

SULFUR AS A MEANS TO REDUCE NITRATE ACCUMULATION AND IMPROVE  
STATEWIDE FORAGE BARLEY QUALITY

by

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## ABSTRACT

Nitrate accumulation in cereal forages is a serious concern for livestock health that can potentially be reduced through sulfur application, yet there has been limited research on this topic in the northern Great Plains. This study aimed to develop an understanding of the effect of sulfur on forage barley nitrate accumulation and quality in Montana. Field experiments were conducted across four Montana locations with varying soil types and climates. The study found that sulfur additions generally had little impact on yield, crude protein, and relative forage quality. The findings suggest that when Montana State University yield-based nitrogen rates of 35 lbs. ton<sup>-1</sup> of forage are applied with 10 and 20 lbs. acre<sup>-1</sup> rates of sulfur nitrate concentrations can be reduced without reductions in yield or nutritive quality. These findings could provide an additional proactive management strategy that is not solely reliant on nitrogen-management. Reductions seen over the two years of this study have the potential to reduce costs for producers incurred from both low-nitrate replacement hay and calf and lamb losses due to nitrate toxicity. Further research is needed to validate these results across a wider range of conditions.

## CHAPTER ONE

## LITERATURE REVIEW

Introduction

Producers in Montana rely on harvested forage to weather the characteristically long winters of the Northern Great Plains when nutritionally poor-quality roughage dominates the rangelands which cover 70% of Montana (Delcurto et al., 2000; Wyffels et al., 2020). In addition to poor quality forage on winter range, absence of water systems, extreme cold, reduced animal performance, winter calving, and crusting of snow also contribute to difficulties of winter grazing which lead producers to feed harvested forages (Hibbard et al., 2021). On average, producers require two to four months of hay to feed over the winter (NASS,2023) which is generally the largest cost to ranches (Goosey, 2024). Due to this high cost of hay, slight improvements to the crop can provide significant reductions in cost (NASS, 2023).

Montana's hay crop is valued at about \$325 million annually with 1.48 million acres of hay production under dryland production and 1.12 million acres under irrigated management (NASS, 2023). Of this \$325 million hay crop, 90% of the tonnage produced is fed to livestock on the ranch where it was grown, re-emphasizing the importance of hay quality to Montana producers (NASS, 2023). Annual cereal forages are well adapted to Montana's short growing seasons, especially its dryland areas, provide increased production per acre compared to perennial forages, and provide quality forage for livestock (Meccage et al., 2019)

Despite the ability of annual cereal hay to provide quality roughage for overwintering livestock, annual forages have greater risk of nitrate accumulation than leguminous forages or

perennial grasses (Wright & Davison, 1964). Nitrate accumulation in cereal forages is a result of stressful growing conditions such as drought, frost damage, high soil nitrate, or nutrient deficiencies. Nitrate accumulations decrease forage quality, which presents livestock health concerns, with nitrate toxicity resulting in decreased conception rates, increased early term abortions, and possibly death (Bush et al., 1979; Hall, 2018) From 2000-2005, the economic impact of nitrate accumulation and subsequent nitrate toxicity in Montana cereal forages was \$13 million to \$39 million due to potential calf abortion losses and high nitrate hay replacement value (D. S. Cash et al., 2005) Current methods to manage nitrate toxicity include harvesting when nitrate levels are lowest, increasing cutting height, mixing high nitrate hay with low nitrate hay when feeding, or buying low nitrate hay (Drewnoski et al., 2019) . Recommendations for managing nitrate accumulations in cereal hay are reliant on split applications of nitrogen (N), species selection, weed control, and soil testing for sufficient phosphorus (P) and potassium (K). Little has been done regarding sulfur (S) additions despite laboratory experiments suggesting that S deficiencies caused an accumulation of nitrate and reduction in protein synthesis (Prosser et al., 2001). In the continental US, soil and crop sulfur deficiencies have increased since the passage of the Clean Air Act in 1970 (Hinckley & Driscoll, 2022). A combination of lower sulfur dioxide emissions from fossil fuel power plants due to the introduction of scrubbing technology, the increased use of high-analysis low-S-containing fertilizers, fungicides, and pesticides, as well as high-yielding varieties of crops have all contributed to this deficiency in the continental United States. Despite the trend of increasing S deficiencies in the Midwest and Eastern United States, much of Montana did not experience these drastic deposition changes due to a lack of upwind coal-burning power plants. Regardless, soil tests are still not considered a reliable method for

determining fertilizer S responses (Olson-Rutz and Jones, 2015b), in part due to variable amounts of S mineralization and leaching, where both processes are dependent on weather conditions. (Olson-Rutz and Jones, 2015b). In addition, available S levels often are highly variable across a field, and only one or two high S soils in a field-composite can indicate that no S is needed, when it is needed on much of the field.

Sulfur is a necessary component of essential amino acids and proteins, and its availability has been shown to improve forage quality and can reduce nitrate accumulation (Malhi et al., 2009; Prosser et al., 2001; Puoli et al., 1991; Reuveny et al., 1980). Montana's beef and sheep industries are heavily reliant on stored forages, and small improvements could greatly reduce feed-related costs (Goosey et al., 2022). Regardless of the knowledge that S can improve forage quality and yield, and reduce nitrate accumulation, soil S tests are often unreliable predictors of crop yield (Carciochi et al., 2019; Franzen, 2018; Goh & Pamidi, n.d.; Hu et al., 2005; Ketterings et al., 2011; Shirisha et al., 2011). Plant tissue S is known to be a limiting factor of nitrate reductase activity but due to the current limits of soil (and tissue?) S tests, little is understood about S soil additions effect on nitrate accumulation in forage barley.

### Annual Cereal Forage

Annual cereal forages are well adapted to dryland and the short growing seasons of Montana and can provide high-quality forage necessary to meet the needs of livestock producers. Annual cereal forages include winter cereals (wheat, triticale, and spelt), spring cereals (barley, oat, triticale, wheat, and emmer), and warm season crops (millet, sudangrass, sorghum, sorghum-sudan hybrids, and corn). Of the annual cereal forages, multiple varieties have been developed with increased palatability traits, such as hooded varieties (e.g. Haybet' and 'Lavina') and awnless winter wheat (e.g. 'Willow Creek' and 'Ray'). Cow-

calf operations rely on stored forages during the second and third trimesters which occurs during the winter feeding period. Beef cows can have all their nutritional needs met with cereal hay as opposed to the more nutritionally demanding dairy cattle (National Academics of Science, 2015). The popularity of cereal forages persists because these crops fit well into renovations of declining perennial forage stands, such as alfalfa, and cereal forage production can provide much needed breaks in pest cycles (Meccage et al., 2019). There is very little insect or disease overlap between annual cereal crops and perennial forages. Even the issue of autotoxicity, a condition in which mature alfalfa plants kill or stunt seedlings, has little impact on cereal forages compared to reseeded alfalfa (Moore et al., 2020). Planting and growing cereal forages can be accomplished with the same drills used for planting alfalfa or other cereal crops. Attractive to producers for this reason, cereal forages have similar agronomic requirements as cereal crops grown for grain or feed. Generally, cereal forage seeding rates are increased by 10 to 20% compared to grain, less N fertilizer is required reducing the risk of nitrate toxicity, and often little to no herbicide is needed (Meccage et al., 2019)

Acreage of dry hay from annual crops, now considered “alternative forages”, declined with the introduction of tractors and field machinery, but 13% of the 1.1 million acres of barley seeded in Montana is planted to forage varieties (National Agricultural Statistical Services, 2022). Even some of the barley acreage not designated as forage is often used for emergency forage crops during drought conditions, as well as other salvaged cereal crops like winter and spring wheat. Haybet and Lavina varieties make up over half of the total forage barley acreage in Montana with Lavina being the only forage barley variety grown in each region of the state (National Agricultural Statistical Services, 2022) Generally, barley cultivars have higher forage quality than oats, wheat, or triticale (Cherney et al., 1983; Cherney & Marten, 1982a; Khorasani et al., 1997a; McCartney et al., 1993). Barley is a good candidate for a cereal forage due to lower neutral detergent fiber (NDF), higher yielding digestible dry matter, lower acid detergent fiber (ADF), and crude protein (CP) usually above the minimum acceptable threshold for beef cattle (Meccage et al., 2019).

Barley forage does not have an exact stage of maturity that is recommended for harvest.

Generally, the harvest timing recommendation falls between heading and soft dough (Zadoks 50 – 85; Feekes 10.1 – 11.2)(Brundage et al., 1979; S. D. Cash et al., 2007; Helsel & Thomas, 1987; Meccage et al., 2019). Ideally, harvest occurs at the time of maximized yield and crude protein, minimized fiber, and other anti-quality factors like nitrate. Reports of CP levels ranging from 15-23% between boot to soft-dough (Khorasani et al., 1997b) decreasing to 5.8-7.3% from early milk to early dough (Brundage et al., 1979) demonstrates that barley forage crops experience a decrease in CP through maturity. Yield seems to perform consistently across numerous studies in which yield increased up until the middle of the milk stage, after which, yield plateaued (Brundage et al., 1979; Helsel & Thomas, 1987; Mannerkorpi & Taube, 1995a). Fiber concentrations, both NDF and ADF, also consistently increased with maturity until about early dough, indicating lower dry matter intake (DMI) and total digestible nutrients (TDN) at early dough stage (Brundage et al., 1979; S. D. Cash et al., 2007; Mannerkorpi & Taube, 1995a; Meccage et al., 2019). Nitrate decreases in the plant leaf and stem tissue as the plant matures past anthesis with nitrate concentrations generally peaking at anthesis. (Bowman et al., 2003). When grain fill begins, the process creates a source-sink relationship with the foliar tissue and grain, translocating nitrate from the stems and leaves to the seed for protein formation (Egle et al., 2015). In summation, there are recommendations on when to harvest barley forage, but forage quality estimates at the Zadoks, Feekes, or Haun stages of maturity do not exist for forage barley crops (Table 1).

While cereal hay can provide livestock forage, the risk of nitrate accumulation causes warranted apprehension surrounding the crop. At maturity stage anthesis, nitrate concentrations are highest across all annual cereal hay species but typically decline with time in barley and triticale while remaining stable in oats (Bowman et al., 2003). Oats are notorious for accumulating the highest levels of nitrate among the commonly grown cereal forages and have been replaced in popularity with barley due to the reduced nitrate accumulation (Cash et al., 2006). For the purposes of this research, S deficiency will be the focus

as a plant stressor that can inhibit protein synthesis and subsequently contribute to the accumulation of nitrate and cereal hay (Sorin et al., 2015)

Table 1. Forage quality factors and yield changes associated with stages of maturity.

<b>Forage Quality Factor</b>	<b>Change with Maturity</b>	<b>Growth Stages Affected</b>	<b>Zadoks</b>	<b>Feekes</b>	<b>References</b>
<b>Yield</b>	Increase, then plateau	Boot – Milk	45-75	10 - 11	(Brundage et al., 1979; Helsel & Thomas, 1987; Mannerkorpi & Taube, 1995a)
<b>Crude Protein</b>	Decrease	Boot – Soft Dough	45-85	10 – 11.2	(Brundage et al., 1979; Khorasani et al., 1997b)
<b>Nitrate</b>	Decrease	Boot – Soft Dough	45 - 85	10 – 11.2	(Bowman et al., 2003; Egle et al., 2015)
<b>Fiber (ADF, NDF, ADL)</b>	Increase	Boot – Soft Dough	45 - 85	10 – 11.2	(Brundage et al., 1979; D. S. Cash et al., 2005; Mannerkorpi & Taube, 1995a; Meccage et al., 2019)

### Soil and Plant Tissue Sulfur

#### Plant Tissue Sulfur

Sulfur is a macronutrient that is essential for crop growth and is required in relatively large quantities for adequate yield, development, and forage quality. It is taken up as sulfate ( $\text{SO}_4^{2-}$ ) by the roots and then reduced to sulfide ( $\text{S}^{2-}$ ) in the chloroplasts before it is assimilated into organic sulfur compounds, such as cysteine-containing tripeptide glutathione (GSH). S and N assimilation and uptake are dependent upon one another due to the large proportion of reduced S incorporated into amino acids cysteine and methionine. Both are essential S-containing amino acids which are required for proteins; the major end-products of sulfate assimilation (Dick et al., 2015). Amino acids are precursors not only of proteins but

other S-containing compounds like coenzymes and secondary plant products as either structural constituents or as functional groups involved in metabolic reactions. Sulfate-containing metabolites are also integral to cellular communication that allow plants to upregulate genes to alter molecular pathways and tolerate adverse conditions (Samanta et al., 2020). Cysteine affects structure and function of proteins because of the formation of disulfide bonds between two cysteine residues in the polypeptide chain which is important for the tertiary structure and thereby the function of enzymes, like nitrate reductase (Rengel et al., 2023).

### Soil Sulfur

Sulfur, a necessary nutrient for crop growth and development, has been declining in soil nationwide due to less atmospheric deposition because of the passage of the Clean Air Act, higher purity herbicides and fertilizers, and higher yielding crops removing more sulfate from the soil (Hinckley & Driscoll, 2022b). Sulfate is most often the inorganic form of soil sulfur, which can leach due to excess precipitation, and/or coarse, shallow soils. Calcareous soils, which dominate many soils in the Northern Great Plains of Montana, can immobilize sulfate as it can coprecipitate with the high concentrations of calcium in the soil (Tisdale et al., 1993). Sulfate can also sorb to soil minerals, but with pH greater than 6.5, adsorption is negligible and not a concern for plant-sulfur availability. Generally, soils that are coarse-textured and sandy are likely to have a limited ability to supply S to crops due to leaching and generally lower organic matter contents (Scherer, 2009). In Montana, there is not a ubiquitous sulfur deficiency, however, areas of higher rainfall, like intermountain valleys, and shallower soils found in central Montana frequently have less than adequate plant available sulfur levels (Olson-Rutz & Jones, 2015b) Areas low in soil organic matter are often S deficient because S released from organic matter (“S mineralization”) is the major source of available S in the absence of S fertilizer. Inorganic S, largely sulfate, varies based on the time of year, moisture present, and temperature (Scherer, 2009). The unsatisfactory nature of soil S-sulfate testing has been well established (Dinkins & Jones, 2013; Franzen,

2018; Olson-Rutz & Jones, 2015b; Scherer, 2009; Tabatabai, 2018) despite different methods available. Plant responses to S are less consistent than phosphorus or potassium, particularly in the semi-arid Great Plains, in part due to higher concentrations of deep soil S in the form of gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) that can often provide substantial available S below typical soil sampling depths (six- 24 inches) combined with variable levels of S mineralization (Dinkins & Jones, 2013). Currently, visual symptoms (light green upper leaves) as well as location in the landscape (e.g. eroded ridges with little soil organic matter (SOM)) are the best ways to determine a potential plant S deficiency.

### Practical Plant and Soil Sulfur Applications

Without adequate S available to most forage crops, protein levels, quality, and digestibility decline due to inhibition of protein synthesis (Prosser et al., 2001). An inhibition of protein synthesis is correlated with an accumulation of soluble organic N and nitrate (Sorin et al., 2015). The S requirement for plant growth has the highest demand within the Brassicaceae family and decreases in demand from Brassicaceae to Fabaceae to Poaceae (Hawkesford et al., 2023). Grass forage, including barley, is considered S deficient at tissue levels below 0.20-0.25 percent, but pose possible livestock health risk problems, such as reduced average daily gains and polioencephalomalacia (PEM), when concentrations reach greater than 0.30 percent (Olson-Rutz & Jones, 2015b). The current Montana State University barley production guide does not recommend the application of S fertilizer to malt barley as it is reported to either provide no advantage or a slight negative effect on yield and quality; however, malt barley yield is grain driven as opposed to the biomass driven yield of hay barley (Jackson, 2008). If sulfur deficiency is suspected, rates of 5-10 pounds per acre are suggested to be top-dressed (McVay et al., 2017). For forage barley, there is no recommendation due to the unreliable nature of soil tests for soil sulfate and plant tissue testing is recommended as the most reliable method to detect a deficiency and manage nutrients in-season (Olson-Rutz & Jones, 2015b). Unpublished data (Westcott, n.d.) from two western

Montana research centers suggest that additions of sulfur to forage barley could improve yield and reduce nitrate levels.

### Nitrogen Management and Nitrate Accumulation

Sulfur, as stated above, is an essential plant macronutrient that, when deficient in the tissues of cereal forages, can limit yield and quality. Nitrogen, even more so than S, can limit the yield and quality of a monocot specie like barley. Nitrogen and S are heavily reliant on each other as assimilation and uptake are dependent upon one another. Sulfur deficiencies repress nitrate reductase activity causing nitrate to accumulate in young leaf tissue even when adequate nitrate is present (Reuveny et al., 1980). This tissue process leads to high and often toxic levels of nitrate in monocot stems due to excess absorption of soil nitrate at a rate faster than the nitrate can be converted to proteins, causing a buildup of nitrate within the xylem closest to the soil, the lower stem (Harper, 2015). Amino acids used in protein synthesis are disrupted with large amounts of non-S-containing amino acids which build up within the plant tissue causing neither RNA nor protein content to increase when S is insufficient (Prosser et al., 2001). Ratios of N and S have been developed to predict when S is deficient in monocots, with tissue N:S ratios greater than 15:1 indicating a deficiency (Olson-Rutz and Jones, 2015a). When N is sufficient, but S is deficient, the risk of nitrate accumulation in the plant increases, leading to an increased risk of the livestock disorder, nitrate toxicity.

