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Lentil nitrogen fixation response to fertilizer and inoculant in the northern Great Plains

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Abstract

Lentil (*Lens culinaris* Medikus) production in the semiarid northern Great Plains of the United States has increased dramatically over the past two decades. Lentil in rotation provides agroecosystem benefits of more efficient water use, pest cycle disruption, and biological nitrogen (N) fixation. Increasing N fixation could alleviate soil acidification and groundwater impairment, decrease N fertilizer costs, and increase lentil seed yield. Despite widespread farmer adoption of lentil in the region, little is known about the benefits of fertilizer or inoculant type on N fixation. The aim of this study was to determine how nutrients (potassium (K), sulfur (S), and foliar-applied micronutrients) and rhizobial inoculant types (seed-coat powder and granular) influence N fixation of lentil. The study was conducted at two field sites in Montana from 2019 to 2021. Fixed N amounts were calculated using both an N difference approach and ^{15}N natural abundance method. N fixation was highly responsive to climatic conditions and soil characteristics. The amount of N fixed did not respond to K fertilization, likely because soil test K levels were sufficient. In a moderately dry year at a site with low soil sulfate-S, fertilizer application of 5.6 kg S ha^{-1} increased N fixed by 40%. Inoculated lentil fixed more N than uninoculated lentil in two site-years, but there were no differences in N fixed between inoculant types. Inoculation response was not related to field cropping histories with legumes. The study shows that S fertilization and rhizobial inoculation have potential to increase lentil N fixation amounts in the northern Great Plains.

1 | INTRODUCTION

Lentil (*Lens culinaris* Medikus) has become increasingly popular in the historically cereal-dominated northern Great Plains (NGP), with ~214,000 ha of lentil planted in Montana in 2021 (USDA NASS, 2022). Lentil production has increased in part due to high lentil grain prices resulting from consumer demand for plant-based protein (Warne et al., 2019). Incorporating lentil into NGP cropping systems, often in place of summer fallow, has several benefits. Pulse crops, including lentil, can stabilize profits and minimize financial risk when compared to cereal-only systems (Miller et al., 2015; Zentner et al., 2001). Several studies have found increases in cereal yield when grown after pulse crops (Beckie & Brandt, 1997; Bremer et al., 2011; Miller et al., 2003a). Pulses provide agroecosystem benefits of summer fallow reduction (Long

Abbreviations: fNdfa, fraction of nitrogen derived from atmosphere; LTA, long-term average; NA, ^{15}N natural abundance; NARC, Northern Agricultural Research Center; ND, nitrogen difference; NGP, northern Great Plains of the United States; PF, Arthur H. Post Agronomy Research Farm.

et al., 2014), efficient water use (Miller et al., 2003b), pest cycle disruption (Stevenson & van Kessel, 1996), and biological nitrogen (N) fixation (Beckie & Brandt, 1997). Repeated application of N fertilizer at high rates to support high protein wheat in the NGP has caused elevated groundwater nitrate concentrations (Sigler et al., 2020) and soil acidification (Engel, 2020). Through N fixation, lentil can decrease N fertilizer inputs for producers and potentially improve agricultural sustainability in the region.

While lentil production in the NGP has grown substantially over the past few decades, research on how to best manage soil fertility, both for optimal yield and N fixation, has lagged. Legumes tend to be more sensitive to nutrient deficiencies than other crops because of the high nutrient demand of N fixation. Nutrient deficiencies can reduce N fixation by limiting root nodule numbers or nitrogenase activity (Becana et al., 2018; Divito & Sadras, 2014). Alternatively, N fixation can decrease when nutrient deficiencies limit growth of the host legume and hence reduce photosynthate available to be mobilized to nodules (Divito & Sadras, 2014). Pulse crops respond to phosphorus (P) fertilizer when soil P levels are low (Divito & Sadras, 2014; McKenzie et al., 2001a). Nitrogen fixation of legume crops responds positively to fertilizer sulfur (S) (Cazzato et al., 2012; Scherer et al., 2006; Zhao et al., 1999) and potassium (K) (Abbasi et al., 2012; Abdelhamid et al., 2011; Collins et al., 1986). The micronutrients boron (B), molybdenum (Mo), and zinc (Zn) together increased nodulation of lentil (Hossain et al., 2020), and iron (Fe) had a positive effect on biomass N accumulation (Nasar et al., 2019). Lentil N fixation response to S, K, and micronutrients in the NGP is unknown.

The bacteria associated with leguminous N fixation often need to be introduced to agroecosystems in the form of crop-specific inoculant. In a meta-analysis of grain legumes (Kaschuk et al., 2009), average grain yield increased 16% and average grain protein by 7% when seeds were inoculated at planting. The majority of inoculant used in Montana is peat-based, either in powder form applied as a seed-coat or granular form applied in furrow. Both are used to a relatively equal extent in Montana pea production (Bestwick et al., 2018), but their relative efficacies are unknown. Clayton et al. (2004) found that granular inoculant resulted in greater pea N fixed amounts and seed yield than seed-coat inoculant in fields never previously planted with pea or lentil. Potential yield differences and known cost differences (granular is four times more expensive on a per hectare basis) between inoculant types demonstrate that research in this area could have significant economic impacts for Montana lentil growers.

There is an absence of research on N fixation benefits in lentil associated with fertilizer or inoculant in the NGP. Therefore, we initiated a study to fill this research void. The primary objective of this study was to evaluate the response of lentil N fixation to fertilizer (K, S, and micronutrients) and inoculant type (granular and seed-coat). Other objectives were to assess K, S, and micronutrient fertilizer and inoculant effects on N yield (grain N content × yield) and to investigate the relationships between N fixation and seed yield and protein.

Core Ideas

- Inoculating lentil increased nitrogen (N) fixed by up to 37 kg N ha^{-1} in 40% of site-years, but inoculant type had no effect.
- Sulfur fertilization increased N fixed by lentil in 40% of site-years by up to 38 kg N ha^{-1}.
- Greater N fixed amounts were often associated with greater biomass N accumulation, seed yield, and seed protein.
- Increasing tissue S concentrations may increase N fixed amounts more than seed and N yields.

