PULSE CROP MANAGEMENT TO ENHANCE BIOLOGICAL NITROGEN FIXATION IN THE NORTHERN GREAT PLAINS

by

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ABSTRACT

Pulse crop production has increased dramatically in Montana and the surrounding northern Great Plains over the past few decades. Through N fixation, pulse crops, including pea and lentil, can reduce N fertilizer requirements, both by replacing non N-fixing crops and positively contributing to soil N pools for subsequent crop uptake. Three studies were conducted in Montana over three years to investigate pulse crop management practices that enhance N fixation of pea and lentil.

The first study investigated lentil N fixation response to fertilizers and inoculant types. Inoculant and S fertilizer each increased N fixed by lentil in 40% of site-years, but response was not well correlated with cropping history or soil sulfate-S levels. In one site-year, N fixation appeared to continue increasing at the highest tissue S concentration while seed yield plateaued below that highest level, indicating that S fertilizer could increase the soil N benefit of lentil even if a yield response is not expected. Potassium fertilizer nor inoculant type influenced N fixed. The second study assessed differences in N fixation among lentil and pea varieties. Amounts of N fixed varied in 75% and 50% of site-years for lentil and pea, respectively. Differences among varieties were sometimes large, up to 45 kg N ha^{-1}. Two lentil varieties, CDC Richlea and Riveland, were frequently among the top N-fixers, while no pea varieties consistently fixed more N than others. Correlations between N fixed and seed yield ranged from weak to moderate, and pea more frequently had positive relationships than lentil. The third study evaluated N fixation response of two pea varieties to inoculant formulations. Inoculant formulation did not impact N fixed by either variety, and the uninoculated treatment performed as well as the inoculated treatments. This suggests producers may not need to inoculate pulse crops to achieve sufficient N fixation when effective rhizobia populations exist in their fields.

These results contribute to the researchers’ understanding of N fixation by pulse crops in the region. Together, these studies can help producers in the northern Great Plains better manage pea and lentil, improving both economic and environmental sustainability of the region’s cropping systems.

CHAPTER ONE

REVIEW OF LITERATURE

Background

Nitrogen (N) is widely accepted as the most limiting nutrient to plant growth. Consequently, humans globally apply over 108 teragrams (Tg) of N fertilizer to crops each year at a cost of over $28 billion USD (FAO, 2018). In addition to the significant economic costs, reliance upon synthetic N fertilizers is plagued by inefficiencies and environmental concerns. It is estimated that only 50% of inorganic N applied to agricultural lands is taken up by crops (Fowler et al., 2013). Denitrification (Galloway et al., 2003), volatilization (Pan et al., 2016), and leaching (Galloway et al., 2003) are mechanisms for N to be potentially lost from agroecosystems. Leached N can cause elevated nitrate levels in groundwater (Sigler et al., 2020), both a concern for aquatic environments and human health. Fertilizer N can denitrify to nitrous oxide, a powerful greenhouse gas (Grant et al., 2006). Repeated application of inorganic N fertilizer can cause soil acidification, which can lead to crop failure from aluminum or manganese toxicity (Brown et al., 2008; Engel, 2020). Additionally, high inorganic N inputs disrupt natural soil microbial functions such as N mineralization (Huang et al., 2019). Through biological N fixation, pulse crops incorporated into cereal production systems can decrease the need for N inputs while serving as a profitable commodity for producers.

Pulse Crops in the Northern Great Plains

Historically, agroecosystems of the United States northern Great Plains (NGP) region have been dominated by cereal crop monoculture. These systems are economically and environmentally unsustainable due to low crop diversity and prevalence of summer fallow. Wheat-only production systems often present Montana farmers significant economic uncertainties (Miller et al., 2015). Summer fallow has several negative impacts on agricultural productivity, namely declines in soil organic matter (Romero et al., 2019), inefficient use of moisture, and high susceptibility to soil erosion (Carlyle, 1997). Pulse crops, legumes harvested for their dry seed, have increased in popularity in the NGP where they often replace summer fallow. The area of pulse crops planted (lentil, pea, and chickpea) in Montana increased by more than 17-fold from 2000 to 2021 (NASS, 2022). Montana producers planted at least 400,000 ha of pulse crops each of the past five years (NASS, 2022). Pea in rotation with wheat can stabilize wheat yield and protein, and the inclusion of pea often produces greater net returns than wheat-only systems (Miller et al., 2015).

Pulse crops have been adopted quickly because they fit well agronomically into the existing cereal production systems of the NGP. Several studies have found that pulse-cereal rotations yield greater than monocrop cereal rotations (Beckie and Brandt, 1997; Bremer, 2011; Miller et al., 2003a). Additionally, wheat grown after a pulse crop has greater grain protein than wheat grown after wheat or summer fallow (Bremer et al., 2011). Researchers have attributed these increases in grain yield and quality following pulses to a few different mechanisms. Pulse crops have a shallow rooting depth, which leaves more soil moisture behind for subsequent crops than cereals (Miller et al., 2003b). Stevenson and van Kessel (1996) determined that a reduction in disease severity and weed pressure accounted for yield gains in a pea-wheat rotation. Beckie and Brandt (1997) concluded that the increase in wheat yield when grown after a pulse was primarily an effect of residual nitrate-N contributed by pulse residue. Wheat grown in soil containing pulse root residue yielded more than wheat grown in soil with wheat root residue, suggesting that pulse root N inputs, while difficult to quantify, may play a significant role in soil N dynamics of pulse-cereal rotations (Arcand, 2014).

Aboveground pulse crop residue is more readily mineralized than cereal and oilseed crop residues because of its lower carbon (C) to N ratio, resulting in more timely nutrient mineralization for subsequent crop uptake (Lupwayi et al., 2004). However, it is recognized that soil N contributions from pulse crop residues are highly variable year-to-year and difficult to predict with much certainty on a short-term basis (Walley et al., 2007). Grain harvest removes a high proportion of the N fixed by legumes from the field, so much so that in some cases legumes appear to remove N from the cropping system when root N contributions are not considered (Peoples et al., 2009). In Saskatchewan, 60 to 77% of aboveground plant N was removed as grain for pea and lentil at harvest (Hossain et al., 2014). This means a small fraction of total fixed N returns to the cropping system in crop residue, and only a portion of that residue N will mineralize and be available for the subsequent crop. Consequently, the N credit, the amount that N fertilizer is reduced from typical guideline based on soil nitrate, from growing pulses is relatively low; current Montana fertilizer guidelines recommend N fertilizer be reduced by 0 to 22 kg N ha^{-1} when the previous crop was a legume harvested for grain (Dinkins and Jones, 2019).

Biological Nitrogen Fixation

Like all legumes, pulse crops utilize atmospheric N (N_2) via symbiotic relationships with rhizobia and bradyrhizobia bacteria in the soil (Oldroyd et al., 2011). It is estimated that biological N fixation by legumes and other N-fixing plants contributes 110 Tg of plant-available N to terrestrial systems each year, on par with N fertilization (Gruber and Galloway, 2008). The bacteria-legume relationship is characterized by the formation of root nodules, a process summarized in Oldroyd et al. (2011). Briefly, the first steps of nodulation are a series of chemical signals between bacteria and legume roots that encourage bacteria to move to the roots via flagella. A specific chemical from bacteria, the *Nod* factor, induces the plant to grow an infection thread that runs from the root hair to the root cortex. Here, cortex cells undergo cell division while bacteria travel through the infection thread, eventually colonizing the newly-divided cortex cells. This swollen area of the root containing bacteria is known as a root nodule which is where N fixation occurs.

The process of converting N_2 to plant-available ammonium (NH_4^+) is highly energy- and resource-intensive (Ferguson et al., 2018). For each molecule of N_2 converted to two molecules of NH_4^+, 16 molecules of ATP are required (Vance, 2008). Because this energy is provided to bacteria from the legume as a condition of symbiosis, root nodules can consume 13 to 28% of the total photosynthate produced by the plant (Vance, 2008). Accordingly, it would be expected that legumes fertilized with N would yield more than legumes that rely on N fixation. However, in a comparison of inoculated and N-fertilized pea in Montana, seed yield was not different (McConnell et al., 2002). This could indicate that in the semi-arid NGP, a limiting factor in addition to N such as water limits yield, and the C cost of N fixation does not negatively impact pulse productivity.

Given the substantial energetic cost of N fixation, legumes preferentially use soil N. As soil nitrate concentrations increase, the fraction of plant N derived from the atmosphere (fNdfa) decreases (Voisin et al., 2002). Legume roots can sense nitrate in soil and inhibit nodule formation through signaling pathways in high nitrate soil environments (Murray et al., 2017). When soil nitrate was extremely high (>350 kg N ha^{-1}) at seed harvest, cumulative fNdfa was zero in field-grown pea in France (Voisin et al., 2002). In the same study, N fixation was zero during vegetative growth stages when soil nitrate exceeded around 50 kg N ha^{-1}. Because of soil nitrate's dynamic nature, N fixation rates likely change over a legume's growth cycle as plant available soil N pools fluctuate.

Biological N fixation is also impacted by other environmental conditions. In the eastern Canadian prairies, indigenous rhizobia appeared to nodulate pea roots more effectively when soil pH increased from 6 to 8, suggesting rhizobia may prefer high pH soils (Chemining'wa and Vessey, 2006). In a greenhouse study using Idaho field soils, lentil nodulation was most robust above pH 5.5 and was completely inhibited at pH below 4 (Mohebbi and Mahler, 1989). Based on these findings, the soil acidification currently being experienced in parts of Montana where pulse crops are grown may negatively impact N fixation in addition to plant growth, whereas management practices that increase N fixation could help reduce N fertilization and hence acidification.

Soil Fertility and Legumes

Legumes tend to be more sensitive to nutrient deficiencies than other crops because of high nutrient demands of N fixation. Nutrient deficiencies can directly impact N fixation by limiting nodule numbers, mass, or activity. Alternatively, N fixation can be influenced indirectly by affecting growth of the host legume and hence photosynthate available to be mobilized to nodules (Divito and Sadras, 2014). Despite the documented importance of nutrients to N fixation, only 14% of conventional NGP (majority from Montana) and Pacific Northwest lentil producers report applying inorganic fertilizers (Warne et al., 2019). Pulse crops responded to P fertilizer in Alberta when soil P levels were low (McKenzie et al., 2001a), and P deficiency is well-documented in adversely impacting N fixation of grain and pasture legumes (Divito and Sadras, 2014). There have been few field experiments evaluating pulse crop response to other essential plant nutrients in the greater NGP region, and none evaluating the response of N fixation and yield to sulfur (S) or potassium (K).

Sulfur

Sulfur, an essential nutrient for plant growth and development, is taken up from the soil in the form of sulfate (SO_4^{2-}). The element is physiologically coupled to N in several ways. Sulfur, along with N, is a crucial component of certain amino acids used for protein synthesis (Havlin et al., 2013). Legumes require more S than non-leguminous crops because of greater concentrations of proteins and amino acids, specifically the S-containing methionine and cysteine (Gayler and Sykes, 1985). Sulfur plays an important role in ferredoxin, a protein involved in the reduction pathway during photosynthesis, N fixation, and N assimilation in the roots (Havlin et al., 2013). Additionally, S is a main component of the nitrogenase enzyme that gives rhizobia bacteria the ability to fix N (Howard and Rees, 2006). Research regarding critical soil sulfate-S levels has been historically overlooked because S rarely limited crop growth (Scherer, 2009). However, due to higher yielding crop varieties, high grade fertilizers that are now lower in S, and reduced atmospheric deposition of S from industrial processes, S deficiency is a growing problem faced by farmers (Scherer, 2009).

Soil test sulfate-S and S fertilization have been correlated to legume responses with mixed results. In the northern Corn Belt of the United States, S fertilizer only increased soybean yield at one of four sites (Kaiser and Kim, 2013). The site that had a yield response to S was the lowest in soil organic matter (< 20 g kg^{-1}). This aligns with previous research that concluded low organic matter soils are prone to S deficiency (Freney and Stevenson, 1966). Kaiser and Kim (2013) reported grain protein was increased by S fertilizer at two sites, suggesting that S may boost legume grain quality without increasing yield. Sulfur increased pea protein under moderate drought stress in Montana, but not under normal or severe stress (Bestwick et al., 2018). Soil sulfate-S tests may be unreliable because extractants used by soil testing laboratories can underestimate the S available to the plant over the growing season (Nanthana et al., 1997). This could be due to S being released from mineralized organic matter over the growing season or dissolved gypsum in subsoils not detected by shallow soil samples, both of which increase with higher moisture.

Several experiments suggest that S deficiency limits N fixation in pulses, but this has rarely been evaluated in field experiments and has not been investigated in the NGP. In field pea grown in greenhouses, plants supplied with S fixed more N than plants supplied with no S (Scherer et al., 2006; Zhao et al., 1999). Zhao et al. (1999) monitored N fixed over pea’s growth cycle. Amounts of N fixed doubled with S additions compared to without S at each growth stage, while differences in shoot biomass were not observed until the flowering stage. Based on these findings and fewer nodules observed on S-deficient roots, the authors concluded that S deficiency directly inhibited nodulation and N fixation. In contrast, Scherer et al. (2006) determined that S deficiency had an indirect effect on N fixation. They observed lower carbohydrate levels in roots of S-deficient plants. Because nodules require large amounts of carbohydrates for N fixation, the authors concluded that S deficiency first impacted pea photosynthesis which in turn decreased carbohydrate supply to nodules. In field grown faba bean in a Mediterranean climate, the highest fertilizer rate of 11 kg S ha^{-1} increased N fixed by 50 kg N ha^{-1} over the 0-S control (Cazzato et al., 2012).

Potassium

Potassium is a mobile nutrient in plants taken up from soil in the form of K^{+}. Physiologically, K assists with the processes of photosynthesis, enzyme activation, photosynthate translocation, and water status maintenance (Havlin et al., 2013). Decreased functioning of any of these processes due to K deficiency could negatively impact a legume's ability to fix N because of N fixation's high demand for carbohydrates. The grain K concentration of pulses is double that of cereals (5.6 and 2.8 g K kg^{-1}-grain, respectively; Jacobsen et al., 2003), resulting in similar K removal rates between pulses and cereals even though cereals typically yield much greater. While the soils of the NGP often test high in exchangeable K, low temperature and moisture could cause a disparity between soil test K and the actual amount of K available to the plant during the growing season (Skogley and Haby, 1981). This is highlighted by a wheat yield response to K fertilization even in soils with high soil test K (Skogley and Haby, 1981). There is little research on pulse crop response to K fertilizer. Pea seed protein did not respond to K fertilizer in the NGP (Bestwick et al., 2018).

Response of measures related to N fixation to K fertilizer is most studied in soybean and alfalfa. Nodules increased in mass and number in response to K fertilizer in greenhouse experiments (Premartne and Oertli, 1994; Abdelhamid et al., 2011). The same response was observed in field-grown soybean when soil exchangeable K was 67 mg kg^{-1} (Abbasi et al., 2012). Additionally, aboveground biomass N concentrations and biomass N increased with K fertilizer in the greenhouse and the field (Abdelhamid et al., 2011; Abbasi et al., 2012). Rates of N fixation in alfalfa increased by 2.8-fold in sandy soil and 1.7-fold in silt loam soil with the addition of 224 kg K ha^{-1}, and this was accompanied by increases in aboveground biomass and nodule number (Collins et al., 1986). Potassium inputs had no effect on alfalfa N fixation in a calcareous silt loam with high exchangeable K (>250 mg kg^{-1}) (Rehm and Sorensen, 1974). These findings again highlight the influence of available soil K on fertilizer K response of legumes.

Micronutrients

Micronutrients are needed in smaller quantities than macronutrients and are less likely to be limiting factors to plant growth. However, micronutrients are still essential nutrients for plant growth and development, and they are important to consider when making fertility management decisions, especially in soils known to have low fertility. Field experiments have shown that pulse crops respond to micronutrient fertilization. Islam et al. (2018) evaluated effects of B, Mo, and Zn on field-grown lentil in Bangladesh. Separately and combined, micronutrients increased several yield components: plant height, branches per plant, pods per plant, seeds per pod, and thousand seed weight (Hossain et al., 2020). Maximum lentil grain yield (1807 kg ha^{-1}) and grain protein concentration (266 g kg^{-1}) were achieved when B, Mo, and Zn were applied together (Hossain et al., 2020). Pea response to foliar micronutrient applications of B, Cu, Fe, Mn, Mo, and Zn in Montana was investigated by Mohammed and Chen (2018). All micronutrients tested increased pea grain yield at a very high-yielding site in both study years, but only B and Mo increased yield in one of two years at the low-yielding site (Mohammed and Chen, 2018). Notably, yield responses to Fe and Mn were observed at the high-yielding site even when soil test levels were above critical levels (Mohammed and Chen, 2018), indicating micronutrient soil tests may not accurately predict fertilizer response.

Little research exists for micronutrient fertilizer impacts on N fixation, especially for pulse crops in the NGP. Metal micronutrients play essential roles in N fixation including chemical signaling, enzyme activation, and metalloprotein composition (González-Guerrero et al., 2014). Nodule number was maximized when B, Mo, and Zn were applied together to lentil (Hossain et al., 2020). Molybdenum is a cofactor to nitrogenase, and fertilization of a low-Mo soil increased nodulation, N uptake, and nitrogenase activity in hairy vetch (Alam et al., 2015). Another micronutrient, Fe, had a positive effect on lentil seed yield, protein concentration, and N uptake (Nasar et al., 2019). In addition to plant Fe demands, this may be due in part to Fe's structural roles in ferredoxin, leghemoglobin, and nitrogenase (Howard and Rees, 2006).

Rhizobial Inoculation

The rhizobia bacteria associated with leguminous N fixation often need to be introduced to agroecosystems in the form of inoculant. In a meta-analysis of grain legumes by Kaschuk et al. (2009), average grain yield increased 16% and average grain protein increased 7% when seeds were inoculated at planting. Seed yield for inoculated pea was on average 966 kg ha^{-1} greater than non-inoculated pea grown in fields known to have no history of pulse crops in western Canada (Clayton et al., 2004a). In fields in Montana with no pulse history and no native rhizobia, inoculated pea yielded on average 870 kg ha^{-1} greater than non-inoculated pea (McConnell et al., 2002). While pulse crop area planted has increased dramatically in Montana, there are still many fields that have never been planted with a pulse crop. In Alberta from 1995 to 1998, inoculation increased pea yield in 41% of field trials, with an average yield increase of 14% (McKenzie et al., 2001b). When fields had no history of legumes, inoculation had no effect in 55% of the trials (McKenzie et al., 2001b). This was likely due to native rhizobia populations, as Kucey and Hynes (1989) found 1 to 100 rhizobia per gram of soil in Alberta fields never planted with legumes. A native strain of rhizobia outperformed commercial strains for lentil in Pakistan in nodule number, nodule dry weight, lentil biomass, grain yield, total N, and N fixed (Hafeez et al., 2000). This suggests commercial inoculants may not be necessary if healthy native rhizobia populations exist in soil.

Fields planted with inoculated legumes maintained high rhizobia populations for years after harvest (Kucey and Hynes, 1989). Soil rhizobia populations were found not to vary between crop rotations of pea-pea, wheat-pea, and wheat-wheat-pea when inoculant was applied to each pea crop (Lupwayi et al., 2012). Lentil yields did not significantly differ between inoculated and non-inoculated plots in a Montana field with no history of pulse crops (Huang et al., 2016). This could be because water was yield-limiting (evidenced by low yield across treatments), making the existing soil N sufficient for crop growth and rendering N fixation unnecessary (Huang et al., 2016). Pea biomass and shoot N concentrations were 610 kg ha^{-1} and 10.3 g kg^{-1} greater, respectively, in inoculated than non-inoculated plants in Montana (McConnell et al., 2002). The increase in biomass N was most likely due to higher N fixation.

There are two common types of inoculant used in pulse production in Montana: seed-coat and granular. The seed-coat inoculant is applied to the seed as a peat powder prior to planting. Granular inoculants, which can be peat, clay, or synthetic-based, are placed next to the seed (in-furrow) during planting. Each is used to a relatively equal extent in Montana (Bestwick et al., 2018), but their relative efficacies in Montana are unknown. Clayton et al. (2004b) found that granular inoculant resulted in higher N fixed amounts and seed yield than seed-coat inoculant in fields never planted with pulses before. Granular inoculant is approximately four times more expensive than peat-powder inoculant on a per hectare basis, so research in this area could have significant economic impacts for Montana pulse growers.

Variety Effects on Nitrogen Fixation

Legume crops have historically been intensively bred for greater yield and other beneficial agronomic traits. However, there has been little focus on boosting N fixation and belowground traits that may enhance symbiosis with rhizobia. There is evidence from theoretical models that intensive domestication and breeding, along with high synthetic N inputs in agriculture, may have diminished legume crops' capacity for N fixation (Liu et al., 2020). As a result, cropping systems using modern pulse varieties may have lower rates of N fixation. Herridge and Rose (2000) proposed three strategies for breeding programs to select for higher N fixation in legume crops: maximizing crop biomass and yield and thereby N demand; enhancing the ability to fix N in high nitrate environments; and optimizing nodulation (number, mass, duration, and rhizobia strain selectivity). However, the authors note that while breeding for higher N fixation has been a point of interest for decades, little progress has been made in successfully developing high N-fixing lines that are feasible for large-scale production.

Preliminary attempts in breeding for higher N fixation investigate differences in N fixed amounts among varieties. Zimmer et al. (2016) reported differences among three soybean varieties in fNdfa and N fixed amounts, ranging from 0.43 to 0.55 and 48 to 100 kg N ha^{-1}, respectively. Abi-Ghanem et al. (2011) found the number of nodules was highly correlated to N fixation amounts in a greenhouse setting, suggesting that greater N fixation amounts could be caused by larger rhizobia colonization rates in some varieties. However, the same varieties performed differently in a field experiment, signifying that variety selection and breeding decisions should not be based on greenhouse experiments (Abi-Ghanem et al., 2013). In that field experiment, there were biomass N differences among pea varieties. Heritability was estimated to be 0.57, meaning 57% of variation in total plant N is attributed to genetics (Abi-Ghanem et al., 2013). In field-grown lentil in Pakistan, fNdfa ranged among six varieties from 0.09 to 0.48 and N fixed ranged from 4 to 35 kg N ha^{-1}. Differences in N fixation among field-grown lentil and chickpea varieties in western Canada were only detected when three study years were combined (Hossain et al., 2016). Field pea N fixed amounts did not vary among varieties over three years (Hossain et al., 2016). There were differences among chickpea, pea, and lentil varieties in nitrogen harvest index, a measure of how much of total plant N exists in grain (Hossain et al., 2016); while this is not directly related to N fixation, it does have impacts on N contributions to the following crop from pulse crops. The limited existing research does suggest that N fixation could be a trait among pulse varieties that can be exploited through breeding, but field N fixation results are inconsistent. There have not been field studies in Montana comparing N fixation of different pulse varieties.

Measuring Biological Nitrogen Fixation

Methods of quantifying biological N fixation are summarized by Unkovich et al. (2008). Two of the most common and feasible methods for field experiments are N difference (via an N budget) and ^{15}N natural abundance. These methods are advantageous because they measure the fixed N accumulated by the plant over an entire growing season (Unkovich et al., 2008). Both methods calculate only the N fixed in aboveground plant biomass, so total N fixation is underestimated as root N contributions are not considered. Doing so would add significant assumptions as root measurements in the field are not feasible and estimations of root N contributions require substantial assumptions to be made (Anglade et al., 2015). However, it is likely that the ratio of total N fixed to aboveground N fixed is relatively consistent (Arcand et al., 2014), and therefore both methods are suitable when comparing N fixed among treatments or varieties.

The driving principle of the N difference method (ND) is that the legume will have more biomass N than a non N-fixing reference crop, and that the N difference is largely the result of N fixation. Variation in soil N uptake between the legume and reference crop is accounted for in the Nd method by calculating the difference in soil nitrate-N between legume and reference crop at the end of the growing season. The ND method assumes that the reference crop has characteristics similar to the legume, such as rooting depth and water use. This assumption is largely met if careful consideration is taken when selecting the reference crop for the experiment (Unkovich et al., 2008). The N budget method also assumes that both the pulse and the reference crop scavenge the soil for the same pool of nitrate-N, and large differences in soil N transformation rates between the legume and reference crop would invalidate that assumption. As such, the amounts of N mineralization, leaching, and denitrification are assumed to be similar under the legume and reference crop, and similar water use between legume and reference crops will results in these being similar.

The ^{15}N natural abundance method (NA) uses small variations in the natural, stable isotope of nitrogen (^{15}N) to determine the fraction of legume N derived from the atmosphere (fNdfa). The main principle for NA is that N in the soil has a greater natural abundance of ^{15}N than the atmosphere. The reference crop, which does not fix N, is used to estimate the ^{15}N signature of soil N taken up by the plant. This method requires researchers to know the ^{15}N signature of the legume grown in the absence of soil N, meaning all the plant's N is obtained through N fixation. While theoretically this value would be zero (equal to the ^{15}N composition of the atmosphere), N isotope discrimination by rhizobia during the process of N fixation and differences in ^{15}N partitioning between legume shoots and roots make this value slightly negative (Unkovich et al., 1994). When grown in the field, the legume will derive some of its N from the soil and some from the atmosphere via fixation, meaning its ^{15}N signature will be somewhere between that of the reference crop and the legume grown without soil N.

The NA method also depends on several assumptions. Like the N budget method, it assumes that soil N gains and losses from soil N cycling processes are the same under the legume and reference crop. It assumes that the ^{15}N signature of soil N taken up by the legume and reference crops are the same. There is evidence that soil ^{15}N shifts temporally over the growing season as different N sources become available to plants (Hauggaard-Nielson et al., 2010). Soil ^{15}N also varies spatially, and it is recommended that the distance between a legume and its reference plant be limited to 3 to 5 m (Walley et al., 2001). This minimizes measurement error from natural soil ^{15}N variation while avoiding any belowground N interactions between the legume and reference plants. Differences in N fixation amounts of pea calculated by the NA method could not be correlated with topographic features (Androsoff et al., 1995) or soil parameters (Hauggard-Nielson et al., 2010), suggesting that the variability is due to soil ^{15}N fluctuations of unknown origin. In addition to soil ^{15}N, the NA method also assumes that the legume and reference crop have the same degree of ^{15}N fractionation between roots and aboveground tissues (Dakora et al., 2008). This once again highlights the importance of selecting an appropriate reference crop when quantifying N fixation.