Nitrate is not toxic to livestock, rather the rumen-reduced by-product, nitrite ( $\text{NO}_2^-$ ). Interestingly, in the animal rumen, the reduction from nitrate to nitrite happens rapidly while the change from nitrite to ammonia takes relatively longer (Hall, 2018). So, when large concentrations of nitrate are consumed, rumen microbes are overwhelmed and cannot convert all

the nitrite to ammonia before portions of it are passed to the small intestine where it is diffused into the bloodstream. This is problematic because nitrite forms methemoglobin which is unable to transport oxygen (O<sub>2</sub>) throughout the body causing the blood to become dark brown in color (Whitehead and Moxon, 1952). Chronic nitrate toxicity is marked by watery eyes, reduced appetite, milk production, unthrifty appearance, weight loss or little to no daily gains, night blindness, or abortion. Acute symptoms include accelerated pulse, labored breathing, muscle tremors, weakness, staggering gait, cyanosis, or even death. Treatment for nitrate toxicity is limited and so prevention is mostly relied on through hay testing and subsequently diluting high nitrate hays by mixing with less toxic forages. In cases of lethal levels of nitrate, the only treatment would be an immediate intra-venous injection of methylene blue, an impractical method for producers, as most producers do not have the specific tools or skills necessary for such measures. In cases of sub-lethal nitrate levels, chronic symptoms of poor winter health and abortions may be attributed to several other causes. Leaving the root causes undiscovered but still a concern due to an increased chance of aborted pregnancies and producers' reliance on stored hay during lactation and gestation.

### Management Options

If high N was not managed prior to planting (e.g. soil testing, previously high N application, etc.) and standing forage tests high for nitrates, management is limited to avoidance and dilution of the highest testing material. If tissue is sampled prior to harvest, and nitrate levels are high, it is recommended to: 1) raise cutter heads to avoid the highest nitrate concentrations found in the lower plant stems (Karmoker et al., 1991), 2) harvest at a later maturity stage (Khorasani et al., 1997; Bowman et al., 2003), 3) control weeds that are known to accumulate

nitrate most notably the *Chenopodium* family (Hall, 2018; Goosey et al., 2022), or 4) harvest when plant photosynthetic rates are the highest (Rengel et al., 2023). Ensiling high nitrate hay can reduce nitrate levels by 10 to 60 percent but is often impractical in Montana due to low moisture levels (Goosey et al., 2022). Diluting high nitrate hay prior to feeding is also a solution if low nitrate hay is available, although low nitrate hay can be expensive and difficult to source in drought years when nitrate levels are generally higher. Currently, options to manage nitrate accumulation rely heavily on N management (e.g. soil testing, split fertilizer applications) with solutions to offset high N levels limited to cutting height changes and rationing high nitrate forage. Managing high nitrate forage is a costly expense for an industry already subsisting on margins (Economic Research Service, 2024). Although it is known that foliar applications of S direct soil S uptake, and a balanced soil N:S ratio can improve N assimilation and reduce nitrate accumulation, there is not a defined understanding of the relationship between soil S and plant uptake and subsequent cereal forage yield and quality. This knowledge gap exists partially due to the unreliable nature of soil S tests (Scherer, 2009; Dinkins and Jones, 2013; Olson-Rutz and Jones, 2015; Franzen, 2018; Tabatabai, 2018). Currently, a Montana hay producer cannot rely on a soil test to determine if S additions are necessary to optimize forage yield and/or quality. With these existing problems in mind, the objectives of this study were to: (i) develop models to develop a predictive relationship between forage barley S and nitrate and a relationship between soil S and forage barley tissue S, and (ii) and to better understand the influence of soil S on forage barley S, plant tissue nitrate concentrations, and forage quality.

## CHAPTER TWO

EFFECT OF SULFUR FERTILIZATION ON FORAGE  
QUALITY AND NITRATE CONCENTRATION OF FORAGE  
BARLEY IN MONTANAAbstract

Nitrate accumulation in cereal forages is a serious concern for livestock health that can potentially be reduced through sulfur application, yet there has been limited research on this topic in the northern Great Plains. This study aimed to develop an understanding of the effect of sulfur on forage barley nitrate accumulation and quality in Montana. Field experiments were conducted for two years across four Montana locations with varying soil types and climates. The study found that sulfur additions generally had little impact on yield, crude protein, and relative forage quality; however, the findings do suggest that when Montana State University yield-based nitrogen rates of 35 lbs. N ton<sup>-1</sup> of forage are applied with 10 and 20 lbs. acre<sup>-1</sup> rates of sulfur, nitrate concentrations can be reduced without reductions in forage yield or nutritive quality. These findings could provide an additional proactive management strategy that is not solely reliant on nitrogen-management. Reductions seen over the two years of this study have the potential to reduce costs for producers incurred from both low-nitrate replacement hay and calf and lamb losses due to nitrate toxicity. Further research is needed to validate these results across a wider range of conditions.

## Introduction

Montana producers often rely on harvested forage not only to weather the characteristic long winters of the Northern Great Plains, but also to offset the nutritionally poor-quality roughage available on range, covering 70% of Montana's land area (Delcurto et al., 2000; Wyffels et al., 2020). On average, livestock producers require two to four months of dry hay to feed over winter to meet the nutritional needs of gestating livestock, which also represents the largest variable cost to ranch economics (Goosey, 2024). Hay can be grown on the ranch or purchased and due to this high cost of purchased, slight improvements to the ranch produced crops can provide significant reductions in cost (Goosey, 2024). Montana's hay crop is valued at about \$325 million annually with 2.5 million acres produced under dryland production.

Approximately 90% of this hay crop is produced and fed on the same ranch, re-emphasizing the importance of the ability to produce quality hay on the ranch to Montana producers. Of hay crops suited for short-growing seasons and dryland production, annual cereal forages prove best adapted. Annual cereal forages, such as barley, wheat, and triticale, provide more production per acre than perennial forage as well as provide a quality forage for livestock (Meccage et al., 2019)

Despite the ability of annual cereal hay to provide quality roughage for overwintering livestock, there is an increased risk of nitrate accumulation. Nitrate ( $\text{NO}_3^-$ ) accumulation in cereal forages is a result of stressful growing conditions, whether drought, frost damage, or nutrient deficiencies. Nitrate accumulation decreases the overall forage quality and presents a serious livestock health concern with nitrate toxicity resulting in decreased conception rates, increased early term abortions, and possibly death (Drewnoski et al., 2019; Hall, 2018; Reynolds & Drewnoski, 2022). From 2000-2005, the economic impact of nitrate accumulation and

subsequent nitrate toxicity in Montana cereal forages was \$13 million to \$39 million, an estimation of potential calf abortion losses and high nitrate hay replacement value (Cash et al., 2005). Current methods to manage nitrate toxicity include harvesting when nitrate levels are lowest, increasing cutting height, mixing high nitrate hay with low nitrate hay when feeding, or buying low nitrate hay. Managing nitrate accumulation in cereal hay is mostly limited to split applications of N, managing weeds known to accumulate nitrate, managing P and K deficiencies, but little research has been done on additions of S. In laboratory experiments, S-deficiencies caused an accumulation of nitrate and a reduction in protein synthesis (Prosser et al., 2001). Soil sulfur deficiencies have been on the rise since the passing of the Clean Air Act of 1970 and later amendments across much of the Midwest and Eastern United States (Hinckley and Driscoll, 2022). A combination of less sulfur dioxide emissions, the increased use of high-analysis low-S-containing fertilizers, fungicides, and pesticides, as well as high-yielding varieties of crops have all contributed to this deficiency. Montana, unlike much of the Midwest, has not experienced significant declines in atmospheric S-deposition due to a lack of proximity to coal burning powerplant and large population centers with more vehicle emissions (Hinckley & Driscoll, 2022). Despite these increasing deficiencies, soil sulfur tests are still not considered a consistent method for determining plant available sulfur in the soil due to the dynamic nature of S portions soil organic matter and plant-available sulfate-S (Scherer, 2009; Dinkins and Jones, 2013; Olson-Rutz and Jones, 2015; Franzen, 2018; Tabatabai, 2018).

Sulfur is a necessary component of essential amino acids and proteins that improve forage quality and can reduce nitrate accumulation greatly decreasing the large cost of purchased low nitrate replacement hay of cow-calf and ewe-lamb producers in Montana. Regardless of the

knowledge that sulfur can improve forage quality, yield, and reduce nitrate accumulation, the predictive ability of soil sulfur tests still falls short of a reliable predictor of yield and plant tissue S. Deficient plant tissue S concentrations are a limiting factor of nitrate reductase synthesis (Rengel et al., 2023b) but due to the current variable results from soil S tests, nitrate accumulation in forage crops in Montana has not been proven to respond to S. To capture the range of Montana climatic regions and soil types, four dryland locations were chosen. The objective was to better understand the influence of soil and fertilizer N and S on forage barley S, plant tissue nitrate concentrations, and forage nutritive quality.

### Materials and Methods

#### Sites

The experimental sites were located at the Northwestern Agricultural Research Center (NWARC) in Creston, MT (48.188251, -114.129211; altitude 2930 ft); the Central Agricultural Research Center (CARC) located 2.2 mi west of Moccasin, MT (47.060356, -109.965257; altitude 4255 ft); the Ft. Ellis (FE) Research and Teaching Farm located 3.1 mi east of Bozeman, MT (45.666874, -110.978001; altitude 4902 ft); and Broadview (BV) private land located 7.8 mi west of Broadview, MT in 2023 (46.078017, -109.007966; altitude 4098 ft ) and 11.2 mi southwest of Broadview, MT in 2024 (46.034316, -109.011682; altitude 4144 ft). NRCS mapped soil survey data varied by site and only varied by year at the BV site (Table 3). Yearly average precipitation also varied by site with FE having the highest yearly average precipitation and CARC and BV sites having the lowest yearly average precipitation (Figure 1).

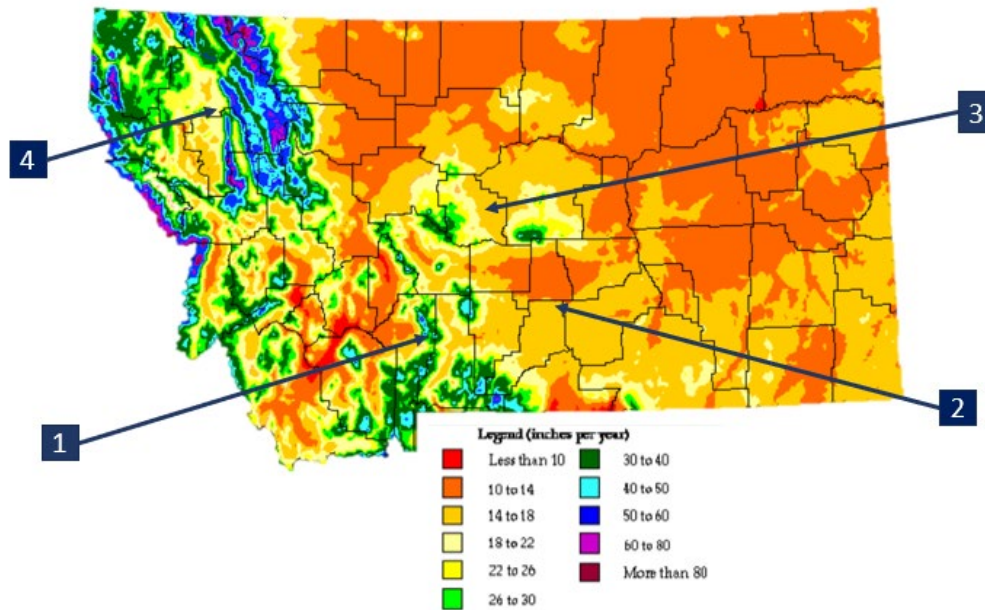


Figure 1. Mean annual precipitation for Montana (in).<sup>1</sup>

<sup>1</sup> (Oregon State University, 2014) 1.) Ft. Ellis Research and Teaching Farm (FE), Bozeman, MT; 2.) Broadview (BV), MT, 3.) Central Agriculture Research Center (CARC), Moccasin, MT; 4.) Northwestern Agriculture Research Center (NWARC), Creston, MT

Table 2. NRCS site soil descriptions by location and year.<sup>2</sup>

Site	Year	Mapped Soil Type	Soil Type Description	Parent Material
NWARC	2023, 2024	Flathead very sandy loam	coarse-loamy, mixed, superactive, frigid Pachic Haplustolls 0-3 % slopes	Glacial outwash or alluvium
CARC	2023,2024	Danvers-Judith silt loam	fine-loamy, carbonatic, frigid Typic Calciustolls, 0-2% slopes	Calcareous alluvium or colluvium derived mainly from limestone
FE	2023,2024	Blackmore silt loam	fine-silty, mixed, superactive frigid Typic Argiustolls, 0-4% slopes	loess on relict stream terraces
BV	2023	Tanna clay loam	fine, smectitic, frigid Aridic Argiustolls, 0-3% slope	residuum weathered from semiconsolidated shale, mudstone, or siltstone, or glaciofluvial deposits or alluvium over bedrock
BV	2024	Bonfri-Lambeth Complex	fine-loamy, mixed superactive, frigid Aridic Haplustalfs, 0-3% slopes	alluvium, colluvium, or residuum from semiconsolidated interbedded sandstone and shale

### Experimental Design

The experimental design was a randomized complete block with four blocks, each with a block size of nine, with nine treatment combinations of N and S (FE and BV). A split-plot in a randomized complete block (NWARC and CARC) with four blocks, with a block size of nine,

<sup>2</sup> (NRCS, 1998, 1999b, 1999a, 2002, 2009, 2013)

where N treatment was randomized to main plots (three) and S treatment was randomized to split-plots (three). The fertilization method was to broadcast N and S prior to planting, as a means of minimal incorporation with the planting equipment. Further incorporation occurred via natural rainfall. N rates were calculated based on Montana Agriculture Research Center reports yield goal-based N-rates (Table 4) and soil nitrate-N with rates 0x, 1.0x, and 1.5x (low, medium, high N) the calculated rate. In 2023, 25 lbs. Nton<sup>-1</sup> was used as the yield-based N rate (Olson-Rutz & Jones, 2015) and was increased to 33.8 lbs. N ton<sup>-1</sup> in 2024 based on work by Lenssen et al. (2021) and to increase the odds of eliciting a nitrate accumulation response in the test plots (Table 5). Sulfur rates were 0, 10, and 20 lbs. acre<sup>-1</sup> (low, medium, high S) (Table 5). All sites were planted with Lavina barley at a rate of 100 lbs. acre<sup>-1</sup>. Row spacing, and openers varied due to equipment variation at each site (Table 5). Planting and harvest started earliest at NWARC and culminated at FE both years (Table 4).

Table 3. Yield goals, planting, and harvest dates by site and year.

Site	Year	Yield Goal (tons acre <sup>-1</sup> )	Planting Date	Harvest Date
NWARC	2023	6.5	4/24/2023	7/5/2023
NWARC	2024	4.0	4/23/2024	7/15/2024
CARC	2023	1.5	5/13/2023	7/21/2023
CARC	2024	2.5	5/15/2024	7/19/2024
FE	2023	4.0	5/16/2023	7/25/2023
FE	2024	5.0	5/16/2024	7/21/2024
BV	2023	1.5	5/2/2023	7/18/2023
BV	2024	3.0	5/14/2024	7/15/2024

Table 4. Planting equipment, row spacing, and planting rate by site.

Site	Row Spacing (in)	Make	Model	Openers	Planting Rate (lbs. ac <sup>-1</sup> )
NWARC	6	H & N Equipment	Custom fabricated	Custom fabricated	100
CARC	12	Zeromax	Y41 Special Drive	AcraPlant	100
Ft. Ellis	8	Great Plains	3P806NT	D.D. Assembly - Argentina	100
Broadview	10.5	Hege	1000	AcraPlant	100

Table 5. Fertilizer treatment weight for N (lbs. ton<sup>-1</sup>) and rate for S (lbs.acre<sup>-1</sup>) by year and location.

<b>Location - Year</b>	<b>N x S</b>	
<b>NWARC 23</b>	[0, 141, 223]	[0,10,20]
<b>NWARC 24</b>	[0, 110, 165]	[0,10,20]
<b>CARC 23</b>	[0, 21, 39]	[0,10,20]
<b>CARC 24</b>	[0, 63, 131]	[0,10,20]
<b>FE 23</b>	[0, 51, 101]	[0,10,20]
<b>FE 24</b>	[0, 151, 227]	[0,10,20]
<b>BV 23</b>	[0, 21, 39]	[0,10,20]
<b>BV 24</b>	[0, 69, 104]	[0,10,20]

### Soil and Tissue Sampling

Soils were sampled to the restrictive depth at all sites in the fall and spring. Four cores block<sup>-1</sup> were sampled. All cores for a single block were divided by depth (0-6, 6-12, 12-24, 24-36 inches) and composite samples were created by the four same depth segments by block. Soil at CARC could not be sampled past 24 inches. Fall sampling was done to establish plot locations, where the lowest plant available-S and adequate P and K levels were selected for test plot locations. Spring soil samples were collected for most accurate plant available N and S at planting.

Table 6. Spring soil analyses by site and year<sup>3</sup>

Site	Year	pH	SOM (%)	Nitrate-N (lbs. ac <sup>-1</sup> ) <sup>4</sup>	Potassium (ppm)	Phosphorus (ppm)	Sulfate-S (lbs. ac <sup>-1</sup> ) <sup>5</sup>
NWARC	2023	6.5	2.0	25.3	113	22	124.5
NWARC	2024	7.9	2.6	33.3	162	19	50.7
CARC	2023	6.9	5.0	34.5	342	19	72.5
CARC	2024	8.2	4.4	18.0	255	21	33.0
FE	2023	6.8	5.6	41.8	437	61	81.0
FE	2024	6.2	6.6	47.3	507	62	69.5
BV	2023	8.1	2.2	18.8	207	9	38.5
BV	2024	6.5	1.6	39.0	418	25	87.5

Plant tissue samples were collected when the plot reached milk – soft dough stage in 2023 and at anthesis in 2024 to capture the highest nitrate concentrations (Feekes 11.1-11.2). From the center of each plot, two, one-meter sections were sampled at a cutting height of 4-inches above the soil surface using a hand-sickle. Fresh biomass was weighed and bagged in the field and transported to Montana State University – Bozeman to dry in a forced air-drying oven at (109.4° F, 3.0% humidity) for approximately 48 hours. Dry masses were then recorded, and samples were individually ground to 1 mm using a Wiley Mill (Thomas Scientific, 2023). From

<sup>3</sup> pH, percent SOM, P, and K are based on the top six inches of soil. Nitrate-N and sulfate-S pools are the result of adding the pools of all depths from the composite samples from a single block, for each block, then averaging these sums across the four plots for a mean N and S total.