2 | MATERIALS AND METHODS

2.1 | Site selection

This study was part of a larger lentil fertility study conducted at seven sites in Montana and North Dakota from 2019 to 2021 (Miller et al., 2022). Two of those sites, Montana State University's Arthur H. Post Agronomy Research Farm (PF) near Bozeman, MT (45°40′48″ N latitude; 111°9′00″ W longitude) and Northern Agricultural Research Center (NARC) near Havre, MT (48°29′24″ N latitude; 109°47′60″ W longitude), were selected to evaluate N fixed amounts. Historically, these two sites span the precipitation gradient of Montana's dryland agricultural areas, with PF and NARC averaging 417 and 306 mm per year, respectively. Different fields were used at the two sites each year to imitate the typical Montana crop rotation of lentil after a cereal. The soil type at PF was Amsterdam silt loam (Fine-silty, mixed, superactive, frigid Typic Haplustolls) in all years. Soil types at NARC were Telstad–Joplin loam (Fine-loamy, mixed, superactive, frigid Aridic Argiustolls) in 2019 and 2020 and Kenilworth-Fortbenton fine sandy loam (Fine-loamy, mixed, superactive, frigid Aridic Argiustolls) in 2021. Site selection was based on fall-sampled soil parameters of nitrate-N (0.12 M KCl, cadmium reduction), exchangeable K (1 M ammonium acetate, inductively coupled plasma), sulfate-S (0.12 M KCl, turbidimetric), Olsen P (0.5 M $NaHCO_3$, colorimetric), and soil organic matter (loss on ignition) to identify locations within suitable fields that had relatively similar fertility levels. Soil at each field

TABLE 1 General soil properties (sampled in spring of each year) for Arthur H. Post Agronomy Research Farm (i.e., "Post Farm," Bozeman, MT) and Northern Agricultural Research Center (Havre, MT) in lentil nitrogen (N) fixation study fields, 2019–2021.

Soil property	2019	2020	2021
	Post Farm		
Pea/lentil history (year)[a]	2017	None	None
Previous crop	Fallow	Spring malt barley	Oat
Soil texture[b]	Silt loam	Silt loam	Silt loam
Soil pH (1:1 soil:water)	7.7	7.9	7.0
Organic matter (g kg^{-1})	28	25	26
Olsen P (mg kg^{-1})	9.3	12.3	18.8
Exchangeable K (mg kg^{-1})	414	334	370
Sulfate-S (mg kg^{-1})	4.4	3.2	1.7
Sulfate-S (mg kg^{-1}), 15–60 cm	1.0	1.6	1.0
Nitrate-N (kg N ha^{-1}), 0–90 cm	18	33	61
	Northern Agricultural Research Center		
Pea/lentil history (year)	2016	2016	None
Previous crop	Spring wheat	Spring malt barley	Forage barley
Soil texture	Loam	Loam	Sandy loam/loam
Soil pH (1:1 soil:water)	8.1	6.5	8.3
Organic matter (g kg^{-1})	21	15	12
Olsen P (mg kg^{-1})	13.5	30.5	20.5
Exchangeable K (mg kg^{-1})	366	308	334
Sulfate-S (mg kg^{-1})	14.3	20.3	3.1
Sulfate-S (mg kg^{-1}), 15–60 cm	3.7	17.4	1.7
Nitrate-N (kg N ha^{-1}), 0–90 cm	49	261	45

Note: Pre-seed average of 10 soil sample locations (two samples per block), 0–15 cm unless noted.
[a]Most recent in the 10 previous years.
[b]*Source*: NRCS (2023).

site was sampled (two locations per block) in the spring of each growing season and analyzed for pH (0–15 cm; 1:1 soil/water), organic matter (0–15 cm), Olsen P (0–15 cm), exchangeable K (0–15 cm), sulfate-S (0–60 cm), and nitrate-N (0–90 cm). Fall and spring pre-study soil samples were analyzed by an independent laboratory (AGVISE Laboratories). Soil characteristics and pulse crop histories are summarized in Table 1.

2.2 | Treatments and experimental design

Treatments included eight combinations of inoculant and fertilizer formulations (Table 2). Inoculants included granular (Verdesian Primo GX2) and seed-coat powder (Verdesian N-Charge). The foliar micronutrient solution (Micro1000, Agroliquid) contained all the micronutrients required for plant growth except for chlorine, which has not been documented as needed for N fixation, plus cobalt (Table 3). The micronutrient treatment was abandoned in 2019 because application at five-fold the label rate (to increase potential for response) resulted in crop injury. Experiments were conducted in a randomized complete block design with five replicates for each location per year.

2.3 | Plot management

Avondale lentil, a commonly grown green variety with a medium-sized seed, was planted into re-crop, no-till managed fields with standing cereal stubble at a target rate of 120 pure live plants m^{-2} (approximately 70 kg seed ha^{-1}) in 23-cm rows at PF and 30-cm rows at NARC. Plot sizes were 8 m long by 2 m wide. Dates for planting and other field activities are shown in Table S1. Plots were seeded in the following order of inoculant formulations to prevent rhizobia contamination: control, granular, and seed-coat. Inoculants of *Rhizobium leguminosarum* biovar *viceae* were applied at the recommended label rate (6.7 kg ha^{-1} for granular and 0.31 kg per 100 kg seed for seed-coat). Flax, used as a non-N fixing reference crop, was mechanically seeded in a 2-m strip adjacent to all lentil plots. Flax was selected because of its similar root biomass and vertical distribution to lentil (Gan et al., 2009). Monoammonium phosphate (11-52-0) was

TABLE 2 Inoculant types (*Rhizobium leguminosarum* biovar *viceae*), fertilizer sources, and rates for each treatment in Montana lentil rhizobial inoculant and fertility studies, 2019–2021.

Treatment	CFU[a] content (CFU g^{-1})	Fertilizer source	K (kg ha^{-1})	S (kg ha^{-1})
Control	–	–		
Granular[b]	1×10^8	–		
Seed-coat[c]	2×10^8	–		
Granular + K	1×10^8	KCl	14	
Seed-coat + K	2×10^8	KCl	14	
Granular + K + S	1×10^8	Potassium sulfate	14	5.6
Seed-coat + K + S	2×10^8	Potassium sulfate	14	5.6
Granular + K + S + Micros[d]	1×10^8	Potassium sulfate + Micro1000[e]	14	5.6

[a]Colony forming units of *Rhizobium leguminosarum* biovar *viceae*.
[b]Granular inoculant applied at 6.7 kg ha^{-1}.
[c]Seed-coat powder inoculant applied at 0.31 kg per 100 kg seed.
[d]Micronutrient Solution, 2020 and 2021 study years only.
[e]AgroLiquid.

TABLE 3 Nutrient concentrations in Micro1000 foliar product for use in Montana lentil rhizobial inoculant and fertility studies, 2019–2021.

Nutrient	Nutrient (%)	Rate (g ha^{-1})[a]
Boron	0.02	0.6
Copper	0.25	7
Iron	0.37	10
Manganese	1.0	28
Zinc	1.0	28
Calcium	1.0	28
Magnesium	0.5	14
Cobalt	0.1	3
Molybdenum	0.1	3
Nickel	0.001	0.003

[a]Micro1000 applied at rate of 2.3 L ha^{-1} near first flower.

applied in furrow at planting at a rate of 50 kg ha^{-1} to provide 5.6 kg ha^{-1} of starter N and 11.2 kg ha^{-1} of starter P to both lentil and flax. Appropriate pest management practices were conducted throughout each growing season (Table S2).