Given the many assumptions that are required in both methods, spatial and temporal variability in N fixation measurements is expected. Additionally, external environmental factors and inherent soil variability can cause significant inaccuracies and imprecision when estimating N fixation (Unkovich et al., 1994; Walley et al., 2001). Measuring N fixation using both methods at a given site strengthens confidence in results (McCauley et al., 2012). It is unlikely that all the assumptions are met in the field. While this could cause an over or underestimation of amounts of N fixed, the errors created by incorrect assumptions for each treatment should be approximately equal except in cases of high nitrate concentrations and extreme spatial variability. Therefore, while using these methods in this project may not determine the actual quantities of N fixed in aboveground biomass, they are able to determine relative differences in N fixation among experimental treatments.

Research Objectives

The studies presented in this thesis focus on management practices of pulse crops in the NGP with the goal of increasing biological N fixation. In addition to N fixed amounts, I also investigate impacts of treatment on biomass N, fNdfa, residual soil nitrate-N, seed yield, and seed protein. In Chapter Two, I evaluate the response of lentil N fixation to fertilizer applications and inoculant types. In Chapter Three, I assess differences in N fixation among regionally-grown pea and lentil varieties. In Chapter Four, I investigate the N fixation response of two pea varieties to different inoculant formulations. Given the rapid expansion of pulse crops in the NGP and their general lack of representation in published literature, the findings of these studies on fertilizer, inoculant, and variety effects on pulse N fixation and productivity have far-reaching impacts to the region’s pulse producers.

References

Abbasi, M.K., M.M. Tahir, W. Azam, Z. Abbas, and N. Rahim, 2012. Soybean yield and chemical composition in response to phosphorus-potassium nutrition in Kashmir. *Agron J*. 104(5):1476-1484.

Abdelhamid M.T., H.A. Kamel, and M.G. Dawood, 2010. Response of non-nodulating, nodulating, and super-nodulating soybean genotypes to potassium fertilizer under water stress. *J of Plant Nutr*. 34(11): 1675-1689.

Abi-Ghanem, R., L. Carpenter-Boggs, and J.L. Smith. 2011. Cultivar effects on nitrogen fixation in peas and lentils. *Biol Fertil Soils*. 47:115-120.

Abi-Ghanem, R., E.T. Bodah, M. Wood, and K. Braunwart. 2013. Potential breeding for high nitrogen fixation in *Pisum sativum* L.: Germplasm phenotypic characterization and genetic investigation. *Am J Plant Sci*. 4:1597-1600.

Alam, F., T.Y. Kim, S.Y. Kim, S.S. Alam, P. Pramanik, P.J. Kim, Y.B. Lee. 2015. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* Roth). *Soil Sci and Plant Nutr*. 61(4):664-675.

Androsoff, G.L., C. Kessel, and D.J. Pennock. 1995. Landscape-scale estimates of dinitrogen fixation by *Pisum sativum* by nitrogen-15 natural abundance and enriched isotope dilution. *Biol Fert Soils*. 20(1):33-40.

Anglade, J., G. Billen, and J. Garnier. 2015. Relationships for estimating N2 fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. *Ecosphere*. 6(3):1-24.

Arcand, M.M., R. Lemke, R.E. Farrell, J.D. Knight. 2014. Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. *Biol Fert Soils*. 50:507-515.

Beckie H.J., and S.A. Brandt (1997) Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Can J Plant Sci*. 77:311-322.

Bestwick, M., K. Olson-Rutz, C. Jones, and P. Miller. 2018. Environmental and Management Effects on Pea Protein in Montana: A Research Report from the Montana Research and Economic Development Initiative. https://landresources.montana.edu/soilfertility/documents/PDF/reports/Bestwick2018MREDIEnvironMgtPeaProt.pdf

Bremer E., H.H. Janzen, B.H. Ellert, R.H. McKenzie. 2011. Carbon, nitrogen, and greenhouse gas balances in an 18-year cropping system study on the northern Great Plains. *Soil Sci Soc Am J*. 75:1493-1502.

Brown, T.T., R.T. Koenig, D.R. Huggins, J.B. Harsh, and R.E. Rossi, 2008. Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. *Soil Sci Soc Am J*. 72:634-640. doi:10.2136/sssaj2007.0061.

Carlyle, W.J. 1997. The decline of summerfallow on the Canadian prairies. *The Canadian Geographer*. 3:267-280.

Cazzato, E., V. Tufarelli, E. Ceci, A.M. Stellacci and V. Laudadio, 2012. Quality, yield and nitrogen fixation of faba bean seeds as affected by sulphur fertilization. *Soil & Plant Sci*. 62(8):732-738. doi: 10.1080/09064710.2012.698642

Chemining'wa, G.N. and J.K. Vessey. 2006. The abundance and efficacy of *Rhizobium leguminosarum* bv. *viciae* in cultivated soils of the eastern Canadian prairie. *Soil Biol Biochem*. 38:294-302.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Crop yield and seed quality. *Can J Plant Sci*. 84:89-96.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Can J Plant Sci*. 84:79-88.

Collins, M., D.J. Lang, and K.A. Kelling. 1986. Effects of phosphorus, potassium, and sulfur on alfalfa nitrogen-fixation under field conditions. *Agron J*. 78(6):959-963.

Dakora F.D., A.C. Spriggs, P.A. Ndakidemi, and A. Belane. 2008. Measuring N_2 Fixation in Legumes Using ^{15}N Natural Abundance: Some Methodological Problems Associated with Choice of Reference Plants. In: Dakora F.D., Chimphango S.B.M., Valentine A.J., Elmerich C., Newton W.E. (eds) Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture, vol 42. Current Plant Science and Biotechnology in Agriculture. Springer, Dordrecht, The Netherlands, pp 45-45.

Divito, G.A. and V.O. Sadras. 2014. How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crops Research*. 156:1161-171.

Engel, R. 2020. Soil acidity management of long-term no-till fields in Montana to prevent crop failure. USDA Sustainable Agriculture Research & Education. https://projects.sare.org/?export=1&project_report=644340

FAO, 2018. Inputs: Fertilizers by nutrient. http://www.fao.org/faostat/en/#data/RFN (accessed 16 September 2020).

Fowler, D., M. Coyle, U. Skiba, M.A. Sutton, J.N. Cape, S. Reis, L.J. Sheppard, A. Jenkins, B. Grizzetti, J.N. Galloway, P. Vitousek, A. Leach, A.F. Bouwman, K. Butterbach-Bahl, F. Dentener, D. Stevenson, M. Amann, and M. Voss. 2013. The global nitrogen cycle in the twenty-first century. *Phil Trans R Soc B.* 368:1-13.

Freney, J.R. and F.J. Stevenson. Organic sulfur transformations in soils. 1966. *Soil Sci*. 101(4):307-316.

Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, B.J. Cosby. 2003. The nitrogen cascade. *BioScience.* 53(4):341-356.

Gayler, K.R., and G.E. Sykes. 1985. Effects of nutritional stress on the storage proteins of soybeans. *Plant Phys.* 78(3):582-585.

Grant, R.F., E. Pattey, T.W. Goddard, L.M. Kryzanowski, and H. Puurveen. 2006. Modeling the effects of fertilizer application rate on nitrous oxide emissions. *Soil Sci Am J.* 70:235-248.

Gruber, N and J.N. Galloway. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*. 451 (7176):293-296.

González-Guerrero, M., A. Matthiadis, A. Sáez, and T.A. Long. 2014. Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. *Front Plant Sci*. 5:45.

Hafeez, F.Y., N.H. Shah, and K.A. Malik. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biol Fert Soils*. 31:65-69.

Hauggaard-Nielson H, Holdensen L, Wulfsohn D, Jensen ES. 2010. Spatial variation of N-2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the N-15 natural abundance method. *Plant Soil*. 327 (1-2):167-184.

Havlin, J.L, S.L. Tisdale, W.L. Nelson, J.D. Beaton, 2013. Soil Fertility and Fertilizers: An Introduction to Nutrient Management, 8th Edition. Pearson.

Herridge, D. and I. Rose. 2000. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Research*. 65(2-3):229-248.

Hossain, A., A. Quddus, K. Alam, H.M. Naser, B. Anwar, F. Khatun, H. Rashid, F. Khatun, and A. Siddiky. 2020. Application of zinc, boron, and molybdenum in soil increases lentil productivity, nutrient uptake, and apparent balance. *Can J Soil Sci*. doi: https://doi.org/10.1139/cjss-2019-0141

Hossain, Z., X. Wang, C. Hamel, J.D. Knight, M.J. Morrison, and Y. Gan. 2016. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can J Plant Sci*. 97:119-131.

Howard, J.B. and D.C. Rees. 2006. How many metals does it take to fix N_2? A mechanistic overview of biological nitrogen fixation. *Proceedings of the National Academy of Sciences of the United States of America*. 103(46):17088-17093.

Huang, J., R.K. Afshar, and C. Chen. 2016. Lentil response to nitrogen application and rhizobia inoculation. *Communications in Soil Science and Plant Analysis*. 47(21):2458-2464.

Huang, L., C.W. Riggins, S. Rodriguez-Zas, M.C. Zabaloy, and M.B. Villamil, 2019. Long-term N fertilization imbalances potential N acquisition and tranformations by soil microbes. *Sci of the Total Environ*. 691:562-571.

Jacobsen J., G. Jackson, and C. Jones. 2003. Fertilizer guidelines for Montana crops. MSU Extension Bulletin EB161. Bozeman, MT. http://animalrange.montana.edu/documents/extension/eb161.pdf

Kaiser, D.E. and K.I. Kim, 2013. Soybean response to sulfur fertilizer applied as a broadcast or starter using replicated strip trials. *Agron J*, 105(4):1189-1198.

Kaschuk G., P.A. Leffelaar, K.E. Giller, O. Alberton, M. Hungria, and T.W. Kuyper. 2009. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. *Soil Biol & Biochem*. 42:125-127.

Kucey, R.M.N. and M.F. Hynes. 1989. Populations of *Rhizobium leguminorsarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. *Can J Microbiol*. 35(6):661-667.

Liu, J., X. Yu, Q. Qin, R.D. Dinkins, and H. Zhu. 2020. The impacts of domestication and breeding on nitrogen fixation symbiosis in legumes. *Front Genet*. https://doi.org/10.3389/fgene.2020.00973

Lupwayi, N.Z., G.W. Clayton, J.T. O'Donovan, K.N. Harker, T.K. Turkington, and W.A. Rice. 2004. Decomposition of crop residues under conventional and zero tillage. *Can J Soil Sci*. 84(4):403-410.

Lupwayi, N.Z., Lafond G.P., W.E. May, C.B. Holzapfel, R.L. Lemke. 2012. Intensification of field pea production: Impact on soil microbiology. *Agron J*. 104(4):1189-1196.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: Effect of planting and termination timing. *Nutr Cycl Agroecosys*. 92(3):305-314.

McConnell, J.T., P.R. Miller, R.L. Lawrence, R. Engel, and G.A. Nielsen. 2002. Managing inoculation failure of field pea and chickpea based on spectral responses. *Can J Plant Sci*. 82(2):273-282.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001a. Response of pea to rate and placement of triple superphosphate fertilizer in Alberta. *Can J Plant Sci*. 81(4):645–649.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001b. Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Can J Plant Sci.* 81:637–643.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003a. Pulse crops for the northern Great Plains: II. Cropping sequence effects on cereal, oilseed, and pulse crops. *Agron J*. 95(4): 980-986

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003b. Pulse crops for the northern Great Plains: I. Grain productivity and residual effects on soil water and nitrogen. *Agron J*. 95(4):972-979.

Miller, P.R., Bekkerman, A., C.A. Jones, M.H. Burgess, J.A. Holmes, R.E. Engel. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. *Agron J.* 107(2):541-550.

Mohebbi, S., and R. Mahler. 1989. The effect of soil pH on wheat and lentils grown on an agriculturally acidified northern Idaho soil under greenhouse conditions. *Communications in Soil Science and Plant Analysis*. 20(3-4):359-381.

Murray, J.D., C.W. Liu, Y. Chen, A.J. Miller. 2017. Nitrogen sensing in legumes. *J Exper Bot*. 68(8):1919-1926.

Nanthana, C., R. Lefroy, and G.J. Blair, 1997. Effect of crop duration and soil type on the ability of soil sulfur tests to predict plant response to sulfur. *Aus J Soil Research*. 35(5)1131-1142.

Nasar, J., A. Roshan, A. Alam, M.Z. Khan, B. Ahmad. 2019. The impact of foliage fertilization of iron and molybdenum on yield, N uptake and root nodulation of lentil (*Lens culinaris* Medic) crop. *Indian J Ag Research*. 53(5):628-631.

Oldroyd, Giles E.D., J.D. Murray, P.S. Poole, and J.A. Downie, 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics*. 45:119-44.

Pan, B., S.K. Lam, A. Mosier, Y. Luo, and D. Chen. 2016. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: A global synthesis. *Agric, Ecosys and Environ*. 232:283-289.

Peoples, M.B., J. Brockwell, D.F. Herridge, I.J. Rochester, B.J.R. Alves, S. Urquiaga, R.M. Boddey, F.D. Dakora, S. Bhattarai, S.L. Maskey, C. Sampet, B. Rerkasem, D.F. Khan, H. Hauggaard-Nielson, and E.S. Jensen. 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis*. 48:1-17.

Premaratne, K.P. and J.J. Oertli, 1994. The influence of potassium supply on nodulation, nitrogenase activity and nitrogen accumulation of soybean (*Glycine max* L. Merrill) grown in nutrient solution. *Fert Research*. 38:95-99.

Rehm, G.W. and R.C. Sorensen. 1974. Effects of the application of phosphorus, potassium, and sulfur to alfalfa grown on a calcareous silt loam. *Soil Sci*. 117:58-65.

Romero, C.M, R.E. Engel, J. D'Andrilli, P.R. Miller, and R. Wallander. 2019. Compositional tracking of dissolved organic matter in semiarid wheat-based cropping systems using fluorescence EEMs-PARAFAC and absorbance spectroscopy. *J Arid Environs*. 167:34-42.

Scherer, H.W., S. Pacyna, N. Manthey, M. Schulz, 2006. Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N_2 fixation. *Plant Soil Environ*. 52(2):72-77.

Scherer, H.W., 2009. Sulfur in soils. *J. Plant Nutr*. *Soil Sci.,* 172:326–335.

Sigler, W.A., S.A. Ewing, C.A. Jones, R.A. Payn, P. Miller, and M. Maneta. 2020. Water and nitrate loss from dryland agricultural soils is controlled by management, soils, and weather. Agriculure, Ecosystems & Environment. https://doi.org/10.1016/j.agee.2020.107158

Skogley, E.O, and V.A. Haby. 1981. Predicting crop responses on high-potassium soils of frigid temperature and ustic moisture regimes. *Soil Sci Am J*. 45(3):533-536.

Stevenson, F.C. and C. van Kessel. 1996. A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. *Soil Sci Soc Am J*. 60:1797-1805.

Unkovich, M.J., J.S. Pate, P. Sanford, E.L. Armstrong. 1994. Potential precision of the delta-N-15 natural-abundance method in-field estimates of nitrogen-fixation by crop and pasture legumes in south-west Australia. *Aust J Agric Res*. 45(1):119-132.

Unkovich M., D.F. Herridge, M.B. Peoples, G. Cadisch, R.M. Boddey, K.E. Giller, B. Alves, and P.M. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph. Australian Centre for International Agricultural Research, Canberra, AU.

Vance C.P., 2008. Carbon and Nitrogen Metabolism in Legume Nodules. In: Dilworth M.J., James E.K., Sprent J.I., Newton W.E. (eds) Nitrogen-fixing Leguminous Symbioses. Nitrogen Fixation: Origins, Applications, and Research Progress, vol 7. Springer, Dordrecht.

Voisin, A., C. Salon, N.G. Munier-Jolain, and B. Ney. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 243:31-42.

Walley, F.L., G.M. Fu, J.W. van Groenigen, and C. van Kessel. 2001. Short-range spatial variability of nitrogen fixation by field-grown chickpea. *Soil Sci Soc Am J*. 65(6):1717-1722.

Walley, F.L., G.W. Clayton, P.R. Miller, P.M. Carr, and G.P. Lafond. 2007. Nitrogen economy of pulse crop production in the Northern Great Plains. *Agron J.* 99:1710-1718.

Warne, T., S. Ahmed, C.B. Shanks, and P. Miller. 2019. Sustainability dimensions of a North American lentil system in a changing world. *Front Sustain Food Syst*. https://doi.org/10.3389/fsufs.2019.00088

Zhao, F.J., A.P. Wood and S.P. McGrath, 1999. Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 212(2):209-219.

Zimmer, S., M. Messmer, T. Haase, H.P. Piepho, A. Mindermann, H. Schulz, A. Habekuß, F. Ordon, K.P. Wilbois, and J. Heß. 2016. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Europ J Agronomy*. 72:38-46.

CHAPTER TWO

LENTIL NITROGEN FIXATION RESPONSE TO FERTILIZER AND INOCULANT IN THE NORTHERN GREAT PLAINS

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Abstract

Lentil production in the semi-arid northern Great Plains has increased dramatically over the past two decades, providing agroecosystem benefits of efficient water use, pest cycle disruption, and biological nitrogen (N) fixation. Increasing N fixation could help alleviate soil acidification and groundwater impairment by reducing N fertilizer needs. Despite widespread farmer adoption of lentil in the region, little is known about the benefits of fertilizer or inoculant type on N fixation. The primary aim of this study was to determine how nutrients (potassium (K), sulfur (S), and foliar-applied micronutrients), and rhizobial inoculant types (seed-coat 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 the ^{15}N natural abundance method. N fixation proved to be 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 indicated sufficiency. In a moderately dry year at a site with low soil sulfate levels, S fertilization increased N fixed by 40%. Inoculated lentil fixed more N than uninoculated lentil in two site-years, and no differences in N fixed between inoculant types were found. Inoculation response was not related to field cropping histories. The study shows that sulfur fertilization and rhizobial inoculation have potential to significantly increase lentil N fixation amounts in the northern Great Plains.

Introduction

Lentil (*Lens culinaris* Medikus) has become increasingly popular in the historically cereal-dominated northern Great Plains (NGP), with approximately 214,000 ha of lentil planted in Montana in 2021 (USDA NASS, 2021). Lentil production has increased in part due to high 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 (Zentner et al., 2001; Miller et al., 2015). Several studies have found increases in cereal yield when grown after pulse crops (Beckie and Brandt, 1997; Miller et al., 2003a; Bremer et al., 2011). Pulses provide agroecosystem benefits of summerfallow reduction (Long et al., 2014), efficient water use (Miller et al., 2003b), pest cycle disruption (Stevenson and van Kessel, 1996), and biological N fixation (Beckie and Brandt, 1997). Repeated application of N fertilizer at high rates 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 input requirements for producers and 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 impact N fixation by limiting nodule numbers or nitrogenase activity (Divito and Sadras, 2014). Alternatively, N fixation can decrease when nutrient deficiencies limit growth of the host legume and hence photosynthate available to be mobilized to nodules (Divito and Sadras, 2014). Pulse crops respond to phosphorus (P) fertilizer when soil P levels are low (McKenzie et al., 2001a; Divito and Sadras, 2014). Nitrogen fixation of legume crops responds positively to fertilizer S (Zhao et al., 1999; Scherer et al., 2006; Cazzato et al., 2012) and K (Collins et al., 1986; Abdelhamid et al., 2011; Abbasi et al., 2012). 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 inoculant. In a meta-analysis of grain legumes by Kaschuk et al. (2009), average grain yield increased 16% and average grain protein increased 7% when seeds were inoculated at planting. There are two common types of peat-based inoculant used in lentil production: seed-coat and granular. Seed-coat peat powder inoculant is applied to the seed surface before planting. Granular inoculant is placed near the seed (in-furrow) during planting. Each is used to a relatively equal extent in Montana in 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-ha 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 of inoculant or fertilizer in the NGP. Therefore, a study was initiated 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 seed yield and protein, investigate the relationship between N fixation and seed yield and protein, and evaluate how soil nitrate-N pools following lentil are affected by fertilizer and inoculant.

Materials and Methods

Site Selection

This study was part of a larger lentil yield 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 (latitude and longitude: 45.68°N, 111.15°W) and Northern Agricultural Research Center (NARC) near Havre, MT (latitude and longitude: 48.49°N, 109.80°W), were selected to evaluate N fixed amounts. Historically, these two sites span the precipitation gradient of Montana's dryland agricultural areas (Figure 2-1). The soil type at PF was Amsterdam silt loam (frigid Typic Haplustoll) in all years. Soil types at NARC were Telstad-Joplin loam (frigid Aridic Argiustoll) in 2019 and 2020 and Kenilworth-Fort Benton fine sandy loam (frigid Aridic Argiustoll) in 2021. Site selection was based on fall-sampled soil parameters of nitrate-N (0.12M KCl, Cd reduction), exchangeable K (1M ammonium acetate, ICP), sulfate-S (0.12M KCl, turbidimetric), Olsen P (0.5M $NaHCO_3$, colorimetric), and soil organic matter (loss on ignition) to identify locations within suitable fields that had relatively similar fertility levels (analysis by AgVise Laboratories, Northwood, ND, USA). Soil at each field 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; AgVise Laboratories, Northwood, ND, USA). Soil characteristics and pulse crop histories are summarized in Table 2-1.

Treatments and Experimental Design

Treatments included eight combinations of inoculant and fertilizer formulations (Table 2-2). The foliar micronutrient solution contained all the micronutrients required for plant growth except for Cl, which has not been documented as needed for N fixation, plus cobalt (Table 2-3). The micronutrient treatment was not included in 2019 analyses due to application above label rate that resulted in crop injury. Experiments were conducted in a randomized complete block design with five replicates for each location per year.

Plot Management

Avondale lentil, a medium-sized green variety, was seeded into no-till managed fields with standing cereal stubble at a target rate of 120 pure live plants m^{-2} 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 2-4. Plots were seeded in the following order of inoculant formulations to prevent rhizobia contamination: control, granular (Verdesian Primo GX2, Cary, NC, USA), seed-coat (Verdesian N-Charge, Cary, NC, USA). Inoculants of *Rhizobium leguminosarum* biovar *viceae* were applied at the recommended label rate (6.7 kg ha^{-1} for granular and 0.31 g kg-$seed^{-1}$ for seed-coat). Flax, used as a non N-fixing reference crop, was mechanically seeded in a 2-m strip adjacent to the 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 applied in furrow 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. Appropriate pest management practices were conducted throughout each growing season (Table 2-5).

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, from each lentil plot was sampled for 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 lentil plots. Based on high and variable soil nitrate-N observed in 2021, and issues encountered with variable soil nitrate-N pools in 2020 at NARC, a flax sample (and corresponding soil sample) was taken for each lentil plot at both sites in 2021 to limit error from spatial variation. Biomass samples were dried (50°C, 72h), weighed, and ground (< 0.5 mm). Subsamples were analyzed for N concentration via combustion (LECO, St. Joseph, MI, USA), and approximately 10% of samples were analyzed in duplicate to assess precision (RSD averaged 2.5%). At PF in 2020, biomass samples for the K and K + S treatments for both inoculant types were analyzed for S and Zn concentration (ICP; AgVise Laboratories, Northwood, ND, USA). Naturally occurring isotope ^{15}N was analyzed using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (RSD averaged 2.2%; Sercon Ltd., Cheshire, UK).

Plots were harvested with a combine, excluding edge rows, and lentil seeds were cleaned and weighed. Seed yield (kg ha^{-1}) was determined on a dry matter basis. Seed N concentrations were determined by combustion (LECO, St. Joseph, MI, USA), and seed protein concentration was reported as N x 6.25 (Coyne et al., 2005). Shortly after lentil harvest, soil was sampled to 90-cm depth (3.1-cm diameter core) in each lentil plot and at each location where flax was sampled and split in the field into three 30-cm segments. Soils were dried (50°C, 72h), weighed, and bulk density calculated. The soils were ground (< 2 mm), extracted using 1M KCl, and analyzed for nitrate-N with a flow injection colorimetric analyzer (RSD averaged 7.0%; Lachat, Loveland, CO, USA). The nitrate-N pool (kg N ha^{-1}) for each 90-cm sample was calculated.

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}.

$$N\ fixed = \left(Biomass\ N_{pulse} - Biomass\ N_{flax}\right) + \left(Soil\ N_{pulse} - Soil\ N_{flax}\right) \quad [1]$$

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 was used because it is not possible to have negative N fixed amounts. 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 (9.0% of ND observations) were omitted from analysis.

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):

$$fNdfa = \left(\frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - B}\right) \quad [2]$$

Where $\delta^{15}N$ is:

$$\delta^{15}N\ (‰) = \left(\frac{atom\%N_{sample} - atom\%N_{atmosphere}}{atom\%N_{atmosphere}}\right) * 1000 \quad [3]$$

The B in Eq. 2 refers to the $\delta^{15}N$ of lentil grown without soil N, meaning it reflects conditions in which all plant N is fixed. The specific B-value for Avondale lentil used in our study (-0.99) was obtained in a greenhouse experiment detailed in Appendix A. Amounts of N fixed (kg N ha^{-1}) for the NA method were calculated using Eq. 4.

$$N\ fixed = Biomass\ N_{pulse} * fNdfa \qquad [4]$$

Amounts of N fixed less than 0 were omitted from analysis (1.3% of NA observations).

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 N, fNdfa from NA, seed yield, seed protein concentration, protein yield, and post-harvest soil nitrate-N) 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 likely obscured. Therefore, site-years were analyzed independently for treatment effects using analysis of variance (ANOVA). Differences among treatments were further evaluated using pre-planned orthogonal contrasts with the ‘emmeans’ package (Lenth, 2021) and Tukey-Kramer 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, fNdfa from NA) and seed parameters (yield, protein concentration, protein yield).

Results and Discussion

Weather

Yearly weather varied substantially at both sites over the course of the study (Table 2-6). 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.

Evaluation of N Fixation Methods

Both the ND and NA methods of estimating N fixed amounts require assumptions in the field to be met. The ND method is invalidated when the legume and reference crops have access to different pools of soil nitrate-N; this would happen 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). At NARC in 2020, spring soil nitrate-N was high and variable (Table 2-1), which resulted in a majority of N fixed amounts calculated using ND falling outside of the established bounds; therefore, this 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). Additionally, precision of the NA method is affected when there are only small differences between legume and reference crop $\delta^{15}N$ (Bremer et al., 1993), especially when the difference is less than 2‰ (Unkovich et al., 1994). Based on high observed nitrate-N variability and differences between lentil and flax $\delta^{15}N$ of only 1.0‰ at NARC in 2020, confidence in results was compromised and results were not reported here.

Using both methods strengthen confidence in N fixed amounts (McCauley et al., 2012). Overall, both methods were often in agreement when differences among treatments were detected. The ND method frequently resulted in higher N fixed amounts than the NA method, but relative differences among treatments were similar for each method. Estimates of N fixed at our field sites were impacted by variable soil nitrate-N, highlighting the importance of careful site selection when conducting N fixation field experiments.