<sup>4</sup> NWARC 2023 – 36 in.; NWARC 2024 – 36 in.; CARC 2023 – 24 in; CARC 2024 – 12 in.; FE 2023 – 24 in.; FE 2024 – 36 in; BV 2023 – 36 in.; BV 2024 – 36 in.

<sup>5</sup> NWARC 2023 – 36 in.; NWARC 2024 – 36 in.; CARC 2023 – 24 in; CARC 2024 – 12 in.; FE 2023 – 24 in.; FE 2024 – 36 in; BV 2023 – 36 in.; BV 2024 – 36 in.

the original sample, a sub-sample was partitioned to be ground to 0.5 mm sieve using a UDY cyclone sample mill (UDY Corporation, 2023)

### Analyses

Soil samples were dried at 221° F for 24 hours prior to analysis by Agvise Laboratories, Northwood, ND. Nutrient concentration and pool calculations were based on an assumed bulk density used by Agvise Laboratories. Nitrate-N<sup>6</sup> (Gelderman & Beegle, 2015), sulfate-S<sup>7</sup> (Franzen, 2015), P<sup>8</sup> (Frank et al., 2015), K<sup>9</sup> (Warnacke & Brown, 2015), SOM<sup>10</sup> (Combs & Nathan, 2015), and pH<sup>11</sup> (Peters et al., 2015) were all analyzed using Recommended Chemical Soil Test Procedures for the North Central Region (NCR-13, 2015)

Plant tissue was analyzed for total N and S, nitrate concentrations, and forage nutritive parameters.

The 0.5 mm subsamples were submitted to the Montana State Land Resources Soil Fertility Laboratory and analyzed for total tissue N (TN) and S (TS) using a LECO CNS combustion analyzer (LECO Corporation, 2023).

The 1.0 mm samples were analyzed using a FOSS NIR DS2500 spectroscopy for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), ether extract (EE), ash, 48-hour in-vitro NDF digestibility (NDFD), neutral detergent fiber crude protein (NDFCP), all reported as percent dry matter (% DM) (FOSS Analytical, 2023)(FOSS Analytical, 2023)(FOSS

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<sup>6</sup> Nitrate-N 0.012 M KCl, Cd reduction

<sup>7</sup> Sulfate-S 0.12 M KCl, turbidmetric BaCl<sub>2</sub>

<sup>8</sup> P – Olsen

<sup>9</sup> K – 1.0 M NH<sub>4</sub>OAc (pH 7.0), ICP-AES, AAS

<sup>10</sup> SOM – loss on ignition (680°F)

<sup>11</sup> 1:1 soil 0.01 M CaCl<sub>2</sub>

Analytical, 2023). From these values, fatty acids (FA), N free NDF (NDFn), non-fibrous carbohydrate (NFC), dry matter intake (DMI), total digestible nutrients (TDN), and relative forage quality (RFQ) were calculated:

$$FA = EE - 1$$

$$NDFn = NDF - NDFCP$$

$$NFC = 100 - (NDFn + CP + EE + ash)$$

$$TDN_{grass} = (NFC * 0.98) + (CP * 0.87) + (FA * 0.97) + \left( NDFn * \frac{NDFD}{100} \right) - 10$$

$$DMI_{grass} = -2.318 + 0.442 * CP - 0.0100 * CP^2 - 0.0638 * TDN + 0.000922 * TDN^2 \\ + 0.180 * ADF - 0.00196 * ADF^2 - 0.00529 * CP * ADF$$

$$RFQ_{grass} = DMI * TDN / 1.23$$

A subsample of ten grams of 1.0-mm samples was analyzed by the Montana State Agricultural Analytical laboratory for nitrate analysis. A qualitative screening test (strip test) is performed to determine the presence of nitrate. If this test is positive, quantitative nitrate determination is performed by extracting nitrate in a buffered aqueous solution and measuring with a nitrate selective ion electrode (Carlson & Schneider, 1986). Results are reported as the nitrate ion on an air-dried basis, and the reporting limit for nitrate in forages is 0.15% as the nitrate ion. From the reported results, nitrate levels were converted to parts per million nitrates.

### Statistical Analyses

Statistical analysis was completed utilizing the Fisher's LSD and GLIMMIX procedures of SAS 9.4 (SAS Institute, 2013). Experimental unit was identified as individual plot ( $n = 36$ ) for Ft. Ellis and Broadview and individual split-plot for NWARC and CARC. Fixed effects were N, S, and the N x S interaction. The random effect for all sites was block, with N main plot nested within the block random effect for NWARC and CARC. Due to environmental differences, the main effects of N, S, and the N x S interaction were all analyzed within location and year. Nitrate levels that were reported as  $< 1500$  ppm were removed from the datasets for analysis of nitrate concentrations only. For most analyses, most notably nitrate, N rate was the only significant effect. The relationship between available N and nitrate levels is well established (Bowman et al., 2003; Crawford et al., 1961; George et al., 1973; Hall, 2018) and the purpose of this study was to examine the relationship between S and the N x S interaction on nitrate concentrations. For this reason, in the RCBD sites (FE and BV), N effects are not ignored but rather removed from the models for a separate analysis to better understand the S effects and the N x S interaction (Bolker et al., 2009; Burnham et al., 2011; Gbur et al., 2012; Ruiz et al., 2024; SAS Institute, 2013; Stroup, 2012). Statistical differences were determined at  $P \leq 0.05$ .

## Results and Discussion

### Statistical Results and Justification

When N is removed from the full models, the variation explained by N is reallocated into the N x S interaction (Table 7). The N x S term captures the combined effect of N and S on the dependent variable. Once N is removed, the model can no longer separate the independent contribution of N from N x S interaction. Because of this, N x S absorbs the variability in the

dependent variable that was previously attributed to the N term (Bolker et al., 2009; Burnham et al., 2011; Gbur et al., 2012; Ruiz et al., 2024; SAS Institute, 2013; Stroup, 2012). This is validated in that AIC values generated from models with and without N are identical suggesting that all N variability is reallocated to NXS when N is removed. (Table 7).

In years when all nitrate levels were reported as < 1500 ppm, CP was used as a proxy to predict effects of N, S, and N x S on nitrate. CP is determined based on protein-N and non-protein-N (NPN), which includes nitrate-N. Pearson correlation coefficients for nitrate and CP provide strong evidence for a correlation between CP and nitrate for locations and years where nitrate levels were not all <1500 ppm (Table 8, 9, 10, 11, 12).

Table 7. FE and BV full models, reduced models, associated AIC values, and associated p-values for N, S, and N x S effects on nitrate for both 2023 and 2024.

Location	Year	Full model	AIC	p-value	Reduced model	p-value	AIC
FE	2023	N	452.39	0.001	-	-	452.39
		S		0.6529	S	0.6529	
		N x S		0.7547	N x S	0.0014	
FE	2024	N	472.95	0.0583	-	-	472.95
		S		0.3544	S	0.3537	
		N x S		0.0838	N x S	0.0368	
BV	2023	N	NA <sup>12</sup>	NA	-	NA	NA
		S		NA	S	NA	
		N x S		NA	N x S	NA	
BV	2024	N	584.28	0.1531	-	-	584.28
		S		0.0794	S	0.0794	
		N x S		0.1647	N x S	0.1282	

<sup>12</sup> All nitrate levels were reported less than 1500 ppm resulting in models that could not be fit.

Table 8. Ft. Ellis 2023 Pearson correlation coefficient values for forage nitrate and CP.

	CP	Nitrate
CP	1.00000	0.82315
Nitrate	0.82315	1.00000

Table 9. Ft. Ellis 2024 Pearson correlation coefficients for forage nitrate and CP.

	CP	Nitrate
CP	1.00000	0.90536
Nitrate	0.90536	1.00000

Table 10. NWARC 2023 Pearson correlation coefficients for forage nitrate and CP.

	CP	Nitrate
CP	1.00000	0.81143
Nitrate	0.81143	1.00000

Table 11. NWARC 2024 Pearson correlation coefficients for forage nitrate and CP.

	CP	Nitrate
CP	1.00000	0.76643
Nitrate	0.76643	1.00000

Table 12. CARC 2024 Pearson correlation coefficients for forage nitrate and CP.

	CP	Nitrate
CP	1.00000	0.82557
Nitrate	0.82557	1.00000

### Climate Context

Due to year-to-year and site-to-site variation, it is proposed that climate greatly contributed to differences in forage quality, yield, and nutritive value. Most notably, nitrate results appear to be dependent on precipitation totals and drought conditions experienced in the given areas during a growing season. Figures 2-8 provide daily precipitation context from March 30 through the sampling date (Coop, 2024; United States Department of Agriculture, 2024a, 2024b), whereas Figures 10 and 11 display drought and abnormally dry conditions between the planting dates and sampling dates (Fuchs & National Drought Mitigation Center, 2024). In 2023, CARC, FE, and BV did not experience drought or abnormally dry conditions during the growing period, but NWARC did (Figure 10). In 2024, CARC, FE, and NWARC did experience drought and abnormally dry conditions, but BV did not (Figure 11).

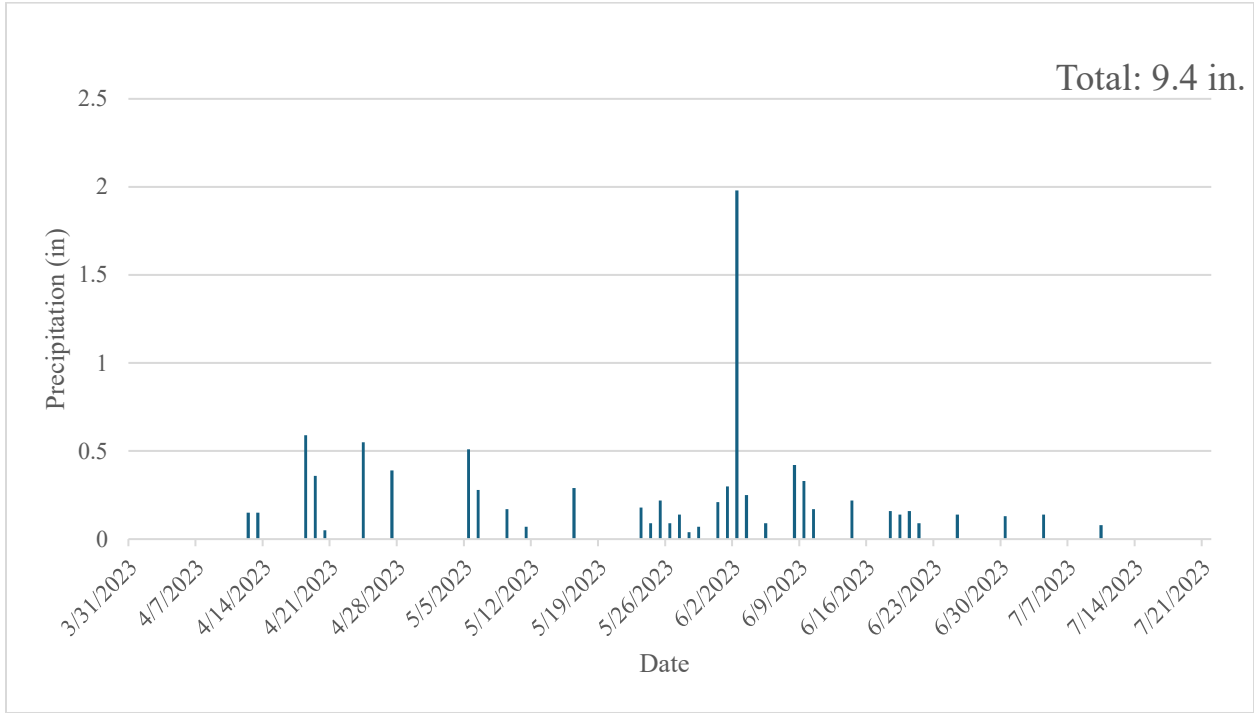


Figure 2. CARC 2023 daily precipitation (in).<sup>13</sup>

<sup>13</sup> (United States Department of Agriculture, 2024a)

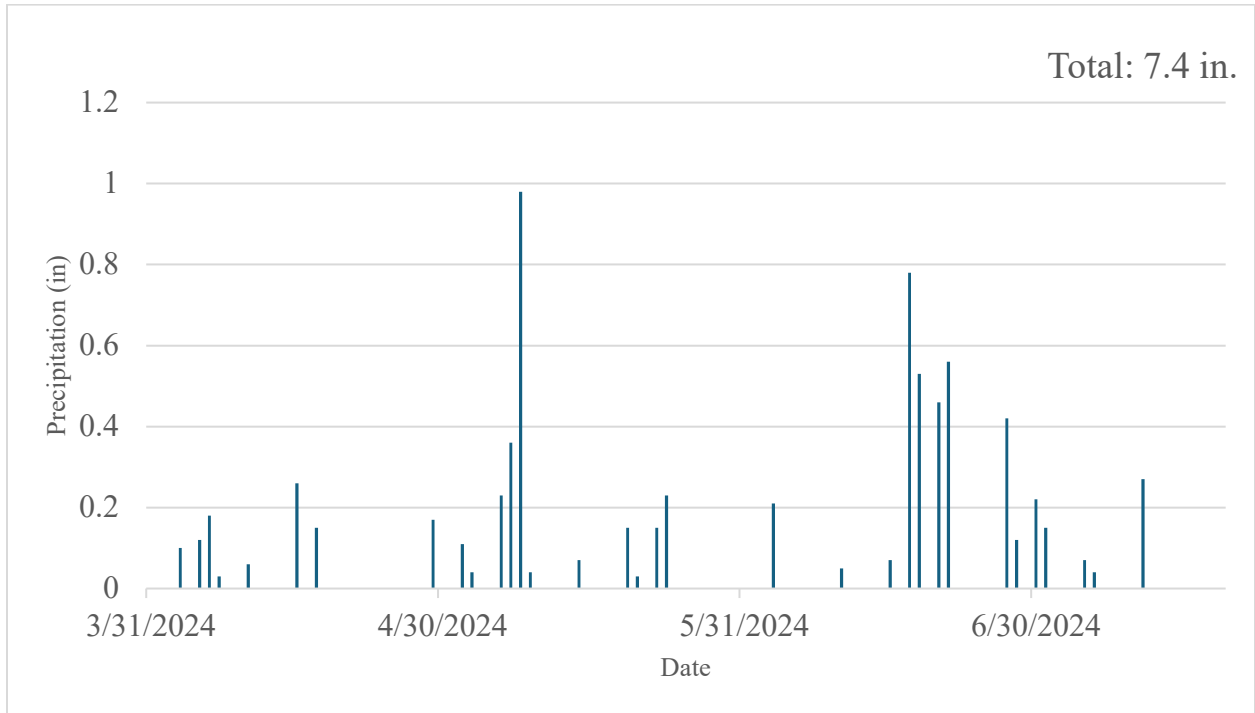


Figure 3. CARC 2024 daily precipitation (in).<sup>14</sup>

<sup>14</sup> (United States Department of Agriculture, 2024a)

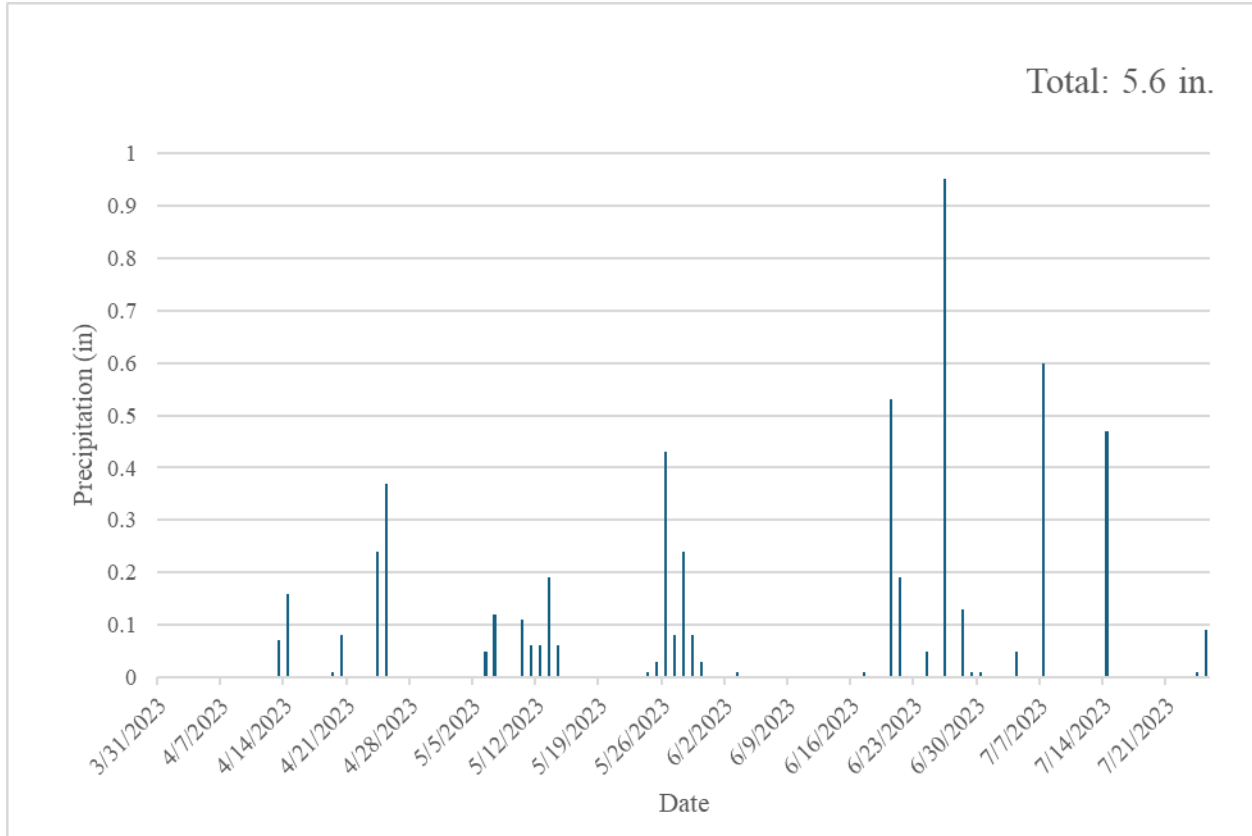


Figure 4. Ft. Ellis 2023 daily precipitation (in).<sup>15</sup>

<sup>15</sup> (Coop, 2024)

