

BARLEY YIELD AND PROTEIN RESPONSE TO NITROGEN AND SULFUR  
FERTILIZER RATES AND APPLICATION TIMING

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## ABSTRACT

The introduction of new barley varieties and changes in management practices necessitate re-evaluating nitrogen (N) and sulfur (S) nutrient management and application timing guidelines. Nitrogen has a significant impact on barley grain quality and yield. However, overapplication of N can result in yield reduction, groundwater pollution, and high protein content, resulting in lower end-use quality of barley, while underapplication of N results in reduced grain quality and yield. Because S improves N utilization and enhances protein synthesis and split N application improves yield and N use efficiency in winter barley, split N application timing and the interaction of N and S may be a valuable tool to reduce N loss, increase yield, improve grain quality, and improve N use efficiency for agronomically optimal spring barley production. In a bid to provide barley growers in the Western US with an optimal N application timing, as well as appropriate N and S rates for improved yields and grain quality and reduced input costs and environmental contamination, we evaluated the effects of N and S fertilizer rates and application timing on malt, feed, and food barley grain yield and quality for four site-years in Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Idaho for the 2021 and 2022 growing seasons. Three barley varieties: malt (Moravian 179), feed (Claymore), and food (Julie) were grown at 1,980,000 seeds ha<sup>-1</sup>. Nitrogen fertilizer treatments included urea (46-0-0) applied at 0, 45, 90, 135, or 180 kg N ha<sup>-1</sup> at planting or a split application of 45 kg N ha<sup>-1</sup> done at planting and top-dressed with 23, 45, or 90 kg N ha<sup>-1</sup> at heading. Sulfur fertilizer treatments included three S rates of potassium sulfate (0-0-53-18) fertilizer applied at 0, 17, or 34 kg S ha<sup>-1</sup> at planting. Data was collected on grain yield, protein concentration, plant height, harvest heads, test weight, kernel plumpness, and N use efficiency.

We investigated fertilizer rates for N and S, but S did not affect yield and yield components due to the high S concentration in the irrigation water. Plant height, harvest heads, and grain yield increased with increasing N rate for all varieties except at the Aberdeen 2021 field site, where grain yield was non-responsive to N due to the high preplant N at this location. The linear responses indicate N fertilizer insufficiency to maximize yield. Claymore had a quadratic response at Rexburg, with a maximum yield at approximately 120 kg N ha<sup>-1</sup> rate.

At the Aberdeen 2021 site, Julie responded to N and had a quadratic response with a maximum yield between 135-180 kg N ha<sup>-1</sup>. Grain protein concentration, test weight, and kernel plumpness were similar to those reported for Moravian 179, Claymore, and Julie in the southeastern and southcentral Idaho variety trials. Single N application produced similar or greater yields than split N application, contributing a 6-46% yield advantage over split N application across all varieties. Similarly, single N applications improved N use efficiency compared to split N applications and contributed a 9-25% N use efficiency advantage. For malt barley at Kimberly and Rexburg, split N application produced grains with 0.6-1.4% higher protein concentrations than acceptable for malting, suggesting an economic loss for growers as grains are sold as feed. This study demonstrated how pre-plant soil N content and N treatment timing affect spring barley yield and quality responses to N. Furthermore, we showed that the high S concentration in the irrigation water in this area negates the need for additional S fertilizer to maximize barley productivity and quality on the Snake River Plain. Split N applications are not an efficient way to increase yield, and N use efficiency for spring barley production and should be avoided in favor of a single N application at planting.

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## **DEDICATION**

I dedicate this thesis to my parents, Pastor and Deaconess S.F. Adeyemi, who have always stood by me and encouraged me to pursue my dreams. Their prayers, support, and staunch belief in my abilities have been essential for completing this degree. I am forever grateful for their sacrifices.