N Fixation

Inoculant. Inoculation had no impact on N fixed in any year at PF (Table 2-7), but increased N fixed in both years that N fixed data were collected at NARC (Table 2-8). Using the ND method, inoculated plots fixed 37 and 13 kg N ha^{-1} more N than control plots at NARC in 2019 and 2021, respectively. The NA method also found higher N fixed in inoculated plots at NARC, with estimated differences of 23 and 9 kg N ha^{-1} in 2019 and 2021, respectively. The observed inoculant response appeared to be unrelated to previous cropping history. At NARC, the 2019 field site was planted in pea three years before the study and N fixed was still substantially higher when lentil was inoculated. At PF, no inoculant effect was observed in any year, even though 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 and Hynes, 1989) from the region that found 1 to 100 rhizobia per gram of soil in fields never planted with legumes. In our study, nodules were observed on all lentil roots regardless of treatment, 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 granular or seed-coat inoculants seemingly outperformed the existing strain(s) in the control plots. However, at PF, the existing strain(s) appeared to be equally effective at fixing N as the commercial inoculants. There is evidence that indigenous rhizobia strains 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 than seed-coat inoculant based on previous research in the NGP. Clayton et al. (2004) found that pea with granular inoculant on average fixed 43 kg N ha^{-1} more than 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. Common bean N-fixed amounts were shown to increase 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. Distribution of root nodules were not visibly different between granular and seed-coat inoculants, but hard, dry soils made it difficult to collect full root systems.

Fertilizer. Potassium fertilizer did not increase N fixed in any year at either site. Soils in all site-years had high baseline levels of 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 slightly 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. A strong S response was observed at PF in 2020, where S fertilizer increased N fixed by 43 and 38%, respectively, using the ND and NA methods. Soil sulfate-S in the upper 15 cm was relatively low for this site-year (3.2 mg kg^{-1}), and moderate drought developed towards the end of the growing season. Lentil plots not fertilized with S became noticeably less green than fertilized plots by early July. The chlorosis was consistent in upper and lower leaves, making it difficult to distinguish between N and S deficiency. Shoot tissue analysis revealed that S fertilizer increased S concentration by 64% and N concentration by 18% (Table 2-9), suggesting that plants were severely S deficient and the likely cause of the greenness difference. The S deficiency likely reduced plant productivity, indirectly impacting N fixation by reducing carbohydrate supply to root nodules. Sulfur 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).

Other field sites had similarly low sulfate-S levels as PF in 2020, 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 are prone to S deficiency (Freney and Stevenson, 1966; Kaiser and 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. In 2021, severe drought at both sites prevented S from becoming a limiting growth factor, apparently negating any benefits of S fertilizer on 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, but no studies were located that evaluated N fixed amounts (Alam et al., 2015; Nasar et al., 2019; Hossain et al., 2020). 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 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 than that is absorbed by plants. 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 considering that most of the product likely hit the soil rather than plant leaves or dripped off leaves and strongly sorbed to the soil surface.

Biomass N and fNdfa

Biomass N varied significantly in three of six site-years (Table 2-10). The three years with biomass N differences also had differences in N fixed. In those years, treatments that resulted in higher biomass N generally aligned with treatments with higher N fixed amounts. This agrees with McCauley et al. (2012), where pea and lentil had strong positive relationships between biomass N accumulation and N fixed amounts over a two-year study. Given the intensive time and expenses for measurements required by the ND and NA methods, using biomass N as a surrogate for N fixation may be possible, especially when resources are limited. Variation in fNdfa values was less related to N fixed amounts (Table 2-11). At NARC in 2019, inoculated lentil had fNdfa values 0.17 higher than the control, reflecting the higher N fixed amounts in inoculated treatments. Sulfur fertilizer increased fNdfa by 0.16, but a corresponding difference in N fixed amounts was not detected. At PF in 2021, seed-coat inoculant increased fNdfa 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 compared to at PF.

Seed Yield, Protein Concentration, and Protein Yield

Seed yield varied among treatments in four of six site-years (Table 2-12). At NARC in 2019, inoculated lentil yielded 403 kg ha^{-1} (56%) greater than the control. At NARC in 2021, inoculation increased yield compared to the control by an average of 212 kg ha^{-1} (45%). The low yields in control treatments reflect the inoculant effect found in N fixed amounts at NARC in 2019 and 2021. In both years at NARC when inoculation increased yield, seed-coat inoculant resulted in greater yields than granular inoculant. Granular inoculant resulted in greater yield than seed-coat inoculant only at PF in 2020. In 2020, treatments with S yielded 278 kg ha^{-1} more than treatments with only K, aligning with differences found in N fixed.

Seed protein concentrations differed among treatments in three of four site-years with data (Table 2-13). At NARC in 2019, the control had the lowest protein concentration overall and was 20 g kg^{-1} lower than inoculated lentil. Seed-coat inoculant resulted in protein concentrations 10 g kg^{-1} higher than granular inoculant. Sulfur increased seed protein concentration at PF in 2019 and at NARC in 2020 by 7 and 2 g kg^{-1}, respectively. At PF in 2020, where S fertilizer increased N fixed and yield, surprisingly there was no response in protein concentration.

Protein yield, the product of seed yield and protein concentration, varied significantly in three of four site-years using orthogonal contrasts (Table 2-14). Inoculation increased protein yield at PF and NARC in 2019 by 54 and 133 kg ha^{-1}, respectively. The effect of inoculant type was inconsistent between sites. At PF in 2019 and 2020, granular inoculant resulted in greater protein yield than seed-coat inoculant (32 and 13 kg ha^{-1}, respectively). At NARC in 2019, similar to the response observed in yield and protein concentration, protein yield was 29 kg ha^{-1} greater using seed-coat inoculant than granular inoculant. At PF in 2020, S fertilizer increased protein yield by 75 kg ha^{-1}.

Inconsistencies in lentil yield and protein response to inoculant and fertilizer have been found at other sites in the NGP. For the larger seven-site lentil yield and protein study, inoculation increased seed yield in six of 20 site-years and seed protein concentration in two of 13 site-years (Miller et al., 2022). Additional studies in Montana have found inconsistent impacts of inoculation on pulse crop yield, with no effect in lentil (Huang et al., 2016) and large benefits in pea (McConnell et al., 2002). As discussed previously, inoculation benefits may be affected by rhizobia persistence and indigenous strains.

In our study, the lack of yield or protein response to K fertilizer is again unsurprising given the high exchangeable K levels each site-year. Lentil yield increased at a site with soil K below critical levels but also had no response at a different site with even lower soil K, indicating there may be a more complex relationship between K availability, environment, and lentil yield (Miller et al., 2022). Yield and protein responses to S were 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 protein under moderate drought (Bestwick et al., 2018). Weather variation could explain some of the inconsistency of S responses observed in our study. Miller et al. (2022) reported inconsistent responses across seven sites in Montana and North Dakota in 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.

N Fixation and Seed Yield, Protein, and Protein Yield Relationships

Relationships between N fixation parameters (N fixed using both methods, biomass N, fNdfa from NA) and lentil seed measurements (yield, protein concentration, protein yield) were inconsistent (Table 2-15). In our study, greater N fixed parameters both increased and decreased with seed yield and protein, as correlation coefficients ranged 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 C for yield formation when N fixation demands are high yet N is not greatly limiting. Of the 47 correlations, 23 were different from 0 ($P<0.1$). Correlation analysis was likely affected by sampling area, as seed measurements were 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. 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.

At PF in 2020, N removed by grain harvest plateaued around aboveground tissue S concentrations of 9 g kg^{-1}, while N fixed maintained a linear relationship to the highest S concentration near 12 g kg^{-1} (Figure 2-2). The difference between N fixed and N removed represents lentil residue N that remains in the field to decompose and mineralize for future crops, defined previously as the 'net N benefit' (Gollner et al., 2019). Our results suggest that while N removed and yield leveled off with increasing tissue S concentrations, and hence S availability, the net nitrogen benefit continued to increase above that point. Therefore, producers might want to fertilize with S even when a yield response 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 net N benefit, thereby lowering the fertilizer N needed for the subsequent, could help alleviate soil acidification, nitrate-N leaching, and nitrous oxide emissions. This could be especially important for those relying upon legume cover crops for their N benefit. However, this was only measured at one site-year and to our knowledge no similar research has been conducted.

Residual Soil Nitrate-N

Post-harvest soil nitrate-N levels only varied at two of six site-years (Table 2-16). In 2019 at PF, granular inoculant had an average soil nitrate-N level 6 kg N ha^{-1} greater than seed-coat inoculant. In 2020 at PF, the control had soil nitrate-N levels 12 kg N ha^{-1} greater than inoculated treatments. These differences were unrelated to N fixed amounts. Pulse crops can influence soil mineral N in the long-term (Hamel et al., 2018). In our study, soil nitrate-N was measured shortly after harvest for use in the ND method, so much of the fixed N existed in lentil shoot and root residues. Soil N inputs from pulse crops are difficult to assess in the short-term as residue decomposition and N mineralization are dependent upon various environmental conditions (Walley et al., 2007). As such, we did not expect higher N fixed amounts to translate to higher soil nitrate-N immediately after the growing season. It was hypothesized that more N fixed would be achieved by healthier lentil plants, and increased plant health would allow for more soil N scavenging and lower residual nitrate-N levels. However, it is recognized that nitrate-N is also affected by mineralization, and therefore several environmental conditions. In site-years with differences in N fixed amounts, no variation in post-harvest soil-N levels was detected. Soil sampling the following spring when some residue N has had time to mineralize may better reflect impacts of lentil N fixed amounts on soil N pools. Miller et al. (2003b) demonstrated that over-winter mineralization influences soil nitrate-N, as residual nitrate-N differences increased from fall to the following spring.

Conclusion

In this three-year study, inoculation and S fertilizer each increased N fixed by lentil in 40% of site-years. Effects of inoculation were unrelated to previous cropping histories. 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 40% 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 Montana could increase N fixation for many producers. Responses to inoculation and S fertilizer were sometimes relatively large, 37 and 38 kg N ha^{-1} using the ND method, respectively, which could have substantial benefits to lentil productivity and 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.

References

Abbasi, M.K., M.M. Tahir, W. Azam, Z. Abbas, and N. Rahim, 2012. Soybean yield and chemical composition in response to phosphorus-potassium nutrition in Kashmir. *Agron J*. 104(5):1476-1484.

Abdelhamid M.T., H.A. Kamel, and M.G. Dawood, 2010. Response of non-nodulating, nodulating, and super-nodulating soybean genotypes to potassium fertilizer under water stress. *J of Plant Nutr*. 34(11): 1675-1689.

Alam, F., T.Y. Kim, S.Y. Kim, S.S. Alam, P. Pramanik, P.J. Kim, Y.B. Lee. 2015. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* Roth). *Soil Sci and Plant Nutr*. 61(4):664-675.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J Stat Software*. 67(1):1-48.

Beckie H.J., and S.A. Brandt (1997) Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Can J Plant Sci*. 77:311-322.

Bestwick, M., K. Olson-Rutz, C. Jones, and P. Miller. 2018. Environmental and Management Effects on Pea Protein in Montana: A Research Report from the Montana Research and Economic Development Initiative. https://landresources.montana.edu/soilfertility/documents/PDF/reports/Bestwick2018MREDIEnvironMgtPeaProt.pdf

Bremer, E., H. Gehlen, G.D.W. Swerhone, and C. van Kessel. 1993. Assessment of reference crops for the quantification of N_2 fixation using natural and enriched levels of N-15 abundance. *Soil Biol Biochem.* 25(9):1197-1202.

Bremer, E., H.H. Janzen, B.H. Ellert, R.H. McKenzie. 2011. Carbon, nitrogen, and greenhouse gas balances in an 18-year cropping system study on the northern Great Plains. *Soil Sci Soc Am J*. 75:1493-1502.

Cazzato, E., V. Tufarelli, E. Ceci, A.M. Stellacci and V. Laudadio, 2012. Quality, yield and nitrogen fixation of faba bean seeds as affected by sulphur fertilization. *Soil & Plant Sci*. 62(8):732-738. doi: 10.1080/09064710.2012.698642

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Can J Plant Sci*. 84:79-88.

Collins, M., D.J. Lang, and K.A. Kelling. 1986. Effects of phosphorus, potassium, and sulfur on alfalfa nitrogen-fixation under field conditions. *Agron J*. 78(6):959-963.

Coyne, C., M. Grusak, L. Razai, and B. Baik. 2005. Variation for pea protein concentration in the USDA *Pisum* core collection. *Pisum Genetics*. 37:5-9.

Divito, G.A. and V.O. Sadras. 2014. How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crops Research*. 156:1161-171.

Engel, R. 2020. Soil acidity management of long-term no-till fields in Montana to prevent crop failure. USDA Sustainable Agriculture Research & Education. https://projects.sare.org/?export=1&project_report=644340

Freney, J.R. and F.J. Stevenson. Organic sulfur transformations in soils. 1966. *Soil Sci*. 101(4):307-316.

Gan, Y.T., C.A. Campbell, H.H. Janzen, R. Lemke, L.P. Liu, P. Basnyat, and C.L. McDonald. 2009. Root mass for oilseed and pulse crops: growth and distribution in the soil profile. *Can J Plant Sci.* 89:883-893.

Gollner, G., W. Starz, and J.K. Friedel. 2019. Crop performance, biological N fixation and pre-crop effect of pea ideotypes in an organic farming system. *Nutr Cycl Agroecosyst*. 115:391-405.

Hafeez, F.Y., N.H. Shah, and K.A. Malik. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biol Fert Soils*. 31:65-69.

Hamel, C., Y. Gan, S. Sokolski, and L.D. Bainard. 2018. High frequency cropping of pulses modifies soil nitrogen level and the rhizosphere bacterial microbiome in 4-year rotation systems of the semiarid prairie. *Applied Soil Eco*. 126:47-56.

Hauggaard-Nielson H, Holdensen L, Wulfsohn D, Jensen ES. 2010. Spatial variation of N-2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the N-15 natural abundance method. *Plant Soil*. 327 (1-2):167-184.

Hossain, A., A. Quddus, K. Alam, H.M. Naser, B. Anwar, F. Khatun, H. Rashid, F. Khatun, and A. Siddiky. 2020. Application of zinc, boron, and molybdenum in soil increases lentil productivity, nutrient uptake, and apparent balance. *Can J Soil Sci*. doi: https://doi.org/10.1139/cjss-2019-0141

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical J*. 50(3):346-363.

Huang, J., R.K. Afshar, and C. Chen. 2016. Lentil response to nitrogen application and rhizobia inoculation. *Communications in Soil Science and Plant Analysis*. 47(21):2458-2464.

Jacobsen J., G. Jackson, and C. Jones. 2003. Fertilizer guidelines for Montana crops. MSU Extension Bulletin EB161. Bozeman, MT. http://animalrange.montana.edu/documents/extension/eb161.pdf

Kaiser, D.E. and K.I. Kim, 2013. Soybean response to sulfur fertilizer applied as a broadcast or starter using replicated strip trials. *Agron J*, 105(4):1189-1198.

Kaschuk G., P.A. Leffelaar, K.E. Giller, O. Alberton, M. Hungria, and T.W. Kuyper. 2009. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. *Soil Biol & Biochem*. 42:125-127.

Kucey, R.M.N. and M.F. Hynes. 1989. Populations of *Rhizobium leguminorsarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. *Can J Microbiol*. 35(6):661-667.

Lenth, R.V. 2021. emmeans: Estimated Maginal Means, aka Least-Squares Means. R package version 1.6.3. https://CRAN.R-project.org/package=emmeans

Long, J.A., R.L. Lawrence, P.R. Miller, L.A. Marshall and M.C. Greenwood. 2014. Adoption of cropping sequences in northeast Montana: A spatio-temporal analysis. *Agric. Ecosys. Environ.* 197:77-87.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: Effect of planting and termination timing. *Nutr Cycl Agroecosys*. 92(3):305-314.

McConnell, J.T., P.R. Miller, R.L. Lawrence, R. Engel, and G.A. Nielsen. 2002. Managing inoculation failure of field pea and chickpea based on spectral responses. *Can J Plant Sci*. 82(2):273-282.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001. Response of pea to rate and placement of triple superphosphate fertilizer in Alberta. *Can J Plant Sci*. 81(4):645–649.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003a. Pulse crops for the northern Great Plains: II. Cropping sequence effects on cereal, oilseed, and pulse crops. *Agron J*. 95(4):980-986.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003b. Pulse crops for the northern Great Plains: I. Grain productivity and residual effects on soil water and nitrogen. *Agron J*. 95(4):972-979.

Miller, P.R., A. Bekkerman, C.A. Jones, M.H. Burgess, J.A. Holmes, and R.E. Engel. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. *Agron J*. 107(2):541-550.

Miller, P.R., S. Atencio, C. Jones, E. Eriksmoen, B. Franck, J. Rickertson, …, K. Baber. 2022. Lentil inoculant, potassium, sulfur, and micronutrient effects on yield and protein in the northern Great Plains. *Great Plains Soil Fertility Conference Proceedings*.

Nasar, J., A. Roshan, A. Alam, M.Z. Khan, B. Ahmad. 2019. The impact of foliage fertilization of iron and molybdenum on yield, N uptake and root nodulation of lentil (*Lens culinaris* Medik.) crop. *Indian J Ag Research*. 53(5):628-631.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Robinson, D. 2001. δ^{15}N as an integrator of the nitrogen cycle. Trends in Ecology & Evolution. 16(3):153-162.

Shearer, G. and D.H. Kohl. 1986. N-2 fixation in field settings – estimations based on natural N-15 abundance. *Aust J Plant Physiol*. 13(6):699-756.

Scherer, H.W., S. Pacyna, N. Manthey, M. Schulz, 2006. Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N_2 fixation. *Plant Soil Environ*. 52(2):72-77.

Sigler, W.A., S.A. Ewing, C.A. Jones, R.A. Payn, P. Miller, and M. Maneta. 2020. Water and nitrate loss from dryland agricultural soils is controlled by management, soils, and weather. Agriculure, Ecosystems & Environment. https://doi.org/10.1016/j.agee.2020.107158

Stevenson, F.C. and C. van Kessel. 1996. A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. *Soil Sci Soc Am J*. 60:1797-1805.

Unkovich, M.J., J.S. Pate, P. Sanford, E.L. Armstrong. 1994. Potentical precision of the delta-N-15 natural-abudannce method in-field estimates of nitrogen-fixation by crop and pasture legumes in south-west Australia. *Aust J Agric Res.* 45(1):119-132.

Unkovich, M., D.F. Herridge, M.B. Peoples, G. Cadisch, R.M. Boddey, K.E. Giller, B. Alves, and P.M. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph. Australian Centre for International Agricultural Research, Canberra, AU.

USDA National Agriculture Statistics Service. 2022. https://www.nass.usda.gov.

Vance C.P., 2008. Carbon and Nitrogen Metabolism in Legume Nodules. In: Dilworth M.J., James E.K., Sprent J.I., Newton W.E. (eds) Nitrogen-fixing Leguminous Symbioses. Nitrogen Fixation: Origins, Applications, and Research Progress, vol 7. Springer, Dordrecht.

Voisin, A., C. Salon, N.G. Munier-Jolain, and B. Ney. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 243:31-42.

Walley, F.L., G.M. Fu, J.W. van Groenigen, and C. van Kessel. 2001. Short-range spatial variability of nitrogen fixation by field-grown chickpea. *Soil Sci Soc Am J*. 65(6):1717-1722.

Walley, F.L., G.W. Clayton, P.R. Miller, P.M. Carr, and G.P. Lafond. 2007. Nitrogen economy of pulse crop production in the Northern Great Plains. *Agron J.* 99:1710-1718.

Warne, T., S. Ahmed, C.B. Shanks, and P. Miller. 2019. Sustainability dimensions of a North American lentil system in a changing world. *Front Sustain Food Syst.* https://doi.org/10.3389/fsufs.2019.00088

Wolyn, D.J., J. Attewell, P.W. Ludden, and F.A. Bliss. 1989. Indirect measures of N_2 fixation in common bean (*Phaseolus vulgaris* L.) under field conditions: The role of lateral root nodules. *Plant Soil*. 113:181-187.

Zentner, R., C. Campbell, V. Biederbeck, P. Miller, F. Selles, and M. Fernandez. 2001. In search of a sustainable cropping system for the semiarid Canadian prairies. *J Sustain Ag*. 18(2-3):117-136.

Zhao, F.J., A.P. Wood and S.P. McGrath, 1999. Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 212(2):209-219.

Tables

Table 2-1. General soil properties (sampled in spring of each year) for Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT) N fixation study sites, 2019-2021.

Soil property[a]	2019	2020	2021
	--------------------------- Post Farm ---------------------------		
Pea/lentil history (year)[b]	2017	None	None
Soil texture[c]	Silt loam	Silt loam	Silt loam
Soil pH (1:1 soil:water)	7.7	7.9	7.0
Organic C (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
Soil texture	Loam	Loam	Sandy loam/loam
Soil pH (1:1 soil:water)	8.1	6.5	8.3
Organic C (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

[a] Pre-seed average of 10 soil sample locations (2 samples per block), 0-15 cm unless noted
[b] 10 previous years
[c] From USDA-NRCS Web Soil Survey

Table 2-2. Fertilizer sources and rates for lentil fertility treatments.

Treatment	CFU[d] Rate	Fertilizer Source	K	S
	----- CFU g^{-1} -----		--- kg ha^{-1} ---	
Control	--	--		
Granular[a]	1 x 10^8	--		
Seed-coat[b]	2 x 10^8	--		
Granular+K	1 x 10^8	KCl	14	
Seed-coat+K	2 x 10^8	KCl	14	
Granular+K+S	1 x 10^8	Potassium sulfate	14	5.6
Seed-coat+K+S	2 x 10^8	Potassium sulfate	14	5.6
Gran+K+S+Micros[c]	1 x 10^8	Potassium sulfate + Micro1000	14	5.6

[a] Granular inoculant applied at 6.7 kg ha^{-1}

[b] Seed-coat inoculant applied at 0.31 kg per 100 kg seed

[c] 2020 and 2021 study years only

[d] Colony forming units of *Rhizobium leguminosarum* biovar *viceae*

Table 2-3. Nutrient concentrations in Micro1000 foliar product (AgroLiquid, St. Johns, MI).

Nutrient	Nutrient %	Rate (kg ha^{-1})[a]
Boron	0.02	0.0006
Copper	0.25	0.007
Iron	0.37	0.01
Manganese	1.0	0.028
Zinc	1.0	0.028
Calcium	1.0	0.028
Magnesium	0.5	0.014
Cobalt	0.1	0.003
Molybdenum	0.1	0.003
Nickel	0.001	0.00003

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

Table 2-4. Field activities and dates for Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT), 2019-2021 study sites.

Field Activity	Post Farm			Northern Agricultural Research Center		
	2019	2020	2021	2019	2020	2021
Spring soil sampling	25 Apr	20 Apr	7 May	15 Apr	20 Apr	23 Mar
Planting	3 May	21 Apr	8 Apr	22 Apr	25 Apr	8 Apr
Micro application	--[a]	2 July	21 Jun	--	1 July	17 Jun
Biomass sampling	12 Aug	30 July	9 July	25 July	17 July	25 Jun
Harvest	26 Aug	24 Aug	26 July	1 Aug	2 Aug	7 Aug
Fall soil sampling	28 Aug	28 Aug	30 July	25 Sep	10 Aug	12 Aug

[a] Foliar micronutrient treatment was not analyzed in 2019 due to application above label rate that caused plant injury

Table 2-5. Pesticide applications at Post Farm (PF) and Northern Agricultural Research Center (NARC), 2019-2021 study sites.

Site-Year	Timing/ Use	Date	Product	Company	Active Ingredient	Product Rate (mL ha^{-1})
PF 2019	Pre-plant Herbicide	23 Mar	Makaze	Loveland	Glyphosate	2339
	Pre-plant Herbicide	3 Apr	Prowl	BASF	Pendimethalin	1754
	Pre-plant Herbicide	3 Apr	Sharpen	BASF	Saflufenacil	73
	In-crop Insecticide	31 May	Warrior II	Syngenta	Lambda-cyhalothrin	143
PF 2020	Pre-plant Herbicide	20 Apr	Prowl	BASF	Pendimethalin	1754
	Pre-plant Herbicide	20 Apr	Sharpen	BASF	Saflufenacil	73
	In-crop Herbicide	23 Jun	Assure II	Dupont	Quizalofop p-ethyl	585
PF 2021	Pre-plant Herbicide	17 Mar	GlystarPlus	Albaugh	Glyphosate	2339
	In-crop Insecticide	25 May	Warrior II	Syngenta	Lambda-cyhalothrin	143
	In-crop Herbicide	7 Jun	Assure II	Dupont	Quizalofop p-ethyl	731
Havre 2019	None --	--	--	--	--	--
Havre 2020	Pre-plant Herbicide	20 Apr	Prowl	BASF	Pendimethalin	1754
	Pre-plant Herbicide	20 Apr	Sharpen	BASF	Saflufenacil	73
	In-crop Herbicide	4 Jun	Quiz	AgriCity	Quizalofop p-ehtyl	731
Havre 2021	Pre-plant Herbicide	16 Apr	RT3	Bayer	Glyphosate	2339
	Pre-Plant Herbicide	16 Apr	Prowl	BASF	Pendimethalin	1754
	Pre-Plant Herbicide	16 Apr	Sharpen	BASF	Saflufenacil	73
	In-crop Herbicide	4 Jun	Quiz	AgriCity	Quizalofop p-ehtyl	731

Table 2-6. Precipitation and temperature data for 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 ----------							
Sep-Mar[b]	150	210	120	161	-0.7	1.7	1.9	1.5
Apr	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 ----------							
Sep-Mar[b]	107	125	119	110	-2.3	0.0	0.9	0.7
Apr	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] 1 Apr – 31 July

Table 2-7. Means, summary of analysis of variance, and orthogonal contrasts for N fixed using N difference (ND) and ^{15}N natural abundance (NA) methods at Post Farm.