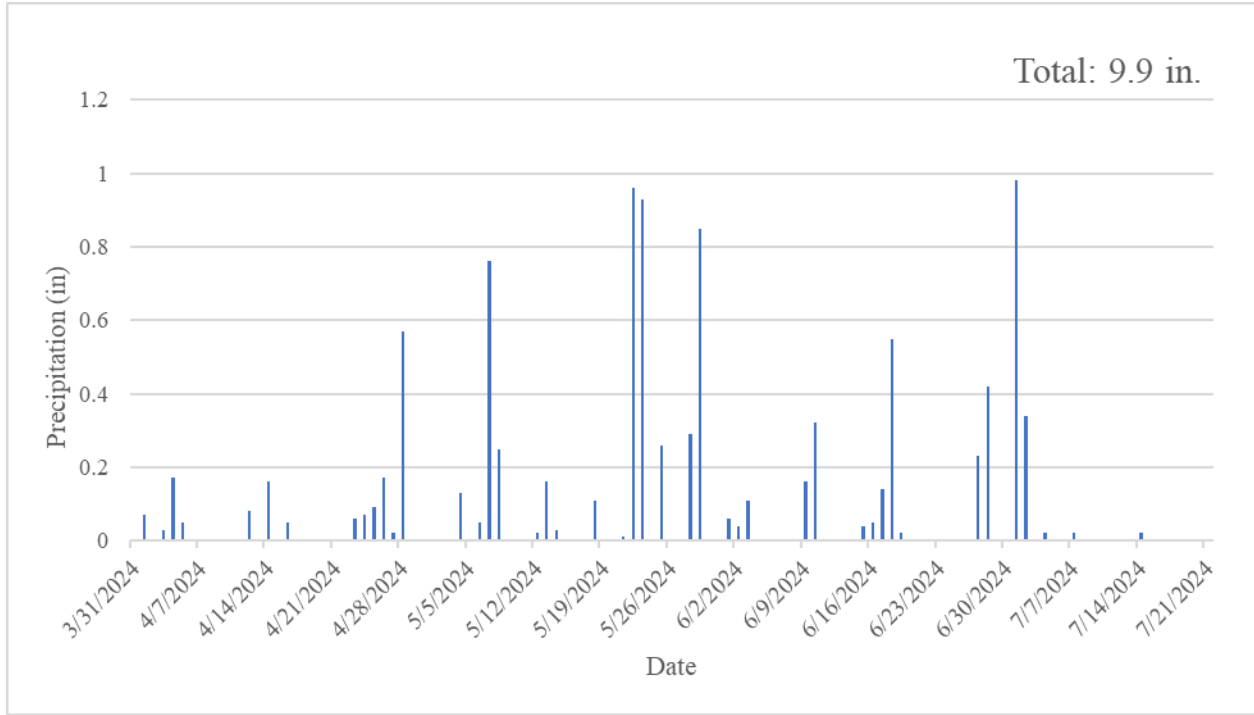


Figure 5. Ft. Ellis 2024 daily precipitation (in).<sup>16</sup>

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<sup>16</sup> (Coop, 2024)

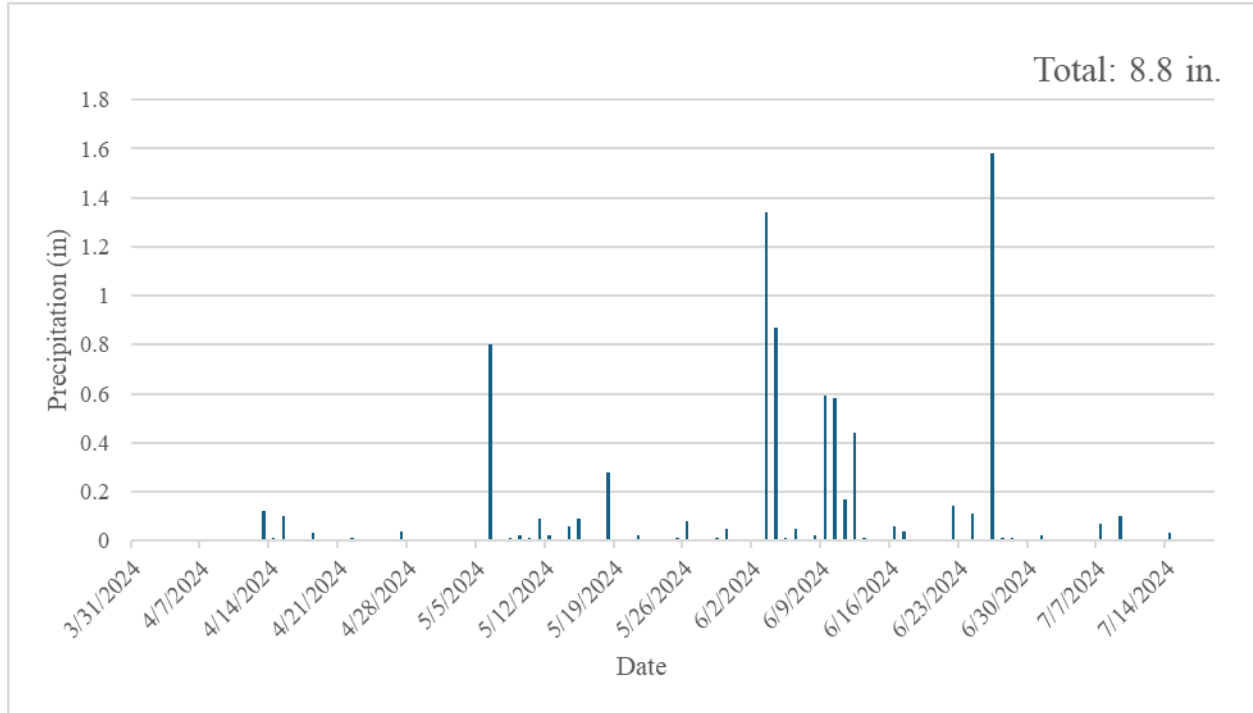


Figure 6. Broadview 2023 daily precipitation (in.).<sup>17</sup>

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<sup>17</sup> (Coop, 2024)

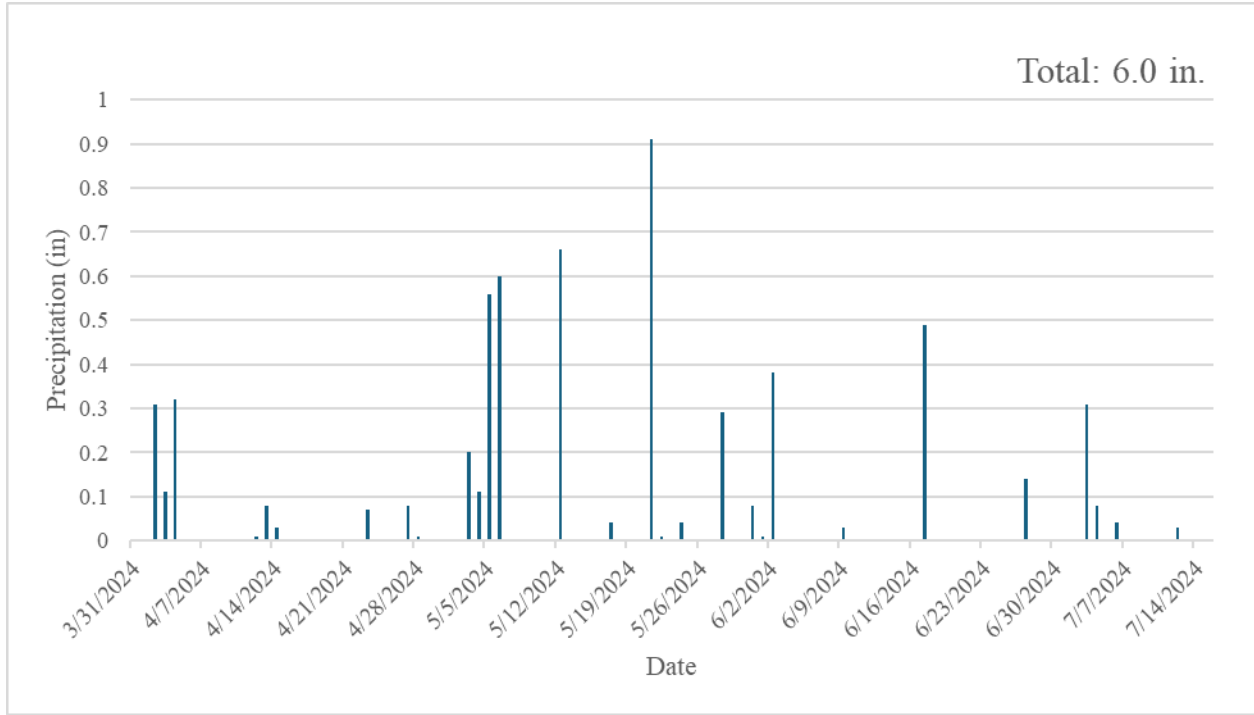


Figure 7. Broadview 2024 daily precipitation (in.)<sup>18</sup>

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<sup>18</sup> (Coop, 2024)

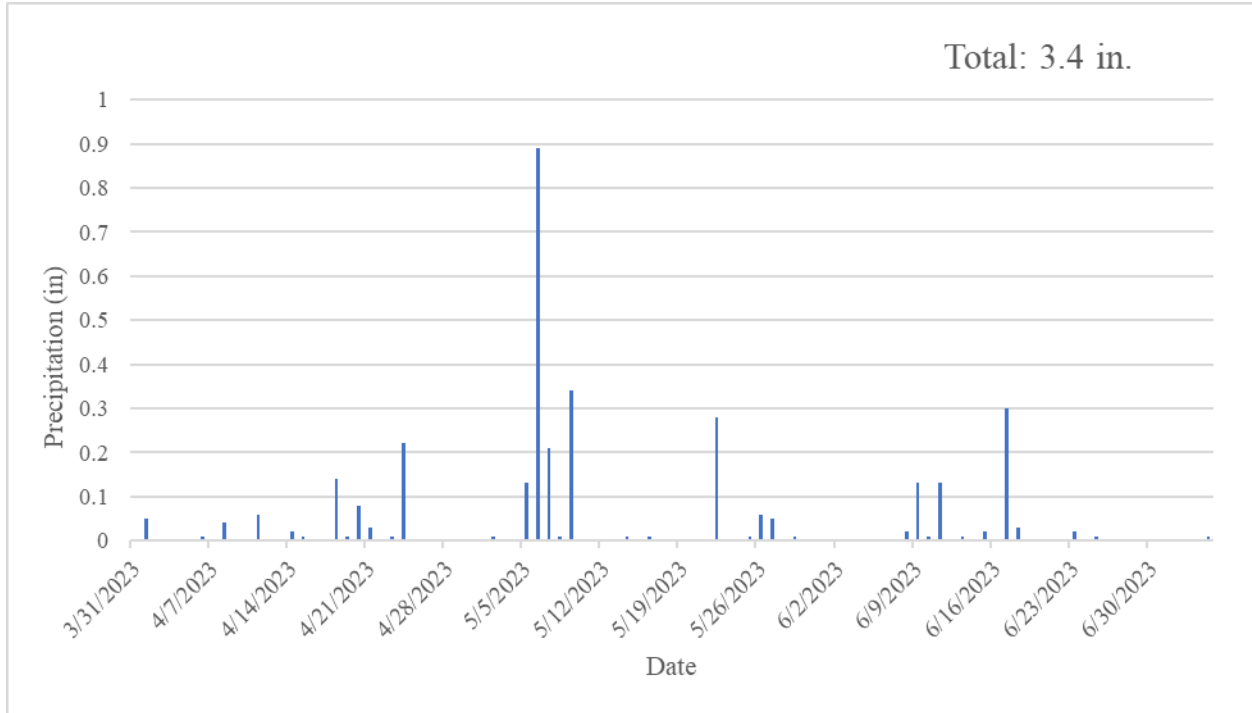


Figure 8. NWARC daily precipitation (in)<sup>19</sup>.

<sup>19</sup> (United States Department of Agriculture, 2024b)

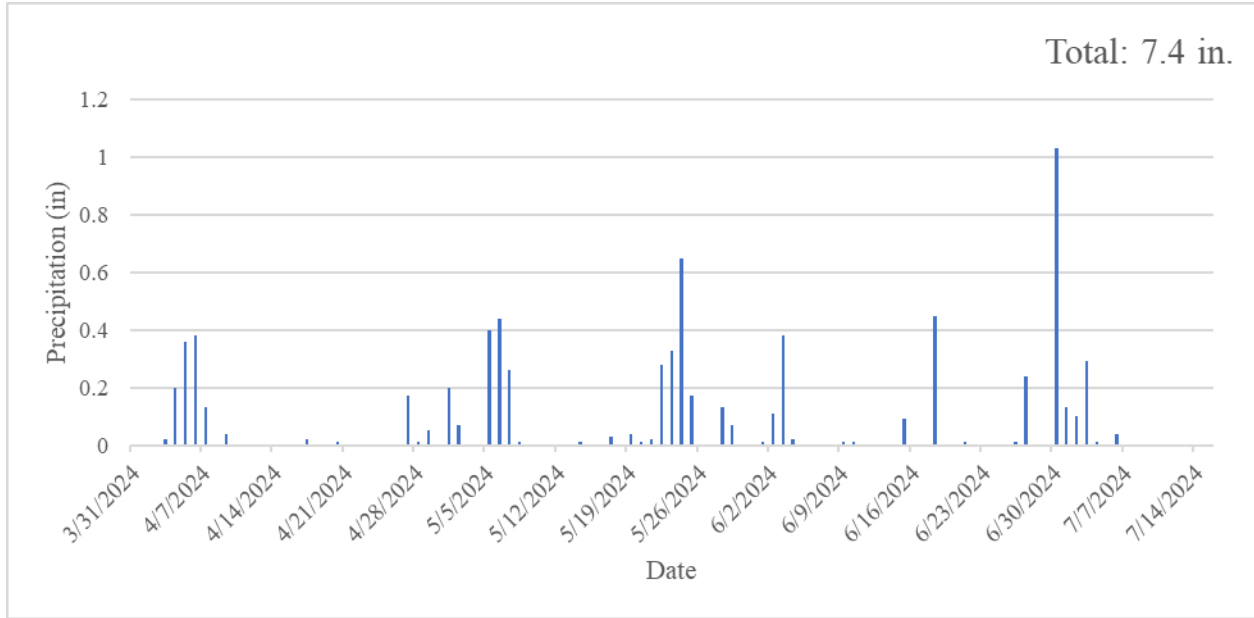


Figure 9. NWARC daily precipitation (in)<sup>20</sup>.

<sup>20</sup> (United States Department of Agriculture, 2024b)

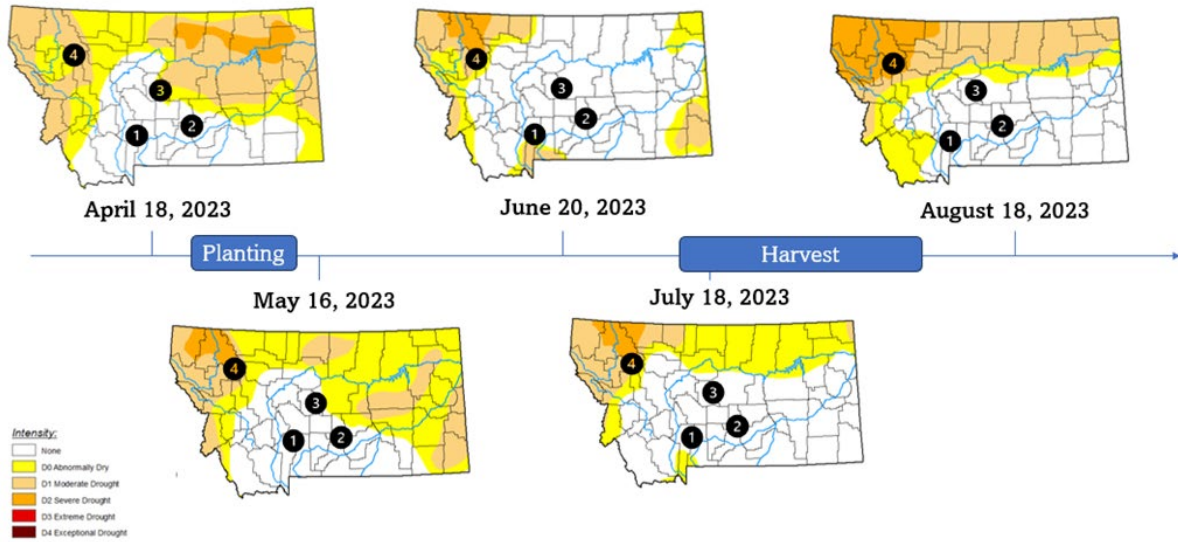


Figure 10. Drought and abnormally dry conditions during the 2023 growing season ((Fuchs & National Drought Mitigation Center, 2024).