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## **CHAPTER 1: BARLEY YIELD AND PROTEIN RESPONSE TO NITROGEN AND SULFUR FERTILIZER RATES AND APPLICATION TIMING**

### **1.1 INTRODUCTION**

Barley (*Hordeum vulgare*) is one of the world's oldest crops (Nevo, 1992) and, in terms of global grain quantity produced and area cultivated, ranks fourth after wheat, rice, and corn (Zhou, 2009a). Barley has the widest geographical range of any crop species, with estimated global production of approximately 150 million metric tons cultivated primarily in North America, Europe, Russia, China, and Australia (Paulitz & Steffenson, 2011; Statista, 2023). The wide ecological adaption of barley distinguishes it from other cereals and a large portion of the world's barley is grown outside of areas where cereals like corn or rice are abundant (Hellewell et al., 2000). Barley flourishes best in cool, moderately dry climates but can survive high humidity in a cool climate or high heat in a dry region. Although heat and humidity are part of the soil-plant-atmosphere system and they dynamically interact to improve crop yield (Asseng et al., 2015; Semenov et al., 2014), barley struggles when the climate is both hot and humid (Klages, 1942; Wezel et al., 2009). For example, heat and humidity negatively impact yield by reducing transpiration rate and reproductive development, increasing disease pressure, and accelerating senescence rate (Asseng et al., 2011; Huntingford et al., 2005; Poehlman, 1985). Hence, to meet the optimal growing conditions, spring barley is grown during long summer days in the northern latitudes of North America (e.g., Idaho, Montana, and North Dakota) and Europe while it is cultivated during short winter days in places like California and Mediterranean climate regions (e.g., Australia, Turkey, and Algeria). In places such as Washington and Oregon where fall-sown barley cannot withstand the harsh winter weather, spring barley is used as a substitute (Hellewell et al., 2000). Idaho is the highest barley-producing state in the United States with over 59 million bushels produced on 540,000 harvested acres at an average yield of 110 bushels per acre in 2022 (USDA NASS, 2022). Idaho's high-elevation, arid conditions, and high quality supplemental irrigation allow growers to consistently produce high-yielding and high-quality barley that meets end-user quality requirements.

Barley is distributed globally and has significant economic importance primarily as animal feed and for malting purposes, with secondary consideration as human food (Newton et al., 2011a). Recent interest has been exploring new applications of barley for food, nutritional, and health benefits (Newton et al., 2011a) due to the discovery of the cholesterol-lowering effect of  $\beta$ -glucan, a cell-wall polysaccharide found in whole-grain barley (Newman & Newman, 2006; Wood et al., 1989). As a result, barley flour is now being increasingly used for products such as bread (Izydorczyk et al., 2001), porridge (Zhou, 2009a), pasta (Cavallero et al., 2000), malted syrups, and pet food (Scoular, 2021). Asian countries (e.g., Japan, Korea, and Taiwan) import barley which is used as a rice extender (US Grains Council, 2022). Further, barley flour contains the alpha-amylase enzyme that is used to increase the enzymatic activity of bread dough (Al-Attabi et al., 2017) and aids in converting complex sugars and starches in the dough to simple sugars like maltose, making them simpler for yeast consumption (Sullivan et al., 2010). Due to barley's superior water use efficiency compared to other small grains, barley is also grown as forage for animals during droughts when barley is typically harvested at the milk to soft dough stage before seedhead development (Schaffer et al., 1993). In contrast to malt, food, and feed barley cultivars, forage barley cultivars are awnless. Awned barley cultivars targeted for forage must be harvested prior to the development of mature awns to enhance digestibility (Rosser et al., 2016) and prevent lump-jaw or sore eyes in livestock (Cash et al., 2004).

Based on spike morphology, barley varieties are classified into two-rowed (barley with two rows of seed on each head) and six-rowed (barley with six rows of seed on each head) types. Six-rowed barley type was more popular than the two-rowed type until the 1990s due to its relatively higher yields that favored animal production and high demand from large industrial brewers (AMBA, 2018). Today, however, about 85% of reported acreage was planted with two-rowed barley type due to greater plumps, greater water absorption properties, uniform kernel size, and malt yields with 1-2% higher extract that represents an increased economic return for maltsters and brewers (Heisel, 2017; Schwarz & Horsley, 2001). Food and forage barley can be either two or six-rowed barley type, and in many instances, their final application depends on the characteristics of the crop at harvest.