Treatment		N fixed (kg N ha^{-1}) 2019 ND	2019 NA	2020 ND	2020 NA	2021 ND	2021 NA
1	Control	136	109	87 c[a]	85 b	81	54
2	Granular	134	104	86 c	82 b	67	59
3	Seed-coat	160	124	104 b	85 b	78	57
4	Granular+K	151	114	91 bc	86 b	60	59
5	Seed-coat+K	145	117	86 c	84 b	74	57
6	Granular+K+S	157	129	125 a	116 a	69	58
7	Seed-coat+K+S	151	119	128 a	118 a	43	55
8	Gran+K+S+Micros	--	--	130 a	116 a	66	58
		P value					
		0.57	0.26	**<0.01**	**<0.01**	0.25	0.96
		Coefficient of Variation (%)					
		16.4	14.3	23.2	20.4	32.3	14.1
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]					
2, 3 vs. 1	Inoculant – Control	--	--	--	--	--	--
2, 4, 6 vs. 3, 5, 7	Granular – Seed-coat	--	--	--	--	--	--
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	--	--	38	32	--	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

[b] Only if significant at P=0.10

Table 2-8. Means, summary of analysis of variance, and orthogonal contrasts for N fixed using N difference (ND) and ^{15}N natural abundance (NA) methods at Northern Agricultural Research Center.

		N fixed (kg N ha^{-1})					
	Treatment	2019		2020[a]		2021	
		ND	NA	ND	NA	ND	NA
1	Control	28 b[b]	12 b	--	--	22	16 b
2	Granular	63 a	25 ab	--	--	34	25 ab
3	Seed-coat	66 a	38 a	--	--	35	26 ab
4	Granular+K	61 a	24 ab	--	--	26	23 ab
5	Seed-coat+K	41 a	14 ab	--	--	37	25 ab
6	Granular+K+S	58 a	28 ab	--	--	42	24 ab
7	Seed-coat+K+S	64 a	35 a	--	--	36	30 a
8	Gran+K+S+Micros	--	--	--	--	27	21 ab
		P value					
		<0.01	**0.06**	--	--	0.14	**0.06**
		Coefficient of Variation (%)					
		39.9	55.1	--	--	36.5	25.0
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})					
2, 3 vs. 1	Inoculant – Control	37	20	--	--	13	9
2, 4, 6 vs. 3, 5, 7	Granular – Seed-coat	--	--	--	--	--	--
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	--	12	--	--	--	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)
[b] 2020 data not included due to high and variable soil N

Table 2-9. Means, summary of analysis of variance, and orthogonal contrasts of aboveground N and S concentrations for +K and +K+S treatments at Post Farm, sampled 30 Jul 2020.

	Treatment	Shoot N (g kg^{-1})	Shoot S (g kg^{-1})
1	Granular+K	24.8 b[a]	0.58 b
2	Seed-coat+K	25.5 b	0.61 b
3	Granular+K+S	29.6 a	0.94 a
4	Seed-coat+K+S	29.7 a	1.01 a
		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})	
3, 4 vs. 1, 2	K+S – K only	4.5	0.38

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Table 2-10. Means, summary of analysis of variance, and orthogonal contrasts for biomass N at Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT).

Treatment		Biomass N (kg N ha^{-1})					
		Post Farm			Northern Agricultural Research Center		
		2019	2020	2021	2019	2020	2021
1	Control	164	120 b[a]	77	47 b	107	35 b
2	Granular	162	115 b	85	70 a	110	48 ab
3	Seed-coat	183	131 b	81	81 a	102	47 ab
4	Granular+K	170	121 b	85	76 a	102	41 ab
5	Seed-coat+K	179	118 b	80	81 a	112	47 ab
6	Granular+K+S	185	161 a	82	65 ab	108	54 a
7	Seed-coat+K+S	178	161 a	76	83 a	107	51 a
8	Gran+K+S+Micros	--	163 a	81	--	95	41 ab
		P value					
		0.55	**<0.01**	0.55	**<0.01**	0.55	**0.07**
		Coefficient of Variation (%)					
		12.8	23.8	11.9	23.8	11.9	26.8
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})					
2,3 vs. 1	Inoculant – Control	--	--	--	28	--	13
2,4,6 vs. 3,5,7	Gran. – Seed-coat	--	--	--	-9	--	--
4,5 vs. 2,3	K – No fertilizer	--	--	--	--	--	--
6,7 vs. 4,5	K+S – K only	--	42	--	--	--	8

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, $P < 0.1$)

Table 2-11. Means, summary of analysis of variance, and orthogonal contrasts for fraction of N derived from atmosphere (fNdfa; from ^{15}N natural abundance) at Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT).

Treatment		fNdfa					
		Post Farm			Northern Agricultural Research Center		
		2019	2020	2021	2019	2020	2021
1	Control	0.66	0.70	0.70	0.25	--[a]	0.49 bc[b]
2	Granular	0.64	0.71	0.70	0.32	--	0.52 abc
3	Seed-coat	0.67	0.65	0.70	0.41	--	0.54 abc
4	Granular+K	0.68	0.71	0.70	0.31	--	0.57 ab
5	Seed-coat+K	0.66	0.71	0.72	0.19	--	0.55 abc
6	Granular+K+S	0.69	0.73	0.71	0.42	--	0.47 c
7	Seed-coat+K+S	0.67	0.74	0.74	0.45	--	0.60 a
8	Gran+K+S+Micros	--	0.72	0.72	--	--	0.51 bc
		P value					
		0.77	0.19	0.79	0.21	--	**0.02**
		Coefficient of Variation (%)					
		7.8	7.0	6.2	43.3	--	13.2
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					
2,3 vs. 1	Inoculant – Control	--	--	--	0.17	--	--
2,4,6 vs. 3,5,7	Gran. – Seed-coat	--	--	-0.05	--	--	--
4,5 vs. 2,3	K – No fertilizer	--	--	--	--	--	--
6,7 vs. 4,5	K+S – K only	--	--	--	0.16	--	--

[a] 2020 data not included due to high and variable soil N

[b] Columns with the same letter are not significantly different (Tukey-Kramer HSD, $P < 0.1$)

Table 2-12. Means, summary of analysis of variance, and orthogonal contrasts for lentil seed yield at Post Farm and Northern Agricultural Research Center, 2019-2021.

Treatment		Seed Yield (kg ha^{-1})					
		Post Farm			Northern Agricultural Research Center		
		2019	2020	2021	2019	2020	2021
1	Control	2549 ab[a]	1975 c	789	721 c	2295	469 b
2	Granular	2803 a	2016 bc	808	1094 ab	2321	586 ab
3	Seed-coat	2692 a	1923 c	701	1154 a	2242	777 a
4	Granular+K	2677 ab	1926 c	710	1097 ab	2253	613 ab
5	Seed-coat+K	2604 ab	1898 c	730	1124 ab	2252	778 a
6	Granular+K+S	2571 ab	2251 a	773	1003 b	2230	692 ab
7	Seed-coat+K+S	2404 b	2129 ab	773	1153 a	2258	710 a
8	Granular +K+S+Micros	--	2239 a	750	--	2219	574 ab
		P value					
		0.03	**<0.01**	0.13	**<0.01**	0.48	**0.02**
Contrast		*P-value*					
2, 3 vs. 1	Inoc. – Control	0.12	1.00	0.37	**<0.01**	0.72	**<0.01**
2,4,6 vs. 3,5,7	Gran. – Seed-coat	0.21	**0.01**	0.21	**0.02**	0.99	**0.02**
4, 5 vs. 2, 3	K – No fertilizer	0.49	0.31	0.24	0.99	0.96	0.81
6, 7 vs. 4, 5	K+S – K only	0.16	**<0.01**	**0.07**	0.80	1.00	0.93
		Estimated Difference (kg ha^{-1})					
2, 3 vs. 1	Inoc. – Control	--	--	--	403	--	212
2,4,6 vs. 3,5,7	Gran. – Seed-coat	--	81	--	-79	--	-125
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	--	221	53	--	--	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Table 2-13. Means, summary of analysis of variance, and orthogonal contrasts for seed protein concentration at Post Farm and Northern Agricultural Research Center, 2019-2021.

Treatment		Protein Concentration (g kg^{-1})					
		Post Farm			Northern Agricultural Research Center		
		2019	2020	2021	2019	2020	2021
1	Control	250 b[a]	254	--[b]	196 c	252 ab	--
2	Granular	252 b	258	--	211 b	252 ab	--
3	Seed-coat	252 b	258	--	222 ab	252 ab	--
4	Granular+K	252 b	259	--	219 ab	250 b	--
5	Seed-coat+K	252 b	264	--	225 ab	252 ab	--
6	Granular+K+S	258 a	260	--	217 b	253 a	--
7	Seed-coat+K+S	260 a	266	--	230 a	254 a	--
8	Granular+K+S+Micros	--	264	--	--	255 a	--
		P value					
		<0.01	0.42	--	**<0.01**	**0.02**	--
Contrast		*P-value*					
2, 3 vs. 1	Inoc. – Control	0.79	0.90	--	**<0.01**	0.84	**--**
2,4,6 vs. 3,5,7	Gran. – Seed-coat	1.00	0.64	--	**<0.01**	0.22	--
4, 5 vs. 2, 3	K – No fertilizer	1.00	0.86	--	0.24	0.14	--
6, 7 vs. 4, 5	K+S – K only	**<0.01**	0.99	--	0.99	**<0.01**	--
		Estimated Difference (g kg^{-1})					
2, 3 vs. 1	Inoc. – Control	--	--	--	20	--	--
2,4,6 vs. 3,5,7	Gran. – Seed-coat	--	--	--	-10	--	--
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	7	--	--	--	2	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

[b] 2021 protein data not available at time of writing

Table 2-14. Means, summary of analysis of variance, and orthogonal contrasts for protein yield at Post Farm and Northern Agricultural Research Center, 2019-2021.

Treatment		Protein Yield (kg ha^{-1}) Post Farm 2019	Post Farm 2020	Post Farm 2021	Northern Agricultural Research Center 2019	Northern Agricultural Research Center 2020	Northern Agricultural Research Center 2021
1	Control	638	502 b[a]	--[b]	156 c	498	--
2	Granular	706	520 b	--	255 ab	523	--
3	Seed-coat	677	496 b	--	284 a	505	--
4	Granular+K	676	499 b	--	267 ab	503	--
5	Seed-coat+K	656	501 b	--	266 ab	507	--
6	Granular+K+S	664	583 a	--	241 b	504	--
7	Seed-coat+K+S	620	566 a	--	294 a	511	--
8	Granular+K+S+Micros	--	590 a	--	--	498	--
				P value			
		0.12	**<0.01**	--	**<0.01**	0.81	--
Contrast				*P-value*			
2, 3 vs. 1	Inoc. – Control	**0.03**	0.58	--	**<0.01**	0.27	--
2,4,6 vs. 3,5,7	Gran. – Seed-coat	**0.07**	**0.08**	--	**<0.01**	0.81	--
4, 5 vs. 2, 3	K – No fertilizer	0.20	0.37	--	0.91	0.42	--
6, 7 vs. 4, 5	K+S – K only	0.24	**<0.01**	--	0.73	0.78	--
				Estimated Difference (kg ha^{-1})			
2, 3 vs. 1	Inoc. – Control	54	--	--	113	--	--
2,4,6 vs. 3,5,7	Gran. – Seed-coat	32	13	--	-29	--	--
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	--	75	--	--	--	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

[b] 2021 protein data not available at time of writing

Table 2-15. Pearson correlation coefficients (r values) between N fixation parameters and seed yield and/or protein at Post Farm (PF) and Northern Agricultural Research Center (NARC).

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	--[a]	--
		NA	0.04	--	--
		Biomass N	0.21	--	--
		fNdfa	**0.35**	--	--
	NARC	ND	**0.64**	--	--
		NA	**0.58**	--	--
		Biomass N	**0.73**	--	--
		fNdfa	**-0.39**	--	--

Bold values indicate correlation coefficient different from 0 ($P < 0.1$)

[a] Protein and protein yield data not available at time of writing

Table 2-16. Means, summary of analysis of variance, and orthogonal contrasts for post-harvest nitrate-N at Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT).

Treatment		Nitrate-N (kg N ha^{-1})					
		Post Farm			Northern Agricultural Research Center		
		2019	2020	2021	2019	2020	2021
1	Control	13.5 ab[a]	6.0	62.7 a	35.6	163.2	14.6
2	Granular	18.9 ab	5.0	48.9 ab	40.3	183.1	17.7
3	Seed-coat	9.2 b	7.1	46.6 ab	36.2	150.1	15.8
4	Granular+K	19.9 a	5.2	43.2 ab	34.9	151.1	14.4
5	Seed-coat+K	12.6 ab	6.4	58.1 ab	38.5	160.3	19.0
6	Granular+K+S	14.7 ab	6.5	48.3 ab	36.3	167.0	18.7
7	Seed-coat+K+S	14.7 ab	6.9	32.0 b	34.1	168.8	18.3
8	Gran+K+S+Micros	--	4.7	41.8 ab	--	200.7	16.0
		P value					
		0.10	0.68	**0.08**	0.97	0.85	0.44
		Coefficient of Variation (%)					
		42.2	40.1	42.8	22.9	35.2	21.7
Contrast		*P value*					
2, 3 vs. 1	Inoculant – Control	0.85	0.99	**0.10**	0.64	0.91	0.35
2,4,6 vs. 3,5,7	Gran. – Seed-coat	**0.01**	0.18	0.45	0.79	0.72	0.61
4, 5 vs. 2, 3	K – No fertilizer	0.40	0.80	0.86	0.70	0.66	0.98
6, 7 vs. 4, 5	K+S – K only	0.55	0.41	0.12	0.80	0.62	0.32
		Estimated Difference (kg N ha^{-1})					
2, 3 vs. 1	Inoculant – Control	--	--	-12	--	--	--
2,4,6 vs. 3,5,7	Gran. – Seed-coat	6	--	--	--	--	--
4, 5 vs. 2, 3	K – No fertilizer	--	--	--	--	--	--
6, 7 vs. 4, 5	K+S – K only	--	--	--	--	--	--

[a] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Figures

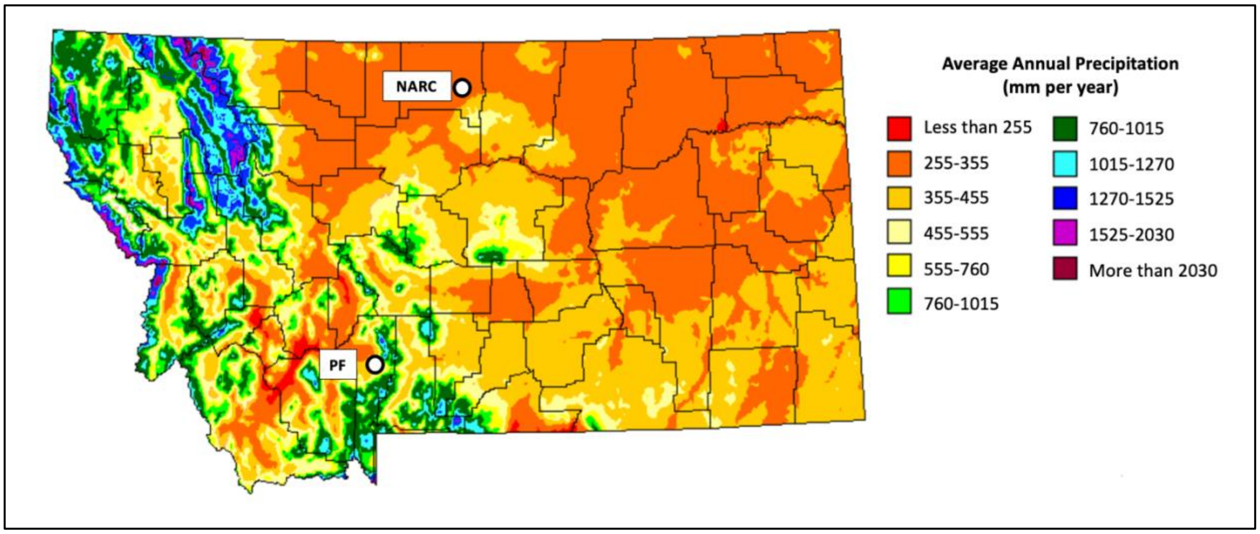

Figure 2-1. Annual precipitation map for Montana, USA, with study sites PF (Post Farm near Bozeman) and NARC (Northern Agricultural Research Center near Havre). Data and map from PRISM Climate Group, Oregon State University.

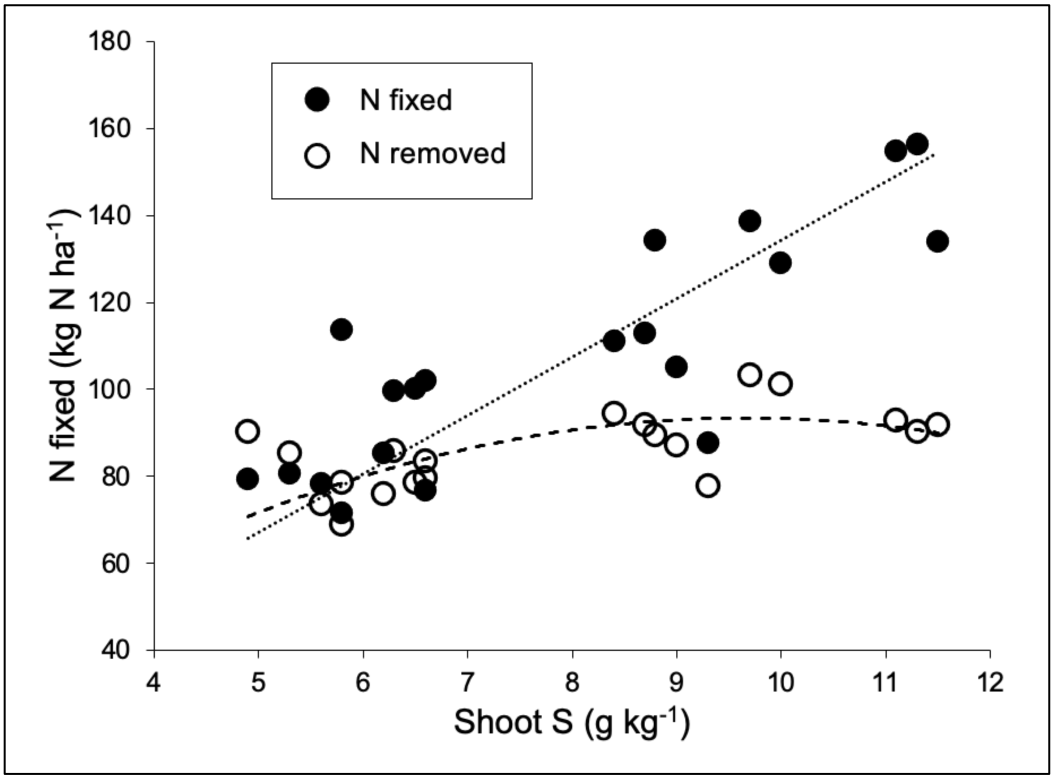

Figure 2-2. Relationship between shoot S concentration and N fixed (ND method) and N removed in grain at Post Farm in 2020. The difference between the two curves can be viewed as the net benefit to the soil from N fixation.

CHAPTER THREE

ASSESSMENT OF NITROGEN FIXATION AMONG PEA AND LENTIL VARIETIES IN THE SEMIARID NORTHERN GREAT PLAINS

Contribution of Authors and Co-Authors

Manuscript in Chapter 3

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Contributions: Literature review, data collection, data analysis

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Abstract

Pulse crops, including lentil and pea, can improve the sustainability of northern Great Plains cropping systems, namely through biological nitrogen (N) fixation. By increasing N fixed by lentil and pea, producers can achieve greater seed yield and decrease N fertilizer inputs for subsequent crops. The objective of this study was to assess differences in N fixation among lentil and pea varieties. Studies were conducted at two sites in Montana from 2019 to 2021. Amounts of N fixed were calculated using an N difference approach and the ^{15}N natural abundance method. Nitrogen fixation was largely influenced by environmental conditions, as more precipitation resulted in greater N fixed amounts. Lentil varieties varied in N fixed in three of four site-years, while pea varieties varied in two of four site-years. Differences were as large as 45 kg N ha^{-1} for pea. CDC Richlea and Riveland were frequently high N-fixing lentil varieties, but no pea variety emerged as being consistently different from the others. Correlations between N fixation parameters and seed yield were stronger for pea than lentil, suggesting that breeding for greater yields may increase N fixation for pea but not lentil. Breeding lentil and pea based on these results is likely difficult as varieties performed differently under variable environmental conditions.

Introduction

Nitrogen (N) is widely accepted as the most limiting nutrient to plant growth. Consequently, humans globally apply over 108 teragrams 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, which can lead to crop failure from aluminum or manganese toxicity (Brown et al., 2008). Through biological N fixation, pulse crops such as pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medikus) incorporated into cereal production systems can decrease N input needs while serving as a profitable commodity for producers.

Pulse crops, legumes harvested for their dry seed, are well adapted to NGP semiarid growing conditions, and have increased in popularity in recent years, with planted area in Montana increasing ten-fold from 2000 to 2020 (NASS, 2020). Pulse crops are often grown in place of 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 (Stevenson and van Kessel, 1996; Beckie and Brandt, 1997; Miller et al., 2003; Bremer, 2011; Arcand et al., 2014) and protein (Miller et al., 2003; Bremer, 2011) when grown after pulse crops, and some have attributed the benefits to N fixation by pulse crops (Beckie and Brandt, 1997; Arcand et al., 2014). While a majority of fixed N is removed from the system during grain harvest (Hossain et al., 2017), pulse residues left in the field are more readily mineralized than cereal residues because of their lower C to N ratio, resulting in more N available for subsequent crop uptake (Lupwayi et al., 2004). By increasing N fixed by pulse crops, producers may be able to decrease N fertilizer rates for the subsequent crop. Additionally, growing a pulse in place of a cereal or oilseed results in fertilizer N savings because pulses require little to no N inputs. Producers can 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 been 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 total N total N derived from the atmosphere (fNdfa; Abi-Ghanem et al., 2011; Zimmer et al., 2016; Hossain et al., 2017), number of root nodules (Abi-Ghanem et al., 2011), total plant N (Abi-Ghanem et al., 2013), and N fixed amounts (Hossain et al., 2017). This provides evidence that N fixation could be a trait among pulse varieties that could be increased through breeding.

Despite the promise of focusing on N fixation amounts as a measurable trait for lentil and pea breeding programs, only one such study 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 amounts of N fixed, biomass N accumulation, and fNdfa among regionally-grown lentil and pea varieties. Other objectives were to investigate the relationship between N fixation and seed yield and to evaluate differences in post-harvest soil nitrate-N pools among lentil and pea varieties.

Materials and Methods

Site Selection and Experimental Design

Field experiments were conducted at Montana State University's Arthur H. Post Agronomy Research Farm (PF) near Bozeman, MT (45.68°N, 111.15°W) and Northern Agricultural Research Center (NARC) near Havre, MT (48.49°N, 109.80°W) from 2019 to 2021.

Historically, these two sites approximately span the precipitation gradient of Montana's dryland agricultural areas (Figure 3-1). The soil type at PF was Amsterdam silt loam (frigid Typic Haplustoll) in all years. Soil types at NARC were Telstad-Joplin loam (frigid Aridic Argiustoll) in 2019 and 2020 and Kenilworth-Fort Benton fine sandy loam (frigid Aridic Argiustoll) in 2021. Site selection was based on soil parameters of nitrate-N (0.12M KCl, Cd reduction), exchangeable K (1M ammonium acetate, ICP), sulfate-S (0.12M KCl, turbidimetric), Olsen P (0.5M $NaHCO_3$, colorimetric), and soil organic matter (loss on ignition) to identify locations within suitable fields collected the fall prior that had relatively similar fertility levels (analysis by AgVise Laboratories, Northwood, ND, USA). 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, sulfate-S, and nitrate-N (AgVise Laboratories, Northwood, ND, USA). Soil characteristics and pulse crop histories are summarized in Table 3-1. Ten regionally-grown varieties of lentil and pea were each grown in a randomized complete block design with four replicates (Table 3-2).

<u>Plot Management</u>

Pulses were seeded into no-till managed fields with standing cereal stubble 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 8 by 2 m. Dates for planting and other field activities are shown in Table 3-3. Seeds were inoculated with commercial *Rhizobia leguminosarum* biovar. *viceae* and treated with fungicide (mefenoxam and fludioxonil) according to manufacturer label. A reference crop of flax was grown adjacent to pulse plots. 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

($CaSO_4 \cdot 2H_2O$) was applied in furrow to provide 8 kg S ha^{-1} to prevent S deficiencies in these low soil sulfate-S soils (1-2 mg kg^{-1}). Pre-plant (glyphosate, pendimethalin, saflufenacil) and in-crop (quizaloflop p-ethyl) herbicides were applied to lentil and pea plots.

Data Collection

A representative area, consisting of three 1-m rows at PF for an area 0.69 m^2 or two 1-m rows at NARC for an area of 0.61 m^2, from each pulse plot was sampled for 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 in 2021 and issues encountered with variable soil nitrate-N pools in 2020 at NARC, a flax sample was harvested for each pea plot at PF in 2021. Biomass samples were dried (50°C, 72h), weighed, and ground (< 0.5 mm). Subsamples were analyzed for N concentration via combustion (LECO, St. Joseph, MI, USA), and approximately 10% of samples were analyzed in duplicate to assess precision (RSD averaged 2.5%). Isotope ^{15}N was analyzed using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (RSD averaged 2.4%; Sercon Ltd., Cheshire, UK).

Plots were harvested with a combine, excluding edge rows, and pulse seeds were cleaned and weighed. Seed yield (kg ha^{-1}) was determined on a dry matter basis accounting for seed moisture. Shortly after pulses were harvested, soil was sampled to 90 cm depth (3.1-cm diameter core) in each pulse plot, and at each location where flax was sampled, and split in the field into three 30-cm segments. Soil was also sampled in the same manner from each location where flax biomass was harvested. Soils were dried (50°C, 72h), weighed, and bulk density calculated. The soils were ground (< 2 mm), extracted using 1M KCl, and analyzed for nitrate-N with a flow injection colorimetric analyzer (Lachat, Loveland, CO, USA). Nitrate-N pool (kg N ha^{-1}) for each 90-cm sample was calculated.

Measuring N Fixation

Aboveground N fixed, in kg N ha^{-1}, 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 biomass N of flax was unable to be quantified in 2019 due to poor biomass production. The ND method used the following equation as reported by Unkovich et al. (2008) with all units in kg N ha^{-1}.

$$N\ fixed = \left(Biomass\ N_{pulse} - Biomass\ N_{flax}\right) + \left(Soil\ N_{pulse} - Soil\ N_{flax}\right) \quad [1]$$

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 pulse harvest.
A lower bound of 0 was used because it is not possible to have negative N fixed amounts. An upper bound on N fixed was set as the pulse plot's aboveground biomass N amount. Amounts of N fixed outside the set bounds (12.5% of ND observations) were omitted from analysis.

