INFLUENCE OF ENVIRONMENT, GENETICS, AND MANAGEMENT ON FIELD PEA GRAIN NITROGEN CONCENTRATION AND YIELD RESPONSE AND SUBSEQUENT EFFECT OF LEGUME-BASED CROP ROTATIONS ON SOIL ORGANIC CARBON

by

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DEDICATION

This dissertation is dedicated to the following people and ideas. To my lovely and beautiful wife, Josie Koeshall, for all the love, kindness, and encouragement you have provided me with during my education and scientific pursuits. Thank you for all you have been to me during this chapter of life. To my parents, Tim and Regena Koeshall, for guiding me in my quest for knowledge, meaningful experiences, and aiming to live well. To my dear friends, Jonathan Ray, Zach Fox, Jacob Clay, John Edwards, Tyler Zimmer, and Clay Mullins, for friendships that are true, honest, and full of goodness. To my friend and Alpha Gamma Rho-Alpha Kappa big brother, Billy Rochelle who mentored me in the fraternity and was a role model to being dedicated to agricultural pursuits, whom I will see again. To the landscapes of Montana that have provided countless reprieves from academic rigor and endless adventure. To the idea that a high degree of richness in life is experienced through pursuing questions and research that may seem to have no end, because it is about the process and just showing up to the challenge. And to the goodness of God the Creator and Christ Jesus that have been endlessly revealed to me through research and outdoor pursuits across Montana, of which I hope my research efforts reveal to others.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF TABLES CONTINUED

Table Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES CONTINUED

Figure Page

ABSTRACT

Field pea (*Pisum sativum* L). is a cool-season legume, commonly referred to as a pulse crop, that is rotated with cereal and oilseed crops across the northern Great Plains (NGP). Field pea domestic markets have benefited from the growth of the plant-based protein market. North American plant-based protein markets commonly reward pea farmers with value-added contracts for producing high protein pea grain. Previous field pea research has focused on environment, genetics, or management factors that increase yield, while ignoring protein. Additionally, carbon (C) markets are emerging that reward farmers for utilizing practices such as diverse crop rotations to sequester atmospheric C into soil organic carbon (SOC). Pea farmers in the NGP need guidance on how to manage pea for elevated protein while also understanding how long-term production practices influence SOC sequestration. This dissertation provides an approach to understanding how environmental, genetic, and management factors influence pea protein and yield, and how diverse crop rotations affect SOC accrual. An interdisciplinary team conducted research across the USA and Canada from 2016 – 2024. Across the NGP, environment explained 70.5% of the variation in protein concentration observed across 23 environments, while genetics explained 10.5%. Precipitation accumulation during late vegetative growth followed by heat accumulation during grain fill predicted protein concentration. Intra-plant protein variation revealed that bottom pods frequently have a greater protein concentration across the NGP and that the environment × genetic interaction explained 50.7% of intra-plant protein variation. Analysis of genotypes revealed that stability of protein concentration is not associated with mean protein or yield stability, suggesting trait stability should be considered when evaluating pea genotypes. A management study revealed that application of seed-coat insecticide to mitigate pea leaf weevil infestations increased protein by 7 g kg^{-1}. A six-year study revealed that diverse crop rotations that rotate pea and lentil with spring wheat optimize SOC accrual, at a rate of 0.41 Mg SOC ha^{-1} yr^{-1}. These findings provide foundational information to inform pea growers and the larger plant-based protein industry how pea protein formation is controlled and the subsequent benefit of including field pea in diverse crop rotations to increase ecosystem services.

CHAPTER ONE

LITERATURE REVIEW

Classification, History, and Market Overview of Pea

Field pea (Pisum sativum L.) is a cool-season legume characterized by a brief growing season, and it has been widely adopted across various cropping systems in the northern and central Great Plains of North America (Padbury et al., 2002). It is commonly integrated into crop rotations with cereals such as spring or winter wheat (Triticum aestivum L.) and oilseeds such as spring canola (Brassica napus L.) (Padbury et al., 2002). Pea is a member of the Fabaceae or Legume family, and related to chickpea (Cicer arietinum), and lentil (Lens culinaris), which are grown in similar crop rotations across North America as field pea. Field pea was one of the first crops cultivated by humans, with its center of origin in central and southwest Asia (Oelke et al., 1991; Pavek & NRCS Pullman Plant Materials Center, 2012). Wild field pea lines can still be found in Afghanistan, Iran, and Ethiopia (Oelke et al., 1991). Field pea has been used as a food source for humans for approximately 9,500 years, with cultivation existing for approximately 8,500 years (Elzebroek & Wind, 2008; Pavek & NRCS Pullman Plant Materials Center, 2012). Ancient authors from Greece and Rome discussed field peas; however, cultivated varieties were not comprehensively documented until approximately the sixteenth century. (N. I. Vavilov, 1992).

Currently, the primary regions for global field pea production are Russia, France, China, India, Canada, and the United States (Oelke et al., 1991). Over 10 million ha of field pea are

grown globally, with leading countries producing primarily for export markets (Schatz & Endres, 2009). It is presently estimated that Canada produces 2.9 billion kg of pea, while the United States produces approximately 838 million kg. Both countries possess robust domestic markets that are fueled by protein ingredient processing and pasta production, complemented by international export markets to Asia. (StatsCan, 2025; *USDA/NASS QuickStats*, 2025).

Within the United States, field pea production occurs throughout the northern Great Plains and Pacific Northwest (commonly referred to as the Palouse region), with recent emerging markets in the central Great Plains in states such as Nebraska (11,300 ha) and portions of western Minnesota (19,481 ha) (Pavek & NRCS Pullman Plant Materials Center, 2012; Schatz & Endres, 2009). The provinces of Alberta, Manitoba, and Saskatchewan comprise Canada's pea-growing regions. Most Canadian pea grain is exported to India, China, Bangladesh, Pakistan, and the United States (*Pulse Weekly: Canadian Pea/Lentil Exports Slow to Start 2025/26 | The Western Producer*, 2026). Adoption of pea across various cropping systems has resulted in total planted ha of 1,424,000 and 360,000 in Canada and the United States, respectively, in 2024 (NASS, 2025; StatsCan, 2025). Since the turn of the century, pea production in the United States has expanded from 121,000 ha in 2002 to 362,200 ha in 2024, almost exclusively within the NGP (NASS, 2025). Global demand for pea protein, starch, and fiber products has driven the global market value of pea to $3.27 billion for raw, dried pea grain, while the cumulative global market value is estimated at $17.3 billion as of 2025 (Mordor Intelligence, 2025). These trends, combined with the persistent expansion of both domestic and international markets for pea grain, suggest rising global demand for plant-based protein products and other pea-derived food

ingredients, alongside the growing popularity of the crop among farmers in North America and beyond.

Morphology of Pea

Field pea is commonly grown as a summer annual or winter annual legume, with winter pea cultivars being successfully established in Idaho, Montana, and Washington (SARE, 2012). Spring-seeded field pea (ei, yellow field pea or green field pea) comprises the largest market share of pea production in North America. In contrast, winter pea production serves specialty markets, primarily forage and cover crop applications, and occasionally yields greater benefits for soil nutrient levels than spring pea (J. McDonnell, personal communication, January 2018). Field pea usually develops as a single stem, but can branch from nodes below the first flower, most often after enduring cold injury to the apical meristem (Schatz & Endres, 2009). Two types of field pea are commonly grown in the United States: the common vine pea, which displays normal leaves with shoots ranging from 0.9 to 1.8 m, and the semi-leafless pea that expresses shorter vine lengths with modified leaflets and tendrils and grows to a height of 0.6 to 1.2 m (Schatz & Endres, 2009). Most determinate and indeterminate field peas express one or several oval leaflets, terminal tendrils, and alternate, pinnately compound leaves (Pavek & NRCS Pullman Plant Materials Center, 2012). Nearly all spring-seeded yellow pea cultivars follow a determinate growth habit (Kevin McPhee, personal communication, January 2022).

Field pea pods are located on the top two-thirds of the plant and develop from the bottom to the top. Click or tap here to enter text.Indeterminate field pea varieties usually flower longer than determinate field peas, especially under cool, wet conditions with a maturity range of 90 to

100 days after planting (DAP) (Schatz & Endres, 2009). Determinate field pea varieties mature at 80 to 90 DAP and flower for only a limited period (Oelke et al., 1991; Schatz & Endres, 2009). The flowering period of determinate field pea flowers will occur for two to four weeks if the plant is free from abiotic stress such as drought or high air temperature (Pavek & NRCS Pullman Plant Materials Center, 2012). All yellow field pea types are sensitive to abiotic stress such as elevated air temperatures and low soil moisture, especially during flower formation, pollination, pod set, and seed set (Oelke et al., 1991; Pavek & NRCS Pullman Plant Materials Center, 2012; SARE, 2012; Schatz & Endres, 2009). However, indeterminate field peas have the potential to compensate for hot, dry periods and overall arid climates because of their extended reproductive period compared to determinate varieties (Schatz & Endres, 2009). Pea often develops via a determinate growth pattern due to environmental conditions, such as heat stress, that halt flowering and restrict continued vegetative growth. Field pea varieties that are semi-leafless and determinate should be used in growing regions where soil moisture will not be limited during critical reproductive stages, and plant microclimate conditions that favor plant disease development will be reduced (Benjamin & Nielsen, 2006; Oelke et al., 1991). If field pea experiences high air temperatures during seed fill, the grain may have a higher protein concentration, accompanied by lower fiber and starch content, and overall reduced grain yield potential (Pavek & NRCS Pullman Plant Materials Center, 2012). However, if pea experiences early-season drought stress, grain protein can be greatly reduced (Cutforth et al., 2007a; P. R. Miller et al., 2002; Mohammed et al., 2018a).

Even though heat stress and drought stress can be detrimental to field pea growth, field peas can withstand considerable cold periods and freeze injury without the apical meristem

becoming injured or necrotic (Meyer & Badaruddin, 2001). If the field pea shoot apical meristem is terminated by a freeze event or prolonged cold conditions before the development of the fifth node, new auxiliary shoots will form from hypogeal emergence (P. R. Miller et al., 2002; Pavek & NRCS Pullman Plant Materials Center, 2012; Schatz & Endres, 2009). Pea can withstand low temperatures during planting and germinate in cold, wet soil conditions, with most field pea types germinating at a soil temperature of 4.4° C (Meyer & Badaruddin, 2001; P. Miller et al., 2001; Schatz & Endres, 2009; Welbaum et al., 1997). However, field pea prefers well-drained soils, as waterlogged soils can impede root development and rhizobium survival (Cannel et al., 1979). Most field pea types will emerge 10 to 14 days after planting (Schatz & Endres, 2009). Field peas grow at an optimum temperature between 12° and 18° C during the vegetative stage. Spring-planted field peas are tolerant of harsh temperatures throughout late winter and early spring, with exposed plants tolerating -10 °C and, if covered with a layer of snow, -30 °C (Elzebroek & Wind, 2008). In 2003, Bourion *et al.* found that the frost tolerance of most winter and spring pea is related to the high concentration of soluble sugars in the leaves, especially if the pea selected was developed as a winter-hardy variety (Pavek & NRCS Pullman Plant Materials Center, 2012). In general, yellow field pea will continue to be adapted to a wider array of eco-regions as climate trends evolve and the demand for field pea grain ingredients increases in domestic and international markets (Cutforth et al., 2007a; Joshi & Rao, 2017; P. R. Miller et al., 2002).

Ecological Benefits of Pea

Ecological and economic benefits to cropping systems and rural communities have fostered the continued global and domestic growth of pea markets. Pea has become a popular rotational legume in cereal- and oilseed-based cropping systems due to the ecosystem services it provides. Generally, the most significant ecological benefit to cropping systems via field pea establishment originates from direct N benefits due to atmospheric N fixation and subsequent N release from pea residues (Beckie & Brandt, 1997a; Burgess et al., 2012a; Lory et al., 1995; Stevenson & Van Kessel, 1996a, 1996b). Pea requires little to no fertilizer N to attain full yield potential due to atmospheric N fixation via symbiotic *Rhizobium leguminosarum* bv. *viciae*, located in the rhizosphere, that infects the root system and provides the pea with ammonia in exchange for C (Salon et al., 2001a; Schiltz et al., 2005a). Click or tap here to enter text.Many grain producers across the northern Great Plains (NGP) region have adopted pea into their respective crop rotations due to the positive N economy that pulse crops such as pea have been shown to foster in the soil ecosystem, which has been shown to benefit subsequent cereal and oilseed crop yields while simultaneously reducing the amount of fertilizer N that a producer needs to maintain cereal or oilseed yield potential (Burgess et al. 2012a.).

Previous studies have shown that pea residues continue to release N and C via decomposition in the soil ecosystem beyond the growth of the first subsequent crop, thereby benefiting the N economy of the cropping system for multiple years (Lupwayi & Soon, 2015a, 2016a). In addition to the direct N-benefits from pea to cropping systems, pea also fosters positive non-N benefits to subsequent crops, such as a low soil water withdrawal due to a short growing season and compact effective rooting zone, increasing water availability and total soil

water for subsequent crops, which potentially can have multi-year implications to crop management (Stevenson & Kessel, 1996). Other non-N benefits of pea include an expansion of herbicide options to control problematic weed species found in cereals and oilseed crops, the addition of rhizobia root exudates that benefit the soil microbiome, rapid residue N release to the soil ecosystem, increased soil organic C stocks, and crucial disease breaks via legume incorporation in monocot-based systems (Cutforth et al., 2007; Engel et al., 2017; Koeshall et al., 2022; Miller et al., 2003).

Potential Benefit of Legumes to Soil Organic Carbon

Advancements in global agriculture, including crop management, the availability of fertilizer N sources, increased access to pest management tools, and the advent of precision technologies, have led to greater food stability, improved crop yields, and enhanced crop quality (Tilman et al., 2002a). These advancements in global crop production have increased food availability and reduced the per capita share of income spent on annual food purchases (Gollin, 2023). However, modern agricultural practices that have increased food production have unintended consequences for global and regional ecosystem soils.

Soil organic carbon (SOC), a foundational component of soils and the building block of soil organic matter (SOM), has decreased across both tillable and grazable land under agricultural management compared to native ecosystems such as prairie grasslands (Cambardella & Elliott, 1994; Cotton & Acosta-Martínez, 2018; Sanderman et al., 2017). Global SOC balance study by Sanderman et al. (2017) estimated that the global SOC debt was 133 Pg C in the top 2 m of soil due to agricultural land use, primarily from the utilization of marginal soil ecosystems in production agriculture. A research study by Cotton & Acosta-Martínez (2018) found that

grasslands converted to tillable row crop production lost 33% of organic C and 30% of total N, along with SOC decreasing 20% in the top 30cm of the soil profile following one growing season. Analysis of the Haas long-term soil archive by Liebig et al. (2024a) found that, over 71 years, two of three sampling locations had experienced 15% and 36% reductions in SOC, attributed to management and tillage practices in dryland cropping systems. These findings suggest opportunities to improve agricultural practices and land management to reduce SOC losses, remediate depleted soils, and restore SOC stocks across ecosystems.

Voluntary C markets in agricultural crop production worldwide have attracted attention over the last 20 years, with a recent increase in program availability, research interest, and farmer accessibility. Carbon markets offer a unique opportunity for agricultural producers and land managers to monetize management practices that have been shown to sequester atmospheric C in SOC stocks. These programs reward crop producers for either 1) switching to management practices such as reduced tillage or growing cover crops that have been shown to foster SOC sequestration or 2) are paid per unit of SOC accrual per year via soil sampling analysis, with sampling occurring every 5-10 yr (Johansson et al., 2025; Souza et al., 2025). However, many of these programs have low participation, as producers have limited information on which specific agronomic practices will increase SOC accrual in their ecosystems and face program complexity (Pudasaini et al., 2025). Additionally, many programs communicate and market different pay schedules and rates for different practices and report different SOC sequestration rates for the same practices (Pudasaini et al., 2025; Sellars et al., 2021; Souza et al., 2025). Furthermore, the value of C can vary widely across program types and with annual fluctuations in C credit prices (Pudasaini et al., 2025; Souza et al., 2025). These issues present a need to refine and understand

what practices, such as crop rotation, soil disturbance, species diversity, and crop residue management, result in SOC accrual and at what rate in specific ecoregions.

Previous research on long-term agriculture practices has shown that C and N losses can be reduced by crop rotation and species selection. A 15-year study by Drinkwater et al. (1998) reported that SOC increased when various legume species were incorporated into the crop rotation, resulting in higher SOC accumulation rates than in a conventional corn (*Zea mayes*) – soybean cropping system. Shrestha et al. (2013) discovered that increased biomass production was associated with increased SOC accrual, suggesting that increasing cropping intensity dries SOC accrual. Sainju et al. (2017) explored the benefits of diverse crop rotations and species diversity, which have been shown to reduce pest infestations and their effects on SOC, finding that alternating-year crop rotations that include field pea enhance SOC and crop yields compared to a stacked-year crop rotation that repeats cereal species. A ten-year crop rotation study in southwest Montana by Engel et al. (2017) found that SOC accrual was 0.09 Mg ha^{-1} yr^{-1} in a no-till pea/oilseed-wheat crop rotation, while SOC losses were greatest in the tilled fallow-wheat crop rotation, showing that fallow replacement with legumes increased SOC accrual. These findings suggest that properly selected crop management practices and species diversity in grain production systems can reduce SOC degradation and foster SOC accrual, potentially increasing producer net returns through C market opportunities.

In addition to potential C market profits, other studies have shown the specific potential of pulse crop species to increase producer net returns through synergistic relationships with a wheat-based cropping system. A short-term crop sequence study by Koeshall et al. (2022) in western Nebraska found that replacing chemical summer fallow in a fallow-wheat rotation with

field pea resulted in greater net returns and reduced net losses across three grain pricing schedules while also reporting increased N release without reducing soil organic C stocks, suggesting that pea benefits the producer both ecologically and economically (Koeshall et al., 2022). A long-term study by Miller et al. (2015) reported that economic variability was reduced in two pea 'fallow'-wheat crop rotations compared to tilled and no-till fallow-wheat systems, when considered under reduced N fertilizer rates and under a variable pricing program that rewards producers for high-protein wheat grain. Furthermore, a study by Lenssen et al. (2007) showed that the inclusion of field pea in a wheat-based cropping system resulted in the least reduction in wheat yield and soil water loss in water-stressed environments in Montana.

Pea and other pulse crops are commonly grown in rotation with cereals, such as winter wheat, across a large portion of the northern and central Great Plains. This has resulted in pulse crops being seeded into variable-height cereal stubble due to contrasting direct-cut harvesting methods used across this region. A study by Cutforth & McConkey (1997) found that subsequent wheat yields and estimated water-use efficiency increased by 6% in 30 cm tall stubble compared with wheat seeded into cultivated stubble and by 12% compared to tilled soil. A subsequent study by Cutforth et al. (2006) found that tall cereal stubble increased canola yield by 24% and improved water use efficiency by 19%. Much of the benefit to subsequent crop yields from very tall stubble comes from reduced microclimate temperatures and increased water availability and storage, resulting from reduced evaporation rates and reduced soil surface temperatures (Cutforth & McConkey, 1997; Cutforth et al., 2006). A more recent study by Cutforth et al. (2011) found that crop yields of pea, canola, and wheat increased linearly with stubble height, reporting that extra-tall stubble increased subsequent crop yields by 17%. The timing of this study was

fortuitous in that stripper harvesters of appropriate width (12.8 m) for wheat harvest first became commercially available from Shelbourne-Reynolds in 2012. Furthermore, a thesis study by Barnes (2025) found that pulse crop yield increased in specific site-year observations, and that basal pod height in pea, lentil, and chickpea increased by 4 – 5.5 cm when grown in very tall cereal stubble.

Stripper harvesters require using no-till disc air-seeders adapted to seed into tall stubble (Chaudhuri, 2001; Siemens et al., 2004). Hoe opener seeders have remained the standard seed method, though in cropping systems that still utilize standard direct-cut draper header technologies. This contrasting methodology practically pairs stripper harvesters with no-till air seeders and hoe-opener seeders with standard draper headers. This difference in seeding and residue management practices has created unique differences in soil disturbance at crop establishment and at harvest. These diverse management practices during seeding and harvest may result in variations in soil health, particularly concerning SOC. These contrasting practices may have microclimate, soil moisture, and soil biological implications that alter SOC stocks and accrual rates in crop rotations common to the Great Plains. Although previous research has detailed the benefits of growing subsequent crops in tall stubble and the morphological benefits to pulse crop species via improved micro-climate conditions, little is known about how the presence of very tall cereal stubble in diverse crop rotations found in the Great Plains regions influences SOC stocks and accrual compared to traditional stubble and residue management.

Overall, previous research has shown that the inclusion of pulse crops, reduced tillage, and diverse crop rotations can benefit soil health (e.g., SOC). Additionally, the advent of voluntary C markets and the social and ecological need to reduce atmospheric C via C

sequestration details the need to understand and explore crop production practices that promote SOC sequestration and accrual. Furthermore, the ability to access the C market for grain producers across the northern Great Plains enables them to increase net returns to their farming system via annual C credit payments, thereby creating an additional revenue stream. However, there is little to no research detailing the potential for SOC accrual and cropping system rates across the northern Great Plains, which is also the main growing region for pulse crops in North America. This research gap demands long-term crop rotation research to estimate how SOC accrual and stocks are influenced by crop rotation, legume inclusion, soil disturbance, and residue management in dryland cropping systems. This research would inform producers about which practices increase SOC and help the broader C market sector with improved SOC sequestration for the NGP.

Potential Benefit of Pea and Pulse Crops to Subsequent Crops and Soil Nitrogen Cycling

Fertilizer N inputs, such as urea, have continued to increase (Burgess et al., 2012). In addition to high input costs, fertilizer N application is among the most energy-intensive agricultural inputs used globally, particularly in developed countries (Burgess et al., 2012). Agricultural production and food availability have benefited from the use of fertilizer N, as total global cereal production doubled from 1960 to 2000 (Tilman et al., 2002b). There have been ecological issues associated with this increased use of fertilizer N across our landscapes. Emissions from agricultural production are one of the leading causes of increased concentrations of greenhouse gas emissions, with nitrous oxide being one of the main culprits (Canfield et al., 2010; Eagle et al., 2020; Sebilo et al., 2013; Tilman et al., 2002b; Van Meter et al., 2016).

Dependence on fertilizer N sources has led to agricultural soils becoming the largest N sink through the soil organic nitrogen (SON) cycle, thereby contributing to unprecedented levels of N pollution in groundwater and surface water sources, especially in major water bodies such as the Missouri River Basin (Van Meter et al., 2016). In agricultural soils across North America, increased fertilizer N use has also caused harmful changes in the chemical composition of the soil profile. Liebig et al. (2024b) detailed in a review of the Hass soil series archive across three locations across the Great Plains, from 1947 to 2008, at a soil depth of 0 - 15.2 cm, a reduction in soil pH, with one site displaying pH changes from 7.4 to 5.8. Schroder et al. (2011) reported that increased N applications in winter wheat production resulted in soil pH below 5.0. This threshold often leads to aluminum toxicity in most crops and inhibits proper nodulation in pulse crops. soil pH levels have also been falling in Montana due to fertilizer N use (Jones et al., 2019). Due to the direct short- and long-term N benefits of pea and other pulse crops to soil ecosystem fertility, the regular integration of pea and various pulse crop species into cereal- or oilseed-based rotations offers the potential to reduce ammonium-based fertilizer N applications. Previous research, primarily conducted in the central and northern regions of the Canadian prairies, has extensively examined various aspects of the potential N and non-N benefits of pulses, with particular emphasis on field pea as the legume of interest. Stevenson & Van Kessel (1996) found in a short-term study that when pea was established the prior year before wheat, compared to a continuous wheat rotation, the pea crop resulted in 6-14 kg N ha^{-1} of accumulated N in the soil, directly from the decomposition of pea residues. In the same study, wheat grain yield following pea was increased by 43%, although most of that yield advantage came from non-N benefits (disease break). Beckie & Brandt (1997a) found that the residual N effect of pea averaged 27, 12,

and 28 kg N ha^{-1} across three unique sites, with site-specific environmental conditions influencing the final realized N benefit. Seed yield of pea was used to quantify residual N, in which the authors concluded that for every 1000 kg ha^{-1} pea seed produced, farmers should assume 15 kg N ha^{-1} was deposited by pea. In a longer-term study, Lupwayi & Soon (2016) found that the use of various legume crops, including pea and fava bean, resulted in positive rotational benefits to subsequent cereal and oilseed crops three years following legume establishment while also finding that belowground N factors, likely from decomposing legume roots and root/nodule exudates, also contributed to net positive rotational effects.

Fostering an understanding of potential N credits in Montana cropping systems could lead our cropping systems to increase NUE and take advantage of a growing N sink of SON found within our local cropland soils. Yan et al. (2020) detailed that much of the fertilizer N used in crop production is not utilized by the crop within the same year of application, of which only 37% of cereal N uptake was found to be from fertilizer N sources. This finding suggests that a different pool of N, other than fertilizer N, is responsible for driving non-legume crop production. (Van Meter et al., 2016) discussed the dynamics of the growth of SON and the legacy N residing within SON pools from historic fertilizer N application. Previous research has also detailed the positive N and C release from pulse crop residues back into the soil profile, with many observations showing that pulse residues release N and C still at the 3-yr mark and possibly longer (Engel et al., 2017c; Lupwayi & Soon, 2015b, 2016b).

Accurately quantifying the annual N credits from field pea and general pulse crops in common crop rotations across North America could enable a region-wide reduction in annual fertilizer N application, thereby positively affecting other soil fertility parameters, such as soil

pH. Overall, quantifying N credits that Montana crop producers could capitalize on could increase legacy soil fertility, reduce long-term fertilizer N applications, and increase our utilization of N in our SON pools, thereby increasing NUE for cropping systems over time and possibly leading to increased annual yield or grain quality.

Economic Benefits of Pea to Cropping Systems

The application of pea has been shown to improve economic parameters in farming systems across North America, in addition to the ecological benefits the species provides. Much of the economic advantage of pea comes from its synergistic relationship with cereal-based rotations, in part through the positive N economy and the direct N credits it provides, resulting in reduced fertilizer N application. Miller et al. (2015) evaluated economic returns across common crop rotations in southwestern Montana. Findings from this study revealed that a pea-wheat rotation yielded the greatest 4-year net returns and provided the best economic stability among rotations, particularly in scenarios where the rotation was subjected to reduced fertilizer N applications and when wheat was heavily discounted due to below-threshold grain protein concentration (P. Miller et al., 2015). Furthermore, when pea replaced fallow periods in a crop sequence, the economic variability of the farming system decreased because a non-production period was replaced by a cash crop (Koeshall et al., 2022; P. Miller et al., 2015). Koeshall et al. (2022) found that a pea-wheat sequence resulted in greater net profit or reduced net losses in five out of nine site-year comparisons via three unique grain pricing models for dryland cropping systems in western Nebraska. Gross revenue was increased on average by \$113 ha^{-1} via the replacement of chemical summer fallow with pea (Koeshall et al., 2022). Furthermore, the inclusion of pea elsewhere in the NGP has been shown to increase subsequent cereal yields,

except in instances of below-average growing season precipitation, while also increasing annualized yields in many observations compared to traditional fallow-wheat rotations (Gan et al., 2003; Koeshall et al., 2022; P. Miller et al., 2015; Nielsen & Vigil, 2014, 2017, 2018).

Overall, the ecological benefits of peas, mainly through direct N gains, combined with reduced economic variability from higher, more stable yields and increased grain protein in subsequent wheat crops, have led to widespread adoption of peas across various cropping systems and boosted their popularity among grain farmers in North America. This increased adoption among grain producers across North America has also partly resulted from the rising use of pulse crop grains, such as peas, in human and livestock diets.

Consumption of Pea Products in Human Diets

Pea grains have demonstrated positive effects on human health when incorporated into various food products. Research indicates that consuming pea protein or fiber supports effective blood sugar management, weight loss, enhanced gut health, and a lower risk of chronic diseases (Garousi et al., 2017; Mancabelli et al., 2025). Previous research has reported that pea protein and fiber can decrease short-term food intake and blood glucose levels in healthy individuals (Arnoldi et al., 2015; Wu et al., 2023). It was also found that pea-derived protein and fiber boost beneficial gut bacteria, such as *Lactobacillus* (Mancabelli et al., 2025). Additionally, various reviews and studies suggest that grain legumes may help prevent hypercholesterolemia and hypertension and might lower the risk of certain cancers (Arnoldi et al., 2015; Hall et al., 2017; Ren et al., 2021).

Peas and other grain legumes are used in various forms across the culinary and food industries. Much of the prior growth and adoption of pea-sourced food ingredients stemmed from

the popularity of pea protein isolates in plant-based protein powders and meat analogs, as evidenced by products from Beyond Meat (*Kerry Releases Report on Plant-Based Food & Beverage | Food Dive*, 2020). Pea-based protein ingredients have enabled consumers to avoid soy-based products, thereby reducing the allergenic risk associated with plant-based protein products (Dahl et al., 2012; Hall et al., 2017; Wu et al., 2023). Furthermore, pea flour has grown in popularity among consumers seeking gluten-free pasta, particularly those with celiac disease or gluten sensitivity (Wu et al., 2023). The adoption of pea-based flours in human diets is revealed in a global market value of $19 billion as of 2024, with pasta, baked goods, and snack foods being the top three food categories in which pea flour is utilized (*Pea Flour Market Share, Size, Growth & Forecast 2025-2035*, 2025; *Pea Flour Market Size & Share | Industry Outlook 2025-2034*, 2025).

Consumption of Pea Products in Livestock Diets

In addition to benefiting human health, the consumption of pea grain in livestock diets has been shown to improve the overall health of beef cattle, swine, dairy cattle, and poultry (Anderson et al., 2007; Kandel et al., 2025; Panaite et al., 2025; Pulido et al., 2024). Pulido et al. (2024) examined the opportunity to replace soybean meal with pea grain meal in grazing dairy cattle diets. Anderson et al. (2007) found that including pea grain in beef cattle finishing diets at 10% or more improves meat tenderness without detracting from other carcass quality traits. In swine production, Panaite et al. (2025) found that the inclusion of pea grain in diets of growing male pigs of 10 to 20% positively contributed to intestinal health and morphology, N retention, and enhanced growth performance. They found that milk quality is not affected when dairy cattle are supplied with soybean meal and maintain similar milk production levels. Finally, findings by

Kandel et al. (2025) showed that broiler chickens increased body weight gain and reduced feed conversion ratio while maintaining carcass yield when diets include 5-12% pea grain. Overall, consumption of pea grain ingredients by both human and livestock subjects resulted in net positive health benefits and attributes.

Additionally, pea protein ingredients were widely adopted by the North American dog pet food industry, leading to increased demand for plant-based proteins and driving premium protein markets for pea growers (*Once a Sidekick in Food, the Pea Finds Itself the Star of the Show | Food Dive*, n.d.). However, a preliminary, incorrect health notice from the USDA and an investigation by the FDA led to the decline and eventual disappearance of the pea-based dog food market based on a claim that consumption of pea proteins resulted in canine heart disease (*Questions & Answers: FDA Center for Veterinary Medicine's Investigation into a Possible Connection Between Diet and Canine Heart Disease (Updated June 27, 2019) | FDA*, n.d.). However, the pet food industry's adoption of pea contributed to the initial growth and broader adoption of the pea protein market.

Although incorporating pea into various cropping systems has demonstrated improvements in ecological and economic outcomes, and consuming pea grain ingredients benefits both human and livestock health, there are gaps in our understanding. Specifically, the mechanisms by which environmental and genetic factors affect pea grain protein formation, development, and relationship with grain yield remain unclear. These gaps threaten the long-term market growth and sustainability of peas for farmers in North America, while leaving pea processors with little knowledge of which regions consistently produce high-protein pea grain.

Pea Protein

Increasing pea yield directly leads to higher net returns for farmers. Therefore, most previous research has focused on understanding how environmental, genetic, and management factors influence biomass or grain yield. Historically, public and private programs have aimed to improve aboveground biomass and grain yield through breeding, agronomic practices, disease resistance, and pest management. However, grain quality parameters, such as grain protein concentration, have received less attention (Cousin, 1997; French, 1990; Huang et al., 2023; Koeshall et al., 2021; Sagan et al., 1993). For instance, previous studies on optimizing planting time and seeding rates in various ecoregions aimed to increase grain yield at the cropping system level, often neglecting how these management practices affected grain quality metrics such as protein content (Baird et al., 2009; Carr et al., 2024; Koeshall et al., 2021; Stepanovic & Werle, 2016). Additionally, the food industry's processing techniques and consumer preferences for pea grain ingredients or products did not prioritize improving grain protein concentration, as most quality issues were cosmetic, like off-colored, split, or cracked grains, which could typically be managed during harvesting or seed cleaning (Pea Harvest Management | Saskatchewan Pulse Growers, 2025; Zhang et al., 2025).

The demand for research on pea protein has surged since 2014, driven by food processors. These premiums varied by region, but high-protein pea grains with more than 24% protein often fetched an additional $0.0276 per kg ($0.75 per US bushel) over base market prices for nearby domestic delivery. Understanding how to manage pea cultivation to achieve high-protein grains became crucial, as buyers sometimes apply discounts or reject pea batches with protein below 21-22% (R. Edinger, pers. comm., 13 Dec 2024). This risk of rejection, combined

with the opportunity to establish private contracts with processors for high-protein pea production, has heightened the need to identify both biotic and abiotic factors that simultaneously influence pea grain protein concentration and yield.

Potential Environmental Influences on Pea Protein

Pea is cultivated across ecoregions and climate gradients within the NGP, exposing it to diverse environmental conditions (Padbury et al., 2002). Previous research on pea grain protein concentration has aimed to understand how and to what extent environmental factors influence the formation and development of pea grain protein concentration and grain yield (Bestwick et al., 2018; Franck et al., 2023; Larmure et al., 2005; Mohammed et al., 2018b; Tao et al., 2017). Several previous studies have found that the environment is a determining and controlling factor of pea protein and yield potential (Franck et al., 2023; Mohammed et al., 2018b; Tao et al., 2017). Tao et al. (2017) evaluated nine pea cultivars at five locations across Montana in 2013 and 2014 and, using additive main effect and multiplicative interaction (AMMI) analysis, concluded that environmental effects explained 92.5% and 93.1% of the variation in protein concentration and grain yield, respectively. The study by Tao et al. (2017) found that, using the modified Palmer Drought Index scores for each site-year, precipitation across various plant life-cycle stages accounted for most of the environmental control on protein and yield. Abnormally dry and wet soil conditions during pea establishment and vegetative growth were found to limit protein concentration. A subsequent multi-site field-based study conducted in Montana and North Dakota by the same research team (Mohammed et al.,2018) also found that environment influenced protein concentration (ranged from 16.6% to 26.4%) to a greater degree than cultivar variation (protein from 20.7% to 22.3%). Mohammed et al. (2018) suggested that, given the

meaningful genetic control over yield potential and grain quality, there is an opportunity to develop environment-specific cultivars. However, this study did not identify which specific environmental factors influenced pea protein concentrations, except that it showed that different site-years significantly affected protein concentration. Growing-season precipitation, soil properties, flowering temperature, and growing-degree days were identified as potential environmental variables that may explain variation in grain protein concentration. Temperature patterns during the growing season are another environmental factor that has been shown to influence pea protein concentration and grain yield. Larmure et al. (2005) presented findings on the influence of temperature on C and N allocation to pea seeds and on final seed N concentrations. In a greenhouse study, pea plants subjected to a day/night temperature regime of 15/10°C, compared with 25/20°C, were found to optimize C availability by increasing C fixation, resulting in more vegetative structures and a greater N demand (Larmure et al., 2005). During the seed-filling period, pea plants grown at warmer temperatures produced pea grain with higher N concentrations (Larmure et al., 2005). This study suggests that when pea was subjected to higher air temperatures during the growing season produce higher N concentrations in pea grain occurred. Overall, lower temperatures drive C fixation efficiency through optimized respiration, which, given that pea N fixation was equal in the two temperature treatments, results in the plant being able to partition a greater pool of C and drive greater vegetative production, resulting in greater competition for N sources to a greater number of sinks. Pea grain yield has also been shown to be influenced by temperature. In Saskatchewan, Bueckert et al. (2015) reported that pea grain yield and time in the reproductive growth cycle were reduced when pea was subjected to more than 20 days of maximum daily temperatures above 28 °C. That study

also found that precipitation was correlated with grain yield, with peak yield observed when pea was received cumulative precipitation above 350 mm. Heat stress during the reproductive cycle can severely reduce pea yield potential via the abortion of flowers and developing pods, particularly at temperatures above 30 °C, with reproductive organ abortion rates between 20 and 50% (Guilioni et al., 1997). Previous research and findings on the environmental control of pea protein and yield show that more work is necessary to improve our understanding for pea growers, crop advisors, and pea processors.

Influence of Heat Stress on Pea

Heat stress can alter photosynthate production, C and N allocation throughout and during the plant life cycle, flowering and pollination success, and net primary productivity, all leading to potential influence on observed intra-plant protein and field-scale protein variation (Larmure et al., 2005; Salon et al., 2001b). A greenhouse study by Larmure et al. (2005) found that increasing maximum daily temperatures from 15 °C to 25 °C reduced C fixation per degree-day, resulting in fewer N-sink structures formed via reduced aboveground biomass and fewer viable pods that require N. Furthermore, this study found that pea plants prioritize C allocation to developing seeds over N assimilation, suggesting that starch dilution and the overall source-sink ratio for C and N influence observed grain protein concentration. Guilioni et al. (1997) detailed how heat stress influences abortion of buds and flowers in pea, which suggests that if heat stress is first experienced during flower formation, the N sink structures for developing pea pods are reduced while total N source for a single plant remains the same, likely resulting in greater grain N concentrations.

Huber et al. (2016) reported that intra-plant grain protein concentrations in soybean were altered by stand density, suggesting that microclimate alterations influence N dynamics, resulting in varying grain protein concentrations across pod-nodal positions and stand-density treatments. This observation in soybean aligns with other work on the effects of heat on N and C fixation, partitioning, and remobilization. It underscores the need to examine the variation in protein between pod positions within a pea plant. Overall, lower temperatures drive C fixation efficiency through optimized respiration, which, given that pea N fixation is equal from 15 °C to 25 °C, results in the plant being able to partition a greater pool of C and drive greater vegetative production, resulting in greater competition for N sources to a greater number of sinks (Guilioni et al., 2003b; Larmure et al., 2005; Jiang et al., 2017). Although research has shown the influence that heat stress has on N and C accumulation and partitioning, which affects grain protein concentration, little research has been conducted on how variable air temperature observed during the growing season and heat stress, influence the variation in protein between pod positions within a pea plant, field-scale protein concentration, and grain yield through the lens of contrasting pea genotypes.

Influence of Water Stress on Pea

Water stress experienced by pea throughout the growing season can alter N accumulation and partitioning, thereby influencing intra-plant grain protein concentration and field-scale protein. When pea experiences water stress early in the growing season, both yield and protein concentration may be affected by biological N-fixation. Serraj et al. (1999) also observed that N-fixation in legume crops is altered by the incidence of water stress during the growing season, attributing reduced N-fixation in water-stressed environments to possibly a variety of factors

such as carbon shortage from the plant, oxygen limitations, or feedback regulation by nitrogen accumulation, with phloem flow likely resulting in explaining why N-fixation is severely limited in drying soil environments. Hossainet al. (2016) reported that low-precipitation growing seasons across contrasting cultivars reduced N fixation relative to historically normal-precipitation growing seasons, suggesting that the efficiency and health of rhizobia nodules in the rhizosphere can be decreased under dry soil conditions.

The timing of water stress influences whether grain protein concentration increases, decreases, or remains constant. Lhuillier-Soundélé et al. (1999b) detailed how water stress reduces aboveground biomass in pea, thereby reducing total N-sink structures and potentially decreasing N accumulation in shoots and leaves, ultimately reducing N remobilized to seeds. Guilioni et al. (2003a) found that water stress incurred at any point during the growing season reduces total seed production per plant, thereby increasing grain protein concentration. A soybean study by Huber et al. (2016) examined how intra-plant grain protein concentration varied across stand densities, suggesting that water availability and intra-plant competition for resources influence N accumulation and partitioning ratios, thereby shaping final grain protein concentrations across nodal positions within individual pods. Overall, early-season water stress can prematurely halt or reduce N-fixation and limit total aboveground biomass accumulation. In contrast, water stress later in the growing season can decrease seed production. Although previous research has detailed how water stress influences N accumulation and distribution throughout pea plants, little to no research has examined how contrasting regions and environments, and growing-season precipitation within those environments, influence intra-plant

grain protein concentration, field-scale protein concentration, and grain yield among contrasting pea genotypes.

Influence of Pea Leaf Weevil Infestations on Pea

Pea leaf weevil (PLW) has emerged as the primary economic pest of concern in most pea production regions across North America, owing to its persistent and widespread presence in diversified crop production systems and its capacity to induce significant yield reductions. (Cárcamo et al., 2011; Seidenglanz et al., 2010; Van De Steene et al., 1999; Vankosky et al., 2009; Wanner, 2007; Williams et al., 1995). Previous research has shown that the economic threshold for PLW occurs when 25 – 30 percent of pea plants have any leaflet or growth point (i.e. 'clamshell') feeding evidence (Cárcamo et al., 2011; Williams et al., 1995; Willsey et al., 2021). Although foliar feeding can reduce grain yield potential, unmanaged adult PLW infestations early in the growing season are a precursor to greater damage from larval feeding to rhizobia nodules. Infestations cause plant damage in varying ways, depending upon the life cycle of the PLW that is active. Adults cause initial foliar feeding of peas immediately following plant emergence which is evident by U-shaped notches created on the margins of pea leaflets (Cárcamo et al., 2011; Wijerathna et al., 2021; Willsey et al., 2021). Pea plants are most at risk of foliar feeding and yield potential loss by adult PLW before the 5th node stage, even though later foliar feeding can occur during later reproductive stages of pea, commonly in late July (Wanner, 2007; Williams et al., 1995). If PLW infestations are not controlled early, oviposition begins in pea fields, which fosters the most insidious feeding from PLW larvae that target rhizobium nodules, resulting in decreased N fixation and hence N availability to develop grain protein (Van De Steene et al., 1999; Wanner, 2007; Williams et al., 1995; Willsey et al., 2021). Pea plants

depend predominantly on N fixation to fulfill most of their N requirements. Consequently, they are susceptible to potential yield reduction and a decrease in grain protein concentration when PLW larvae are not effectively managed. (Gauer et al., 1992; Jones, 2016; Mariotti et al., 2008).

Chemical controls are available commercially that can be applied before seeding through an insecticidal seed coat or sprayed as a foliar product during plant growth (Willsey et al., 2021). Seed-coat applied products commonly contain Group 4A insecticides (Nicotinic acetylcholine receptor [nAChR] antagonists) such as imidacloprid or thiamethoxam, and are an effective residual control that mitigates economic damage from both PLW adults and larvae throughout the growing season (Cárcamo et al., 2011). Previous research has shown that foliar and nodule feeding is reduced with the use of seed-coat insecticide, even though yield may not always be increased (Cárcamo et al., 2011; Willsey et al., 2021). However, little to no research has been conducted on the yield and grain protein response to varying rates of seed-coat-applied insecticide.

Foliar-applied insecticides commonly contain Lambda-cyhalothrin as the active ingredient and result in the highest efficacy if applied before the development of the 6^{th} node in the pea plant (Cárcamo et al., 2011; Wanner, 2007; Willsey et al., 2021). Foliar-applied insecticides are less effective in controlling PLW adults due to the difficulty in timing application before oviposition occurs, which results in below-ground nodule feeding by PLW larvae if applied too late (Wanner, 2007). Proper timing of Lambda-cyhalothrin application is important to pea growers as the half-life of Lambda-cyhalothrin is 5 days (National Pesticide Information Center, 2001), with the active ingredient having a detectable presence on plant tissue up to 14 days post-application (Djouaka et al., 2018). Previous research has shown that a greater yield

response in pea is obtained with a seed-coat-applied product compared with a foliar-applied product (Willsey et al., 2021). The use of a seed-coat insecticide, such as thiamethoxam, can provide long-term plant protection by allowing the active ingredient to be absorbed through the roots and transported to various plant tissues via the xylem (Tian et al., 2022). Although foliar insecticide can be a valuable tool to control acute PLW infestations, other crops in the same farming system can serve as hosts to PLW, allowing for multiple waves of adults (Wanner, 2007; Wijerathna et al., 2024; Williams et al., 1995). However, little to no research has been conducted on the yield and grain protein responses in peas due to the varying foliar application timing of insecticides used to manage PLW. With the continued presence of PLW populations across the pea-producing regions of the NGP, it is important to evaluate the influence that both seed-coat and foliar-applied insecticide applications have in field pea to maintain or increase grain yield and protein content. Furthermore, it is important to understand the rate of seed-coat insecticide and the timing of foliar-applied insecticide that results in the greatest yield and protein advantage.

Potential Genetic Influence on Pea Protein

Genetic variation (e.g., cultivar) has also been demonstrated to impact pea grain protein concentration and yield substantially. Research by Tao et al. (2017) showed that variation among cultivars had little influence on pea grain protein concentration relative to the environment. However, the study indicated that cultivar performance ranks varied substantially across environments, suggesting site-specific adaptability of pea cultivars. Tao et al. (2017) showed that grain protein concentration varied among cultivars due to inherent inter-cultivar advantages arising from optimized biological N fixation and rhizobium strain associations. Tao et al. (2017)

showed that resistant starch concentration is controlled to a greater extent by genetics than by environment, as AMMI analysis indicated that genetics accounted for 9.1% of the variation in resistant starch, compared with 1.7% and 1.9% for yield and grain protein, respectively. This finding shows that breeding programs may be able to advance grain quality parameters more than yield via breeding techniques. A study by Franck et al. (2023) evaluated seven pea cultivars at seven locations across Montana over two years and concluded that genetics exert consistent control over grain protein concentration, as evidenced by the absence of a significant cultivar × location interaction. This finding indicates that genetic factors have a greater impact on pea grain protein concentration than environmental factors, contrasting with conclusions from other studies conducted in the same North American region (Mohammed et al., 2018b; Tao et al., 2017). Franck et al. (2023) also found that three distinct mega-environments described the variation in pea grain yield and grain quality parameters, suggesting that unique eco-regions could be identified that consistently produce better yields or high-protein pea grain, presenting the possibility for certain eco-regions of the NGP to specialize in their pea production and management for a specific end product. A three-year multi-location trial on soybean by Omari et al. (2025) observed that genotype explained only a small proportion of the variation in grain yield (1.4%), protein yield (1.3%), and crude protein concentration (10.5%), compared with the influence of environment on these response variables. However, the AMMI analysis by Omari et al. (2025) suggests that breeding efforts focused on increasing grain protein concentration and proper site-specific selection of genetics can positively affect realized protein concentration. Previous work and contrasting findings on how genetic variation influences pea protein and yield

suggest that further research is needed to elucidate the extent to which genetic variation affects these traits, particularly in relation to environmental factors.

Grain Yield and Grain Protein Concentration Relationships

The relationship between pea grain yield and protein concentration, along with other grain quality parameters, has been examined in prior studies. These investigations typically involve extensive, multi-environment, multi-year experiments designed to assess the influence of environmental factors such as precipitation and temperature, contrasting cultivars, or differing management practices on protein concentration and yield. Much of the agronomic research across crop species has concluded that increasing yield potential is associated with reduced grain protein concentration (Mohammed et al., 2018b; Simmonds, 1996; Tao et al., 2017). Although breeding efforts, refined management practices, and proper environmental conditions have been identified that have historically improved yield or protein potential, rarely have specific techniques been identified that increase grain yield without resulting in an observed reduction in grain protein concentration (Bogard et al., 2010; Crosta et al., 2025; Huang et al., 2023; Mohammed et al., 2018; Omari et al., 2025; Tao et al., 2017). A study by Mohammed et al. (2018) found no meaningful relationship ($r = -0.074$, $p = 0.1057$) between pea grain yield and protein concentration across 22 environments, each with six contrasting cultivars. Franck et al. (2023) observed a similar relationship ($r = 0.075$, $p = 0.18$) between pea grain yield and protein across 13 environments and seven cultivars. However, a study by Tao et al. (2017) found that increasing pea grain yield was positively associated with protein concentration ($r = 0.39$, $p = <0.0001$). Observational findings from pea grower reports have further complicated the relationship between yield and grain protein concentration. Pea growers in northeast Montana

and northwest North Dakota have experienced unique environmental occurrences, particularly during the exceptionally dry 2017 growing season, in yellow field pea that resulted in historically low grain yield (< 600 kg ha^{-1}) paired with abnormally low grain protein concentration levels (< 200 g kg^{-1}, commonly ranging from 150-170 g kg^{-1}). This suggests that plant stress, both biotic and abiotic, that limits yield can also influence grain quality, including protein concentration, likely by interfering with rhizobia N fixation. Further research is needed on the relationship between yield and grain protein concentration, particularly to identify specific regions, environmental conditions, or genetic factors that tend to yield high grain protein concentrations while maintaining full yield potential.