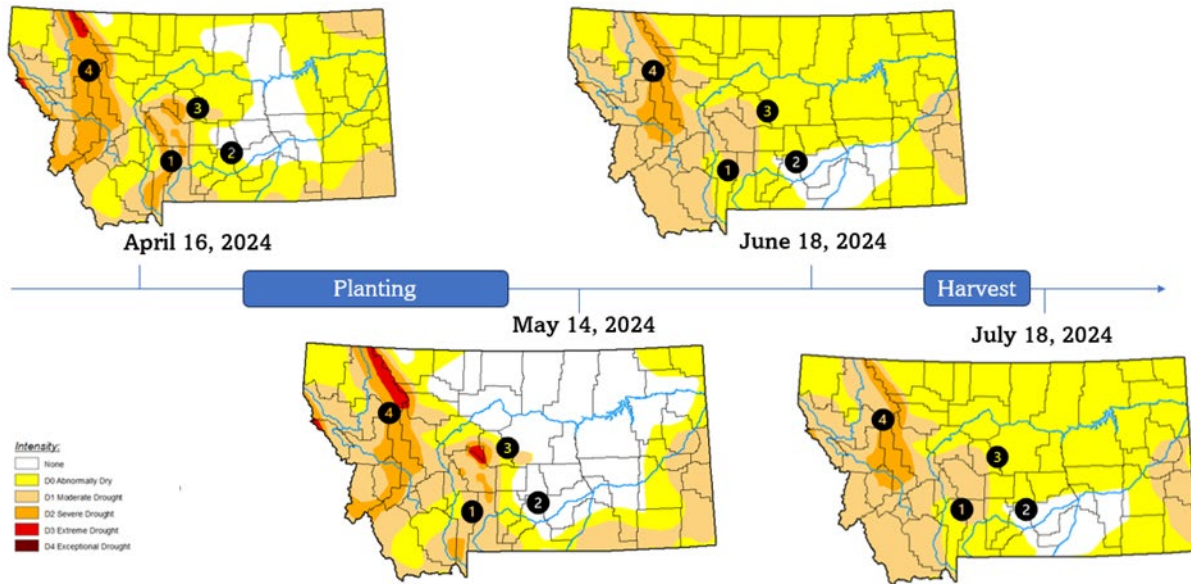


Figure 11. Drought and abnormally dry conditions during the 2024 growing season (Fuchs & National Drought Mitigation Center, 2024).

### Ft. Ellis

In 2023, the reduced model, excluding nitrate observations below the detection limit ( $n = 24$ ), nitrate levels after S additions were numerically below standard restriction levels (Table 13). The only reduction in nitrate for 2023 was due to a lower N rate, made evident in the original full model (Table 9). Nitrate levels in 2024 were all above 10,000 ppm, the upper nitrate concentration limit restricting any feeding (Goosey et al., 2022). These elevated nitrate levels were most likely due to an assumed increased yield potential for 2024 based on yields observed in 2023, higher potential for N mineralization due to higher SOM, and drought conditions for much of the growing season (Figure 11). Despite these extremely high concentrations, reductions, like the trends observed in 2023 (Figure 3), were detected in 2024 (Figure 4) related to S additions. Nitrate levels were reduced when high rates of S (20 lbs. acre<sup>-1</sup>) were applied with

medium N (35 lbs. N ton<sup>-1</sup>) compared to medium N with only a medium S rate (10 lbs. acre<sup>-1</sup>). S rates did not decrease nitrate at high N rates in both years (Table 14).

Medium N treatments resulted in lower nitrate levels both years when compared to high N with additions of S; N x S interaction was significant in both 2023 (P = 0.001) and 2024 (P = 0.037). With less N applied, there were no reductions in yield for either year, with medium N, resulting in a greater yield than high N treatments in 2024 (Figure 12). This response is most likely due to diminishing returns from applying one and a half times the recommended N rate for spring forage barley (Lenssen et al., 2021) (Figure 6). Predictive yield curves for both 2023 (Figure 13) and 2024 (Figure 14) suggest that a yield-based N-rate of 33.8 lbs. N ton<sup>-1</sup> would be appropriate for maximizing yield if nitrate was not a concern. Because of climate uncertainty and the role it plays in nitrate accumulation, yield goals become less important in order to produce a usable crop.

The difference among forage nutritive values (e.g., NDF, ADF, CP, TDN, DMI, RFQ) in samples with a nitrate reduction could only be attributed to N treatment (P < 0.0001) in 2023 and all but ADF were affected by N rates in 2024. ADF decreased with larger additions of S (P=0.027). RFQ was used as a summation of overall forage quality to compare year-to-year variability because all previously mentioned nutritive metrics are used to calculate this RFQ (Ward & Beth De Ondarza, 2008). Of the differences in RFQ, due to increasing available-N, that were observed in 2023 (P<0.0001) and 2024 (P<0.0001), more available-N decreased RFQ between a more nutritionally demanding gestating ewe to a less nutritionally demanding gestating beef cow regardless of S treatment (Figure 15) (National Academies of Sciences, 2016;

National Research Council, 2007). RFQ for both years was within ranges adequate for gestating mature beef cows (Figure 12)

Table 13. Forage nitrate concentrations (ppm) in response to N x S interactions at Ft. Ellis, 2023 Letter groupings provide statistical significance for both row and column comparisons. Low S = 0 lbs. S acre<sup>-1</sup>, Medium S = 10 lbs. S acre<sup>-1</sup>, High S = 20 lbs. S acre<sup>-1</sup>; Medium N = 1.0\*25 lbs. N ton<sup>-1</sup>, High N = 1.5 \* 25 lbs. N ton<sup>-1</sup>; with a yield goal of 4 tons/acre.

	Low S	Medium S	High S
Medium N	5075bc	3400c	3450c
High N	8825a	8800a	8475ab

Table 14. Forage nitrate concentrations (ppm) in response to significant N x S interactions at Ft. Ellis, 2024 Letter groupings provide statistical significance for both row and column comparisons. Low S = 0 lbs. S acre<sup>-1</sup>, Medium S = 10 lbs. S acre<sup>-1</sup>, High S = 20 lbs. S acre<sup>-1</sup>; Medium N = 1.0\*33.8 lbs. N ton<sup>-1</sup>, High N = 1.5 \* 33.8 lbs. N ton<sup>-1</sup>; with a yield goal of 5 tons acre<sup>-1</sup>

	Low S	Medium S	High S
Medium N	14667ab	16750a	11375b
High N	15500ab	17000a	18200a

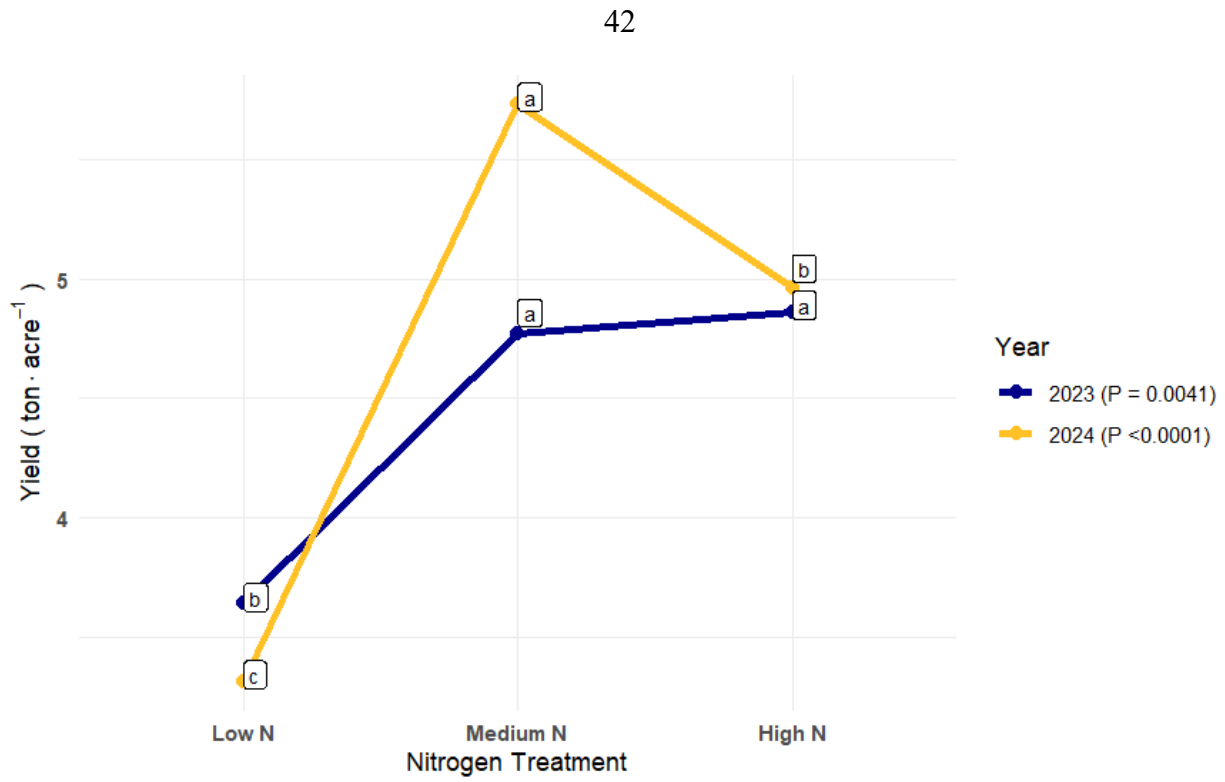


Figure 12. Yield (ton acre<sup>-1</sup>) response to N treatments at the Ft. Ellis site.<sup>21</sup>

<sup>21</sup> Letters indicate differences between treatment levels in a single year. Low N = 0x lbs. N ton<sup>-1</sup>, medium N = 1x lbs. N ton<sup>-1</sup>, high N = 1.5 x lbs. N ton<sup>-1</sup>. 2023 yield-based rate – 25 lbs. N ton<sup>-1</sup> \* 4 tons; 2024 yield-based rate – 33.8 lbs. N ton<sup>-1</sup> \* 5 tons.

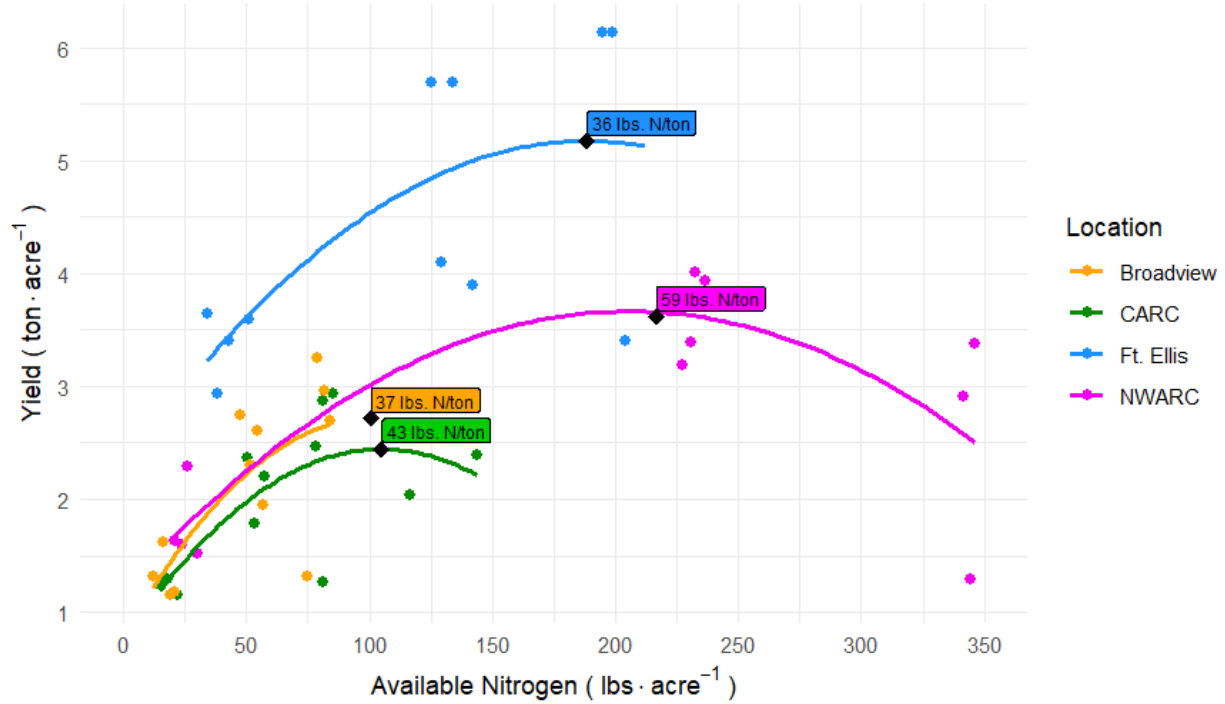


Figure 13. Site yield (tons acre<sup>-1</sup>) responses to plant available N (soil + fertilizer N) during the 2023 field season.<sup>22</sup>

<sup>22</sup> (0x, 1.0x, 1.5x 25 lbs. N ton<sup>-1</sup> + nitrate-N from spring 2023 soil, 4 cores sampled to a depth of 24 inches x 4 blocks). A quadratic trendline was fit to determine or predict the N rate that maximized yield. Based on (Lenssen et al., 2021) 33.8 lbs. N ton<sup>-1</sup> maximizes tonnage for forage winter wheat. Ft. Ellis and Broadview closely align with this value, but CARC and NWARC do not potentially due to the leaching potential of the soils.

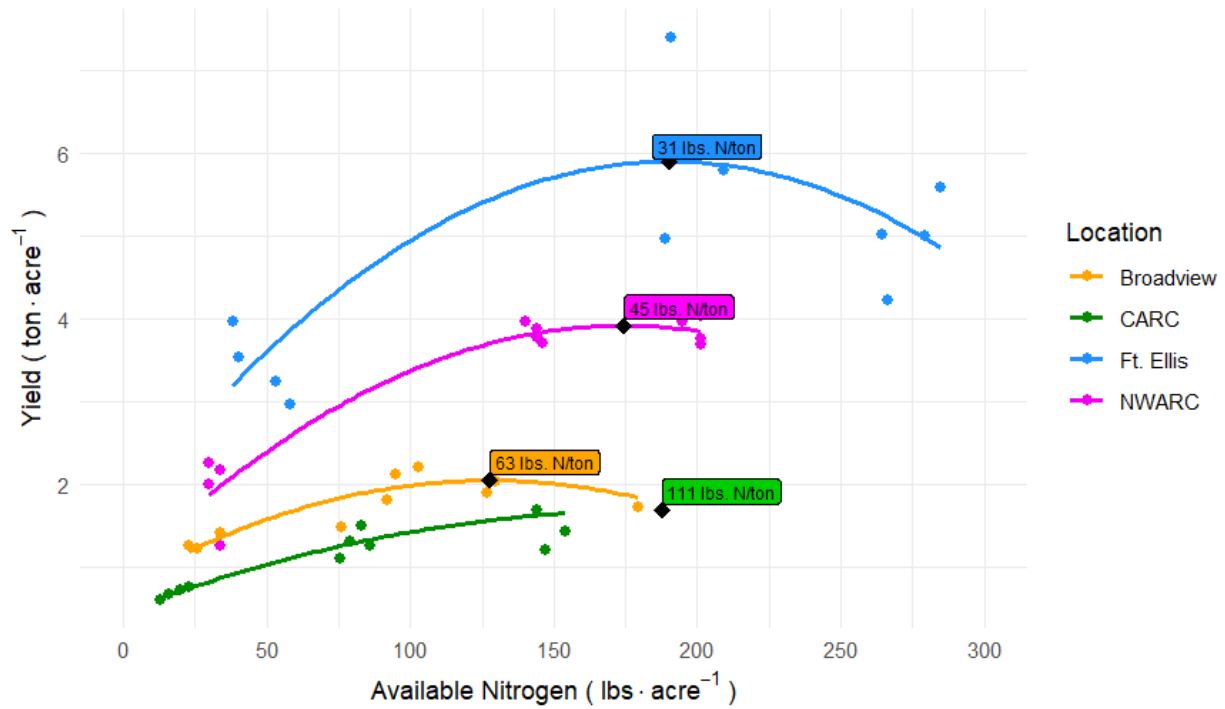


Figure 14. Site yield (tons acre<sup>-1</sup>) responses to plant available N (soil + fertilizer N) during the 2024 field season.<sup>23</sup>

<sup>23</sup> (0x, 1.0x, 1.5x 35 lbs. N ton<sup>-1</sup> + nitrate-N from spring 2024 soil, 4 cores sampled to a depth of 24 inches x 4 blocks). A quadratic trendline was fit to determine or predict the N rate that maximized yield. Based on (Lenssen et al., 2021) 35 lbs. N/ton/acre maximizes tonnage for forage winter wheat. Ft. Ellis aligns with this value, but all other sites most likely had moisture-limited yield responses in 2024.