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):

$$fNdfa = \left(\frac{\delta^{15}N_{reference} - \delta^{15}N_{legume}}{\delta^{15}N_{reference} - B}\right) \quad [2]$$

Where $\delta^{15}N$ is:

$$\delta^{15}N\ (‰) = \left(\frac{atom\%N_{sample} - atom\%N_{atmosphere}}{atom\%N_{atmosphere}}\right) * 1000 \quad [4]$$

The B in Eq. 2 refers to the $\delta^{15}N$ 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, obtained in a greenhouse experiment detailed in Appendix A, were used in our study. Amounts of N fixed for the NA method were calculated using Eq. 4.

$$N\ fixed = Biomass\ N_{pulse} * fNdfa \qquad [4]$$

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 N, fNdfa, yield, and post-harvest soil nitrate-N) were fit using variety as a fixed effect and block as a random 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. Therefore, site-years were analyzed independently for variety effects using analysis of variance (ANOVA). Differences among varieties were further evaluated using Tukey-Kramer 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) between N fixation parameters (N fixed using both methods, fNdfa, biomass N) and yield.

Results and Discussion

Site Characterization

Yearly weather varied substantially at both sites over the course of the study (Table 3-4). 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 were droughty, with 2021 being more intense due to low over-winter precipitation and hotter than average temperatures. Drought was particularly severe at NARC in 2021, where the rainfall total for June, a pivotal month for pulse growth in the NGP, was 9% of the long-term average. Pea data at NARC in 2019 were not collected due to heavy deer grazing, and at PF in 2020 lentil data 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 residue and cool April temperatures.

Evaluation of N Fixation Methods

Both the ND and NA methods of estimating N fixed amounts require assumptions in the field to be met. The ND method is invalidated when the pulse and reference crops have access to different pools of soil nitrate-N; this would happen when pre-seed nitrate-N are different between pulse and reference crops (Unkovich et al., 2008). At NARC in 2020 and PF in 2021, spring soil nitrate-N was high and variable (Table 3-1), which resulted in large differences in soil N pools between pulse and flax. There was little confidence in results using the ND method for these two site-years and they were 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. Additionally, accuracy of the NA method is affected when there are only small differences between pulse and reference crop $\delta^{15}N$ (Bremer et al., 1993). Based on observed nitrate-N variability and low flax $\delta^{15}N$ at NARC in 2020, confidence in results was compromised and results are not reported here for either crop.

When used with confidence, amounts of N fixed between methods in the same site-year were similar. Using both methods increases confidence in N fixed amounts (McCauley et al., 2012). Overall, the NA method seemed to better predict differences among varieties in our study, whereas McCauley et al. (2012) favored the ND method when estimating N fixed at different times over the growing season. Estimations of N fixed at our field sites were often impacted by variable soil nitrate-N, especially using the ND method.

N Fixation, Biomass N, and fNdfa

Climate and site influenced N fixed amounts of lentil and pea (Tables 3-5 and 3-6). Overall, amounts of N fixed by lentil and pea varieties trended higher at PF than NARC, which corresponds to the wetter climate at PF. The 2019 growing season was favorable at both sites and resulted in high N fixed amounts (NA method) by lentil at PF, with a mean of 126.5 kg N ha^{-1}. In 2021, a drought year, the mean lentil N fixed amount (NA method) at PF was 40.6 kg N ha^{-1}. The difference between years was less drastic at NARC, with a 2019 N fixed (NA method) mean of 36.2 kg N ha^{-1} and 2021 means of 22.6 and 23.0 kg N ha^{-1}, for ND and NA, respectively. At PF, N fixed by pea trended higher in 2019 and 2020 than in 2021. While 2020 and 2021 had similar cumulative precipitation over the growing season, 2020 likely had higher N fixed amounts because of timely rainfall in June. Under water stress, N fixation decreases before other plant physiological processes (King and Purcell, 2001; McCauley et al., 2012). 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), and under water stress pulses likely prioritize C allocation to other tissues over nodules to successfully complete their growth cycle, since N is likely less limiting.

Lentil variety had a significant effect ($P < 0.1$) on N fixed in three of four site-years using NA but not in the one site-year using ND. In 2019 at NARC, CDC Richlea fixed an average of

19.8 kg ha^{-1} more N by NA than CDC Robin and ND Eagle, and CDC Maxim fixed 18.9 kg ha^{-1} more N than CDC Robin. In 2021, CDC Richlea and Riveland were among the top N-fixing varieties at both sites. Riveland and CDC Richlea fixed 12.0 and 8.2 kg ha^{-1} more N by NA, respectively, than CDC Impala at NARC in 2021, and 18.3 and 21.7 kg ha^{-1} more N than CDC Impala at PF in the same year.

Biomass N amounts differed among lentil varieties in three of five site-years (Table 3-7). 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 groupings. CDC Richlea and Riveland were two of the larger-seeded varieties tested, which may explain the robust growth in the early growing season at NARC in 2021 compared to other varieties (Figure 3-2). 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, there was little overlap between varieties with high fNdfa values and greater N fixed amounts.

The amount of N fixed by pea varieties varied in two of four site-years using NA but did not vary in any site-year using ND. In 2019 at PF, Arvika (the only forage pea tested) fixed on average 24.5 more kg N ha^{-1} than the five lowest varieties (Carousel, Cruiser, Delta, DS Admiral, Majoret) by NA. Arvika, a variety that flowers later in the season, likely benefitted from consistent precipitation later in the 2019 growing season and accumulated more biomass N. In 2020 at PF, N fixed varied among pea varieties using NA but not ND. In this drier year, Arvika was not among the high or low N-fixing varieties, while Delta fixed much more N (47.2 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 it was actively fixing N compared to other varieties.

At PF in 2019 and 2020, where differences in N fixed among pea varieties were observed using NA, fNdfa values also varied with similar trends (Table 3-8). 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 five site-years, yet this did not translate to differences in N fixed using either method.

Two lentil varieties (Pennell and Riveland) and two pea varieties (Delta and Lifter) studied by Abi-Ghanem et al. (2011) were also 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 ours was a field study and crops accessed more mineral N. Comparing overlapping varieties in the two studies, both found no differences in fNdfa between the two lentil varieties and the two pea varieties in any year, indicating that the lower fNdfa values in our study likely did not affect differences in fNdfa among varieties.

Amounts of N fixed can vary among varieties within legume crop species (Hossain et al., 2017; Zimmer et al., 2016). Nitrogen fixation of organic pea varieties did not vary in a temperate environment, resulting in fNdfa < 0.50 (Gollner et al., 2019). Estimated N fixed amounts are influenced by fNdfa (using NA) and biomass N (using both methods). 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 amounts of N fixed when the crops accumulated more biomass N. In our study, lentil varieties with greater biomass N accumulation had greater amounts of fixed N 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 (by NA) when they had higher fNdfa. In a western Canada field study, trends in fNdfa often matched trends in quantities of N fixed (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, 2011). In our study, no variety qualitatively had more or less root nodules based on visual observations. However, successfully digging up pulse root systems proved difficult given the hard, dry soil conditions at both field sites.

Seed Yield

Seed yield varied ($P < 0.1$) for all four site-years with data among lentil and pea varieties (Table 3-9). 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. In 2021 at the same site, CDC Impala, CDC Rosetown, and ND Eagle were the three highest yielding varieties. The site-year with the greatest lentil yields overall was PF in 2019, highlighting the 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. 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 years, but Cruiser had greater yield relative to other varieties only at PF in 2019, the wettest site-year. 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 three-year study, but cultivar characteristics that may have explained the interaction were not addressed.

N Fixation and Yield Relationships

Overall, correlation coefficients of N fixation parameters (biomass N, fNdfa, N fixed using ND and NA) and seed yield were inconsistent in direction and strength, ranging from -0.19 to 0.65 (Table 3-10). This suggests that factors other than N fixation also influenced seed yield. This is especially surprising in 2019, when favorable growing conditions encouraged robust growth and N would be expected to be a limiting growth factor. 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 23 correlations (11 lentil and 12 pea), nine were different from 0 ($P < 0.1$). For lentil, 36% of regressions resulted in negative correlations and occurred in both wet (2019) and dry (2021) growing seasons. 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, 75% of pea N fixation parameters had positive relationships with yield, while just 29% of lentil N fixation parameters were positively related with 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. Theoretical models, however, indicate that intensive breeding and domestication has diminished legume crops' capacity for N fixation, as breeding programs often utilize soils with high mineral N and inadvertently select lower N-fixing lines (Liu et al. 2020). Our results suggest that modern pea varieties in the NGP may fix more N when they yield higher. Higher yielding varieties will have higher N demand, driving N fixation in the absence of soil N. For lentil varieties, the relationship between N fixation and yield is less predictable, indicating a potential physiological difference between pea and lentil yield formation. Overall, our results show that N fixation and yield relationships for pulse crops are complex and likely impacted by environmental conditions.

Residual Soil Nitrate-N

Post-harvest soil nitrate-N levels rarely varied among varieties (Table 3-11). In 2019 at NARC, soils from plots where lentil varieties CDC Maxim and CDC Robin were grown had nitrate-N levels 7.8 kg ha^{-1} less than plots where CDC Viceroy was grown. At PF in 2019, plots with Aragorn and Lifter had the highest nitrate-N levels (23.4 and 24.5 kg ha^{-1}, respectively), and these were greater than all other pea varieties. Measuring soil nitrate-N levels after harvest (typically late summer) likely did not detect the majority of soil N contributions from N fixation, meaning these data do not reflect soil N inputs from that year's pulse crop. Increasing the frequency of pulse crops in a cropping rotation increases soil mineral N in the long-term (Hamel et al., 2018); however, residues must first decompose for N to mineralize. Mineralization can be highly variable and is dependent on soil and environmental conditions, so soil N inputs from pulse crops are difficult to assess, especially in the short-term (Walley et al., 2007). In our study, we expected that healthier pulse crops would be more robust soil N scavengers and would have lower post-harvest soil nitrate-N levels. However, the varieties with the highest seed yields and biomass production did not align with the varieties with the lowest soil nitrate-N levels after harvest. We soil sampled post-harvest primarily to calculate N fixed using the ND method. Soil sampling in the spring, after some organic N including pulse residue N has had time to mineralize, may be a better approach to observe varietal effects on soil nitrate-N levels.

Conclusion

In this three-year study, lentil and pea varietal effects on N fixed amounts and seed yield varied considerably depending on environmental conditions. CDC Richlea and Riveland emerged as the most consistent high N-fixing lentil varieties and may have benefitted from an early nutritive advantage from their relatively large seed sizes, although Pennell, which has a large seed size, had intermediate amounts of N fixed. Overall, there was less variation in N fixed among pea varieties than lentil varieties, and no pea variety was consistently found to perform particularly poorly or well. Correlations between N fixation parameters and seed yield were weak to moderate, with correlation coefficients ranging from -0.19 to 0.65. Correlations trended stronger for pea than lentil, suggesting pea N fixation may benefit from breeding for higher yield.

This study was the first field evaluation of N fixed amounts by lentil and pea varieties in Montana, and among the first in the NGP. When N fixed amounts varied among varieties, differences were sometimes relatively large (up to 45 kg N ha^{-1}), which could have substantial benefits to agroecosystem N availability. However, because varieties performed inconsistently among site-years, breeding for N fixation based on these results will be challenging. Producers can reference our results when selecting optimal varieties depending on production goals and their specific growing environment. If growing pulses for grain, producers could use N fixation data to help decide between varieties with similar yields. For those growing pulses as cover crops, the highest N-fixing variety would be best if their goal is to build soil N.

References

Abi-Ghanem, R., L. Carpenter-Boggs, and J.L. Smith. 2011. Cultivar effects on nitrogen fixation in peas and lentils. *Biol Fertil Soils*. 47:115-120.

Abi-Ghanem, R., E.T. Bodah, M. Wood, and K. Braunwart. 2013. Potential breeding for high nitrogen fixation in *Pisum sativum* L.: Germplasm phenotypic characterization and genetic investigation. *Am J Plant Sci*. 4:1597-1600.

Andersen, L., T. Warkentin, O. Philipp, A. Xue, and A. Sloan. 2002. DS Admiral field pea. *Can J Plant Sci*. 82:751-752.

Arcand, M.M., R. Lemke, R.E. Farrell, and J.D. Knight. 2014. Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. *Biol Fertil Soils*. 50:507-515.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J Stat Software*. 67(1):1-48.

Beckie H.J., and S.A. Brandt (1997) Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Can J Plant Sci*. 77:311-322.

Bremer E., H.H. Janzen, B.H. Ellert, R.H. McKenzie. 2011. Carbon, nitrogen, and greenhouse gas balances in an 18-year cropping system study on the northern Great Plains. *Soil Sci Soc Am J*. 75:1493-1502.

Bremer, E., H. Gehlen, G.D.W. Swerhone, and C. van Kessel. 1993. Assessment of reference crops for the quantification of N_2 fixation using natural and enriched levels of N-15 abundance. *Soil Biol Biochem.* 25(9):1197-1202.

Brown, T.T., R.T. Koenig, D.R. Huggins, J.B. Harsh, and R.E. Rossi, 2008. Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. *Soil Sci Soc Am J*. 72:634-640. doi:10.2136/sssaj2007.0061.

Carlyle, W.J. 1997. The decline of summerfallow on the Canadian prairies. *The Canadian Geographer*. 3:267-280.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Can J Plant Sci*. 84:79-88.

FAO, 2018. Inputs: Fertilizers by nutrient. http://www.fao.org/faostat/en/#data/RFN (accessed 16 September 2020).

Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, B.J. Cosby. 2003. The nitrogen cascade. *BioScience.* 53(4):341-356.

Gollner, G., W. Starz, J.K. Friedel. 2019. Crop performance, biological N fixation and pre-crop effect of pea ideotypes in an organic farming system. *Nutr Cycl Agroecosyst*. 115:391-405.

Hamel, C., Y. Gan, S. Sokolski, and L.D. Bainard. 2018. High frequency cropping of pulses modifies soil nitrogen level and the rhizosphere bacterial microbiome in 4-year rotation systems of the semiarid prairie. *Applied Soil Eco*. 126:47-56.

Hauggaard-Nielson H, Holdensen L, Wulfsohn D, Jensen ES. 2010. Spatial variation of N-2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the N-15 natural abundance method. *Plant Soil*. 327 (1-2):167-184.

Herridge, D. and I. Rose. 2000. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Research*. 65(2-3):229-248.

Hossain, Z., X. Wang, C. Hamel, J.D. Knight, M.J. Morrison, and Y. Gan. 2017. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can J Plant Sci*. 97:119-131.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical J*. 50(3):346-363.

John, A.A., C.A. Jones, S.A. Ewing, W.A. Sigler, A. Bekkerman and P.R. Miller. 2017. Fallow replacement is more important than nitrogen fertilizer management at reducing nitrate leaching in a semiarid region. *Nutr Cycl Agroecosys*. 108:279- 296.

King, C.A., and L.C. Purcell. 2001. Soybean nodule size and relationship to nitrogen fixation response to water deficit. *Crop Sci*. 41:1099–1107.

Liu, A., C.A. Contador, K. Fan, and H.M. Lam. 2018. Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes. *Front Plant Sci*. doi: 10.3389/fpls.2018.01860

Liu, J., X. Yu, Q. Qin, R.D. Dinkins, and H. Zhu. 2020. The impacts of domestication and breeding on nitrogen fixation symbiosis in legumes. *Front Genet.* https://doi.org/10.3389/fgene.2020.00973

Long, J.A., R.L. Lawrence, P.R. Miller, L.A. Marshall and M.C. Greenwood. 2014. Adoption of cropping sequences in northeast Montana: A spatio-temporal analysis. *Agric Ecosys Environ*. 197:77-87.

Lupwayi, N.Z., G.W. Clayton, J.T. O'Donovan, K.N. Harker, T.K. Turkington, and W.A. Rice. 2004. Decomposition of crop residues under conventional and zero tillage. *Can J Soil Sci*. 84(4):403-410.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: effect of planting and termination timing. *Nutr Cycl Agroecosys*. 92(3):305-314.

McPhee, K.E. and F.J. Muehlbauer. 2002. Registration of 'Lifter' green dry pea. *Crop Sci*. 42(4):1377-1378.

McPhee, K.E. and F.J. Muehlbauer. 2009. Registration of 'Riveland' lentil. *J Plant Registrations*. 3(1):5-9.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003. Pulse crops for the northern Great Plains: II. Cropping sequence effects on cereal, oilseed, and pulse crops. *Agron J*. 95(4):972-979.

Muehlbauer, F.J. and K.E. McPhee. 2004. Registration of 'Pennell' lentil. *Crop Sci*. 44(4):1488.

National Agriculture Statistics Service. 2020. https://www.nass.usda.gov.

Pan, B., S.K. Lam, A. Mosier, Y. Luo, and D. Chen. 2016. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: A global synthesis. *Agric, Ecosys and Environ*. 232:283-289.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Romero, C.M, R.E. Engel, J. D'Andrilli, P.R. Miller, and R. Wallander. 2019. Compositional tracking of dissolved organic matter in semiarid wheat-based cropping systems using fluorescence EEMs-PARAFAC and absorbance spectroscopy. *J Arid Environs*. 167:34-42.

Shearer, G. and D.H. Kohl. 1986. N-2 fixation in field settings – estimations based on natural N-15 abundance. *Aust J Plant Physiol*. 13(6):699-756.

Unkovich M., D.F. Herridge, M.B. Peoples, G. Cadisch, R.M. Boddey, K.E. Giller, B. Alves, and P.M. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph. Australian Centre for International Agricultural Research, Canberra, AU.

Unkovich, M.J., J. Baldock, and M.B. Peoples. 2010. Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. *Plant Soil*. 329(1-2):75-89.

Vandenberg, A. and A.E. Slinkard. 2002. CDC Mozart field pea. *Can J Plant Sci*. 82:429-431.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, J. Wahab, and A.E. Slinkard. 2002. CDC Robin lentil. *Can J Plant Sci.* 82:111-112.

Vandenberg, A., S. Banniza, T.D. Warkentin, S. Ife, B. Barlow, S. McHale, B. Brolley, Y. Gan, C. McDonald, M. Bandara, and S. Dueck. 2006. CDC Redberry lentil. *Can J Plant Sci.* 86: 497–498.

Walley, F.L., G.M. Fu, J.W. van Groenigen, and C. van Kessel. 2001. Short-range spatial variability of nitrogen fixation by field-grown chickpea. *Soil Sci Soc Am J*. 65(6):1717-1722.

Walley, F.L., G.W. Clayton, P.R. Miller, P.M. Carr, and G.P. Lafond. 2007. Nitrogen economy of pulse crop production in the Northern Great Plains. *Agron J.* 99:1710-1718.

Zimmer, S., M. Messmer, T. Haase, H.P. Piepho, A. Mindermann, H. Schulz, A. Habekuß, F. Ordon, K.P. Wilbois, and J. Heß. 2016. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Europ J Agronomy*. 72:38-46.

Tables

Table 3-1. General soil properties for Post Farm (PF) and Northern Agricultural Research Center (NARC) N fixation study sites, 2019-2021.

Soil property[a]	2019	2020	2021
		PF	
Pea/lentil history (year)[b]	2017	None	None
Soil texture[c]	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 (kg ha^{-1}), 0-90 cm	20	33	74
		NARC	
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 (kg ha^{-1}), 0-90 cm	46	95	19

[a] Average of 16 soil samples (8 samples each from lentil and pea), 0-15 cm unless otherwise noted

[b] 10 previous years

[c] From USDA-NRCS Web Soil Survey

Table 3-2. Lentil and pea varieties grown at Post Farm (Bozeman, MT) and Northern Agricultural Research Center (Havre, MT), 2019-2021.

		Days to Flower[e]		Days to Maturity		Registration
Lentil Variety	Type	2019	2020[f]	2019	2020	Article
CDC[a] Impala	Extra small red	69	--	111	--	--
CDC Maxim	Small red	61	--	111	--	--
CDC Redberry	Small red	65	--	111	--	Vandenberg et al., 2006
CDC Richlea	Medium green	61	--	112	--	--
CDC Robin	Small red	60	--	110	--	Vandenberg et al., 2002
CDC Rosetown	Extra small red	69	--	112	--	--
CDC Viceroy	Small green	63	--	111	--	--
ND Eagle[b]	Small green	60	--	112	--	--
Pennell	Large green	67	--	112	--	Muehlbauer and McPhee, 2004
Riveland	Large green	60	--	114	--	McPhee and Muehlbauer, 2009
Pea Variety		2019	2020	2019	2020	
Aragorn[c]	Green	59	67	98	104	--
Arvika	Forage	67	72	105	113	--
Carousel[c]	Yellow	61	70	100	108	--
CDC Mozart	Yellow	61	68	102	108	Vandenberg and Slinkard, 2002
Cruiser[c]	Green	62	68	103	105	--
Delta	Yellow	61	71	99	106	--
DS Admiral	Yellow	62	70	101	108	Andersen et al., 2002
Hampton[d]	Green	66	73	105	110	--
Lifter	Green	64	70	105	105	McPhee and Muehlbauer, 2002
Majoret	Green	62	69	100	106	--

[a] Crop Development Centre, Saskatoon, SK, CA
[b] North Dakota Crop Improvement and Seed Association, West Fargo, ND, USA
[c] ProGene Plant Research, Othello, WA, USA
[d] Washington State Crop Improvement Association, Pullman, WA, USA
[e] All flower and maturity data collected at Post Farm
f No 2020 lentil data due to herbicide damage

Table 3-3. Field activities and dates for Post Farm (PF) and Northern Agricultural Research Center (NARC), 2019-2021 N fixation study sites.

Field activity	2019	2020	2021
	---------------------------------- PF ----------------------------------		
Spring soil sampling	25 Apr	10 Apr	7 May
Planting	7 May	21 Apr	15 Apr
Lentil biomass sampling	7 Aug	N/A[a]	13 July
Pea biomass sampling	31 July	28 July	13 July
Lentil harvest	3 Sept	N/A	N/A[b]
Pea harvest	20 Aug	19 Aug	N/A
Fall soil sampling	4 Sept	21 Aug	16 Aug
	-------------------------------- NARC --------------------------------		
Spring soil sampling	15 Apr	20 Apr	24 Mar
Planting	22 Apr	23 Apr	8 Apr
Lentil biomass sampling	25 July	17 Jul	25 Jun
Pea biomass sampling	N/A[c]	17 Jul	25 Jun
Lentil harvest	1 Aug	1 Aug	7 Aug
Pea harvest	N/A	1 Aug	20 July
Fall soil sampling	25 Sept	11 Aug	11 Aug

[a] Lentil at PF in 2020 not sampled due to suspected herbicide injury

[b] Seed yield not measured at PF in 2021 because of poor stand

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

Table 3-4. Precipitation and temperature data for 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							
Sep-Mar[b]	150	210	120	161	-0.7	1.7	1.9	1.5
Apr	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							
Sep-Mar[b]	107	125	119	110	-2.3	0.0	0.9	0.7
Apr	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] Apr – July

Table 3-5. Means and summary of analysis of variance for nitrogen (N) fixed by lentil varieties as determined by the N difference (ND) and ^{15}N natural abundance (NA) methods by year and site.

Lentil Variety	N fixed (kg N ha^{-1})		
	2019[a]	2021	
	NA	ND[d]	NA
	Post Farm[b]		
CDC Impala	109	--	29 b[e]
CDC Maxim	138	--	42 ab
CDC Redberry	116	--	35 ab
CDC Richlea	128	--	51 a
CDC Robin	122	--	36 ab
CDC Rosetown	118	--	36 ab
CDC Viceroy	128	--	49 ab
ND Eagle	154	--	42 ab
Pennell	118	--	39 ab
Riveland	134	--	48 a
	P-value		
	0.13	--	**0.04**
	2019	2021	
	NA	ND	NA
	Northern Agricultural Research Center[c]		
CDC Impala	32 abc	21	18 c
CDC Maxim	43 ab	21	21 bc
CDC Redberry	35 abc	20	21 bc
CDC Richlea	45 a	29	26 ab
CDC Robin	24 c	25	18 c
CDC Rosetown	39 abc	29	24 abc
CDC Viceroy	42 abc	17	21 bc
ND Eagle	26 bc	23	26 ab
Pennell	36 abc	18	25 ab
Riveland	41 abc	24	30 a
	P-value		
	0.03	0.12	**<0.01**

[a] ND not used in 2019 because of reference crop seeding design
[b] No 2020 data at Post Farm due to herbicide damage
[c] No 2020 data at Northern Agricultural Research Center due to high/variable nitrate
[d] ND not used in 2021 at Post Farm due to high/variable nitrate
[e] Columns with the same letter are not significantly different (Tukey-Kramer HSD, $P < 0.1$)

Table 3-6. Means and summary of analysis of variance for nitrogen (N) fixed by pea varieties as determined by the N difference (ND) and ^{15}N natural abundance (NA) methods by year and site.

Pea Variety	N fixed (kg N ha^{-1})				
	2019[a]	2020		2021	
	NA	ND	NA	ND	NA
	Post Farm				
Aragorn	91 bcd[e]	98	109 ab	--[b]	53
Arvika	110 a	109	112 ab	--	65
Carousel	77 d	113	104 ab	--	57
CDC Mozart	96 abc	129	129 ab	--	53
Cruiser	87 bcd	74	88 b	--	63
Delta	95 bc	146	135 a	--	43
DS Admiral	89 bcd	116	110 ab	--	55
Hampton	99 ab	101	118 ab	--	63
Lifter	98 ab	96	113 ab	--	70
Majoret	82 cd	94	91 ab	--	56
	P-value				
	0.07	0.15	**0.10**	--	0.13
	Northern Agricultural Research Center				
Aragorn	--[c]	--[d]	--	52	47
Arvika	--	--	--	50	43
Carousel	--	--	--	42	40
CDC Mozart	--	--	--	49	43
Cruiser	--	--	--	42	39
Delta	--	--	--	50	42
DS Admiral	--	--	--	56	40
Hampton	--	--	--	49	47
Lifter	--	--	--	57	48
Majoret	--	--	--	57	46
	P-value				
	--	--	--	0.75	0.74

[a] No 2019 ND data because of reference crop seeding design to only assess NA
[b] No 2021 ND data at Post Farm due to high/variable nitrate
[c] No 2019 NA data at Northern Agricultural Research Center due to deer grazing
[d] No 2020 data at Northern Agricultural Research Center due to high/variable nitrate
[e] Columns with the same letter are not significantly different (Tukey-Kramer HSD, $P < 0.1$)

Table 3-7. 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.

Lentil Variety	Biomass N (kg ha^{-1})			fNdfa	
	2019	2020	2021	2019	2021
	Post Farm				
CDC Impala	145 b[c]	--[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-value				
	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-value				
	0.28	0.64	**<0.01**	0.12	0.11

[a] No 2020 data at Post Farm due to herbicide damage

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

[c] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Table 3-8. 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.