Intra-plant Grain Formation and Development Dynamics

Evidence from previous research suggests that grain protein concentration varies in relation to the nodal position of individual pods within a single pea plant (Atta et al., 2004; Bestwick et al., 2016, 2018; Guilioni et al., 1997). Modern, spring-seeded yellow field pea cultivars that comprise much of the domestic North American pea market exhibit a determinate growth pattern, with flowering initiated at lower branching nodes and progressing up the stem (Atta et al., 2004; Bestwick et al., 2018; Guilioni et al., 2003a). This continual development of flowering up the pea plant results in lower pods having a prolonged ability to receive endogenous and exogenous N via remobilization to pods located towards the top of the plant, leading to lower pods possibly containing a greater N concentration than upper pods (Salon et al., 2001b; Schiltz et al., 2005b). However, due to lower pods having a prolonged development period compared to upper pods, lower pods also can receive more photosynthate products before seed maturity, resulting in a higher C concentration, thus a reduced grain N concentration

(Bestwick et al., 2016, 2018; Hörtensteiner & Feller, 2002; Lecoeur & Sinclair, 2001; Lhuillier-Soundélé et al., 1999; Salon et al., 2001b; Schiltz et al., 2005b). Cultivar variation, water stress, heat stress, and other factors can influence N partitioning and accumulation among pods within a single plant.

Research across various legume species has demonstrated that cultivars or genotypes can influence the extent and variability of grain protein concentration at different pod locations within the plant. Lhuillier-Soundélé et al. (1999b) reported that cultivars that tend to produce fewer seeds per plant were more likely to produce high-protein grain as the total N source within the plant was concentrated into fewer N sinks. Atta et al. (2004) found that among six differing pea genotypes, grain protein concentration patterns varied across nodal positions within a single plant, suggesting that specific cultivars can produce different grain protein concentrations in upper and lower pods. Larmure & Munier-Jolain (2004) reported similar findings: different pea cultivars yielded contrasting pod protein concentrations, attributed to some cultivars that were more efficient at remobilizing shoot and leaf N to developing pods. A study by Huber et al. (2016) in soybean found that pod location, as determined by the vertical position of the connected nodal position along the main stem, resulted in varying grain protein concentrations, like findings by Atta et al. (2004). Preliminary research conducted via on-farm collaboration with pea growers across Montana suggested that grain protein concentrations were greater for bottom pods in both irrigated and dryland systems (S. Koeshall, 2020, unpub. data). This observation contradicts other research detailing intra-plant protein variation in legume species (Huber et al., 2016). Although cultivar selection can influence the distribution of grain N and observed protein concentration at the field scale, environmental factors may exert a more

substantial influence on intra-plant pod protein dynamics, field-scale protein, and field-scale yield (Bestwick et al., 2018; Mohammed et al., 2018b; Tao et al., 2017).

While previous research has documented how cultivar differences and environmental stressors affect whole-plant and field-scale grain protein formation, there is limited research on how varying and contrasting growing environments and genetics influence intra-plant variation in grain protein concentration. Although the global pea market value is expected to continue growing, and planted acres are likely to persist across the NGP and surrounding regions in North America, there is a need to further understand the environmental and genetic factors that affect pea grain N concentrations, grain yield, and grain N yield.

Research Objectives

The objectives of this dissertation research are as follows:

1) Study the influence of commonly used insecticides in field pea on pea leaf weevil infestation and resultant grain protein concentration and grain yield differences via varying rates of seed-coat-applied insecticide and different timings of foliar-applied insecticide.
2) Quantify and measure the influence that contrasting environmental factors and varying genetics have on field pea grain N concentration and grain yield; identification of specific crop growth windows that predict pea grain N concentration and grain yield; identify unique growing regions via climate characteristics that influence field pea; investigate the relationship between pea grain N concentration and grain yield.

3) Explore intra-plant grain N concentration via contrasting pod raceme positions through quantifying the influence the environment and genetics have on intra-plant grain N variation to further understand how N is allocated to seeds.
4) Evaluate the relationship of field pea trait stability of grain N concentration and grain yield with trait means, explore the value of utilizing modern AMMI- and best linear unbiased prediction (BLUP)-based stability indices to evaluate pea genotypes, and utilize BLUP-based modeling to identify locations that balance trait stability and trait mean of grain N concentration and grain yield.
5) Study the influence of timing of heat and drought stress on field pea grain N concentration and grain yield via the use of open-top climate chambers and rain-off shelters to impose warming and reduced precipitation.
6) Investigate how crop rotations, soil disturbance, and stubble management that are common to the NGP influence SOC at depth, SOC accrual rates over time, and the identification of optimum cropping systems strategies to maximize potential C sequestration that would benefit producers enrolled in voluntary C credit markets.

Figures

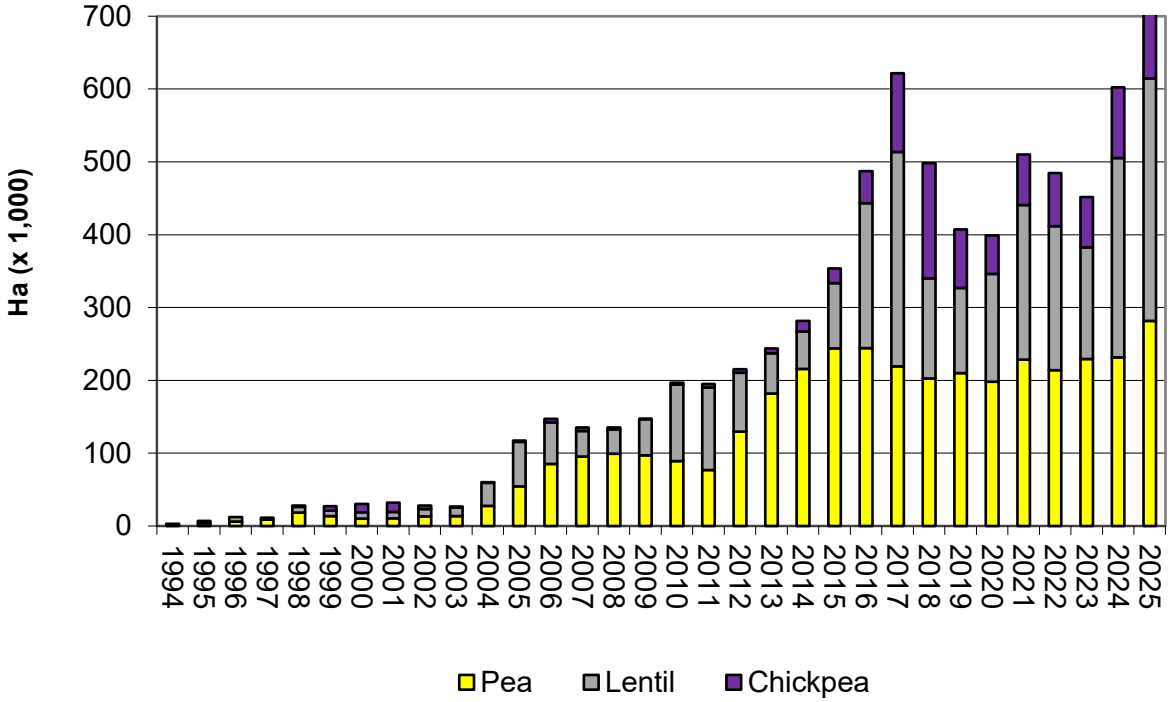

Figure 1. Production of major pulse crop species, including pea, lentil, and chickpea, in Montana from 1994 to 2025 (courtesy of Perry Miller) (NASS, 2025).

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CHAPTER TWO

INSECTICIDES IN FIELD PEA BENEFIT GRAIN YIELD AND PROTEIN CONCENTRATION

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Abstract

Pea leaf weevil (*Sinoa lineatus* L.) (PLW) has established robust populations throughout the Northern Great Plains (NGP) as field pea (Pisum sativum L) has become a popular legume species in cropping systems across the region and has established itself as a major economic insect of concern for pea growers. Adult PLW feed on leaflet margins after plant emergence, reducing yield potential. Following oviposition, PLW larvae utilize rhizobium nodules as their main food source, decreasing N fixation, N availability, and grain protein concentration. Chemical controls are available to control PLW infestations via seed-coat or foliar insecticide. Previous research has shown the potential for reducing plant damage and increasing yield with chemical tools; however, little research has been conducted on the benefit that these chemical controls have on improving yield and protein. An experiment was established in Bozeman, MT, for three years to evaluate three rates (control, low, high) of seed-coat-applied thiamethoxam and three application timings (control, early, late) of foliar-applied lambda-cyhalothrin on pea grain yield, grain protein concentration, and protein yield. Spraying lambda-cyhalothrin increased grain yield by 162 (early) and 128 (late) kg ha^{-1}, resulting in a mean net profit increase of \$68.68 ha^{-1}. Applying seed-coat thiamethoxam resulted in elevated grain protein ($P = .098$) and protein yield levels ($P = .072$), with a \$14 ha^{-1} net profit increase at the high rate via protein premiums. This study shows the agronomic benefits to pea producers of using seed-applied and foliar insecticides to mitigate PLW, thereby increasing yield and grain protein concentration.

1. Introduction

Field pea (Pisum sativum L.) was planted on an average of 216,000 ha from 2019 to 2023 in Montana, whereas only 9,900 ha of pea was planted in 2000, signaling a positive adoption of this crop species in Montana cropping systems (NASS, 2020). Pea has gained popularity among farmers in the NGP due to soil health, soil fertility, and economic benefits that complement wheat production in many dryland cropping systems (Acosta-Martínez et al., 2007; N. Z. Lupwayi & Soon, 2009; Newton Z. Lupwayi et al., 2012; P. R. Miller, Engel, et al., 2006; Perry R. Miller et al., 2015; Walley et al., 2007). Pea is a spring-seeded annual legume that is well adapted to semi-arid cropping systems, given that the crop requires little to no fertilizer N to obtain complete yield potential (Baber et al., 2023; Beckie & Brandt, 1997; Newton Z. Lupwayi & Soon, 2015; Stevenson & Van Kessel, 1996). Many cereal producers have been drawn to pea as a beneficial rotational crop due to the positive N economy from atmospheric N fixation that leads to residual soil N, reducing the amount of urea (46-0-0) required to grow high yielding, high-protein cereal crops such as winter or spring wheat (Triticum aestivum L.) (N. Z. Lupwayi & Soon, 2009; Peoples et al., 2009; Walley et al., 2007). In addition to the positive N benefit, pea fosters non-N benefits such as lower growing season water use compared to other crops which can benefit subsequent cereal or oilseed crop water availability and total soil water content which can have multi-year crop sequence and rotation consequences (Koeshall et al., 2022; P. R. Miller et al., 2002; Perry R. Miller et al., 2015). Furthermore, the addition of legumes in cropping systems has been shown to benefit soil microbiology due to rhizobium nodule exudates (Perry R. Miller et al., 2002; Peoples et al., 2009). Pea has become a desired grain crop for producing plant-based protein products such as pea-based protein powder via grain fractionation for human

consumption or pea meal for livestock rations (USA Pulses, 2018). Additionally, pea grain consumption has been found to impart positive health effects in both humans and livestock, aiding in crop adoption (Liponi et al., 2007; Marinangeli & Jones, 2011; Ndiaye et al., 2012; Neumann et al., 2016). The overall agronomic and social benefits that peas contribute have led to a global market value of $4.37 billion in 2021, with growth expected to surpass $5.5 billion by 2025 (Wood, 2021). Although the global pea market value is expected to continue to grow along with planted acres in North America, there are management issues that have emerged with this growing grain market that jeopardize its long-term sustainability for farmers and fractionation facilities.

Pea crop damage from pea leaf weevil (Sinoa lineatus L.) (PLW) is common in many of the pea-growing regions within the USA and Canada. The PLW was introduced to North America during the 1920s from Europe, Asia, and Africa with the first introduction to the west and east coasts of North America (Wanner, 2007). Adult PLW persists in winter cereal crops and forages such as alfalfa and perennial grasses commonly found in neighboring fields during the winter months (Prochaska et al., 2018; Wijerathna et al., 2024). Adult PLW migrates to emerging pea fields once the ambient air temperatures surpass 13 ^{o}C (Prochaska et al., 2018) and begin feeding on emerging seedlings, commonly targeting the main growth point (Wanner, 2007; Williams et al., 1995). Oviposition begins in May once adult PLW populations have been established in pea fields and continues through June with peak egg densities occurring 2-3 weeks following first infestation, aligning with the early vegetative stages of spring-sown field pea across growing regions in the US and Canada based on recommended seeding dates for pea (Koeshall et al., 2020; P. R. Miller, Brandt, et al., 2006; Prochaska et al., 2018)(Williams et al.,

1995). The oviposition potential of PLW is great, with a single female adult laying between 1,000 and 3,300 eggs after migration to new pea fields (Bogdan, 2018; Cárcamo et al., 2011; Otani, 2013; Prochaska et al., 2018; Wanner, 2007). Following egg deposition, PLW larvae will hatch within 2 – 3 weeks, which leads to the most concerning aspect of PLW infestations which is root nodule damage due to feeding and consequently reduced N fixation potential (Bogdan, 2018; Prochaska et al., 2018; Williams et al., 1995; Willsey et al., 2021).

PLW has become the main economic insect of concern in most pea production areas of North America due to their consistent and common occurrence in diversified crop production systems and their ability to cause substantial yield reduction (Cárcamo et al., 2011; Seidenglanz et al., 2010; Van De Steene et al., 1999; Vankosky et al., 2009b; Wanner, 2007; Williams et al., 1995). Previous research has shown that the economic threshold for PLW occurs when 25 – 30 percent of pea plants have any leaflet or growth point (i.e. 'clamshell') feeding evidence (Cárcamo et al., 2011; Williams et al., 1995; Willsey et al., 2021). Although foliar feeding can result in grain yield potential reduction, unmanaged adult PLW infestations early in the growing season are a precursor to more damage via larvae feeding. Infestations cause plant damage in varying ways, depending upon the life cycle of the PLW that is active. Adults cause initial foliar feeding of peas immediately following plant emergence which is evident by U-shaped notches created on the margins of pea leaflets (Cárcamo et al., 2011; Wijerathna et al., 2021; Willsey et al., 2021). Pea plants are most at risk of foliar feeding and yield potential loss by adult PLW before the 5th node stage, even though later foliar feeding can occur during later reproductive stages of pea, commonly in late July (Wanner, 2007; Williams et al., 1995). If PLW infestations are not controlled early, oviposition begins in pea fields, which fosters the most insidious feeding

from PLW larvae that target rhizobium nodules, resulting in decreased N fixation and hence N availability to develop grain protein (Van De Steene et al., 1999; Wanner, 2007; Williams et al., 1995; Willsey et al., 2021). Pea plants rely on N fixation for the majority of their N requirements, leaving pea plants vulnerable to potential yield loss and grain protein concentration reduction when PLW larvae are not controlled (Gauer et al., 1992; Mariotti et al., 2008).

Pea grain purchasers in the USA and Canada provide price premiums for pea grain with a protein concentration above 22 percent, although most pea grain purchasers will reject pea grain deliveries if grain protein content is below 22 percent protein (Ryan Edinger, pers comm, Dec 13, 2024). The yield and grain protein reduction from uncontrolled PLW infestations pose a substantial threat to the future viability of dry pea production across the NGP, especially if pea crops are unable to be sold due to low grain protein content from uncontrolled PLW larvae feeding.

Chemical controls are commercially available that can be applied before seeding via an insecticidal seed coat or sprayed as a foliar-applied product during plant growth (Willsey et al., 2021). Seed-coat applied products commonly contain Group 4A insecticides (Nicotinic acetylcholine receptor [nAChR] antagonists) such as imidacloprid or thiamethoxam and are an effective residual control that mitigates economic damage from both PLW adults and larvae throughout the growing season (Cárcamo et al., 2011). Previous research has shown that foliar and nodule feeding is reduced with the use of seed-coat insecticide, even though yield may not always be increased (Cárcamo et al., 2011; Willsey et al., 2021). However, little to no research has been conducted on the yield and grain protein response of varying the rate of seed-coat applied insecticide.

Foliar-applied insecticides commonly contain Lambda-cyhalothrin as the active ingredient and result in the highest efficacy if applied before the development of the 6th node in the pea plant (Cárcamo et al., 2011; Wanner, 2007; Willsey et al., 2021). Foliar-applied insecticides are less effective in controlling PLW adults due to the difficulty in timing application before oviposition occurs, which results in below-ground nodule feeding by PLW larvae if applied too late (Wanner, 2007). Proper timing of Lambda-cyhalothrin application is important to pea growers as the half-life of Lambda-cyhalothrin is 5 days (National Pesticide Information Center, 2001), with the active ingredient having a detectable presence on plant tissue up to 14 days post-application (Djouaka et al., 2018). Previous research has shown that a greater yield response in pea results from the use of a seed-coat-applied product compared to a foliar-applied (Willsey et al., 2021). The use of a seed-coat insecticide such as thiamethoxam can result in long-term plant protection due to root absorption of the active ingredient and subsequent transportation to various plant tissues via the xylem (Tian et al., 2022). Although foliar insecticide can be a valuable tool to control acute PLW infestations, other crops in the same farming system can serve as hosts to PLW, allowing for multiple waves of adults (Wanner, 2007; Wijerathna et al., 2024; Williams et al., 1995). However, little to no research has been conducted on the yield and grain protein responses in peas due to varying foliar application timing of insecticide to manage PLW. With the continued presence of PLW populations across the pea-producing regions of the NGP, it is important to evaluate the influence that both seed-coat and foliar-applied insecticide applications have in field pea to maintain or increase grain yield and protein content. Furthermore, it is important to understand the rate of seed-coat insecticide and the timing of foliar-applied insecticide that results in the greatest yield and protein advantage.

The objective of this experiment was to evaluate the effects of varying rates of seed-coat-applied thiamethoxam and varying application timings of foliar-applied lambda-cyhalothrin, and thereby evaluate the effect on grain yield, protein content, and the treatment effect on yield vs. protein relationships. The results from this experiment may provide pea growers across the NGP with PLW chemical management recommendations that result in increased farm profit via yield potential protection and/or access to protein premiums at the time of sale via elevated grain protein concentrations.

2. Materials and Methods

2.1 Study site, design, and management

Studies were conducted in Bozeman, MT, at the Arthur H. Post Agronomy Farm (8.5 km west of Bozeman) (45.672, -111.154) during 2020-22. Each experiment was managed under a no-till system, planted into canola stubble in 2020 and 2021, and spring wheat stubble in 2022. The predominant soil series for all years of the study was an Amsterdam Typic Haplustoll. The Köppen-Geiger climate classification for Bozeman, MT is Dfa (*World maps of Köppen-Geiger climate classification*, 2017). Monthly ambient air temperature and precipitation for Bozeman from Jan 2020 to Dec 2022 is detailed in Figure 1, along with 30-year average temperature and precipitation. Before seeding, weeds were controlled using Glystar Plus [glyphosate, N-(phosphonomethyl)glycine, in the form of its isopropylamine salt] at a rate of 1,542 g ai ha^{-1} as a pre-emergent herbicide. Each experimental plot was 1.83-m wide and 7.62-m long. Field pea (cv. Delta) was used for the entirety of the 3-year study. Pea seed was inoculated using *Verdesian N-Charge* peat powder (*Rhizobium leguminosarum biovar viceae* 2 x 10^8 CFU g^{-1}) at 0.27 kg per 100 kg of seed. Pea was seeded in each experimental plot in rows spaced 22.86 cm apart with the

seed placed approximately 3 cm below the soil surface. The seeding rate for each experimental plot was adjusted for germination to target a pure live seedling density of 80 PLS m^{-2}. The experiment was designed as a randomized complete block with four replications, with nine unique treatment combinations present within each replication. Thiamethoxam was applied to pea seed before planting following the targeted active ingredient rates using *CruiserMaxx® Vibrance® Pulse* (treatment = 0.3 g ai kg^{-1} seed) for the 'low' thiamethoxam rate *and CruiserMaxx® Vibrance® Pulse + Cruiser® 5FS* (treatment = 0.5 g ai per kg^{-1} seed) for the 'high' thiamethoxam rate. We used *VibranceMaxx®* Pulse RTA Pulse at a rate of 3.26 ml kg^{-1} seed for the 'control' treatment level to provide all plots with fungicide protection. For the 'early' and 'late' application spray timings of lambda-cyhalothrin, the foliar insecticide *Warrior II* with *Zeon Technology®* (ai: lambda-cyhalothrin), was used at a rate of 35.7 g ai ha^{-1}. Tables 1 and 2 detail the specific treatment combinations of thiamethoxam insecticide rates and lambda-cyhalothrin application timing for each year of the study. *Assure® II* (quizalofop-P-ethyl) post-emergent herbicide was applied at a rate of 77 g ai ha^{-1} before pea flowering to control grass species during the study. Following flowering and pod formation, the edge rows (rows 1 and 8) were removed from each experimental plot to facilitate harvesting. Following grain maturity, plots were harvested with a *Zurn 130* small plot combine (ZURN USA Inc., Brooklyn Park, MN) to obtain plot yield and grain moisture measured with a mini-GAC handheld grain moisture analyzer (Dickey-john, Auburn, IL) to express grain yield on a dry matter basis. The total harvested grain from each experimental plot was subsampled for grain protein analysis via CNS combustion using a Leco Truspec CNS analyzer (Leco Corp., Saint Joseph, MI). Grain protein is obtained using a common conversion factor of 6.25, resulting in the following equation:

[6.25 × grain N concentration = grain protein concentration %]

Protein yield was calculated via dry matter grain yield (kg ha^{-1}) and grain protein concentration, from the following equation:

[dry matter grain yield (kg ha^{-1}) × (grain protein concentration / 100) = protein yield (kg ha^{-1})]

2.2 Statistical Analysis

Year of study, thiamethoxam rate, and lambda-cyhalothrin spray timing were treated as explanatory fixed factors with replication treated as an explanatory random factor. Model selection indicated that including an interaction term between thiamethoxam and lambda-cyhalothrin did not aid in explaining response variable patterns. Grain yield, grain protein concentration, and protein yield response were our response variables of interest. Treatment effects were analyzed using *nlme* (RDocumentation, 2021b), *lme4*, and *stats* packages to obtain main and interaction effects, least-square means, and Tukey Kramer 'Honest Significant Difference' means comparisons in R 4.1.2 (R Development Core Team, 2022). Means comparisons and yield vs. protein relationship by treatment type graphs were created using *ggplot2* (RDocumentation, 2021a) and *tidyverse* (RDocumentation, 2021c) in R 4.1.2 (R Development Core Team, 2022). Pearson's correlation coefficients for treatment effect on yield vs. relationships were calculated using the *corr* package in R 4.1.2 (R Development Core Team, 2022). Significance was evaluated at an alpha = 0.10 level for all models and means comparisons. There were no significant interactions between seed-coat thiamethoxam and foliar lambda-cyhalothrin by grain yield, protein, or protein yield ($P>0.10$). Therefore, the main effects of our explanatory variables will be presented as only main effects on our response variables.

3. Results and Discussion

3.1 Thiamethoxam Treatment Effect

Across all years, the main effect of the thiamethoxam treatment rate influenced grain protein concentration (P = .098), with the high treatment rate obtaining 254 g kg^{-1} protein, whereas the control led to 247 g kg^{-1} protein, resulting in a 7 g kg^{-1} protein difference (Table 3). A 7 g kg^{-1} protein concentration difference may increase the value-added protein premium per unit of grain sold that a farmer receives at the point of sale, depending upon current market dynamics and the regionally-specific protein premium schedule (Ryan Edinger, pers comm, Dec 13, 2024). Previous protein premium markets in the NGP were paying up to an additional \$0.0275 kg^{-1} (equivalent to \$0.75 dry US $bushel^{-1}$) for peas with a grain protein concentration above 240 g kg^{-1} (Ryan Edinger, pers comm, Dec 13, 2024). Additionally, the use of the high rate of thiamethoxam increased observed protein yield (P = .072), resulting in a 36 kg ha^{-1} increase in harvested protein compared to the control, thereby increasing the crop value to fractionation companies.

Overall, we found that the use of seed-coat applied thiamethoxam increases grain protein concentration and protein yield (Table 3). The grain protein benefit from thiamethoxam is most likely directly linked to rhizosphere mitigation of PLW larvae feeding, thereby allowing rhizobium nodules to fix atmospheric N further into the growing season for vegetative growth and seed development (Seidenglanz et al., 2010; Willsey et al., 2021). The management decision to use a seed-coat applied thiamethoxam before seeding to improve or protect grain protein concentration potential is tactically a simple alteration to spring seeding operations as most producers already treat commonly grown crop seed with at least a fungicide product, resulting in

only a seed treatment product change. Although precise seed treatment input costs are difficult to obtain due to supplier cost variations and most pea protein premium schedules are not publicly posted, if thiamethoxam is used compared to our control (fungicide-only) a producer would increase his net return ha^{-1} by $14 due to the realization of a grain protein premium at the time of grain delivery to market. The assumption of protein premium net return via the use of thiamethoxam vs the control (fungicide only) was calculated from the following equations and market assumptions:

Base grain yield = 3000 kg ha^{-1}

Pea grain market price = \$0.551 kg^{-1}

Pea grain market price with a \$0.01837 kg^{-1} seed protein premium = × \$0.56937 kg^{-1}

Thiamethoxam cost = \$40.64 ha^{-1}

Fungicide alone: [3000 kg ha^{-1} × \$0.551 kg^{-1} = \$1653 ha^{-1}]

Thiamethoxam: [(3000 kg ha^{-1} × \$0.56937 kg^{-1}) - \$40.64 ha^{-1} = \$1667 ha^{-1}]

Thiamethoxam advantage: [\$1667 ha^{-1} - \$1653 ha^{-1} = \$14 ha^{-1}]

3.2 Lambda-cyhalothrin Spray Timing Effect

Across all years, the main effect of lambda-cyhalothrin spray timing increased grain yield (P = .0099), with the Early (2681 kg ha^{-1}) and Late (2647 kg ha^{-1}) spray treatments yielding 162 and 128 kg ha^{-1} greater than the Control (2519 kg ha^{-1}), resulting in a 6.4 and 5.1% yield increase with the use of thiamethoxam compared to the control treatment (Table 3). These grain yield advantages align with the early season presence of adult PLW on young pea plants across the study in all years, especially following the emergence of seedlings. The effective yield advantage of applying lambda-cyhalothrin to peas before the initiation of the 6^{th} node stage (Early or Late

spray timing treatment) suggests that an economic threshold of PLW (presently defined as 25-33% of seedlings showing clamshell leaf feeding damage) was present in all years of our study based on previous research and observed clam leaf feeding damage (Wanner, 2007) (Figure 2). This grain yield advantage of 162 and 128 kg ha^{-1} obtained by spraying lambda-cyhalothrin on pea seedlings can result in a net profit increase realized by the producer of $78.18 (Early) and $59.18 (Late) net profit increase ha^{-1}.

Applying a foliar application of lambda-cyhalothrin resulted in a mean increase to grain yield of 145 kg ha^{-1} ('Early' and 'Late' vs 'Control'). This yield advantage aligns with previous research that shows adult PLW populations are negatively associated with plant productivity, suggesting that applying an early-season foliar insecticide should improve plant productivity (Vankosky et al., 2009a; Williams et al., 1995). Although there is a cost-to-benefit balance that should be considered when weighing the cost of applying foliar insecticides to peas under varying grain market conditions and the observed density of PLW adults counted during field scouting, the yield advantage gained from insecticide application paired with above-average grain market prices resulted in a mean net profit gain of $68.68 ha^{-1} as described in the previous section (Prochaska et al., 2018; Wanner, 2007). The assumption of net return via the use of lambda-cyhalothrin (in the form of Warrior II with Zeon Technology®) compared to the control (no lambda-cyhalothrin application) was calculated with the following equations and observations:

Yield of the Control treatment: 2519 kg ha^{-1}

Yield of the Early spray treatment: 2681 kg ha^{-1}

Yield of the Late spray treatment: 2647 kg ha^{-1}

Cost of Lambda-cyhalothrin: \$10.82 ha^{-1}

Pea Market Price: \$0.551 kg^{-1}

Control gross revenue: [2519 kg ha^{-1} × \$0.551 kg^{-1} = \$1388 ha^{-1}]

Early spray gross revenue: [2681 kg ha^{-1} × \$0.551 kg^{-1} = \$1477 ha^{-1}]

Late spray gross revenue: [2647 kg ha^{-1} × \$0.551 kg^{-1} = \$1458 ha^{-1}]

Early spray partial net return: [(\$1477 ha^{-1} - \$10.82 ha^{-1}) - \$1388 ha^{-1} = \$78.18 ha^{-1}]

Late spray partial net return: [(\$1458 ha^{-1} - \$10.82 ha^{-1}) - \$1388 ha^{-1} = \$59.18 ha^{-1}]

3.3 Study Year Effect

Across all treatments, the main effect of year influenced both grain yield (P = <.0001) and grain protein concentration (P = <.0001), with 2020 obtaining the greatest grain yield (3458 kg ha^{-1}) although 2021 produced the greatest grain protein concentration (26.2%). The discrepancy between yield and protein optimization as influenced by the study year is a result of a potential grain N dilution during 2020 due to elevated grain starch composition which is driven by advantageous growing season conditions such as precipitation or cool ambient air temperatures (Figure 3)(Salon et al., 2001; Schiltz et al., 2005). The 2021 study year experienced severe drought conditions, limiting grain yield and potentially concentrating available N from above and belowground biomass through remobilization to seed during seed formation and development (Figure 1)(Schiltz et al., 2005). This observation supports previous research findings that growing season precipitation influences grain yield and protein concentration results, with the yield vs protein relationship, usually being a negative association, which is driven through varying levels of grain starch concentration and total N remobilized to the pod structures before senescence (Guilioni et al., 2003; Kibite & Evans, 1984; Larmure et al., 2005).

Furthermore, 2020 resulted in the greatest protein yield (859 kg ha^{-1}), which was a function of the large yield advantage of 486 kg ha^{-1} over 2022 and 2041 kg ha^{-1} over 2021 created by above-average June 2020 rainfall (Figure 1). These grain yield, grain protein concentration, and protein yield results describe the antagonistic yield vs protein relationships present in many common dryland grain crops (Larmure et al., 2005). Varying levels of growing season precipitation influence overall plant productivity, which controls total C assimilation, thereby influencing total seed dry matter and seed N accumulation at harvest (Larmure et al., 2005).

4. Conclusions

This experiment increased our understanding of pea plant productivity responses to the use of common insecticide tools and evaluated different rates and application timing of these chemical tools when used in spring-seeded dryland field pea production. Overall, results and findings from this three-year study show that the use of insecticidal tools at seeding and during the early vegetative stages of pea results in improved grain yield, grain protein concentration, and protein yield. These conclusions suggest that pea producers in Montana would benefit by proactively using insecticidal tools to mitigate insect infestations in spring-planted pea, especially if insect populations such as PLW are commonly found in their farming system.

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during the entirety of this study, who are as follows: Rosie Wallander, Isaac Aasgaard, Tristan Hoyer, Sydney Atencio, Jenna Anderson, and Piper Lacy.

Figures

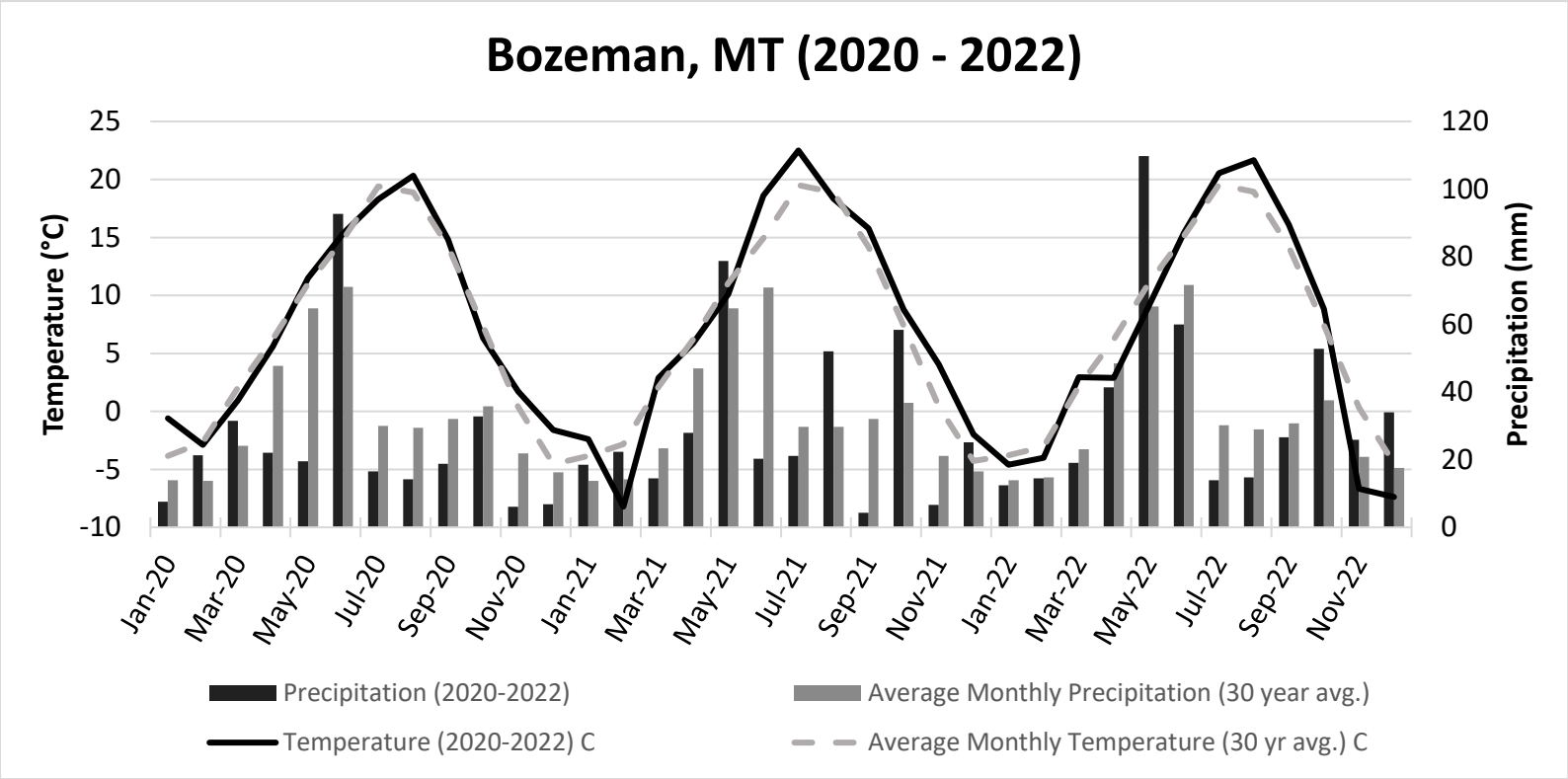

Figure 1. Monthly mean precipitation and ambient air temperature with monthly 30-year mean precipitation and ambient air temperature for the study site located near Bozeman, MT. Annual and 30-year monthly mean climate data were obtained through the High Plains Climate Center ACIS-CLIMOD online data service, utilizing the Bozeman 6 W EXP FARM climate station.

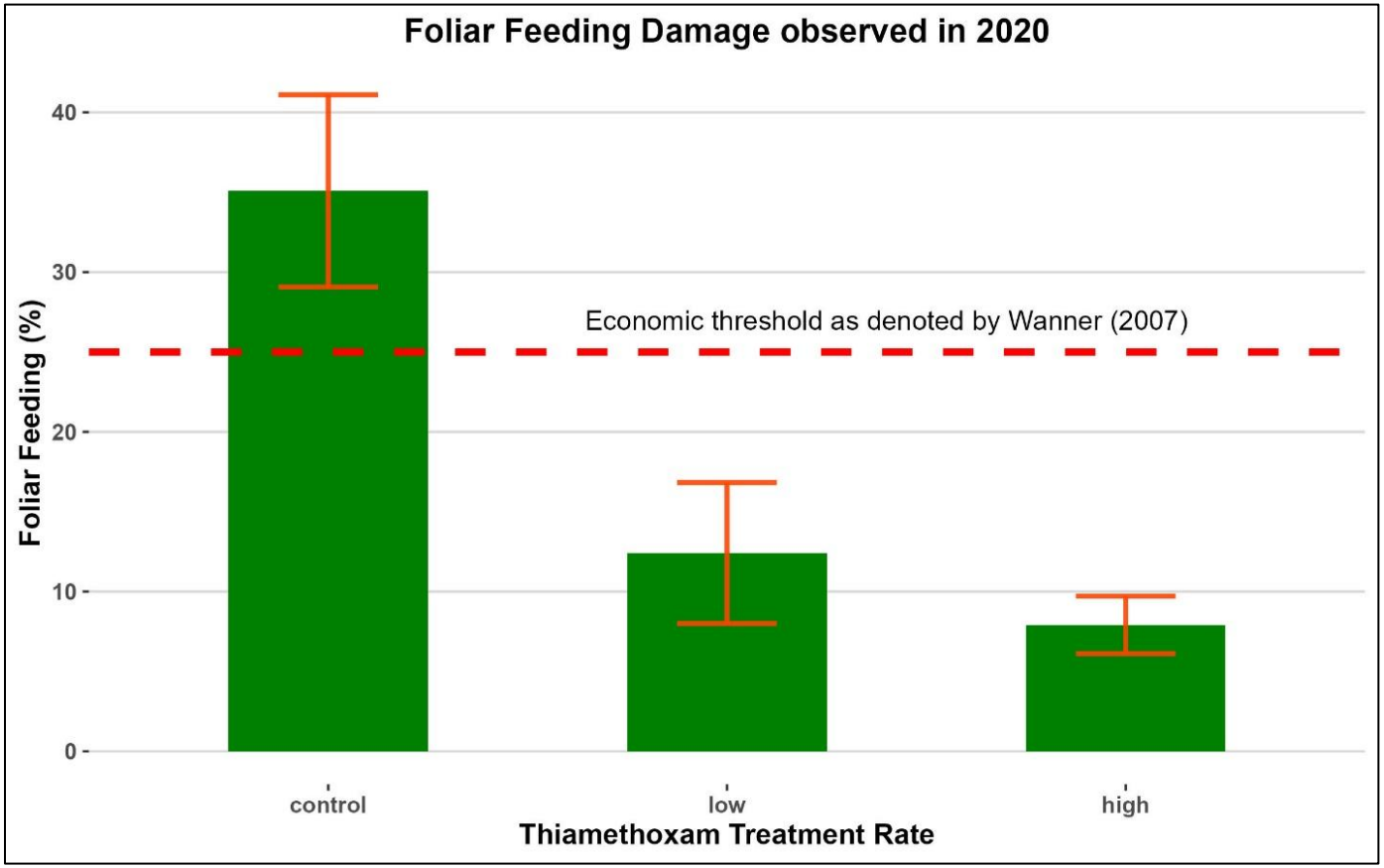

Figure 2. Percent of foliar feeding that was present on seedlings in 2020 among the three thiamethoxam treatments prior to any foliar insecticide applications. The level of seedling damage observed in the control treatment aligns with previous economic thresholds stated by Wanner (2007).

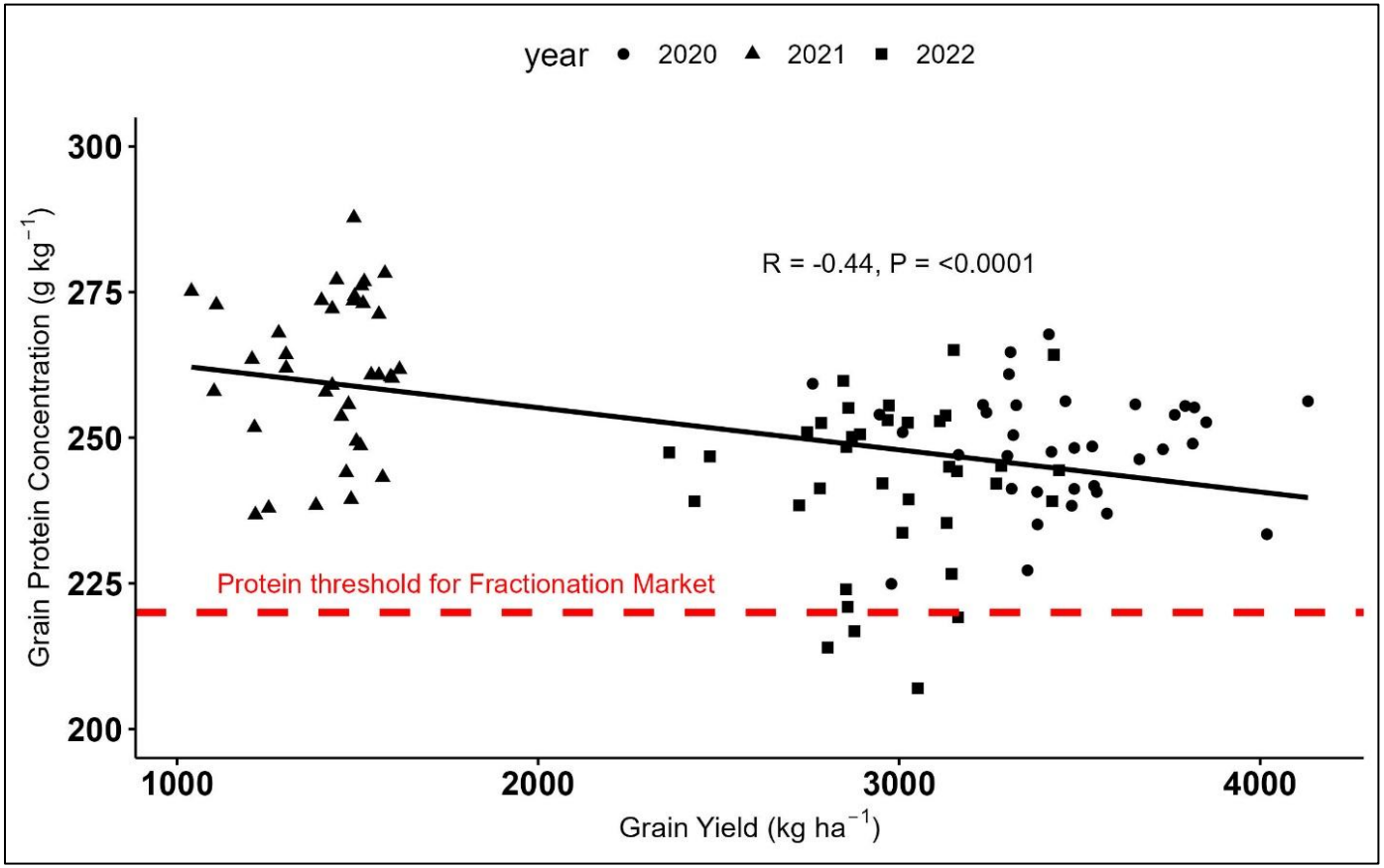

Figure 3. The linear relationship between grain protein concentration and grain yield (kg ha^{-1}) of field pea across three study years (2020 – 2022) of harvest data from Bozeman, MT. The dashed horizontal red line denotes 220 g kg^{-1} (22%) grain protein concentration.

Tables

Table 1. Insecticide treatments on spring pea for pea leaf weevil control, Bozeman MT.

Treatment Combinations **Thiamethoxam Rate - Lambda-cyhalothrin Timing**	**Thiamethoxam Rate** gram ai kg^{-1}	**Lambda-cyhalothrin Spray Timing** vegetative stage of application[a]
1. Base Control	-	-
2. Low Thiamethoxam rate	0.3	-
3. High Thiamethoxam rate	0.5	-
4. Early Lambda–cyhalothrin	-	2nd node
5. Low Thiamethoxam rate – Early Lambda–cyhalothrin	0.3	2nd node
6. High Thiamethoxam rate – Early Lambda–cyhalothrin	0.5	2nd node
7. Late Lambda-cyhalothrin	-	5th node
8. Low Thiamethoxam rate – Late Lambda–cyhalothrin	0.3	5th node
9. High Thiamethoxam rate – Late Lambda–cyhalothrin	0.5	5th node

[a]target application timing for foliar spraying of lambda–cyhalothrin

Table 2. Date of Lambda-cyhalothrin application for each treatment level for each year of this study. Early application treatments targeted the 2nd node stage of plant development and late application targeted the 5th node stage which is in conjunction with the optimal application window for application of this insecticide product.

	Lambda-cyhalothrin Timing by Treatment Level		
Year[a]	**~ Application Dates ~**		
	control	**early**	**late**
2020	NA	26-May	05-Jun
2021	NA	19-May	01-Jun
2022	NA	25-May	01-Jun

[a]all years of this study were conducted in Bozeman, MT at the Arthur M. Post Agronomy Research Center

Table 3. Pea grain yield and grain protein concentration response to seed-coat applied Thiamethoxam and Lambda-cyhalothrin foliar applications, 2020-2022, Bozeman, MT.

Source of Variation (S.V)	**Grain Yield**	**Grain Protein**	**Protein Yield**
	kg ha^{-1}	g kg^{-1}	kg ha^{-1}
Year (P-value)	**<.0001**	**<.0001**	**<.0001**
Thiamethoxam Rate (P-value)	.54	**.0982**	**.072**
Lambda-cyhalothrin Timing (P-value)	**.0099**	.77	**.0127**
Year			
2020	**3458 a**	248 b	**859 a**
2021	1417 c	**262 a**	371 c
2022	2972 b	242 c	720 b
Thiamethoxam Rate			
Control	2590	247 b	633 b
Low (0.3 gram ai kg^{-1})	2609	251 ab	647 ab
High (0.5 gram ai kg^{-1})	2650	**254 a**	**669 a**
Lambda-cyhalothrin Timing			
Control	2519 b	250	623 b
Early (2nd node stage)	**2681 a**	250	**668 a**
Late (5th node stage)	**2647 a**	252	**658 a**

Note: differing Tukey HSD common display letters denote a significant least square means difference at alpha = 0.10

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CHAPTER THREE

ENVIRONMENT AND GENETIC INFLUENCE ON FIELD PEA GRAIN NITROGEN AND YIELD IN NORTH AMERICA

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Abstract

Pea (Pisum sativum L.) is cultivated across North America and has been established in diverse environments. We evaluated five pea genotypes across eight sites in North America over three years for grain nitrogen (N) concentration and grain yield. A non-hierarchical cluster analysis was used to classify environments based on growing-season precipitation and temperature. Pearson correlation analysis was applied to evaluate the relationships of precipitation and heat accumulated per-degree-day during specific growth windows on grain N concentration and grain yield. Additive main effect and multiplicative interaction analysis (AMMI) was utilized to evaluate the control of environment and genotype. Cluster analysis revealed that classifying environments into four unique "growing-season environments" influenced grain yield but not grain N concentration. Precipitation was the best predictor of grain N concentration, while heat accumulated per-degree-day was the better predictor of grain yield. Precipitation and heat accumulation during seed fill initiation and maturity were more correlated with grain yield than for other growth windows. The AMMI analysis revealed that environment was the most important factor in determining grain N concentration and yield, explaining 70.5% and 97.1% of observed variation. Genotype significance in AMMI analysis revealed site-specific ability to increase grain N concentration. Grain N concentration was negatively correlated with yield; however, results suggest that growing-season environments that exhibited warmer temperatures and reduced precipitation resulted in a greater grain N concentration reduction per unit of grain yield increase. This study determined that environment exerted strong control over pea grain N concentration and yield, whereas genetic effects were weak.

1. Introduction

Spring pea (*Pisum sativum* L.) is a cool-season legume with a short growing season that has been adopted across cropping systems in the northern and central Great Plains of North America, commonly in rotation with cereals such as spring or winter wheat (*Triticum aestivum* L.) and oilseeds such as spring canola (*Brassica napus* L.) (MacWilliam et al., 2014; Tanaka et al., 2010). Pea has become a popular rotational legume in cereal- and oilseed-based cropping systems due to the ecosystem services it provides. Pea requires little to no fertilizer N to attain full yield potential due to atmospheric N_2 fixation via symbiotic *Rhizobium leguminosarum* bv. *viciae*, located in the rhizosphere, that infects the root system and provides the plant with available N in exchange for carbon (C) (Salon et al., 2001a; Schiltz et al., 2005a). Generally, the most significant ecological benefit from peas comes from direct and indirect N benefits due to atmospheric N_2 fixation and subsequent N and C release from pea residue following harvest (Beckie & Brandt, 1997b; Burgess et al., 2012; Lory et al., 1995; Peoples et al., 2009; Stevenson & Van Kessel, 1996a, 1996c). Adoption of pea across various cropping systems has resulted in total planted hectares of 360,000 and 1,424,000 in the United States and Canada, respectively, in 2024 (NASS, 2025; StatsCan, 2025). As of 2025, Montana and North Dakota alone produced 321,000 ha of dry yellow pea, with new growing regions in Minnesota and Nebraska producing 19,480 and 11,370 ha (NASS, 2025). Furthermore, pea has provided positive economic benefits to cropping systems. Much of the economic advantage of pea stems from its synergistic relationship with cereal-based rotations, in part through the positive N economy and the direct N credits it provides. In southwestern Montana, pea-wheat produced greater 4-year net returns and provided more economic stability than other common rotations (Miller et al., 2015). When pea

replaces fallow periods in a crop sequence, the economic variability of the farming system decreased because a cash crop replaced a non-production period and expanded market access (S. Koeshall et al., 2022; Miller et al., 2015).

Pea grain products have beneficial effects on human health when incorporated into various food products. The intake of pea protein or fiber contributes to effective glycemic control and weight loss, improved gut health, and a reduced likelihood of developing chronic diseases (Akin et al., 2025; Dahl et al., 2012; Mancabelli et al., 2025). Pea protein and fiber can reduce short-term food intake and blood glucose levels in healthy adults (Dahl et al., 2012). The presence of pea-derived protein and fiber increases the levels of beneficial gut bacteria, such as *Lactobacillus* (Akin et al., 2025). Furthermore, grain legumes prevent hypercholesterolemia and hypertension, as well as potentially reduce the risk of various cancers in humans (Arnoldi et al., 2015; Hall et al., 2017; Ren et al., 2021).