2.4 | Data collection

A representative area, consisting of three 1-m rows at PF for an area 0.69 m^2 and two 1-m rows at NARC for an area of 0.61 m^2, was sampled for biomass from each lentil plot at early pod fill 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 lentil plots. In 2021, one flax sample and corresponding soil sample was collected for each lentil plot at both sites to minimize potential error from spatial variation. Biomass samples were dried (50˚C, 72 h), weighed, and ground (<0.5 mm). Subsamples were analyzed for N concentration via combustion with a LECO TruSpecCN Analyzer and approximately 10% of samples were analyzed in duplicate to assess precision (CV averaged 2.5%). At PF in 2020, there were visual signs of nutrient deficiency, and biomass samples for the K and K + S treatments for both inoculant types were also analyzed for S and Zn concentrations (inductively coupled plasma; AgVise Laboratories). Naturally occurring isotope ^{15}N was analyzed at the University of California–Davis Stable Isotope Facility using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (CV averaged 2.2%; Sercon Ltd.).

Plots were harvested with a small-plot combine (Model 130, ZURN Harvesting), excluding edge rows, and lentil seeds were cleaned and weighed. Seed yield was determined on a dry matter basis (kg ha^{-1}) and not adjusted for a standard moisture. Seed N concentrations were determined by combustion (LECO TruSpecCN), and seed protein concentration was reported as N × 6.25 (Coyne et al., 2005). Shortly after lentil harvest, soil was sampled to 90-cm depth (3.0-cm diameter core) in each lentil plot and at each location where flax was sampled. Each soil sample was split into three 30-cm segments. Soils were dried (50˚C, 72 h), weighed, and bulk density was calculated. The soils were ground (<2 mm), extracted using 1 M KCl, and analyzed for nitrate-N with a flow injection colorimetric analyzer (Lachat). Approximately 10% of samples were analyzed in duplicate, and CV averaged 7.0%. The nitrate-N pool (kg N ha^{-1}) for each 90-cm sample was calculated.

2.5 | Measuring N fixation

Aboveground N fixed was estimated using two methods: an N-difference approach (ND) and the ^{15}N natural abundance (NA) method. The ND method used the following equation as reported by Unkovich et al. (2008), with all units in kg N ha^{-1}.

$$\text{Nfixed} = \left(\text{biomassN}_{\text{legume}} - \text{biomassN}_{\text{reference}}\right) + \left(\text{soilN}_{\text{legume}} - \text{soilN}_{\text{reference}}\right) \quad (1)$$

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

A lower bound of 0 for N fixed was used because N fixed amounts cannot be negative. An upper bound on N fixed was set as the lentil plot's aboveground biomass N amount. Amounts of N fixed outside the set bounds were omitted from analysis (9.0% of observations).

For the NA method, the fraction of N derived from the atmosphere (fNdfa) was calculated using the following equation, first published by Shearer and Kohl (1986):

$$\text{fNdfa} = \frac{\delta^{15}\text{N}_{\text{reference}} - \delta^{15}\text{N}_{\text{legume}}}{\delta^{15}\text{N}_{\text{reference}} - B}, \quad (2)$$

where δ^{15}N is given as:

$$\delta^{15}\text{N}(\%_o) = \frac{\text{atom }\%\text{N}_{\text{sample}} - \%\text{N}_{\text{atmosphere}}}{\text{atom }\%\text{N}_{\text{atmosphere}}} \quad (3)$$

The B in Equation 2 refers to the δ^{15}N of lentil grown without N, meaning it reflects conditions in which all plant N is fixed. The B-value for Avondale lentil used in our study (−0.99) was obtained in a greenhouse experiment (Baber, 2022). Amounts of N fixed (kg N ha^{-1}) for the NA method were calculated using Equation 4.

$$\text{Nfixed} = \text{biomassN}_{\text{legume}} \times \text{fNdfa} \quad (4)$$

Amounts of N fixed less than 0 were omitted from analysis (1.3% of observations) and there were no N fixed amounts above Biomass N_{legume}.

2.6 | Statistical analyses

All statistical procedures were conducted using R (R Core Team, 2021). Linear mixed models for response variables (N fixed using both methods, biomass, biomass N, fNdfa from NA, seed yield, seed protein concentration, protein yield) were fit using treatment as a fixed effect and block as a random effect in the "lme4" package (Bates et al., 2015). A model combining site-years was attempted, but variance among site-years was large such that treatment variance was obscured. Therefore, site-years were analyzed independently for treatment effects using analysis of variance. Differences among treatments were further evaluated using pre-planned orthogonal contrasts with the "emmeans" package (Lenth, 2021) and Tukey–Kramer honestly significant difference (HSD) familywise comparisons of means ($\alpha = 0.1$) with the "multcomp" package (Hothorn et al., 2008). Correlation analysis was conducted to analyze relationships (Pearson correlation coefficients) among N fixation parameters (N fixed using both methods, biomass N, and fNdfa from NA) and seed parameters (yield, protein concentration, and protein yield). Correlations were conducted on a plot basis for each site-year.

3 | RESULTS AND DISCUSSION

3.1 | Weather

Yearly weather varied substantially at both sites during the study (Table 4). The 2019 growing season was more favorable than 2020 and 2021 at both sites, with cumulative 2019 growing season precipitation representing 113% and 84% of the long-term average (LTA) for PF and NARC, respectively. The 2020 growing season was drier than 2019 but benefitted from pre-season precipitation and timely rainfall during the growing season. The 2021 growing season was drier and hotter than the LTA at both sites. Drought was particularly severe at NARC in 2021, where the rainfall total for June, a pivotal month for lentil growth in the NGP, was 9% of the LTA.

3.2 | Biomass, biomass N, and fNdfa

Biomass varied among treatments in four of six site-years (Table 5). Inoculation increased lentil biomass by 1042 and 438 kg ha^{-1} at NARC in 2019 and 2021, respectively. In 2019 at NARC, seed-coat inoculant resulted in 419 kg ha^{-1} more biomass than granular inoculant. Sulfur fertilizer increased biomass at PF in 2020 by 657 kg ha^{-1} and at NARC in 2021 by 444 kg ha^{-1}. There was a small negative response to K fertilizer at PF in 2021, where biomass decreased by 260 kg ha^{-1} with K fertilizer.