Pea Variety	Biomass N (kg ha^{-1})			fNdfa		
	2019	2020	2021	2019	2020	2021
	---- Post Farm ----					
Aragorn	132	130	74 ab[c]	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-value					
	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-value					
	--	**0.04**	0.92	--	--	0.43

[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
[c] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Table 3-9. 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	2374 ab[d]	948 d	1896 cd	578 ab
CDC Maxim	2564 a	1211 abcd	2118 bcd	473 d
CDC Redberry	2393 ab	1215 abcd	2042 bcd	506 cd
CDC Richlea	2382 ab	1393 a	2228 ab	549 bc
CDC Robin	2297 ab	992 cd	2023 bcd	385 e
CDC Rosetown	2485 a	1045 bcd	1814 e	626 a
CDC Viceroy	2313 ab	1283 ab	2423 a	457 d
ND Eagle	2529 a	1262 abc	2163 bac	567 abc
Pennell	2465 a	1134 abcd	1765 d	301 f
Riveland	2129 b	1209 abcd	1953 bcd	378 e
	P-value			
	0.01	**<0.01**	**<0.01**	**<0.01**
	Post Farm[b]		Northern Agricultural Research Center[c]	
Pea Variety	2019	2020	2020	2021
Aragorn	1824 bc	2840 bc	2285 abc	761 cd
Arvika	2037 ab	2879 bc	2006 cde	545 e
Carousel	2075 ab	3255 bac	2464 a	838 bcd
CDC Mozart	2118 ab	3577 a	1961 cde	1002 a
Cruiser	2406 a	2887 cd	2265 abcd	629 e
Delta	1820 bc	3490 ab	2148 bcde	851 bc
DS Admiral	1920 abc	3358 ab	2117 bcde	739 d
Hampton	1729 bc	3127 abc	2159 abcde	981 a
Lifter	1369 c	3481 ab	1885 e	936 ab
Majoret	1818 bc	2000 d	2392 ab	619 e
	P-value			
	<0.01	**<0.01**	**<0.01**	**<0.01**
	Coefficient of Variation (%)			
	18.9	21.8	10.2	21.5

[a] No 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

[d] Columns with the same letter are not significantly different (Tukey-Kramer HSD, P < 0.1)

Table 3-10. Pearson correlation coefficients (r values) between N fixation parameters and seed yield at Post Farm (PF) and Northern Agricultural Research Center (NARC).

			Yield	
Year	Site	Parameter	Lentil	Pea
2019	PF	NA fixed N[a]	-0.17	0.12
		fNdfa[b]	-0.19	**0.54**
		Biomass N	0.05	**0.34**
	NARC	NA fixed N	**0.31***	--[d]
		fNdfa	0.04	--
		Biomass N	**0.62**	--
2020	PF	ND fixed N[c]	--	**0.41**
		NA fixed N	--	**0.58**
		fNdfa	--	**0.54**
		Biomass N	--	**0.65**
	NARC	Biomass N	0.14	0.24
2021	NARC	ND fixed N	**0.28**	0.02
		NA fixed N	-0.05	0.19
		fNdfa	-0.19	0.19
		Biomass N	0.06	0.11

*Bold indicates different from 0 (P< 0.1)

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

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

[c] N fixed using N difference method

[d] No data

Table 3-11. Means and summary of analysis of variance for post-harvest nitrate-N for lentil and pea varieties by year and site.

	Post-harvest soil nitrate-N (kg ha^{-1})[a]					
	Post Farm			Northern Agricultural Research Center		
Lentil Variety	2019	2020	2021	2019	2020	2021
CDC Impala	13.2	--[b]	42.7	13.2 ab[d]	76.2	18.1
CDC Maxim	8.4	--	42.4	8.4 b	71.5	17.7
CDC Redberry	13.0	--	30.1	13.0 ab	60.9	18.7
CDC Richlea	12.1	--	51.4	12.1 ab	56.9	19.4
CDC Robin	8.8	--	49.8	8.8 b	43.0	21.9
CDC Rosetown	13.4	--	55.0	13.4 ab	67.3	16.5
CDC Viceroy	16.4	--	44.3	16.4 a	61.1	15.1
ND Eagle	12.5	--	42.3	12.5 ab	45.5	15.2
Pennell	10.8	--	48.7	10.8 ab	49.3	14.3
Riveland	9.9	--	55.7	9.9 ab	59.2	13.3
			P-value			
	0.10	--	0.79	**0.08**	0.80	0.47
Pea Variety						
Aragorn	23.4 a	7.0	38.8	--[c]	74.1	25.0
Arvika	9.0 c	5.9	65.3	--	75.2	19.2
Carousel	12.6 bc	6.9	54.2	--	82.6	19.8
CDC Mozart	16.5 b	5.8	60.4	--	70.5	20.4
Cruiser	14.6 b	7.0	51.4	--	98.4	24.1
Delta	13.9 b	7.9	49.2	--	62.6	20.3
DS Admiral	13.7 b	5.6	89.2	--	52.4	25.2
Hampton	14.9 b	3.6	54.2	--	62.0	23.5
Lifter	24.5 a	5.4	51.8	--	63.8	19.9
Majoret	12.3 bc	8.3	50.3	--	80.6	24.0
			P-value			
	<0.01	0.33	0.35	--	0.65	0.69

[a] Top 90 cm of soil
[b] No data due to lentil herbicide damage
[c] No data due to deer grazing
[d] Columns with the same letter are not significantly different (Tukey-Kramer HSD, $P < 0.1$)

Figures

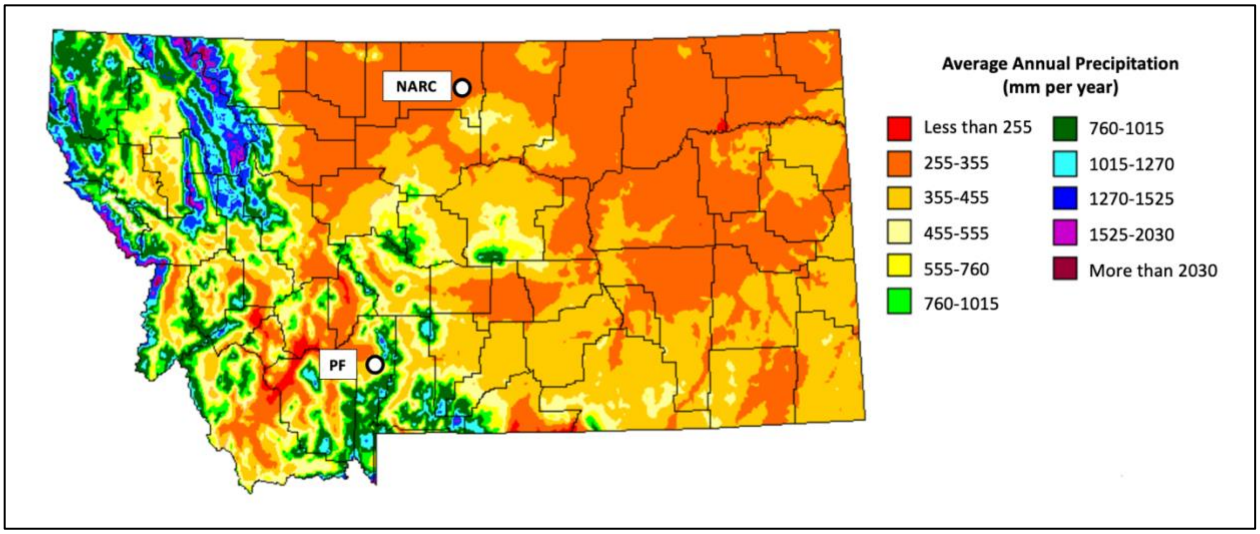

Figure 3-1. Annual precipitation map for Montana, USA, with study sites PF (Post Farm near Bozeman) and NARC (Northern Agricultural Research Center near Havre). Data and map from PRISM Climate Group, Oregon State University.

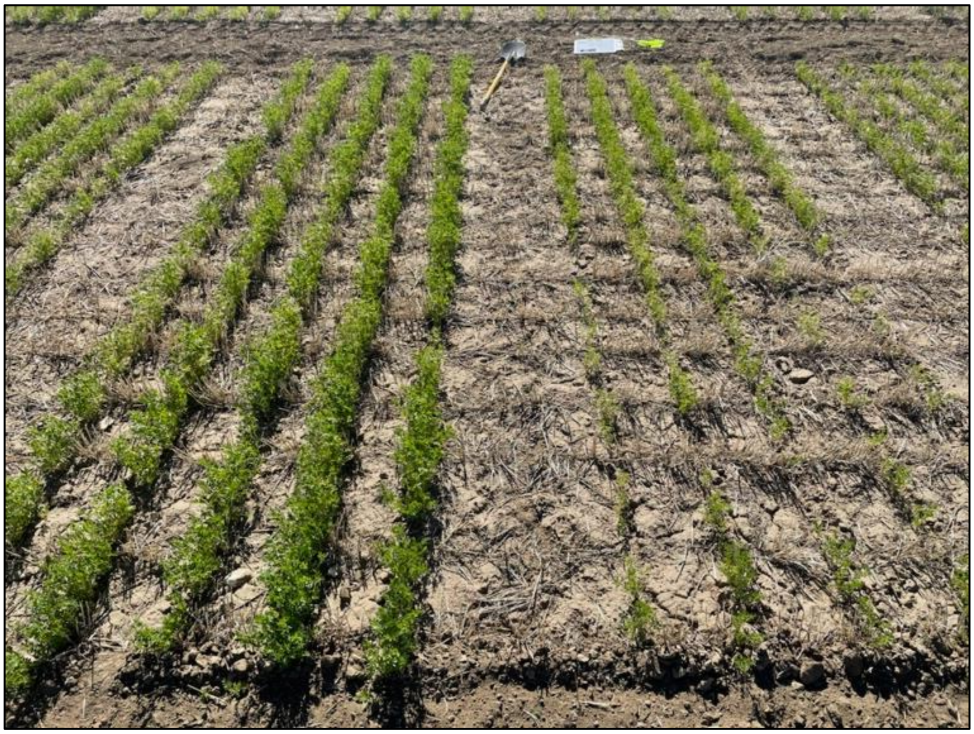

Figure 3-2. Large green lentil variety Riveland (left) and small red variety CDC Robin (right), 7 Jun 2021 at Northern Agricultural Research Center.

CHAPTER FOUR

PEA NITROGEN FIXATION RESPONSE TO INOCULANT FORMULATION

Abstract

Field pea is an important crop in Montana that provides increased agroecosystem biodiversity, stabilized and increased economic returns for producers, and is capable of biological N fixation. Through N fixation, pea can reduce fertilizer N inputs by replacing cereal and oilseed crops in rotation and positively contributing to available soil N. Objectives of this study were to evaluate how different inoculant formulations (granular, seed-coat, granular plus seed-coat, and double seed-coat) influence N fixation, seed yield, and protein of two pea varieties compared to an uninoculated control. Field experiments were conducted from 2019 to 2021 in southwest Montana. Amounts of N fixed were often higher for the Delta (yellow) variety than the Viper (green) variety. Inoculation generally did not impact N fixation, indicating high N fixed amounts in controls evidenced by plentiful nodules on uninoculated pea. Granular inoculant resulted in greater seed yield than seed-coat inoculant in one year. Results suggest that inoculating pea is not always necessary to achieve optimal N fixation, seed yield, and protein when effective rhizobia populations exist in the soil. Correlations between N fixation parameters and seed yield and protein were generally positive but weak.

Introduction

Field pea (*Pisum sativum*) is a significant pulse crop in the northern Great Plains (NGP), grown on 231,000 ha in Montana in 2021 (USDA NASS, 2022). In crop rotations, pea reduces financial volatility and increases profit (Miller et al., 2015), disrupts pest cycles (Stevenson and van Kessel, 1996), and increases productivity of subsequent crops (Beckie and Brandt, 1997; Miller et al., 2003; Bremer et al., 2011). A major benefit of pea in crop rotations is its ability to biologically fix N through its symbiotic relationship with rhizobia. In place of non N-fixing crops or fallow, pea can positively contribute to soil N pools (Beckie and Brandt, 1997) and decrease fertilizer N inputs, reducing soil acidification (Engel, 2020) and nitrate leaching (John et al., 2017; Sigler et al., 2020) that can result from N fertilization.

Inoculation of pea is often needed to introduce the bacteria responsible for N fixation, *Rhizobium leguminosarum* biovar *viceae*, to the agroecosystem. Not inoculating risks nodulation failure, which can decrease seed yield and protein (McConnell et al., 2002). In a 2016 survey of 82 Montana farms, two inoculant types, granular and seed-coat, were almost equally used in pea production (Bestwick et al., 2018). Seed-coat inoculant is applied to the seed as a peat powder before planting. Granular inoculants, which can be peat, clay, or synthetic-based, are placed near the seed (in-furrow) during planting. In western Canada fields with no history of pulse crops, granular inoculant resulted in 60% higher N fixed amounts (Clayton et al., 2004a) and 17% greater seed yield (Clayton et al., 2004b) than seed-coat inoculant. On a per-hectare basis, granular inoculant is approximately four times more expensive than seed-coat inoculant. Despite the cost difference and potential advantages of granular inoculants to N fixation and seed yield, the efficacies of inoculant formulations in pea production have not been investigated in Montana.

The primary objective of this study was to determine how inoculant formulations affect N fixation, seed yield, and seed protein of a green and yellow pea variety in Montana. The relationship between pea N fixation and seed yield and protein were also investigated, as well as how soil nitrate-N pools following pea are affected by pea variety and inoculant formulations.

Materials and Methods

Site Selection and Experimental Design

Field experiments were conducted at Montana State University's Arthur H. Post Agronomy Research Farm (PF) near Bozeman, MT (45.68°N, 111.15°W) from 2019 to 2021. The soil type was Amsterdam silt loam (frigid Typic Haplustoll) in all years. Site selection was based on soil parameters of nitrate-N (0.12M KCl, Cd reduction), exchangeable K (1M ammonium acetate, ICP), sulfate-S (0.12M KCl, turbidimetric), Olsen P (0.5M $NaHCO_3$, colorimetric), and soil organic matter (loss on ignition) to identify locations within suitable fields that had relatively similar fertility levels (analysis by AgVise Laboratories, Northwood, ND, USA). Soil at each selected field site was sampled (two locations per block) in the spring of each growing season prior to planting and analyzed for pH (1:1 soil:water), organic matter, Olsen P, exchangeable, sulfate-S, and nitrate-N (AgVise Laboratories, Northwood, ND, USA). Soil characteristics and pulse crop histories are summarized in Table 4-1.

Treatments were a full factorial of five inoculant formulations (no inoculant, granular, seed-coat, granular+seed-coat, 2x seed-coat) and two pea varieties (Viper, a green variety, and Delta, a yellow variety) for a total of ten treatments (Table 4-2). The granular+seed-coat formulation was included because some producers in Montana choose to double inoculate to increase chance of nodulation success. The 2x seed-coat formulation is still less expensive than granular inoculant and was included to determine if it could match the performance of granular inoculant. The experiment was conducted in a randomized complete block design with four replicates.

Plot Management

Pea was seeded into standing cereal stubble at a rate of 80 plants m^{-2}. Dates for seeding and other field activities are shown in Table 4-3. Control plots were seeded first, granular second, and seed-coat last to minimize rhizobia contamination. Row widths were 23 cm and plot sizes were 8 x 2 m. A reference crop of flax was seeded in a 2-m perpendicular strip adjacent to the pea plots. Monoammonium phosphate (11-52-0) was applied in furrow at planting at a rate of 50 kg ha^{-1} to provide 5.5 kg ha^{-1} of starter N and 26 kg ha^{-1} of starter P. Insecticide was applied each year to control pea leaf weevil, which feeds on root nodules and can limit N fixation (Cárcamo and Vankosky, 2011), and other appropriate pest management practices were carried out throughout the growing season (Table 4-4).

Data Collection and N Fixation Measurements

See Ch. 2 for descriptions of data collection, analyses, and the N difference (ND) and ^{15}N natural abundance (NA) N fixation methods. In this study, 6.7% of ND observations fell outside the bounds of N fixed (< 0 kg N ha^{-1} or > biomass N) and were omitted. No NA observations were omitted. Seed N concentrations were determined by combustion (LECO, St. Joseph, MI, USA), and seed protein concentration was reported as seed N x 6.25 (Coyne et al., 2005).

Statistical Analyses

All statistical procedures were conducted using R (R Core Team, 2021). Analysis of variance (ANOVA) was performed for response variables of N fixed (ND and NA), biomass N, fNdfa (from NA), seed yield, protein, and protein yield, and post-harvest soil nitrate-N using linear mixed models with the 'lme4' package (Bates, et al., 2015). Inoculant formulation was a fixed effect and block was a random effect. Based on an interaction between inoculant formulation and variety detected using two-way analysis of variance (ANOVA), Viper and Delta varieties were analyzed separately to evaluate each variety's response to inoculant using ANOVA. Treatment differences were further investigated using Tukey-Kramer familywise comparisons of means (alpha = 0.1) with the 'multcomp' package (Hothorn et al., 2008) and pre-planned orthogonal contrasts with the 'emmeans' package (Length, 2021). Correlation analyses combining varieties were conducted between N fixation parameters (N fixed using both methods, biomass N, fNdfa from NA) and seed yield, protein concentration, and protein yield.

Results and Discussion

Weather

Weather for each year at PF is discussed in Ch. 2 and summarized in Table 4-5. Importantly, growing season weather ranged from cooler and wetter than normal (2019) to drier and warmer than normal (2021).

N Fixation

Assumptions of the ND and NA methods are discussed in Ch. 2. The ND method tended to result in higher N fixed amounts than the NA method, but relative differences among treatments using each method were generally in agreement. Mean standard errors of mixed linear models were on average 42% smaller for the NA method than the ND method, which may indicate the NA method is better at detecting differences in N fixed in the semiarid NGP. The average difference between pea and flax $\delta^{15}N$ ranged from 1.6 in 2019 to 3.3 in 2021. Large ^{15}N differences between legume and reference crops increases accuracy of the NA method (Bremer et a., 1993), and accuracy is greatly impacted when differences are less than 2‰ (Unkovich et al., 1994). While sites had generally low and uniform soil nitrate-N each year, inherent spatial variation of soil N may have increased error using the ND method.

Delta fixed more N than Viper in 2019 and 2021 using both methods, but in 2020 the varieties did not differ in N fixed (Table 4-5). In 2019, differences between varieties were 16 and 12 kg N ha^{-1} using ND and NA, respectively. In 2021, differences between varieties were 9 and 6 kg N ha^{-1} using ND and NA, respectively. Amounts of N fixed can vary among pea varieties (Ch. 3 and Abi-Ghanem et al., 2011). It is not clear why there was no difference between varieties in 2020, as growing season precipitation was similar to that of 2019 (especially in June, an important month for pea growth and development) and soil nitrate-N levels were similar to 2019 and 2021.

Uninoculated pea fixed as much N as inoculated pea in all years using both methods, except in 2021 when uninoculated pea fixed slightly more N using the ND method (Table 4-6). This may be because introduced rhizobia were concentrated at planting depth and were impacted by drought, while rhizobia in control plots existed in deeper, moister soil. There were no differences in N fixed between granular and seed-coat inoculants. For Viper, N fixed varied ($P < 0.1$) among inoculant formulations in two years using ND and one year using NA (Table 4-7). Overall, there was no inoculant formulation that consistently outperformed the others. Interestingly, the control was in the top statistical grouping each time. For Delta, N fixed only varied among treatments in 2019 using the NA method, and the control was again in the top statistical grouping.

The lack of consistent differences among inoculant formulations is unsurprising given inoculation itself did not impact N fixed amounts. In 2019, this could have been due to a recent (2017) inoculation of lentil in that field. In 2020 and 2021 fields, however, there was no previous history of pea or lentil production. Active nodules were found on pea roots in control plots in each year roots were dug (2020 and 2021; Figure 1). Given the research farm environment, rhizobia may have been introduced from adjacent fields via farm equipment or wind. The Gallatin Valley had a significant pea production and canning industry until 1962, so rhizobia could have persisted in area soils until present day. Another explanation could be that indigenous strain(s) of rhizobia exist at PF and can form successful symbioses with pea. Studies in the NGP have found effective indigenous strains (Kucey and Hynes, 1989; McKenzie et al., 2001; McConnell et al., 2002). Indigenous rhizobia strains have even been reported to result in more N fixed than commercial strains in lentil (Hafeez et al., 2000), similar to our finding using the ND method in 2021. The 2021 growing season was much warmer and drier than average, which could suggest that indigenous strains were more resilient to the more extreme conditions that year than introduced commercial strains.

Biomass N and fNdfa

Biomass N accumulation only varied ($P < 0.1$) among inoculant formulations for Viper in 2021 but not in any year for Delta (Table 4-8). In 2019 and 2021, uninoculated pea accumulated more N than inoculated pea, by 11 and 8 kg N ha^{-1}, respectively (Table 4-8). Comparing varieties, Delta accumulated more biomass N than Viper in 2019 and 2021, aligning with differences found in N fixed amounts. Greater biomass N accumulation was directly related to more N fixed over a two-year study in Montana (McCauley et al., 2012), not surprising as pulse biomass N plays a large role in both the ND and NA methods.

Values for fNdfa varied ($P < 0.1$) in 2019 among Delta treatments, but not in any year among Viper treatments (Table 4-8). In 2019 for Delta, the granular + seed-coat treatment had a lower average fNdfa than the other three inoculant formulations. In 2019, inoculated pea had an fNdfa value 0.04 higher than uninoculated pea (Table 4-9). Comparing varieties, fNdfa of Viper was 0.09 higher than Delta in 2020. Even though fNdfa is a component in the NA equation used to estimate N fixed, differences in fNdfa did not translate to differences in N fixed in any year. Further, N fixed amounts using the NA method with variety-specific B-values resulted in the same statistical trends as using a species-average B-value. Variation in biomass N, the other component in the NA equation, likely negated any effect of fNdfa, which had small differences and less variation, on N fixed amounts.

Seed Yield and Protein

Yield and protein data for Delta in 2021 is not reported due to herbicide damage that did not affect Viper. In 2019, Delta yielded 251 kg ha^{-1} more than Viper, but Viper had seed protein concentrations 7 g kg^{-1} higher than Delta (Table 4-10). Protein yield was 35 kg ha^{-1} greater for Delta. Granular inoculant yielded 137 kg ha^{-1} greater than seed-coat inoculant in 2020. In 2021, inoculated pea yielded 50 kg ha^{-1} greater than uninoculated pea. Among Viper treatments, seed yield and protein yield varied in 2021 (Table 4-11). The 2x seed-coat was the highest-yielding inoculant formulation and was among the top treatments for protein yield. No differences were found in any year in seed and protein yield among Delta treatments, and protein did not vary for either pea variety in any year. Seed yield can vary among pea varieties depending on environmental conditions, most notably precipitation (Hossain et al., 2017). Inoculant type and drought severity had an interactive effect on pea protein in Montana (Bestwick et al., 2018), but no such response was observed in our study even though precipitation varied greatly over the three-year study. Pea yields increased by 56% in western Canada with inoculation (Clayton et al., 2004b). In an environment more similar to Montana, inoculation increased seed yield to a lesser degree, in nine of 22 trials by an average of 14% (McKenzie et al., 2001). In our study, the 2020 and 2021 fields did not have recent histories of pea or lentil, but inoculation only increased yield in 2021.

N Fixation and Seed Yield, Protein, and Protein Yield Relationships

Correlations between N fixation parameters (N fixed using both method, biomass N, and fNdfa from NA) and seed yield, protein concentration, and protein yield were generally weak (Table 4-12). Correlation coefficients ranged from -0.07 to 0.53. Of the 36 correlation coefficients, 39% were different from 0 ($P < 0.1$). Yield and protein yield more often had positive correlations with N fixation parameters than protein concentration. This could suggest that pea seed protein concentration is somewhat static in relation to N fixation. Of the N fixation parameters, only fNdfa was not correlated with yield, protein concentration, or protein yield in any year. This is not surprising, as there was not much variation in fNdfa among treatments according to ANOVA and orthogonal contrasts. Environmental conditions, in addition to N fixation, likely influenced strength of correlations. Generally, correlations were strongest in 2019 when precipitation was well above average. In 2021, there was unusually severe drought and correlations were weak, indicating that moisture was the major constraint to yield and protein instead of N. Based on three years of data, results indicate that when pea fixes more N, producers can expect higher seed and protein yield, but not necessarily higher seed protein concentrations.

Residual Soil Nitrate-N

Pea variety and inoculant formulations had little impact on residual soil nitrate-N. Post-harvest soil nitrate-N varied among Viper treatments in 2019, with the control having the lowest average nitrate-N level (Table 4-13). Also in 2019, inoculated pea resulted in nitrate-N levels 3 kg N ha^{-1} greater than uninoculated pea. In 2020, seed-coat inoculant had higher nitrate-N levels than granular inoculant, but the difference was just 2 kg N ha^{-1}. Given the minimal differences in N fixed amounts among treatments, it is not surprising that nitrate-N was not meaningfully affected. Additionally, measuring nitrate-N shortly after pea harvest likely does not accurately portray the influence of pea variety and inoculant formulation because residue has not had time to decompose and mineralize N (discussed in more detail in Ch. 2 and 3).

Conclusion

In this three-year study at one site in southwest Montana amounts of N fixed by two pea varieties did not benefit from inoculation, nor were there differences in fixed N amongst inoculation formulations. Active root nodules were observed on uninoculated pea, suggesting an effective population of rhizobia existed in the soil and was able to fix N as effectively as commercial inoculants. Inoculation increased pea yield in one year, while protein concentration and protein yield were not affected. As pea has become an economically significant crop in Montana, research on its management is of great value to regional producers. Our results suggest that inoculation may not always be needed to achieve optimal pea N fixation and seed yield and protein. However, producers who are unsure whether their soils have effective rhizobia populations take on the risk of inoculation failure if they choose not to inoculate pea. Further investigation into rhizobia distribution and persistence in Montana’s agricultural areas would help producers make more informed decisions regarding pea inoculation.

References

Abi-Ghanem, R., L. Carpenter-Boggs, and J.L. Smith. 2011. Cultivar effects on nitrogen fixation in peas and lentils. *Biol Fertil Soils*. 47:115-120.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J Stat Software*. 67(1):1-48.

Beckie H.J., and S.A. Brandt (1997) Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Can J Plant Sci*. 77:311-322.