Although growing pea can improve ecological, economic, and human health parameters, gaps in our understanding of how environmental and genetic factors influence the formation and production of pea grain protein and grain yield jeopardize cost-efficient market development and expansion of pea in North America. Increasing the yield potential of pea directly correlates with higher net returns to the farming system; thus, understanding how environmental, genetic, and management factors influence biomass or grain yield has attracted most research efforts. However, grain quality parameters such as grain protein concentration have been largely unstudied (Huang et al., 2023; Koeshall et al., 2021).

Pea is cultivated across ecoregions and climate gradients within the NGP, exposing it to diverse environmental conditions (Padbury et al., 2002). Previous research has aimed to

understand how and to what extent environmental factors influence the formation and development of pea grain protein concentration and grain yield (Bestwick et al., 2018; Franck et al., 2023; Mohammed et al., 2018; Tao et al., 2017a). Several previous studies have found that environment is a determining and controlling factor of pea protein and yield potential (Franck et al., 2023; Mohammed et al., 2018; Tao et al., 2017a). In a greenhouse study, pea plants subjected to a day/night temperature regime of 15/10 °C, compared with 25/20 °C, were found to optimize C availability by increasing C fixation, resulting in more vegetative structures and a greater N demand (Larmure et al., 2005). During the seed-filling period, pea plants that were subjected to warmer temperatures produced pea grain with greater N concentrations; however, grain yield was reduced (Larmure et al., 2005). Bueckert et al. (2015) reported that pea grain yield and time in the reproductive growth cycle were reduced when pea was subjected to more than 20 days of maximum daily temperatures above 28 °C.

Variation in genetics (e.g., cultivar, variety, or genotype) has also been shown to influence pea grain protein concentration and yield significantly (Chen et al., 2021; Mohammed et al., 2018; Tao et al., 2017b). Findings by Tao et al. (2017a) and Mohammed et al. (2018) suggest that environment exerts a more substantial influence on pea grain protein concentration than genetic factors, in contrast to conclusions from other studies conducted in the same region of North America. A study by Franck et al. (2023) evaluated seven pea cultivars at seven locations across Montana over two years and concluded that genetics exert consistent control over grain protein concentration, as evidenced by the absence of a significant cultivar × location interaction.

Agronomic research on field pea has reported mixed findings on the relationship between pea grain yield and grain protein concentration (Mohammed et al., 2018; Simmonds, 1996; Tao et al., 2017a). For example, Tao et al. (2017) found that increasing pea grain yield was positively associated with protein concentration ($r = 0.39$, $p = <0.0001$), whereas there was no meaningful relationship between pea grain yield and protein concentration ($p > 0.10$) found by Mohammed et al. (2018). Franck et al. (2023) observed a similar non-significant relationship ($r = 0.075$, $p = 0.18$, $n = 317$) between pea grain yield and protein across 13 environments and seven cultivars.

Certain environments throughout the Great Plains may also consistently experience heat or drought stress from year to year (Padbury et al., 2002). Heat stress can alter photosynthate production, C and N allocation throughout and during the plant life cycle, flowering and pollination success, and net primary productivity. Guilioni et al. (1997) detailed how heat stress influences abortion of buds and flowers in pea, which suggests that if heat stress is first experienced during flower formation, the N sink structures for developing pea pods are reduced. At the same time, the total N contained per plant remains constant, likely leading to higher grain N concentrations (Larmure et al., 2005). Yang et al. (2018) demonstrated that there is differential sensitivity of C and N assimilatory enzyme in maize that is associated with heat stress. Increasing maximum daily temperatures from 15 ºC to 25 ºC reduced C fixation per degree-day, resulting in fewer N-sink structures formed via reduced aboveground biomass and fewer viable pods that require N, as reported by Larmure et al. (2005). Furthermore, key enzymes utilized in starch synthesis are limited by heat stress (Singletary et al., 1994). Zhao et al. (2008) observed a similar C and N sensitivity in heat stress applied to wheat. However, heat stress can also decrease aboveground biomass production, resulting in decreased N demand, and hence total N source

(McCauley et al., 2012; Unkovich et al., 2010). Water stress experienced by pea throughout the growing season can alter N accumulation and partitioning. Serraj et al. (1999) observed that N_2 fixation in legume crops is altered by the incidence of water stress during the growing season, attributing reduced N-fixation in water-stressed environments to a variety of factors such as C shortage from the plant, oxygen limitations, or feedback regulation by N accumulation, with phloem flow likely explaining why N_2 fixation is severely limited in drying soil environments. Hossain et al. (2016) reported that low-precipitation growing seasons across contrasting cultivars reduced N fixation relative to historically normal-precipitation growing seasons, suggesting that the efficiency and health of rhizobia nodules in the rhizosphere can be decreased under dry soil conditions.

Although the global pea market value is expected to continue growing, and planted acres are likely to persist across the NGP and surrounding regions in North America, there is a need to understand further the environmental and genetic factors that affect pea grain N concentration and grain yield to refine producer practices, management, and broadly inform the domestic pea market in North America what factors determine grain N formation. The objectives of this study were to 1) classify environments into broad growing-season environments that are characterized by climate variables that drive pea grain N concentration or grain yield, 2) evaluate the relationship between environment and genotype, and 3) identify pea growth windows that are useful in predicting pea grain N concentration and grain yield using cumulative precipitation and heat accumulation rates.

2. Materials and Methods

2.1 Site Description

Field trials were conducted at eight locations across the northern Great Plains of North America (Table 1) in 2022, 2023, and 2024, a region that accounts for most of North America's pea production. Soils ranged from course to fine-textured, with dominantly udic and ustic moisture regimes, and generally had thick, organic-rich surface horizons that qualified them as Mollisols in the US Taxonomy (Table 1). Harvest dates ranged from mid-July to early September, growing periods ranged from 83 to 113 days, and growing degree-days (GDD) accumulation rates ranged from 13.4 to 19.5 per day (Table 2). Growing degree days, commonly referred to as heat units, are the cumulative heat units experienced by the plant from the day of seeding to the day of harvest. GDD was calculated using the following equation (McMaster & Wilhelm, 1997):

$$GDD = \sum_{1}^{n} \frac{T_{max} + T_{min}}{2} - T_{base}$$

where T_{max} is the maximum daily temperature, T_{min} in the minimum daily temperature, and the T_{base} temperature for pea is 0 °C. April to August cumulative precipitation ranged from 139 mm to a high of 618 mm and mean temperature ranged from 13.5 to 19.4 C (Tables 3 – 4).

2.2 Experimental Design

All experiments were conducted in a randomized complete block design with five replications and five cultivar entries during the three-year study. All experimental locations received the same five yellow pea cultivars (AAC Profit, CDC Amarillo, CDC Dakota, Delta, and DS Admiral) each year (Table 5), which were chosen based on protein and yield potential,

growth habit, and breeding background (Andersen et al., 2011; Bing et al., 2022; Limagrain, 2025; Warkentin, 2010; Warkentin et al., 2014). Further, all five pea cultivars were grown from a common seed source which was increased at Bozeman, MT during the 2021 growing season to avoid variation among seed lots. All pea seed was treated with *CruiserMaxx Vibrance* for pulse crops at a rate of 3.26 mL kg^{-1}, a seed-coat treatment that combines insecticides and fungicides for plant protection. All cultivars were seeded at a target pure live stand (PLS) density of 80 PLS m^{-2}. *Exceed Granulated Peat for Pea, Vetch, and Lentil* (*Rhizobium leguminosarum biovar viceae* 1×10^{8} colony forming units [CFU] g^{-1}) inoculant was applied at all experimental locations at a rate of 5.7 kg ha^{-1} in-furrow. Pea roots were dug to confirm infection of root systems with rhizobia during the growing season. Monoammonium phosphate (11-52-0-0) and potassium sulfate (0-0-50-18) were blended at equal ratios, resulting in an NPKS starter fertilizer blend (5.5-26-26-9) applied at a rate of 100 kg ha^{-1} in-furrow at the time of seeding. Plot dimensions at the experimental locations ranged from 1.28 to 2.0 m in width and were 6.1 m long. Row spacing ranged from 22.8 to 30.5-cm among experimental locations. Experimental locations targeted recommended seeding dates based on their respective climate and soil conditions (Table 2). At each experimental location, pesticides were applied as needed to control weeds, insects, and pathogens, in accordance with best management practices for that site. There were no notable pest issues at these research locations during this study. Each experimental location harvested plots with research-specific small-plot combine harvesters, with a 50-g subsample of harvested grain saved from each experimental unit. All grain yield observations were adjusted to represent yield on a dry matter basis.

Grain subsamples were ground in a Udy high-speed centrifugal mill to a particle size of < 2 mm to represent the whole plot. All milled grain samples were analyzed for N concentration using a *Leco Truspec CNS Combustion Analyzer* (Leco Corp., St. Joseph, MI). Although it is common to report grain protein concentration with a N to protein conversion factor of 6.25, we report grain N concentration (g kg^{-1}) due to the inaccuracy of this conversion, which does not account for the specific amino acid composition of grain legumes. See Mariotti et al. (2008) for proper conversion values for obtaining grain protein concentration values from grain N concentration observations reported in this article.

2.3 Statistical Analysis

To accurately evaluate environmental and genetic influence on pea grain N concentration and grain yield, "location" will be referred to as a geographical location, and "environment" will be referred to as an individual site-year in this study. A single site-year will be considered a single "environment" as each site-year represents a unique growing season climate, particularly in timing and accumulation of precipitation and heat throughout the life cycle of pea. Significance of modeling results was assessed at the level of $p \leq 0.05$, unless otherwise noted. Grouping similar environments into larger growing-season environments that represent larger growing regions is key to potentially understanding how growing season climates alter grain N concentration and yield, via agronomically valid weather variables. Thus, due to the range of locations across three years, totaling 23 unique site-years, separating and grouping environments became challenging when multiple weather variables were considered simultaneously. A clustering analysis was conducted using a combination of temperature and precipitation parameters recorded and calculated throughout the growing season for each environment (Jain,

2010; Maechler et al., 2025a). Temperature and precipitation histories for each environment were obtained from the High Plains Climate Center data service for all environments in the United States and the Canadian Climate data service for the two SK environments (*Canadian Climate*, 2025; *High Plains Regional Climate Center*, 2016). All statistical analyses were performed using R (R Core Team, 2025). Precipitation (mm), mean minimum temperature (°C), mean maximum temperature (°C), and the number of days above 28 °C during the growing season were the four climate variables used to characterize the environments for clustering analysis. Previous multi-environment research has shown that these climate variables are valid for classifying environments via clustering analysis (Camargos, 2025). These four climate variables were standardized using the Bayes Classification Method and the *scale* function in R to ensure equal contributions from all variables to the clustering algorithm (R Core Team, 2025). The growing season for clustering analysis was defined as the period from the day of seeding to the day of harvest. Two primary clustering methods were used to ensure the validity and accuracy of the clustering classification and to compare clustering results for consistency. A hierarchical cluster analysis was performed using the R packages *cluster* (Maechler et al., 2025b) and *factoextra* (Kassambara & Mundt, 2020) with Ward's minimum-variance method and squared Euclidean distance, as described by Ward (1963). Secondly, a K-means analysis was applied, following Jain (2010), as this algorithm is a non-hierarchical method based on Euclidean distance that minimizes within-cluster variance, using the R *stats* package (R Core Team, 2025). Silhouette validation was performed using the R packages *cluster* (Maechler et al., 2025) and *factoextra* (Kassambara & Mundt, 2020) to determine the optimal number of clusters for the cluster modeling methods listed above.

Grain N concentration and grain yield data were analyzed using the additive main effect and multiplicative interaction (AMMI) methodology, as detailed by the protocol explained by Gauch (2013). The AMMI analysis was performed using the *agricolae* R package (de Mendiburu, 2023) in the R statistical software (R Core Team, 2025) and considering the following equation:

$$Y_{ij} = u + g_i + e_j + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij} + \epsilon_{ij}$$

where Y_{ij} is the mean of i^{th} cultivar in the environment j; u is the overall mean; G is the effect of cultivar i; and ϵ is the effect of the environment j. The GE interaction is explained by the multiplicative term of the equation where λ is the singular value of the principal component (PC) of $k, \varepsilon,$ and n are scores of cultivar i and environment j, respectively, for the principal component k, n ranges from 0 to the number of PC required to describe the AMMI model appropriately. The sums of squares (SS) for the interaction of cultivar × environment and the interaction of cultivar × environment noise were calculated. There was a meaningful amount of SS for environment, genotype, and the environment-by-genotype interaction in the model for grain N concentration and grain yield ($p \leq 0.05$).

Pearson correlation analyses were performed to assess relationships among grain N concentration, grain yield, and various growing-season weather variables, with significance at $p \leq 0.05$. Confidence intervals or error bars displayed in figures are shown as the standard error of the treatment or group mean, unless otherwise noted.

Multiple linear regression was performed on GDD accumulation rates and precipitation for individual growth-stage windows to predict grain N concentration and grain yield.

Precipitation and GDD accumulation rates were treated as fixed factors. Adjusted R^2 results from each model were used to evaluate the use of precipitation and GDD accumulation rates as predictors of grain N concentration or grain yield.

Analysis of precipitation and heat accumulation during specific pea growth windows was performed using Pearson correlation analyses and multiple linear regression on the response variables, grain N concentration and grain yield, in R statistical software (R Core Team, 2025). Cumulative precipitation and GDD accumulation rates were calculated for each crop growth window within each site-year. Pea growth windows were also chosen by using GDD crop staging results and predicted crop development reported in Miller et al. (2001). Four individual crop growth windows were identified using Miller et al. (2001), which informed the creation of an additional six larger composite crop growth windows that capture two or more individual crop growth windows, for a total of ten pea growth windows. The individual growth windows captured precipitation and heat accumulation rate from 1) the day of seeding (GDD = 0) to four leaves open (GDD = 321) (BBCH: 0 – 14), 2) four leaves open to the day that 50% of plants have at least one open flower (GDD = 780) (BBCH: 14 – 65), 3) from the day when 50% of plants have at least one open flower to the start of seed fill (GDD = 1093) (BBCH: 65 – 71), and 4) from the day that plants start seed fill to completion of physiologically mature seed (GDD = 1378) (BBCH: 71 – 79).

Analysis of variance (ANOVA) mixed models were performed on the response variables grain N concentration and grain yield to describe general differences among environments, among locations, among genetics, genetics within environments and locations, and environments

across individual genetics. Location, environment, and genetics were treated as explanatory fixed factors, with replication within each environment treated as an explanatory random factor.

3. Results and Discussion

3.1 Environment classification by growing season precipitation and heat

Results from the hierarchical cluster analysis using Ward's method, based on the eigenvalues of the covariance matrix, indicated that the first three principal components accounted for 98% of the total variability (Table 6). One site-year (Mead 2024) was excluded from all climate or weather classification analyses and from analyses of resultant grain N concentration and grain yield due to excessive precipitation during the growing season, which led to waterlogged root systems and impaired pea growth. The K-means algorithm with four pre-determined groups explained 74% of the total variance and yielded moderate separation among the four distinct growing-season environment clusters.

Cluster analysis of the 23 environments used in this study classified 9 environments into Cluster 1, which can generally be described as a dry growing-season environment (Table 7, Figure 1). Eight environments comprise Cluster 2, which includes a cool-night growing-season environment with low precipitation. Cluster 3 contains four environments that collectively produce a hot growing-season environment. Cluster 4 comprises only two environments, collectively representing a cool/wet growing-season environment. The daily mean minimum temperature (Tmin), daily mean maximum temperature (Tmax), days above 28 °C during the growing season, and growing season precipitation are presented in Table 7 for each growing-season environment identified via the clustering methodology.

Site specific factors may contribute to effects of weather conditions categories. Fine soil texture, soil thickness, vertical textural contrast, or development of clay-rich B horizons, as seen at the Williston and Havre sites (Table 1, Argiustoll classification), may facilitate soil water storage that buffers limited growing season water (Sigler et al. 2020). Cluster 4 weather conditions included only two years at the same site in Minnesota, where subirrigation may contribute to sustained water supply (Figure 1, Table 1, Endoaquoll classification).

3.2 Effect of weather on grain N concentration and grain yield

An ideal growing-season environment would maintain yield potential while consistently producing pea grain with a grain N concentration above 38.4 g kg^{-1}, thereby enabling access to elevated protein premiums and resulting in high net returns over time. The hot growing-season environment (Cluster 3) had the greatest daily mean maximum temperature (26 °C) and the greatest number of days above 28 °C (44 d) (Table 7). The hot growing-season environment presents characteristics that lead to heat stress consequences, such as reduced C fixation efficiency, fewer reproductive N sink structures, flower and bud abortion, and reduced grain yield potential. Thereby, the hot growing-season environment presents climate conditions that would tend to depress grain yield potential while increasing grain N concentration (Bueckert et al., 2015; Larmure et al., 2005). The wet/cool growing-season environment (Cluster 4) displayed the fewest days above 28 °C (4 d). This temperature characteristic of the cool/wet growing-season environment suggests that the two environments within this growing-season environment cluster could optimize grain yield, given that this growing-season environment also has the greatest growing-season precipitation (322 mm) (Larmure et al., 2005; Tao et al., 2017a). The cold-night growing-season environment may enhance grain yield potential by increasing C

availability through greater C fixation per degree-day. However, it may also reduce grain N concentrations at the expense of increased grain yield, as Cluster 2 had the second-lowest growing-season precipitation (179 mm) (Bueckert et al., 2015; Guilioni et al., 1997; Larmure et al., 2005). The dry growing-season environment experienced a mean of 27 days above 28 °C, potentially reducing the potential for grain yield (Bueckert et al., 2015; Larmure et al., 2005). The cold night growing-season environment (Cluster 2) exhibited the lowest mean minimum night-time temperature (8 °C), suggesting that environments within this growing-season environment cluster would have lower C photosynthate costs for operating night-time respiration, thereby increasing photosynthetic products that drive yield or grain quality potential due to little night-time heat stress. However, Cluster 2 still contained 25 days with temperatures above 28 °C on average across eight environments, leaving this growing-season environment at risk of reduced grain yields due to cumulative heat stress during the growing season.

The cool/wet, dry, and cold night growing-season environments exhibit climate parameters that may balance grain N concentration and grain yield. In contrast, the hot growing-season environment contains climate parameters that may favor the growth of high-grain N-concentration pea crops, but at the expense of overall grain yield potential. However, the dry growing-season environment may be at risk of depressed yield potential from reduced precipitation and could experience reduced or stunted N fixation due to dry soil conditions that could cause rhizobia death or cause the pea plant to discontinue C transport to root nodules during drought stress (McCauley et al., 2012; Streeter, 2003). Overall, each growing-season environment exhibits unique growing-season climate parameters that may lead to specialization in either high-grain N-concentration or high-grain-yield pea crops.

3.3 Grain N concentration and grain yield differences by growing-season environment cluster

Growing-season environment classification did not influence grain N concentration ($p = 0.44$) among the four unique climate clusters (Table 8). This finding suggests that C fixation was largely affected by heat or water stress, whereas the plant's ability to remobilize N to pod structures or to support growing-season N fixation was not reduced, thereby maintaining grain N concentration among growing-season environment clusters. Growing-season environment classification influenced grain yield ($p = <0.0001$) among the four climate clusters (Table 8). Grain yield was greatest for the cool/wet growing-season environment cluster, with a mean grain yield of 6091 kg ha^{-1}, followed by the dry and cold night growing-season environments, at 3447 and 2893 kg ha^{-1}. The hot growing-season environment had the lowest grain yield (2052 kg ha^{-1}), suggesting that elevated daytime temperatures, as indicated by days above 28 °C, and higher mean daytime temperatures reduced yield potential (Bueckert et al., 2015). Comparisons among growing-season environments indicate that cooler growing seasons and greater precipitation were associated with higher grain yield across genotypes, while consistently elevated daytime temperatures were associated with reduced grain yield. These results suggest that a detailed analysis of precipitation and heat accumulation patterns throughout the growing season was needed to understand better how growing-season climate conditions influence grain N concentration and grain yield. While comparisons of the four identified clusters provide insight into general patterns controlling grain yield, these comparisons do not inform us on how environments or genotypes influence pea grain N concentration.

3.4 Precipitation and heat accumulation rate effects on grain N concentration and yield

Pea growth windows were identified based on the influence that precipitation and heat accumulation have on N fixation, flower and bud abortion, seed fill, and N source/sink dynamics during seed formation (Bueckert et al., 2015; Guilioni et al., 1997; Huang et al., 2023; Seddigh & Jolliff, 1986). Pearson correlation coefficient results from the four individual pea crop growth windows indicate that precipitation during the second growth window (GDD: 321 – 780, BBCH: 14 – 65) was the strongest predictor of grain N concentration ($r = 0.27$, $p < 0.0001$) (Table 9). These findings suggest that increased precipitation and, hence, soil moisture during vegetative growth to the start of the reproductive cycle are associated with increased grain N concentration. The next best predictor of grain N concentration among the four individual growth windows was the GDD accumulation rate during the fourth growth window (BBCH: 71 – 79; $r = 0.25$, $p < 0.0001$) (Table 9). Early-season precipitation may enhance N_2 fixation rates and extend the effective lifespan of rhizobia nodules, thereby enabling greater N accumulation throughout the plant. Importantly, in previous work in Montana, pea N_2 fixation had ceased by first flower when May plus June precipitation was 27% below average but continued past first flower when May plus June precipitation was 18% above average, suggesting that precipitation prolongs N_2 fixation and increases N availability (McCauley et al., 2012). The timing of drought stress in relation to crop development has also been found to affect N_2 fixation (Streeter, 2003). Nitrogen fixation is affected by soil water content, not only because rhizobia require moisture, but also because higher moisture levels increase plant growth, thereby increasing N demand and, in turn, plant-microbe signaling (Unkovich et al., 2010). The presence of drought stress likely also reduced C transport to rhizobia nodules. Additionally, our findings show that increased heat

accumulation rates during seed formation (GDD: 1093 – 1378, BBCH: 71 – 79) are associated with increased grain N concentration, likely due to increased heat accumulation limiting starch formation, the building block of seed mass and thus yield, resulting in an increased grain N concentration. Heat and drought stress during seed development typically accelerates leaf senescence, limiting the plants photosynthetic capacity while accelerating N remobilization from leaf tissue to seed, resulting in a greater N:C seed ratio (Streeter, 2003; Wallwork et al., 1998; Yang et al., 2018; Zhao et al., 2008). Furthermore, increased heat accumulation rates may have increased flower and bud abortion rates, thereby limiting total pod formation per plant and the total number of N sinks in each plant that required N remobilization from plant N sources (Guilioni et al., 1997). However, MLR results of precipitation and heat accumulation rate explained little of the model variation of grain N concentration ($R^2 = 0.08$; Table 10) for the second growth window (BBCH: 14 – 65). This finding suggests that precipitation and heat accumulation rates within specific growth windows may not be the best explanatory variables for predicting grain N concentration, and that other environmental or climate variables, such as soil fertility, soil type, or precipitation frequency, may also be valuable explanatory variables to include in future studies, in addition to precipitation amount and heat accumulation rates.

Among the four individual growth windows, Pearson correlation results indicated that heat accumulation rate during the first growth window was the strongest predictor of grain yield (BBCH: 0 – 14; $r = 0.47$, $p < 0.0001$) (Table 9). The next best predictor of grain yield was heat accumulation rate during the fourth growth window (BBCH: 71 – 79) from initiation of seed fill to completion of physiological seed maturity (GDD: 1093 – 1378) ($r = -0.45$, $p < 0.0001$). These findings suggest that early-season heat accumulation after seeding positively contributes to

increased yield potential, likely through increased seedling vigor and earlier seedling emergence. The finding that higher heat accumulation rates during the fourth growth stage window are associated with lower yield potential suggests that increased heat may decrease flower viability, pod formation, seeds per pod, and overall seed mass. Increased temperatures, likely assaociated with low plant available water, accelerate leaf senescence, reducing overall plant photosynthetic capacity while accelerating N remobilization from senescing leaves to seeds (Sita et al., 2018; Yang et al., 2018). Furthermore, MLR analysis reported that the greatest variation in grain yield was explained by precipitation and heat accumulation during the first (GDD: 0 – 321) and second (GDD: 321 – 780) growth windows (Table 10), which supports the Pearson correlation results. These results indicate that cumulative precipitation and heat accumulation rates during specific growth periods may be adequate predictors of grain yield, particularly when little information about the production environment is known or when crop production history is limited.

Pearson correlation coefficient results among the six composite growth windows show that the best predictor of grain N concentration is precipitation during the four leaves open to the initiation of seed fill growth period (BBCH: 14 - 71) ($r = 0.29$, $p < 0.0001$) (Table 11). The next best composite growth window that predicts grain N concentration is from four leaves open to completion of physiological seed maturity (BBCH: 14 – 79; $r = 0.25$, $p < 0.0001$) (Table 11). However, MLR analysis showed that among the six composite growth windows, cumulative precipitation and heat explained little variation in grain N concentration (Tables 11 – 12). Other environmental parameters, such as varying soil nutrient profiles, soil texture, and unique biotic stressors in the growing region, may also be needed to explain grain N concentration patterns.

Among the six composite growth windows, heat accumulation rate from the initiation of flowering to the completion of physiologically mature seed (GDD: 780 – 1378, BBCH: 65 – 79) is the best predictor of grain yield based on Pearson correlation coefficients ($r = -0.50$, $p < 0.0001$) (Table 11). This composite growth window captured grain variation more accurately, by 19%, when compared to the best individual growth window (Tables 10 and 12) Legume species utilize stomatal conductance restriction as a primary defense mechanism to limit water loss, though this also limits the rate of photosynthesis, suggesting that periods of elevated evapotranspiration results in reduced photosynthate production that likely limits yield potential (Haghpanah et al., 2024; Khatun et al., 2021). The second-best predictor of grain yield among the six composite growth windows was heat accumulation rate from the day of seeding to initiation of flowering (GDD: 0 – 780) ($r = 0.31$, $p <0.0001$) (Table 11). In contrast, greater heat accumulation from the start of flowering to the completion of seed maturity is associated with decreased grain yield. These results align with individual growth window results for grain yield and previous research conducted by Bueckert et al. (2015) that reported reduced grain yield and days in reproductive growth with increased temperature, with Huang et al. (2017) reporting that pea yield decreased as mean air temperatures increased during flowering (Tables 9 – 12). MLR analysis revealed that, among the composite growth windows, cumulative precipitation and heat accumulation rate during the flowering-to-physiological seed maturity growth window explained 48% of the variation in grain yield (Table 12). This finding is meaningful, as this climate variables analysis encompasses 23 environments, including four climate categories, seven soil types, soil nutrient profiles at planting, differing insect, weed, and pathogen management, and precipitation frequency within each location. Explaining half of the variation in grain yield with

two climate variables during a specific growth-stage window offers an opportunity to forecast grain yield based on easily obtainable growing-season weather variables within a growing region. These findings detail the unique timing of precipitation and heat accumulation throughout the pea life cycle and resultant interplay between grain N concentration and grain yield.

3.5 AMMI – Grain N Concentration

Environment was the most important source of variation in grain N concentration, explaining 70.5% (p = <0.0001) of the total (G + E + G×E) sums of squares (Table 13, Figure 2). The contribution of genetics (p = <0.0001) and the interaction of environment × genetics (p = 0.0001) were meaningful factors in explaining the total variation of grain N concentration within this study, with genetics and the interaction of environment × genetics explaining 10.5% and 18.9% of the total sums of squares from this analysis (Table 13).

Grain N concentration had a grand mean of 39.6 g kg^{-1}, with pea genotypes centered around the narrow band on the AMMI biplot (Figure 2). At the same time, environments covered a larger grain N concentration range, from 34.3 g kg^{-1} observed at Sutherland in 2023 to 44.0 g kg^{-1} at Havre in 2023, reflecting a 6.0 g kg^{-1} spread in grain N concentration (Table 14). The ANOVA results from the mixed model show that, across years and genotypes, locations differed ($p < 0.0001$), suggesting that grain N concentration was influenced by environment (Table 14). Grain N concentration among genotypes ranged from 38.3 g kg^{-1} for DS Admiral to 41.0g kg^{-1} for CDC Dakota (Table 15) (Andersen et al., 2011; Warkentin, 2010). The results of the mixed-model ANOVA also suggest that genotypes differ in grain N concentration across environments ($p < 0.0001$). Additionally, the AMMI results on grain N concentration suggest that the

adaptability and performance of pea genotypes are environment-specific, as 18.9% of the variation in grain N concentration was explained by the interaction between genotype and environment.

The AMMI results suggest that unique environmental characteristics, such as growing-season climate variables, exert a more substantial influence on observed grain N concentration than contrasting genotypes. Furthermore, these results show that grain N concentration is overwhelmingly controlled by environmental factors, unique among the 23 environments tested, rather than by genotype. Tao et al. (2017a) conducted a similar analysis on the environment vs genetic control on pea protein concentration in Montana (380,000 km^2), reporting that environment explained 92.5% of observed protein variation. Our results indicate that environment explains 70.5% of observed grain N concentration variation across a larger geographic area (~ 878,000 km^2) of the broader central and northern Great Plains. This finding aligns with previous research, reinforcing the importance of the environment, and hence unique precipitation and temperature gradients and patterns present across North America have on production of protein (grain N concentration) in field pea (Omari et al., 2025; Tao et al., 2017). There is evidence that grain N concentration performance is also controlled by genotype, which aligns with AMMI results from soybean protein by Omari et al. (2025). Additionally, there is evidence that grain N concentration performance is also controlled by pea genotypes' adaptability to specific environments, as indicated by the significant genotype-by-environment interaction. AMMI results, in conjunction with the growing-season environment classification of environments, indicated that growing-season climate characteristics, such as precipitation or temperature, that characterize a growing-season environment cluster are poor predictors of grain

N concentration. However, heat accumulation and precipitation during specific crop growth windows show promise as predictors of grain N concentration.

3.6 AMMI – Grain Yield

Environment explained 97.1% (p = <0.0001) of grain yield variation via AMMI analysis (Table 13, Figure 3). The contribution of genetics (p = <0.0001) and the interaction of environment × genetics (p = <0.0001) were meaningful factors due to the large number of grain yield observations in this study. However, genetics and the interaction between environment × genetics explained only 0.5% and 2.4% of the total sums of squares in this analysis (Table 13). This shows that genetics and genetic × environmental interaction have limited predictive power with respect to grain yield. Environments tested in this study span a wide range of grain-yield values, from 6805 kg ha^{-1} at Roseau in 2023 to 625 kg ha^{-1} at Mead in 2022 (Figure 3). Across all locations, years, and genotypes, the observed mean grain-yield spread was 5571 kg ha^{-1}, whereas across environments the mean grain-yield spread among genotypes was only 339 kg ha^{-1} (Tables 14 – 15). This result alone suggests that yield potential is primarily determined by growing-season climate and other local conditions, such as soil type, fertility, and regionally specific management practices, such as variable seeding dates and contrasting plant populations, which have been proven to influence grain yield (Carr et al., 2024; Koeshall et al., 2021). In contrast, even optimized pea genotypes in poor environments cannot overcome environmental effects on observed final yield potential. In comparison to grain N concentration AMMI results, genotype has a 21-fold stronger control on grain N concentration compared to grain yield (Table 13). This result suggests that advancement of protein production (grain N concentration) can be optimized via proper genotype selection to specific locations or ecoregions and that breeding

efforts can have a greater effect on pea protein production than yield (Table 13). This finding also presents an opportunity for pea protein processors to recommend specific genotypes to clients to optimize protein production within specific locations.

Across years and genotypes, location influenced grain yield ($p < 0.0001$), suggesting that, across variable multi-year climates, certain growing locations are better suited to fostering high yield potential (Table 14). Williston in 2022 exhibited the highest grain-yield stability, with a yield of 2304 kg ha^{-1}, followed by Lucky Lake in 2022, with a yield of 1167 kg ha^{-1} (Figure 3). Roseau in 2023 and 2024 achieved the highest grain yields at 6805 and 6533 kg ha^{-1}, respectively. Pea grain yield at Roseau likely benefited from a Endoaquoll soil type that is characterized by saturation deep in the soil profile, allowing for elevated evapotranspiration rates during warmer temperatures that coincide with flowering and seed formation (Table 1). Williston in 2022 is associated with the hot growing-season environment, while Lucky Lake is associated with the dry growing-season environment (Figure 1). Among environments, Roseau in 2022 and 2024 was classified as the cool/wet growing-season environment, suggesting that high yield potential was associated with growing-season precipitation and few days above 28 °C. CDC Dakota exhibited the highest grain-yield stability, with a mean grain yield across environments of 3239 kg ha^{-1}, followed by AAC Profit (3423 kg ha^{-1}) and DS Admiral (3140 kg ha^{-1}) (Figure 3) (Andersen et al., 2011; Bing et al., 2022; Warkentin, 2010). AAC Profit achieved the highest grain yield across environments at 3423 kg ha^{-1}, followed by CDC Amarillo (3339 kg ha^{-1}) (Bing et al., 2022; Warkentin et al., 2014). Delta exhibited the lowest grain yield across environments (3084 kg ha^{-1}) and the lowest grain-yield stability among all genotypes tested in this study (Limagrain, 2025). Although genotypes have little control over grain yield in comparison to

environmental influence, evidence from this study suggests that recent breeding programs have been successful at improving both grain yield performance and stability, as the most recently publicly released genotypes (e.g., AAC Profit) in this study scored above older pea genotypes (e.g., DS Admiral and Delta). Overall, grain-yield results suggest that avoiding high-temperature days during the growing season and achieving optimal precipitation foster the greatest grain-yield potential, as the two highest-yielding environments were associated with the wet/cool growing-season environment. Selection of appropriate genotypes can improve overall grain yield and stability.

3.7 Correlation between grain yield and grain N concentration

Pearson's correlation coefficient across all observations (n=569) between pea grain yield and grain N concentration (Figure 4) indicated a weak negative relationship ($r = -0.28$, $p = <0.0001$). Additionally, the nearly perfect relationship ($r = 0.98$, $p <0.0001$) between grain yield and grain N yield and the poor relationship between grain N concentration and grain N yield ($r = -0.12$, $p = 0.0045$) suggest that grain yield is a clear representation of total grain N yield (e.g. protein produced per unit of land) (Table 16). Hence, grain N yield can be easily deduced from grain yield results (Table 16). In this correlation analysis, only 23 data points (4.0%) had grain N concentrations below 33.6 g kg^{-1}, which would not meet the threshold for acceptable grain N concentration under common protein industry standards (using the 6.25 correction factor), potentially leading to rejection or dockage of the pea crop at the delivery site. Fifty-four data points (9.5%) had a grain N concentration under 35.2 g kg^{-1}, representing the total number of grain N concentration samples that would not qualify for a protein premium under common industry protein standards. However, 338 data points exceeded 38.4 g kg of grain N

concentration, representing the highest protein premium bracket per industry standards, suggesting that 59.4% of all grain yield observations would qualify for a prime protein premium.

Pearson correlation analysis was applied to the four distinct growing-season-environments identified in the environmental climate clustering analysis to assess whether growing-season environmental cluster influence alters the relationship between grain yield and grain N concentration (Figures 5 – 8). There was a significant relationship ($r = -0.42$, $p < .0001$) between grain yield and grain N concentration within the dry growing-season environment (Cluster 1), comprising 9 environments (Figure 5). Drought-induced premature leaf senescence precipitates a decline in photosynthetic capacity and accelerates the remobilization of foliar N to the developing grain, thereby reducing the C:N ratio available for seed filling (Sita et al., 2018). Within this growing-season environment, 13 grain N concentration observations were below 33.6 g kg^{-1}, and 33 observations were below the grain N concentration level of 35.2 g kg^{-1} (data not shown). This result suggests that 5.8% of peas grown in this growing-season environment would be at risk of rejection or dockage, and 14.5% would not qualify for a protein premium. Within the dry growing-season environment, 133 grain N concentration observations were above 38.4 g kg, resulting in 59.4% of all pea grain produced in the dry growing-season environment would qualify for a prime protein premium (Figure 5).

The cold night growing-season environment (Cluster 2), comprising eight environments, contained a correlation coefficient of 0.07 ($p = 0.32$), indicating a flat, non-significant relationship between grain yield and grain N concentration within this growing-season environment cluster (Figure 6). This result for the cool/wet growing-season environment revealed that 4.1% of peas grown in this growing-season environment would be at-risk of

dockage or rejection at the time of sale, and 7.7% would not qualify for a protein premium (Figure 6). In the cold night growing-season environment, 62.6% of all pea grain produced in this growing-season environment would qualify for a prime protein premium.

Correlation results revealed a weak negative relationship ($r = -0.36$, $p = 0.0003$) between grain yield and grain N concentration in the hot growing-season environment (Cluster 3) (Figure 7). Within the hot growing-season environment, no grain N concentration observations were below 33.6 g kg^{-1}, and five were below 35.2 g kg^{-1} (Figure 7). These findings suggest that no peas grown in the hot growing-season environment would be at risk of rejection or dockage at the time of sale, 5% would not qualify for a protein premium, and 59.0% of pea grain grown in this growing-season environment would qualify for a prime protein premium (Figure 7).

The cool/wet growing-season environment composed of only two environments, and therefore perhaps most appropriately considered an outlier, showed no relationship between grain yield and grain N concentration (p-value = 0.09) (Figure 8). Within the cool/wet growing-season environment, only two grain N concentration observations were below 33.6 g kg^{-1}, and 5 were below 35.2 g kg^{-1} (Figure 8). These findings suggest that 4% of peas grown in this growing-season environment would be at-risk of rejection or dockage at the time of sale, and 10% would not qualify for a protein premium. Finally, 48.0% of pea grain produced in this growing-season environment cluster would qualify for a prime protein premium (Figure 8).

Overall, the likelihood of rejection or dockage varies among growing-season environments (0% to 5.8%); however, the proportion of peas in the four growing-season environments that do not qualify for a protein premium was larger and more variable (5.0% to 14.4%) compared to the proportion with grain N concentrations below 33.6 g kg^{-1}. The proportion of grain N

observations that would not qualify for a protein premium was highest within the dry growing-season environment, indicating that abiotic stress from reduced precipitation and hence reduced soil moisture limits grain N concentration development, likely from reduced N fixation or premature rhizobium nodule death from stressful rhizosphere conditions. This observation and finding contradict previously held beliefs that high-yield potential environments are more likely to produce pea grain with reduced grain N concentrations. This is evident in the mean results of the growing-season environment climate variables. The cool/wet growing-season environment received 322 mm of precipitation, while the dry growing-season environment received only 164 mm. Research by Ahmed et al. (2015) and Wallwork et al. (1998) aligns with the grain N concentration and grain yield relationship across 23 environments as N assimilation enzymes are increased under stress while starch formation decreases. However, across all growing-season environment clusters, 59.4% of pea grain had a grain N concentration above 38.4 g kg^{-1}, suggesting that all growing-season environments can produce high-protein pea grain; however, certain growing-season environment clusters are more at risk of producing grain that might result in rejection or dockage at the time of sale. These findings suggest that, in certain situations, the absence of moisture is a greater problem for pea producers managing for high protein potential than in environments with ample moisture, where high yield potential is the primary goal and outcome, as elevated precipitation can offset increased evapotranspiration demand from elevated temperatures.

4. Conclusion

This study revealed that classifying pea-growing regions into growing-season environment clusters based on growing-season climate variables resulted in grain yield

differences among clusters, largely a function of growing season precipitation, followed by temperature. Sufficient moisture during the growing season allowed for enhanced leaf evaporative cooling, even during temperatures that have been found to cause heat stress. Grain N concentration did not differ among growing-season environments. This study found that the environment explained 71% and 97% of the variation in grain N concentration and grain yield. However, genotype and the interaction between environment and genotype were meaningful factors, suggesting that genotypes exhibit site-specific adaptability across environments. Our study revealed through AMMI analysis that genotype has a 21-fold stronger control over grain N concentration than grain yield, suggesting that genotype selection is crucial for optimizing protein production within a specific region or location. This study revealed that the timing of precipitation and the rate of heat accumulation throughout the pea plant's life cycle are predictive of grain yield and, to a lesser extent, grain N concentration, as predicted grain yield was best modeled using precipitation received from seed fill initiation to seed maturity. Grain N concentration had a weak, negative correlation with grain yield across all environments.

This study increases our understanding of how, and to what extent, environmental and genetic factors influence grain N concentration and grain yield across North America. Findings from this study inform the broader pea-processing industry about the growing-season environmental characteristics that control grain N concentration and grain yield, and their relationship, thereby providing both industry and pea growers with opportunities to specialize in either high-yield or high-grain N (protein) concentration production. Results suggest that environment determines grain yield, although findings suggest that proper genotype selection is vital to maintaining protein production in high-yield environments. Results from this study

indicate that grain N concentration is maintained in growing-season environments that can generally be described by cooler growing season temperatures and increased precipitation. Overall, the northern Great Plains states of Montana and North Dakota balance grain N concentration production above 38.4 g kg^{-1} while producing adequate grain yield, suggesting that future pea protein processing facilities should consider these areas for future expansion, given that a majority of pea production in the United States is centered in Montana and North Dakota. Results from this study provide pea growers and processors with valuable insights into which regions promote high-protein, high-yield, or both outcomes, as well as the specific climate conditions responsible for these outcomes across contrasting genotypes.

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Figures

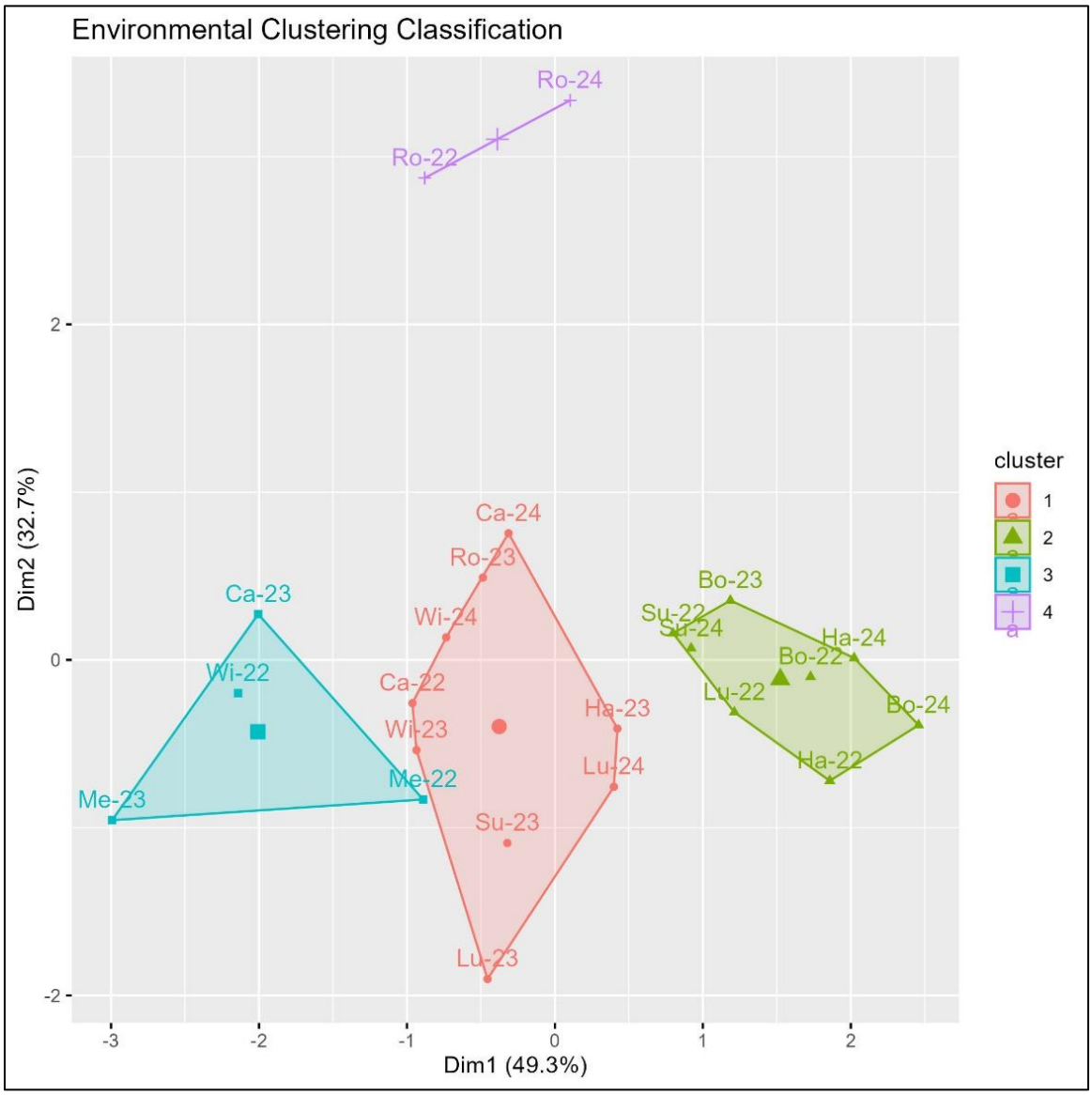

Figure 1: Mega-environment climate classification of 23 environments tested across the USA and Canada based on growing season precipitation and temperature variables that identified four unique mega-environment clusters.

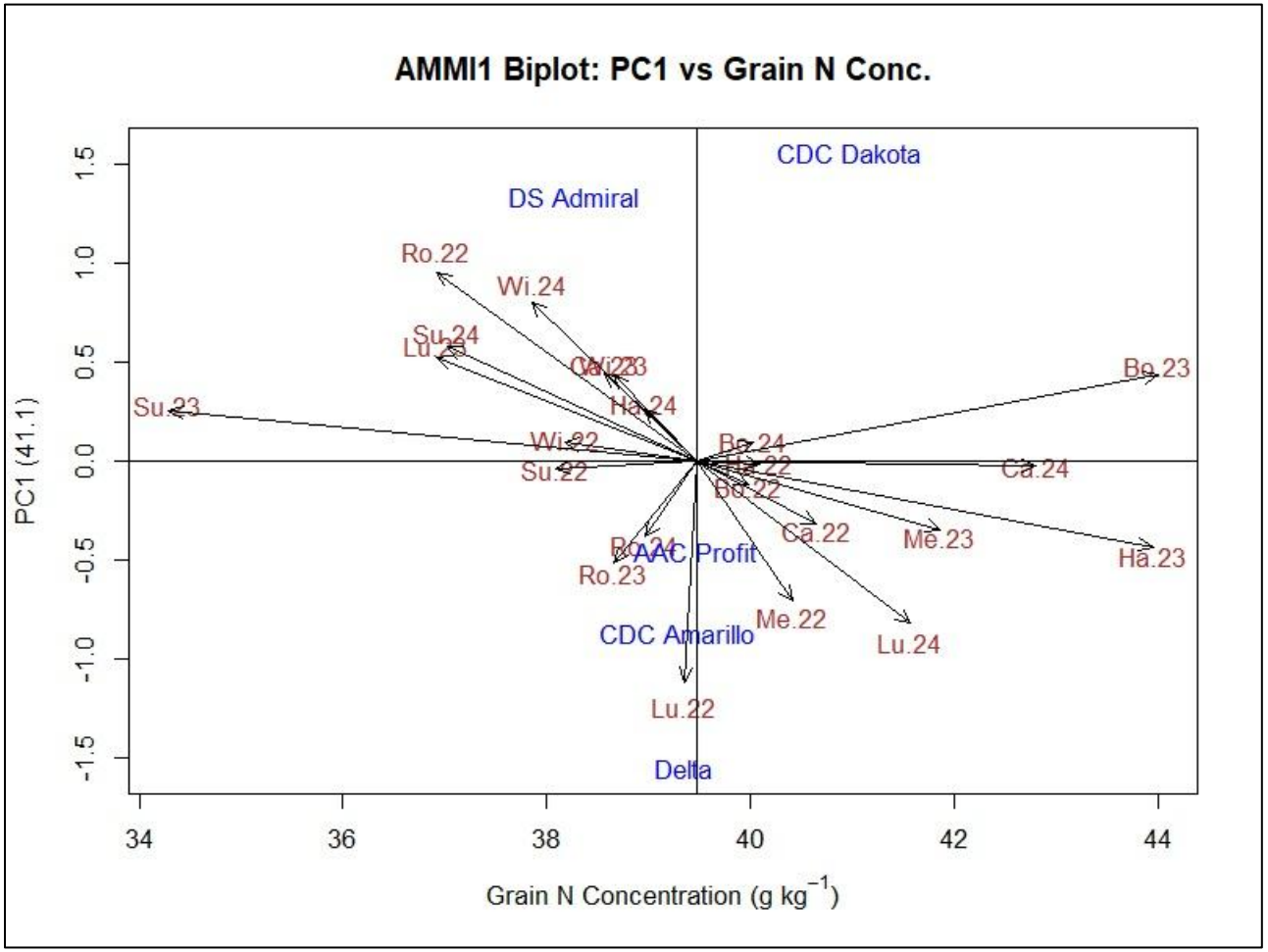

Figure 2: Additive main effect and multiplicative interaction (AMMI) biplot showing grain N concentration vs PC1 of 23 environments (red color) and five genotypes (blue color).

