge and Rose (2000). For lentil varieties, the relationship between N fixation and yield is less predictable, indicating a potential physiological difference between pea and lentil yield formation likely caused by apparent differences in growth habits under water stress experienced in the NGP. Overall, our results show that N fixation and yield relationships for pulse crops are complex and likely impacted by environmental conditions. Based on our study, it is recommended that pulse breeders should continue to breed for high yielding varieties to directly help producers with a good possibility this will indirectly increase N fixation, especially for pea.

4 | CONCLUSION

This 3-year study was the first field evaluation of N fixation by lentil and pea varieties in Montana, and among the first in the NGP. In this study, lentil and pea varietal effects on N fixation and seed yield varied considerably depending on environmental conditions. Overall, there was less variation in N fixation among pea varieties than lentil varieties, and no pea variety consistently performed particularly poorly or well. Greater N fixation in lentil was correlated with varieties that had a shorter time to flowering, whereas, greater N fixation in pea was correlated with longer time to maturity. Correlations between N fixation parameters and seed yield were weak to moderate, with correlation coefficients ranging from −0.19 to 0.65, but significant correlations were always positive, suggesting N fixation may benefit from breeding for higher yield. Further research into physiological differences between the two crops is urgently needed to help with breeding and making appropriate management decisions.

ACKNOWLEDGMENTS

This work was funded by the United States Department of Agriculture—Agricultural Research Service—Pulse Crop Health Initiative (Agreement Number: 58-3060-8-025). We would like to thank Terry Rick for her efforts on this study.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Kaleb Baber: Formal analysis; investigation; methodology; writing—original draft. **Clain Jones**: Conceptualization; funding acquisition; investigation; methodology; supervision; writing—review and editing. **Kevin McPhee**: Conceptualization; funding acquisition; investigation; methodology; writing—review and editing. **Perry R. Miller**: Writing—review and editing. **Peggy Lamb**: Investigation; methodology; writing—review and editing.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Baber, K., Jones, C., McPhee, K., Miller, P. R., & Lamb, P. (2023). Nitrogen fixation among pea and lentil varieties in the Northern Great Plains. *Agronomy Journal*, *115,* 2325–2338. https://doi.org/10.1002/agj2.21419