Bestwick, M., K. Olson-Rutz, C. Jones, and P. Miller. 2018. Environmental and Management Effects on Pea Protein in Montana: A Research Report from the Montana Research and Economic Development Initiative. https://landresources.montana.edu/soilfertility/documents/PDF/reports/Bestwick2018MREDIEnvironMgtPeaProt.pdf

Bremer, E., H. Gehlen, G.D.W. Swerhone, and C. van Kessel. 1993. Assessment of reference crops for the quantification of N_2 fixation using natural and enriched levels of N-15 abundance. *Soil Biol Biochem*. 25(9):1197-1202.

Bremer E., H.H. Janzen, B.H. Ellert, R.H. McKenzie. 2011. Carbon, nitrogen, and greenhouse gas balances in an 18-year cropping system study on the northern Great Plains. *Soil Sci Soc Am J*. 75:1493-1502.

Cárcamo, H.A. and M. Vankosky. 2011. Managing the pea leaf weevil in peas. *Prairie Soils & Crops J*. 4:77-85.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004a. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Can J Plant Sci*. 84:79-88.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Crop yield and seed quality. *Can J Plant Sci*. 84:89-96.

Coyne, C., M. Grusak, L. Razai, and B. Baik. 2005. Variation for pea protein concentration in the USDA *Pisum* core collection. *Pisum Genetics*. 37:5-9.

Engel, R. 2020. Soil acidity management of long-term no-till fields in Montana to prevent crop failure. USDA Sustainable Agriculture Research & Education. https://projects.sare.org/?export=1&project_report=644340

Hafeez, F.Y., N.H. Shah, and K.A. Malik. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biol Fert Soils*. 31:65-69.

Hossain, Z., X. Wang, C. Hamel, J.D. Knight, M.J. Morrison, and Y. Gan. 2016. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can J Plant Sci*. 97:119-131.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical J*. 50(3):346-363.

Huang, J., R.K. Afshar, and C. Chen. 2016. Lentil response to nitrogen application and rhizobia inoculation. *Comm Soil Sci and Plant Anal*. 47(21):2458-2464.

John, A.A., C.A. Jones, S.A. Ewing, W.A. Sigler, A. Bekkerman and P.R. Miller. 2017. Fallow replacement is more important than nitrogen fertilizer management at reducing nitrate leaching in a semiarid region. *Nutr Cycl Agroecosys*. 108:279- 296.

Kucey, R.M.N. and M.F. Hynes. 1989. Populations of *Rhizobium leguminorsarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. *Can J Microbiol*. 35(6):661-667.

Lenth, R.V. 2021. emmeans: Estimated Maginal Means, aka Least-Squares Means. R package version 1.6.3. https://CRAN.R-project.org/package=emmeans.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: Effect of planting and termination timing. *Nutr Cycl Agroecosys*. 92(3):305-314.

McConnell, J.T., P.R. Miller, R.L. Lawrence, R. Engel, and G.A. Nielsen. 2002. Managing inoculation failure of field pea and chickpea based on spectral responses. *Can J Plant Sci*. 82(2):273-282.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001. Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Can J Plant Sci.* 81:637–643.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003a. Pulse Crops for the Northern Great Plains: II. Cropping Sequence Effects on Cereal, Oilseed, and Pulse Crops. *Agron J*. 95(4): 980-986.

Miller, P.R., A. Bekkerman, C.A. Jones, M.H. Burgess, J.A. Holmes, and R.E. Engel. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. *Agron J*. 107(2):541-550.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Sigler, W.A., S.A. Ewing, C.A. Jones, R.A. Payn, P. Miller, and M. Maneta. 2020. Water and nitrate loss from dryland agricultural soils is controlled by management, soils, and weather. Agriculure, Ecosystems & Environment. https://doi.org/10.1016/j.agee.2020.107158

Stevenson, F.C. and C. van Kessel. 1996. A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. *Soil Sci Soc Am J*. 60:1797-1805.

USDA National Agriculture Statistics Service. 2022. https://www.nass.usda.gov.

Tables

Table 4-1. General soil properties for 2019-2021 study sites (Post Farm, Bozeman, MT).

Soil property[a]	2019	2020	2021
Pea/lentil history (year)[b]	2017	None	None
Soil texture[c]	Silt loam	Silt loam	Silt loam
Soil pH (1:1 soil:water)	8.1	7.8	6.6
Organic matter (g kg^{-1})	25	26	30
Olsen P (mg kg^{-1})	6	17	18
Exchangeable K (mg kg^{-1})	412	317	434
Sulfate-S (mg kg^{-1}), 0-15 cm	4	4	3
Sulfate-S (mg kg^{-1}), 15-60 cm	2	3	1
Nitrate-N (kg N ha^{-1}), 0-90 cm	18	29	39

[a] Average value of 8 samples (2 locations per block), 0-15 cm unless otherwise noted
[b] 10 previous years
[c] From USDA-NRCS Web Soil Survey

Table 4-2. Inoculant formulations and rates used on Viper and Delta pea varieties.

Inoculant Formulation	Application Rate ($kg\ ha^{-1}$)	CFU Rate ($CFU\ ha^{-1}$)
Control	--	--
Granular	6.72	6.7×10^{11}
Seed-coat	0.23	4.6×10^{10}
Granular + Seed-coat[b]	6.72 + 0.23	7.2×10^{11}
2x Seed-coat	0.46	9.2×10^{10}

[a] Colony forming units of *Rhizobium leguminosarum* biovar *viceae*

[b] Application rates expressed as (Granular) + (Seed-coat)

Table 4-3. Field activities and dates at Post Farm (Bozeman, MT), 2019-2021 study sites.

Field Activity	2019	2020	2021
Spring soil sampling	25 Apr	21 Apr	7 May
Planting	3 May	21 Apr	8 Apr
Insecticide application[a]	31 May	22 May	25 May
Biomass sampling	31 July	27 July	2 July
Harvest	9 Aug	11 Aug	21 July
Fall soil sampling	19 Aug	14 Aug	27 July

[a] Syngenta Warrior II (active ingredient Lambda-cyhalothrin Synthetic pyrethroid) at 0.15 L ha^{-1}

Table 4-4. Pesticide applications from 2019 to 2021.

Product	Manufacturer	Active Ingredient	Use	Application	Rate mL ha^{-1}	Date
------ 2019 ------						
Makaze	Loveland	Glyphosate	Herbicide	Pre-plant	2339	23 Apr
Prowl	BASF	Pendimethalin	Herbicide	Pre-plant	1754	23 Apr
Sharpen	BASF	Saflufenacil	Herbicide	Pre-plant	55	23 Apr
ApronMaxx	Syngenta	Mefenoxam, Fludioxonil	Fungicide	Seed trt.	--[a]	3 May
Warrior II	Syngenta	Lambda-cyhalothrin	Insecticide	In-crop	146	31 May
------ 2020 ------						
Prowl	BASF	Pendimethalin	Herbicide	Pre-plant	1754	20 Apr
Sharpen	BASF	Saflufenacil	Herbicide	Pre-plant	73	20 Apr
ApronMaxx	Syngenta	Mefenoxam, Fludioxonil	Fungicide	Seed trt.	--	21 Apr
Warrior II	Syngenta	Lambda-cyhalothrin	Insecticide	In-crop	146	22 May
Warrior II	Syngenta	Lambda-cyhalothrin	Insecticide	In-crop	146	26 May
------ 2021 ------						
GlystarPlus	Albaugh	Glyphosate	Herbicide	Pre-plant	2339	25 Mar
CruiserMaxx Vibrance	Syngenta	Thiamethoxam, Thiabendazole, Sedaxane, Mefenoxam, Fludioxonil	Insecticide, Fungicide	Seed trt.	--	8 Apr
Warrior II	Syngenta	Lambda-cyhalothrin	Insecticide	In-crop	146	25 May
Assure II	Dupont	Quizlofop p-ethyl	Herbicide	In-crop	731	7 Jun

[a] All seed treatments applied at rate of 3.26 mL kg-seed^{-1}

Table 4-5. Precipitation and temperature data for Post Farm (Bozeman, MT), 2019-2021 study sites.

	Precipitation (mm)				Mean temperature (°C)			
	2019	2020	2021	LTA[a]	2019	2020	2021	LTA
Sep-Mar[b]	150	210	120	161	-0.7	1.7	1.9	1.5
Apr	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

[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 4-6. Orthogonal contrast summaries of nitrogen (N) fixed using N difference (ND) and ^{15}N natural abundance (NA) methods by year for variety and inoculant treatment groupings at Post Farm (Bozeman, MT).

Contrast	ND method		NA method	
	P value	Difference (kg N ha^{-1})	P value	Difference (kg N ha^{-1})
		2019		
Delta – Viper[a]	**<0.01**	16	**<0.01**	12
Inoculated – Control[b]	0.19	--	0.50	--
Granular – Seed-coat	0.79	--	0.78	--
		2020		
Delta – Viper	0.68	--	0.10	--
Inoculated – Control	0.19	--	0.36	--
Granular – Seed-coat	0.77	--	0.85	--
		2021		
Delta – Viper	**0.01**	9	**0.05**	6
Inoculated – Control	**0.02**	-12	0.26	--
Granular – Seed-coat	0.56	--	0.41	--

[a] All Delta treatments minus all Viper treatments

[b] All Viper and Delta inoculated treatments minus Viper and Delta controls

Table 4-7. Means and summary of analysis of variance for nitrogen (N) fixed as determined by the N difference (ND) and ^{15}N natural abundance (NA) methods by year and variety at Post Farm (Bozeman, MT).

Treatment	N fixed (kg N ha^{-1})					
	2019		2020		2021	
	ND	NA	ND	NA	ND	NA
	---------- Viper ----------					
Control	101 ab[a]	85	105	111	98 a	78 a
Granular	111 a	96	82	95	75 b	71 ab
Seed-coat	92 ab	88	90	102	80 ab	63 b
Granular+Seed-coat	108 ab	98	104	108	74 b	64 b
2x Seed-coat	81 b	78	99	105	84 ab	74 ab
	P value					
	0.04	0.17	0.48	0.62	**0.08**	**0.05**
	---------- Delta ----------					
Control	126	110 a	109	98	94	75
Granular	100	96 ab	112	103	95	77
Seed-coat	115	100 ab	97	99	92	77
Granular+Seed-coat	112	91 b	90	89	82	70
2x Seed-coat	119	109 a	87	87	90	82
	P value					
	0.34	**0.09**	0.37	0.61	0.44	0.71

[a] Same letter within column indicates not significantly different (Tukey HSD, alpha=0.1)

Table 4-8. Means and summary of analysis of variance for aboveground biomass N and fNdfa by pea variety and year at Post Farm (Bozeman, MT).

Treatment	Biomass N (kg N ha^{-1})			fNdfa		
	2019	2020	2021	2019	2020	2021
	Viper					
Control	128	132	99 a[a]	0.66	0.85	0.78
Granular	133	108	88 ab	0.72	0.88	0.80
Seed-coat	118	115	82 b	0.75	0.89	0.76
Granular+Seed-coat	131	130	86 b	0.75	0.85	0.75
2x Seed-coat	115	127	92 ab	0.68	0.83	0.79
	P value					
	0.25	0.47	**0.04**	0.32	0.66	0.58
	Delta					
Control	154	132	103	0.71 ab	0.74	0.72
Granular	130	138	102	0.74 a	0.75	0.75
Seed-coat	135	126	97	0.75 a	0.80	0.79
Granular+Seed-coat	137	115	93	0.66 b	0.78	0.75
2x Seed-coat	143	115	103	0.76 a	0.76	0.79
	P value					
	0.31	0.64	0.74	**0.02**	0.31	0.30

[a] Same letter within column indicates not significantly different (Tukey HSD, alpha=0.1)

Table 4-9. Orthogonal contrast summaries of biomass N and fNdfa (by ^{15}N natural abundance) by year for variety and inoculant treatment groupings at Post Farm (Bozeman, MT).

Contrast	Biomass N		fNdfa	
	P value	Difference (kg N ha^{-1})	P value	Difference
	------------------------------- 2019 -------------------------------			
Delta – Viper[a]	**<0.01**	15	0.43	--
Inoculated – Control[b]	**0.08**	-11	**0.08**	0.04
Granular – Seed-coat[c]	0.52	--	0.48	--
	------------------------------- 2020 -------------------------------			
Delta – Viper	0.76	--	**<0.01**	-0.09
Inoculated – Control	0.29	--	0.37	--
Granular – Seed-coat	0.83	--	0.24	--
	------------------------------- 2021 -------------------------------			
Delta – Viper	**<0.01**	10	0.24	--
Inoculated – Control	**0.06**	-8	0.29	--
Granular – Seed-coat	0.28	--	0.95	--

[a] All Delta treatments minus all Viper treatments

[b] All Viper and Delta inoculated treatments minus Viper and Delta controls

Table 4-10. Orthogonal contrast summaries of yield, protein concentration, and protein yield by year for variety and inoculant treatment groupings at Post Farm (Bozeman, MT).

Contrast	Seed Yield		Protein Concentration		Protein Yield	
	P value	Difference (kg ha^{-1})	P value	Difference (g kg^{-1})	P value	Difference (kg ha^{-1})
	------------------ 2019 ------------------					
Delta – Viper[a]	**<0.01**	251	**<0.01**	-7	**0.01**	35
Inoculated – Control[b]	1.00	--	0.14	--	0.70	--
Granular – Seed-coat	0.58	--	0.39	--	0.43	--
	------------------ 2020 ------------------					
Delta – Viper	0.87	--	0.44	--	0.58	--
Inoculated – Control	0.29	--	0.11	--	0.15	--
Granular – Seed-coat	**0.07**	137	0.47	--	0.26	--
	------------------ 2021[c] ------------------					
Inoculated – Control	**0.06**	50	0.36	--	0.49	--

[a] All Delta treatments minus all Viper treatments

[b] All Viper and Delta inoculated treatments minus Viper and Delta controls

[c] No variety and inoculant type contrasts due to no Delta data

Table 4-11. Means and summary of analysis of variance for seed yield, protein concentration, and protein yield by pea variety and year at Post Farm (Bozeman, MT).

Treatment	Yield (kg ha^{-1})			Protein Concentration (g kg^{-1})			Protein Yield (kg ha^{-1})		
	2019	2020	2021	2019	2020	2021	2019	2020	2021
	---------- Viper ----------								
Control	2423	2033	1196 bc[a]	215	283	271	521	475	324 b
Granular	2716	2135	1174 c	216	236	265	586	503	310 b
Seed-coat	2516	2026	1253 b	212	239	269	533	483	337 ab
Gran+Seed-coat	2719	2117	1208 bc	214	236	266	582	500	321 b
2x Seed-coat	2512	2134	1346 a	215	237	266	538	505	358 a
	P value								
	0.14	0.75	**<0.01**	0.84	0.87	0.82	0.12	0.85	**0.04**
	---------- Delta ----------								
Control	2982	2053	--[b]	211	232	--	628	478	--
Granular	2767	2147	--	206	235	--	570	504	--
Seed-coat	2865	1982	--	206	238	--	590	474	--
Gran+Seed-coat	2713	2202	--	208	243	--	565	536	--
2x Seed-coat	2813	2100	--	207	243	--	582	513	--
	P value								
	0.32	0.25	--	0.22	0.27	--	0.17	0.33	--

[a] Same letter within column indicates not significantly different (Tukey HSD, alpha=0.1)

[b] Delta pea data not analyzed in 2021 due to herbicide damage

Table 4-12. Correlations (r values) of pea N fixation parameters and seed yield and protein at Post Farm (Bozeman, MT).

Year	N Fixation Parameter	Yield	Protein Concentration	Protein Yield
2019	ND	**0.52***	0.14	**0.51**
	NA	**0.45**	0.18	**0.43**
	Biomass N	**0.45**	0.11	**0.44**
	fNdfa	0.10	0.14	0.07
2020	ND	**0.41**	0.16	**0.39**
	NA	**0.53**	0.24	**0.51**
	Biomass N	**0.50**	**0.29**	**0.51**
	fNdfa	0.11	0.22	0.16
2021[a]	ND	0.26	**0.39**	0.34
	NA	0.25	0.05	0.24
	Biomass N	0.19	0.11	0.23
	fNdfa	0.18	-0.07	0.10

* Bold indicates correlation coefficient different from 0 ($P < 0.1$)

[a] Viper data only in 2021 due to herbicide damage to Delta

Table 4-13. Means and summary of analysis of variance for post-harvest soil nitrate-N by pea variety and year at Post Farm (Bozeman, MT).

Treatment	Nitrate-N (kg N ha^{-1})[a]		
	2019	2020	2021
	------------------------ Viper ------------------------		
Control	8 b	9	30
Granular	18 a	7	23
Seed-coat	11 a	10	38
Granular+Seed-coat	11 a	9	28
2x Seed-coat	10 a	7	32
		P value	
	0.01	0.33	0.17
	------------------------ Delta ------------------------		
Control	12	9	31
Granular	12	8	48
Seed-coat	16	10	35
Granular+Seed-coat	13	9	35
2x Seed-coat	14	8	32
		P value	
	0.55	0.60	0.51
Contrast		*P value*	
Delta – Viper[b]	0.11	0.49	0.14
Inoculated – Control[c]	**0.05**	0.63	0.53
Granular – Seed-coat[d]	0.56	**0.03**	0.84
		Estimated Difference (kg N ha^{-1})	
Delta – Viper	--	--	--
Inoculated – Control	3	--	--
Granular – Seed-coat	--	-2	--

[a] Soil nitrate-N in top 90 cm

[b] All Delta treatments minus all Viper treatments

[c] All Viper and Delta inoculated treatments minus Viper and Delta controls

[d] Viper and Delta granular treatments minus Viper and Delta seed-coat (1x rate) treatments

Figures

Figure 4-1. Uninoculated pea roots in 2020 (left) and 2021 (right) with active root nodules.

CHAPTER FIVE

SUMMARY

Pulse crops, including pea and lentil, have potential to increase sustainability of cropping systems in the northern Great Plains (NGP) through biological N fixation, which can reduce costly N inputs, decrease soil acidification, and minimize water quality impairment. The goal of this thesis project was to investigate how different pea and lentil management practices influence N fixation, yield, and protein. Three studies were conducted for three years. The first study evaluated inoculant types (granular and seed-coat) and fertilizers (potassium (K), sulfur (S), and micronutrients) on lentil; the second compared several lentil and pea varieties; and the third tested different inoculant formulations in pea production. All studies were conducted at the Post Agronomy Research Farm (PF) near Bozeman, Montana, and only the first two at Northern Agricultural Research Center (NARC) near Havre, Montana.

Pea and lentil responded inconsistently to inoculation between sites. Inoculated lentil fixed more N and yielded greater than uninoculated lentil at NARC in two years. At PF, neither lentil nor pea N fixation was increased with inoculation, and pea yield only increased with inoculation in one year. At PF, active root nodules were observed on uninoculated lentil and pea plants when roots were dug in 2020 and 2021; both fields had no history of lentil or pea in the past 10 years. The source of rhizobia is not clear, though it is possible that effective indigenous rhizobia strains existed in the 2020 and 2021 fields at PF, or that rhizobia had been spread from adjacent fields at the research farm. Conducting research on producer fields with no history of lentil or pea could provide better insights into inoculant efficacies, as rhizobia are less likely to be spread via wind or equipment compared to at a research farm, where fields are small, and equipment is used on multiple studies.

Amounts of N fixed by lentil were increased by S addition at PF in 2020. The response was strongest at PF in 2020 when soil sulfate-S was low and there was moderate drought. However, soil sulfate-S tests did not accurately predict responses. Soil sulfate-S was similarly in all three years at PF, but in 2019 and 2021 S fertilizer did not influence lentil N fixation. Our results suggest that S can drastically increase N fixed (up to 40%) and yield (up to 15%) under certain conditions, so research on how to better understand and predict S response should benefit Montana lentil producers. Critical S concentrations for lentil tissue are unknown, so further research in that area could add to our knowledge of managing S fertility. Similar to inoculant, research on S may also be more relevant if conducted in producer fields. Research farms likely have different soil available nutrient levels than producer fields. We do not know how soil fertility of research farms generally compares to the average producer field, but soil fertility could be higher from frequent nutrient applications for field trials or lower due to high nutrient removal rates on irrigated fields (e.g. at PF).

At PF in 2020, N fixed by lentil increased with increasing tissue S concentrations while N removed as grain plateaued, meaning that the net nitrogen input (N fixed – N harvested) from N fixation continued to increase above the optimum available S level for yield. These findings suggest that even small amounts of S fertilizer can benefit N returns to the soil, resulting in subsequent advantages of reduced fertilizer N costs and less soil acidification. This could be especially valuable information to producers interested in growing pulses as cover crops. The observation was only made at PF in 2020 in a field with relatively low soil S, and to my knowledge there is no other literature on the subject. More research on how S influences the net nitrogen input of pulse crops could confirm our findings and potentially allow producers to decrease fertilizer N requirements of crops following pulses.

Amounts of fixed N varied among lentil and pea varieties by as much as 45 kg N ha^{-1}. No pea variety consistently fixed more N than others, while two lentil varieties emerged as high N-fixers. Breeding for high N fixation might be challenging based on this study. Two lentil varieties, CDC Richlea and Riveland, were often among the top N-fixing varieties and both were among the larger-seeded varieties, possibly giving them a nutritive advantage in the early growing season. The effect of seed size on lentil N fixation may be interesting area to research in the future. Varieties were tested as a single variable. It is likely that several management practices could interact to influence N fixation. I would encourage future researchers to investigate how N fixation differs among varieties when managed differently (fertilizer, tillage, etc.). There could potentially be ideal combinations of varieties and management decisions that result in higher amounts of N fixed. This idea was somewhat evaluated in Ch. 4, with two pea varieties and different inoculant formulations, but there is certainly more work to be done.

Across the three studies, correlations between N fixation parameters and seed yield and protein were inconsistent in strength and direction. Lentil frequently had negative correlations between N fixation and seed yield and protein, while pea mostly had positive correlations. Specific to the variety trials, this could suggest that higher yielding pea varieties fix more N and that breeding pea for greater yield can also increase N fixation. The relationship for lentil is more complex and may be a result of its indeterminate growth. Strength of correlations for all studies ranged from weak to moderate, highlighting the challenging NGP growing environment where precipitation likely limits pulse productivity more than N fixation. Measuring N fixed at the smaller subplot scale has inherently higher error than measuring yield at the plot scale, due to higher variability at smaller spatial scales (Perry Miller, personal communication, 2022). In addition, the nitrogen difference and ^{15}N natural abundance calculations are based on five and four measurements, respectively, increasing variability further, making it less likely to detect N fixation than yield differences among varieties.

Moving forward, I envision climate to become an even greater challenge to agriculture in the NGP as precipitation becomes less predictable. Over the course of my project, we experienced some of the least droughty (2019) and most droughty (2021) years on record, and patterns in results varied substantially year-to-year as a result. It will be important to take climate change into account when conducting agronomic research in the region to help producers prepare for anticipated challenges. Across the three studies, precipitation clearly influenced amounts of N fixed. This makes it difficult to inform producers of the net nitrogen input of growing pea or lentil, as it could be negative or positive depending on whether the growing season has low or high precipitation. Variable precipitation will also make soil fertility decisions more difficult, as soil moisture influences nutrient mineralization and availability in the soil.

My results add to the existing knowledge of pulse crop management in the NGP. Consequences of substantial fertilizer N use in the region, such as soil acidification and nitrate leaching, are quickly becoming apparent. Pea and lentil production can reduce fertilizer N inputs and costs, both by replacing cereal or oilseed crops and positively contributing to soil N pools. This benefits both economic and environmental sustainability. I hope that these results can help producers better manage pea and lentil to increase N fixation, yield, and protein, and serve as groundwork for future research regarding pulse management in the NGP.

APPENDIX A

DETERMINATION OF ^{15}N NATURAL ABUNDANCE B-VALUES

Introduction

The purpose of Appendix A is to explain how B-values for the pea and lentil varieties grown in the field studies (Ch. 2-4) were determined. B-values are used in the ^{15}N natural abundance (NA) method of nitrogen (N) fixation calculation to represent $\delta^{15}N$ of the legume when it obtains all its N from N fixation. When a legume obtains all of its N from the atmosphere, the whole-plant $\delta^{15}N$ is expected to be close to zero. However, the difficulty of harvesting roots from the field forces researchers to only collect aboveground biomass, and a correction is needed to account for the fractionation of ^{14}N and ^{15}N between roots and shoot tissues. Published B-values range from -0.71 to -0.51 for lentil and -1.0 to -0.41 for pea (Unkovich et al., 2008; McCauley et al., 2012). Small variations in B-values among varieties of the same species are often ignored (Unkovich et al., 2008). However, recent studies have found that N fixation amounts calculated using a variety-specific B-value are significantly different than N fixation amounts calculated using the average B-value for the species (Woldekirstos et al., 2014: Balboa and Ciampitti, 2019). Additionally, when N fixation in the field is high (>70%), error from B-value variation can have large impacts on the fraction of biomass N derived from the atmosphere (fNdfa) and calculated N fixation amounts (Balboa and Ciampitti, 2019). Since variety is an explanatory variable of interest in Ch. 3 and 4, it was important to consider potential variations in ^{15}N fractionation between shoots and roots, indicated by the B-value, among lentil and pea varieties.

Materials and Methods

The greenhouse experiment was conducted in the Plant Growth Center West Wing greenhouse at Montana State University in Summer 2021. Treatments were the 11 pea and 11 lentil varieties grown in the field studies (see Ch. 2-4 for variety names). The experiment was set up in a randomized complete block design with four replications. Pots within blocks were systematically rotated weekly.

Plants were grown in pots (20.3-cm diameter x 15.2-cm depth) filled with approximately 500 g mixture of horticultural perlite and vermiculite (1:1 volume, pH: 8.5, nitrate-N: 0.21 mg kg^{-1}). Growing media was brought to saturation with de-ionized (DI) water prior to seeding. Seeds were sown at double the field seeding rate for each species and thinned 14 d after emergence to match field seed rates. This resulted in three plants per pot for pea and four plants per pot for lentil. Peat-based seed-coat rhizobia inoculant was applied to seeds at the label recommend rate to ensure healthy root nodulation. Pots were watered to saturation when gravimetric soil water content dropped to 75% of greenhouse ‘field capacity’. Field capacity was arbitrarily determined by weighing a plant-free pot 24h after watering to saturation. Pots were watered with DI water until plants were fully emerged, after which the watering solution was an N-free McKnight nutrient solution (Unkovich et al., 2008) that was diluted with DI water to 0.25-strength. Lentils displayed adverse growth symptoms due to the high pH of the growing medium and were watered more frequently for the first three weeks in an attempt to lower the pH. Aboveground biomass of all pea varieties was harvested when half of the varieties had full pods. Lentil biomass was harvested when half of the varieties had at least one full pod. These developmental stages on average mirror development stages at which biomass was harvested in the field studies. Biomass samples were oven-dried (50°C, 72h) and ground (<0.5 mm). Subsamples were analyzed for isotope ^{15}N using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at UC David Stable Isotope Facility.