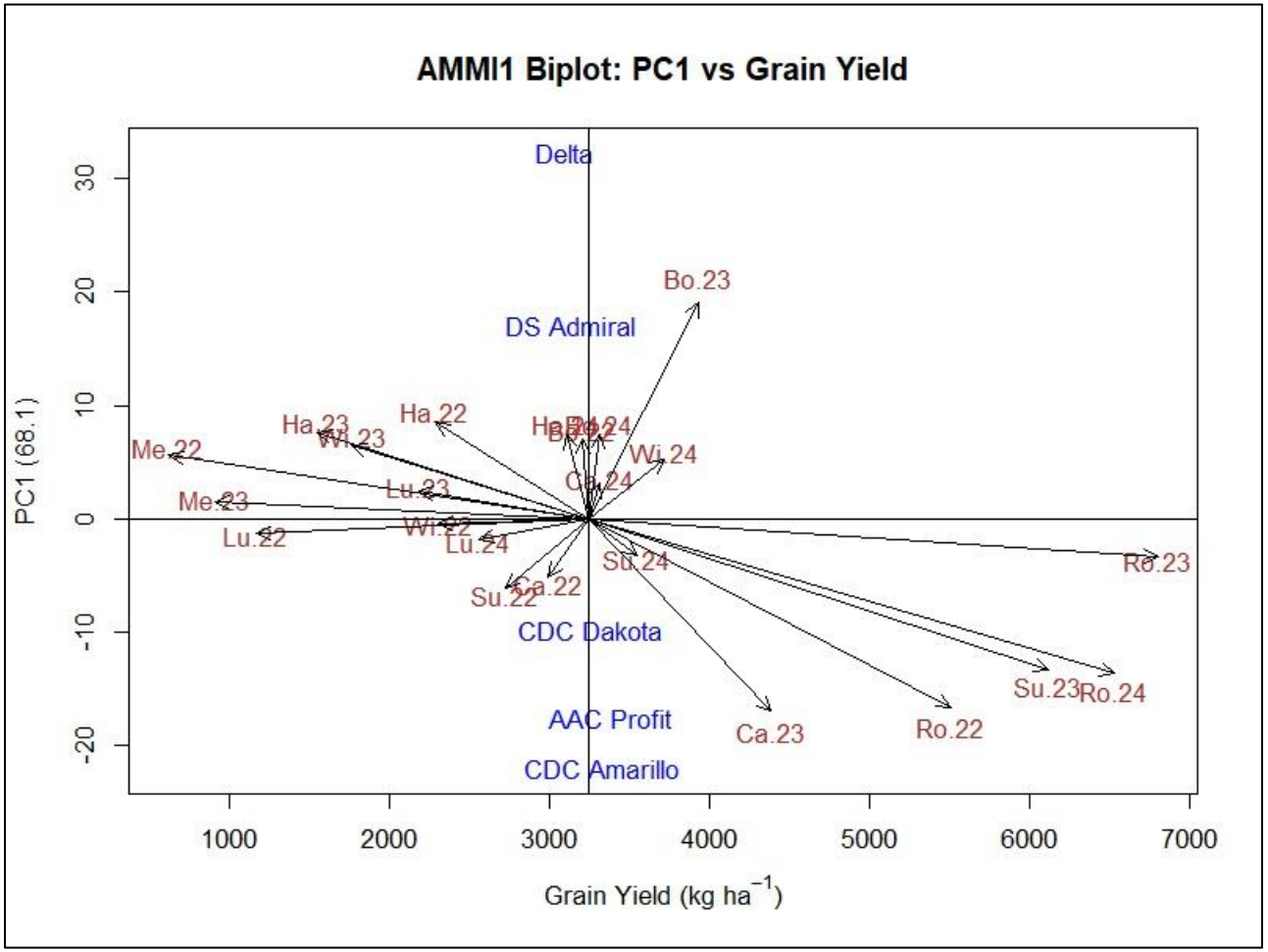

Figure 3: Additive main effect and multiplicative interaction (AMMI) biplot showing grain yield vs PC1 of 23 environments (red color) and five genotypes (blue color).

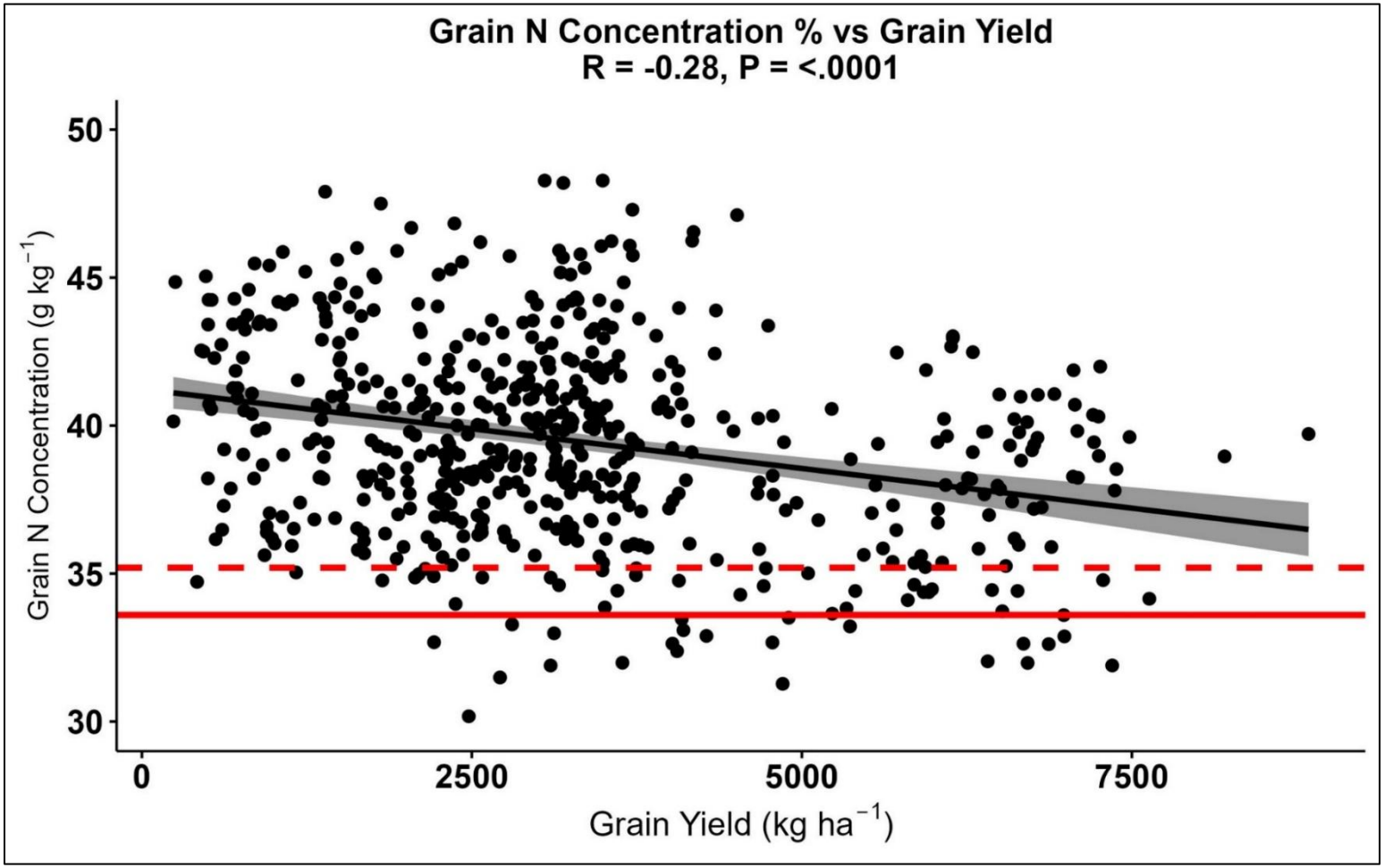

Figure 4: Relationship between pea grain N concentration and grain yield across 23 environments (n = 569). The red dashed line represents the 35.2 g N kg-1 threshold that marks the start of protein premiums, while the 33.6 g N kg-1 solid red line represents an approximate point that pea grain could be subjected to dockage or rejection at time of sale.

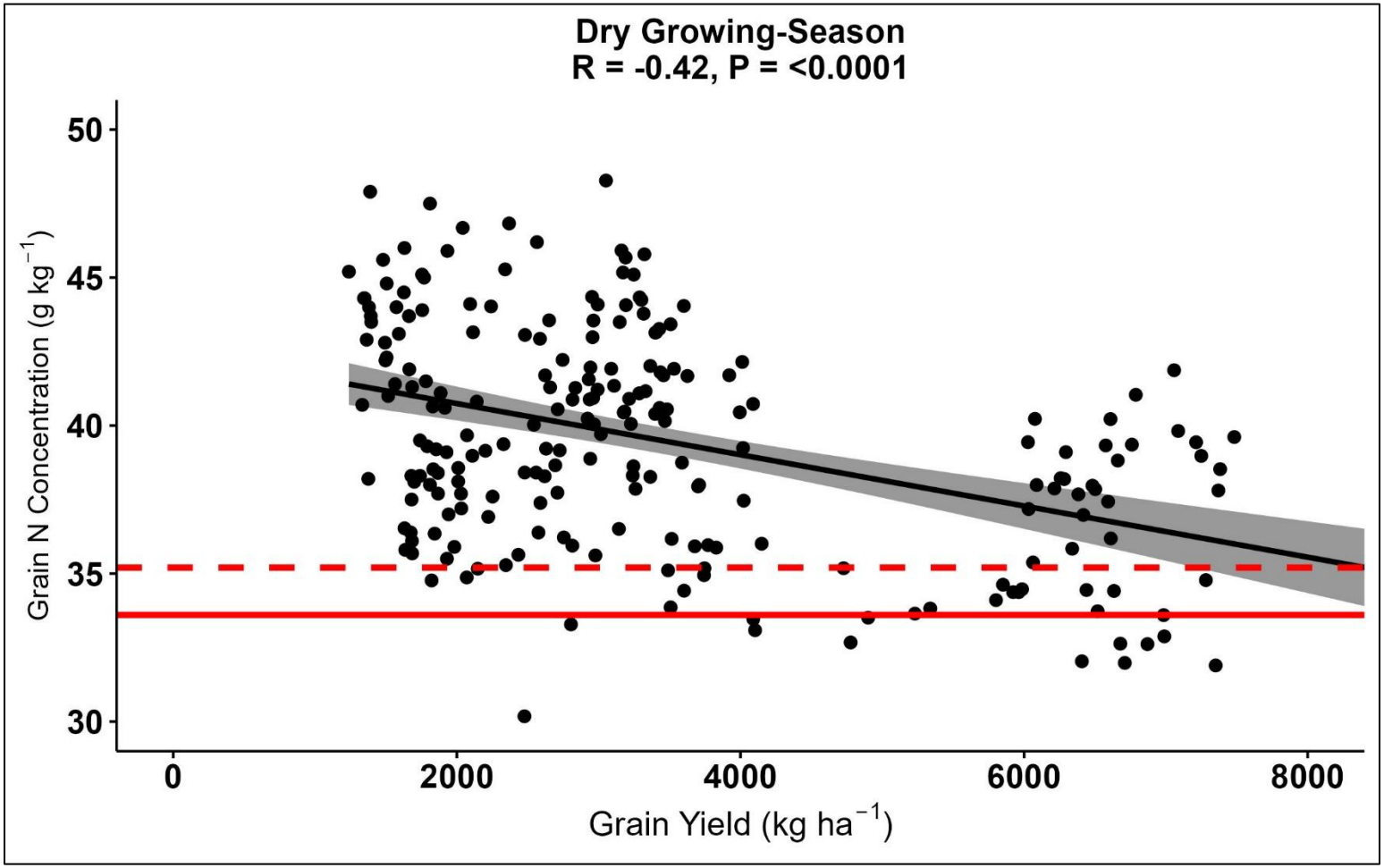

Figure 5: Relationship between pea grain N concentration and grain yield across nine environments classified within the dry mega-environment (n = 224). The red dashed line represents the 35.2 g N kg-1 threshold that marks the start of protein premiums, while the 33.6 g N kg-1 solid red line represents an approximate point that pea grain could be subjected to dockage or rejection at time of sale.

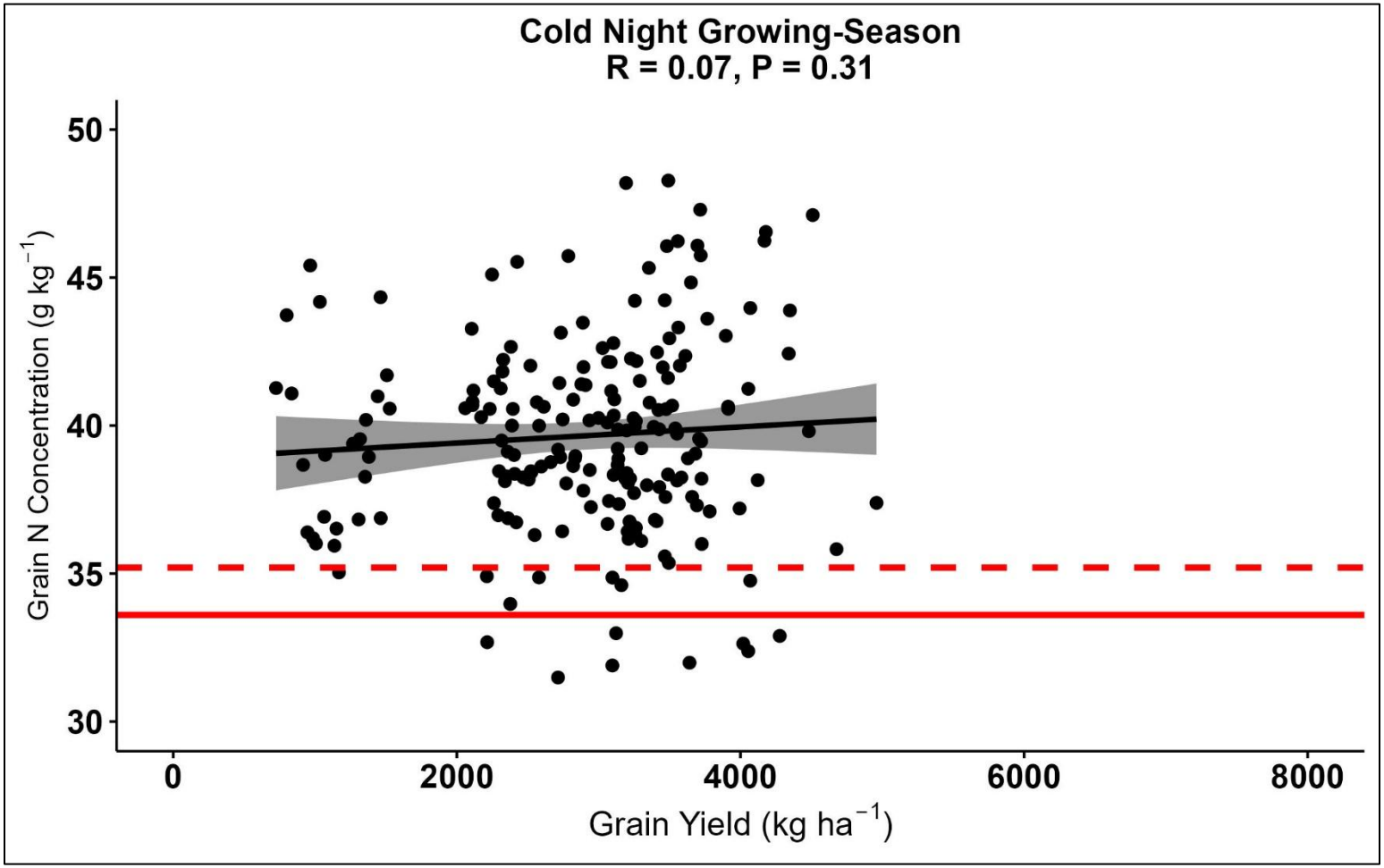

Figure 6: Relationship between pea grain N concentration and grain yield across eight environments classified within the cold night mega-environment (n = 195). The red dashed line represents the 35.2 g N kg-1 threshold that marks the start of protein premiums, while the 33.6 g N kg-1 solid red line represents an approximate point that pea grain could be subjected to dockage or rejection at time of sale.

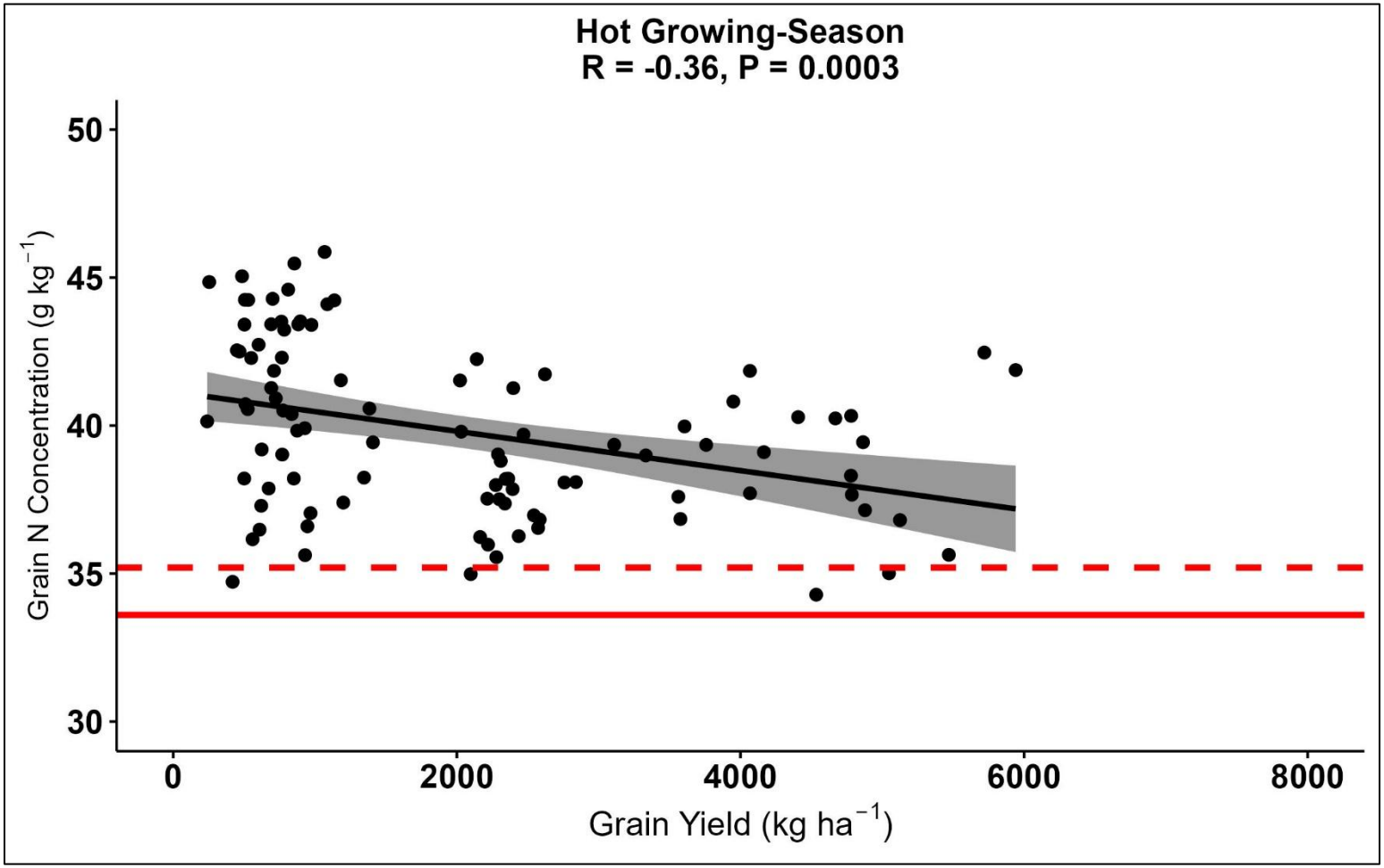

Figure 7: Relationship between pea grain N concentration and grain yield across four environments classified within the hot mega-environment (n = 100). The red dashed line represents the 35.2 g N kg-1 threshold that marks the start of protein premiums, while the 33.6 g N kg-1 solid red line represents an approximate point that pea grain could be subjected to dockage or rejection at time of sale.

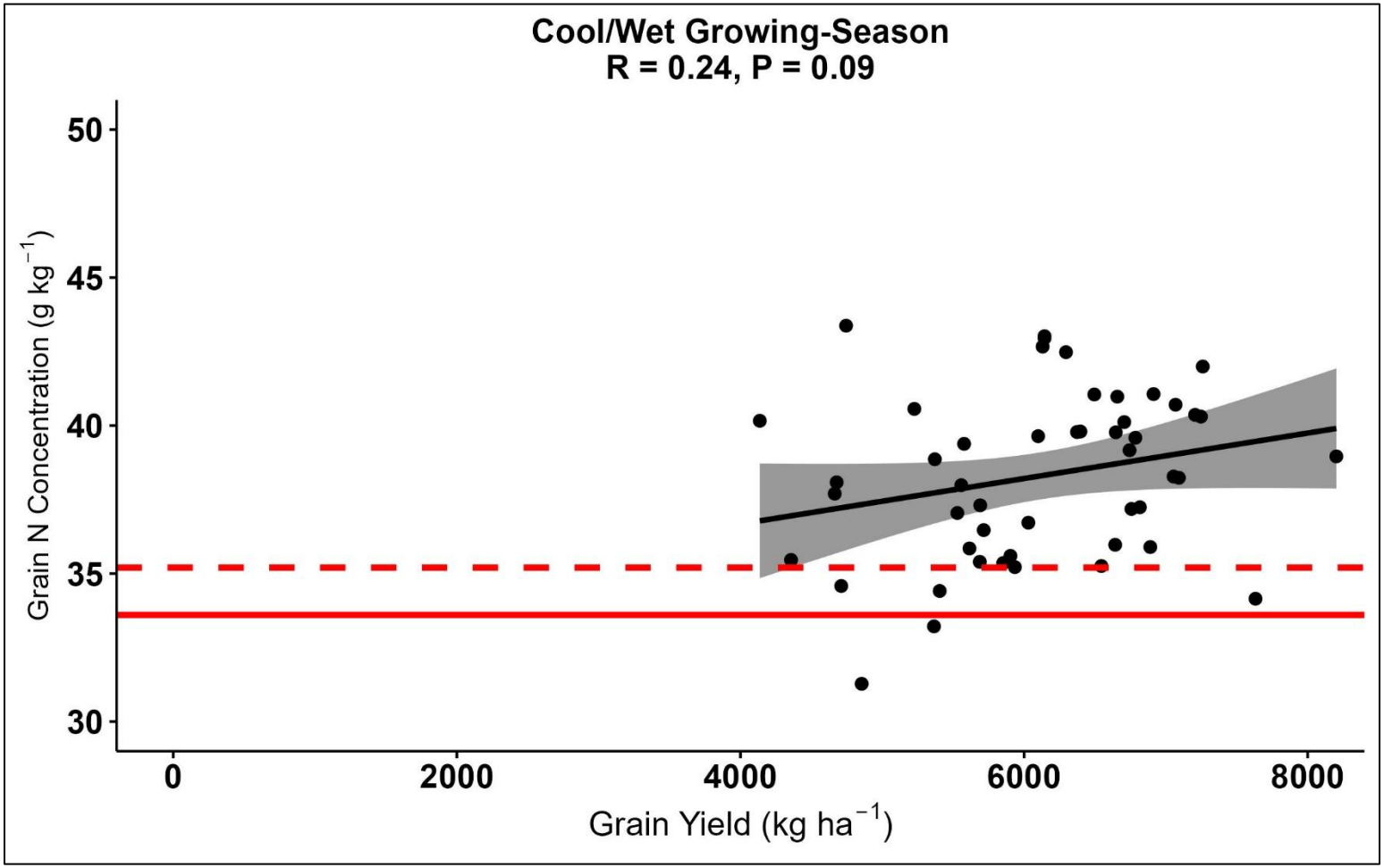

Figure 8: Relationship between pea grain N concentration and grain yield across four environments classified within the hot mega-environment (n = 50). The red dashed line represents the 35.2 g N kg-1 threshold that marks the start of protein premiums, while the 33.6 g N kg-1 solid red line represents an approximate point that pea grain could be subjected to dockage or rejection at time of sale.

Tables

Table 1: Summary of study locations from 2022 to 2024 across the USA and Canada with location coordinates, location altitude, and location soil type information.

Location	Geographic Coordinate lat/long	Altitude m	Dominant Soil Type*
Sutherland, SK**	52.166, -106.504	482	Aridic Argiustoll
Lucky Lake, SK**	51.103, -107.241	555	Aridic Argiustoll
Havre, MT	48.494, -109.802	820	Fine-loamy, mixed, superactive, frigid Aridic Argiustolls
Bozeman, MT	45.678, -111.148	1455	Typic Haplustolls, Typic Calciustolls (frigid)
Carrington, ND	47.505, -99.121	476	Calcic Hapludolls, Pachic Hapludolls
Williston, ND	48.134, -103.740	642	Typic Argiustolls, Pachic Argiustolls
Roseau, MN	48.853, -95.800	319	Typic Endoaquolls, glaciolacustrine
Mead, NE	41.183, -96.467	361	Mollic Hapludalfs, terraces

*Soil information for USA locations obtained via University of California - Davis SoilWeb

**Canada soil information obtained from Saskatchewan Soil Information System

Table 2: Summary of seeding date, harvest date, growing period, accumulated growing degree days from seeding to harvest, and growing degree day accumulation rate for each study location from 2022 to 2024 across the USA and Canada.

Location	Year	Seeding Date	Harvest Date	Growing Period	Accumulated GDD	GDD Accumulation Rate
				days		GDD day^{-1}
Sutherland, SK	2022	9 May	16 August	99	1630	16.5
	2023	4 May	15 August	103	1830	17.8
	2024	11 May	21 August	102	1620	15.9
Lucky Lake, SK	2022	5 May	10 August	97	1580	16.3
	2023	9 May	14 August	97	1780	18.4
	2024	15 May	21 August	98	1520	15.5
Havre, MT	2022	21 April	27 July	97	1470	15.2
	2023	26 April	25 July	90	1530	17.0
	2024	15 April	29 July	105	1560	14.9
Bozeman, MT	2022	20 April	8 August	110	1620	14.7
	2023	28 April	14 August	108	1720	15.9
	2024	11 April	2 August	113	1550	13.7
Carrington, ND	2022	16 May	9 August	85	1580	18.6
	2023	5 May	9 August	96	1840	19.2
	2024	13 May	13 August	92	1630	17.7
Williston, ND	2022	4 May	4 August	92	1760	19.1
	2023	26 April	18 July	83	1530	18.4
	2024	26 April	2 August	98	1740	17.8
Roseau, MN	2022	28 May	7 September	102	1880	18.4
	2023	16 May	28 August	104	1910	18.4
	2024	13 May	28 August	107	1850	17.3
Mead, NE	2022	1 April	21 July	111	1940	17.5
	2023	25 April	19 July	85	1690	19.9
	2024	12 April	31 July	110	2160	19.6

Note: Growing degree-day data for each location were obtained via the High Plains Climate Center ACIS-CLIMOD and Climate Canada data services.

Table 3: Summary of monthly cumulative precipitation, cumulative precipitation from April 01 to August 31, and the 30-year mean precipitation accumulation from April 01 to August 31 for each study location in the USA and Canada.

Location	Year	Apr	May	June	July	Aug	Apr - Aug	30-yr Apr - Aug Average
		precipitation (mm)						
Sutherland, SK	2022	3	26	38	47	26	139	242
	2023	8	52	44	20	50	174	
	2024	12	46	111	27	45	241	
Lucky Lake, SK	2022	14	20	49	71	10	163	238
	2023	17	25	30	12	59	141	
	2024	24	91	61	23	6	204	
Havre, MT	2022	4	10	81	44	23	163	198
	2023	14	74	57	16	25	186	
	2024	22	110	63	18	37	250	
Bozeman, MT	2022	41	110	60	14	15	240	253
	2023	19	19	129	25	47	239	
	2024	36	89	41	19	26	211	
Carrington, ND	2022	61	162	80	38	31	372	309
	2023	43	99	92	58	41	333	
	2024	60	128	145	89	122	545	
Williston, ND	2022	72	166	50	52	13	354	250
	2023	20	75	77	48	71	292	
	2024	15	77	82	44	14	232	
Roseau, MN	2022	88	134	53	90	149	513	343
	2023	16	46	35	67	38	201	
	2024	37	91	122	116	63	430	
Mead, NE	2022	30	110	77	26	74	316	493
	2023	38	6	130	199	62	436	
	2024	67	186	71	166	129	618	

Note: 30-year April to August precipitation averages at each location are representative of 1991 to 2020, and precipitation data were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada online data services.

Table 4: Summary of monthly mean temperature during the growing season and mean growing season temperature from April 01 to August 31 for each study location in the USA and Canada from 2022 to 2024.

Location	Year	Apr	May	June	July	Aug	Apr - Aug
		Temperature °C					
Sutherland, SK	2022	1.8	11.0	15.7	19.3	19.6	13.5
	2023	1.4	15.5	19.2	17.7	18.1	14.4
	2024	6.5	11.2	13.7	19.9	18.3	13.9
Lucky Lake, SK	2022	1.9	11.2	16.0	19.6	20.7	13.9
	2023	0.9	15.0	18.7	18.7	18.8	14.4
	2024	6.8	10.7	14.2	20.3	18.6	14.1
Havre, MT	2022	2.9	11.4	16.0	21.7	22.8	15.0
	2023	4.7	14.9	17.3	21.1	21.1	15.8
	2024	7.3	11.0	15.3	21.8	20.1	15.1
Bozeman, MT	2022	2.9	9.1	15.4	20.6	21.7	13.9
	2023	4.2	13.3	14.4	19.7	19.4	14.2
	2024	7.4	9.5	16.2	20.4	18.8	14.5
Carrington, ND	2022	-0.2	11.5	18.4	20.9	20.0	14.1
	2023	-0.9	15.3	21.0	19.2	19.3	14.8
	2024	5.6	11.8	17.3	21.3	18.8	14.9
Williston, ND	2022	1.6	12.8	18.9	23.3	23.8	16.1
	2023	5.2	16.0	21.1	21.8	21.8	17.2
	2024	9.7	13.4	17.2	23.3	21.4	17.0
Roseau, MN	2022	1.0	12.6	18.9	21.2	19.7	14.7
	2023	0.7	16.7	21.7	19.6	19.7	15.7
	2024	6.5	13.0	17.7	21.6	20.3	15.8
Mead, NE	2022	8.2	16.0	22.4	24.8	23.6	19.0
	2023	10.2	17.8	22.6	22.8	23.1	19.3
	2024	11.2	16.8	22.7	23.2	23.1	19.4

Note: 30-year April to August temperature means at each location are representative of 1991 to 2020, and temperature data were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada online data services.

Table 5: Summary of study locations by year across the USA and Canada, environment code, mega-environment classification code, pea genotypes used across all environments, and genotype codes used in cluster and AMMI analysis.

Location	Year	ENV Code	Mega-ENV Cluster ID	Genotypes	Genetic Code (GEN)
Sutherland, SK	2022	Su.22	2	AAC Profit	G1
	2023	Su.23	1	CDC Amarillo	G2
	2024	Su.24	2	CDC Dakota	G3
Lucky Lake, SK	2022	Lu.22	2	Delta	G4
	2023	Lu.23	1	DS Admiral	G5
	2024	Lu.24	1		
Havre, MT	2022	Ha.22	2		
	2023	Ha.23	1		
	2024	Ha.24	2		
Bozeman, MT	2022	Bo.22	2		
	2023	Bo.23	2		
	2024	Bo.24	2		
Carrington, ND	2022	Ca.22	1		
	2023	Ca.23	3		
	2024	Ca.24	1		
Williston, ND	2022	Wi.22	3		
	2023	Wi.23	1		
	2024	Wi.24	1		
Roseau, MN	2022	Ro.22	4		
	2023	Ro.23	1		
	2024	Ro.24	4		
Mead, NE	2022	Me.22	3		
	2023	Me.23	3		
	2024	Me.24	NA		

Table 6: Principal components used to inform mega-environment classification, scaled eigenvalues, difference of scaled eigenvalues, proportion of environment variation by principal component, and cumulative environment variation by principal components.

Principal Components	Scaled Eigenvalue	Difference	Proportion	Cumulative
1	1.97	0.66	0.49	0.49
2	1.31	0.65	0.33	0.82
3	0.65	0.59	0.16	0.98
4	0.07		0.02	1.00

Table 7: Summary of climate variable means for four mega-environments that were identified during environmental cluster classification across 23 environments.

Clustering Variable	Cluster 1 (n=9)	Cluster 2 (n=8)	Cluster 3 (n=4)	Cluster 4 (n=2)
	Dry	Cold Nights	Hot	Cool/Wet
Precipitation (mm)	164	179	251	322
Daily Minimum Temperature °C	10.6	8.0	11.6	11.8
Daily Maximum Temperature °C	25.0	22.8	26.0	23.7
Days above 28 °C	27	25	44	4

Table 8: Pea grain N concentration and grain yield response among four mega-environment clusters identified during environmental cluster classification across 23 environments.

Mega-Environment	Grain N Conc.	Grain Yield
	g kg^{-1}	kg ha^{-1}
p-value	0.44	<0.0001
Cluster 1 (Dry) (n=9)	39.5	3447 b
Cluster 2 (Cold Nights) (n=8)	39.7	2893 bc
Cluster 3 (Hot) (n=4)	39.8	2052 c
Cluster 4 (Cool/Wet) (n=2)	38.3	6091 a

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 9: Pearson's correlation coefficients with associated p-values for grain N concentration, grain yield, and growing degree-day accumulation rate for four pea growth windows.

Growth Window	BBCH Window	Explanatory Variable	Grain N Conc.	Grain Yield
			g kg^{-1}	kg ha^{-1}
0 – 321	0 – 14	**GDD Accumulation Rate**	-0.14	0.47
		p-value	0.0009	<0.0001
		Precipitation mm	-0.05	0.14
		p-value	0.25	0.0009
321 – 780	14 – 65	**GDD Accumulation Rate**	-0.12	0.04
		p-value	0.0034	0.36
		Precipitation mm	0.27	-0.13
		p-value	<0.0001	0.0014
780 – 1093	65 - 71	**GDD Accumulation Rate**	0.01	-0.31
		p-value	0.80	<0.0001
		Precipitation mm	0.05	0.21
		p-value	0.21	<0.0001
1093 - 1378	71 - 79	**GDD Accumulation Rate**	0.25	-0.45
		p-value	<0.0001	<0.0001
		Precipitation mm	-0.14	0.25
		p-value	0.0010	<0.0001

Note: Significance of Pearson correlation coefficients evaluated at $\alpha = 0.05$.

Table 10: Summary of multiple linear regression analysis within each pea growth window on grain N concentration and grain yield using accumulated precipitation and growing degree-day accumulation rates across 23 environments.

Growth Window	BBCH Window	Source of Variation (S.V)	Grain N Conc.	Grain Yield
			g kg^{-1}	kg ha^{-1}
0 - 321	0 - 14	**Adjusted R-Squared**	0.02	0.29
		Precipitation (mm)	0.25	<0.0001
		GDD Accumulation Rate	0.0002	<0.0001
		PPT x GDD Interaction	NS	NS
321 - 780	14 – 65	**Adjusted R-Squared**	0.08	0.29
		Precipitation (mm)	<0.0001	0.0002
		GDD Accumulation Rate	0.95	0.43
		PPT x GDD Interaction	0.0067	<0.0001
780 – 1093	65 – 71	**Adjusted R-Squared**	-0.002	0.20
		Precipitation (mm)	0.21	<0.0001
		GDD Accumulation Rate	0.89	<0.0001
		PPT x GDD Interaction	0.83	<0.0001
1093 – 1378	71 - 79	**Adjusted R-Squared**	0.07	0.27
		Precipitation (mm)	0.0008	<0.0001
		GDD Accumulation Rate	<0.0001	<0.0001
		PPT x GDD Interaction	NS	<0.0001

Note: Significance of multiple linear regression evaluated at $\alpha = 0.05$

Table 11: Pearson's correlation coefficients with associated p-values for grain N concentration, grain yield, and growing degree-day accumulation rate for six composite pea growth windows.

Growth Window	BBCH Window	Explanatory Variable	Grain N Conc.	Grain Yield
			g kg^{-1}	kg ha^{-1}
0 – 780	0 – 65	**GDD Accumulation Rate**	-0.15	0.31
		p-value	0.0005	<0.0001
		Precipitation mm	0.15	-0.13
		p-value	0.0004	0.0015
321 – 1093	14 – 71	**GDD Accumulation Rate**	-0.10	-0.07
		p-value	0.0213	0.10
		Precipitation mm	0.29	0.01
		p-value	<0.0001	0.87
780 – 1378	65 – 79	**GDD Accumulation Rate**	0.17	-0.50
		p-value	<0.0001	<0.0001
		Precipitation mm	-0.03	0.28
		p-value	0.45	<0.0001
0 – 1093	0 – 71	**GDD Accumulation Rate**	-0.14	0.25
		p-value	0.0013	<0.0001
		Precipitation mm	0.16	0.08
		p-value	0.0001	0.0587
321 – 1378	14 – 79	**GDD Accumulation Rate**	0.01	-0.25
		p-value	0.88	<0.0001
		Precipitation mm	0.25	0.09
		p-value	<0.0001	0.0251
0 – 1378	0 - 79	**GDD Accumulation Rate**	-0.08	0.16
		p-value	0.04	0.0002
		Precipitation mm	0.13	0.14
		p-value	0.0021	0.0008

Note: Significance of Pearson correlation coefficients evaluated at $\alpha = 0.05$.

Table 12: Summary of multiple linear regression analysis within each composite pea growth window on grain N concentration and grain yield using accumulated precipitation and growing degree-day accumulation rates across 23 environments.

Growth Window	BBCH Window	Source of Variation (S.V)	Grain N Conc.	Grain Yield
			g kg^{-1}	kg ha^{-1}
0 - 780	0 - 65	**Adjusted R-Squared**	0.04	0.23
		Precipitation (mm)	0.0004	0.98
		GDD Accumulation Rate	0.0323	<0.0001
		PPT x GDD Interaction	0.0047	<0.0001
321 - 1093	14 - 71	**Adjusted R-Squared**	0.08	0.13
		Precipitation (mm)	<0.0001	0.86
		GDD Accumulation Rate	0.69	0.0714
		PPT x GDD Interaction	NA	<0.0001
780 - 1378	65 - 79	**Adjusted R-Squared**	0.03	0.48
		Precipitation (mm)	0.49	<0.0001
		GDD Accumulation Rate	<0.0001	<0.0001
		PPT x GDD Interaction	NA	<0.0001
0 - 1093	0 - 71	**Adjusted R-Squared**	0.04	0.12
		Precipitation (mm)	0.0001	0.0446
		GDD Accumulation Rate	0.0349	<0.0001
		PPT x GDD Interaction	0.0478	<0.0001
321 - 1378	14 - 79	**Adjusted R-Squared**	0.06	0.11
		Precipitation (mm)	<0.0001	0.0180
		GDD Accumulation Rate	0.80	<0.0001
		PPT x GDD Interaction	NA	<0.0001
0 - 1378	0 - 79	**Adjusted R-Squared**	0.02	0.06
		Precipitation (mm)	0.0020	0.0006
		GDD Accumulation Rate	0.0775	<0.0001
		PPT x GDD Interaction	NA	0.0015

Note: Significance of multiple linear regression evaluated at $\alpha = 0.05$

Table 13: AMMI analysis for the effect of environment, genotype, and their interaction on grain N concentration and grain yield of pea grown across the USA and Canada from 2022 to 2024. PC1, PC2, PC3, and PC4 are the first, second, third, and fourth principal components of the interaction term, respectively.

Source of Variation	Grain N Conc.			Grain Yield		
	%SS	p-value	% G x E explained	%SS	p-value	% G x E explained
Environment	70.5	<0.0001		97.1	<0.0001	
Genotype	10.5	<0.0001		0.5	<0.0001	
G x E	18.9	0.0001		2.4	<0.0001	
PC1		0.0003	41.7		<0.0001	67.7
PC2		0.0071	31.1		<0.0001	18.5
PC3		0.11	20.9		0.0125	8.8
PC4		0.98	6.3		0.29	5.0

Note: AMMI results are evaluated at $\alpha = 0.05$.

Table 14: Response of pea grain yield and grain N concentration among eight locations from the USA and Canada from 2022 to 2024.

Location	Grain Yield	Grain N Concentration
	kg ha^{-1}	g kg^{-1}
p-value	<0.0001	<0.0001
Sutherland, SASK	4131 b	36.5 d
Lucky Lake, SASK	1973 d	39.3 bc
Havre, MT	2316 d	41.0 ab
Bozeman, MT	3473 bc	41.4 a
Williston, ND	2597 cd	38.2 cd
Carrington, ND	3564 bc	40.7 ab
Roseau, MN	6330 a	38.4 cd
Mead, NE	759 e	41.1 ab

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 15: Response of pea grain yield and grain N concentration among five genotypes grown across 23 environments from the USA and Canada from 2022 to 2024.

Genotype	Grain Yield	Grain N Concentration
	kg ha^{-1}	g kg^{-1}
p-value	<0.0001	<0.0001
AAC Profit	3423 a	39.6 b
CDC Amarillo	3339 ab	39.3 b
CDC Dakota	3239 bc	41.0 a
Delta	3084 d	39.3 b
DS Admiral	3140 cd	38.3 c

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 16: Pearson's correlation coefficients with associated p-values for 23 environments and five pea genotypes for grain N concentration, grain yield, and grain N yield.

Variables		Grain N Concentration	Grain Yield	Grain N Yield
Grain N Concentration		1		
	p-value	NA		
Grain Yield		-0.28	1	
	p-value	<0.0001	NA	
Grain N Yield		-0.12	0.98	1
	p-value	0.0045	<0.0001	NA

Note: Significance of Pearson correlation coefficients evaluated at $\alpha = 0.05$.

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CHAPTER FOUR

ENVIRONMENT FOLLOWED BY GENOTYPE DRIVE INTRA-PLANT GRAIN NITROGEN CONCENTRATION VARIABILITY IN FIELD PEA

Contribution of Authors and Co-Authors

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Abstract

Pea (*Pisum sativum* L.) is cultivated across the northern and central Great Plains (GP) and has been established in diverse environments. Breeding programs have released improved pea genotypes for North American pea production; however, older genotypes are still utilized. Additionally, peas develop flowers continuously along the stem, resulting in pods with varying developmental times between the top and bottom pods. These developmental differences represent an opportunity for variable nitrogen (N) remobilization within the plant, variable grain N concentration among pods, and an opportunity for breeding to address intra-plant N variation. We evaluated five pea genotypes across eight sites in the GP over three years to assess intra-plant variability in grain N concentration, denoted as delta (Δ) pod grain N concentration. Additive main effect and multiplicative interaction analysis (AMMI) was utilized to evaluate the control of environment (site-year) and genotype on intra-plant grain N concentration. AMMI analysis revealed that environment explained 40.6% of intra-plant grain N concentration variation across three years. An genotype by environment interaction in 2022 revealed that genotypes expressed site-specific adaptations among environments that controlled Δ pod grain N concentration. AAC Profit expressed the greatest Δ pod grain N concentration, while CDC Dakota had the least across three years. Across 22 environments, bottom pods contained a greater amount of grain N concentration (50%) than top pods (18%). This study details how environment and genetics influence intra-plant grain N concentration in pea across the GP.

1. Introduction

Spring pea (*Pisum sativum* L.) is a cool-season legume grown within the agricultural landscapes of the Great Plains (GP) of North America. Primarily integrated into rotations featuring cereals, such as spring or winter wheat (*Triticum aestivum* L.), and oilseeds, including spring canola (*Brassica napus* L.), pea has gained prominence due to the extensive ecosystem services it facilitates (Padbury et al., 2002). Pea requires minimal to no nitrogen (N) fertilization to achieve optimal grain yield, a direct consequence of biological atmospheric N_2 fixation. The broader ecological value of the spring pea is derived from these direct N inputs and the subsequent indirect benefits associated with the release of N and C from crop residues post-harvest (Beckie & Brandt, 1997; Gan et al., 2003; Stevenson & Kessel, 1996). Pea positively contributes to increasing partial net returns and decreasing farm-scale economic variability, in addition to benefiting cereal-based systems ecologically (Koeshall et al., 2022; Miller et al., 2015). The strategic importance of this legume is reflected in its widespread adoption; as of 2024, total planted areas reached approximately 360,000 ha in the United States and 1,424,000 ha in Canada (NASS, 2025; StatsCan, 2025).

Cultivation of pea spans diverse ecoregions and climatic gradients within the GP, exposing the crop to a broad range of environmental conditions (Padbury et al., 2002). Consequently, extensive research has sought to elucidate the extent to which environmental variables influence the physiological development of grain protein concentration and overall yield (Atta et al., 2004; Bestwick et al., 2018; Franck et al., 2023; Mohammed et al., 2018; Stoddard et al., 1993; Tao et al., 2017). Existing literature identifies the environment as a

primary determinant and controlling factor of both protein content and yield potential in peas (Mohammed et al., 2018; Tao et al., 2017).

Empirical evidence from controlled environments suggests that temperature regimes significantly modulate resource allocation; for instance, pea plants grown under a 15/10 °C day/night cycle, as opposed to 25/20 °C, optimized C availability through enhanced carbon fixation, leading to increased vegetative biomass and a corresponding elevation in N demand (Larmure et al., 2005). While elevated temperatures during the seed-filling stage have been shown to increase grain N concentrations, increased temperature also reduces yield (Larmure et al., 2005). This sensitivity to heat stress is further evidenced by findings that grain yield and the duration of the reproductive cycle are significantly truncated when the crop is subjected to more than 20 days of maximum daily temperatures exceeding 28 °C (Bueckert et al., 2015; Huang et al., 2023).

Genetic variation (e.g. cultivar, variety, and genotype) significantly influences both grain protein concentration and yield in pea (Franck et al., 2023). Genetic factors can exert a more substantial influence on grain protein concentration than environmental conditions (Tao et al., 2017; Mohammed et al.; 2018), representing a departure from divergent conclusions reached in other studies conducted within the same North American region Furthermore, Franck et al. (2023) evaluated seven pea cultivars across seven locations in Montana over a two-year period, concluding that genetics maintains consistent control over grain protein concentration. This consistency was underscored by the absence of a significant cultivar-by-location interaction, suggesting that genotypic performance for protein remains relatively stable across varying environmental contexts.

Environments across the Great Plains frequently encounter heat and water stress, both of which fundamentally alter C and N dynamics in pea. Heat stress disrupts photosynthate production and induces reproductive abortion, reducing N-sink capacity while restricting essential C and N assimilatory enzymes (Guilioni et al., 1997; Yang et al., 2018; Zhao et al., 2008). Although reduced sink capacity may increase grain N concentrations if the N source remains stable, heat also limits starch synthesis and overall biomass, potentially decreasing total N demand (Singletary et al., 1994; Unkovich et al., 2010). Concurrently, water stress impairs N_2 fixation efficiency by compromising rhizobia health and restricting phloem transport of water and nutrients in drying soils (Serraj et al., 1999).

Grain protein concentration varies in relation to the raceme position of individual pods within a single pea plant (Atta et al., 2004; Bestwick et al., 2016, 2018; Guilioni et al., 1997). Continual development of flowering along the main stems, with bottom pods being formed first, results in lower pods having a prolonged ability to receive endogenous and exogenous N via remobilization within the plant and biologically fixed N_2 compared to pods located towards the top of the plant, possibly leading to a greater N concentration in lower pods (Salon et al., 2001; Schiltz et al., 2005). However, due to lower pods having a longer development period than upper pods, lower pods also can receive more photosynthate products before seed maturity, resulting in a higher C concentration, thus a reduced grain N concentration (Bestwick et al., 2016, 2018; Hörtensteiner & Feller, 2002; Lecoeur & Sinclair, 2001; Lhuillier-Soundélé et al., 1999; Salon et al., 2001; Schiltz et al., 2005). Cultivar variation, water stress, heat stress, and other factors can influence N partitioning and accumulation among pods within a single plant. While previous research has documented how cultivar differences and environmental stressors affect whole-plant

and field-scale grain protein formation, there is limited research on how varying and contrasting growing environments and genetics influence intra-plant variation in grain protein concentration.

Due to a lack of literature on intra-plant grain N concentration dynamics, combined with a range of precipitation and temperature gradients across the common pea-growing regions in North America, there is a need to understand better the environmental and genetic factors that affect intra-plant grain N concentration variability in pea. The objectives of this study were to 1) quantify the influence environment and genetics have on explaining intra-plant grain N concentration, and 2) investigate if bottom or top pod structures have a greater probability of containing greater N concentration.

2. Materials and Methods

2.1 Site Description

Field trials were conducted at eight locations across the northern and central GP of North America (Table 1) in 2022, 2023, and 2024, a region that accounts for most of North America's pea production. Soils ranged from course to fine-textured, with dominantly udic and ustic moisture regimes, and generally had thick, organic-rich surface horizons that qualified them as Mollisols in the US Taxonomy (Table 1). Harvest dates ranged from mid-July to early September, growing periods ranged from 83 to 113 days, and growing degree-days (GDD) accumulation rates ranged from 13.4 to 19.5 per day (Table 2). Growing degree days, commonly referred to as heat units, are the cumulative heat units experienced by the plant from the day of seeding to the day of harvest. GDD was calculated using the following equation (McMaster & Wilhelm, 1997):

$$GDD = \sum_{1}^{n} \frac{T_{max} + T_{min}}{2} - T_{base}$$

where T_{max} is the maximum daily temperature, T_{min} in the minimum daily temperature, and the T_{base} temperature for pea is 0 °C. April to August cumulative precipitation ranged from 139 mm to a high of 618 mm and mean temperature ranged from 13.5 to 19.4 C (Tables 3 – 4).