Biomass N varied among treatments in three of six site-years (Table 6), and differences in biomass N mostly mirrored differences in biomass. Inoculation increased biomass N accumulation by 28 and 13 kg N ha^{-1} at NARC in 2019 and 2021, respectively, and at NARC in 2019 lentil with seed-coat inoculant accumulated 9 kg N ha^{-1} more than lentil with granular inoculant. S fertilizer increased biomass N accumulation by 42 and 8 kg N ha^{-1} at PF in 2020 and NARC in 2021, respectively. There were fewer differences in fNdfa among treatments (Table 7). At NARC in 2019, inoculated lentil's fNdfa was 46% higher (fNdfa 0.12 higher) than the control and S fertilizer increased fNdfa by 72% (0.18 fNdfa increase). At PF in 2021, seed-coat inoculant increased fNdfa

TABLE 4 Precipitation and temperature data for Arthur H. Post Agronomy Research Farm (i.e., "Post Farm," Bozeman, MT) and Northern Agricultural Research Center (Havre, MT) at lentil inoculation and fertility study sites, 2019–2021.

	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 WRCC (2023).
[b]Over-winter preceding the growing season.
[c]April 1–July 31.

by 0.05 over granular inoculant. Overall, fNdfa values trended lower at NARC than at PF. Soil nitrate-N pools were generally higher at NARC, and high soil nitrate-N has been shown to limit N fixation (Voisin et al., 2002). This, paired with lower biomass production at NARC due to lower precipitation, likely decreased the degree to which lentil relied upon N fixation at NARC than at PF.

3.3 | Evaluation of N fixation methods

Both the ND and NA methods of estimating N fixed amounts require assumptions to be met. The ND method is invalidated when the legume and reference crops have access to different pools of soil nitrate-N, which could happen if rooting depths were different. Flax was selected as a reference crop because it closely matches the rooting depth of lentil (Gan et al., 2009). The assumption would also be invalidated when pre-plant nitrate-N pools are different between crops or soil N processes of mineralization, leaching, and denitrification occur at different rates under each crop (Unkovich et al., 2008). Nitrate pools were relatively low and uniform in 2019 and 2021. However, at NARC in 2020, postharvest soil nitrate-N between lentil and flax plots was highly variable (ranges of 59–352 and 29–224 kg N ha^{-1}, respectively, Table 1). This resulted in a majority of N fixed amounts calculated using ND falling outside of the established bounds (mostly > biomass N). Therefore, the NARC 2020 site-year was omitted from analysis. 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 as ^{15}N can be enriched or depleted during microbially mediated N transformations (Robinson, 2001). Natural abundance ^{15}N values for lentil and flax are summarized in Table S3. Separation between flax ^{15}N and *B* values were generally large enough to have reasonable precision (Unkovich et al., 1994). However, due to the highly variable nitrate-N at NARC in 2020, confidence in NA results was compromised and results are not reported here.

Confidence in N fixed amounts is strengthened by using both methods (McCauley et al., 2012). Overall, results from the two methods were generally in agreement when differences among treatments were detected. The ND method frequently resulted in numerically higher N fixed amounts than the NA method, but relative differences among treatments were similar for each method. Our study was more focused on relative differences than absolute amounts.

3.4 | N fixation

3.4.1 | Inoculant

Inoculation had no impact on N fixed in any year at PF (Table 8), but increased N fixed in both years reported at NARC (Table 9). Using the ND method, inoculated lentil

TABLE 5 Means, summary of analysis of variance, and orthogonal contrasts for biomass for each site-year at Arthur H. Post Agronomy Research Farm (PF; Bozeman, MT) and Northern Agricultural Research Center (NARC; Havre, MT) lentil rhizobial inoculant and fertility study sites, 2019–2021.

		Biomass (kg ha^{-1})					
		PF			NARC		
Treatment no.	Treatment	2019	2020	2021	2019	2020	2021
1	Control	6794	4724bc	3145ab	2471c	4588	1359b
2	Granular	6702	4879abc	3600a	3279ab	4646	1746ab
3	Seed-coat	7600	4874abc	3554ab	3745a	4289	1849ab
4	Granular + K	6935	4913abc	3234ab	3344ab	4405	1459ab
5	Seed-coat + K	7459	4604c	3399ab	3358ab	4485	1702ab
6	Granular + K + S	7061	5438ab	3367ab	3044bc	4612	2073a
7	Seed-coat + K + S	7004	5393ab	3127b	3820a	4441	1976ab
8	Granular + K + S + Micros[a]	–	5514a	3231ab	–	4267	1561ab
		p-value					
		0.53	**0.02**	**0.05**	**<0.01**	0.47	**0.08**
Contrast		*p*-value					
2, 3 vs. 1	Inoculant–control	0.42	0.53	0.24	**<0.01**	0.51	**0.04**
2, 4, 6 vs. 3, 5, 7	Gran–seed-coat	0.13	0.46	0.67	**<0.01**	0.22	0.54
4, 5 vs. 2, 3	K–no fertilizer	0.90	0.55	**0.03**	0.37	0.88	0.20
6, 7 vs. 4, 5	K + S—K only	0.65	**<0.01**	0.56	0.67	0.58	**0.02**
		Estimated difference (kg ha^{-1})[b]					
2, 3 vs. 1	Inoculant—control	–	–	–	1042	–	438
2, 4 , 6 vs. 3, 5, 7	Gran—seed-coat	–	–	–	−419	–	–
4, 5 vs. 2, 3	K—No fertilizer	–	–	−260	–	–	–
6, 7 vs. 4, 5	K + S—K only	–	657	–	–	–	444

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$) and bold p values indicate $p < 0.10$.
[a]Micronutrient solution (Micro1000, Agroliquid).
[b]Only shown if significant at $p < 0.10$.

fixed 37 and 13 kg N ha^{-1} more N than uninoculated lentil at NARC in 2019 and 2021, respectively. Increases in N fixed translated to increased grain yield as well, with inoculation increasing yield by 56% and 45% at NARC in 2019 and 2021, respectively (Miller et al., 2022). The NA method also found higher N fixed in inoculated plots at NARC, with estimated differences of 20 and 9 kg N ha^{-1} in 2019 and 2021, respectively. The observed inoculant response appeared to be unrelated to previous pea or lentil history. At NARC, the 2019 field site was planted in pea 3 years before the study and N fixed was still substantially higher when lentil was inoculated. At PF, the lack of an inoculant response was somewhat uprising given that no pulse crop had been grown in at least the previous 10 years, and likely never, at the 2020 and 2021 field sites. Possible explanations are that rhizobia have persisted at PF from widespread pea production in the region until the 1960s, rhizobia have been more widely spread throughout PF via wind or farm equipment, or that there is an effective indigenous rhizobia strain at PF.

Clayton et al. (2004) consistently found that inoculated pea fixed more N than non-inoculated pea in western Canada field experiments. Similar to results in our study, some N fixation was still observed in non-inoculated plots. Clayton et al. (2004) attributed this to indigenous rhizobia in the soil, based on another study (Kucey & Hynes, 1989) from the region that found up to 100 rhizobia per gram of soil in fields never planted with legumes. In our study, active nodules were observed on lentil roots in control plots, suggesting that indigenous rhizobia strains exist at both sites or rhizobia introduced in previous years have been moved by equipment or wind. At NARC, the commercial rhizobia strains applied as inoculants seemingly outperformed the existing strain(s) in the control plots. However, at PF, the existing strain(s), whether indigenous or introduced and moved from other fields, appeared to be equally effective at fixing N as the newly applied commercial inoculants. There is evidence that indigenous rhizobia strains in Pakistan can form effective symbioses with lentil and perform as well as commonly used commercial strains (Hafeez et al., 2000).