Statistical analyses were conducted using R software (R Core Team, 2021). Analysis of variance (ANOVA) was performed on a linear mixed model, with variety as a fixed effect and block as a random effect. The Tukey-Kramer HSD post-hoc test was used to ascertain differences among varieties (P=0.1).

Results

Lentil varieties CDC Rosetown, CDC Robin, and Redberry were most affected by high pH of the growing medium. These varieties displayed poor emergence and seedlings were brown and had narrow leaves. All lentil varieties suffered from greenhouse thrips damage later in the experiment. Pea varieties displayed healthy growth over the entire experiment. Healthy root nodulation was observed for lentils and peas, indicating that N fixation was occurring.

There is strong evidence that B values of lentil and pea differed among varieties ($P<0.01$; Table A-1). Lentil varieties had a range of $\delta^{15}N$ (-0.69 to -1.17) lower than the published range, while pea $\delta^{15}N$ results (-0.29 to -0.85) were mostly within the published range. The varieties that struggled with the growing medium high pH nearly spanned the total range of lentil variety $\delta^{15}N$, suggesting that poor early growth did not impact the final $\delta^{15}N$. McCauley (2012) determined CDC Richlea lentil had a $\delta^{15}N$ of -0.71, which is close to what was found in this experiment (CDC Richlea $\delta^{15}N$ = -0.82). Similarly, this experiment determined the $\delta^{15}N$ of Arvika pea to be -0.85 and McCauley (2012) reports a $\delta^{15}N$ of -0.72. The slight differences may be explained by termination timing, as $\delta^{15}N$ decreases as plants matures and pulse biomass has harvested earlier in McCauley (2012). This strengthens confidence in the methods and results of this experiment.

References

Balboa G.R. and I.A. Ciampitti. 2019. Estimating biological nitrogen fixation in field-grown soybeans: impact of B value. *Plant Soil*. 446:195-210.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: effect of planting and termination timing. *Nutrient Cycling in Agroecosystems*. 92(3):305-314.

Rose, T.J., L.J. Kearney, D.V. Erler, and M.T. Rose. 2018. Influence of growth stage and seed nitrogen on B values and potential contributions to error in estimating biological N2 fixation using the 15N natural abundance method. *Plant Soil*. 425:1-2.

Unkovich M., D.F. Herridge, M.B. Peoples, G. Cadisch, R.M. Boddey, K.E. Giller, B. Alves, and P.M. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph. Australian Centre for International Agricultural Research, Canberra, AU.

Woldekirstos, A.N., D. Huygens, H.R. Upadhayay, J. Diels, and P. Boeckx. 2014. Importance of correct B value determination to quantify biological N_2 fixation and N balances of faba beans (*Vicia faba* L.) via ^{15}N natural abundance. *Biology and Fertility of Soils*. 50:517-525.

Table A-1. Summary of means and analysis of variance for lentil and pea variety $\delta^{15}N$ values when grown in the absence of mineral N (B-values).

Lentil Variety	$\delta^{15}N$	Pea Variety	$\delta^{15}N$
Avondale	-0.99 ab[a]	Aragorn	-0.36 cd
CDC Impala	-1.16 ab	Arvika	-0.85 a
CDC Maxim	-0.85 bc	Carousel	-0.54 bc
CDC Redberry	-0.83 bc	CDC Mozart	-0.55 b
CDC Richlea	-0.82 bc	Cruiser	-0.33 d
CDC Robin	-0.99 ab	Delta	-0.57 b
CDC Rosetown	-1.17 a	DS Admiral	-0.44 bcd
CDC Viceroy	-0.92 abc	Hampton	-0.47 bcd
ND Eagle	-0.84 bc	Lifter	-0.53 bc
Pennell	-0.69 c	Majoret	-0.55 bc
Riveland	-0.87 bc	Viper	-0.29 d
	P-value		*P-value*
	<0.01		**<0.01**

[a] Varieties of same species with same letter determined to not be statistically different (Tukey-Kramer HSD, alpha=0.1)

REFERENCES CITED

Abbasi, M.K., M.M. Tahir, W. Azam, Z. Abbas, and N. Rahim, 2012. Soybean yield and chemical composition in response to phosphorus-potassium nutrition in Kashmir. *Agron J*. 104(5): 1476-1484.

Abdelhamid M.T., H.A. Kamel, and M.G. Dawood, 2010. Response of non-nodulating, nodulating, and super-nodulating soybean genotypes to potassium fertilizer under water stress. *J of Plant Nutr*. 34(11): 1675-1689.

Abi-Ghanem, R., L. Carpenter-Boggs, and J.L. Smith. 2011. Cultivar effects on nitrogen fixation in peas and lentils. *Biol Fertil Soils*. 47:115-120.

Abi-Ghanem, R., E.T. Bodah, M. Wood, and K. Braunwart. 2013. Potential breeding for high nitrogen fixation in *Pisum sativum* L.: Germplasm phenotypic characterization and genetic investigation. *Am J Plant Sci*. 4:1597-1600.

Alam, F., T.Y. Kim, S.Y. Kim, S.S. Alam, P. Pramanik, P.J. Kim, Y.B. Lee. 2015. Effect of molybdenum on nodulation, plant yield and nitrogen uptake in hairy vetch (*Vicia villosa* Roth). *Soil Sci and Plant Nutr*. 61(4):664-675.

Andersen, L., T. Warkentin, O. Philipp, A. Xue, and A. Sloan. 2002. DS Admiral field pea. *Can J Plant Sci*. 82:751-752.

Androsoff, G.L., C. Kessel, and D.J. Pennock. 1995. Landscape-scale estimates of dinitrogen fixation by *Pisum sativum* by nitrogen-15 natural abundance and enriched isotope dilution. *Biol Fert Soils*. 20(1):33-40.

Anglade, J., G. Billen, and J. Garnier. 2015. Relationships for estimating N2 fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. *Ecosphere*. 6(3):1-24.

Arcand, M.M., R. Lemke, R.E. Farrell, J.D. Knight. 2014. Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. *Biol Fert Soils*. 50:507-515.

Balboa G.R. and I.A. Ciampitti. 2019. Estimating biological nitrogen fixation in field-grown soybeans: impact of B value. *Plant Soil*. 446:195-210.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J Stat Software*. 67(1):1-48.

Beckie H.J., and S.A. Brandt (1997) Nitrogen contribution of field pea in annual cropping systems. 1. Nitrogen residual effect. *Can J Plant Sci*. 77: 311-322

Bestwick, M., K. Olson-Rutz, C. Jones, and P. Miller. 2018. Environmental and Management Effects on Pea Protein in Montana: A Research Report from the Montana Research and Economic Development Initiative.

Bremer, E., H. Gehlen, G.D.W. Swerhone, and C. van Kessel. 1993. Assessment of reference crops for the quantification of N_2 fixation using natural and enriched levels of N-15 abundance. *Soil Biol Biochem.* 25(9):1197-1202.

Bremer E., H.H. Janzen, B.H. Ellert, R.H. McKenzie. 2011. Carbon, nitrogen, and greenhouse gas balances in an 18-year cropping system study on the northern Great Plains. *Soil Sci Soc Am J*. 75:1493-1502.

Brown, T.T., R.T. Koenig, D.R. Huggins, J.B. Harsh, and R.E. Rossi, 2008. Lime effects on soil acidity, crop yield, and aluminum chemistry in direct-seeded cropping systems. *Soil Sci Soc Am J*. 72:634-640. doi:10.2136/sssaj2007.0061.

Carlyle, W.J. 1997. The decline of summerfallow on the Canadian prairies. *The Canadian Geographer*. 3:267-280.

Cazzato, E., V. Tufarelli, E. Ceci, A.M. Stellacci and V. Laudadio, 2012. Quality, yield and nitrogen fixation of faba bean seeds as affected by sulphur fertilization. *Soil & Plant Sci*. 62(8):732-738. doi: 10.1080/09064710.2012.698642

Chemining'wa, G.N. and J.K. Vessey. 2006. The abundance and efficacy of *Rhizobium leguminosarum* bv. *viciae* in cultivated soils of the eastern Canadian prairie. *Soil Biol Biochem*. 38:294-302.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Crop yield and seed quality. *Can J Plant Sci*. 84:89-96.

Clayton, G.W., W.A. Rice, N.Z. Lupwayi, A.M. Johnston, G.P. Lafond, C.A. Grant, and F. Walley. 2004b. Inoculant formulation and fertilizer nitrogen effects on field pea: Nodulation, N_2 fixation and nitrogen partitioning. *Can J Plant Sci*. 84:79-88.

Collins, M., D.J. Lang, and K.A. Kelling. 1986. Effects of phosphorus, potassium, and sulfur on alfalfa nitrogen-fixation under field conditions. *Agron J*. 78(6):959-963.

Coyne, C., M. Grusak, L. Razai, and B. Baik. 2005. Variation for pea protein concentration in the USDA *Pisum* core collection. *Pisum Genetics*. 37:5-9.

Dakora F.D., A.C. Spriggs, P.A. Ndakidemi, and A. Belane. 2008. Measuring N_2 Fixation in Legumes Using ^{15}N Natural Abundance: Some Methodological Problems Associated with Choice of Reference Plants. In: Dakora F.D., Chimphango S.B.M., Valentine

A.J., Elmerich C., Newton W.E. (eds) Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture, vol 42. Current Plant Science and Biotechnology in Agriculture. Springer, Dordrecht, The Netherlands, pp 45-45.

Divito, G.A. and V.O. Sadras. 2014. How do phosphorus, potassium and sulphur affect plant growth and biological nitrogen fixation in crop and pasture legumes? A meta-analysis. *Field Crops Research*. 156:1161-171.

Engel, R. 2020. Soil acidity management of long-term no-till fields in Montana to prevent crop failure. USDA Sustainable Agriculture Research & Education. https://projects.sare.org/?export=1&project_report=644340

FAO, 2018. Inputs: Fertilizers by nutrient. http://www.fao.org/faostat/en/#data/RFN (accessed 16 September 2020).

Fowler, D., M. Coyle, U. Skiba, M.A. Sutton, J.N. Cape, S. Reis, L.J. Sheppard, A. Jenkins, B. Grizzetti, J.N. Galloway, P. Vitousek, A. Leach, A.F. Bouwman, K. Butterbach-Bahl, F. Dentener, D. Stevenson, M. Amann, and M. Voss. 2013. The global nitrogen cycle in the twenty-first century. *Phil Trans R Soc B*. 368:1-13.

Freney, J.R. and F.J. Stevenson. Organic sulfur transformations in soils. 1966. *Soil Sci*. 101(4):307-316.

Galloway, J.N., J.D. Aber, J.W. Erisman, S.P. Seitzinger, R.W. Howarth, E.B. Cowling, B.J. Cosby. 2003. The nitrogen cascade. *BioScience.* 53(4):341-356.

Gan, Y.T., C.A. Campbell, H.H. Janzen, R. Lemke, L.P. Liu, P. Basnyat, and C.L. McDonald. 2009. Root mass for oilseed and pulse crops: growth and distribution in the soil profile. *Can J Plant Sci.* 89:883-893.

Gayler, K.R., and G.E. Sykes. 1985. Effects of nutritional stress on the storage proteins of soybeans. *Plant Phys.* 78(3):582-585.

Gollner, G., W. Starz, and J.K. Friedel. 2019. Crop performance, biological N fixation and pre-crop effect of pea ideotypes in an organic farming system. *Nutr Cycl Agroecosyst*. 115:391-405.

González-Guerrero, M., A. Matthiadis, A. Sáez, and T.A. Long. 2014. Fixating on metals: new insights into the role of metals in nodulation and symbiotic nitrogen fixation. *Front Plant Sci*. 5:45.

Gruber, N and J.N. Galloway. 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*. 451 (7176): 293-296.

Hafeez, F.Y., N.H. Shah, and K.A. Malik. 2000. Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosarum* bv. *viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *Biol Fert Soils*. 31:65-69.

Hamel, C., Y. Gan, S. Sokolski, and L.D. Bainard. 2018. High frequency cropping of pulses modifies soil nitrogen level and the rhizosphere bacterial microbiome in 4-year rotation systems of the semiarid prairie. *Applied Soil Eco*. 126:47-56.

Hauggaard-Nielson H, Holdensen L, Wulfsohn D, Jensen ES. 2010. Spatial variation of N-2-fixation in field pea (*Pisum sativum* L.) at the field scale determined by the N-15 natural abundance method. *Plant Soil*. 327 (1-2): 167-184.

Havlin, J.L, S.L. Tisdale, W.L. Nelson, J.D. Beaton, 2013. Soil Fertility and Fertilizers: An Introduction to Nutrient Management, 8th Edition. Pearson.

Herridge, D. and I. Rose. 2000. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Research*. 65(2-3):229-248.

Hossain, A., A. Quddus, K. Alam, H.M. Naser, B. Anwar, F. Khatun, H. Rashid, F. Khatun, and A. Siddiky. 2020. Application of zinc, boron, and molybdenum in soil increases lentil productivity, nutrient uptake, and apparent balance. *Can J Soil Sci*. doi: https://doi.org/10.1139/cjss-2019-0141

Hossain, Z., X. Wang, C. Hamel, J.D. Knight, M.J. Morrison, and Y. Gan. 2017. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can J Plant Sci*. 97:119-131.

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical J*. 50(3):346-363.

Howard, J.B. and D.C. Rees. 2006. How many metals does it take to fix N_2? A mechanistic overview of biological nitrogen fixation. *Proceedings of the National Academy of Sciences of the United States of America*. 103(46):17088-17093.

Huang, J., R.K. Afshar, and C. Chen. 2016. Lentil response to nitrogen application and rhizobia inoculation. *Communications in Soil Science and Plant Analysis*. 47(21):2458-2464.

Huang, L., C.W. Riggins, S. Rodriguez-Zas, M.C. Zabaloy, and M.B. Villamil, 2019. Long-term N fertilization imbalances potential N acquisition and tranformations by soil microbes. *Sci of the Total Environ*. 691: 562-571.

Jacobsen J., G. Jackson, and C. Jones. 2003. Fertilizer guidelines for Montana crops. MSU Extension Bulletin EB161. Bozeman, MT. http://animalrange.montana.edu/documents/extension/eb161.pdf

John, A.A., C.A. Jones, S.A. Ewing, W.A. Sigler, A. Bekkerman and P.R. Miller. 2017. Fallow replacement is more important than nitrogen fertilizer management at reducing nitrate leaching in a semiarid region. *Nutr Cycl Agroecosys*. 108:279- 296.

Kaiser, D.E. and K.I. Kim, 2013. Soybean response to sulfur fertilizer applied as a broadcast or starter using replicated strip trials. *Agron J,* 105(4): 1189-1198.

Kaschuk G., P.A. Leffelaar, K.E. Giller, O. Alberton, M. Hungria, and T.W. Kuyper. 2009. Responses of legumes to rhizobia and arbuscular mycorrhizal fungi: A meta-analysis of potential photosynthate limitation of symbioses. *Soil Biol & Biochem*. 42:125-127.

King, C.A., and L.C. Purcell. 2001. Soybean nodule size and relationship to nitrogen fixation response to water deficit. *Crop Sci*. 41:1099–1107.

Kucey, R.M.N. and M.F. Hynes. 1989. Populations of *Rhizobium leguminorsarum* biovars *phaseoli* and *viceae* in fields after bean or pea in rotation with nonlegumes. *Can J Microbiol*. 35(6):661-667.

Lenth, R.V. 2021. emmeans: Estimated Maginal Means, aka Least-Squares Means. R package version 1.6.3. https://CRAN.R-project.org/package=emmeans

Liu, A., C.A. Contador, K. Fan, and H.M. Lam. 2018. Interaction and regulation of carbon, nitrogen, and phosphorus metabolisms in root nodules of legumes. *Front Plant Sci*. doi: 10.3389/fpls.2018.01860

Liu, J., X. Yu, Q. Qin, R.D. Dinkins, and H. Zhu. 2020. The impacts of domestication and breeding on nitrogen fixation symbiosis in legumes. *Front Genet*. https://doi.org/10.3389/fgene.2020.00973

Long, J.A., R.L. Lawrence, P.R. Miller, L.A. Marshall and M.C. Greenwood. 2014. Adoption of cropping sequences in northeast Montana: A spatio-temporal analysis. *Agric. Ecosys. Environ.* 197:77-87.

Lupwayi, N.Z., G.W. Clayton, J.T. O'Donovan, K.N. Harker, T.K. Turkington, and W.A. Rice. 2004. Decomposition of crop residues under conventional and zero tillage. *Can J Soil Sci*. 84(4):403-410.

Lupwayi, N.Z., Lafond G.P., W.E. May, C.B. Holzapfel, R.L. Lemke. 2012. Intensification of field pea production: Impact on soil microbiology. *Agron J*. 104(4):1189-1196.

McCauley, A.M., C.A. Jones, P.R. Miller, M.H. Burgess, and C.A. Zabinski. 2012. Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: Effect of planting and termination timing. *Nutr Cycl Agroecosys*. 92(3):305-314.

McConnell, J.T., P.R. Miller, R.L. Lawrence, R. Engel, and G.A. Nielsen. 2002. Managing inoculation failure of field pea and chickpea based on spectral responses. *Can J Plant Sci*. 82(2):273-282.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001a. Response of pea to rate and placement of triple superphosphate fertilizer in Alberta. *Can J Plant Sci*. 81(4):645–649.

McKenzie, R.H., A.B. Middleton, E.D. Solberg, J. DeMulder, N. Flore, G.W. Clayton, and E. Bremer. 2001b. Response of pea to rhizobia inoculation and starter nitrogen in Alberta. *Can J Plant Sci.* 81:637–643.

McPhee, K.E. and F.J. Muehlbauer. 2009. Registration of 'Riveland' lentil. *J Plant Registrations*. 3(1):5-9.

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003a. Pulse Crops for the Northern Great Plains: II. Cropping Sequence Effects on Cereal, Oilseed, and Pulse Crops. *Agron J*. 95(4): 980-986

Miller P.R., Y. Gan, B.G. McConkey, C.L. McDonald. 2003b. Pulse Crops for the Northern Great Plains: I. Grain Productivity and Residual Effects on Soil Water and Nitrogen. *Agron J*. 95(4): 972-979

Miller, P.R., Bekkerman, A., C.A. Jones, M.H. Burgess, J.A. Holmes, R.E. Engel. 2015. Pea in rotation with wheat reduced uncertainty of economic returns in southwest Montana. *Agron J.* 107(2):541-550.

Miller, P.R., S. Atencio, C. Jones, E. Eriksmoen, B. Franck, J. Rickertson, …, K. Baber. 2022. Lentil inoculant, potassium, sulfur, and micronutrient effects on yield and protein in the northern Great Plains. *Great Plains Soil Fertility Conference Proceedings*.

Mohebbi, S., and R. Mahler. 1989. The effect of soil pH on wheat and lentils grown on an agriculturally acidified northern Idaho soil under greenhouse conditions. *Communications in Soil Science and Plant Analysis*. 20(3-4):359-381.

Muehlbauer, F.J. and K.E. McPhee. 2004. Registration of 'Pennell' lentil. *Crop Sci*. 44(4):1488.

Murray, J.D., C.W. Liu, Y. Chen, A.J. Miller. 2017. Nitrogen sensing in legumes. *J Exper Bot*. 68(8):1919-1926.

Nanthana, C., R. Lefroy, and G.J. Blair, 1997. Effect of crop duration and soil type on the ability of soil sulfur tests to predict plant response to sulfur. *Aus J Soil Research*. 35(5) 1131-1142.

Nasar, J., A. Roshan, A. Alam, M.Z. Khan, B. Ahmad. 2019. The impact of foliage fertilization of iron and molybdenum on yield, N uptake and root nodulation of lentil (*Lens culinaris* Medic) crop. *Indian J Ag Research*. 53(5):628-631.

Oldroyd, Giles E.D., J.D. Murray, P.S. Poole, and J.A. Downie, 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics*. 45:119-44.

Pan, B., S.K. Lam, A. Mosier, Y. Luo, and D. Chen. 2016. Ammonia volatilization from synthetic fertilizers and its mitigation strategies: A global synthesis. *Agric, Ecosys and Environ*. 232: 283-289.

Peoples, M.B., J. Brockwell, D.F. Herridge, I.J. Rochester, B.J.R. Alves, S. Urquiaga, R.M. Boddey, F.D. Dakora, S. Bhattarai, S.L. Maskey, C. Sampet, B. Rerkasem, D.F. Khan, H. Hauggaard-Nielson, and E.S. Jensen. 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. *Symbiosis*. 48:1-17.

Premaratne, K.P. and J.J. Oertli, 1994. The influence of potassium supply on nodulation, nitrogenase activity and nitrogen accumulation of soybean (*Glycine max* L. Merrill) grown in nutrient solution. *Fert Research*. 38: 95-99.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rehm, G.W. and R.C. Sorensen. 1974. Effects of the application of phosphorus, potassium, and sulfur to alfalfa grown on a calcareous silt loam. *Soil Sci*. 117:58-65.

Robinson, D. 2001. $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution*. 16(3): 153-162.

Romero, C.M, R.E. Engel, J. D'Andrilli, P.R. Miller, and R. Wallander. 2019. Compositional tracking of dissolved organic matter in semiarid wheat-based cropping systems using fluorescence EEMs-PARAFAC and absorbance spectroscopy. *J Arid Environs*. 167: 34-42.

Shearer, G. and D.H. Kohl. 1986. N-2 fixation in field settings – estimations based on natural N-15 abundance. *Aust J Plant Physiol*. 13(6):699-756.

Scherer, H.W., S. Pacyna, N. Manthey, M. Schulz, 2006. Sulphur supply to peas (*Pisum sativum* L.) influences symbiotic N_2 fixation. *Plant Soil Environ*. 52(2): 72-77.

Scherer, H.W., 2009. Sulfur in soils. *J. Plant Nutr*. *Soil Sci.*, 172: 326–335.

Sigler, W.A., S.A. Ewing, C.A. Jones, R.A. Payn, P. Miller, and M. Maneta. 2020. Water and nitrate loss from dryland agricultural soils is controlled by management, soils, and weather. Agriculure, Ecosystems & Environment. https://doi.org/10.1016/j.agee.2020.107158

Skogley, E.O, and V.A. Haby. 1981. Predicting crop responses on high-potassium soils of frigid temperature and ustic moisture regimes. *Soil Sci Am J*. 45(3):533-536.

Stevenson, F.C. and C. van Kessel. 1996. A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. *Soil Sci Soc Am J*. 60:1797-1805.

Unkovich, M.J., J.S. Pate, P. Sanford, E.L. Armstrong. 1994. Potential precision of the delta-N-15 natural-abundance method in-field estimates of nitrogen-fixation by crop and pasture legumes in south-west Australia. *Aust J Agric Res*. 45(1):119-132.

Unkovich M., D.F. Herridge, M.B. Peoples, G. Cadisch, R.M. Boddey, K.E. Giller, B. Alves, and P.M. Chalk. 2008. Measuring plant-associated nitrogen fixation in agricultural systems. ACIAR Monograph. Australian Centre for International Agricultural Research, Canberra, AU.

Unkovich, M.J., J. Baldock, and M.B. Peoples. 2010. Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. *Plant Soil*. 329(1-2):75-89.

USDA National Agriculture Statistics Service. 2022. https://www.nass.usda.gov.

Vance C.P., 2008. Carbon and Nitrogen Metabolism in Legume Nodules. In: Dilworth M.J., James E.K., Sprent J.I., Newton W.E. (eds) Nitrogen-fixing Leguminous Symbioses. Nitrogen Fixation: Origins, Applications, and Research Progress, vol 7. Springer, Dordrecht.

Vandenberg, A. and A.E. Slinkard. 2002. CDC Mozart field pea. *Can J Plant Sci*. 82:429-431.

Vandenberg, A., F.A. Kiehn, C. Vera, R. Gaudiel, L. Buchwaldt, S. Dueck, J. Wahab, and A.E. Slinkard. 2002. CDC Robin lentil. *Can J Plant Sci.* 82:111-112.

Vandenberg, A., S. Banniza, T.D. Warkentin, S. Ife, B. Barlow, S. McHale, B. Brolley, Y. Gan, C. McDonald, M. Bandara, and S. Dueck. 2006. CDC Redberry lentil. *Can J Plant Sci.* 86: 497–498.

Voisin, A., C. Salon, N.G. Munier-Jolain, and B. Ney. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 243:31-42.

Walley, F.L., G.M. Fu, J.W. van Groenigen, and C. van Kessel. 2001. Short-range spatial variability of nitrogen fixation by field-grown chickpea. *Soil Sci Soc Am J*. 65(6):1717-1722.

Walley, F.L., G.W. Clayton, P.R. Miller, P.M. Carr, and G.P. Lafond. 2007. Nitrogen economy of pulse crop production in the Northern Great Plains. *Agron J.* 99:1710-1718.

Warne, T., S. Ahmed, C.B. Shanks, and P. Miller. 2019. Sustainability dimensions of a North American lentil system in a changing world. *Front Sustain Food Syst*. https://doi.org/10.3389/fsufs.2019.00088

Woldekirstos, A.N., D. Huygens, H.R. Upadhayay, J. Diels, and P. Boeckx. 2014. Importance of correct B value determination to quantify biological N_2 fixation and N balances of faba beans (*Vicia faba* L.) via ^{15}N natural abundance. *Biol Fert Soils*. 50:517-525.

Wolyn, D.J., J. Attewell, P.W. Ludden, and F.A. Bliss. 1989. Indirect measures of N_2 fixation in common bean (*Phaseolus vulgaris* L.) under field conditions: The role of lateral root nodules. *Plant Soil*. 113:181-187.

Zentner, R., C. Campbell, V. Biederbeck, P. Miller, F. Selles, and M. Fernandez. 2001. In search of a sustainable cropping system for the semiarid Canadian prairies. *J Sustain Ag*. 18(2-3):117-136.

Zhao, F.J., A.P. Wood and S.P. McGrath, 1999. Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). *Plant Soil*. 212(2): 209-219.

Zimmer, S., M. Messmer, T. Haase, H.P. Piepho, A. Mindermann, H. Schulz, A. Habekuß, F. Ordon, K.P. Wilbois, and J. Heß. 2016. Effects of soybean variety and *Bradyrhizobium* strains on yield, protein content and biological nitrogen fixation under cool growing conditions in Germany. *Europ J Agronomy*. 72:38-46.