2.2 Experimental Design

All experiments were conducted in a randomized complete block design with five replications and five cultivar entries during the three-year study. All experimental locations received the same five yellow pea genotypes (AAC Profit, CDC Amarillo, CDC Dakota, Delta, and DS Admiral) each year, as shown in Table 5, which were chosen based on protein and yield potential, growth habit, and breeding background (Andersen et al., 2011; Bing et al., 2022; Limagrain, 2025; Warkentin, 2010; Warkentin et al., 2014). Further, all five pea cultivars were grown from a common seed source, which was increased at Bozeman, MT, during the 2021 growing season to avoid variation among seed lots. All pea seed was treated with *CruiserMaxx Vibrance* for pulse crops at a rate of 3.26 mL kg^{-1} seed. All cultivars were seeded at a target pure live stand (PLS) density of 80 PLS m^{-2}. *Exceed Granulated Peat for Pea, Vetch, and Lentil* (*Rhizobium leguminosarum biovar viceae* 1×10^{8} colony forming units [CFU] g^{-1}) inoculant was applied at all experimental locations at a rate of 5.7 kg ha^{-1} in-furrow. Monoammonium phosphate (11-52-0-0) and potassium sulfate (0-0-50-18) were blended at equal ratios, resulting in an NPKS starter fertilizer blend (5.5-26-26-9) applied at a rate of 100 kg ha^{-1} in-furrow at the time of seeding. Plot dimensions at the experimental locations ranged from 1.28 to 2.0 m in width and were 6.1 m long. Row spacing ranged from 22.8 to 30.5 cm among experimental

locations. Experimental locations targeted recommended seeding dates based on their respective climate and soil conditions (Table 2). At each experimental location, pesticides were applied as needed to control weeds, insects, and pathogens, in accordance with best management practices for that site. There were no notable pest issues at these research locations during this study. Before the mechanical harvest of each experimental unit with small-plot combine harvesters, 10 pea plants were randomly selected within each experimental unit to obtain individual pod samples from the top and bottom racemes. Then, the bottom and top pods on each selected plant were removed and saved separately for individual analysis of grain N concentration. A pod was considered viable and selected if it contained at least two seeds. Pod samples were dried in an oven at 55 °C for 72 hr. Within a single site-year experimental unit, the 10 top pods were shelled and combined into a single grain sample to represent the grain N concentration for all top pods in that unit. The same process was applied to the bottom pods. This process yielded 25 top-pod and 25 bottom-pod grain N-concentration observations across five cultivars at each site-year, for a total of 1200 top- and bottom-pod grain samples. Each pod grain sample was ground in a *Udy* high-speed centrifugal mill (Udy Cyclone Sample Mill, model 3010-014) to a particle size of < 2-mm. All milled grain samples were analyzed for N concentration using a *Leco Truspec CNS Combustion Analyzer* (Leco Corp., St. Joseph, MI). Although it is common to report grain protein concentration with a N to protein conversion factor of 6.25, we report grain N concentration (g kg^{-1}) due to the inaccuracy of this conversion, which does not account for the specific amino acid composition of grain legumes. See Mariotti et al. (2008) for proper conversion values for obtaining grain protein concentration values from grain N concentration observations reported in this article.

2.3 Statistical Analysis

To accurately evaluate environmental and genetic influences on pod grain N concentration, "location" will be used to refer to a geographical location, and "environment" to an individual site-year in this study. Significance of modeling results was assessed at the level of $p \leq 0.05$, unless otherwise noted. Grain N concentration data were analyzed using the additive main effects and multiplicative interactions (AMMI) methodology, as detailed in the protocol by Gauch (2013). The AMMI analysis was performed using the *agricolae* R package (de Mendiburu, 2023) in the R statistical software (R Core Team, 2025) and considering the following equation:

$$Y_{ij} = u + g_i + e_j + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij} + \epsilon_{ij}$$

where Y_{ij} is the mean of ith cultivar in the environment j; u is the overall mean; G is the effect of cultivar i; and ϵ is the effect of the environment j. The GE interaction is explained by the multiplicative term of the equation where λ is the singular value of the principal component (PC) of $k, \varepsilon,$ and n are scores of cultivar i and environment j, respectively, for the principal component k, n ranges from 0 to the number of PC required to describe the AMMI model appropriately. The sums of squares (SS) for the interaction of cultivar × environment and the interaction of cultivar × environment noise were calculated. There was a meaningful amount of SS for environment, genotype, and the environment-by-genotype interaction in the model for Δ delta pod N concentration ($p \leq 0.05$).

Analysis and exploration of intra-plant pod grain N concentrations were calculated by obtaining a delta (Δ) pod grain N concentration value from the mean grain N concentration

values for the top and bottom pod concatenated samples from each experimental unit at each site-year, considering the following equation:

$$\Delta\, pod\ grain\ N\ concentration$$
$$= bottom\ pod\ grain\ N\ concentration - top\ pod\ grain\ N\ concentration$$

Pod raceme position grain N concentration data encompasses individual top and bottom pod samples from 22 environments, resulting in 544 top and bottom pod sample pairs to inform this analysis on Δ pod N concentration, how the environment and genotypes influence intra-plant variation, and in what situations bottom or top pods contain a greater N concentration. The Carrington 2023 and Mead 2024 site-years were excluded from this pod N concentration analysis because pod samples were not collected at Carrington 2023, and Mead 2024 had symptoms of waterlogged root systems during the reproductive period.

3. Results

3.1 AMMI analysis across 22 environments and five genotypes on Δ pod N concentration

The main effect of environment and genotypes influence the intra-plant grain N concentration variation found between pods located at the top and bottom raceme (Table 6, Figure 1). The contributions of genotype ($p = 0.0031$) and the environment ($p = < 0.0001$) were the most meaningful sources of variation within the AMMI analysis, accounting for 8.7% and 40.6% of the total variation across all Δ pod N concentration (bottom grain N concentration – top grain N concentration) observations, respectively. The environment by genotype interaction explained 50.7% of the delta (Δ) pod N concentration; however, it was not meaningful ($p = 0.23$) (Table 6).

Across all environments and genotype Δ pod N concentration observations, the mean Δ pod grain N concentration was found to be +0.5 g kg^{-1} (i.e. 3.1 g kg^{-1} protein assuming 6.25 protein conversion factor) indicating that grain N concentrations were slightly greater in bottom than in top pods. The Δ pod grain N concentrations for environment encompassed a wider range than for genotypes based on the AMMI biplot (Figure 1). Genotype Δ pod N concentrations ranged from +1.36 g kg^{-1} for AAC Profit (G1) to +0.22 g kg^{-1} for CDC Dakota (G3). Environment Δ pod N concentrations ranged from +2.64 g kg^{-1} at Bozeman 2024 to -1.27 g kg^{-1} at Havre 2023, a spread of 3.92 g kg^{-1} (Figure 1). Bozeman 2024, Sutherland 2024, and Bozeman 2023 were the top three environments that fostered a mean positive Δ pod N concentration across genotypes, indicating that bottom pods accumulated a greater N concentration compared to top pods (Figure 1). Havre 2023, Roseau 2024, and Havre 2024 were the top three environments that fostered the most negative mean Δ pod N concentrations, indicating that top pods contained a greater grain N concentration than bottom pods (Figure 1).

Across environments, CDC Dakota (G3), followed by DS Admiral (G5), were the genotypes plotted closest to the mean Δ pod N concentration of 0, resulting in nearly homogeneous grain N concentration across raceme positions (Figure 1) (Andersen et al., 2011; Warkentin, 2010). AAC Profit (G1) exhibited the greatest variation in Δ pod N concentrations across environments, with a mean Δ pod N concentration of +1.36 g kg^{-1}, indicating that among all environments, pods developed on a lower raceme position within the canopy are partitioned a larger proportion of endogenous and exogenous N during the reproductive cycle compared to top pod counterparts (Figure 1) (Bing et al., 2022).

Across all genotypes, 11 of 22 environments had an estimated mean Δ pod N concentration that indicated bottom pods contained a greater concentration of N compared to top pods (Figure 2). Seven of 22 environments had an estimated mean Δ pod N concentration that indicated there was not a meaningful difference in grain N concentration between top and bottom pods at those environments (Figure 2). Four of 22 environments displayed an estimated mean Δ pod N concentration that indicated top pods contained a greater level of grain N concentration compared to bottom pods (Figure 2). Overall, 50% of environments expressed a Δ pod N concentration that suggested bottom pods contained more N, while 18% of environments contained more N in top pods. In order to reduce the effect of contrasting climate years on the possible effect that environment has on the AMMI analysis, AMMI analysis was conducted on each year to understand environment and genotype control over intra-plant grain N concentrations.

3.2 Environment and genotype influence on Δ pod N concentration in 2022

The AMMI analysis in 2022 indicated that the environment-by-genotype interaction explained 69.4% of the Δ pod N concentration ($p = 0.0301$) (Table 7). The main effects of environment and genotype were not useful in explaining Δ pod N concentration variability (Table 7). Among the eight environments in 2022, AAC Profit (G1) and Delta (G4) expressed the greatest Δ pod N concentration variability among genotypes. AAC Profit was associated with Sutherland and Carrington, Delta (G4) was associated with Roseau, CDC Dakota (G2) was associated with Mead, Havre, and Williston, while CDC Amarillo (G3) and DS Admiral (G5) were associated with Bozeman and Lucky Lake (Figure 3). In 2022, in the Roseau environment,

identified as one of the higher-grain-yield environments, Delta (G4) was the only genotype to produce a top-pod grain N concentration below 33.6 g kg^{-1}, with an estimated top-pod grain N concentration of 33.5 g kg^{-1} (data not shown). No bottom-pod grain N concentration observations below 33.6 g kg^{-1} were recorded in 2022 (data not shown).

3.3 Environment and genotype influence on Δ pod N concentration in 2023

Environment explained 38.3% of Δ pod N concentration ($p = 0.0012$) in 2023 (Table 7 and Figure 4). Genotype explained 15.8% and the environment by genotype interaction explained 45.9% of the Δ pod N concentration, however, were not significant (Table 7). Delta (G4) and AAC Profit (G1) expressed the greatest Δ pod N concentration variability, while DS Admiral (G5) expressed the lowest Δ pod N concentration variability in 2023 (Figure 4) (Andersen et al., 2011; Bing et al., 2022; Limagrain, 2025). Delta (G4) and AAC Profit (G1) were associated with Sutherland, while CDC Dakota (G3) was associated with Havre and Mead (Figure 4). Two genotypes grown at Sutherland in 2023 had bottom-pod grain N concentrations below 33.6 g kg^{-1}: CDC Amarillo (G2) and DS Admiral (G5), at 33.2 g kg^{-1} and 32.1 g kg^{-1}, respectively (data not shown). Additionally, in 2023, Sutherland was the only environment that exhibited top-pod grain N concentrations below 33.6 g kg^{-1} (data not shown). In contrast, all genotypes except CDC Dakota (G3) expressed top- and bottom-pod grain N concentrations above 35.3 g kg^{-1}, qualifying for a protein premium (data not shown) (Warkentin, 2010).

3.4 Environment and genotype influence on Δ pod N concentration in 2024

The environment largely explained observed Δ pod N concentration variability in 2024 compared to genotype and the environment by genotype interaction (Table 7). Environment

explained 51.8% ($p = <0.0001$) of Δ pod N concentration variation, while genotype and the environment by genotype interaction explained only 8.8 ($p = 0.20$) and 39.4% ($p = 0.49$) (Table 7). AAC Profit (G1) and CDC Amarillo (G2) expressed the greatest Δ pod N concentration variability (Figure 5) (Bing et al., 2022; Warkentin et al., 2014). AAC Profit (G1) was associated with Sutherland, while CDC Amarillo (G2) was associated with Lucky Lake (Figure 5) (Bing et al., 2022; Warkentin et al., 2014). There were no bottom- or top-pod grain N concentration observations in 2024 were below 33.6 g kg^{-1}. However, in 2024 at Williston, DS Admiral (G5) had an estimated bottom-pod grain N concentration below 35.2 g kg^{-1} (Andersen et al., 2011).

4. Discussion

The AMMI results suggest that variation in intra-plant grain N concentration is primarily controlled by environmental factors and, to a lesser degree, by genotype (Table 6, Figure 1). In two of three years, environment explained the majority Δ pod N concentration variation, 38.3% in 2023 and 51.8% in 2024, respectively (Table 7). This finding suggests that conditions and characteristics that are unique to an environment, such as precipitation, heat accumulation, soil fertility, or site-specific management factors, such as seeding date, influence intra-plant grain N concentration more than genotype or the interplay between environment and genotype. In 2022, the overwhelming significance of the genotype by environment interaction from AMMI results suggests that environmental conditions paired with our panel of genotypes resulted in a high degree of site-specific adaptability among genotypes regarding Δ pod N concentration variability (Table 7).

The majority of environments observed in this study indicate that across a diverse set of genotypes, the bottom pods contain a greater concentration of N, suggesting that earlier

developed pods during the reproductive cycle tend to have an N accumulation advantage compared to top pods, even though bottom pods also have a greater time period to accumulate C to create starch products and hence 'dilute' grain N. Environments with either low growing-season moisture or more than 20 growing-season days with daily temperatures over 28 °C may be experiencing several drought or heat-stress consequences, resulting in observed intra-plant grain N variation. These consequences to pod grain N concentration dynamics from lack of moisture or elevated heat stress may be either 1) prioritization of N remobilization to older pod structures, 2) lack sufficient moisture to develop starch within pods, thereby increasing Δ pod grain N concentration, or 3) experience soil moisture conditions that reduce N fixation or pre-maturely halt N fixation, limiting N accumulation within later maturing pods. In specific environments (e.g., Sutherland and Roseau) that would be conducive to high-yield potential based on precipitation and heat accumulation patterns, older genotypes occasionally produced top- or bottom-pod grain N concentrations below key thresholds of 33.6 g kg^{-1} and 35.3 g kg^{-1}, posing a potential marketability issue for pea growers in those regions who select high yield genotypes. A grain N concentration below 33.6 g kg^{-1} would result in possible dockage at the time of sale or rejection of the pea grain, as many pea markets desire to only purchase pea for the protein market with a grain N concentration above 33.6 g kg^{-1}. Most pea protein markets across North America do not begin to offer premium pricing until pea grain N concentration is above 35.2 g kg^{-1}. Intra-plant grain N concentration observations suggest that locations with a high-yield potential should select genotypes that balance grain N accumulation with grain yield to reduce possible marketability issues. Overall, environmental characteristics exerted the strongest control on Δ pod N concentrations.

5. Conclusion

Across all years, the environment explained most of the observed variation in Δ pod N concentration between bottom and top pods. Intra-plant grain N concentration varied among environments and genotypes, with the majority of intra-plant grain N concentration variation explained by environment in two of three years. In one of three years, the interaction between environment and genotype explained most of the variation in Δ pod N concentration, suggesting unique conditions that resulted in a high degree of site-specific adaptation. Across 22 environments, bottom pods had a greater frequency of containing a greater grain N concentration than top pods. This study provides insight into intra-plant variation in grain N concentration across environments and genotypes, identifies which raceme locations are more likely to produce higher grain N concentrations, and quantifies the extent to which environments and genotypes control this variation.

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Figures

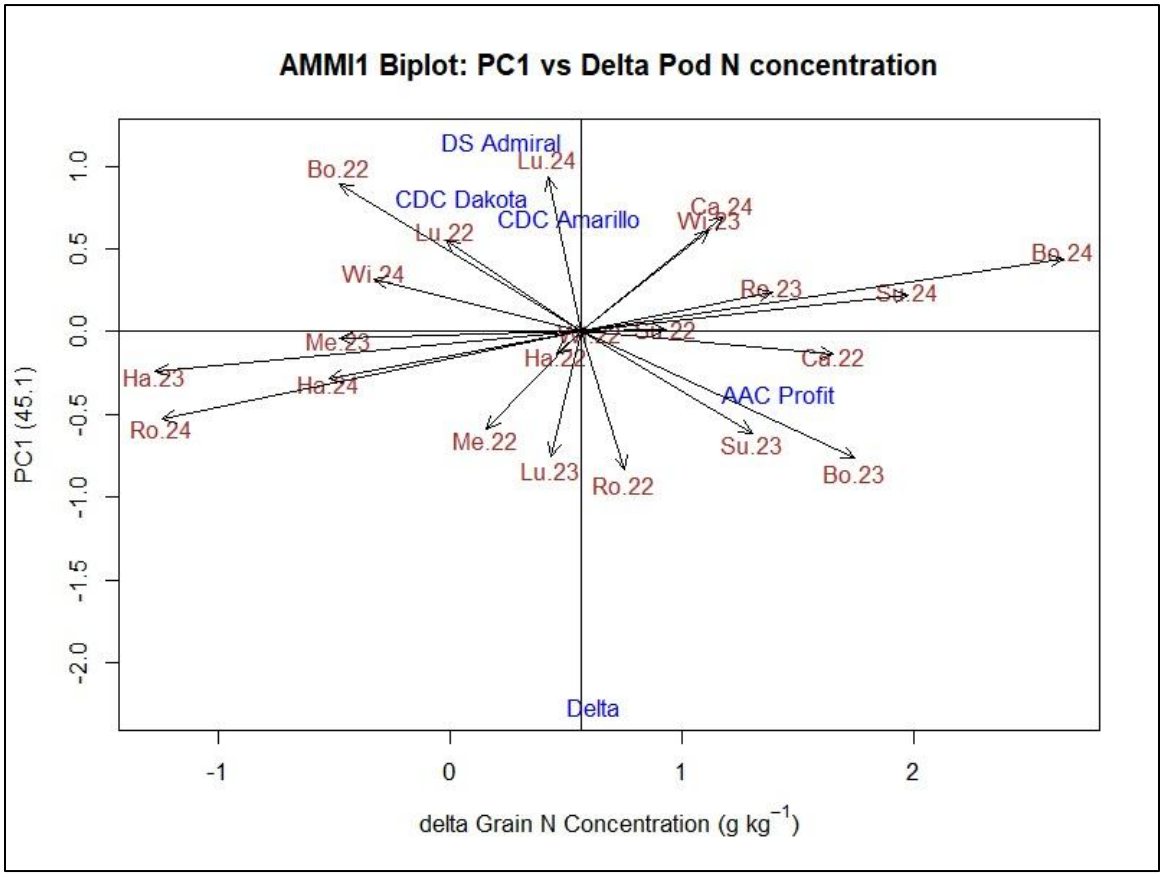

Figure 1: Additive main effect and multiplicative interaction (AMMI) biplot showing Δ pod N concentration vs PC1 of 23 environments (red color) and five genotypes (blue color).

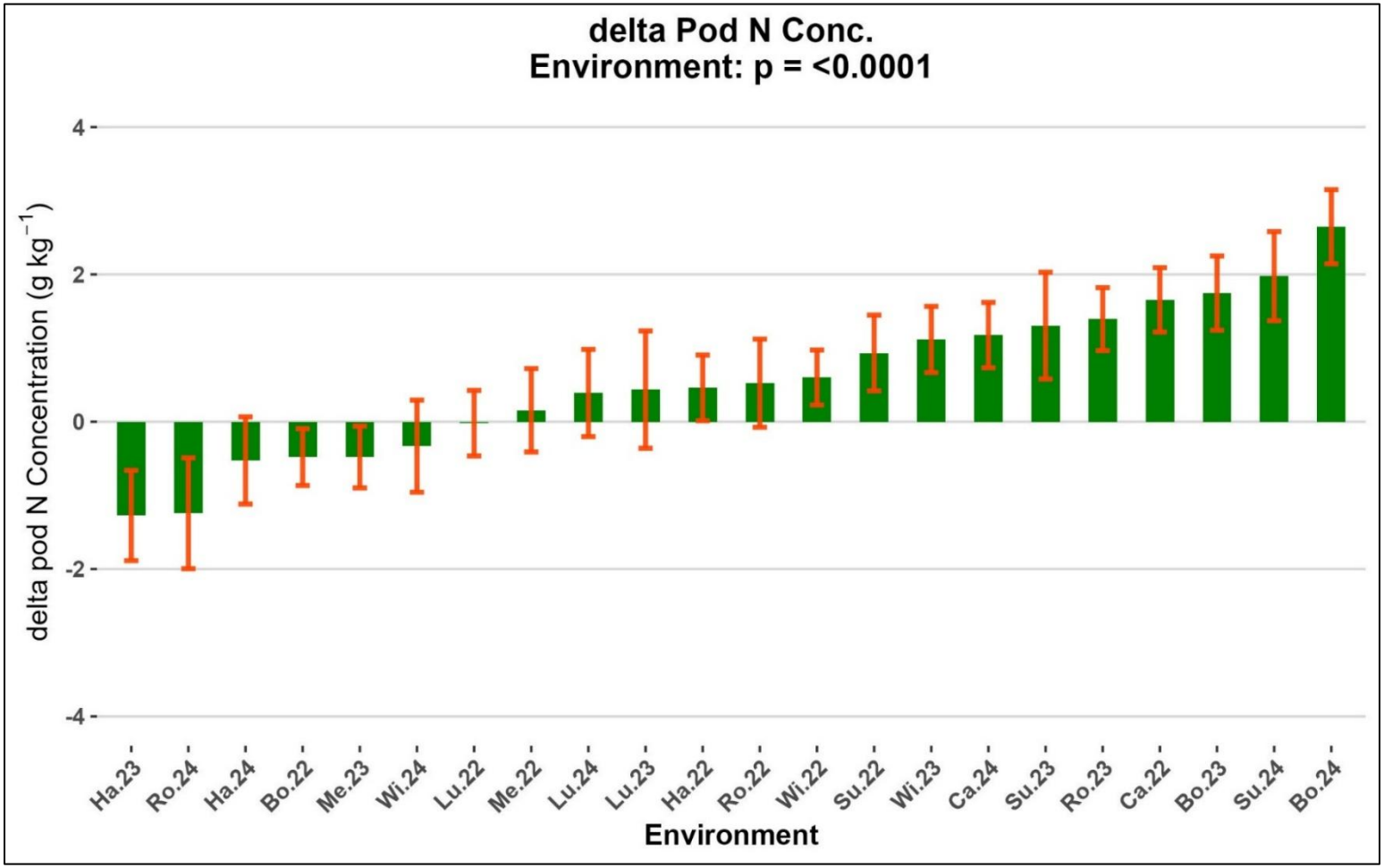

Figure 2: Delta Δ pod N concentration for 22 environments with error bars indicating the standard error of the individual environment. A positive value indicates that bottom pods had a greater grain N concentration than top pods.

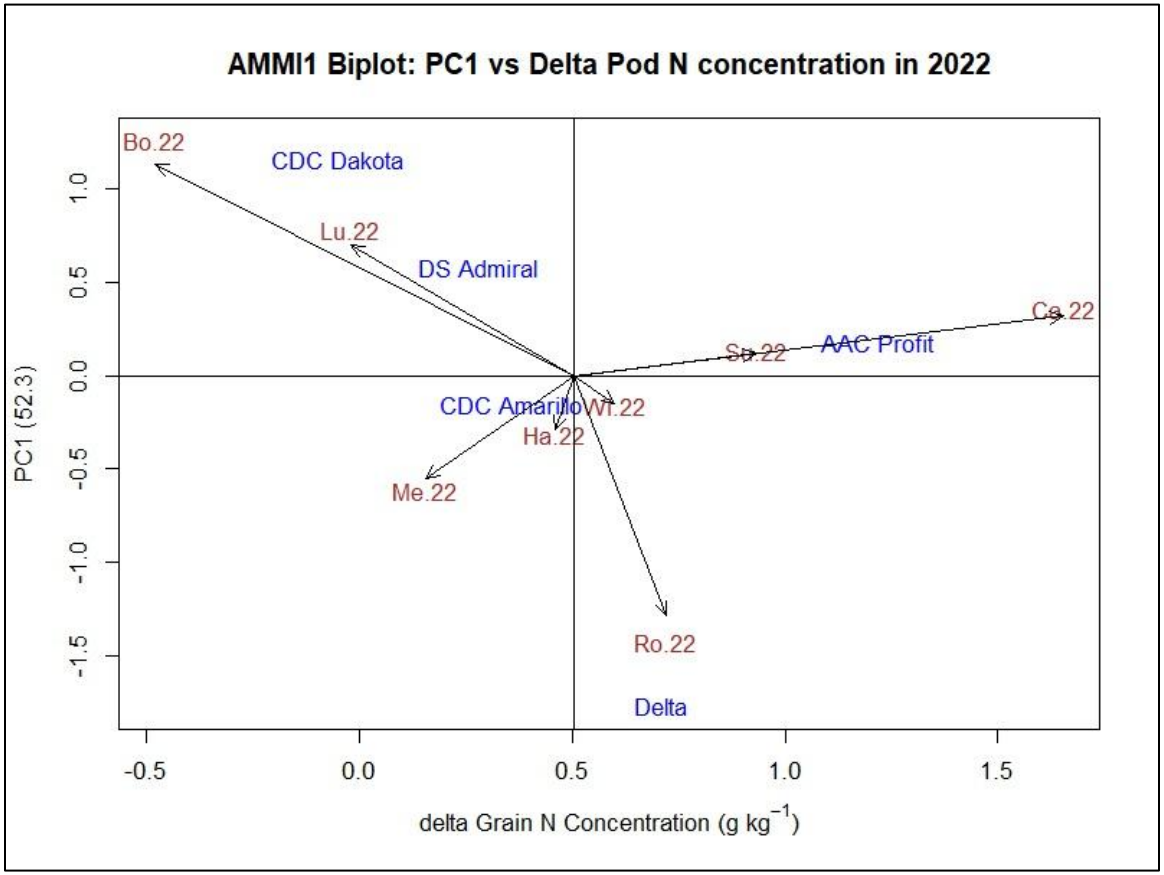

Figure 3: Additive main effect and multiplicative interaction (AMMI) biplot showing Δ pod N concentration vs PC1 of eight environments (red color) and five genotypes (blue color) in 2022.

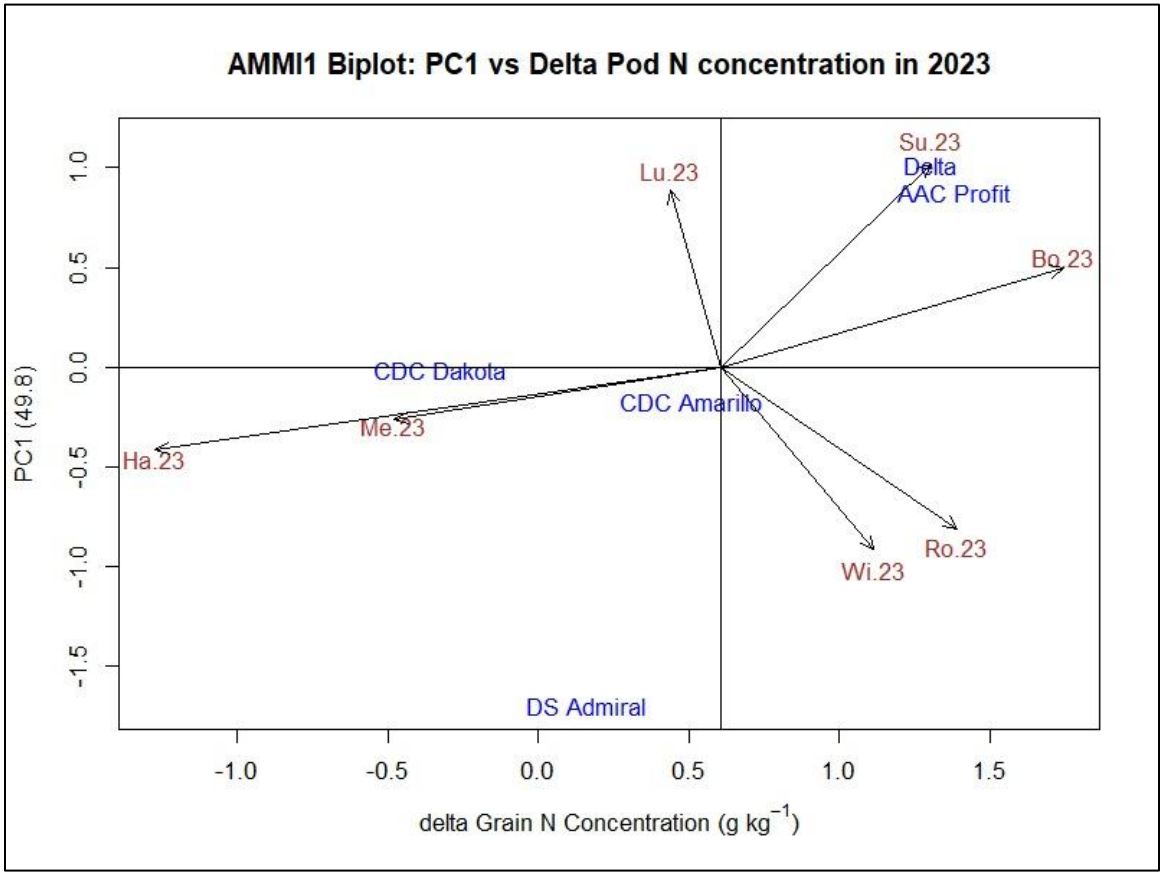

Figure 4: Additive main effect and multiplicative interaction (AMMI) biplot showing Δ pod N concentration vs PC1 of seven environments (red color) and five genotypes (blue color) in 2023.

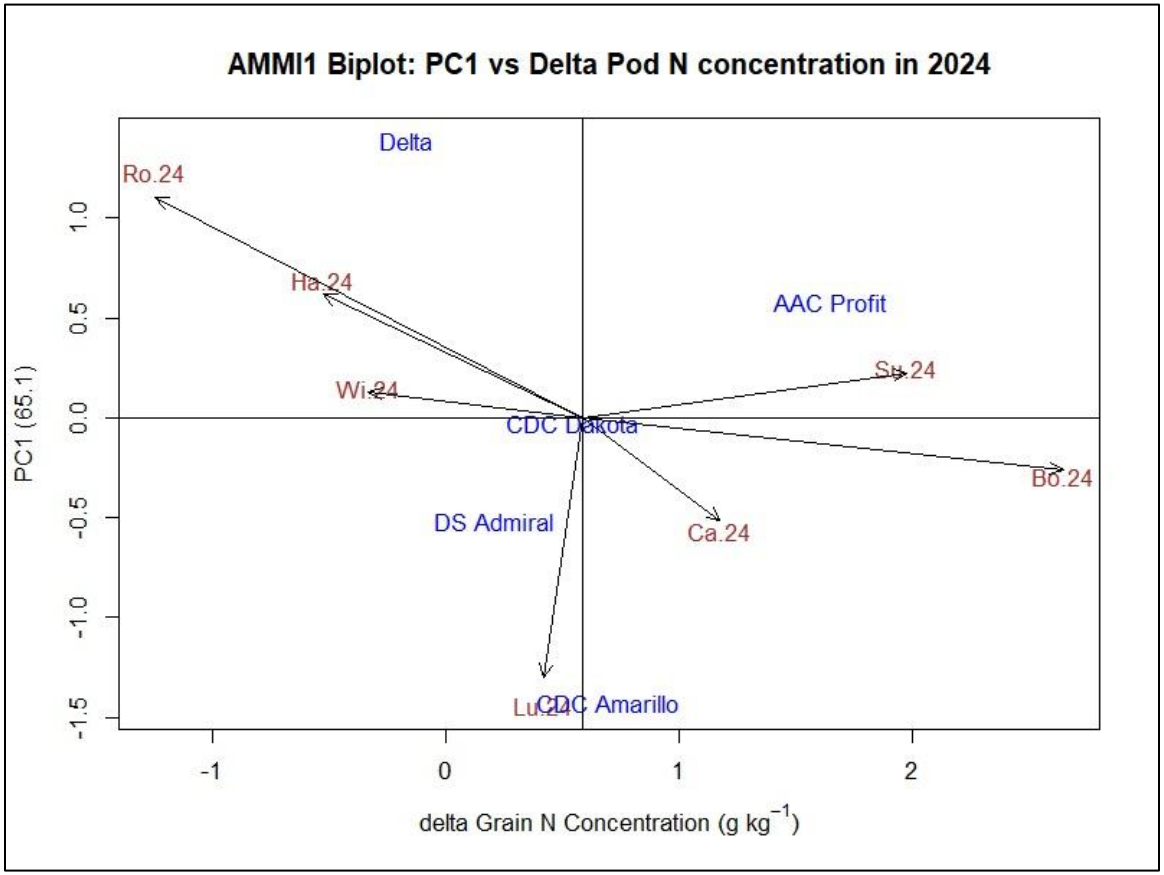

Figure 5: Additive main effect and multiplicative interaction (AMMI) biplot showing Δ pod N concentration vs PC1 of seven environments (red color) and five genotypes (blue color) in 2024.

Tables

Table 1: Summary of study locations from 2022 to 2024 across the USA and Canada with location coordinates, location altitude, and location soil type information.

Location	Geographic Coordinate lat/long	Altitude m	Soil Type*
Sutherland, SK**	52.166, -106.504	482	Aridic Argiustoll
Lucky Lake, SK**	51.103, -107.241	555	Aridic Argiustoll
Havre, MT	48.494, -109.802	820	Fine-loamy, mixed, superactive, frigid Aridic Argiustolls
Bozeman, MT	45.678, -111.148	1455	Typic Haplustolls, Typic Calciustolls (frigid)
Carrington, ND	47.505, -99.121	476	Calcic Hapludolls, Pachic Hapludolls
Williston, ND	48.134, -103.740	642	Typic Argiustolls, Pachic Argiustolls
Roseau, MN	48.853, -95.800	319	Typic Endoaquolls, glaciolacustrine
Mead, NE	41.183, -96.467	361	Mollic Hapludalfs, terraces

*Soil information for USA locations obtained via University of California - Davis SoilWeb

**Canada soil information obtained from Saskatchewan Soil Information System

Table 2: Summary of seeding date, harvest date, growing period, accumulated growing degree days from seeding to harvest, and growing degree day accumulation rate for each study location from 2022 to 2024 across the USA and Canada.

Location	Year	Seeding Date	Harvest Date	Growing Period	Accumulated GDD	GDD Accumulation Rate
				days		GDD day^{-1}
Sutherland, SK	2022	9 May	16 August	99	1630	16.5
	2023	4 May	15 August	103	1830	17.8
	2024	11 May	21 August	102	1620	15.9
Lucky Lake, SK	2022	5 May	10 August	97	1580	16.3
	2023	9 May	14 August	97	1780	18.4
	2024	15 May	21 August	98	1520	15.5
Havre, MT	2022	21 April	27 July	97	1470	15.2
	2023	26 April	25 July	90	1530	17.0
	2024	15 April	29 July	105	1560	14.9
Bozeman, MT	2022	20 April	8 August	110	1620	14.7
	2023	28 April	14 August	108	1720	15.9
	2024	11 April	2 August	113	1550	13.7
Carrington, ND	2022	16 May	9 August	85	1580	18.6
	2023	5 May	9 August	96	1840	19.2
	2024	13 May	13 August	92	1630	17.7
Williston, ND	2022	4 May	4 August	92	1760	19.1
	2023	26 April	18 July	83	1530	18.4
	2024	26 April	2 August	98	1740	17.8
Roseau, MN	2022	28 May	7 September	102	1880	18.4
	2023	16 May	28 August	104	1910	18.4
	2024	13 May	28 August	107	1850	17.3
Mead, NE	2022	1 April	21 July	111	1940	17.5
	2023	25 April	19 July	85	1690	19.9
	2024	12 April	31 July	110	2160	19.6

Note: Growing degree-day data for each location were obtained via the High Plains Climate Center ACIS-CLIMOD and Climate Canada data services.

Table 3: Summary of monthly cumulative precipitation, cumulative precipitation from April 01 to August 31, and the 30-year mean precipitation accumulation from April 01 to August 31 for each study location in the USA and Canada.

Location	Year	Apr	May	June	July	Aug	Apr - Aug	30-yr Apr - Aug Average
		precipitation (mm)						
Sutherland, SK	2022	3	26	38	47	26	139	242
	2023	8	52	44	20	50	174	
	2024	12	46	111	27	45	241	
Lucky Lake, SK	2022	14	20	49	71	10	163	238
	2023	17	25	30	12	59	141	
	2024	24	91	61	23	6	204	
Havre, MT	2022	4	10	81	44	23	163	198
	2023	14	74	57	16	25	186	
	2024	22	110	63	18	37	250	
Bozeman, MT	2022	41	110	60	14	15	240	253
	2023	19	19	129	25	47	239	
	2024	36	89	41	19	26	211	
Carrington, ND	2022	61	162	80	38	31	372	309
	2023	43	99	92	58	41	333	
	2024	60	128	145	89	122	545	
Williston, ND	2022	72	166	50	52	13	354	250
	2023	20	75	77	48	71	292	
	2024	15	77	82	44	14	232	
Roseau, MN	2022	88	134	53	90	149	513	343
	2023	16	46	35	67	38	201	
	2024	37	91	122	116	63	430	
Mead, NE	2022	30	110	77	26	74	316	493
	2023	38	6	130	199	62	436	
	2024	67	186	71	166	129	618	

Note: 30-year April to August precipitation averages at each location are representative of 1991 to 2020, and precipitation data were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada online data services.

Table 4: Summary of monthly mean temperature during the growing season and mean growing season temperature from April 01 to August 31 for each study location in the USA and Canada from 2022 to 2024.

Location	Year	Apr	May	June	July	Aug	Apr - Aug
		Temperature °C					
Sutherland, SK	2022	1.8	11.0	15.7	19.3	19.6	13.5
	2023	1.4	15.5	19.2	17.7	18.1	14.4
	2024	6.5	11.2	13.7	19.9	18.3	13.9
Lucky Lake, SK	2022	1.9	11.2	16.0	19.6	20.7	13.9
	2023	0.9	15.0	18.7	18.7	18.8	14.4
	2024	6.8	10.7	14.2	20.3	18.6	14.1
Havre, MT	2022	2.9	11.4	16.0	21.7	22.8	15.0
	2023	4.7	14.9	17.3	21.1	21.1	15.8
	2024	7.3	11.0	15.3	21.8	20.1	15.1
Bozeman, MT	2022	2.9	9.1	15.4	20.6	21.7	13.9
	2023	4.2	13.3	14.4	19.7	19.4	14.2
	2024	7.4	9.5	16.2	20.4	18.8	14.5
Carrington, ND	2022	-0.2	11.5	18.4	20.9	20.0	14.1
	2023	-0.9	15.3	21.0	19.2	19.3	14.8
	2024	5.6	11.8	17.3	21.3	18.8	14.9
Williston, ND	2022	1.6	12.8	18.9	23.3	23.8	16.1
	2023	5.2	16.0	21.1	21.8	21.8	17.2
	2024	9.7	13.4	17.2	23.3	21.4	17.0
Roseau, MN	2022	1.0	12.6	18.9	21.2	19.7	14.7
	2023	0.7	16.7	21.7	19.6	19.7	15.7
	2024	6.5	13.0	17.7	21.6	20.3	15.8
Mead, NE	2022	8.2	16.0	22.4	24.8	23.6	19.0
	2023	10.2	17.8	22.6	22.8	23.1	19.3
	2024	11.2	16.8	22.7	23.2	23.1	19.4

Note: 30-year April to August temperature means at each location are representative of 1991 to 2020, and temperature data were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada online data services.

Table 5: Summary of study locations by year across the USA and Canada, environment code, pea genotypes used across all environments, and genotype codes used in AMMI and BLUP analysis.

Location	Year	ENV Code	Cultivar	Genetic Code (GEN)
Sutherland, SK	2022	Su.22	AAC Profit	G1
	2023	Su.23	CDC Amarillo	G2
	2024	Su.24	CDC Dakota	G3
Lucky Lake, SK	2022	Lu.22	Delta	G4
	2023	Lu.23	DS Admiral	G5
	2024	Lu.24		
Havre, MT	2022	Ha.22		
	2023	Ha.23		
	2024	Ha.24		
Bozeman, MT	2022	Bo.22		
	2023	Bo.23		
	2024	Bo.24		
Carrington, ND	2022	Ca.22		
	2023	Ca.23		
	2024	Ca.24		
Williston, ND	2022	Wi.22		
	2023	Wi.23		
	2024	Wi.24		
Roseau, MN	2022	Ro.22		
	2023	Ro.23		
	2024	Ro.24		
Mead, NE	2022	Me.22		
	2023	Me.23		
	2024	Me.24		

Table 6: AMMI analysis for the effect of environment, genotype, and their interaction on Δ pod N concentration of pea grown across the USA and Canada from 2022 to 2024. PC1, PC2, PC3, and PC4 are the first, second, third, and fourth principal components of the interaction term, respectively.

Source of Variation	delta Δ pod N	
	%SS	p-value
Environment	40.6	<0.0001
Genotype	8.7	0.0031
Genotype x Environment Interaction	50.7	0.23

Note: AMMI results are evaluated at $\alpha = 0.05$.

Table 7: AMMI analysis by year for the effect of environment, genotype, and their interaction on Δ pod N concentration of pea grown across the USA and Canada from 2022 to 2024. PC1, PC2, PC3, and PC4 are the first, second, third, and fourth principal components of the interaction term, respectively.

Source of Variation	2022			2023			2024		
	%SS	p-value	% G x E	%SS	p-value	% G x E	%SS	p-value	% G x E
Environment	21.4	0.11		38.3	**0.0012**		51.8	**<0.0001**	
Genotype	9.3	0.19		15.8	0.07		8.8	0.20	
G x E	69.4	**0.0301**		45.9	0.36		39.4	0.49	
PC1		0.0132	52.3		0.17	49.8		0.10	59.0
PC2		0.13	28.0		0.34	30.6		0.68	20.2
PC3		0.26	17.4		0.57	14.6		0.76	11.9
PC4		0.90	2.4		0.73	4.9		0.70	8.8

Note: AMMI results are evaluated at $\alpha = 0.05$.

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CHAPTER FIVE

STABILITY OF GRAIN NITROGEN CONCENTRATION AND YIELD IN FIELD PEA ACROSS NORTH AMERICA

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Abstract

Pea (*Pisum sativum* L.) is an important source of protein, with a large portion of production occurring in North America across the northern and central Great Plains (GP). To understand the influence of the environment (site-year) across this landscape and its interaction with different pea genotypes, a three-year multi-location trial was conducted across the GP. The objectives were to 1) quantify genetic and environmental variability for pea grain N concentration and grain yield, 2) utilize stability indices to evaluate if trait means are associated with trait stability, and 3) evaluate and rank locations for grain N concentration and grain yield trait mean and stability across a panel of genotypes. The mean grain N concentration was 39.6 g kg^{-1}, and the mean grain yield was 3130 kg ha^{-1}. The largest portion of variance in grain N concentration was due to environment (70.5%), followed by the genotype-environment interaction (18.9%) and genotype (10.5%). Environment explained 97.1% of grain yield variation. Additive main effect and multiplicative interaction (AMMI) and best linear unbiased prediction (BLUP)-based models found that trait means are not associated with trait stability among genotypes. Williston, ND, was the most suitable location for stable and elevated grain N concentration among all locations. The study reveals that environment explains most of the grain N concentration and yield variation while detailing the importance of genetics to mean grain N concentration and stability. This study also provides a framework for future genotype trials to utilize stability indices to increase genotype evaluation robustness across the GP.

1. Introduction

Field pea (Pisum sativum L.) is a cool-season legume characterized by a brief growing season, and it has been widely adopted across various cropping systems in the northern and central Great Plains of North America (Padbury et al., 2002). It is commonly integrated into crop rotations with cereals such as spring or winter wheat (Triticum aestivum L.) and oilseeds such as spring canola (Brassica napus L.) (Padbury et al., 2002). In North America, field pea (*Pisum sativum* L.) cultivation is concentrated within the northern Great Plains and Pacific Northwest, with recent expansion into the central Great Plains and Minnesota. Canada remains the dominant producer, with 1.4 million ha planted in 2024—primarily in Saskatchewan and Alberta—to support extensive export markets in Asia and the United States. U.S. production has nearly tripled since 2002, reaching 360,000 ha. This growth is propelled by escalating global demand for pea-derived protein, starch, and fiber, resulting in a 2025 raw grain market value of $3.27 billion and a total sector valuation of approximately $17.3 billion (Mordor Intelligence, 2025).

Field pea integration into cereal- and oilseed-based rotations provides significant ecological and economic advantages, primarily through symbiotic N fixation by *Rhizobium leguminosarum* bv. *viciae* (Beckie & Brandt, 1997; Lupwayi & Soon, 2016; Peoples et al., 2009; Stevenson & Kessel, 1996). This process facilitates high yield potential with minimal external N inputs while improving the soil N economy through atmospheric fixation and the subsequent release of N and C from residues (Jensen, 1997; Lupwayi & Soon, 2015; Sharma & Behera, 2009). These N contributions enhance subsequent crop yields and reduce fertilizer requirements across the northern Great Plains (Miller et al., 2015), with benefits extending over multiple years due to sustained residue decomposition (Lupwayi & Soon, 2015, 2016). Beyond N dynamics,

peas offer critical non-N benefits, including improved soil water availability for subsequent crops due to their short growing season and shallow rooting depth (Stevenson & Kessel, 1996). Furthermore, pea inclusion supports diversified weed management, enriches the soil microbiome through rhizobia exudates, increases soil organic C stocks, and provides essential disease breaks in monocot-dominated systems (Cutforth et al., 2007; Koeshall et al., 2022; Miller et al., 2003).

Beyond ecological services, field pea significantly enhances the economics of North American cropping systems. This advantage is largely derived from its synergy with cereal rotations, where positive N dynamics and direct N credits reduce synthetic fertilizer applications. In southwestern Montana, a pea-wheat rotation achieved the highest four-year net returns and superior economic stability, particularly during scenarios of reduced N availability or wheat protein discounts (Miller et al., 2015). Substituting traditional fallow periods with pea increases the economic output of the farming system by replacing a non-productive interval with a cash crop (Koeshall et al., 2022; Miller et al., 2015). In dryland systems of western Nebraska, the replacement of chemical fallow with pea increased average gross revenue by \$113 ha^{-1} and yielded higher net profits or reduced losses in most of the evaluated site-years (Koeshall et al., 2022). Across the northern Great Plains, pea integration typically enhances subsequent cereal yields and total annualized production, though these benefits may be limited during periods of below-average precipitation (Anderson et al., 1999; Nielsen & Vigil, 2014, 2017, 2018).

Extensive research indicates that environmental factors are the primary determinants of grain protein concentration and yield potential (Bestwick et al., 2016, 2018; Franck et al., 2023; Tao et al., 2017). Specifically, Tao et al. (2017) demonstrated that environmental effects accounted for approximately 93% of the variance in both protein and yield, with precipitation

patterns serving as a critical control, which encompassed research sites across Montana, accounting for ~ 380,000 km^2. Moisture extremes during vegetative stages specifically constrain protein development (Tao et al., 2017). Similarly, Mohammed et al. (2018) observed that environmental influence vastly exceeded genetic variation, though the presence of genetic control suggests potential for environment-specific cultivar development, specifically regarding grain protein advancement, across a similar sized geographic region.

Genetic variation significantly influences pea grain yield and protein concentration, although its impact relative to environmental factors varies across studies. Tao et al. (2017) found environmental effects to be the primary drivers of protein variation, though shifting cultivar ranks across locations indicated site-specific adaptability. This variation was linked to inter-cultivar differences in biological N fixation and rhizobium associations (Tao et al., 2017). Furthermore, while yield and protein were highly sensitive to the environment, resistant starch was found to be under stronger genetic control (9.1% of variation), suggesting that breeding programs may more effectively advance specific quality parameters than yield (Tao et al., 2017). Similarly, research in Germany by Walter et al. (2022) identified cultivar as a leading influence on protein levels and noted that environmental stressors such as high temperatures and drought increased protein concentrations. Franck et al. (2023) demonstrated that genetics exerted consistent control over protein concentration, with no significant genotype-by-environment (GEI) interaction, suggesting that genetic factors may outweigh environmental influences. Their identification of three distinct mega-environments implies that certain regions can specialize in specific production goals, such as high-protein or high-yield pea (Franck et al., 2023). Omari et al. (2025) observed that genotype accounted for only a minor portion of the variation in yield

(1.4%) and protein (10.5%) compared to the environment yet maintained that site-specific genetic selection is crucial for maximizing protein concentration. Collectively, these contrasting results underscore the need for further investigation into the precise interplay between genetic and environmental factors in determining pea grain yield and quality.

Selection of suitable pea genotypes that show high yield and grain quality and high adaptability to a wide range of environments is important to both the pea grower and breeder (Olivoto, Lúcio, da Silva, Marchioro, et al., 2019; Olivoto, Lúcio, da Silva, Sari, et al., 2019). Standardized variety trials (SVT) of crop species over the last century have enabled the identification of site-specific cultivars best adapted to regional growing-season climates and soil conditions, thereby maximizing crop production and profitability (Araus & Cairns, 2014; Araus & Kefauver, 2018; Cooper et al., 2022). However, most SVT have continued to utilize dated statistical methodology, lack integration of advanced environmental data such as spatial soil or precipitation records, and focus only on mean (e.g., mean grain yield within a location or across locations) without reporting stability of a given trait such as grain yield or grain quality within a location or across a panel of locations (Araus & Cairns, 2014; Ding et al., 2008; Olivoto, Lúcio, da Silva, Marchioro, et al., 2019; Xu, 2016). Additionally, SVT reports utilize traditional statistical methods such as Least Significant Difference (LSD) that have a greater probability of reporting false positive among genotypes to rank trait such as grain yield (Nicholson et al., 2022). Furthermore, traditional statistical methods commonly found in SVT and equivalent multiple environment trials (MET) do not test trait stability among genotypes or locations. Previous research has shown how traditional SVT methods fall short and propose methods to increase SVT robustness.

Zobel et al. (1988) detailed how traditional means-comparison statistical models do not properly address genetic-by-environment interaction, proposing that more modern and advanced methods, such as additive main effect and multiplicative interaction (AMMI) and best linear unbiased prediction (BLUP), can enhance the value of yield trials. Grausgruber et al. (2000) detailed the value of quantifying stability parameters in winter wheat in Australia and how stability indices can be useful for ranking genotype trait consistency. WeiKai et al. (2002) detailed GGE biplot analysis methods that enhance the value of multiple-environment trials (MET), allowing the ranking of both genotypes and environments based on a combination of mean and stability. These methods have been successfully implemented in MET studies in crop research in Europe to assess genotype and environment and stability simultaneously (Karges et al., 2022).