Barley is an important component of cover cropping and crop rotation, contributing to cropland soil quality, fertility, productivity, species diversity, and weed, pest, and disease control (Magdoff & van Es, 2021). As a fast-growing annual grass, barley outcompetes weeds by providing shade and absorbing water and nutrients from the soil. Barley also reduces weed germination by generating allelopathic chemicals, which are toxic to other plants (USDA, 2012). According to research conducted in Ohio, planting barley as a cover crop significantly reduced the emergence of yellow foxtail (*Setaria glauca*) by 81% (Creamer et al., 1996). Barley reduces nitrate and phosphorus pollution as it has a deep rooting system that scavenges nitrogen (N), phosphorus, and potassium among other nutrients from deeper soil depths and recycles them to the upper soil profile (Magdoff & van Es, 2021). The nutrient recovery process can save producers money on fertilizer expenses and help preserve groundwater quality (Hipke et al., 2022; IDEQ, 2021). Barley's deep rooting system allows it to draw water from deep in the soil profile when water availability is low. Decomposing barley biomass increases soil organic matter, soil aggregate stability, soil drainage, and soil aeration (Valenzuela & Smith, 2002).

The challenges restricting global barley production are poor nutrient management and soil fertility, drought, cultivation of low-yielding varieties, erosion, frost, low soil pH, disease pressure, insect attacks, and weeds (Hayes et al., 2022; Verma, 2018). Agegnehu et al., (2014) and Assefa et al., (2018) reported that inadequate macronutrients are the major constraint to the production of barley and other cereal crops. Nitrogen is one of the most important factors influencing barley growth, grain yield, and grain protein concentration (Khan et al., 2017). Nitrogen aids photosynthesis and promotes the growth and development of plant roots, leaves, stems, and other vegetative parts (Leghari et al., 2016). Despite the importance of N to soil fertility and crop productivity (Miao et al., 2006; SO & VO, 2007), misuse or overuse can result in lodging and reduced grain yield (Shafi et al., 2011), increased grain protein- potentially making malt barley less valuable for its end use market, and leaching of excess nitrate into ground or surface waters (Lamb et al., 2014) which negatively impacts drinking water supplies and natural ecosystems (IDEQ, 2020; Yunseop Kim et al., 2005a). The increasing population and a worldwide surge towards a more protein-rich diet in developing countries are two of the main drivers of annually increasing chemical N fertilizer



demand (Lassaletta et al., 2016). Furthermore, extensive greenhouse gas emissions are linked to the use of N, either directly via the manufacture of N fertilizers or indirectly through the release of nitrous oxide gas ( $\text{N}_2\text{O}$ ) by denitrification processes in the soil (Schaufler et al., 2010).