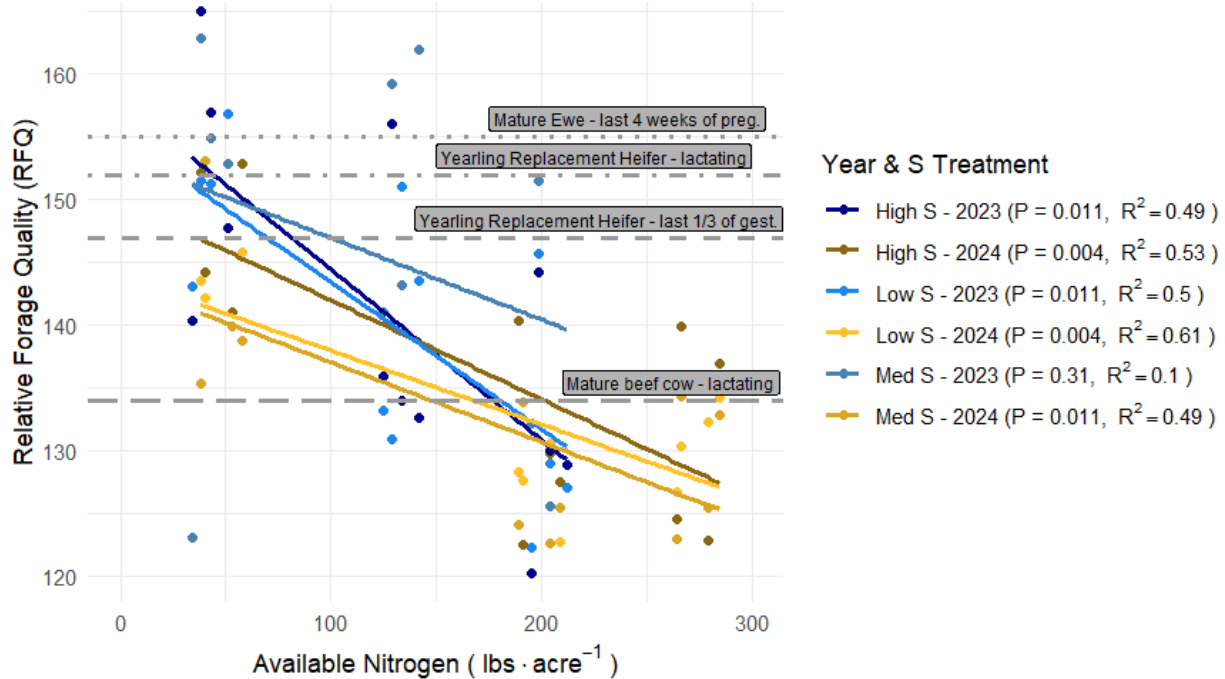


Figure 15. Relative forage quality response to increasing available N (soil + fertilizer N) over low, medium, and high S treatments.<sup>24</sup>

### Broadview

Nitrate levels at the Broadview site were all below the 1500 ppm detection limit in 2023, most likely due to harvest at soft-dough and nitrate-N translocation from foliage to grain (Buxton, 1996; Egle et al., 2015; Khorasani et al., 1997b; Mannerkorpi & Taube, 1995b). In 2024, more nitrate levels were reported above 1500 ppm compared to 2023, with a tendency towards an S effect (P = 0.080) for nitrate in both the full and reduced models (Table 7). High N treatments resulted in higher nitrate levels compared to low N treatments in 2024. However, when all treatments are compared, medium N, medium S treatments resulted in a mean nitrate concentration of < 1500 ppm compared to high N, high S treatment, but the N x S interaction

<sup>24</sup> 0, 1.0, 1.5x lbs. N ton<sup>-1</sup> + nitrate-N from spring soil, 4 cores sampled to a depth of 36 inches x 4 blocks = Available N. Low S = 0 lbs. acre<sup>-1</sup>; medium S = 10 lbs. acre<sup>-1</sup>; high S = 20 lbs. acre<sup>-1</sup>

was not significant ( $P = 0.130$ ) despite this observation. Broadview 2023 nitrate levels provided little information on the relationship between N, S, or N x S effects on nitrate due to concentrations being below the detection limit. Crude protein is composed of both protein-N and non-protein-N, which includes nitrate. Pearson correlation coefficients at other sites, FE 2023 and 2024, NWARC 2023 and 2024, and CARC 2024, strongly suggest a correlation between nitrate concentrations and percent CP (Tables 8, 9, 10, 11, 12). Because of this, CP was used as a proxy for nitrate concentrations for BV in 2023 only. In 2023, CP was affected by N ( $P < 0.001$ ) and S ( $P = 0.012$ ), with high N treatments resulting in higher CP concentrations, but low S treatments resulting in higher CP concentrations (Figure 16). This relationship, though speculative, may be a result of a reduction in nitrate levels. Sulfur deficiencies would generally not result in higher CP because of a decrease in the protein-N portion but was most likely due to a decrease in the NPN portion. Sulfur-containing essential amino acids are the precursors to proteins, enzymes, and plant structural proteins, and with less available S, S-containing amino acids, cysteine and methionine, would generally be deficient, limiting protein levels (Reuveny et al., 1980; Thompson et al., 2015). The results observed for 2023 did not reflect this relationship (medium and high S additions resulted in lower CP compared to no S additions) suggesting a reduction in the NPN portion of CP (Figure 16).

In both 2023 and 2024 yield was impacted by N only, ( $p < 0.001$ ) with high N treatments contributing to higher yields than medium or low N treatments in 2023 and higher yields with additions of N in 2024 (Figure 13). In neither year did S or N x S seem to play a role in increasing or decreasing yield. In 2023, the N rate to maximize yield was predicted to be 37 lbs. N  $\text{ton}^{-1}$ , only three pounds greater than the recommended N rate for spring annual cereal forage

(Figure 14). Perhaps this similar predicted N-rate could be due to less moisture related stress or loss of N through volatilization at application or leaching below root accessible depths. The 2024 crop experienced more water stress, compared to 2023 crop (Figure 19), where the predicted N needed to maximize yield was 39 lbs. greater than the established rate, suggesting a water-limited yield response, potential N volatilization due to broadcast application as opposed to incorporating, or leaching (Lenssen et al., 2021).

Forage nutritive value was impacted differently from 2023 to 2024. NDF and ADF increased with N additions ( $P < 0.001$ ;  $< 0.001$ ), decreased with S additions ( $P = 0.015$ ;  $0.022$ ), and only NDF decreased with a combination of lower N and higher S ( $P = 0.035$ ) (Table 8). TDN and DMI, also indicative of nutritive value, followed the inverse of the relationships of fiber, with TDN decreasing with N additions ( $P < 0.001$ ) and increasing with S additions ( $P = 0.047$ ). DMI, had the same relationship with N ( $P < 0.001$ ), S ( $P = 0.013$ ), and N x S ( $P = 0.02$ ) as NDF, as DMI is calculated from NDF. Overall, both digestibility (TDN & ADF) and dry matter intake (NDF & DMI) decreased with N and S additions. Relative forage quality values were also significantly affected by N ( $P < 0.001$ ), S ( $P = 0.016$ ), and N x S ( $P = 0.027$ ), with lower N resulting in higher RFQ values, medium S treatments with significantly higher RFQ values, and interactions of low N, high S resulting in the best forage quality. Differences in quality are most likely due to the more mature harvest stage (i.e., soft dough) that resulted in less nutritive value compounding with a greater growth rate of high N treatments resulting in more fiber, less digestibility, and available energy (Brundage et al., 1979; Buxton, 1996; Khorasani et al., 1997b; Mannerkorpi & Taube, 1995b). Nitrogen additions had the opposite effect on fiber percentages in 2024, with NDF and ADF lower ( $P = 0.039$ ;  $< 0.001$ ) at high N than low N treatments, but with

no S or N x S effects. Relative forage quality was decreased with increased N-availability in 2023 ( $P < 0.001$ ) (Figure 18), but no change in RFQ was detected as a result of N ( $P = 0.34$ ) or S ( $P = 0.60$ ). N x S ( $P = 0.064$ ) had a tendency towards an increasing RFQ with an increase in available-N with no S additions in 2024 (Figure 18). Year – to – year differences in RFQ are likely due to forage quality dependence on factors such as maturity, daily temperature, and moisture potentially affecting quality more than N or S (Brundage et al., 1979; Buxton, 1996; Cherney & Marten, 1982b; Egle et al., 2015; Khorasani et al., 1997c; Mannerkorpi & Taube, 1995b).

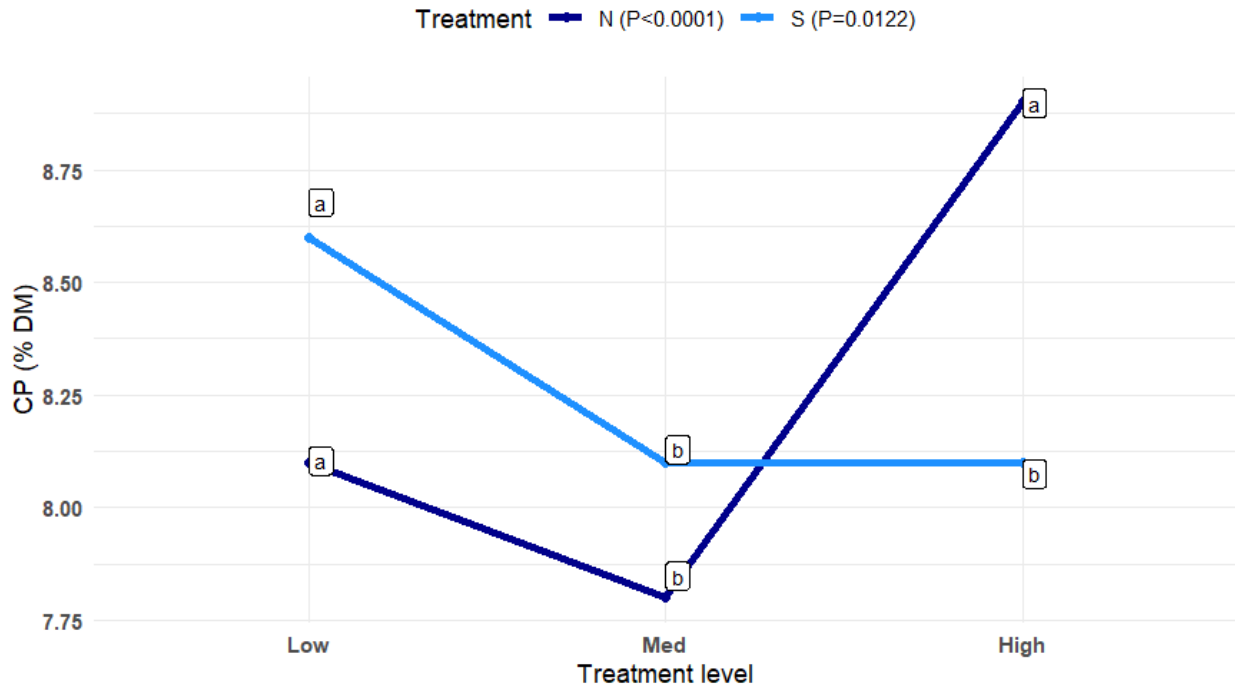


Figure 16. Broadview 2023 crude protein (% dry matter) in response to N and S treatments.<sup>25</sup>

<sup>25</sup> Letters indicate differences between treatment levels under a single treatment (S or N). Low N = 0x lbs. N ton<sup>-1</sup>; medium N = 1.0x lbs. N ton<sup>-1</sup>; high N = 1.5x lbs. N ton<sup>-1</sup>. 2023 yield-based N-rate = 25 lbs. N ton<sup>-1</sup> \* 1.5 tons; 2024 yield-based N-rate = 33.8 lbs. N ton<sup>-1</sup> \* 3 tons. Low S = 0 lbs. S acre<sup>-1</sup>; medium S = 10 lbs. S acre<sup>-1</sup>; high S = 20 lbs. S acre<sup>-1</sup>.

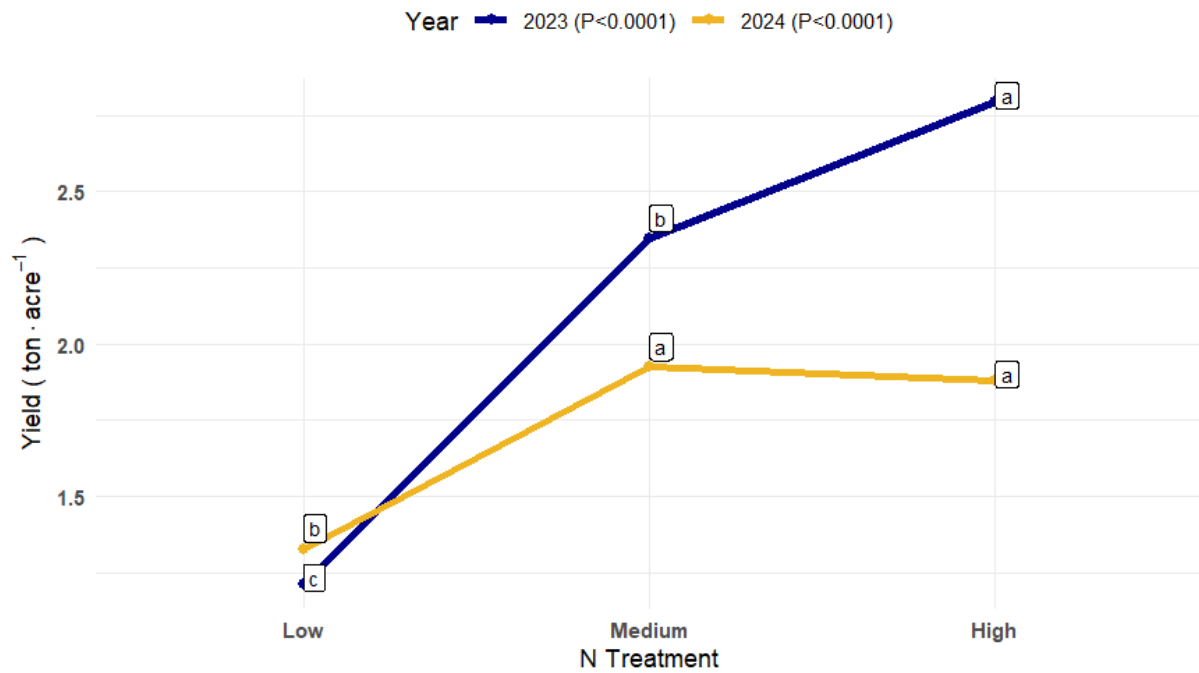


Figure 17. Broadview 2023 and 2024 yield (tons acre<sup>-1</sup>) by N treatments.<sup>26</sup>

<sup>26</sup> Letters indicate differences between treatment levels in a single year. Low N = 0x lbs. N ton-1; medium N = 1.0x lbs. N ton-1; high N = 1.5x lbs. N ton-1. 2023 yield-based N-rate = 25 lbs. N ton-1 \* 1.5 tons; 2024 yield-based N-rate = 33.8 lbs. N ton-1 \* 3 tons.

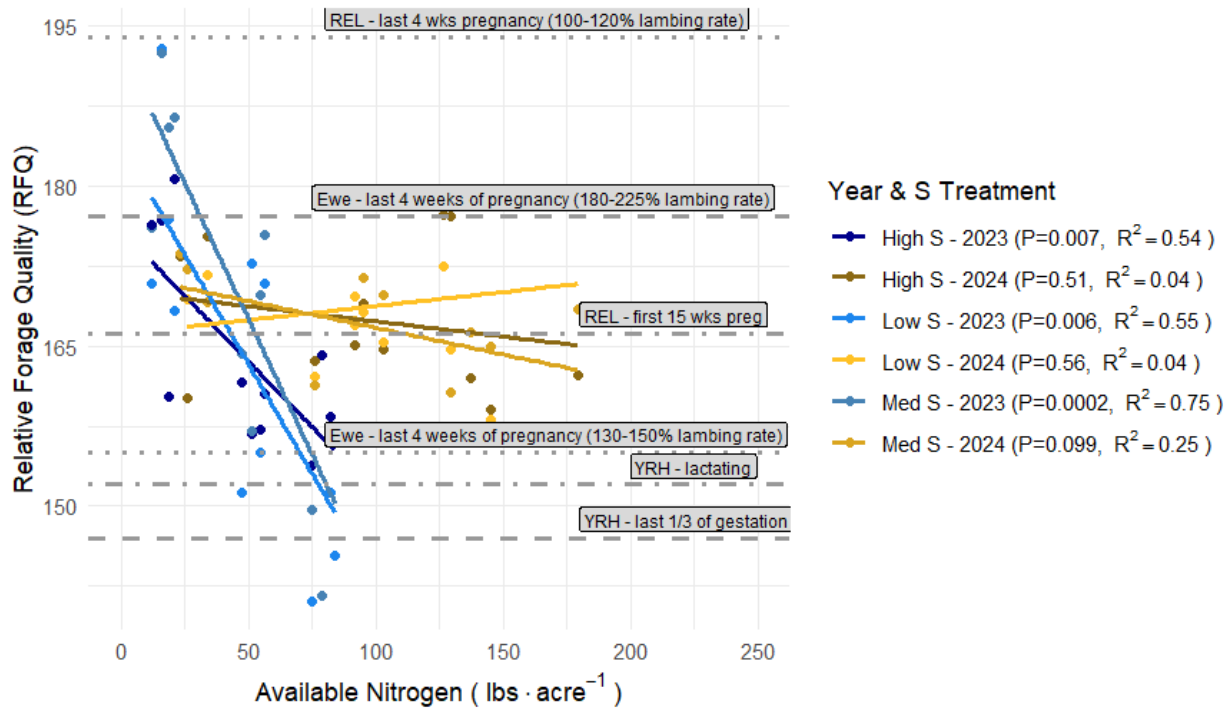


Figure 18. The response of relative forage quality to increasing available N (soil + fertilizer N) at the Broadview site during the 2023 and 2024 field seasons.<sup>27</sup>

### CARC

Similar to the 2023 Broadview site, all nitrate levels for CARC 2023 were below the detection threshold of 1500 ppm. Again, this was most likely due to a later maturity stage that resulted in nitrate-N translocation to protein formation in grain fill of the barley crop instead of elevated levels of nitrate in the foliage (Bulman & Smith, 1993; Egle et al., 2015), but could also be due to adequate moisture levels in 2023 compared to 2024 (Figure 2,3,10,11). Nitrogen rates needed to maximize yields varied by a drastic 43 lbs. N ton<sup>-1</sup> in 2023 to 111 lbs. N ton<sup>-1</sup> in 2024, suggesting lack of moisture limited yield in 2024 as opposed to N, which would also contribute

<sup>27</sup> 0, 1.0, 1.5x lbs. N ton<sup>-1</sup> + nitrate-N from spring soil, 4 cores sampled to a depth of 36 inches x 4 blocks = Available N. YRL = Yearling Replacement Heifer; REL = Replacement Ewe Lamb

to a higher nitrate accumulation potential (Figure 13,14)(Crawford et al., 1961). To estimate potential nitrate levels in 2023 and the effects of N, S, and N x S on nitrate, CP concentrations were compared with a N x S effect ( $P = 0.003$ ). Because high CP can reflect elevated nitrate levels (Moore et al., 2020), it is interesting to note that there were effects of N ( $P = 0.022$ ), S ( $P = 0.001$ ), and N x S ( $P = 0.003$ ) on CP in 2023, again with no S additions resulting in higher CP and the interaction of high N with no S additions resulted in the highest CP concentrations and medium N, medium S resulting in the lowest (Figure 14). While CP is not entirely indicative of nitrate, nitrate is included in measurements and additions of S generally would not decrease CP because of depressed protein synthesis as S is necessary to this process (Hawkesford et al., 2023; Thompson et al., 2015). Yield responded positively to additions of not only N ( $P < 0.001$ ), but also S ( $P = 0.03$ ). With higher additions of S, yield increased, suggesting S could improve yield at sites with low SOM and highly leachable soils (Figure 15).