To assess grain N concentration and grain yield and trait stability, a variety of methods are available, and the use of a combination of statistical methods is suggested to avoid biased conclusions (Reckling et al., 2021). Additive main effect and multiplicative interaction and best linear unbiased prediction statistical models are widely used in understanding genotype-environment trials. AMMI uses a fixed-effects model, whereas BLUP uses a mixed linear model to interpret genotype-environment interaction (GEI). AMMI modeling has been useful to MET studies to visualize the GEI via easily integrated graphical tools (Bocianowski et al., 2019; Tao et al., 2017). AMMI falls short as not all interaction principal component axes (IPCA) are utilized, while BLUP utilizes all the estimated model IPCA axes to obtain a more accurate and refined model (Olivoto, Lúcio, da Silva, Marchioro, et al., 2019; Olivoto, Lúcio, da Silva, Sari, et al., 2019).

The objectives of this study were to 1) utilize stability indices to evaluate if trait means are associated with trait stability using AMMI and BLUP-based models and 2) evaluate and rank locations for grain N concentration and grain yield trait mean and stability across genotypes using BLUP modeling.

2. Materials and Methods

2.1 Site Description

Field trials were conducted at eight locations across the northern Great Plains of North America (Table 1) in 2022, 2023, and 2024, a region that accounts for most of North America's pea production. Soils ranged from course to fine-textured, with dominantly udic and ustic moisture regimes, and generally had thick, organic-rich surface horizons that qualified them as Mollisols in the US Taxonomy (Table 1). Harvest dates ranged from mid-July to early September, growing periods ranged from 83 to 113 days, and growing degree-days (GDD) accumulation rates ranged from 13.4 to 19.5 per day (Table 2). Growing degree days, commonly referred to as heat units, are the cumulative heat units experienced by the plant from the day of seeding to the day of harvest. GDD was calculated using the following equation (McMaster & Wilhelm, 1997): Click or tap here to enter text.

$$GDD = \sum_{1}^{n} \frac{T_{max} + T_{min}}{2} - T_{base}$$

Where T_{max} is the maximum temperature, T_{min} in the minimum temperature, and the T_{base} temperature for pea is 0° C. Weather parameters for each experimental location by year, including monthly cumulative precipitation, historical 30-year precipitation, average temperature during the growing season at each location, and historical temperature, are shown in Tables 2

and 3. GDD accumulation rates in each environment were calculated from seeding to the nearest calendar day at which the cumulative GDD reached 1378. 1378 GDD is the mean reported GDD value by Miller et al. (2001) that results in seed physiological maturity. Total growing-season precipitation was calculated similarly to the GDD accumulation rate, with total growing-season precipitation representing the amount of precipitation received between the day of seeding and the nearest whole day after the environment reached 1378 GDD.

2.2 Experimental Design

All experimental locations were conducted in a randomized complete block design with five replications and five cultivar entries during the three-year study. All experimental locations received the same five yellow pea genotypes (AAC Profit, CDC Amarillo, CDC Dakota, Delta, and DS Admiral) each year, as shown in Table 4, which were chosen based on protein and yield potential, growth habit, and breeding background to overall maximize genetic diversity via commercially available yellow pea varieties (Andersen et al., 2011; Bing et al., 2022; Limagrain, 2025; Warkentin, 2010; Warkentin et al., 2014). Further, all five pea genotypes were grown from a common seed source, which was increased at Bozeman, MT, during the 2021 growing season to avoid variation among seed lots. All pea seed was treated with *CruiserMaxx Vibrance* for pulse crops at a rate of 3.26 ml kg^{-1} seed before seeding to mitigate potential insect and soil pathogen pressure that may develop at experimental locations. All cultivars were seeded at a target pure live stand density of 80 PLS m^{-2}. *Exceed Granulated Peat for Pea, Vetch, and Lentil* (*Rhizobium leguminosarum biovar viceae* 1×10^8 colony forming units [CFU] g^{-1}) inoculant was applied to all cultivars at all experimental locations at a rate of 5.7 kg ha^{-1} in-furrow. Monoammonium phosphate (11-52-0-0) and potassium sulfate (0-0-50-18) were blended at equal

ratios, resulting in an NPKS starter fertilizer blend (5.5-26-26-9) that was distributed to all experimental locations and applied at a rate of 100 kg ha^{-1} in-furrow at the time of seeding at each experimental location. Plot dimensions at the experimental locations ranged from 1.3 to 2.0 m in width and were 6.1 m long. Row spacing ranged from 22.8 to 30.5 cm among experimental locations. Experimental locations targeted recommended seeding dates based on their respective climate and soil conditions, as shown in Table 2. At each experimental location, pesticides were applied as needed to control weeds, insects, and pathogens, in accordance with best management practices for that site. There were no notable pest issues at these research locations during this study. Experimental units were harvested with research-specific small-plot combine harvesters at each site-year and sub-sampled for analysis of grain N concentration. Each experimental unit grain sample was ground in a *Udy* high-speed centrifugal mill (Udy Cyclone Sample Mill, model 3010-014) to a particle size of < 2mm. All grain yield observations were adjusted to represent yield on a dry matter basis. Although it is common to report grain protein concentration with a conversion factor of 6.25, we report grain N concentration (g kg^{-1}) due to the inaccuracy of this conversion, which does not account for the specific amino acid composition of grain legumes. See Mariotti et al. (2008) for proper conversion values for obtaining grain protein concentration values from grain N concentration observations reported in this article.

2.3 Statistical Analysis

To accurately evaluate environmental and genetic influence on pea grain N concentration and grain yield, “location” will be referred to as a geographical location, and “environment” will be referred to as an individual site-year in this study. A single site-year will be considered a single “environment” as each site-year represents a unique growing season climate, particularly

in timing and accumulation of precipitation and heat throughout the life cycle of pea. Analysis of variance (ANOVA) mixed models were performed on the response variables grain N concentration and grain yield using R statistical software (R Core Team, 2025) with significance assessed at $p \leq 0.05$ to describe general differences among environments, among locations, among genetics, genetics within environments and locations, and environments across individual genetics. Location, environment, and genetics were treated as explanatory fixed factors, with replication within each environment treated as an explanatory random factor.

Grain N concentration and grain yield data were analyzed using the additive main effect and multiplicative interaction (AMMI) methodology, as detailed by the protocol explained by Gauch (2013) to calculate the AMMI stability value (ASV) to compare the stability of grain N concentration and grain yield among genotypes. Lower ASV values indicate a more stable genotype across environments. The AMMI analysis was performed using the *agricolae* R package (de Mendiburu, 2023) in the R statistical software (R Core Team, 2025) and considering the following equation:

$$Y_{ij} = u + g_i + e_j + \sum_{k=1}^{n} \lambda_k \alpha_{ik} \gamma_{jk} + \rho_{ij} + \epsilon_{ij}$$

where Y_{ij} is the mean of ith cultivar in the environment j; u is the overall mean; G is the effect of cultivar i; and ϵ is the effect of the environment j. The GE interaction is explained by the multiplicative term of the equation where λ is the singular value of the principal component (PC) of $k, \varepsilon,$ and n are scores of cultivar i and environment j, respectively, for the principal component k, n ranges from 0 to the number of PC required to describe the AMMI model appropriately.

A modern stability index, the weighted average of absolute scores (WAASB), employs the best linear unbiased prediction (BLUP) method based on a mixed-effects model. The WAASB score shows the stability of genotypes across environments. A lower WAASB score denotes a genotype that deviates least from the mean (grain N concentration or grain yield). WAASB is calculated in the following equation:

$$WAASB_i = \frac{(\sum(n=1)^p |IPCA_{in} \times EP_n|)}{(\sum(n=1)^p EP_n)}$$

where $WAASB_i$ is the weighted average of absolute scores for the $i-th$ genotype, and $IPCA_{in}$ is the score of the $i-th$ genotype in the $n-th$ interaction principal component axis, and p is the total number of IPCA axes retained in the model, respectively.

A modern metric, the weighted average of weighted average of absolute scores (WAASBY), employs the BLUP method based on a mixed-effect model. The WAASBY index allows selection of superior genotypes based on average yield (or grain N concentration) and stability across environments. A higher WAASBY index value indicates greater combined and stability for grain N concentration or grain yield. The WAASBY index is calculated according to Olivoto et al. (2019) in the following equation:

$$WAASBY_{\mathrm{i}} = \frac{[(rY_{\mathrm{i}} \times w_{\mathrm{Y}}) + (rW_{\mathrm{i}} \times w_{\mathrm{s}})]}{(w_{\mathrm{Y}} + w_{\mathrm{s}})}$$

where $WAASBY_{\mathrm{i}}$ is the superiority index of the ith genotype, w_{Y} and w_{s} are the weights for the response variable and stability, respectively; rY_{i} and rW_{i} are the rescaled values, respectively, of response and WAASB. We assigned weights of 50 and 50 to the response variable and WAASB, respectively, to equally weight the contributions of trait performance and stability to the

WAASBY index. All AMMI and BLUP-based stability statistics were calculated using the *metan* package in R statistical software (R Core Team, 2025).

The genetic × environment (GGE) biplots were constructed using the *metan* R package (Olivoto & Dal'Col Lúcio, 2020) to illustrate the interactive effects of genotype × environment, versus stability of cultivars across environments, rank of environments and locations against a modeled ideal environment, and rank of genotype against a modeled ideal genotype across environments using the following equation:

$$Y_{ij} - \mu - \beta_j = \sum_{k=1}^{n} \lambda_k \, \alpha_{ik} \gamma_{jk} + \epsilon_{ij}$$

where Y_{ij} is the observed mean response of the genotype i in environment j; μ is the response grand mean; β_j is the environment main effect; $Y_{ij} - \mu - \beta_j$ is the term representing the environment-centered data, thus containing the genotype and genotype × environment interaction; λ_k is the singular value for the principal component axis κ; α_{ik} is the score of the genotype i for axis κ; γ_{jk} is the score of the environment j for axis κ; and ϵ_{ij} is the residual error not captured by the first n principal components. The application of GGE modeling and visualization via GGE biplots allows the identification of environmental and cultivar dynamics, specifically genotype adaptation to specific environments, in conjunction with ASV, WAASB, and WAASBY index results.

3. Results and Discussion

3.1 Climate characteristics during crop growth

The mean GDD accumulation rate day^{-1} among the 8 locations varied from 14.2 GDD day^{-1} at Bozeman, MT to 18.2 GDD day^{-1} at Carrington, ND, resulting in a spread of 4.2 GDD

day^{-1} across eight locations (Table 1). Across all 23 environments, the mean GDD accumulation rate was 16.6 GDD day^{-1}. Among all 23 environments, Mead 2023 accumulated the greatest growing degree-day rate at 19.5 GDD day^{-1}. Bozeman 2024 experienced the slowest growing season via heat accumulation among all 23 environments at 13.4 GDD day^{-1}, resulting in a heat accumulation spread of 6.1 GDD day^{-1} among 23 environments. Mean precipitation during crop growth across 23 environments was 183 mm. Growing season precipitation varied among the eight locations from 142 mm at Lucky Lake, SK, to 215 mm at Williston, ND, a difference of 73 mm. Among 23 environments, growing season precipitation was greatest at Roseau 2024 at 307 mm, while Lucky Lake 2023 received only 64 mm, resulting in a growing season precipitation spread of 243 mm. Across all 23 environments, pea took an average of 84 days to accumulate 1378 GDD. Among eight locations, Bozeman, MT had the longest growing season, with 98 days to maturity, while Carrington, ND had, on average, the shortest growing season. Among 23 environments, Bozeman 2024 had the longest observed growing season at 103 days to maturity, while Mead 2023 had the shortest at 71 days, representing a 32-day range in days to physiological maturity. The range and variation in physiological days to maturity varied across environments and locations due to differences in GDD accumulation rates, suggesting that environments and locations with a greater number of days to physiological maturity experienced, on average, a cooler growing season.

3.2 Grain N concentration and grain yield across environments

Grain N concentration varied among locations and years ($p < 0.0001$). Mean grain N concentration across years was 39.6 g kg^{-1} and ranged from 34.3 g kg^{-1} at Sutherland 2023 to 44.0 g kg^{-1} at Havre 2023 (Table 5). Across locations, the greatest grain N concentration

observed was 39.6 g kg^{-1} in 2024, while the lowest was in 2022 at 39.3 g kg^{-1}. Grain yield varied significantly across years and locations ($p < 0.0001$), with the greatest yields observed at Roseau. Across years, Roseau expressed the greatest grain yield at 6330 kg ha^{-1}, followed by Sutherland at 4131 kg ha^{-1}. Grain yields ranged from 6533 kg ha^{-1} at Roseau 2024 to 625 kg ha^{-1} at Mead 2022. Across locations, grain yield was greatest during 2024 at 3729 kg $ha^{-1,}$ while grain yield was lowest during 2022 at 2623 kg ha^{-1}.

3.3 AMMI analyses for grain N concentration and grain yield

Environment was the most important source of variation in grain N concentration, explaining 70.5% (p = <0.0001) of the total (G + E + G×E) sums of squares (Table 6, Figure 1). The contribution of genetics (p = <0.0001) and the interaction of environment × genetics (p = 0.0001) were meaningful factors in explaining the total variation of grain N concentration within this study, with genetics and the interaction of environment × genetics explaining 10.5% and 18.9% of the total sums of squares from this analysis (Table 6). Environment was the only important source of grain yield variation, explaining 97.1% (p = <0.0001) of the total (G + E + G×E) sums of squares (Table 6, Figure 2). The contribution of genetics (p = <0.0001) and the interaction of environment × genetics (p = <0.0001) were meaningful factors due to the large number of grain yield observations in this study. However, genetics and the interaction between environment × genetics explained only 0.5% and 2.4% of the total sums of squares (Table 6). These results show that the environment has the main effect on grain N concentration and grain yield, with genotype and GEI having a meaningful influence on grain N concentration, explaining 10.5% and 18.9% of the variation across 23 environments.

The partitioning of GEI revealed that the first two multiplicative terms (IPCA1 and IPCA2) of AMMI were significant for grain N concentration and grain yield (Table 6). The third multiplicative term (IPCA3) of the AMMI analysis on grain yield was also significant (Table 6). The cumulative partitioning of IPCA1 and IPCA2 to the total GEI variance was 72.8% for grain N concentration and 86.2% for grain yield. With the addition of IPCA3, the cumulative partitioning variance captured by GEI increases to 95%. Environments and pea genotypes with greater grain N concentration or grain yield stability are plotted closer to the origin of the bi-plot, and vice versa (Figures 2 – 3).

Grain N concentration stability varied among the tested pea genotypes, with AAC Profit (G1) and CDC Dakota (G3) showing greater stability (close to origin) than the other genotypes (Figure 1). The genotype Delta (G4) showed the greatest instability in grain N concentration among the five genotypes (Figure 1). The AMMI1 biplot showed either positive or negative interactive effects of the five pea genotypes across the 23 environments (Figure 1). Across all locations and years, CDC Dakota (G3) had the greatest grain N concentration of 41.0 g kg^{-1}, followed by AAC Profit (G1) (39.6 g kg^{-1}) (Table 7). DS Admiral (G5) expressed the lowest grain N concentration of 38.3 g kg^{-1} (Table 7). The AMMI stability value (ASV) results show that AAC Profit (G1) has the lowest ASV (2.1), while the genotype with the greatest grain N concentration had the highest ASV (5.9). Additionally, the genotype with the lowest grain N concentration, DS Admiral (G5) did not exhibit the greatest ASV instability (Table 7). These results, from an AMMI analysis of grain N concentration, suggest that genotype trait means are not aligned with genotype trait stability for this response. This presents a potential issue for pea producers, as these two traits do not appear to be synergistic and may force them to choose

genotypes based on the stability of grain quality over time or on the potential for high grain quality. Given that CDC Dakota is not a variety intended for protein ingredients in the food industry, non-traditional pea genotypes may offer valuable genotypic traits to future commercially utilized pea genotypes that are developed for the protein market (Warkentin, 2010).

The genotype AAC Profit (G1) expressed the greatest grain yield (3423 kg ha^{-1}) across all environments, followed by CDC Amarillo (G2) (3339 kg ha^{-1}) (Table 8), suggesting that breeding efforts have advanced grain yield over 30 years given the panel of genotypes used in this study (Andersen et al., 2011; Bing et al., 2022; Limagrain, 2025). However, genotypes that produced greater grain yields expressed greater yield instability as AAC Profit (G1) and CDC Amarillo (G2) were plotted further away from the origin compared to lower-yielding genotypes such as CDC Dakota (G3) or DS Admiral (G5) (Figure 2). ASV results show that CDC Dakota (G3) expressed the greatest yield stability with an ASV of 2541, followed by CDC Amarillo (G2) with an ASV of 4905. Delta (G4) expressed the greatest grain yield instability with an ASV of 8768. The genotype Delta (G4), which showed the lowest grain yield stability, also showed the lowest grain yield (Table 8). Although the genotype AAC Profit (G1) that produced the greatest grain yield did not express the lowest ASV stability index, AMMI analysis of pea grain yield and resultant ASV results suggest that advancement of genotypes via breeding programs has elevated both trait means and stability, although trait mean and trait stability of grain yield do not seem to be perfectly synergistic.

3.4 BLUP analysis for grain N concentration and grain yield

There was a large distribution of pea genotypes based on mean and stability across environments (Figures 3 – 4). Across all environments, AAC Profit (G1) expressed the greatest grain N concentration stability with the lowest WAASB value (1.5), followed by DS Admiral (G5) at 2.3 (Table 7). Additionally, AAC Profit (G1) presented the greatest WAASBY (73.5) among the five genotypes, suggesting that this genotype optimizes the combination of both trait potential and trait stability for grain N concentration among the five genotypes tested across 23 environments. This suggests that breeding over the last 30 years may have increased overall stability and performance for quality traits such as protein across a diverse geographic landscape of the GP region. Genotype Delta (G4) had the lowest WAASBY value (17.9), suggesting that, among the five genotypes tested, Delta (G4) would be the poorest choice when equal weight is given to both trait mean potential and trait stability. However, Delta (G4) did not present the lowest mean grain N concentration. Across all environments, AAC Profit (G1) showed the greatest adaptability to the tested environments, as it was plotted closest to the origin in Figure 3.

Across all environments, CDC Dakota (G3) expressed the greatest grain yield stability with the lowest WAASB value, followed by DS Admiral (G5) (Table 8). CDC Dakota (G3) was also plotted closest to the origin in Figure 4, suggesting that this genotype shows the best grain yield adaptability across the genotypes and environments tested in this study. Across environments, CDC Dakota (G3) resulted in the greatest grain yield WAASBY result (72), followed by CDC Amarillo (G2) at 66, suggesting that these genotypes best balance grain yield mean performance and stability among the genotypes tested (Table 8). Additionally, Delta (G4) expressed the lowest WASSBY value for grain yield among the five genotypes, indicating poor

mean performance and stability across environments. These findings suggest that grain N concentration stability is not linked to yield stability across a broad geographic landscape that captures a variety of environments. The high ranking of CDC Dakota, given that it is a dual-purpose pea, suggest that there may be inherent genotypic traits that could be incorporated into pea genotypes that are developed for the protein ingredient market, such as improved biomass production, that drives N_2 fixation, thereby increasing N availability for grain protein formation . Overall, AAC Profit (G1) was the most stable genotype for grain N concentration, and CDC Dakota (G3) was the most stable genotype for grain yield. However, WAASB stability results indicate that high grain N concentration stability does not align with high grain yield stability, suggesting that selecting for grain yield stability is not associated with elevated grain N concentration stability.

3.5 Selecting pea genotypes using stability indices

The stability indexes detailed large variations in pea grain N concentration and grain yield among the five genotypes. The AMMI-based stability index ASV revealed AAC Profit (G1) to be the most stable genotype for grain N concentration and Delta (G4) the least stable genotype (Table 7, Figure 1). This finding is consistent with the BLUP-based WAASB stability index results (Table 7, Figure 3). Similarly, based on WAASBY index results, AAC Profit (G1) was the most desirable genotype for stable and high grain N concentration. Additionally, both AMMI and BLUP stability index models selected Delta (G4) as the least desirable genotype, which aligns with the BLUP-based WAASBY results (Table 7). However, WAASBY did not rank the greatest grain N concentration genotype, CDC Dakota (G3), as the most desirable

genotype for equally balancing trait means and stability. This indicates that among the genotypes tested across 23 environments, trait mean and stability are not necessarily associated.

CDC Dakota (G3), with the lowest ASV and WAASB, was consistently the most stable and superior genotype for grain yield, as reinforced by the highest WAASBY result among the five genotypes (Table 8). CDC Amarillo (G2) and AAC Profit (G3) were closely ranked as the second- and third-most stable genotypes, as revealed by WAASBY results (Table 8). The AMMI- and BLUP-based stability indices for grain yield largely aligned across genotypes, indicating continuity between the stability indices (Table 8, Figures 1 and 3). Similar to grain N concentration findings, Delta (G4) was the least stable genotype as shown by high AVS and WAASB values (Table 8). This suggests that even though a genotype that exhibits high yield potential, such as AAC Profit (G1), may not be the most desirable genotype due to reduced trait stability. This finding is reinforced via the WAASBY results, which present an equally weighted ranking of genotype mean and stability. Additionally, trait mean and stability results for grain N concentration do not align with those for grain yield, indicating that stability in one trait is not an indicator of stability in another. The comparison among trait stability (ASV and WAASB), trait mean (mean grain N concentration or grain yield), and WAASBY results indicate that traditional SVT, breeder trials, or larger multi-environment trials for field pea are missing a valuable parameter (stability) to inform both pea producers and researchers better as mean of a trait is not an indicator of trait stability nor overall desirability.

4. Conclusion

This study demonstrated a larger environmental effect on the variances in pea grain N concentration and grain yield compared to genotype. The genotype effect on the overall grain

yield variance was negligible, but meaningful for grain N concentration. Grain N concentration and grain yield differed among genotypes and environments. Genotypes (mean grain N concentration and grain yield) varied across environments, and top-yielding genotypes were not stable across environments. Genotypes trait means were not associated with genotype trait stability.

The AMMI and BLUP stability models identified AAC Profit (G1) as the most stable genotype for grain N concentration and CDC Dakota (G3) as the most stable genotype for grain yield. In contrast, CDC Dakota (G3) was the highest yielding genotype for grain N concentration but was only rated as moderately stable. Additionally, AAC Profit (G1) was the highest-yielding genotype for grain yield but expressed poor grain yield stability across environments. Overall, recent breeding efforts seem to have advanced grain N concentration and grain yield, however mean performance and stability are not necessarily synergistic. Additionally, grain N concentration stability is not associated with yield stability as different genotypes were selected as the most stable for each trait. The WAASBY index scores aligned with stability indices (ASV and WAASB) scores for grain N concentration and grain yield among genotypes, suggesting that stability of a trait may be as equally important as trait performance.

These results suggest that there is a greater opportunity for breeding efforts to improve grain quality than grain yield, given AMMI results. These results also underscore the value of integrating robust AMMI- or BLUP-based statistical methods into future crop research utilizing SVT and other MET-based studies. Stability of grain N concentration and grain yield should be considered for the long-term profitability of pea production across GP, particularly for optimizing genotype selection within a growing region.

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Figures

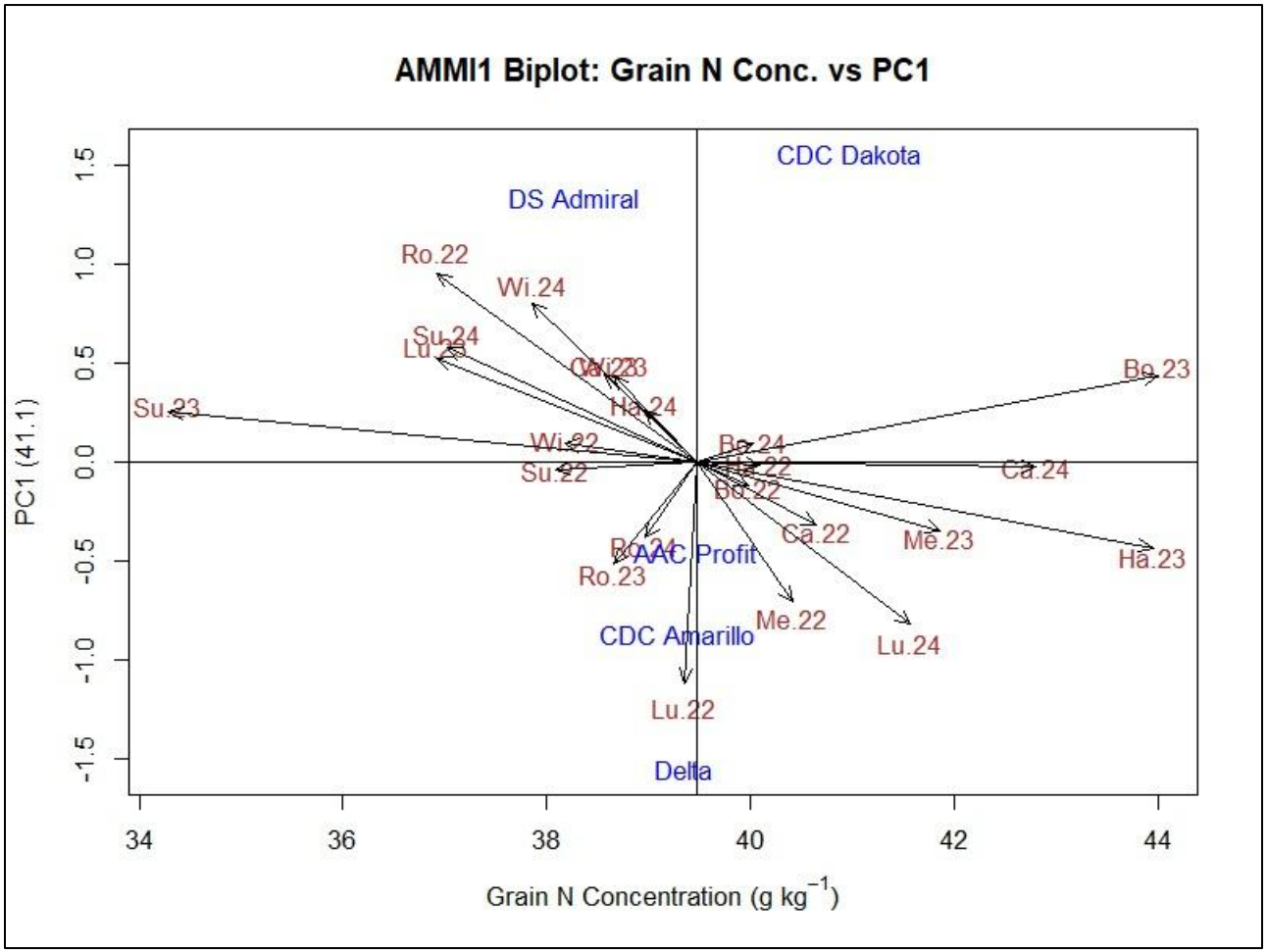

Figure 1: Additive main effect and multiplicative interaction (AMMI) biplot showing grain N concentration vs PC1 of 23 environments (red color) and five genotypes (blue color).

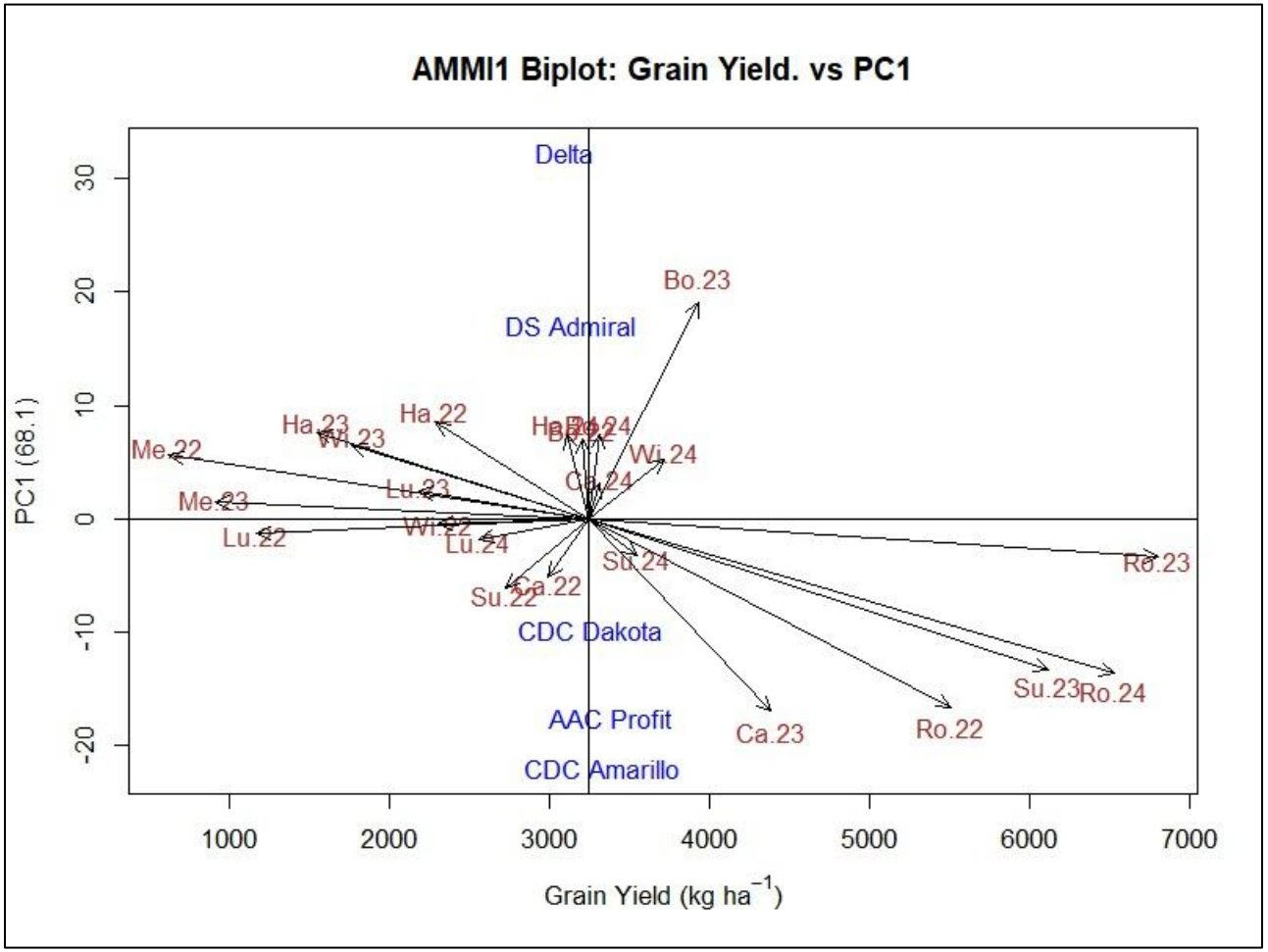

Figure 2: Additive main effect and multiplicative interaction (AMMI) biplot showing grain yield vs PC1 of 23 environments (red color) and five genotypes (blue color).

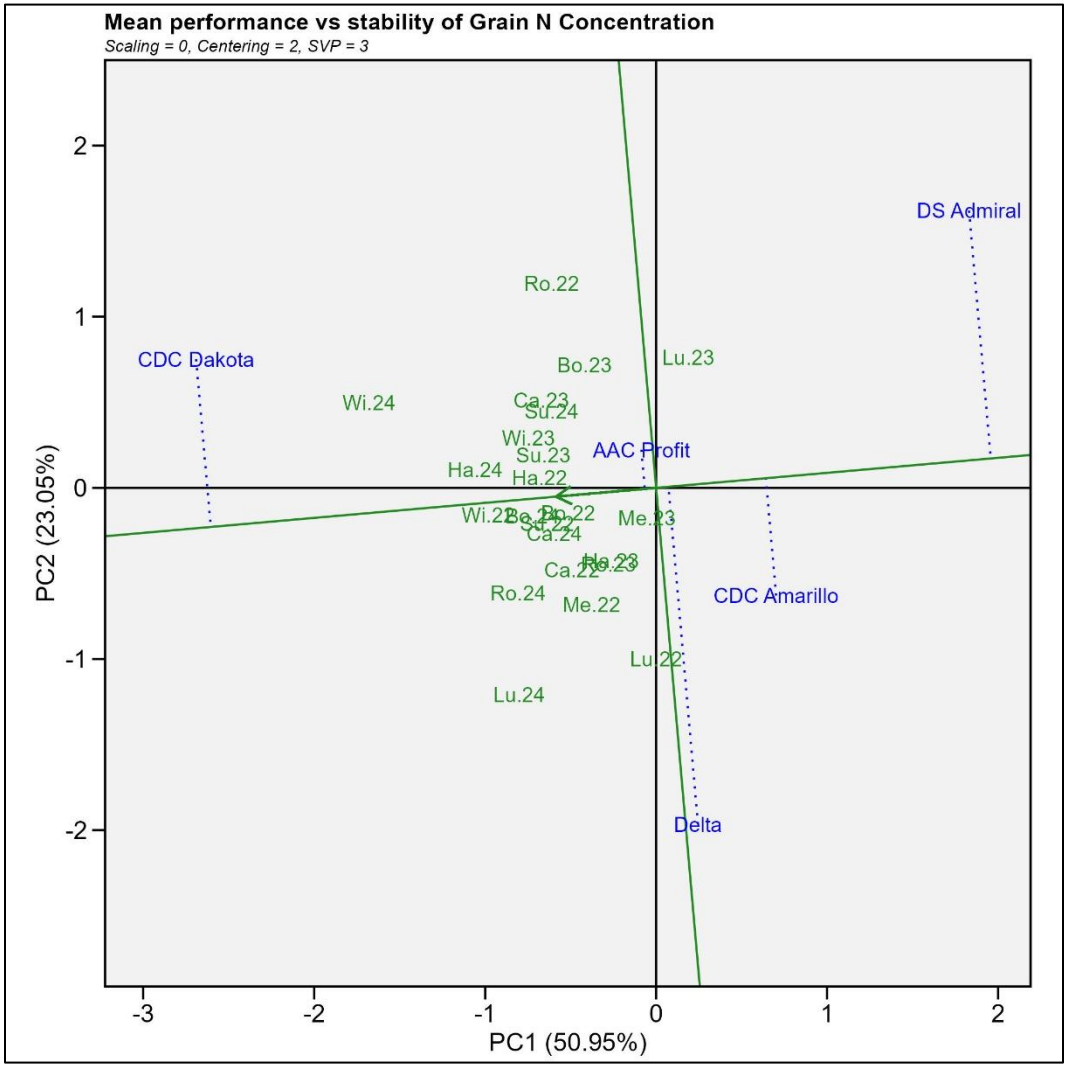

Figure 3: Biplot of environment × genotype displaying the association of genotype mean grain N concentration and stability. CDC Dakota (G3) expressed the greatest grain N concentration while AAC Profit (G1) expressed the greatest grain N concentration stability.

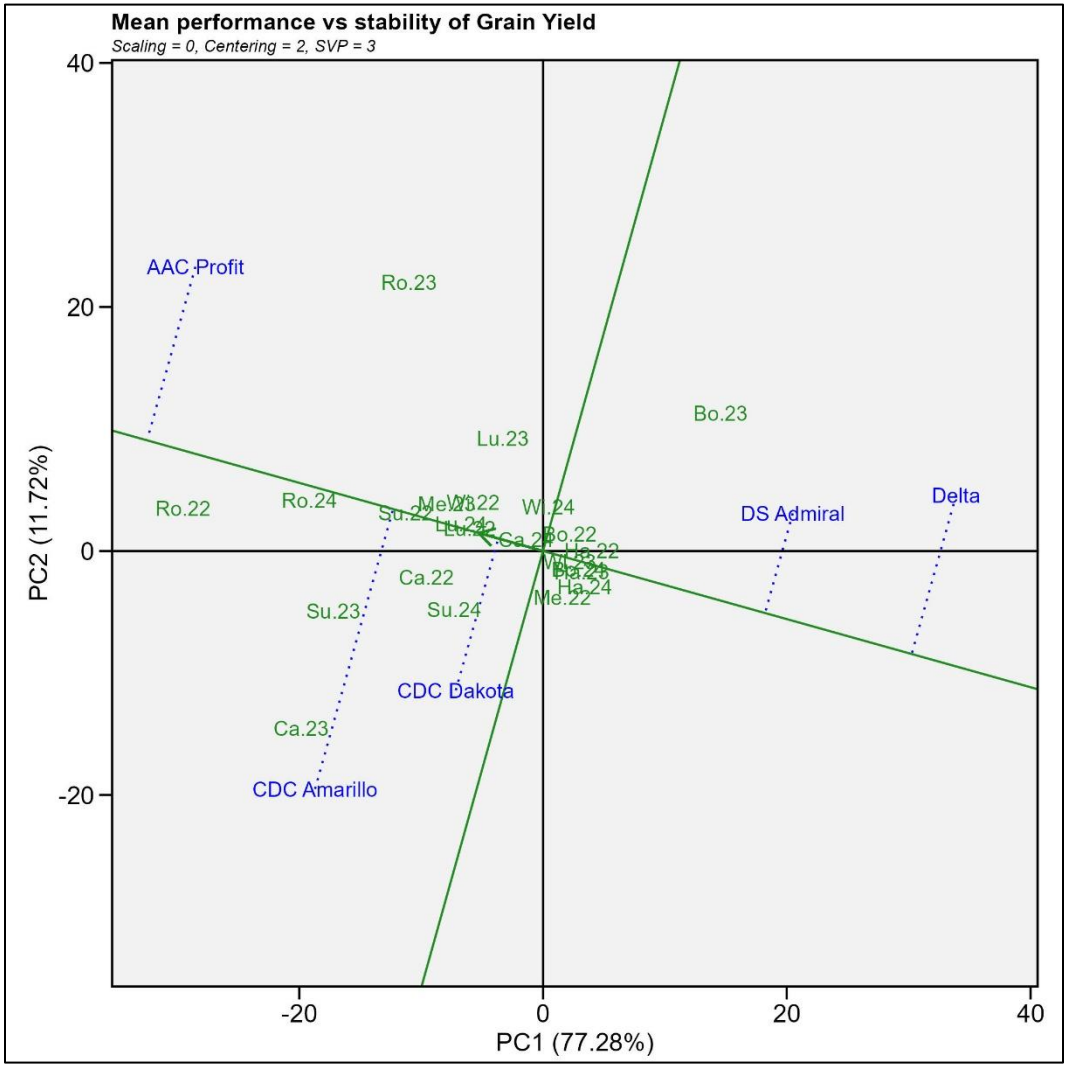

Figure 4: Biplot of environment × genotype displaying the association of genotype mean grain N concentration and stability. AAC Profit (G1) expressed the greatest mean grain yield while Delta (G4) expressed the greatest grain yield stability.

Tables

Table 1: Summary of environmental code, seeding date, harvest date, days to physiological maturity, growing degree days accumulated from seeding to harvest, growing degree-day accumulation rate, and cumulative precipitation for each study location in the USA and Canada from 2022 to 2024.

Location	Year	ENV Code	Seeding Date	Harvest Date	Days to Maturity	GDD rate Day^{-1}	Precipitation (mm)
Bozeman, MT	2022	Bo.22	20 April	8 August	100	13.8	220
Bozeman, MT	2023	Bo.23	28 April	14 August	90	15.3	172
Bozeman, MT	2024	Bo.24	11 April	2 August	103	13.4	174
mean					98	14.2	189
Carrington, ND	2022	Ca.22	16 May	9 August	76	18.1	175
Carrington, ND	2023	Ca.23	5 May	9 August	73	18.8	207
Carrington, ND	2024	Ca.24	13 May	13 August	78	17.6	232
mean					76	18.2	204
Havre, MT	2022	Ha.22	21 April	27 July	93	14.8	133
Havre, MT	2023	Ha.23	26 April	25 July	83	16.5	148
Havre, MT	2024	Ha.24	15 April	29 July	98	14.1	193
mean					91	15.1	158
Lucky Lake, SK	2022	Lu.22	5 May	10 August	87	15.8	130
Lucky Lake, SK	2023	Lu.23	9 May	14 August	77	17.8	64
Lucky Lake, SK	2024	Lu.24	15 May	21 August	83	16.6	234
mean					82	16.7	142
Mead, NE	2022	Me.22	1 April	21 July	90	15.4	217
Mead, NE	2023	Me.23	25 April	19 July	71	19.5	158
mean					81	17.5	188
Roseau, MN	2022	Ro.22	28 May	7 September	75	18.3	185
Roseau, MN	2023	Ro.23	16 May	28 August	75	18.4	131

Roseau, MN	2024	Ro.24	13 May	28 August	82	16.7	307
mean					77	17.8	208
Sutherland, SK	2022	Su.22	9 May	16 August	87	15.8	191
Sutherland, SK	2023	Su.23	4 May	15 August	80	17.3	115
Sutherland, SK	2024	Su.24	11 May	21 August	88	15.7	168
mean					85	16.3	158
Williston, ND	2022	Wi.22	4 May	4 August	77	18.0	266
Williston, ND	2023	Wi.23	26 April	18 July	75	18.2	163
Williston, ND	2024	Wi.24	26 April	2 August	84	16.4	217
mean					79	17.6	215
Study mean					**84**	**16.6**	**183**

Note: Growing degree-day and precipitation data for each location were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada data services.

Table 2: Summary of monthly cumulative precipitation, cumulative growing season precipitation from April 01 to August 31, and 30-year mean growing season precipitation for each study location.

Location	Year	April	May	June	July	August	April - August	30-yr April-Aug Average
		precipitation (mm)						
Sutherland, SK	2022	3	26	38	47	26	139	242
	2023	8	52	44	20	50	174	
	2024	12	46	111	27	45	241	
Lucky Lake, SK	2022	14	20	49	71	10	163	238
	2023	17	25	30	12	59	141	
	2024	24	91	61	23	6	204	
Havre, MT	2022	4	10	81	44	23	163	198
	2023	14	74	57	16	25	186	
	2024	22	110	63	18	37	250	
Bozeman, MT	2022	41	110	60	14	15	240	253
	2023	19	19	129	25	47	239	
	2024	36	89	41	19	26	211	
Carrington, ND	2022	61	162	80	38	31	372	309
	2023	43	99	92	58	41	333	
	2024	60	128	145	89	122	545	
Williston, ND	2022	72	166	50	52	13	354	250
	2023	20	75	77	48	71	292	
	2024	15	77	82	44	14	232	
Roseau, MN	2022	88	134	53	90	149	513	343
	2023	16	46	35	67	38	201	
	2024	37	91	122	116	63	430	
Mead, NE	2022	30	110	77	26	74	316	493
	2023	38	6	130	199	62	436	
	2024	67	186	71	166	129	618	

Note: 30-year April to August precipitation averages at each location are representative of 1991 to 2020, and precipitation data were obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada online data services.

Table 3: Summary of mean monthly temperature and growing season temperature from April 01 to August 31 at each study location from 2022 to 2024.

Location	Year	April	May	June	July	August	April - August
		Temperature °C					
Sutherland, SK	2022	1.8	11.0	15.7	19.3	19.6	13.5
	2023	1.4	15.5	19.2	17.7	18.1	14.4
	2024	6.5	11.2	13.7	19.9	18.3	13.9
Lucky Lake, SK	2022	1.9	11.2	16.0	19.6	20.7	13.9
	2023	0.9	15.0	18.7	18.7	18.8	14.4
	2024	6.8	10.7	14.2	20.3	18.6	14.1
Havre, MT	2022	2.9	11.4	16.0	21.7	22.8	15.0
	2023	4.7	14.9	17.3	21.1	21.1	15.8
	2024	7.3	11.0	15.3	21.8	20.1	15.1
Bozeman, MT	2022	2.9	9.1	15.4	20.6	21.7	13.9
	2023	4.2	13.3	14.4	19.7	19.4	14.2
	2024	7.4	9.5	16.2	20.4	18.8	14.5
Carrington, ND	2022	-0.2	11.5	18.4	20.9	20.0	14.1
	2023	-0.9	15.3	21.0	19.2	19.3	14.8
	2024	5.6	11.8	17.3	21.3	18.8	14.9
Williston, ND	2022	1.6	12.8	18.9	23.3	23.8	16.1
	2023	5.2	16.0	21.1	21.8	21.8	17.2
	2024	9.7	13.4	17.2	23.3	21.4	17.0
Roseau, MN	2022	1.0	12.6	18.9	21.2	19.7	14.7
	2023	0.7	16.7	21.7	19.6	19.7	15.7
	2024	6.5	13.0	17.7	21.6	20.3	15.8
Mead, NE	2022	8.2	16.0	22.4	24.8	23.6	19.0
	2023	10.2	17.8	22.6	22.8	23.1	19.3
	2024	11.2	16.8	22.7	23.2	23.1	19.4

Note: Temperature data was obtained from the High Plains Climate Center ACIS-CLIMOD and Climate Canada data service.

Table 4: Summary of environmental code, genotypes, and genotype code utilized in AMMI and BLUP analysis.

Location	Year	ENV Code	Cultivar	Genetic Code (GEN)
Sutherland, SK	2022	Su.22	AAC Profit	G1
	2023	Su.23	CDC Amarillo	G2
	2024	Su.24	CDC Dakota	G3
Lucky Lake, SK	2022	Lu.22	Delta	G4
	2023	Lu.23	DS Admiral	G5
	2024	Lu.24		
Havre, MT	2022	Ha.22		
	2023	Ha.23		
	2024	Ha.24		
Bozeman, MT	2022	Bo.22		
	2023	Bo.23		
	2024	Bo.24		
Carrington, ND	2022	Ca.22		
	2023	Ca.23		
	2024	Ca.24		
Williston, ND	2022	Wi.22		
	2023	Wi.23		
	2024	Wi.24		
Roseau, MN	2022	Ro.22		
	2023	Ro.23		
	2024	Ro.24		
Mead, NE	2022	Me.22		
	2023	Me.23		
	2024	Me.24		

Table 5: Pea grain N concentration and grain yield response to growing location.

Location	**Year**			
Grain N Conc. (g kg^{-1})	**2022**	**2023**	**2024**	**Average***
Sutherland, SASK	38.1	34.3	37.0	36.5 d
Lucky Lake, SASK	39.4	36.9	41.7	39.3 bc
Havre, MT	40.1	44.0	39.0	41.0 ab
Bozeman, MT	40.0	43.9	40.0	41.4 a
Williston, ND	38.2	38.7	37.9	38.2 cd
Carrington, ND	40.6	38.7	42.8	40.7 ab
Roseau, MN	37.6	38.7	39.0	38.4 cd
Mead, NE	40.4	41.8	NA	41.1 ab
annual mean	39.30	39.61	39.62	
Grain Yield (kg ha^{-1})				
Sutherland, SASK	2724	6119	3550	4131 b
Lucky Lake, SASK	1167	2185	2563	1973 d
Havre, MT	2291	1152	3106	2316 d
Bozeman, MT	3206	3876	3321	3473 bc
Williston, ND	2304	1766	3721	2597 cd
Carrington, ND	2994	4385	3312	3564 bc
Roseau, MN	5676	6805	6533	6330 a
Mead, NE	625	894	NA	759 e
annual mean	2623	3398	3729	

Note: Significance of Tukey HSD results assessed at $\alpha = 0.05$.

Table 6: AMMI analysis for the effect of environment, genotype, and their interaction on pea grain N concentration and grain yield of pea grown across 23 environments and a standard panel of five genotypes. PC1, PC2, PC3, and PC4 are the first, second, third, and fourth principal components of the interaction term, respectively.

Source of Variation	Grain N Conc.			Grain Yield		
	%SS	p-value	% G x E explained	%SS	p-value	% G x E explained
Environment	70.5	<0.0001		97.1	<0.0001	
Genotype	10.5	<0.0001		0.5	<0.0001	
G x E	18.9	0.0001		2.4	<0.0001	
PC1		0.0003	41.7		<0.0001	67.7
PC2		0.0071	31.1		<0.0001	18.5
PC3		0.11	20.9		0.0125	8.8
PC4		0.98	6.3		0.29	5.0

Note: Significance of AMMI analysis results assessed at $\alpha = 0.05$.

Table 7: Pea grain N concentration response to genotype across 23 environments with AMMI-derived stability index ASV (AMMI stability value) and BLUP-derived stability and ranking indices WAASB (weight average of absolute score) and WAASBY (weighted average of weighted average of absolute score).

Genotype	Grain N Conc. g kg^{-1}	ASV	WAASB	WAASBY
G1 (AAC Profit)	39.6 b	2.1	1.5	73.5
G2 (CDC Amarillo)	39.3 b	5.3	2.9	33.8
G3 (CDC Dakota)	41.0 a	5.9	3.1	55.1
G4 (Delta)	39.3 b	7.0	3.3	17.9
G5 (DS Admiral)	38.3 c	4.1	2.3	25.5

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant least square means difference at $\alpha = 0.05$.

Table 8: Pea grain yield response to genotype across 23 environments with AMMI-derived stability index ASV (AMMI stability value) and BLUP-derived stability and ranking indices WAASB (weight average of absolute score) and WAASBY (weighted average of weighted average of absolute score).

Genotype	Grain Yield kg ha^{-1}	ASV	WAASB	WAASBY
G1 (AAC Profit)	3423 a	6567	998	65
G2 (CDC Amarillo)	3339 ab	4905	770	66
G3 (CDC Dakota)	3239 bc	2541	423	72
G4 (Delta)	3084 d	8768	1240	0
G5 (DS Admiral)	3140 cd	5142	759	39

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant least square means difference at $\alpha = 0.05$.