There were no differences in N fixed between granular and seed-coat inoculants for any site-year. Given the existing rhizobia strains at PF, these results are not surprising at that site. At NARC, where lentil N-fixed amounts increased with inoculation, granular inoculant was expected to be more effective

TABLE 6 Means, summary of analysis of variance, and orthogonal contrasts for biomass nitrogen (N) for each site-year at Arthur H. Post Agronomy Research Farm (PF; Bozeman, MT) and Northern Agricultural Research Center (NARC; Havre, MT) lentil rhizobial inoculant and fertility study sites, 2019–2021.

		Biomass N (kg N ha^{-1})					
		PF			NARC		
Treatment no.	Treatment	2019	2020	2021	2019	2020	2021
1	Control	164	120b	77	47b	107	35b
2	Granular	162	115b	85	70a	110	48ab
3	Seed-coat	183	131b	81	81a	102	47ab
4	Granular + K	170	121b	85	76a	102	41ab
5	Seed-coat + K	179	118b	80	81a	112	47ab
6	Granular + K + S	185	161a	82	65ab	108	54a
7	Seed-coat + K + S	178	161a	76	83a	107	51a
8	Gran + K + S + Micros[a]	–	163a	81	–	95	41ab
		p-value					
		0.55	**<0.01**	0.55	**<0.01**	0.55	**0.07**
Contrast		*p*-value					
2, 3 vs. 1	Inoculant—Control	0.48	0.71	0.41	**<0.02**	0.88	**0.02**
2, 4, 6 vs. 3, 5, 7	Gran.—Seed-coat	0.36	0.43	0.26	**0.08**	0.91	0.84
4, 5 vs. 2, 3	K—No fertilizer	0.84	0.56	0.90	0.63	0.87	0.37
6, 7 vs. 4, 5	K+S—K only	0.49	**<0.01**	0.47	0.84	0.87	**0.05**
		Estimated difference (kg N ha^{-1})[b]					
2, 3 vs. 1	Inoculant—Control	–	–	–	28	–	13
2, 4, 6 vs. 3, 5, 7	Granular—Seed-coat	–	–	–	−9	–	–
6, 7 vs. 4, 5	K+S—K only	–	42	–	–	–	8

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$), and bold p values indicate $p < 0.10$.
[a]Micronutrient solution (Micro1000, Agroliquid).
[b]Only shown if significant at $p < 0.10$.

than seed-coat inoculant based on previous research in the NGP. Clayton et al. (2004) found that pea, grown in fields with and without a history of pea or lentil production, fixed 43 kg N ha^{-1} more with granular inoculant than with seed-coat inoculant. Granular inoculants are placed near the seed and inoculate the soil, and hence entire root systems, whereas seed-coat inoculants typically result in root nodules concentrated around the seed. In addition, total rhizobia numbers added with the granular inoculant were greater than for the seed-coat inoculant (Table 2); while inoculant rates we used were typical of producer practices, the rate differences could contribute to a portion of the N fixation difference. Common bean N fixed amounts increased when lateral roots, not just roots around the seed, were colonized by rhizobia (Wolyn et al., 1989). However, our results suggest that granular and seed-coat inoculants are equally effective for lentil N fixation, at least at these two sites in Montana.

3.4.2 | Fertilizer

Potassium fertilizer did not increase N fixed in any year at either site. Soils in all site-years had high baseline exchangeable K (>300 mg kg^{-1}). The established critical level for K in Montana is 250 mg kg^{-1} (Jacobsen et al., 2003), so it is not surprising that K fertilizer did not increase lentil N fixed amounts.

There was moderate evidence ($p = 0.07$) that S fertilizer increased N fixed (12 kg N ha^{-1}) at NARC in 2019 using the NA method, but no difference was detected using the ND method (Table 8). A strong S response was observed at PF in 2020, where S fertilizer increased N fixed by 38 and 32 kg N ha^{-1}, respectively, using the ND and NA methods (Table 8), equating to an increase of approximately 40%. For the same site-year, S fertilizer increased grain yield by 12% (Miller et al., 2022). Soil sulfate-S in the upper 15 cm was relatively low for this site-year (3.2 mg kg^{-1}), with lower amounts in the 15–60 cm zone (ca. 2.5 mg kg^{-1}). Moderate drought developed in July, which may have contributed to S deficiency by limiting root growth and decreasing S mineralization. Bestwick et al. (2018) found an interaction between S fertilizer and water stress, as pea protein only increased with S fertilization under moderate drought conditions. Lentil plots not fertilized with S became noticeably less green than fertilized plots by early July (Figure 1). The chlorosis was consistent in upper

TABLE 7 Means, summary of analysis of variance, and orthogonal contrasts for fraction of nitrogen (N) derived from atmosphere (fNdfa; from ^{15}N natural abundance) at Arthur H. Post Agronomy Research Farm (PF; Bozeman, MT) and Northern Agricultural Research Center (NARC; Havre, MT) lentil rhizobial inoculant and fertility study sites, 2019–2021.

		fNdfa					
		PF			NARC		
Treatment no.	Treatment	2019	2020	2021	2019	2020	2021
1	Control	0.66	0.70	0.70	0.25	–[a]	0.49bc
2	Granular	0.64	0.71	0.70	0.32	–	0.52abc
3	Seed-coat	0.67	0.65	0.70	0.41	–	0.54abc
4	Granular + K	0.68	0.71	0.70	0.31	–	0.57ab
5	Seed-coat + K	0.66	0.71	0.72	0.19	–	0.55abc
6	Granular + K + S	0.69	0.73	0.71	0.42	–	0.47c
7	Seed-coat + K + S	0.67	0.74	0.74	0.45	–	0.60a
8	Gran + K + S + Micros[b]	–	0.72	0.72	–	–	0.51bc
		p-value					
		0.77	0.19	0.79	0.21	–	**0.02**
Contrast		*p*-value					
2,3 vs. 1	Inoculant—Control	0.78	0.31	0.15	**0.10**	–	0.58
2,4,6 vs. 3,5,7	Gran.—Seed-coat	0.79	0.32	**0.03**	0.82	–	0.39
4,5 vs. 2,3	K—No fertilizer	0.63	0.19	0.27	0.23	–	0.71
6,7 vs. 4,5	K + S—K only	0.47	0.24	0.41	**0.05**	–	0.41
		Estimated difference[c]					
2,3 vs. 1	Inoculant—Control	–	–	–	0.12	–	–
2,4,6 vs. 3,5,7	Granular—Seed-coat	–	–	−0.05	–	–	–
6,7 vs. 4,5	K + S—K only	–	–	–	0.18	–	–

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$), and bold *p*-values indicate $p < 0.10$.
[a]2020 data not included due to variable soil nitrate-N.
[b]Micronutrient solution (Micro1000, Agroliquid)
[c]Only shown if significant at $p < 0.10$.

and lower leaves, making it difficult to distinguish between N and S deficiency. Whole plant tissue analysis revealed that S fertilizer increased S concentration by 64% and N concentration by 18% (Table 10), suggesting that plants were severely S deficient and the likely cause of much of the greenness difference. The S deficiency likely reduced plant productivity, indirectly impacting N fixation by reducing carbohydrate supply to root nodules. S fertilizer alleviated the S deficiency, which in turn increased plant growth, N fixed, and shoot N concentration. An indirect effect of S fertilizer through more carbohydrates in root nodules on N fixation was previously observed in field experiments using pea (Scherer et al., 2006).