In 2024, effects of N ( $P < 0.0001$ ), S ( $P = 0.027$ ), and N x S ( $P = 0.011$ ) on nitrate concentrations were observed both with and without observations below the detection limit. While N treatment had the most evident effect on nitrate, S additions were able to reduce the medium N nitrate concentrations from levels unsafe to feed any class of livestock to levels acceptable to feed to non-pregnant livestock and rationing to pregnant livestock (Table 15). While there is not an established trend linking nitrate and available S (Figure 17), the relationship still agrees with differences among treatments. Medium N with S additions did not decrease yields, but reducing N did increase NDF and ADF while decreasing CP.

In 2024, RFQ decreased with greater available N ( $P = 0.01$ ) (Figure 16). Nitrogen and S interaction affected RFQ ( $P = 0.02$ ) with RFQ decreasing despite S treatment, but with medium

and high S and no additional N applied RFQ was generally greater (Figure 16). Relative forage quality decreased with more available N from values adequate for gestating mature ewes to values adequate for gestating yearling replacement heifers (National Academies of Sciences, 2016; National Research Council, 2007).

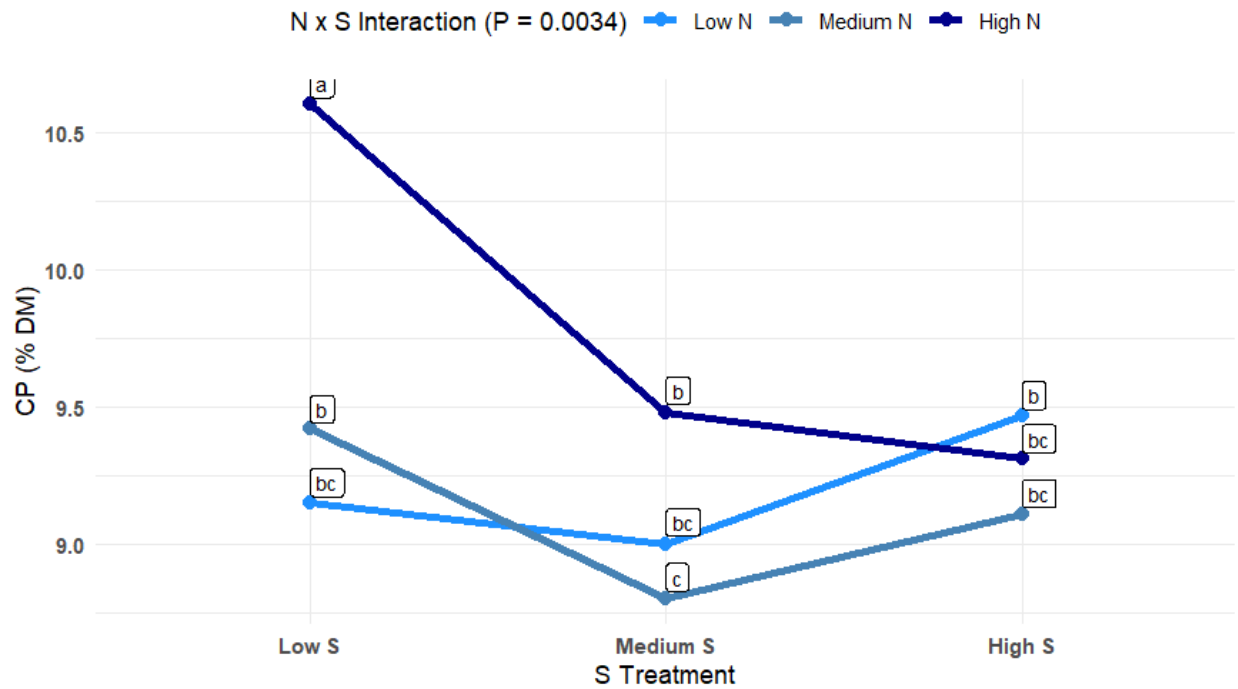


Figure 17. CARC 2023 CP (% DM) in response to an N x S interaction.<sup>28</sup>

<sup>28</sup> Letters indicate differences in treatment levels of the N x S interaction.

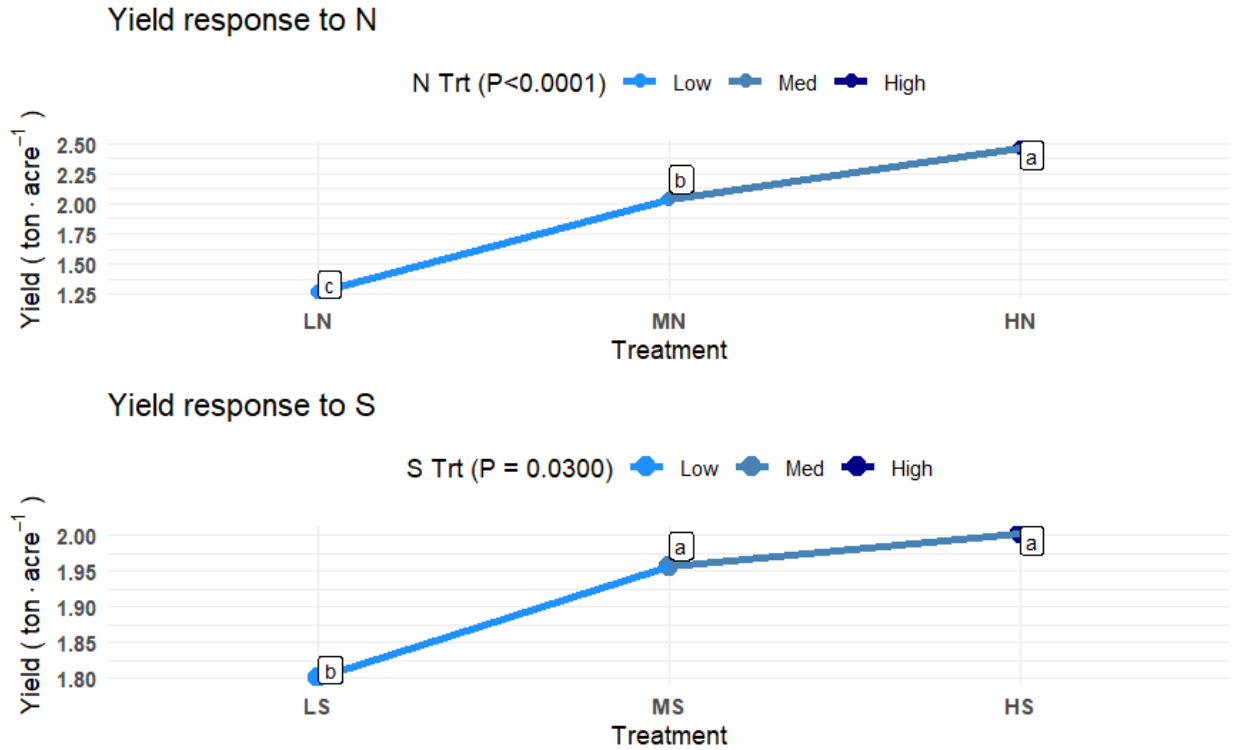


Figure 18. CARC 2023 yield (ton acre<sup>-1</sup>) response to N and S treatments.<sup>29</sup>

Table 15. Nitrate concentrations (ppm) in response to significant N x S interactions at CARC, 2024.<sup>30</sup>

	Low S	Medium S	High S
Medium N	6800a	2100b	2175b
High N	8825a	8150a	9600a

<sup>29</sup> Letters indicate differences between treatments levels.

<sup>30</sup> Medium N, with additions of S significantly reduced nitrate levels. This reduction also allows the ability to feed to more classes of livestock. Letter groupings provide statistical significance for both row and column comparisons. Low S = 0 lbs. S acre<sup>-1</sup>, Medium S = 10 lbs. S acre<sup>-1</sup>, High S = 20 lbs. S acre<sup>-1</sup>; Medium N = 1.0\*33.8 lbs. N ton<sup>-1</sup>, High N = 1.5 \* 33.8 lbs. N ton<sup>-1</sup>; with a yield goal of 2.5 tons acre<sup>-1</sup>

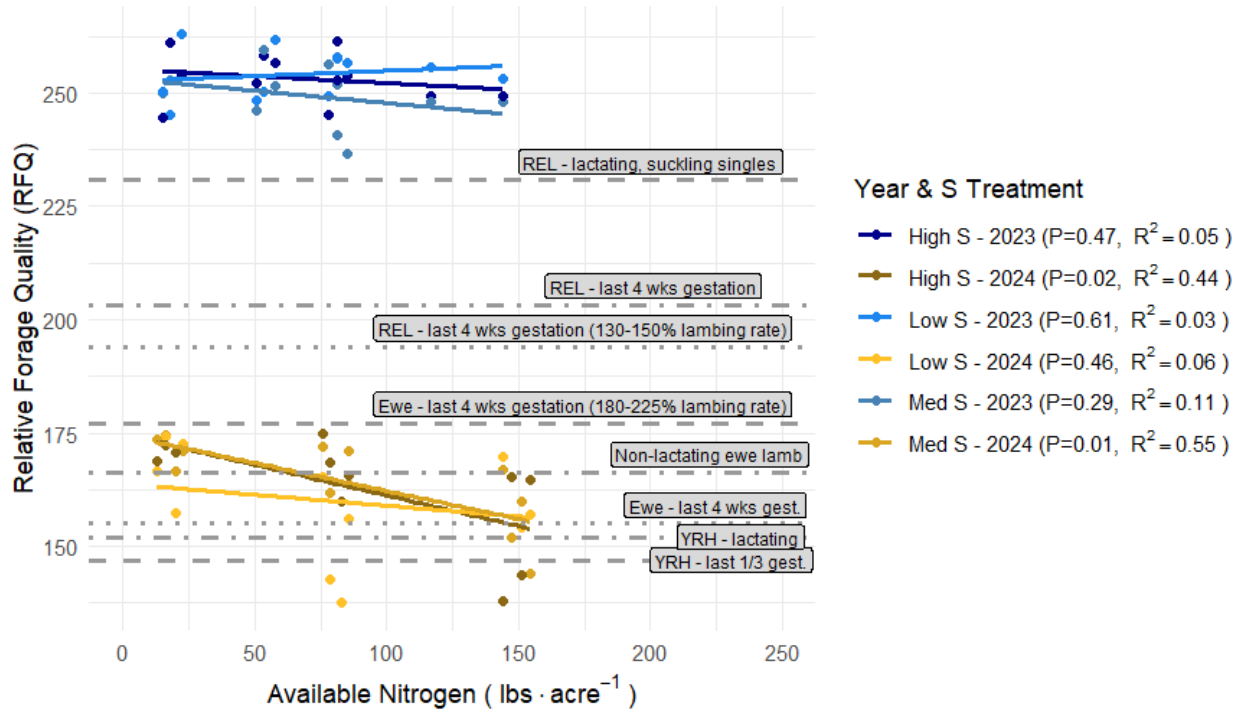


Figure 19. Relative forage quality response to available-N (lbs. acre<sup>-1</sup>) (soil + fertilizer N) across low (0 lbs. acre<sup>-1</sup>), medium (10 lbs. acre<sup>-1</sup>), and high (20 lbs. acre<sup>-1</sup>) S treatments at the CARC site during field season of 2023 and 2024.<sup>31</sup>

<sup>31</sup> 0, 1.0, 1.5x lbs. N ton<sup>-1</sup> + nitrate-N from spring soil, 4 cores sampled to a depth of 36 inches x 4 blocks = Available N. YRL = Yearling Replacement Heifer; REL = Replacement Ewe Lamb

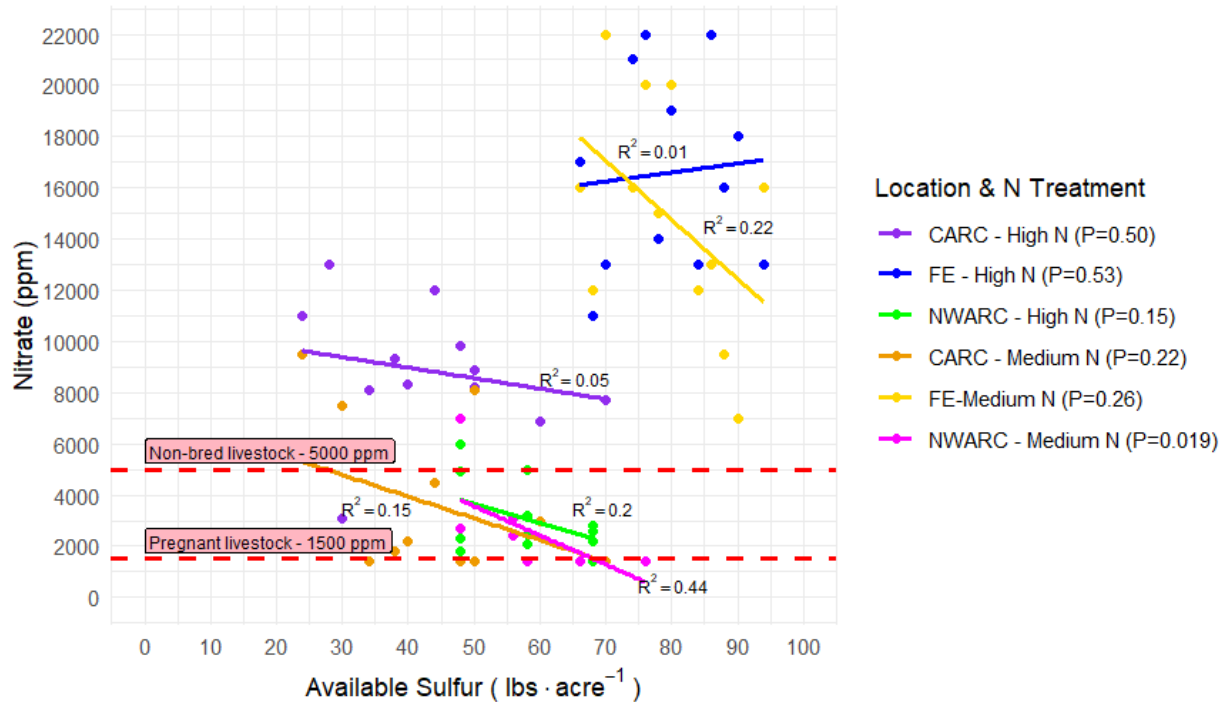


Figure 20. Site locations during the 2024 field season with significant nitrogen treatment effects on nitrate concentrations (ppm) in response to plant-available S<sup>32</sup>

### NWARC

In 2023, N ( $P=0.002$ ) and S ( $P=0.018$ ) had effects on nitrate with observations below the detection threshold, but only had S effects ( $P=0.026$ ) when those observations were removed ( $n=24$ ). High S compared to low S reduced nitrate levels, however, medium S did not increase or decrease these levels (Table 10). A negative linear relationship ( $P=0.008$ ) appears to exist between available S (soil + applied) and nitrate when medium N is applied (Figure 19). With these reductions in nitrate, there were no consequential reductions in yield due to less N, with

<sup>32</sup> (0, 10, 20 lbs. acre<sup>-1</sup> + sulfate-S from spring 2024 soil, 4 cores sampled to a depth of 24 inches x 4 blocks) grouped by locations that had significant S or N x S effects in 2024 or significant trends (Ft. Ellis, CARC, and NWARC) and medium or high N treatment. Thresholds for non-bred and pregnant livestock are provided for practical context. P-values indicate slope differences from zero, R<sup>2</sup> indicates variation explained by available-S on nitrate concentrations.

medium and high N resulting in similar yield ( $P < 0.001$ ). Relative forage quality decreased with an increase in available-N ( $P = 0.016$ ) (Figure 16).

In 2024, there were no full model effects of N, S, or N x S on nitrate concentration and no reduced model effects of S or N x S effects on CP. Out of the 36 total observations, only 3 plots recorded nitrate levels that would require restrictions on feeding to pregnant livestock. Nitrates could have remained low in 2024 due to assumed lower yield-potential derived from 2023 yields or a slight increase in precipitation that did not lessen drought conditions but may have contributed to more nitrate leaching in the very sandy loam soils. However, when nitrate concentrations were plotted against plant-available S, the trend line slope differed from zero, once again suggesting a negative relationship between nitrate and available S (Figure 17). No effects of N, S, or N x S effects were recorded on NDF, ADF, RFQ, TDN, or DMI, with all values suggesting forage quality sufficient to meet the nutritional demands of most classes of livestock. Forage nutritive quality is highly reliant on environmental factors of light, moisture, and temperature (Moore et al., 2020). Based on drought maps as well as precipitation information for NWARC in 2024, there would have been the potential for moisture stress to decrease quality, however, this is not what was observed.