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CHAPTER SIX

INFLUENCE OF ALTERED CLIMATES ON FIELD PEA BIOMASS, GRAIN YIELD, AND NITROGEN CONCENTRATION

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Abstract

Field pea (*Pisum sativum* L.) is a cool-season legume challenged by temperature and precipitation limitations across the North American Great Plains. A three-year field trial was conducted in Bozeman, MT, to evaluate the effects of altered climate conditions on pea biomass, grain yield, and grain nitrogen (N) concentration in two varieties that contrast for determinate vs. indeterminate growth habit. Warming was imposed using open-top chambers (OTC), and a 50% reduction in rainfall was achieved via rain-off shelters, for three plant growth periods. Imposition of altered climates during pre-flowering growth reduced biomass and grain yield in a determinate pea variety and decreased grain N concentration in an indeterminate pea variety in one of three years. Application of climate treatments during pre-flowering growth reduced grain yield by 854 kg ha^{-1} in 2022 for the determinate variety. This treatment also resulted in the highest grain N concentration (41.8 g kg^{-1}), while altered climate imposition during the 6^{th} node stage (BBCH: 36 – 51) resulted in the lowest grain N concentration (38.8 g kg^{-1}). Presence of altered climate reduced grain yield but revealed no difference between warming versus warming plus reduced precipitation for the determinate pea in 2022. June 2023 presented a unique window into pea response to excess precipitation, as reduced precipitation applied at pre-flower and post-flower timings increased grain yield and biomass on average by 26 and 19% over the control. Timing of imposed altered climate revealed that contrasting pea growth habits respond differently to warmer temperatures and reduced precipitation.

1. Introduction

Spring field pea (*Pisum sativum* L.) has been widely adopted in cropping systems across North America (MacWilliam et al., 2014). In 2024, in western Canada and the northwestern United States field pea was grown on 1.4 million and 0.36 million ha, respectively (NASS, 2025; StatsCan, 2025). Pea has been adopted across diverse climates within the northern half of the Great Plains (NGP) of North America (Benjamin & Nielsen, 2006; Carr et al., 2024; S. Koeshall et al., 2021; Miller et al., 2003; Padbury et al., 2002). The inclusion of field pea and other pulse crops in human and animal diets has created strong domestic and international markets, in large part due to the growth and demand in the plant-based protein sector (Mordor Intelligence, 2025).

Field pea and other pulse crops necessitate minimal processing for human consumption in comparison to soybean (*Glycine max* L.) and are regarded as valuable sources of protein, especially within plant-based diets or for individuals with specific allergies to conventional protein sources such as dairy, soybean, or animal meats (Thavarajah et al., 2023). Field pea and various pulse crops fall under category one or two in the NOVA food classification system; they are commonly referred to as ‘clean-label’ or ‘whole’ foods (Sekyi et al., 2023). Additionally, pea is a non-genetically modified crop species, allowing for a marketing advantage compared to soy. Field pea and other pulse crops have been found to have health benefits when included in human diets. Consumption of pea has been shown to prevent hypercholesterolemia and hypertension in human subjects (Arnoldi et al., 2015). Furthermore, research in animal science indicates that field pea products enhance the health and performance of livestock when incorporated into the diets of swine, broiler chickens, and dairy cattle (Prandini et al., 2011; Stein, 2006; Vander Pol et al., 2008).

Given the robust domestic market for field pea products, including protein isolates and pea flour, as well as significant international export markets, grain producers have incorporated field pea as an essential species that alternates with winter or spring wheat (Byington, 2020; *Continued Strong Growth in the Global Pea Protein Market - Eurosoy*, 2021). Generally, the principal ecological advantage of integrating field pea into cropping systems derives from the direct N benefits and soil N credits, which result from atmospheric N_2 fixation and the subsequent release of N from pea residues into agricultural soils (Lupwayi & Soon, 2016; Peoples et al., 2009; Stevenson & Kessel, 1996). Pea requires little to no fertilizer N amendments during the growing season to reach full yield potential because atmospheric N fixation is conducted via *Rhizobium leguminosarum* bv. *viciae* that fosters a symbiotic relationship with the pea plant (Af Geijersstam & Mårtensson, 2006; Frechilla et al., 2000). Across the NGP, grain producers have recognized the N economy benefits from field pea and other pulse crop species, effectively reducing fertilizer N applications across the region (Walley et al., 2007). This has resulted in both an economic benefit to the grain producer via reduced input costs (Burgess et al., 2012) and an ecological benefit to the greater growing region's ecosystem, as reduced fertilizer N applications tend to lower groundwater N concentrations (Sigler, 2020). Although field pea has experienced widespread adoption by grain producers across North America as a profitable cash crop in rotation with cereal and oilseed crops, and has developed substantial domestic and international markets, certain climatic conditions may threaten pea production. Changing climate conditions, such as warmer air temperatures or increasingly variable rainfall, pose a threat to the consistency of grain yield and protein concentration in pea (Cutforth et al., 2007).

Previous research across the NGP region has quantified the influence of growing-season climate conditions and broader environmental differences on field pea grain protein concentration and grain yield. A survey of field pea variety trials by Stoddard et al. (1993) found that grain crude protein varied significantly among research locations, averaged across genotypes. A field-based study conducted across Montana by Tao et al. (2017) reported that environment explained most of the variation in pea grain protein concentration (92.5%), grain yield (93.1%), and starch concentration (66.9%), with most of the environmental contrasts driven by precipitation during seeding and at flowering. Another field-based study from the same research group found that yield potential and grain protein concentration were strongly influenced by growing-season precipitation (Mohammed et al., 2018). Additionally, a cooperative on-farm study in Montana found that yield, grain protein concentration, intra-plant grain protein concentration, and the relationship between yield and protein were influenced by contrasting growing regions and soil moisture availability (Koeshall, 2020). Previous research shows that growing-season climate conditions, which vary across environments and thus growing regions, significantly influence field pea protein productivity (Bueckert et al., 2015).

Precipitation variability, timing, and amount are changing across North America. Holman et al. (2011) reported that precipitation timing across western Kansas over a 56-year study is critical to wheat yield formation, as every 25 mm of precipitation during October resulted in a 229 kg ha^{-1} yield advantage. A study by Basara et al. (2019) detailed the environmental factors that propagated the 2012 flash drought across the central United States, an event which caused an estimated $30 billion loss to agriculture via crop failures, livestock death, and elevated production costs (Basara et al., 2019; “U.S. Billion-Dollar Weather and Climate Disasters, 1980 -

Present (NCEI Accession 0209268)," 2020). Across the northern Great Plains, a drought lasting three to five months has a probability of occurring once every five years, a drought lasting six to eight months has a probability of occurring once every ten years, and a drought lasting nine to 11 months has a probability of occurring once every 20 years (National Integrated Drought Information System, 2025). Amin Preota et al. (2025) found that, from 1904 to 2022, dry-to-dry recurring extreme events have been most prevalent in South Dakota and along the Kansas-Colorado-Texas-Oklahoma border. Additionally, Amin Preota et al. (2025) also found that wet-to-wet recurring extremes are most common in Minnesota, Iowa, Nebraska, and the North Dakota-South Dakota border region.

Long-term temperature patterns have also been changing across the Great Plains regions. A climate study of the Canadian prairies found that, from the 1950s to 2013, the southern Canadian prairie region experienced a shift towards drier conditions, driven by warming, especially in later winter/early spring (Cutforth et al., 1999; Cutforth and Judiesch, 2012; Asong et al., 2018). Baffaut et al. (2025) found that daily minimum temperatures have been increasing faster than maximum temperatures in the central Great Plains, suggesting that nighttime temperatures are warming, likely due to increased atmospheric water vapor. The same study also found that, as the Great Plains have warmed, annual precipitation and rainfall intensity have increased, resulting in precipitation events that deliver greater moisture at higher rates over shorter durations across the landscape (Baffaut et al., 2025). Additionally, a recent article by the Atlantic detailed the oppressive and irregular temperature patterns that the western USA is experiencing, coinciding with the start of the spring 2026 seeding season across the Great Plains

(Boyle, 2026). These significant climate variations and patterns across North America pose challenges for cropping systems and the sustainability of pulse crops, such as field pea.

Heat stress from elevated daytime and nighttime ambient air temperatures throughout the growing season can affect field pea productivity. A study by Guilioni et al. (1997) found that developing field pea pods aborted after only six hours per day for four days at temperatures near 31 °C. Guilioni et al. (2003) reported that moderate heat stress reduced seed number in individual pea plants, a key yield component. These findings suggest that exposure to stressful temperature regimes during the vegetative and reproductive growth stages reduces final yield potential and overall plant productivity. A field-based study by Bueckert et al. (2015) found that field pea grain yield ($r = -0.85$, $p \leq 0.05$) and time in reproductive growth ($r = -0.66$, $p \leq 0.05$) were reduced when field pea was subjected to more than 20 days of daily temperatures above 28 °C throughout the growing season. A greenhouse study found that viability of pea pollen decreased when exposed to daytime temperatures near 36 °C (Jiang et al., 2017). These studies suggest that field pea grain quality and yield potential are strongly associated with growing-season temperature patterns and levels.

Reduced precipitation during both the vegetative and reproductive growth stages of field pea can result in decreased grain protein concentration and yield. Serraj et al. (1999) reported that drought stress reduces N-fixation, attributed to a host of factors, which includes a shortage of carbon substrates available to rhizobium and disruption of phloem flow under dry soil conditions. Lhuillier-Soundélé et al. (1999b) detailed how water stress reduces aboveground biomass in pea, thereby reducing total N-sink structures and potentially decreasing N accumulation in shoots and leaves, ultimately reducing N remobilized to seeds. A field-based

study in the Canadian prairies by Bueckert et al. (2015) found that growing-season precipitation was associated with field pea yield ($r = 0.87$, $p \leq 0.05$), days to maturity ($r = 0.76$, $p \leq 0.05$), and days of reproductive growth ($r = 0.67$, $p \leq 0.05$). Additional studies have also detailed the importance of precipitation timing during the pea plant's life cycle. Reduced precipitation during early vegetative growth of pea can lead to premature cessation of N fixation due to rhizobia death or reduced N fixation (Hossain et al., 2016). Although previous research has elucidated the impact of water stress on grain protein concentration and yield, further investigation is needed to understand the effects of the timing of reduced precipitation during vegetative and reproductive growth stages on field pea productivity. Moreover, additional research is required to enhance understanding of the influence of increased daily mean temperatures on field pea productivity and how the timing of elevated air temperatures throughout the plant's life cycle may affect grain N concentration, grain yield, and grain N yield.

The objective of this study was to evaluate the effects of different timings of imposed warming and rainfall reduction on field pea during three growth periods. We evaluated the effects of altered climate treatments and timing of imposed altered climate conditions on grain N concentration, grain yield, and aboveground biomass through the lens of contrasting genetics for pea growth type; 'determinate' or 'indeterminate'.

2. Materials and Methods

2.1 Site Description

This study was conducted in Bozeman, MT, at the Arthur H. Post Agronomy Farm (8.5 km west of Bozeman) (45.67801, -111.14866) during 2022 – 2024. Each study year was

managed under a no-till soil management system, and all plots were seeded into spring wheat stubble. The predominant soil series for all years of this study was an Amsterdam Typic Haplustoll. The Köppen-Geiger climate classification for Bozeman, MT, is Dfa (Kottek & Rubel, 2017). The study was seeded on 26 Apr 2022, 29 Apr 2023, and 11 Apr 2024. Biomass and grain yield samples were harvested on 2 Aug 2022, 2 Aug 2023, and 31 July 2024. Weather parameters for each study year at Bozeman, including monthly growing-season precipitation, historical 30-year precipitation, and average growing-season temperature, and 30-year historical temperature, are shown in Table 1. All climate data, such as daily temperature, daily precipitation, and growing season precipitation, were obtained from the High Plains Regional Climate Center data service, which was obtained from a weather station located at the Arthur H. Post Agronomy Research Center (station: Bozeman 6 EXP FARM) less than 1 km from our field site each year (*High Plains Regional Climate Center*, 2016).

2.2 Experimental Design

All years of this study were conducted in a randomized complete block design with four replications and 16 experimental units per replication. Before seeding, weeds were controlled using Glystar Plus (glyphosate, N-(phosphonomethyl)glycine, in the form of its isoproplylamine salt) at a rate of 1542 g ai (active ingredient) ha^{-1}, as a pre-emergent herbicide. Each experimental unit was 1.83 m wide and 6.7 m long. Field pea (cv. Delta - determinate and cv. DS Admiral - indeterminate) was used for the entirety of the three-year study (Andersen et al., 2011; Limagrain, 2025). All pea seed was treated with CruiserMaxx Vibrance for pulses at a rate of 3.26 ml kg^{-1} seed before seeding to mitigate potential insect and soil pathogen pressure. All cultivars were seeded at a target pure live stand density of 80 PLS m^{-2}. Pea was seeded in each

experimental unit in rows spaced 23 cm apart with the seed placed approximately 3 cm below the soil surface. *Exceed Granulated Peat for Pea, Vetch, and Lentil* (*Rhizobium leguminosarum biovar viceae* at 1×10^8 colony forming units [CFU] g^{-1}) inoculant was applied to all cultivars at all experimental locations at a rate of 5.7 kg ha^{-1} in-furrow. Monoammonium phosphate (11-52-0-0) and potassium sulfate (0-0-50-18) were blended at equal ratios, resulting in an NPKS starter fertilizer blend (5.5-26-26-9) and applied at a rate of 100 kg ha^{-1} in-furrow at the time of seeding. The foliar insecticide *Warrior II* with *Zeon Technology*® (ai: lambda-cyhalothrin) was used at a rate of 36 g ai ha^{-1} and applied prior to the development of the 5th node growth point to mitigate pea leaf weevil infestations that would decrease yield potential and N fixation (Koeshall et al., 2025). *Assure® II* (quizalofop-P-ethyl) post-emergent herbicide was applied at a rate of 77 g ai ha^{-1} before pea flowering to control grass species during the study.

The study included three treatment factors: pea variety, timing of altered climate imposition, and type of altered climate (warming only vs warming + 50% rainfall reduction). The treatment factor of pea variety included two levels, represented by contrasting growth and development morphology. The cultivar ‘Delta’ was selected to represent a determinate pea type, and cultivar ‘DS Admiral’ was selected to represent an indeterminate pea type. Both cultivars were grown from a common seed source at Bozeman, MT, during the 2021 growing season. The climate alteration treatments had four levels: a control timing that did not receive climate alteration; and growth periods timing coincided with 6th node, pre-flowering, and post flowering. These timings consisted of the BBCH universal plant staging scale of 36 – 51, 51 - 65, and 65 – 79 (Lancashire et al., 1991). Dates of climate-alteration treatments are listed in Table 2 and generally occurred for 14–21 days for each growth period. Warming was imposed using open-

top chambers (OTC) with a design detailed by Marion et al. (1997), each with a basal diameter of 1.5 m and top openings of 1.0 m. The open-top chambers were 0.4 m tall with a 60°-degree incline and were expected to induce 2 ºC warming, as shown by Marion et al. (1997). Comparison of temperatures with a manual thermometer in and outside the OTCs showed daytime temperature differences in the range of 2.0 to 2.1ºC (data not shown). Rainfall reduction (50%) was imposed simultaneously with warming; half the plots that received warming also received rainfall reduction via rain-off shelters (Yahdjian & Sala, 2002). Rain-off shelters measured 2.4 m long and 1.8 m wide and were 45 cm from the ground at the low end, with a 20-degree incline. Rain-off and OTC shelters were placed in the center of each experimental unit.

Following physiological maturity of both field pea cultivars, a 0.5 m sample of two adjacent rows under the center of the OTC was harvested, including all aboveground pea biomass in the sample. The biomass sample was then dried at 55 °C for 72 hr. Dry biomass samples were then weighed to obtain the total dry matter aboveground biomass, and were then threshed with a Wintersteiger harvester (model: Classic; Ried im Innkreis, Austria) as a stationary threshing machine to obtain the total grain yield from each experimental unit biomass sample. A 25-g subsample of harvested grain from each experimental unit was ground in a Udy high-speed centrifugal mill (Udy Cyclone Sample Mill, model 3010-014; Fort Collins, CO) to a particle size of < 2 mm to represent the whole plot. All milled grain samples were analyzed for N concentration using a Leco Truspec CNS Combustion Analyzer (Leco Corp., St Louis, MO). Although it is common to report grain protein concentration, grain N concentration (g kg^{-1}) is used in this study, as the conversion factor (cf = 6.25) applied to most grain quality research is not sufficiently accurate. See Mariotti et al. (2008) for proper conversion values for obtaining

grain protein concentration values from grain N concentration observations reported in this article.

2.3 Statistical Analysis

The timing and type of climate alteration treatments were treated as an explanatory fixed factor, with replication within each study year treated as a random factor. Climate alteration treatments were analyzed separately for each pea type to capture unique genetic effects of the determinate and indeterminate growth habits. The factor of altered climate timing includes both 1) warming and 2) warming with reduced precipitation. The factor of altered climate type includes all levels of timing to test if type of climate alterations influences pea productivity. Modeling of the treatment interactions (timing × type) revealed no significant interaction terms; thus, the interaction terms were removed from the mixed model, and an additive mixed model was applied. Grain N concentration, grain yield, and aboveground biomass were our response variables of interest. Altered climate treatment effects were analyzed using the *nlme*, *lme4*, and *stats* packages in R 4.5.2 (R Core Team, 2025) to obtain main effects, least square means, and Tukey-Kramer "honest significant distance" means comparisons. The significance of all models, contrasts, and mean comparisons was evaluated at an alpha level of 0.10.

3. Results and Discussion

3.1 Effect of Altered Climate Timing for Determinate Pea

In one of three years, the altered climate treatment influenced aboveground biomass ($p = 0.0604$) when applied during the pre-flower treatment (BBCH 51-65) reducing reduced aboveground biomass by 1293 kg ha^{-1} (14.2%) compared to the control for the determinate pea

only (Table 3). Grain yield followed with a reduction ($p = 0.0569$) of 854 kg ha^{-1} (19.4%) in grain yield for the same year and growth period (Table 3). Similarly, the altered climate treatment influenced grain N concentration in one of three years ($p = 0.0732$) with stress timing at the 6th node timing (BBCH 36-51) reducing grain N concentration 3.0 g kg^{-1} compared with the pre-flower (BBCH51-65) timing (Table 3).

These findings reveal potential climate sensitivity during bud formation to early flowering for a determinate growth habit of pea. A review by Fahad et al. (2017) detailed the mechanisms by which heat stress reduces plant productivity, ultimately by increased transpiration, which impairs physiological processes such as nutrient transport and photosynthate production. Additionally, Bueckert et al. (2015) reported how increased temperatures are directly correlated with decreased productivity in pea, in both irrigated and dryland production systems. During 2022, application of warming at the pre-flower timing resulted in a mean daily maximum temperature of 30.7 °C, compared to the control, which experienced a mean daily maximum temperature of 28.6 °C during the same period (data not shown). The application of warming during pre-flower and post-flower resulted in peas experiencing a mean daily maximum temperature above 28 °C (Table 4), which has been shown to reduce plant productivity in previous research (Bueckert et al., 2015).

3.2 Effect of Altered Climate Timing for Indeterminate Pea

The timing of altered climate treatments influenced grain N concentration only in 2022 ($p = 0.0522$) for indeterminate pea, where altered climate timing during the mid-flower to pod fill stage (BBCH 65-79) increased grain N concentration by 3.1-3.2 g kg^{-1} compared with the control and the pre-flower to mid-flower timing (BBCH 51-65). (Table 5). In 2023, unexpectedly,

altered climate treatments increased aboveground biomass ($p = 0.0123$) and pea yield ($p = 0.0182$) (Table 5). Application of altered climate at the pre-flower to mid-flower timing (BBCH 51-65) increased biomass by 3259 kg ha^{-1} (28.6%) and grain yield by 1154 kg ha^{-1} (33.4%) (Table 5). Altered climate timing at the 6^{th} node to early bud stage had a similar increase over the control for grain yield only (27.6%) (Table 5). These results suggest that excess precipitation and cooler temperatures do not always increase plant productivity.

In 2022, the application of altered climate with both warming and reduced rainfall shelters influenced grain N concentration ($p = 0.0522$) for indeterminate pea but not grain yield ($p = 0.4$) or biomass ($p = 0.63$) (Table 5). Application of an altered climate at the pre-flowering stage resulted in the lowest grain N concentration, equal to that of the control (Table 5). Although grain yield was not significantly influenced by timing of altered climate, a reduction in estimated grain yield at the final climate treatment during seed fill aligns with an increase in grain N concentration for the same timing in 2022 (Table 5). Previous work on the relationship between grain yield and grain quality in pea has shown an inverse relationship between yield and protein (Mohammed et al., 2018; Tao et al., 2017). Research by Sita et al. (2018) found that when heat stress reduces both C and N accumulation, the effect on C accumulation is always proportionally greater, suggesting that heat stress affects yield more than grain N concentration as terminal stress typically leads to earlier plant senescence that halts C assimilation while accelerating N remobilization to seeds. Additionally, Ahmed et al. (2015) reported that enzymes responsible for starch formation are limited during heat stress, whereas the N assimilation enzyme glutamine synthetase increases under stress. Furthermore, the pre-flower altered climate treatment in 2024 experienced daily maximum temperatures above 28 °C during the imposition

of heat stress, which has been shown to decrease C fixation (Table 4) (Larmure et al., 2005). Incidence of stress during the pre-flower stage resulted in a grain N concentration that would have received only a \$0.0184 kg^{-1} protein premium. In contrast, all other stress timings would have received the greatest protein premium at \$0.0276 kg^{-1}, in accordance with previous pea protein premium markets accessible in the NGP (Ryan Edinger, personal communication, December 13, 2024).

Application of altered climate during the pre-flower timing in 2023 resulted in the pre-flower treatment experiencing a mean daily maximum temperature of 23.4 °C during application of open-top chambers, while the control experienced 21.3 °C (data not shown). Additionally, warming during the pre-flowering period in 2023 resulted in a mean daily minimum temperature of 9.1 °C, whereas the control experienced 7 °C (data not shown). Previous research has shown that photosynthate fixation rates in pea are not altered between 10 °C and 25 °C (reference needed); thus, these findings in 2023 show that reduced temperatures below 10 °C during initiation of flowering reduced plant productivity (Larmure et al., 2005). Furthermore, this finding suggests that flower and bud formation are reduced when daily minimum temperatures are low, particularly because the control treatment experienced three nights below 4.4 °C and one near 0 °C (data not shown). The increase in temperature during this time due to the warming treatment reduced cold stress during early reproductive development. Thus, these findings indicate that cold stress during flowering reduces grain yield and biomass. A reduction in precipitation of 42 mm during June 2023 benefited biomass and grain yield, as the control treatment may have become waterlogged, thereby reducing plant productivity (Table 5).

3.3 Effect of Altered Climate Type for Determinate Pea

In one of three years, the application of warming and the combination of warming and reduced precipitation influenced grain yield ($p = 0.0563$) for determinate pea (Table 6). In 2022, compared to the control, the application of an altered climate reduced grain yield by 590 and 764 kg ha (Table 6). The presence of warming likely resulted in decreased biomass and grain yield in 2022, as June and July provided mean daily temperatures and maximum temperatures that allowed the heat timing treatments to cause a mean daily temperature above 17.5 °C and maximum temperatures above 28 °C, resulting in an increased growing season stress load that decreased plant productivity (Table 4). Above-average precipitation in June during 2023 and cooler growing-season temperatures likely mitigated any observable warming effects, as increased soil moisture allowed for higher transpiration rates without reducing physiological processes such as photosynthate fixation (Table 1). Furthermore, a larger effect on biomass, grain yield, and grain N concentration may have been revealed because the control treatments experienced, on average, 24 days above 28 °C from seeding to harvest (Table 4).

3.4 Effect of Altered Climate Type for Indeterminate Pea

In one of three years, 2023, climate alteration type influenced grain yield ($p = 0.0208$) and aboveground biomass ($p = 0.0388$) (Table 7) in the indeterminate pea. Climate alteration type did not influence grain yield, biomass in 2022 or 2024, and did not influence grain N concentration in any year (Table 7) in the indeterminate pea. The presence of altered climate treatments increased grain yield and biomass in 2023 by 26 and 19% compared to the control (Table 7) for the indeterminate pea. The increase in biomass and grain yield in 2023 for the indeterminate growth type suggests that above-average precipitation occurred, potentially

resulting in a waterlogged rhizosphere in the control treatment. Biomass and grain yield likely decreased in the control treatment due to 83 mm of precipitation occurring concurrently with the application of reduced precipitation treatments at the 6th node stage in June 2023 (Tables 1 and 2). Furthermore, 129 mm of precipitation was recorded in June 2023, whereas only 60 mm and 41 mm were recorded in 2022 and 2024, respectively (Table 1). The application of drought stress during the 6th node stage in 2023 may have prevented short-term excess soil moisture within the root system, thereby limiting the growth-damaging effects of soil saturation compared with the control treatment. On average, June and July of 2023 were 10% and 4% cooler than June and July of 2022 and 2024, respectively (Table 1).

4. Conclusion

This study revealed that the timing of imposed warming and reduced precipitation affected biomass, grain yield, and grain N concentration differently between determinate and indeterminate pea growth types. The incidence of altered climate during the pre-flower timing resulted in the greatest biomass reduction for a determinate pea. In one of the three years, 2023, the imposition of an altered climate at the pre-flower timing increased grain yield and biomass, largely due to avoidance of late-spring cold temperatures and high rainfall that the control treatment received during an abnormally wet and cool growing season. The reduction in grain N concentration during the pre-flower timing for indeterminate pea was likely due to a reduction in total C transported to rhizobia, due to increased plant stress.

The potential effect of imposing warming via OTC each year may have been confounded by the control plots experiencing daily maximum temperatures of 28 °C for more than 20 days in

2022 and 2024. The timing of reduced precipitation, paired with most of the growing-season precipitation occurring between seeding and the application of reduced precipitation at the 6th node, likely reduced our ability to apply large precipitation reductions between altered climate timings properly. Contrasting growth habits between Delta and DS Admiral reveal varying levels of sensitivity to altered climate, suggesting that reproductive growth habits can be utilized to reduce the effect of warming and drying climates on pea productivity.

Future studies on field pea and imposition of warming or reduced precipitation via the application of OTC or rain-off shelters should implement a study design that applies climate manipulation structures using the following methodology: earlier application of rain-off shelters to impose a greater precipitation gradient in May and June, longer application periods of imposed warming stress, application of reduced precipitation for a longer duration during the growing season, and utilization of rain-off shelters that fully exclude precipitation during altered climate imposition.

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Tables

Table 1: Monthly precipitation and temperature, April to August cumulative growing season precipitation and mean temperature, and the 30-year mean April to August growing season precipitation and temperature for the study site located near Bozeman, MT.

Location	Year	Apr	May	June	July	Aug	Apr - Aug
				Precipitation (mm)			
Bozeman, MT	2022	41	110	60	14	15	240
	2023	19	19	129	25	47	239
	2024	36	89	41	19	26	211
30-year monthly average		**45**	**71**	**71**	**36**	**31**	**253**
				Temperature °C			
Bozeman, MT	2022	2.9	9.1	15.4	21	22	13.9
	2023	4.2	13.3	14.4	20	19	14.2
	2024	7.4	9.5	16.2	20	19	14.5
30-year monthly average		**6.1**	**10.6**	**15.0**	**19.4**	**18.3**	**13.9**

Note: Precipitation and temperature data were obtained through the High Plains Climate Center ACIS-CLIMOD online data service by using the Bozeman 6 EXP FARM climate station, with 30-year monthly climate averages calculated from 1991 to 2020.

Table 2: Timing and duration of imposed altered climate treatments for the study at Bozeman, MT for three years from 2022 to 2024.

Year	Timing of Imposed Altered Climate (BBCH)	Start of Altered Climate	End of Altered Climate
		Date	
2022	Control	NA	NA
	6th Node (36 – 51)*	8 June	29 June
	Pre-Flower (51 – 65)	29 June	21 July
	Post-Flower (65 – 79)	22 July	2 Aug
2023	Control	NA	NA
	6th Node (36 – 51)	24 May	15 June
	Pre-Flower (51 – 65)	15 June	5 July
	Post-Flower (65 – 79)**	5 July	28 July
2024	Control	NA	NA
	6th Node (36 – 51)	12 June	25 June
	Pre-Flower (51 – 65)	25 June	11 July
	Post-Flower (65 – 79)	11 July	24 July

*reduced precipitation shelters were not imposed on the 6th node stage in 2022

**reduced precipitation shelters were not imposed in 2023 during the post-flower stage

Table 3: Determinate pea biomass, grain yield, and grain N concentration response to timing of imposed altered climate treatments from 2022 to 2024 at Bozeman, MT.

Source of Variation (S.V)		Biomass	Grain Yield	Grain N conc.
		kg ha^{-1}	kg ha^{-1}	g kg^{-1}
Altered Climate Timing – 2022 (p-value)		0.06	0.057	0.44
Altered Climate Timing – 2023 (p-value)		0.97	0.71	0.07
Altered Climate Timing – 2024 (p-value)		0.22	0.22	0.11
2022				
	Control	9052 a	4406 a	38.4
	6th Node	8804 ab	4024 ab	39.6
	Pre-Flower	7759 b	3552 b	37.6
	Post-Flower	7980 ab	3700 ab	39.3
2023				
	Control	12580	5294	41.1 ab
	6th Node	12610	5637	38.8 b
	Pre-Flower	12564	5026	41.8 a
	Post-Flower	12141	5084	39.7 ab
2024				
	Control	9378	4763	39.2
	6th Node	9266	4849	38.7
	Pre-Flower	7975	4070	38.6
	Post-Flower	9716	4937	41.3

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.10$.

Table 4: Total days during the growing season during the three-year study at Bozeman, MT that imposition of warming via open-top chambers and control plots experienced daily maximum temperatures above 28 °C and mean daily temperatures above 17.5 °C.

Year	Altered Climate Timing	Daily Maximum Temperature Days above 28 °C	Daily Mean Temperature Days above 17.5 °C
2022	Control	29	41
	6th node	35	43
	Pre-Flower	35	45
	Post-Flower	30	41
2023	Control	17	33
	6th node	18	42
	Pre-Flower	20	35
	Post-Flower	24	34
2024	Control	26	38
	6th node	28	39
	Pre-Flower	28	42
	Post-Flower	26	38

Note: Climate data obtained from the High Plains Climate Center ACIS-CLIMOD online data service by using the Bozeman 6W EXP FARM climate station.

Table 5: Indeterminate pea biomass, grain yield, and grain N concentration response to timing of imposed altered climate treatments from 2022 to 2024 at Bozeman, MT.

Source of Variation (S.V)	Biomass	Grain Yield	Grain N conc.
	kg ha^{-1}	kg ha^{-1}	g kg^{-1}
Altered Climate Timing – 2022 (p-value)	0.63	0.40	0.05
Altered Climate Timing – 2023 (p-value)	0.01	0.02	0.34
Altered Climate Timing – 2024 (p-value)	0.21	0.36	0.16
2022			
Control	9163	3399	38.1 b
6th Node	8893	3355	39.4 ab
Pre-Flower	8665	3192	38.2 b
Post-Flower	8394	2974	41.3 a
2023			
Control	11409 b	3435 b	42.6
6th Node	12921 ab	4383 a	40.9
Pre-Flower	14668 a	4589 a	40.2
Post-Flower	12624 ab	3849 ab	39.7
2024			
Control	9753	4029	39.3
6th Node	8574	3609	40.8
Pre-Flower	10737	4414	39.0
Post-Flower	9783	3986	38.6

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.10$.

Table 6: Determinate pea biomass, grain yield, and grain N concentration response to type of imposed altered climate treatments from 2022 to 2024 at Bozeman, MT.

Source of Variation (S.V)	Biomass	Grain Yield	Grain N conc.
	kg ha^{-1}	kg ha^{-1}	g kg^{-1}
Altered Climate Type – 2022 (p-value)	0.13	0.06	0.20
Altered Climate Type – 2023 (p-value)	0.99	0.90	0.55
Altered Climate Type – 2024 (p-value)	0.83	0.89	0.91
2022			
Control	9052	4406 a	38.4
Warming	8319	3816 b	39.5
Warming + Reduced Precipitation	7905	3642 b	37.5
2023			
Control	12580	5294	41.1
Warming	12457	5176	39.9
Warming + Reduced Precipitation	12401	5395	40.5
2024			
Control	9378	4763	39.2
Warming	9093	4678	39.7
Warming + Reduced Precipitation	8878	4560	39.4

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.10$.

Table 7: Indeterminate pea biomass, grain yield, and grain N concentration response to type of imposed altered climate treatments from 2022 to 2024 at Bozeman, MT.

Source of Variation (S.V)	Biomass	Grain Yield	Grain N conc.
	kg ha^{-1}	kg ha^{-1}	g kg^{-1}
Altered Climate Type – 2022 (p-value)	0.26	0.22	0.38
Altered Climate Type – 2023 (p-value)	0.04	0.02	0.17
Altered Climate Type – 2024 (p-value)	0.98	0.96	0.90
2022			
Control	9163	3399	38.1
Warming	8865	3284	39.6
Warming + Reduced Precipitation	8222	2952	39.7
2023			
Control	11409 b	3435 b	42.6
Warming	13126 a	4134 a	39.9
Warming + Reduced Precipitation	13960 a	4553 a	41.1
2024			
Control	9753	4029	39.3
Warming	9623	3950	39.3
Warming + Reduced Precipitation	9773	4056	39.6

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.10$.

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CHAPTER SEVEN

LEGUME-BASED CROP ROTATIONS INCREASE SOIL ORGANIC CARBON ACCRUAL IN NORTHERN GREAT PLAINS CROPPING SYSTEMS

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Abstract

Soil organic C (SOC) provides the foundation for resilient soil health and crop production. The northern Great Plains (NGP) contains contrasting crop rotations paired with differing seeding and harvesting methods found throughout the region. Carbon markets have created opportunities for agricultural producers to be paid for atmospheric CO_2 sequestered in SOC. There is a need to understand how crop rotations and soil disturbance influence accrual of SOC to inform agricultural producers and C markets. We established a study in Bozeman, MT, in 2016 to evaluate eight crop rotations emblematic of systems throughout Montana and two soil disturbance methods that represent current seeding and harvesting practices. Soil was sampled for SOC in 2016 and 2022 to assess SOC accrual resulting from crop rotation and soil disturbance after six years. Aboveground biomass was sampled for all crop and forage species to estimate plant residue returned to the soil surface. Crop rotation influenced aboveground biomass ($p < 0.0001$) and biomass C ($p < 0.0001$). Crop rotation influenced SOC (Mg ha^{-1}) in the 0-10 cm depth layer ($p = 0.0245$) greater than in the 10-20 and 20-30 cm depth layers. The 'short stubble – hoe opener seeder ' soil disturbance treatment increased SOC mass in the 0-10 cm depth by 0.8 Mg ha^{-1} ($p = 0.061$). Accrual of SOC was optimized under legume-cereal rotations ($p = 0.0003$) at a rate of 0.41 Mg SOC ha^{-1} yr^{-1}. Our results suggest that 1.92 Mg ha^{-1} yr^{-1} of biomass C was needed to maintain SOC in this environment.

1. Introduction

Soil provides the foundation to most modern food and fiber production globally (Paustian et al., 2016a). Recent estimates report that 4.76 billion ha of agricultural land is utilized globally, with 1.5 to 1.6 billion ha for cropland production, which represents approximately one-third of all agriculture land (Tubiello et al., 2023). In North America, the northern Great Plains (NGP) region contains approximately 58.5 million ha of cropland, with this region producing 34% of all US wheat (*Triticum aestivum* L) (AAFC, 2026; USDA, 2026). Across North America, winter wheat yields have increased by 0.79 to 1.1% every year from 1959 to 2008 (Graybosch & Peterson, 2010). Global agriculture systems have successfully increased food and fiber production for a growing population, with global cereal production doubling from 1960 to 2000 (Tilman et al., 2002).

Agricultural soils have experienced a decline in soil organic carbon (SOC) inventory with the adoption of agrarian practices such as tillage and the decline of perennial grasslands globally (Paustian et al., 2016a; Sanderman et al., 2017; Tilman et al., 2002). However, cropland soils across North America have experienced degradation of soil ecosystems over the last century, particularly in SOC inventory, as food and fiber production has increased (Janzen, 2006; M. A. Liebig et al., 2024; Paustian et al., 2016b; Tilman et al., 2002). Aase & Pikul (1995) found that cropping systems in northeast Montana managed under a fallow-crop rotation experienced a 0.4 g kg^{-1} loss per year in SOC over a ten year study. Sanderman et al. (2017) estimated that agriculture has caused a 116 Pg C debt within the top 2 m of soil globally. Engel et al. (2017) found that after ten years of annual crop production, a tilled fallow-wheat rotation lost nearly 3 Mg ha^{-1} of SOC, while a no-till fallow-wheat rotation lost approximately 1.4 Mg ha^{-1}. Engel et

al. (2017) also found that continuous wheat and legume/oilseed-wheat rotations were the only annual crop rotations that maintained SOC. A research study by Cotton & Acosta-Martínez (2018) found that grasslands converted to tillable row-crop production lost 33% of organic C and 30% of total N, with SOC decreasing by 20% in the top 30 cm of the soil profile following one growing season. Hangs et al. (2024) reported that specific agricultural soil types across the Canadian prairies lost 15–30% of their SOC inventory following the adoption of traditional cereal production and tillage. M. A. Liebig et al. (2024) evaluated a 71-year soil archive from locations across the USA Great Plains and found that SOC decreased by 15 and 36% in Montana and Texas within the 0-15.2 cm depth layer, respectively. Soil organic carbon changes documented by M. A. Liebig et al. (2024) found that much of the SOC loss over 71-years was attributed to tillage and reduced additions of biomass C inputs to the soil profile due to adoption of fallow. These findings suggest that alternative and progressive crop rotations for the NGP should be investigated to identify potential species and diverse rotations that limit SOC loss or foster SOC gains over time.

Pea (*Pisum sativum* L.) and other pulse crops are commonly grown in rotation with cereals, such as winter wheat, across a large portion of the northern and central Great Plains. This has resulted in pulse crops being seeded into variable-height cereal stubble due to contrasting direct-cut harvesting methods used across this region. Research by Cutforth & McConkey (1997) demonstrated that wheat grain yields and estimated water-use efficiency (WUE) increased by 12% when seeded into standing stubble compared to cultivated treatments. Subsequent investigations by Cutforth et al. (2006) reported that tall cereal stubble increased canola (*Brassica napus* L.) yields by 24% and improved WUE by 19%. Further evidence

suggests that as stubble height increases, grain yields also increase, as Cutforth et al. (2011) observed that extra-tall stubble increased yields of pea, canola, and wheat by an average of 17%.

Historically, hoe-opener seeders were the predominant seeding technology across the NGP of the USA and the Canadian Prairies, favored for their mechanical reliability, ease of maintenance, and versatility across diverse conditions. While these systems dominated the market through the 1990s, the adoption of stripper harvester technology facilitated a shift toward no-till disc air-seeders designed to operate within tall, high-stubble architectures or heavy surface residues (Chaudhuri, 2001). Conversely, hoe-opener seeders remain the standard in cropping systems utilizing conventional direct-cut draper headers. Hoe-opener configurations often exhibit superior performance in the high-strength, compacted soils characteristic of semi-arid regions, whereas no-till disc seeders are optimized for residue clearance and minimal soil disturbance (Chaudhuri, 2001;). The divergence between the stripper header/disc seeder and draper header/hoe-opener management types create distinct soil disturbance and residue management regimes during seeding and harvest. It is unknown how these contrasting residue management and seeding strategies affect SOC dynamics. Such management-induced shifts in microclimate, moisture retention, and soil biological activity likely alter long-term SOC sequestration and accrual rates.

Long-term agricultural research indicates that C and nitrogen (N) losses can be mitigated through diverse crop rotations and species selection. Drinkwater et al. (1998) demonstrated that integrating low C:N organic residues in combination with increased species diversity into crop rotations over 15 years enhanced soil C and N retention in a Pennsylvania cropping system. Furthermore, diverse rotations and enhanced species diversity have been shown to suppress pest

populations, thereby reducing their deleterious effects on overall system productivity. Engel et al. (2017) reported that a 10-year no-till pea/oilseed-wheat rotation resulted in an SOC accrual rate of 0.09 Mg ha^{-1} yr^{-1}, whereas significant SOC depletion occurred under tilled fallow-wheat. These findings suggest that the addition of biomass C inputs is a crucial driver of SOC accrual or loss over time, as Engel et al. (2017) discovered a strong relationship between biomass C inputs and change in SOC inventory ($R^2 = 0.86$).

Over the past two decades, voluntary C markets within global agricultural systems have garnered significant attention, characterized by a recent expansion in program availability, research interest, and government incentive (Dawes et al., 2023; Dongre et al., 2025; Johansson et al., 2025a). These markets provide a mechanism for land managers to monetize management interventions that sequester atmospheric C within SOC inventory. Incentives are generally structured through two primary pathways: (1) practice-based payments for adopting management suites known to foster SOC accrual, such as conservation tillage or cover cropping, or (2) outcome-based payments tied to measured SOC accrual verified via periodic soil analysis (Johansson et al., 2025b; Souza et al., 2025). Despite this potential, producer adoption remains constrained by program complexity and a lack of ecoregion-specific data regarding SOC sequestration efficacy of various agronomic practices (Pudasaini et al., 2025). Furthermore, inconsistencies in payment schedules, disparate reporting of sequestration rates for identical practices, and market price volatility create significant economic uncertainty (Sellars et al., 2021; Souza et al., 2025). These challenges underscore a critical need to quantify how specific factors, such as crop rotation, soil disturbance intensity, species diversity, and residue management,

influence SOC accrual rates across the NGP, and thus the potential C credits that NGP farmers could monetize to increase farm-scale gross revenue.

The primary objective of this study is to evaluate 1) the influence of contrasting crop rotations common to Montana based on species diversity and cropping intensity, soil disturbance, and stubble management on SOC inventory and change in SOC adjusted for equivalent mass, and 2) the relationship between the change in SOC over time and returned aboveground biomass, and the change in SOC over time and biomass C inputs from returned aboveground crop residue.

2. Materials and Methods

2.1 Site Description

This study was initiated Sep 2016 at the Montana State University- Post Agronomy Research Farm (8.5 km west of Bozeman, MT) (45.672, -111.154). The predominant soil series for all years of this study was an Amsterdam Typic Haplustoll. The Köppen-Geiger climate classification for Bozeman, MT, is Dfa (Kottek, M., & Rubel, 2017). Prior to study initiation the site was uniformly cropped under low-disturbance no-till management with a four-year rotation of yellow mustard (*Sinapis alba* L.) - winter wheat – lentil (*Lens culinaris* Medik.) - spring wheat. Mean seasonal temperature and precipitation were very close to 30-year means (Table 1). The site received an average of 404 mm (15.9 in.) of annual precipitation from 2016 to 2022, with a mean annual temperature of 6.9 °C (44.4°F) (Table 1). All climate data, such as daily temperature, daily precipitation, and growing season precipitation, were obtained from the High Plains Regional Climate Center data service, which was obtained from a weather station located

at the Arthur H. Agronomy Research Center (Bozeman 6W EXP) within 300 m of our field site each year (*High Plains Regional Climate Center*, 2016).

2.2 Experimental Design

This field study was constructed as a randomized complete block design with eight crop rotation treatments, measuring 14 × 7.3 m each, as the main plot factor, replicated four times. The eight crop rotation main plots were divided longitudinally (i.e. 3.65 m wide) into subplots representing two soil disturbance/stubble management levels in a split-plot arrangement. Each crop rotation main-plot treatment was constructed to represent emblematic crop rotations found in different regions of Montana (Table 2). The base crop rotation is the North Central-Traditional (NC-Trad) rotation that alternates chemical fallow with winter wheat. The NC-Trad rotation is found across Montana and the greater NGP, especially in low rainfall semi-arid regions. The NC-Trad rotation was paired with the Southwest-Progressive (SW-Pro), which alternates spring canola and spring lentil with winter wheat. The North Central-Progressive (NC-Pro) rotation includes three grain crops in four years with a single fallow year (fallow – winter wheat – lentil – spring wheat) , which is considered progressive cropping intensity for this major fallow-winter wheat region. The NC-Pro rotation was paired with a duplicate rotation, North Central-Progressive plus cover (NC-Pro+), in order to augment crop species C inputs by adding a three-species 60-day cover crop mixture that contained oat (*Avena sativa* L.), radish (*Raphanus sativus* L.), and faba bean (*Vicia faba* L.) from early May to early July during the fallow season, and established post-season cover crop using winter canola following the lentil season. Northeastern Montana is a major pulse crop producing region; thus the Northeast-Progressive (NE-Pro) rotation focuses on pulse crop production with spring field pea and spring lentil in rotation with

spring wheat. The NE-Pro rotation would more likely grow durum spring wheat (*Triticum turgidum* subsp. *durum*), however this study utilized spring wheat to allow stronger experimental comparisons. The NE-Pro rotation was duplicated in the Northeast-Progressive plus cover (NE-Pro+) rotation that establishes barley (*Hordeum vulgare* L.) following wheat harvest and a fall cover, winter canola, following lentil harvest. The Southeast-Progressive (SE-Pro) rotation includedd Austrian winter pea, to represent winter pea adaptation to the south-central and south-east regions of Montana, combined with warm-season crop diversity utilizing safflower (*Carthamus tinctorius* L) and proso millet (*Panicum miliaceum* L). We recognize that crop and livestock operations are commonly integrated across south-central and south-east Montana, thus, the SE-Pro rotation was complemented by the Southeast-Ranch (SE-Ranch) rotation. The SE-Ranch rotation emphasizes forage production by using a fall barley cover crop to augment volunteer winter wheat, replacing safflower grain production with full-season summer-seeded cover crop for haying that contained millet, spineless safflower, and fava bean and cool-season hay barley relayed with warm-season millet to foster successive hay crops (the millet crop was unsuccessful, consistent with similar treatments in two other Montana locations in earlier experiments, and so this treatment was abandoned after 2020).

The two soil disturbance/stubble management sub-plot treatments were constructed to represent closely associated harvesting and seeding methods commonly found across Montanan and the greater NGP. The first sub-plot treatment, Tall-Disk, replicates the use of tall crop stubble (41-46 cm) seeded with a low-disturbance no-till disk opener to represent the use of stripper for cereal and oilseed crops (*AOF Commodity Outlooks | USDA*, 2026). The second sub-plot treatment, Short-Hoe, replicated the use of traditional hoe-type knife opener seeders paired

with short stubble (10-15 cm) to replicate traditional cutting heights of cereal grain crops, common in areas affected by wheat stem sawfly populations that necessitate the use of low harvest heights. The sub-plot treatment level will be referred to as soil disturbance.

Following harvest of grain or forage crops, all experimental unit subplots were soil sampled to a depth of 1.07 m to obtain soil N requirements for the subsequent crop. Adjustments to available nitrate N before seeding included estimated over-winter mineralization soil organic nitrogen (25 kg N ha^{-1}) and legume N credits (20 kg N ha^{-1}). Urea fertilizer (46-0-0) was applied to satisfy remaining plant N needs according to crop yield goals, mostly commonly by subsurface banding 7-10 cm to the side of the seed furrow at seeding or occasionally (winter wheat only) surface broadcast with a *Valmar* (Salford Group, Cornelia, GA) pneumatic dry fertilizer spreader. A starter fertilizer blend of N-P-K-S (5.5-26-26-9) was applied in-furrow at 100 kg ha^{-1} during seeding of all crops. An additional 89 kg ha^{-1} of monoammonium phosphate (11-52-0-0) was also applied in-furrow in winter wheat only (2018 and 2022), to better match crop removal of P for high yielding winter wheat. The yield targets were 4.5 Mg ha^{-1} for spring wheat, 6.0 Mg ha^{-1} for winter wheat, and 3.0 Mg ha^{-1} for oilseeds.

2.3 Soil Sample Collection, Processing, and Organic Carbon

In 2016 Sep, we collected one 5-cm diam. soil core in three depth increments (0-10, 10-20, and 20-30 cm) with a truck-mounted hydraulic probe (Giddings Machine Co., 10-SCS / Model GSP) from the center of each main plot prior to the initiation of crop rotations in spring 2017 or fall 2016 for the SE rotations (Austrian winter pea). The core samples were weighed to obtain wet mass for soil moisture calculations and then placed in a lab oven at 50 °C for 72 hrs. and then weighed for dry mass, from which bulk density was determined. Dry cores were

crushed to pass through a 2-mm sieve. Cores were crushed and sieved with a motor driven barrel sieve mil assembly. A sub-sample of the 2-mm sieved material (20 – 30 g) was then separated, and identifiable crop residue material, including roots, were removed by hand-picking with tweezers, with 10 minutes allotted to each subsample. The soil subsample was then placed in a jar mill for 12 hours where it was ground into a fine powder (95% passing through a 105 µm sieve). Subsamples of fine milled soil material were analyzed for total carbon (TC) by dry combustion using a Costech ECS 4010 (Costech Analytical Technologies, INC; Valencia, CA). The inorganic C (IC) fraction was determined by the modified pressure calcimeter method of Sherrod et al. (2002).