Nitrogen source, the growing season conditions, and the amount of N carried over from the previous crop all have an impact on barley yield. Barley takes up N as ammonium ( $\text{NH}_4^+$ ) that is readily assimilated into plant metabolites and tissues (Arnon, 1937; Hachiya & Sakakibara, 2017; Vidal et al., 2020) and as nitrate ( $\text{NO}_3^-$ ). Although the total amount of fertilizer N applied has the greatest effect on yield, grain quality, grain protein concentration, and other growth and yield parameters, the number and timing of N applications can have a significant impact (Conry, 1994a; Easson, 1984; McTaggart & Smith, 1995; Needham, 1983). The rate of N uptake is influenced by the stage of crop development, seasonal conditions, soil type, and crop rotational history (Shafi et al., 2011). Nitrogen loss by runoff, leaching, or denitrification may be more likely when all the N fertilizer necessary to maximize yields is applied before, or at the time of planting, especially for winter barley. This is due, in part, to minimal N uptake during the early stages of small grain growth and development (Cameron et al., 2013; Delogu et al., 1998; Jackson et al., 2006). Hence, the application of the appropriate N fertilizer dose at the appropriate time may increase yield and reduce lodging and production costs. Appropriate N fertilizer application improves the overall synchronization of N fertilizer supply and crop N demand (Ladha et al., 2005). Split N applications at tillering or towards the end of the growing season may have an impact on grain yield and quality because a large portion of the plant N is transferred to the grain for protein synthesis. On a global scale, some studies in Canada, Turkey, Ireland, Japan, South Korea, and Ethiopia have reported that split N application increased grain protein concentration and grain weight, and reduced lodging and spike population of cereals (Ayoub et al., 1994; Hackett, 2019; Hattori, 1994; Kim et al., 1998; Kumar, 2021; Tadesse et al., 2021; Tadesse et al., 2013). In California, southwestern Oregon, and some parts of the Midwest with humid climates and longer growing seasons where cereals are fall-planted, split N application in early spring has been reported as a common practice to reduce N loss, increase crop N use efficiency, and increase crop yield (Sullivan et al., 1999). Lorbeer et al., (2000) reported that the efficacy of split N application in the western US is partly dependent

on planting timing and field site soil characteristics. Similarly, Robertson & Stark, (2003) reported that split N application increases NUE for sandy, coarse-textured soils while a single N application is effective for maximum barley yield and quality in loam and silt loam soils. Westcott et al., (1998) reported that split N application increased grain protein by 2% in Montana.

Nitrogen sources for agricultural production include anhydrous ammonia, urea, urea-ammonium nitrate solutions, ammonium sulfate, and calcium nitrate (Sellars & Nunes, 2021). Anhydrous ammonia is 82% N by weight resulting in lower transportation, storage, and distribution costs than other N sources. However, anhydrous ammonia use is declining due to its high toxicity and storage and handling difficulties (Sellars & Nunes, 2021). Urea is the most widely utilized N source globally (Cantarella et al., 2018; Hu et al., 2020; Pan et al., 2016) and in the United States (Woodley et al., 2020) due to its low cost, high N content (46% N), and convenience of transport as a stable dry granular product (Finch et al., 2014; James, 1993). Ammonium nitrate and urea-ammonium nitrate are alternative N sources synthesized by the reaction of urea, nitric acid, and anhydrous ammonia. Ammonium nitrate and urea-ammonium nitrate have shown promise for plant growth, but are more expensive per unit of N than urea and AA due to the additional chemical processes involved in production (Sellars & Nunes, 2021). Further, ammonium nitrate can be more dangerous than other N sources due to its explosive nature upon exposure to high temperatures (Laboureur et al., 2016). Other inorganic N sources such as calcium nitrate, sodium nitrate, and ammonium sulfate have gained popularity in Europe and Latin America (Tur-Cardona et al., 2018; Von Blottnitz et al., 2006). However, these N sources' low N contents (25-28%) are a drawback because they result in higher logistical costs (Turker, 2023). Additionally, it's important to note that although their primary purpose is phosphorus supply, some phosphate fertilizers such mono and diammonium phosphate can be an indirect N source for crop production (Cowman, 1962; Sellars & Nunes, 2021). All ammonium-based fertilizers, including urea, are subject to ammonia volatilization loss. Volatilization losses are accelerated when surface residue is high, soil pH is >7, the soil is moist, and if left near the soil surface can result in up to 50% N loss (Bremner, 1996; Nunes et al., 2020) (Panday et al., 2020; Al-Kanani et al., 1991; Rochette, Angers, et al., 2009).