At PF, the 2019 and 2021 fields had similarly low sulfate-S levels as the 2020 field, but no effect of S fertilizer on N fixed was observed. At PF in 2019, soil sulfate-S was 4.4 mg kg^{-1}, but the 2019 growing season had higher precipitation than 2020, especially in July (68 and 17 mm for 2019 and 2020, respectively). Increased soil moisture may have increased sulfate-S mineralization from soil organic matter in 2019, preventing S deficiency. Soils with low soil organic matter (e.g., <2% in upper 15 cm) are more prone to S deficiency (Kaiser & Kim, 2013), highlighting the importance of S inputs from organic matter mineralization. Additionally, soils in Montana can contain gypsum in subsoil. In 2019, higher precipitation may have allowed lentil roots to grow deeper and access sulfate in subsoil from dissolved gypsum below the depth that was soil-tested (60 cm). In 2021, severe drought at both sites prevented S from becoming a limiting growth factor, apparently negating any benefits of S fertilizer to N fixation.

Micronutrient foliar application did not impact N fixed in any site-year. Several micronutrients (B, Mo, Zn, and Fe) impact nodule number and N uptake in legume crops (Alam et al., 2015; Hossain et al., 2020; Nasar et al., 2019), but no studies were located that evaluated effects of micronutrients on N fixed amounts. Micronutrients were soil-applied in Alam et al. (2015) and Hossain et al. (2020), and foliar-applied in Nasar et al. (2019), as in our study. Because these micronutrients are generally immobile in plants, soil

TABLE 8 Means, summary of analysis of variance, and orthogonal contrasts for nitrogen (N) fixed using N difference (ND) and ^{15}N natural abundance (NA) methods for each site-year at Arthur H. Post Agronomy Research Farm (Bozeman, MT) lentil rhizobial inoculant and fertility study site, 2019–2021.

		N fixed (kg N ha^{-1})					
		2019		2020		2021	
Treatment no.	**Treatment**	**ND**	**NA**	**ND**	**NA**	**ND**	**NA**
1	Control	136	109	87c	85b	81	54
2	Granular	134	104	86c	82b	67	59
3	Seed-coat	160	124	104b	85b	78	57
4	Granular + K	151	114	91bc	86b	60	59
5	Seed-coat + K	145	117	86c	84b	74	57
6	Granular + K + S	157	129	125a	116a	69	58
7	Seed-coat + K + S	151	119	128a	118a	43	55
8	Gran + K + S + Micros[a]	–	–	130a	116a	66	58
		p-value					
		0.57	0.26	**<0.01**	**<0.01**	0.25	0.96
Contrast		*p*-value					
2, 3 vs. 1	Inoculant—Control	0.42	0.60	0.35	0.81	0.25	0.30
2, 4, 6 vs. 3, 5, 7	Granular—Seed-coat	0.60	0.44	0.31	0.82	0.97	0.43
4, 5 vs. 2, 3	K—No fertilizer	0.95	0.80	0.35	0.75	0.62	0.99
6, 7 vs. 4, 5	K + S—K only	0.60	0.26	**<0.01**	**<0.01**	0.24	0.66
		Estimated difference (kg N ha^{-1})[b]					
6, 7 vs. 4, 5	K + S—K only	–	–	38	32	–	–

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$), and bold *p*-values indicate $p < 0.10$.

[a]Micronutrient solution (Micro1000, Agroliquid).

[b]Only shown if significant at $p < 0.10$.

FIGURE 1 Lentil at Arthur H. Post Agronomy Research Farm in 2020 was noticeably greener with sulfur (S) (right) than without S (left).

TABLE 9 Means, summary of analysis of variance, and orthogonal contrasts for nitrogen (N) fixed using N difference (ND) and ^{15}N natural abundance (NA) methods for each site-year at the Northern Agricultural Research Center (Havre, MT) lentil rhizobial inoculant and fertility study site, 2019 and 2021.

		N fixed (kg N ha^{-1})			
		2019		2021	
Treatment no.	Treatment	ND	NA	ND	NA
1	Control	28b	12b	22	16b
2	Granular	63a	25ab	34	25ab
3	Seed-coat	66a	38a	35	26ab
4	Granular + K	61a	24ab	26	23ab
5	Seed-coat + K	41a	14ab	37	25ab
6	Granular + K + S	58a	28ab	42	24ab
7	Seed-coat + K + S	64a	35a	36	30a
8	Gran + K + S + Micros[b]	–	–	27	21ab
		p-value			
		<0.01	**0.06**	0.14	**0.06**
Contrast		*p*-value			
2, 3 vs. 1	Inoculant—Control	**<0.01**	**0.01**	**0.05**	**0.01**
2, 4, 6 vs. 3, 5, 7	Granular—Seed-coat	0.71	0.25	0.66	0.12
4, 5 vs. 2, 3	K—No fertilizer	0.12	0.15	0.52	0.67
6, 7 vs. 4, 5	K + S—K only	0.29	**0.07**	0.16	0.24
		Estimated difference (kg N ha^{-1})[b]			
2, 3 vs. 1	Inoculant—Control	37	20	13	9
6, 7 vs. 4, 5	K + S—K only	–	12	–	–

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$) and bold *p*-values indicate $p < 0.10$.
[a]Micronutrient solution (Micro1000, Agroliquid)
[b]Only shown if significant at $p < 0.10$.

TABLE 10 Means, summary of analysis of variance, and orthogonal contrasts of aboveground N and S concentrations for +K and +K+S treatments at Arthur H. Post Agronomy Research Farm lentil rhizobial inoculation and fertility study site, sampled 30 Jul 2020.