2.4 Aboveground Biomass and Carbon Inputs

Aboveground biomass, crop, and forage residues returned to the soil surface were based on hand-harvests of 0.91 m^2 of the aboveground crop biomass from all sub-plots prior to grain or after forage harvest where approximately 10 cm of basal plant tissue remained. Grain was removed from the crop biomass by threshing with a Wintersteiger harvester (model: Classic; Ried im Innkreis, Austria) used as a stationary threshing machine. All plant residue sub-samples were retained into order to calculate the C concentration using dry combustion with a Leco Truspec CN analyzer (Leco Corp, Saint Joseph, MI) of aboveground biomass residues that were returned to the soil surface. We calculated the quantity of aboveground biomass C inputs to each crop rotation by multiplying crop straw and forage residue yields by the estimated C concentration obtained via Leco analysis. Crop and forage residues commonly had a C concentration of 0.44 for the aboveground residue dry mass that was measured in this study.

2.5 Soil Organic Carbon Equivalent Mass Calculations

Soil organic C concentrations for each depth layer (0-10, 10-20, and 20-30 cm) was converted to a mass per unit area basis multiplying the C concentration, mean bulk density across the study within the specific sample depth, and the sampling depth (10 cm). The SOC mass for the total sampling depth (0-30 cm) was calculated by summing the mass for each depth layer. Due to differences in bulk density among individual experimental units affecting the amount of C in the sample, the principle of equivalent mass was applied to the final comparison of SOC mass among the eight crop rotations (Ellert & Bettany, 1995). In this study, the 100% equivalent mass of all subplots (2016 and 2022) was established from the mean of the lowest bulk density crop rotation or the experimental units in replications one and two in 2016. The following equivalent mass calculations were used to minimize deviations caused by variations in soil mass among the crop rotations and soil disturbance treatments that would affect estimates of SOC inventory based on a fixed depth:

$$SOC_{EM} = SOC_{0-30} - \left[\left(\frac{Mass_{0-30} - 3702}{Mass_{20-30}}\right) \times SOC_{20-30}\right]$$

where SOC_{EM} was the SOC inventory based on equivalent mass (Mg C ha^{-1}), SOC_{0-30} was the SOC inventory based on a fixed sampling depth (Mg C ha^{-1}), $Mass_{0-30}$ was the soil mass in grams of the 0- to 30-cm sampling depth, 3702 grams was the mean mass of the crop rotations in replications one and two in 2022, $Mass_{20-30}$ was the soil mass in grams of the 20- to 30-cm sampling depth, and the SOC_{20-30} was the SOC inventory found in the 20- to 30-cm depth layer. The change in SOC equivalent mass (ΔSOC_{EM}) over the six years of this study was calculated using the equation below as follows:

$$\Delta SOC_{EM} = SOC_{EM2022} - SOC_{EM2016}$$

were SOC_{EM2022} and SOC_{EM2016} was the SOC_{EM} for samples collected in 2022 and 2016, respectively.

2.6 Statistical Analysis

The whole plot unit of crop rotation and the sub-plot unit of soil disturbance were treated as explanatory fixed factors, with replication treated as a random factor. Aboveground biomass, biomass C, SOC inventory in 2022, and ΔSOC_{EM} were our response variables of interest. Linear mixed-models were applied to all response variables to test the main effects of crop rotation and soil disturbance and the interaction effect of crop rotation × soil disturbance using the *nlme*, *lme4*, and *stats* packages in R 4.5.2 (R Core Team, 2025) to obtain main effects, least square means, and Tukey-Kramer "honest significant distance" means comparisons. Significance was accessed at an alpha of 0.10. Aboveground biomass and biomass C were correalted against ΔSOC_{EM} using the *corr* package in R 4.5.2 to conduct Pearson correlation analysis and coefficients. The packages *ggplot2* and *ggthemes* in R 4.5.2 were used to graph and visualize least square means, Tukey-Kramer means comparisons, and Pearson correlations.

3. Results and Discussion

3.1 Returned Aboveground Biomass and Biomass Carbon

Crop rotation type influenced cumulative returned aboveground biomass ($p < 0.0001$) over six years among eight emblematic rotations in Montana (Table 3). Soil disturbance ($p = 0.19$) and the interaction of crop rotation × soil disturbance ($p = 0.68$) did not influence cumulative returned aboveground biomass (Table 3). After six years, the SE-Pro rotation

returned the greatest aboveground biomass (36.7 Mg ha^{-1}) while the SE-Ranch rotation returned the least (27.1 Mg ha^{-1}) due to frequent hay removal.

The SE-Pro rotation included winter pea twice in six years, in 2017 and 2021, and did not include summer fallow (Miller et al., 2018; Miller et al., 2011). Additionally, the NE-Pro and NE-Pro+ rotations utilized an alternating sequence of legumes and cereals, which has been reported to be advantageous compared to a traditional fallow-wheat rotation due to increased annualized returns and increased cereal yields (Koeshall et al., 2022; Miller & Holmes, 2005). Due to fallow every other year, the NC-Trad rotation averaged 5.5 Mg ha^{-1} (16.3%) less aboveground biomass across six years compared to three annually cropped wheat-based systems (NE Pro, NE Pro+, and SW Pro) (Table 3). It is somewhat surprising that this difference wasn't larger, but three of six years were notably drier than normal which likely limited productivity from the annually cropped systems (Table 1). Overall, returned aboveground biomass is positively influenced in crop rotations that avoid fallow periods, alternate crop species among a diverse panel of legume, cereal, and oilseed species, and prioritize grain production over haying.

Returned biomass C was affected by crop rotation ($p < 0.0001$) following six years of management among eight crop rotations (Table 3). Soil disturbance ($p = 0.18$) and the interaction of crop rotation × soil disturbance ($p = 0.63$) did not influence returned biomass C after six years (Table 3). The SE-Pro rotation resulted in the greatest amount of returned biomass C at 16.61 Mg ha^{-1}, followed by NE-Pro (15.7 Mg ha^{-1}), NE-Pro+ (15.5 Mg ha^{-1}), and SW-Pro (15.2 Mg ha^{-1}) (Table 3). The SE-Ranch rotation returned the least amount of biomass C among all rotations at 12.2 Mg ha^{-1} (Table 3). Results among eight crop rotations show that returned biomass and biomass C treatment differences align (Table 3), suggesting that rotation differences, such as use

of fallow, legumes in rotation with cereal and oilseed species, and the presence of grain production rather than haying, positively influenced biomass C. The contrasting C:N ratio found in legume biomass compared to cereal and oilseed biomass may have significantly influenced total biomass C in rotations that contained legumes, compared to a traditional fallow-wheat rotation (NC-Trad) (Lupwayi & Soon, 2015, 2016).

3.2 Soil Organic Carbon at Fixed Sampling Depths (0-10, 10-20, and 20-30 cm)

In 2022, SOC mass was affected by the main effect of crop rotation and soil disturbance, with the interaction of crop rotation × soil disturbance influencing the deepest sampling depth (0-10, 10-20, and 20-30 cm; Tables 4 – 6). Soil organic carbon mass differences among crop rotations and soil disturbance treatments were largest within the 0-10 cm depth, then the 10-20 or 20-30 cm depths, suggesting that most of the C addition to SOC inventory originates at the soil surface as SOC accrual is driven by aboveground biomass C inputs, in addition to belowground C inputs from decaying root systems. SOC mass within the 0-10 cm sampling was influenced by the main effects of crop rotation ($p = 0.0245$) and soil disturbance ($p = 0.037$), but not the interaction term of crop rotation × soil disturbance (Table 5). The NC-Pro+ rotation contained 21.3 Mg ha^{-1} of SOC, while the NC-Trad rotation contained the lowest SOC mass (19.03 Mg ha^{-1}) (Table 4). Among all crop rotations, the use of traditional fallow-wheat hindered SOC inventory in 2022, even after only six years of crop rotation treatment implementation, with the NC-Pro+ rotation containing 2.2 Mg ha^{-1} more SOC mass in the 0-10 cm sampling depth. The short-hoe soil disturbance treatment contained greater SOC mass (20.7 Mg ha^{-1}) in the 0-10 cm sampling depth than the tall-disk soil disturbance treatment (19.9 Mg ha^{-1}) (Table 4). This difference between the two soil disturbance treatments represents a small (0.8 Mg ha^{-1}) but

consistent difference in SOC mass within the 0-10 cm sampling depth. After six years of contrasting soil disturbance and stubble management, crop rotations that were subjected to increased soil disturbance at seeding and traditional harvest methods contained greater SOC mass at the soil surface. This was not expected and suggests that greater residue processing at harvest for grain crops and increased residue mixing with the soil surface increases SOC inventory within the 0-10 cm depth layer.

Soil organic carbon mass within the 10-20 cm sampling depth was affected by crop rotation ($p = 0.0089$) but not soil disturbance ($p = 0.74$) or the interaction of crop rotation × soil disturbance ($p = 0.13$) (Table 5). The NC-Pro, NC-Pro+, and NE-Pro+ annually cropped rotations contained the greatest SOC mass, all greater than the NC-Trad rotation (Table 5). Compared to the three rotations that contained the greatest amount of SOC mass, the NC-Trad rotation contained 5% less SOC mass (Table 5). Increased addition of belowground biomass from root systems likely benefited crop rotations such as NC-Pro and NE-Pro+, which utilized alternating legume-cereal sequences. The addition of more biomass N in rotations that contained legume species likely increased incorporation rates of biomass C due to optimized C:N ratios present in soil surface crop residues (Lupwayi & Soon, 2015, 2016).

Soil organic carbon mass within the 20-30 cm sampling depth was not influenced by the main effect of crop rotation ($p = 0.81$) or soil disturbance ($p = 0.71$) but had interaction of crop rotation × soil disturbance that did not appear to be meaningful ($p = 0.0252$) (Table 6). Therefore, we show each rotation-disturbance combination in Table 6. The combination of SE-Ranch × short-hoe resulted in the greatest SOC mass (11.0 Mg ha^{-1}) at the 20-30 cm depth, while the NC-Pro × tall-disk combination contained the least (9.7 Mg ha^{-1}) (Table 6). Most of the

treatment differences within the 20-30 cm sampling depth may be due to noise captured across the study in 2022 at that depth, as the SE-Ranch crop rotation yielded the least aboveground biomass and biomass C (Table 6).

3.3 Change in SOC Equivalent Mass (2016 – 2022)

Following six years of crop production, the calculated ΔSOC_{EM} was affected by crop rotation ($p = 0.0003$) and soil disturbance ($p = 0.061$) or the interaction of crop rotation × soil disturbance (Table 7). Accrual of SOC_{EM} was observed in six crop rotations, including the NC-Trad rotation (0.17 Mg ha^{-1} yr^{-1}) (Table 8, Figure 1). Accrual of SOC_{EM} was greatest in three rotations as follows: NE-Pro (0.41 Mg ha^{-1} yr^{-1}), NC-Pro+ (0.39 Mg ha^{-1} yr^{-1}), and NC-Pro (0.35 Mg ha^{-1} yr^{-1}) (Table 8, Figure 1). Accrual of SOC_{EM} averaged 0.30 Mg ha^{-1} yr^{-1} among crop rotations that fostered a gain of SOC_{EM} over time (Table 8). Loss of SOC_{EM} was observed in the SE-Pro and SE-Ranch rotations, at -0.13 and -0.14 Mg ha^{-1} yr^{-1} (Table 8). Engel et al. (2017) reported similar modest losses of SOC_{EM} in rotations that utilized fallow, with SOC_{EM} losses similar to the SE-Pro rotation (Table 8). Loss of SOC_{EM} was greatest in the SE-Ranch rotation, which was emblematic of integrated crop-livestock operations in southeast MT and included haying practices that limited return of aboveground biomass to the soil surface. Compared to the NC-Trad rotation, the NE-Pro, NC-Pro+, and NC-Pro rotations accrued 0.24, 0.22, and 0.18 Mg ha^{-1} yr^{-1} more SOC_{EM} (Table 8). Liebig et al. (2005) reported that a no-till cropping system under continuous annual crop production in the northwestern USA will accrue SOC_{EM} at 0.24 ± 0.19 Mg ha^{-1} yr^{-1}, which aligns with the accrual values we observed over six years in this study (Table 8). Both soil disturbance treatments resulted in SOC_{EM} accrual with tall-disk at 0.14 Mg ha^{-1} yr^{-1}

and 0.27 Mg ha^{-1} yr^{-1} for short-hoe (Table 8). However, there was no difference in SOC_{EM} accrual between soil disturbance treatments ($p = 0.061$) (Table 8).

We were surprised by the decline in SOM_{EM} in the SE-Pro rotation, and to a lesser extent, the SE-Ranch rotation (Table 8, Figure 1). The SE-Pro rotation did not use summer fallow, incorporated a variety of grain crop species such as winter pea, safflower, and millet, and included alternate species sequencing. Precipitation and temperature during the 2020 and 2021 growing seasons may have negatively affected the SE-Pro and SE-Ranch systems. Mean annual precipitation during 2020 was 290 mm, 111 mm less than the 30-year annual average from 1991 to 2020 (Table 1). Below-average precipitation in May 2020 and June 2021 may have limited biomass accumulation in the SE-Pro rotation that was growing safflower and millet (Table 1). Over six years, the cumulative precipitation during the April to August growing season in 2020 and 2021 was 78 and 43 mm below the 30-year average for April to August (Table 1). Additionally, four of six growing seasons had above-average temperatures, and two of six had mean annual temperatures greater than the 30-year annual average (Table 1). The change in these climate conditions during these years may have contributed to the loss of SOC_{EM} in the SE-Pro and SE-Ranch rotations, particularly if biomass C additions were limited in 2020 and 2021, paired with elevated growing season temperatures that sustained N mineralization from organic matter pools.

We were also surprised that the ‘tall stubble-disk seeder’ soil disturbance treatment resulted in increased SOC accrual after six years. Although tall stubble does reduce potential wind erosion and increases precipitation use efficiency as lentil grain yields increased by 15% when grown in tall stubble in this study during growing seasons with below-average

precipitation (data not shown), the incorporation of aboveground biomass C via increased soil contact from the hoe-opener seeding method resulted in a 2-fold SOC accrual difference across all eight crop rotations after six years (Barnes, 2025; Cutforth et al., 2011; Cutforth & McConkey, 1997). This finding represents an interesting management choice for NGP crop producers as our results suggest that a traditional seeding and harvesting method increase SOC accrual, while previous research has found that the presence of tall stubble increases crop yields while reducing potential wind erosion (Cutforth et al., 2011; Cutforth et al., 2006; Cutforth & McConkey, 1997).

Among the six crop rotations that presented accrual of SOC_{EM}, the potential value of estimated CO_2 credits varied, utilizing two pricing models (Table 8). Under a low-value model ($25 Mg CO_2 ha^{-1} yr^{-1}), the mean C market value was $27.47 ha^{-1} yr^{-1} across the six crop rotations (Table 8). The NE-Pro rotation would receive $37.45 ha^{-1} yr^{-1} with an annual SOC_{EM} accrual of 0.41 Mg ha^{-1} yr^{-1}, followed by the NC-Pro+ rotation receiving $35.56 ha^{-1} yr^{-1} from an annual SOC_{EM} accrual of 0.39 Mg ha^{-1} yr^{-1} (Table 8). The low pricing model suggests that a traditional no-till fallow-wheat rotation (NC-Trad) would receive $15.58 ha^{-1} yr^{-1} from an annual SOC_{EM} accrual of 0.17 Mg^{-1} ha^{-1} (Table 8). In a high-value model, the mean C market value was $49.45 ha^{-1} yr^{-1} across the six crop rotations. The NE-Pro and NC-Pro+ rotations would receive $67.41 and $64.01 ha^{-1} yr^{-1}, respectively (Table 8). Although the NC-Trad rotation yielded the lowest SOC_{EM} accrual, under the high-value model, this rotation would receive $28.05 ha^{-1} yr^{-1}. These models show that diverse crop rotations that utilize pulse crops for grain, minimize fallow, and alternate legume species with cereal and oilseed crops can sequester atmospheric C through crop production, and the potential value of monetizing progressive crop rotations across the

NGP. Additionally, these findings show that progressive crop rotations have an SOC_{EM} accrual advantage compared to the traditional fallow-wheat system, which can accrue SOC_{EM}, contradicting previous research findings (Engel et al., 2017; M. Liebig et al., 2005; Silva et al., 2026). However, a fallow-wheat rotation (NC-Trad) depends on the biomass C from the winter wheat crop, which could be compromised by growing-season flash droughts or acute pest and pathogen issues. Additionally, a wheat-fallow rotation in North America has been shown to increase exposure to market variability and to a greater variation in annualized net returns at the farm gate (Koeshall et al., 2022; P. Miller et al., 2015; Nielsen et al., 2005).

Across all crop rotation and soil disturbance treatments, post-hoc contrasts were used to assess whether specific management practices were associated with differences in SOC_{EM} accrual. The management practices of growing lentil ($p = 0.0006$) and haying ($p = 0.0197$) influenced SOC_{EM}, but the practice of growing field pea ($p = 0.0662$) and using fallow ($p = 0.57$) did not. Crop rotations that contained lentil as a grain crop (SW-Pro, NC-Pro, NC-Pro+, NE-Pro, and NE-Pro+) accrued 1.96 Mg ha^{-1} of SOC_{EM} following six years of crop production, compared to rotations that lost at a rate of -0.19 Mg ha^{-1} (SE-Pro, SE-Ranch, and NC-Trad) (Table 8). Across all crop rotations, haying (a practice unique to the SE-Ranch rotation) reduced SOC_{EM} over time (-0.81 Mg ha^{-1}), while on average, rotations that did not remove biomass through haying increased SOC_{EM} (1.48 Mg ha^{-1}). This contrast would be greater if the SE-Pro rotation was removed from the post-hoc contrast. The post-hoc contrasts provided limited insight into how a single management practice may influence SOC_{EM} accrual and suggests that a combination of practices, in combination with proper species sequencing, is likely responsible for accrual or loss of SOC_{EM}.

3.4 Change in SOC Equivalent Mass vs Returned Biomass and Biomass Carbon Input

The relationship between ΔSOC_{EM} and returned aboveground biomass was weakly defined ($r = 0.28$, $p = 0.0063$) by the by linear relationships among 12 crop rotation × soil disturbance (excluding the SE-Ranch and SE-Pro observations) (Figure 2). The ΔSOC_{EM} changed about 0.27 Mg ha^{-1} for every Mg unit of returned aboveground biomass (Figure 2). The relationship between ΔSOC_{EM} and returned aboveground biomass revealed that 4.25 Mg ha^{-1} yr^{-1} of returned biomass was needed for maintenance of SOC_{EM} (Figure 2). Engel et al. (2017) reported that 7.0 Mg ha^{-1} yr^{-1} of total net primary productivity was needed for SOC_{EM} stabilization; however, total net primary productivity accounts for total aboveground biomass production (shoot plus grain), while our relationship only accounts for returned aboveground biomass. Aboveground biomass may be a poor predictor of ΔSOC_{EM} across diverse crop rotations, given our results. Rasse et al. (2005) reported that accrual of SOC is more determined by root system C contributions compared to shoot C contributions. Additionally, Mazzilli et al. (2015) found that the humification rate of below-ground C additions into SOC (0-10 cm) averaged 17%, while above-ground additions averaged 0.75% in a no-till corn and soy cropping system.

Consistent with aboveground biomass, we found that the relationship between biomass C and ΔSOC_{EM} was poorly defined ($r = 0.27$, $p = 0.0074$) by the linear relationships among 12 crop rotation × soil disturbance (excluding the SE-Ranch and SE-Pro observations) (Figure 3). The ΔSOC_{EM} changed approximately 0.58 Mg ha^{-1} for every Mg unit of returned biomass C (Figure 3). The relationship between ΔSOC_{EM} and returned biomass C indicated that 1.92 Mg ha^{-1} yr^{-1} of returned biomass C was required to maintain SOC_{EM} (Figure 3). Our biomass C response data is

closely related to previous findings by Engel et al. (2017) and Shrestha et al. (2013) that reported 2.6 and 2.4 Mg ha^{-1} yr^{-1} of total C inputs were needed to maintain SOC in NPG annual cropping systems. However, these reports included estimated below-ground C inputs while we have only reported observed above-ground C inputs. We found little evidence that crop species, including the occasional utilization of fallow, influenced the relationship between ΔSOC_{EM} and returned aboveground biomass or biomass C inputs. Comparing the NC-Trad rotation to NE-Pro rotation, the NE-Pro rotation fostered a 2.4-fold increase in annualized ΔSOC_{EM} accrual, while only producing a 1.22-fold increase in returned aboveground biomass and biomass C. One of the primary differences between the NC-Trad rotation and all other rotations that fostered ΔSOC_{EM} accrual was crop rotation intensity. This single difference of biomass addition every two years compared to largely continuous cropping, paired with the knowledge from previous research on root C influence on SOC, suggests belowground C inputs are likely much more important to ΔSOC_{EM} accrual than aboveground C inputs (Campbell et al., 1991; Lemke et al., 2010). However, our results show that aboveground C inputs do predict ΔSOC_{EM} accrual, revealing that increased biomass C inputs among six annual crop rotations increased ΔSOC_{EM} accrual. These results suggest that continuous addition of total C inputs via crop rotations that minimize fallow periods optimize ΔSOC_{EM} accrual, thereby maximizing potential CO_2 sequestration and subsequent C credits via progressive cropping systems practices.

4. Conclusion

This study revealed that contrasting and diverse crop rotations influence returned aboveground biomass and biomass C to the soil surface. Additionally, the ‘short stubble-hoe seeder’ soil disturbance treatment increased SOC accrual compared to the ‘tall stubble-disk

seeder' treatment, likely due to increased crop residue processing and increased residue incorporation with the soil profile. We found that crop rotations that avoided fallow and rotated with pulse crops and oilseeds in alternate years produced greater biomass and biomass compared to a traditional fallow-wheat rotation. We found that SOC accrual is increased in rotations that grow spring pea and spring lentil in rotation with wheat and include a cover crop sequence. Soil organic carbon accrual results reveal that a traditional fallow-wheat rotation, which is common across the NGP, has the capability to accrue SOC, however diverse crop rotations accrued SOC at a two-fold greater rate. However, both SE-Pro and SE-Ranch rotations resulted in decreased SOC inventory following six years, suggesting that a combination of warm season grasses and use of winter pea may increase N mineralization in the 0-30 cm profile more than C inputs from the cropping system can compensate for. Additionally, our results show that biomass C inputs are not the only driver of SOC accrual, as the SE-Pro rotation decreased SOC inventory while it produced the greatest aboveground biomass C returns to the soil surface. Our correlation analysis revealed that our cumulative returned biomass and biomass C results were poor predictors of SOC accrual, given that the SE-Pro rotation returned the most C inputs to the soil surface among eight crop rotations but presented a SOC loss in 2022. Our results indicate that 1.92 Mg ha^{-1} yr^{-1} of biomass C is needed to maintain SOC, aligning with previous research by Engel et al. (2017) and Shrestha et al. (2013).

This study expanded our understanding of how diverse crop rotations and soil disturbance from contrasting seeding and harvesting methods influence aboveground biomass and biomass C inputs to soil ecosystems and resultant SOC affects to Montana cropping systems and the greater NGP. Findings from this study detail how farming and ranching systems across Montana and the

greater NGP can alter production practices to increase ecosystem services by sequestering atmospheric CO_2 via diverse crop rotations to position themselves for prime C credit payments. This study also provides additional framework for how C markets can value contrasting crop rotations found across NGP to improve precision of C credit payments and incentivize practices that foster SOC accrual.

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Figures

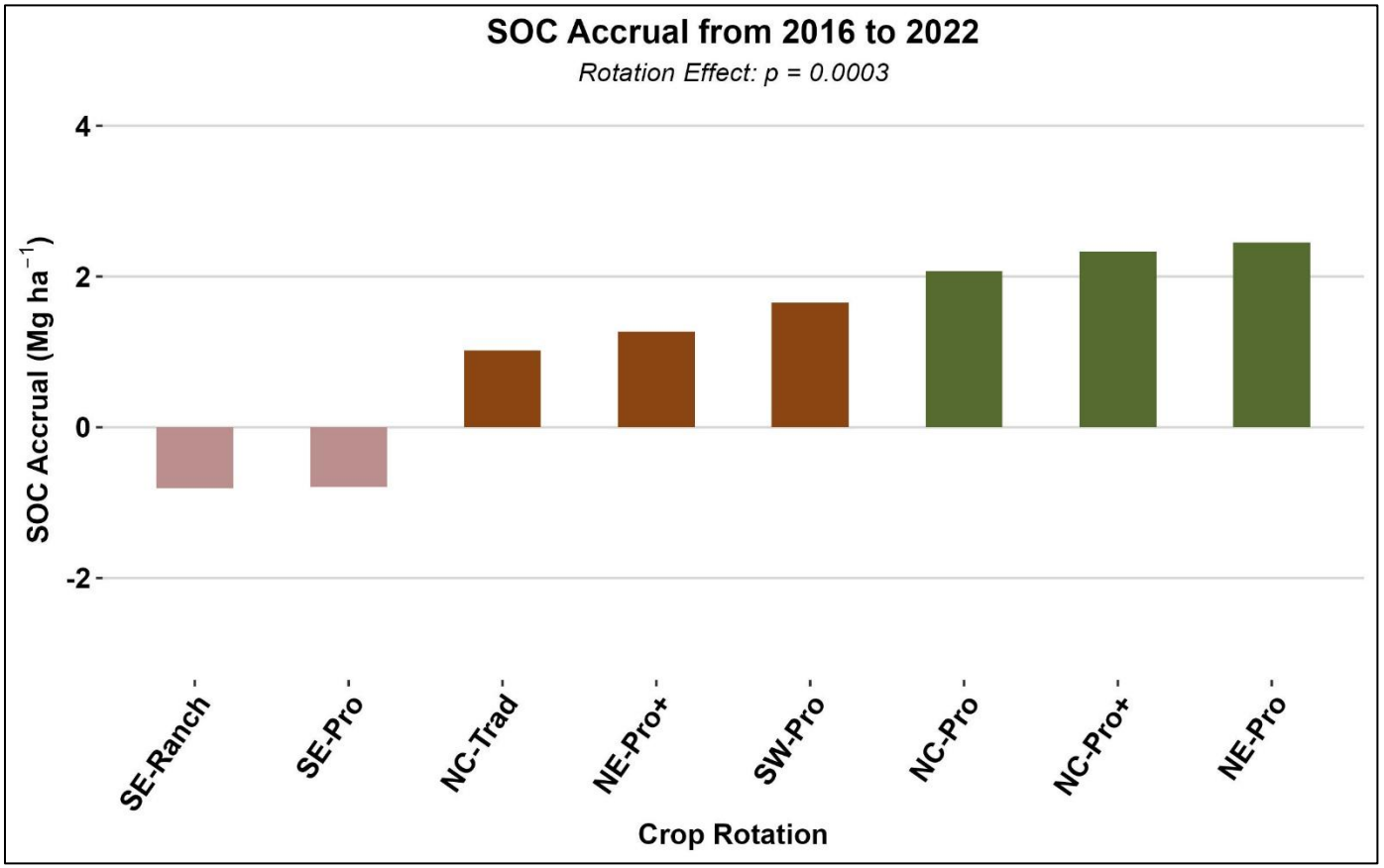

Figure 1: Change in SOC_{EM} (0-30 cm depth) among eight crop rotations after six years of management across two soil disturbance treatments. Error bars indicate the standard error of each treatment's least square means. Differing bargraph colors represent a significant difference among crop rotations at $p \leq 0.05$ via Tukey-Kramer 'honest significant difference' multipule comparisons.

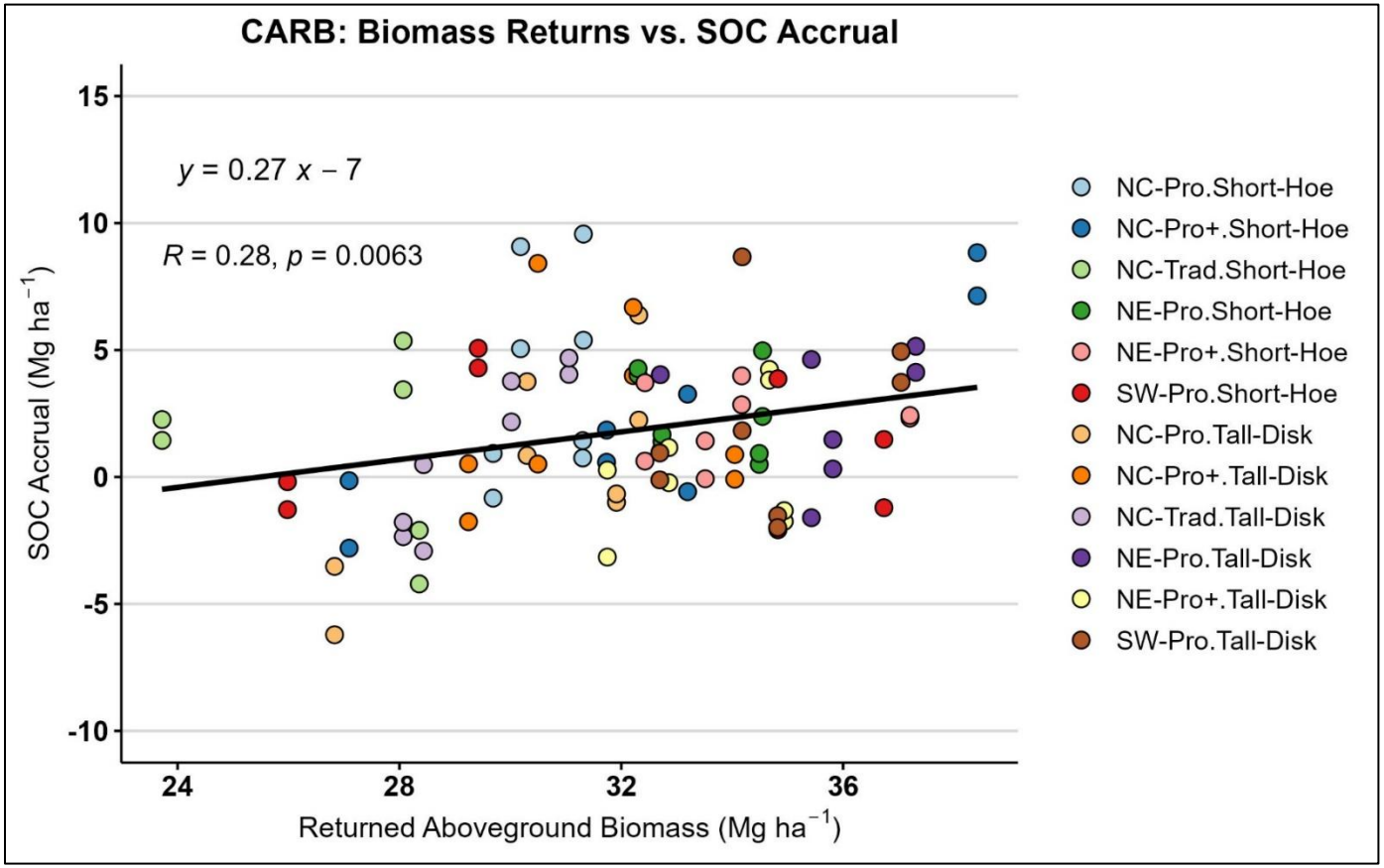

Figure 2: The relationship between returned aboveground biomass and SOC_{EM} (0-30 cm) accrual after six years of management across two soil disturbance methods for the six crop rotation treatments that fostered positive SOC_{EM} accrual.

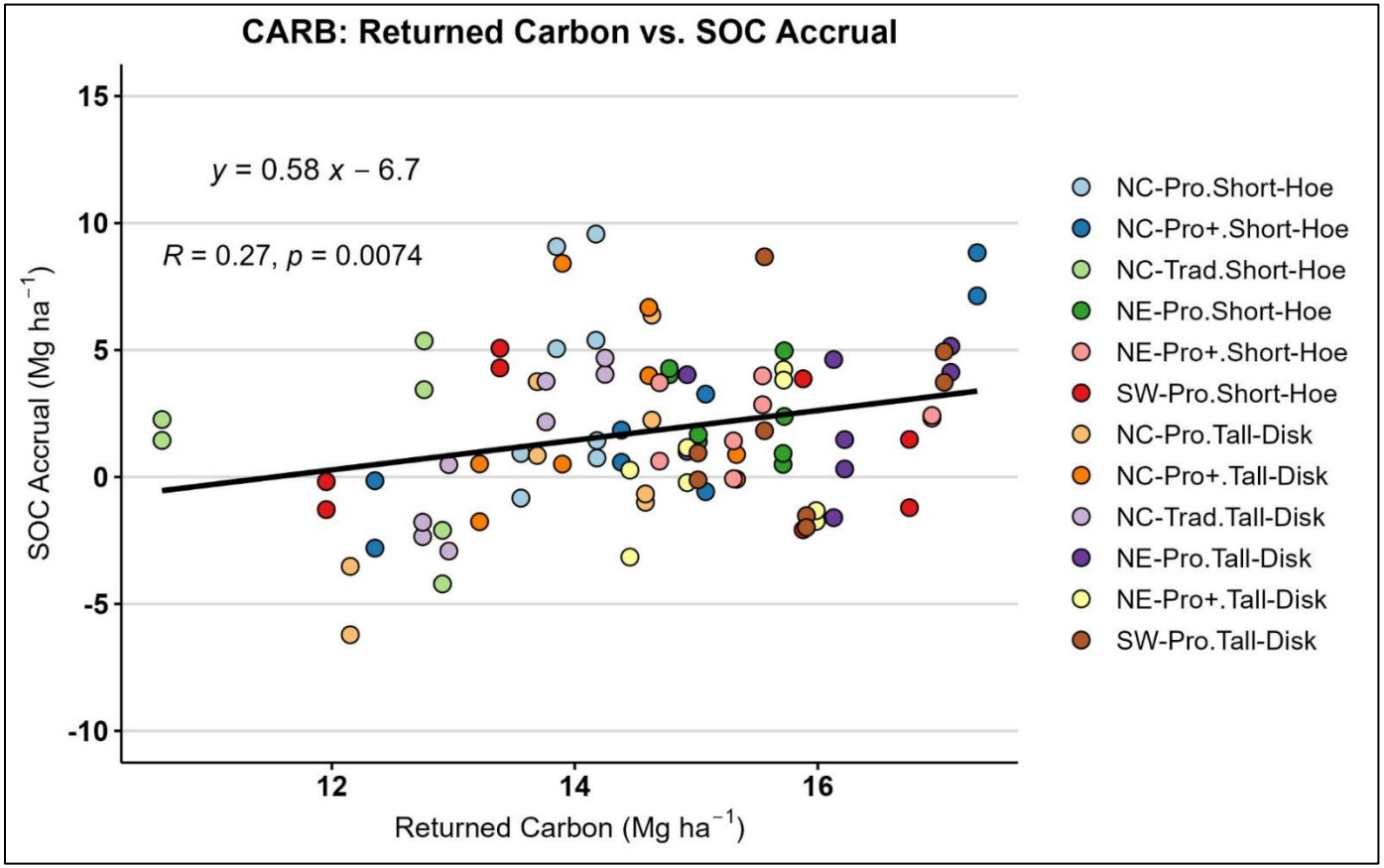

Figure 3: The relationship between returned aboveground biomass carbon and SOC_{EM} (0-30 cm) accrual after six years of management across two soil disturbance methods for the six crop rotation treatments that fostered positive SOC_{EM} accrual.

Tables

Table 1: Monthly precipitation, monthly mean temperature, annual precipitation, annual temperature, April to August growing season precipitation and temperature, and 30-year monthly precipitation and temperature means for the study site near Bozeman, MT.

Year	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan - Dec	Apr - Aug
	Precipitation (mm)													
2016	9	4	33	34	69	20	31	22	61	71	13	22	387	175
2017	13	16	37	66	65	57	3	14	67	25	39	43	445	204
2018	32	15	30	83	74	91	5	32	15	38	25	9	448	285
2019	15	33	15	84	44	52	68	19	92	36	20	2	480	267
2020	8	21	32	22	20	93	17	14	19	33	6	7	290	165
2021	19	22	15	28	79	20	21	52	4	58	7	25	350	200
2022	12	15	19	41	110	60	14	15	27	53	26	34	425	240
study avg	15	18	26	51	66	56	23	24	41	45	19	20	404	220
30-year avg	**14**	**14**	**24**	**50**	**64**	**71**	**30**	**29**	**33**	**36**	**21**	**16**	**401**	**244**
	Temperature °C													
2016	-4.2	1.6	4.1	8.8	10.9	17.6	19.4	19.2	13.4	9.0	4.4	-8.0	8.0	15.2
2017	-8.9	-1.5	5.8	6.6	11.5	15.9	21.8	19.4	13.1	6.8	-0.1	-6.4	7.0	15.0
2018	-2.9	-6.8	1.1	5.4	13.3	15.3	19.1	18.1	13.2	6.6	-0.2	-4.0	6.5	14.2
2019	-3.6	-11.4	-5.5	6.1	9.5	15.1	18.2	18.8	14.4	2.8	-0.4	-2.4	5.1	13.5
2020	-0.6	-2.9	0.9	5.6	11.5	15.3	18.3	20.3	14.8	6.3	1.7	-1.6	7.5	14.2
2021	-2.4	-8.2	2.9	5.9	10.1	18.6	22.5	18.3	15.8	8.8	4.1	-2.0	7.9	15.1
2022	-4.6	-4.0	2.9	2.9	9.1	15.4	20.6	21.7	16.1	8.8	-6.7	-7.4	6.2	13.9
study avg	-3.9	-4.7	1.7	5.9	10.8	16.2	20.0	19.4	14.4	7.0	0.4	-4.5	6.9	14.5
30-year avg	**-4**	**-2.8**	**2**	**6.2**	**11.1**	**14.9**	**19.3**	**19**	**14.0**	**7.3**	**0.3**	**-4.4**	**7.3**	**14.1**

Note: data obtained from the High Plains Climate Center ACIS-CLIMOD data service by using the Bozeman 6 EXP FARM climate station with the 30-year average representing 1991 to 2020.

Table 2: Summary of crop rotation treatments, rotation abbreviations, and species sequence from 2017 to 2022 for all eight crop rotation treatments.

Crop Rotation Treatment	Rotation Abbrev.	Crop Sequence (2017-2022)[a]
North central - Traditional	NC-Trad	F-WW-F-WW-F-WW
Southwest - Progressive	SW-Pro	SpC-WW-Lg-WW-SpC-WW
North central - Progressive	NC-Pro	F-WW-Lg-SW-F-WW
North central - Progressive (plus Cover)	NC-Pro+	CCM-WW-(Lg+Fall Bras CC)-SW-CCM-WW
Northeast - Progressive	NE-Pro	Pg-WW-Lg-SW-Pg-WW
Northeast - Progressive (plus Cover)	NE-Pro+	Pg-WW-(Lg+Fall CC)-(SW+Fall CC)-Pg-WW
Southeast - Progressive	SE-Pro	WPg-WW-SF-M-WPg-WW
Southeast - Ranch	SE-Ranch	WPh-WW-CCM-(Bh+Mh)-WPh-WW

[a]Note: Bh=Barley hay, CCM=Summer Cover Crop Mix, F=Fallow, Fall CC=Fall Cover Crop, Lg=Lentil grain, M=Millet grain, Mh=Millet hay, Pg=Spring Pea grain, SF=Safflower, SpC=Spring Canola, SW=Spring wheat, WPg=Winter Pea grain, WPh=Winter Pea hay, WW=Winter Wheat

Table 3: Cumulative aboveground biomass and biomass carbon response to crop rotation treatments from 2017 to 2022 at Bozeman, MT.

Emblematic Regional Rotation	Rotation Abbrev.	Aboveground Biomass	Biomass Carbon
		Mg ha^{-1}	
North central – Traditional	NC-Trad	28.3 cd	12.9 cd
Southwest – Progressive	SW-Pro	33.2 ab	15.2 ab
North central – Progressive	NC-Pro	30.5 bcd	13.9 bcd
North central– Progressive (+ Cover)	NC-Pro+	32.1 abc	14.5 abc
Northeast – Progressive	NE-Pro	34.4 ab	15.7 ab
Northeast – Progressive (+ Cover)	NE-Pro+	33.9 ab	15.5 ab
Southeast – Progressive	SE-Pro	36.7 a	16.6 a
Southeast – Ranch	SE-Ranch	27.1 d	12.2 d
		Prob > F	
Crop Rotation		<0.0001	<0.0001
Soil Disturbance		0.19	0.18
CR x SD		0.68	0.63

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 4: Soil organic carbon mass in 2022 within the 0-10 cm depth layer for crop rotation and soil disturbance treatments at Bozeman, MT.

Source of Variation	SOC (0-10cm) Mg ha^{-1}
	p-value
Rep	0.83
Rotation	0.0245
Rep x Rotation	<0.0001
Disturbance	0.037
Rotation x Disturbance	0.69
Rotation Means	**Mg ha^{-1}**
NC-Pro+	21.3 a
SW-Pro	21.1 ab
NC-Pro	21.0 ab
NE-Pro+	20.5 ab
SE-Pro	20.1 ab
NE-Pro	19.9 ab
SE-Ranch	19.2 ab
NC-Trad	19.0 b
Disturbance Means	**Mg ha^{-1}**
Short-Hoe	20.7 a
Tall-Disk	19.9 b

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 5: Soil organic carbon mass in 2022 within the 10-20 cm depth layer for crop rotation and soil disturbance treatments at Bozeman, MT.

Source of Variation	SOC (10-20 cm) Mg ha^{-1}
	p-value
Rep	<0.0001
Rotation	0.0089
Rep x Rotation	<0.0001
Disturbance	0.74
Rotation x Disturbance	0.13
Rotation Means	**Mg ha^{-1}**
NC-Pro	14.0 a
NC-Pro+	13.9 a
NE-Pro+	13.9 a
NE-Pro	13.7 ab
SE-Ranch	13.6 ab
SW-Pro	13.6 ab
SE-Pro	13.5 ab
NC-Trad	13.2 b
Disturbance Means	**Mg ha^{-1}**
Tall-Disk	13.7
Short-Hoe	13.7

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 6: Soil organic carbon mass in 2022 within the 20-30 cm depth layer for crop rotation and soil disturbance treatments at Bozeman, MT.

Source of Variation	SOC (20-30 cm) Mg ha^{-1}
	p-value
Rep	0.0569
Rotation	0.81
Rep x Rotation	<0.0001
Disturbance	0.71
Rotation x Disturbance	0.0252
Rotation x Disturbance Means	**Mg ha^{-1}**
SE-Ranch.Short-Hoe	11.0 a
NE-Pro.Tall-Disk	10.9 ab
SW-Pro.Tall-Disk	10.8 ab
NE-Pro+.Short-Hoe	10.8 ab
NC-Pro+.Short-Hoe	10.7 ab
SE-Pro.Tall-Disk	10.6 ab
NC-Pro.Short-Hoe	10.6 ab
NC-Pro+.Tall-Disk	10.5 ab
NC-Trad.Tall-Disk	10.5 ab
SW-Pro.Short-Hoe	10.5 ab
NC-Trad.Short-Hoe	10.2 ab
SE-Ranch.Tall-Disk	10.1 ab
NE-Pro+.Tall-Disk	10.1 ab
NE-Pro.Short-Hoe	10.0 ab
SE-Pro.Short-Hoe	9.8 ab
NC-Pro.Tall-Disk	9.7 b

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

Table 7: Soil organic carbon accrual response to crop rotation and soil disturbance treatments from 2016 to 2022 at Bozeman, MT.

Source of Variation	Δ SOC_{EM} Mg ha^{-1}
	p-value
Rep	0.0005
Rotation	0.0003
Disturbance	0.061
Rep x Rotation	<0.0001
Rotation x Disturbance	0.0871

Note: Significant difference at $\alpha = 0.05$.

Table 8: Soil organic carbon accrual least square means, annualized soil organic carbon accrual, estimated accrued annual carbon credits, and estimated carbon credit value under two pricing models by crop rotation and soil disturbance treatments.

Rotation	**Δ SOC_{EM}**	**Annualized ΔSOC_{EM}**	**CO2 credits**	**Low Price ($25)**	**High Price ($45)**
	Mg ha^{-1}	**Mg ha^{-1} yr^{-1}**	**Mg ha^{-1} yr^{-1}**	**$ ha^{-1} yr^{-1}**	**$ ha^{-1} yr^{-1}**
p-value	0.0003				
NE-Pro	2.45 a	0.41	1.50	37.45	67.41
NC-Pro+	2.33 a	0.39	1.42	35.56	64.01
NC-Pro	2.07 a	0.35	1.27	31.65	56.97
SW-Pro	1.65 ab	0.28	1.01	25.22	45.39
NE-Pro+	1.27 ab	0.21	0.77	19.36	34.84
NC-Trad	1.02 ab	0.17	0.62	15.58	28.05
SE-Pro	-0.79 b	-0.13	NA	NA	NA
SE-Ranch	-0.81 b	-0.14	NA	NA	NA
Disturbance Means	**Mg ha^{-1}**	**Annualized ΔSOC_{EM}**	**CO2 credits**	**Low Price ($25)**	**High Price ($45)**
p-value	0.061				
Tall-Disk	0.82	0.14	0.50	12.52	22.53
Short-Hoe	1.62	0.27	0.99	24.73	44.52

Note: Differing Tukey HSD (honest significant difference), common display letters denote a significant difference at $\alpha = 0.05$.

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CHAPTER EIGHT

SYNTHESIS

This work was made possible by a diverse panel of collaborators across the USA and Canada, particularly regarding the field pea grain N concentration and grain yield research that comprises a majority of this dissertation. The findings, results, and recommendations that this dissertation has made have already improved field pea management across the northern Great Plains region through our evaluation of using insecticides to improve grain protein and grain yield in field pea by mitigating pea leaf weevil infestations. The findings of this dissertation has advanced our knowledge on how environments and genetics control formation of protein and yield in field, which will advance grower knowledge and inform the broader plant-based protein industry. Additionally, this dissertation evaluated how progressive crop rotations and soil disturbance methods influence soil organic carbon accrual, which will inform farmers and ranchers across Montana how they can optimize cropping systems in their region to maximize potential carbon credit payments from voluntary carbon credit markets.

This research significantly advances the understanding of how common insecticidal tools influence pea plant productivity in spring-seeded dryland systems. The study demonstrates that applying insecticides at seeding and during early vegetative stages leads to measurable improvements in grain yield, grain protein concentration, and overall protein yield. These results suggest that producers in Montana can derive substantial benefits from proactive chemical management to mitigate infestations, particularly in farming systems where pea leaf weevil (PLW) populations are consistently present. By evaluating different rates and application

timings, this work provides a clearer framework for using these chemical tools to secure early-season plant health and maximize final output.

The environment remains the primary factor explaining the variation observed in grain yield and nitrogen (N) concentration in field pea. By classifying pea-growing regions into mega-environment clusters based on climate variables, the study revealed that yield differences are largely driven by growing-season precipitation and temperature. Notably, the timing of precipitation and the rate of heat accumulation are highly predictive of grain N concentration and grain yield. Grain yield response to precipitation was best modeled using moisture received during seed fill initiation through seed maturity. Furthermore, while the environment is dominant, meaningful interactions between environment and genotype suggest that certain genotypes possess variable, site-specific adaptability across different North American production regions.

Spatial variation of nitrogen within the plant is also heavily influenced by the growing environment. During the three-year study, environmental factors explained most of the variation in pod N concentration, though genotype-environment interactions occasionally dominated, suggesting unique site-specific adaptations of genotypes. A key finding was the identification of specific raceme locations that produce higher grain N concentrations; across 22 environments, with bottom pods more frequently containing higher N concentrations than top pods. This quantification of intra-plant variation provides deeper insight into how environments and genotypes collaborate to control nitrogen partitioning within the individual pea plant structure.

The analysis of trait stability highlights a larger environmental effect on the variances of grain yield and N concentration compared to the effect of the genotype alone. While the

genotypic effect on yield variance was negligible, it was significant for grain N concentration. Using AMMI and BLUP stability models, the research identified AAC Profit (G1) as the most stable genotype for grain N concentration, while CDC Dakota (G3) was the most stable for grain yield, suggesting that across a broad geographic area, genotypes that express stable grain N concentration do not necessarily also express the most stable grain yields. However, a notable trade-off exists as top-yielding genotypes were often not stable across environments, and trait means were not necessarily associated with stability. These findings suggest that future breeding efforts may have a greater opportunity to improve grain quality (N concentration) than yield and underscore the value of integrating robust statistical methods into multi-environment trial (MET) studies.

The timing of climate stress—such as warming or reduced precipitation—affects biomass, grain yield, and N concentration differently based on the growth habit of the pea variety. Stress imposed during the pre-flower stage often results in the greatest biomass reduction, particularly for determinate types, likely due to reduced carbon transport to rhizobia from plant stress. Conversely, hot and dry conditions during seed fill can accelerate the remobilization of internal nitrogen to the seeds, even while photosynthesis is limited. The study also highlights methodological challenges, noting that high daily maximum temperatures (exceeding 28 °C) in control plots can sometimes confound the effects of manually imposed warming via open-top chambers (OTCs). Future research must account for these contrasting growth habits and implement longer application periods that occur earlier in the plant life cycle for climate manipulation to fully capture precipitation gradients.

Long-term soil health is optimized by diverse crop rotations that avoid fallow and integrate pulse crops, oilseeds, and cover crop sequences. While traditional fallow-wheat rotations in the Northern Great Plains (NGP) can accrue soil organic carbon (SOC), certain diverse rotations do so at 2-fold faster rate. The 0-10 cm soil depth layer experienced the greatest influence from these contrasting rotations. This research established that approximately 1.92 Mg ha^{-1} yr^{-1} of biomass C is required to maintain SOC levels, aligning with previous regional benchmarks. By transitioning to production practices that sequester atmospheric C through increased biomass return, farming and ranching systems in Montana and the NGP can improve ecosystem services and position themselves to receive C credit payments.

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