Sulfur (S) is a secondary macronutrient required by plants in amounts comparable to phosphorus. Sulfur is a necessary component of the essential amino acids cysteine and methionine used in protein synthesis (Assefa et al., 2020; Havlin et al., 2016). Sulfur is also necessary for the synthesis of vitamins, enzymes, and chlorophyll and the processes of photosynthesis and N fixation (Brady & Weil, 2008; Beaton, 1966; Havlin et al., 2016; Taban et al., 1995). S deficiency is manifested as reduced plant growth and interveinal chlorosis that spreads to the entire leaf area of younger leaves. Dobermann, (2000) reported that S-deficient barley plants are stunted with fewer tillers and spikelets, undergo delayed maturity, and are less stress-resistant. Most of the S required by plants is taken by the roots from the soil solution as divalent sulfate anion,  $\text{SO}_4^{2-}$  (Barber, 1995). Aulakh and Chhibba (1992) observed increased root uptake of S when supplied at modest rates. Further, crops have been observed to assimilate and utilize more N when there is an appropriate supply of S (Kumar et al. 2012). When S is deficient, increasing N availability alone results in a reduction in kernel size and weight (Reisenauer & Dickson, 1961). Nitrogen and S modify the grain's protein concentration by controlling the gene expression of prolamin storage proteins (Halford & Shewry, 2007). There is an increased interest in the effects of S, particularly on grain quality and protein content due to reduced atmospheric S deposition.

Sulfur can exist either in organic or inorganic forms. In its inorganic form, S exists in the -2, 0, +2, and +6 oxidation states, with the +6 state (soluble sulfate;  $\text{SO}_4^{2-}$ ) being the principal S source for plants (Wainwright, 1984). Sulfate is typically soluble and rapidly flows with soil water to roots or can move below the root zone in situations of excessive irrigation or areas with high rainfall (Norton, 2012; Ferguson, 2006). Some common  $\text{SO}_4$ -S sources include ammonium sulfate (21% N and 24% S), potassium sulfate (50%  $\text{K}_2\text{O}$  and 17.6% S), gypsum (32.6%  $\text{CaO}$  and 16.8% S), and zinc sulfate (36.4% Zn and 17.8% S) (Oldham, 2011). Other S fertilizer sources include sulfide and elemental S, but these fertilizers must first be oxidized to  $\text{SO}_4$ -S before the S is plant available. Sulfur is incorporated into the soil by fertilizer application, deposition through rainwater, and plants and animal residue (Shaver et al., 2014). It then exits the soil profile through plant absorption, leaching, and volatilization, which increases with higher soil disturbance.

Soil S concentration and availability varies greatly depending on the soil parent material, fertilizer application, soil organic matter content, irrigation, and atmospheric depositions (Scherer, 2008). Less than 10% of the total S in soils exists in inorganic forms (Rehm & Clapp, 2008). Organic S is immobile until it is mineralized (Castellano & Dick, 1991; Fitzgerald, 1978; Scherer, 2008; Strickland et al., 1987). The microbial mineralization rates are influenced by temperature, moisture, and organic matter (Freney et al., 1971; Randlett et al., 1992).

High concentration of available N favors high yield potential which may result in an excessive N content in the grain (Rajala et al., 2007). High grain N levels result in poor fermentable extract yield, which lowers malting quality (Agu, 2003). Sulfur addition has been linked to improvements in endosperm modification during malting. Additionally, dimethylsulphide, an important beer flavoring ingredient, is positively correlated with grain S concentration (F. J. Zhao et al., 2006a). Excess S concentration in malt barley can result in hazy beer, reduced shelf life, and bad flavor (Anheuser Busch, 2022). Previous studies reported that N and S had a beneficial interaction which increased wheat grain yield by 4 – 6%, increased biomass, and N use efficiency (Ladha et al., 2005; Prystupa et al., 2019; Salvagiotti et al., 2009; Shafi et al., n.d.; B. Zhao et al., 2016; F. J. Zhao et al., 2005, 2006b).