Treatment no.	Treatment	Shoot N (g kg^{-1})	Shoot S (g kg^{-1})
1	Granular + K	24.8b	0.58b
2	Seed-coat + K	25.5b	0.61b
3	Granular + K + S	29.6a	0.94a
4	Seed-coat + K + S	29.7a	1.01a
		p-value	
		0.01	**<0.01**
Contrast		*p*-value	
3, 4 vs. 1, 2	K + S—K only	**<0.01**	**<0.01**
		Estimated difference (g kg^{-1})[a]	
3, 4 vs. 1, 2	K + S—K only	4.5	0.38

Note: Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$) and bold *p*-values indicate $p < 0.10$.
[a]Only shown if significant at $p < 0.10$.

applications may be more beneficial to root nodules and directly influence N fixation more than foliar applications. Applying foliar micronutrients at label rate may not provide enough nutrients to impact lentil growth. For example, in this study, Zn was applied at a rate of 0.028 kg Zn ha^{-1}, and likely far less was absorbed by plant leaves. In 2020 at PF, the average shoot Zn concentration was 0.015 g kg^{-1}. Multiplied by average aboveground biomass, this results in lentil Zn uptake of 0.075 kg ha^{-1} (not including Zn in roots), far more than the applied amount that likely was taken up considering that most of the product likely hit the soil rather than plant leaves or dripped off leaves and strongly adsorbed to the soil surface.

3.4.3 | Nitrogen fixed amounts and biomass N

The 3 years with biomass N differences also had differences in N fixed. In those years, treatments with higher biomass N generally aligned with treatments with higher N fixed amounts, which is unsurprising given that legume biomass N is a main component in the equations for NA and ND. This agrees with McCauley et al. (2012). They noted strong positive relationships between pea and lentil biomass N accumulation and N fixed amounts over a 2-year study. Given the intensive time and expense for measurements required by the ND and NA methods, using biomass N as a surrogate for N fixation may be possible. This is especially important when resources are limited and there is prior knowledge that fixed N is heavily relied upon by legumes (e.g., fNdfa values >0.5).

3.5 | N yield

Nitrogen yield, the product of seed yield and grain N concentration, varied in five of six site-years using orthogonal contrasts (Table 11). Inoculation increased N yield in three site-years (PF in 2019, and NARC in 2019 and 2021) by 9, 18, and 11 kg ha^{-1}, respectively. The effect of inoculant type was inconsistent among site-years. Granular inoculant resulted in greater N yield in three site-years. Seed-coat inoculant resulted in lentil with higher N yield than granular inoculant in one site-year. At NARC in 2019, N yield was 5 kg ha^{-1} greater using seed-coat inoculant than granular inoculant. At PF in 2020 and 2021, S fertilizer increased N yield by 12 and 2 kg ha^{-1}, respectively.

In our study, the lack of N yield response to K fertilizer is again unsurprising given the high exchangeable K each site-year. N yield response to S was likely soil and weather dependent. Based on a producer survey, Bestwick et al. (2018) found that S fertilizer interacted with water stress, as fertilization only increased pea protein concentration under moderate drought. Weather variation could explain some of the inconsistency of S responses observed in our study. We found an N yield response to S at PF in 2020, a moderate drought year, but not in 2019 when precipitation was ideal, even though both fields had similar soil sulfate-S levels. Given that S mineralization can substantially increase S availability (Kaiser & Kim, 2013), a moderate drought would impair

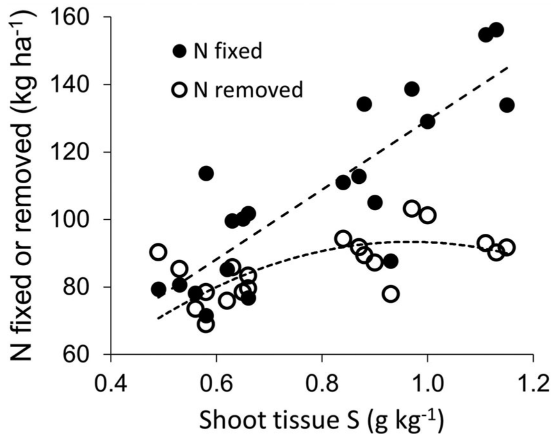

FIGURE 2 Relationship between shoot sulfur (S) concentration and nitrogen (N) fixed (ND method) and N removed in grain at Post Farm lentil rhizobial inoculation and fertility study site in 2020. The difference between the two curves can be viewed as the net benefit to the soil from N fixation. ND, nitrogen difference

mineralization more than in an ideal growing season. Miller et al. (2022) reported inconsistent responses across seven sites in Montana and North Dakota in seed yield and protein that were not predicted by soil sulfate-S tests, including the two sites reported here. This highlights the need for better methods of assessing S fertility requirements for regional producers.

At PF, the average amount of N fixed was greater than the average N yield harvested each year indicating a net “N benefit” from N fixation (Gollner et al., 2019). Using the ND method, the amount of N fixed was on average 33 kg N ha^{-1} greater than N yield, while the average difference between N fixed using the NA method and N yield was 16 kg N ha^{-1}. At NARC, differences between N fixed amounts and N yield were smaller, and sometimes negative, indicating that N fixation from lentil did not result in a net N benefit to the system.

At PF in 2020, N yield, or the amount of N removed from the field during seed harvest, plateaued around aboveground tissue S concentrations of 0.90 g kg^{-1} (Figure 2). Conversely, N fixed maintained a linear positive relationship up to the highest S concentration near 1.15 g kg^{-1}. Our results suggest that while N removed and yield leveled off with increasing tissue S concentrations, and hence greater S availability, the N benefit continued to increase above that point. Tissue S concentrations were not related to fNdfa values ($p = 0.80$). This suggests S deficiency impacted crop growth and biomass N accumulation instead of N fixation directly. Notably, at a tissue S content below 0.60 g kg^{-1}, the N benefit was less than 10 kg N ha^{-1}, whereas at the greatest S content near 1.15 g kg^{-1}, the N benefit approached 60 kg N ha^{-1}, an important difference. Therefore, producers might choose to fertilize with S even when a yield response

TABLE 11 Means, summary of analysis of variance, and orthogonal contrasts for nitrogen (N) yield for each site-year at Arthur H. Post Agronomy Research Farm (i.e., "Post Farm") and Northern Agricultural Research Center lentil rhizobial inoculant and fertility study sites, 2019–2021.