Table 16. Nitrate concentrations (ppm) in response to significant S effect averaged across all N treatments at NWARC 2023<sup>33</sup>

TRT	Nitrate (ppm)
Low S	8675a
Medium S	6213ab
High S	5038b

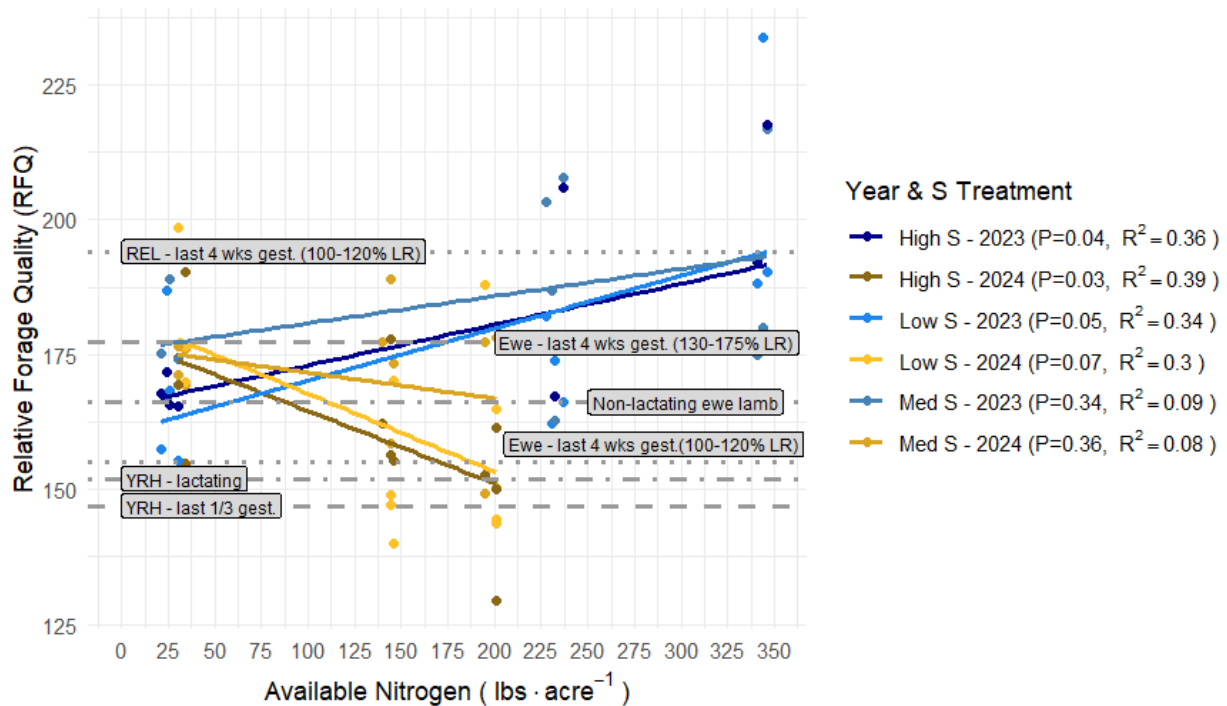


Figure 21. NWARC 2023 and 2024 RFQ in response to available N and S treatment.<sup>34</sup>

<sup>33</sup> Letters indicate significant differences. Low S = 0 lbs. S acre<sup>-1</sup>, Medium S = 10 lbs. S acre<sup>-1</sup>, High S = 20 lbs. S acre<sup>-1</sup>; Medium N = 1.0\*25 lbs. N ton<sup>-1</sup>, High N = 1.5 \* 25 lbs. N ton<sup>-1</sup>; with a yield goal of 6.5 tons acre<sup>-1</sup>

<sup>34</sup> 0, 1.0, 1.5x lbs. N ton<sup>-1</sup> + nitrate-N from spring soil, 4 cores sampled to a depth of 36 inches x 4 blocks = Available N. YRL = Yearling Replacement Heifer; REL = Replacement Ewe Lamb; LR = Lambing Rate

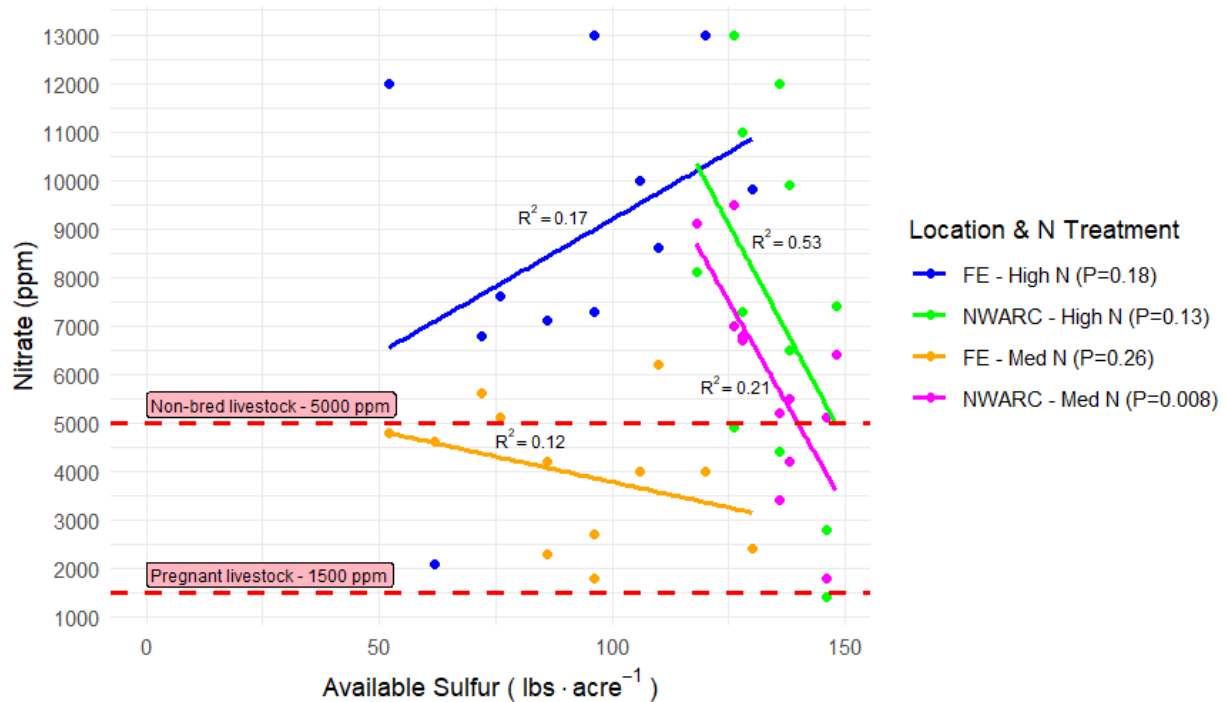


Figure 22. Site locations during the 2023 field season with significant nitrogen treatment effects on nitrate concentrations (ppm) in response to plant-available S<sup>35</sup>

### Sulfur Availability and Response

Plant available-S at sites with nitrate reductions due to additions of S, appear high in both 2023 and 2024 (Figure 17, 19). Upwards of 150 lbs. S acre<sup>-1</sup> were predicted as being plant available, however, based on the results, it was the addition of smaller quantities of sulfur that reduced nitrate. As previously mentioned, S soil tests vary in methodology and S extraction capabilities.

Turbidimetric soil S tests, as used in this study, can dissolve gypsum coated in calcium carbonate

<sup>35</sup> (0, 10, 20 lbs. acre<sup>-1</sup> + sulfate-S from spring 2023 soil, 4 cores sampled to a depth of 24 inches x 4 blocks) grouped by locations that had significant S or N x S effects in 2023 (Ft. Ellis & NWARC) and medium or high N treatment. Thresholds for non-bred and pregnant livestock are provided for practical context. P-values indicate slope differences from zero, R<sup>2</sup> indicates variation explained by available-S on nitrate concentrations.

(Hu et al., 2005; Keren & Kauschansky, 1981). This dissolved gypsum is reflected in soil analyses as higher than what is plant available. In the field, sulfate in gypsum is either coated in calcium carbonate or in drier aggregates, making this S plant unavailable. Turbidimetric soil S tests also overestimate inductively coupled plasma (ICP) spectroscopy S, despite ICP S including both inorganic and organic S, possibly due to S contamination of activated carbon used in turbidimetric testing but not ICP (Ketterings et al., 2011; Shirisha et al., 2011). Sulfur is also incredibly variable even within a consistently managed field, for this reason a single 'high S' soil core in a composite sample of multiple field cores, could cause the entire sample to test high despite only one area actually being high in S. Evidence of this (Figure 25, 26) was seen during both study years, with weak correlation between plant available S and percent total S (TS). Sulfur additions were consistent year-to-year, whereas soil S was not. Nitrate levels responded to additions of S when soil analyses reported pools of 70 – 130 lbs. acre<sup>-1</sup> S in the top 12, 24, or 36 inches, depending on the site (Table 13, 14, 15, 16; Figure 16, 17, 20, 22). These findings agree with current reports for plant-available S in that soil S is variable at a field level and laboratory extraction, like turbidimetric determination, can overpredict S (Carciochi et al., 2019; Franzen, 2015; Goh & Pamidi, n.d.; Hu et al., 2005; Keren & Kauschansky, 1981; Shirisha et al., 2011).

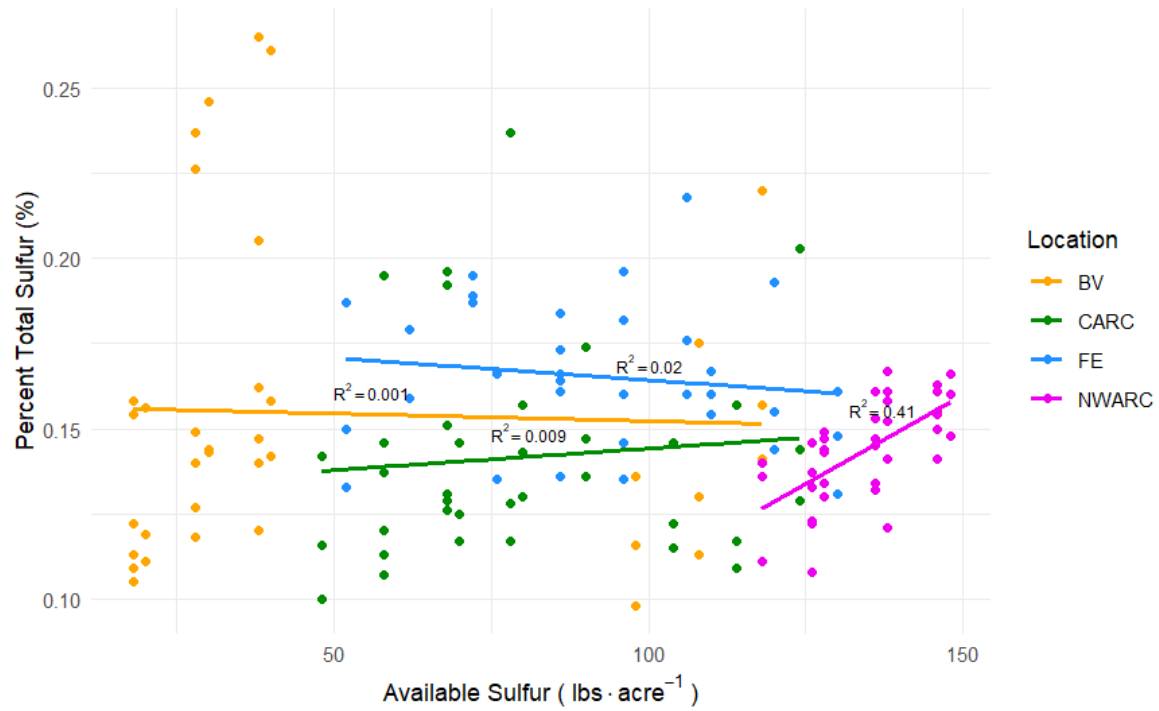


Figure 13. Percent total S response to plant available S at four research sites during the 2023 field season.<sup>36</sup>

<sup>36</sup> 0, 10, 20 lbs. acre<sup>-1</sup> + sulfate-S from spring 2023 soil, 4 cores sampled to a depth of 24 inches x 4 blocks.

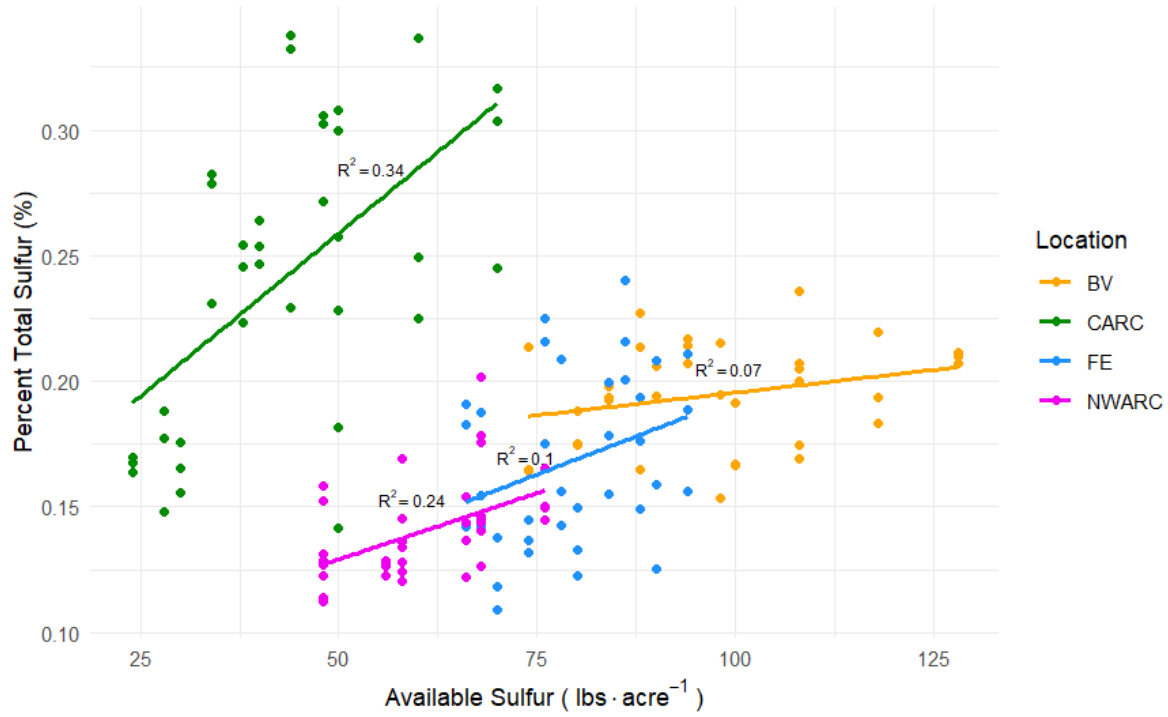


Figure 24. Percent total S response to plant available S at four research sites during the 2024 field season. <sup>37</sup>

<sup>37</sup> 0, 10, 20 lbs. acre<sup>-1</sup> + sulfate-S from spring 2024 soil, 4 cores sampled to a depth of 24 inches x 4 blocks.

## CHAPTER THREE

## CONCLUSION

Soil additions of 20 lbs. acre<sup>-1</sup> S reduced Lavina barley nitrate concentrations when medium N fertilization rates (N - 1.0 x lbs. N ton<sup>-1</sup>) were followed and not exceeded (i.e. high N - 1.5 x lbs. N ton<sup>-1</sup>). While cereal forage nitrates concentrations are more influenced by soil N management (Crawford et al., 1961), the results of this study suggest that N management through soil testing, accurate yield records, and moderate N rates (25 lbs. N ton<sup>-1</sup>), S can be used as a tool to reduce nitrate accumulations and improve the overall value of hay barley. Lenssen et al. fertilization recommendations are for winter wheat as opposed to spring barley. One potential downfall to using the only applicable cereal forage N recommendation for MT could be difference in root mass between the two crops. Winter wheat with a longer growing season and deeper roots would generally require higher N rates to spring barley with a shorter growing season and more shallow roots (Thorup-Kristensen et al., 2009). Drought conditions and lack of soil moisture also played a role in nitrate accumulation as seen in differences among yield, plausibly due to a moisture limiting response, and N rates to maximize yield much greater than the suggested rate in the drier site years. While drought and abnormally dry conditions are often the blame for high nitrates, these conditions cannot be controlled under dryland production environments. For this reason, economic instead of yield-based N fertilization rates would be recommended to producers. Economic rates in the study would equal 25 lbs. N<sup>-1</sup>, which when combined with S, appear to not overpower the plant's ability to process nitrate into plant protein. Lower N may slightly reduce hay yield but it also reduces nitrate, which is economically important because no matter the yield, high nitrate hay is worthless as a livestock forage. .

Fertilizing for maximum yield is not a viable economic strategy under variable climatic conditions.

This improvement to forage quality in Montana could greatly decrease potential winter feeding costs most producers expect each year (Goosey, 2024). Barley hay, a relatively low input forage crop, that is well adapted to Montana growing conditions (Meccage et al., 2019), could provide producers with more consistent low nitrate forage, regardless of climatic conditions. To accomplish this, yield-based N rates need to be decreased, and additions of S need to be made so that the interaction will offset any yield loss with hay that is feedable and within safe concentration ranges for all classes of livestock. Winter feeding costs are generally the largest variable cost to ranches in Montana and a decrease in hay yield is certainly not ideal; however, the above proposed nitrate management strategy helps to remove some of the variability and uncertainty in cereal hay production thus reducing the economic burden associated with winter feeding costs.

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