		N yield (kg N ha^{-1})					
		Post Farm			Northern Agricultural Research Center		
Treatment no.	Treatment	2019	2020	2021	2019	2020	2021
1	Control	102	80b	33	25c	80	19c
2	Granular	113	83b	33	41ab	84	25abc
3	Seed-coat	108	79b	29	45a	81	33a
4	Granular + K	108	80b	29	43ab	80	25abc
5	Seed-coat + K	105	80b	30	43ab	81	32ab
6	Granular + K + S	106	93a	32	39b	81	29ab
7	Seed-coat + K + S	99	91a	32	47a	82	30ab
8	Granular + K + S + Micros[a]	–	94a	31	–	80	24bc
		p-value					
		0.12	**<0.01**	0.16	**<0.01**	0.81	**<0.01**
Contrast		*p*-value					
2, 3 vs. 1	Inoculant—Control	**0.03**	0.58	0.29	**<0.01**	0.27	**<0.01**
2,4,6 vs. 3,5,7	Granular—Seed-coat	**0.07**	**0.08**	0.23	**<0.01**	0.81	**0.02**
4, 5 vs. 2, 3	K—No fertilizer	0.20	0.37	0.23	0.91	0.42	0.95
6, 7 vs. 4, 5	K+S—K only	0.24	**<0.01**	**0.08**	0.73	0.78	0.81
		Estimated difference (kg N ha^{-1})[b]					
2, 3 vs. 1	Inoculant—Control	9	–	–	18	–	11
2,4,6 vs. 3,5,7	Granular—Seed-coat	5	2	–	−5	–	−5
6, 7 vs. 4, 5	K+S—K only	–	12	2	–	–	–

Note. Columns with the same letter are not significantly different (Tukey–Kramer HSD, $p < 0.1$) and bold *p*-values indicate $p < 0.10$.
[a]Micronutrient solution (Micro1000, Agroliquid).
[b]Only shown if significant at $p < 0.10$.

is not expected, as the boost in N fixation can increase soil N inputs from residue and potentially decrease future fertilizer N needs of subsequent crops. Applying S fertilizer to lentil to increase the N benefit, thereby lowering the fertilizer N needed for the subsequent crop, could help alleviate soil acidification, nitrate-N leaching, and nitrous oxide emissions. Aboveground tissue S concentrations were only measured at one site-year and to our knowledge no similar research comparing S concentrations with amounts of N fixed and removed has been conducted. This finding could be especially important for those relying upon legume cover crops for their N benefit.

3.6 | Nitrogen fixation and seed yield, protein, and protein yield relationships

Relationships between N fixation parameters (N fixed using both methods, biomass N, and fNdfa from NA) and lentil seed measurements (yield, protein concentration, and protein yield) were inconsistent (Table 12). In our study, greater N fixed parameters often increased and sometimes decreased with seed yield and protein, with correlation coefficients ranging from −0.39 to 0.80. Greater N fixation may have positive relationships with seed measurements by supplying sufficient N to optimize plant growth. Conversely, relationships could be expected to be negative as N fixation can consume up to 28% of total photosynthate (Vance, 2008), leaving less carbon for yield formation when N fixation demands are high, yet N is not greatly limiting. Of the 63 correlations, 31 were different from 0 ($p < 0.1$). Correlation analysis was likely affected by sampling area, as seed yield and protein were measured at the plot scale (~12 m^2) and N fixation parameters were obtained from biomass subsamples (~0.6 m^2) based on a subjective representative area. Site-years with large differences in N fixed (PF in 2020 and NARC in 2019 and 2021) resulted in the strongest positive correlations with seed measurements. Environmental factors such as precipitation and soil nitrate-N likely influenced the relationships across site-years. The wettest year, 2019, resulted in several negative correlations. Lentil reproductive growth is triggered by environmental stress, so lentil may have fixed more N during prolonged vegetative growth while yield did not increase proportionally due to delayed reproductive growth.

TABLE 12 Pearson correlation coefficients (*r* values) between nitrogen (N) fixation parameters and seed yield and/or protein at Arthur H. Post Agronomy Research Farm (PF) and Northern Agricultural Research Center (NARC) lentil rhizobial inoculant and fertility study sites, 2019–2021.

Year	Site	Parameter	Yield	Protein	Protein Yield
2019	PF	ND	−0.14	0.02	0.17
		NA	−0.31	0.05	**−0.30**
		Biomass N	**−0.31**	−0.05	**−0.34**
		fNdfa	−0.07	0.03	0.01
	NARC	ND	**0.67**	**0.57**	**0.67**
		NA	0.37	**0.59**	**0.46**
		Biomass N	**0.67**	**0.75**	**0.77**
		fNdfa	0.17	**0.43**	0.25
2020	PF	ND	**0.71**	0.11	**0.65**
		NA	**0.78**	0.22	**0.76**
		Biomass N	**0.80**	0.16	**0.75**
		fNdfa	0.09	0.20	0.15
	NARC	Biomass N	0.17	0.06	0.17
2021	PF	ND	−0.07	0.05	<0.01
		NA	0.04	-0.23	<0.01
		Biomass N	0.21	-0.23	0.18
		fNdfa	**−0.35**	<0.01	**−0.35**
	NARC	ND	**0.64**	**0.62**	**0.69**
		NA	**0.58**	**0.61**	**0.63**
		Biomass N	**0.73**	**0.66**	**0.78**
		fNdfa	**−0.39**	−0.09	**−0.39**

Note: Bold values indicate correlation coefficient different from 0 with 90% confidence.

Abbreviations: NA, ^{15}N natural abundance; ND, nitrogen difference; fNdfa, fraction of nitrogen derived from atmosphere.

4 | CONCLUSION

In this 3-year study, inoculation and S fertilizer each increased N fixed by lentil in 40% of site-years. In both site-years at NARC with suitable data, inoculated lentil fixed more N than non-inoculated lentil. For all five site-years, there were no differences in N fixed amounts between granular and seed-coat inoculants. Both K and foliar micronutrient fertilizer applications had no impact on N fixed, while S increased N fixed by 32–38 kg N ha^{-1} at PF in 2020 when soil S was low and there was moderate drought. Amounts of N fixed were associated with greater biomass N accumulation.

This study was the first field evaluation of lentil N fixation response to S fertilizer and inoculant formulations in Montana and the greater NGP region. Given that inoculant and S responses were observed in 40% of site-years, these results extrapolated to lentil fields across the region could increase N fixation for many producers. Responses to inoculation and S fertilizer were sometimes relatively large, up to 37 kg N ha^{-1}, which could have substantial benefits to lentil productivity, agroecosystem N availability, and environmental benefits to soil and water quality. Benefits of inoculant and S fertilizer may be larger in fields where effective rhizobia populations are less likely to exist and S has not been added as standard practice. Responses to S also are more likely on coarse or shallow soils with higher precipitation since sulfate can leach. Lentil has been widely adopted by regional producers, so research focused on fertility management is highly valuable. Our results can help producers as they make management decisions to increase the economic and environmental sustainability of their operations.

AUTHOR CONTRIBUTIONS

Kaleb Barber: Data curation; formal analysis; investigation; methodology; writing—original draft. **Clain Jones**: Conceptualization; funding acquisition; investigation; writing—review and editing. **Perry Miller**: Conceptualization; funding acquisition; writing—review and editing. **Peggy Lamb**: Investigation; methodology; writing—review and editing. **Sydney Atencio**: Data curation; formal analysis; investigation; methodology.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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

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