Nitrogen is an essential macronutrient for barley production and is positively correlated to yield (Rajala et al., 2007), but excess may result in diminished end-use quality (Lamb et al., 2014). For example, since N is negatively correlated to starch concentration, increased N use may reduce the nutrient concentration in feed barley, thereby limiting nutrient and energy supply to animals (Snyder & Bruulsema, 2007). Similarly, increased N use may increase protein concentration above malt quality standards, thus reducing malt extractability and incurring significant economic loss for maltsters (Wilder, 2022). Further, split N application has been reported as a best management practice to increase yield and nitrogen use efficiency in winter barley (Sullivan et al., 1999), which has a more extended growing period than spring barley which is the most prevalent barley type in this region (Robertson & Stark, 2003). Sulfur fertilizer application, on the other hand, has been reported to increase yield and quality by improving N utilization and protein synthesis in barley plants (Zhao et al., 2006a). Hence, the general objectives for this study were: a) to evaluate the response of malt, food,

and feed barley varieties to N and S levels and b) to determine whether split-applying N fertilizer is an effective strategy to increase N use efficiency in spring barley production.

## **CHAPTER 2: EFFECT OF NITROGEN AND SULFUR FERTILIZER RATES AND APPLICATION TIMING ON FEED BARLEY YIELD AND PROTEIN CONCENTRATION**

### **2.1 INTRODUCTION**

Barley is utilized as livestock feed either as processed grain or forage hay in the intensive pig, poultry, dairy, and beef industries (GRDC, 2017). Barley varieties exhibit considerable genetic diversity in terms of nutritional quality and feed suitability (Kling et al., 2004). Generally, the price of feed barley is positively correlated to simple physical characteristics such as high test weight ( $>59 \text{ kg hL}^{-1}$ ) and plump kernels that represent increased nutrient density (Perrott et al., 2018b; Edney, 2010). Most of the barley grain fed to livestock is rolled. A mixture of plump and thin kernels results in an inconsistent rolling output where thin kernels emerge unprocessed and plump kernels are overprocessed producing excess fine particles (Edney et al., 1994; Edney, 2010; Kling et al., 2004). Under-rolling prevents the starch in entire kernels from being available for fermentation by rumen microbial communities while over-rolling may lower feed intake and raise the risk of digestive disturbances (Ahmad et al., 2010; Edney, 2010). Irrespective of the variability in feed barley varieties, the kernels usually contain ~ 55% starch. Hence, the primary use of feed barley is as an energy source for monogastric animals (Kling et al., 2004). Hulled barley kernels impede the digestion rate (Sealey et al., 2008) and constrain the supply of carbohydrates to monogastric animals (Edney, 2010). However, in ruminants, the hull on barley kernels reduces the likelihood of acidity and bloat, indicating its preference as feed over other hullless grains such as wheat (Edney, 2010; Sealey et al., 2008).

Barley, as well as other annual cereal forage crops are considered as “emergency forage” in the Pacific Northwest (PNW) of the US (e.g., Montana and Idaho) due to water scarcity (Cash et al., 2004). Persistent drought led to  $>50\%$  increase in the harvested acreage and a  $>70\%$  increase in the production of small grain hay in Montana since the late 1990’s (Cash et al., 2007). Compared to corn, barley provides animals with a healthier gut and provides farmers with an increased economic return as barley production requires little input cost. Under irrigated conditions, feed barley harvested for hay production at the soft milk to early dough stages has higher crude protein (11.2 to 13.4%), and total digestible nutrients than

corn (Cash et al., 2007). In addition to providing energy, the high protein concentration supplied by barley reduces the need for extra feed supplements and helps to meet livestock's daily protein requirement (Robertson & Stark, 2003). Cash et al., (2007) and Spicer et al., (1986) reported that dry-rolled or steam-flaked barley has a higher rumen organic matter digestibility (61.7%) than dry-rolled maize and grain sorghum (48.5 and 42.6%).

Feed barley quality metrics might potentially be impacted by N fertilizer application (Robertson & Stark, 2003). An appropriate N application rate is essential for feed barley production. Inadequate N levels are associated with reduced yield and low test weight while excess N rates above crop nutrient requirement may negatively impact grain yield due to lodging, feed quality, and result in nutrient imbalances in the feed (O'Donovan, 2015; King, 2020; Berry et al., 2000; Caldwell, 1983; Rajkumara, 2008). Studies carried out over a three-year period at eight sites in Western Canada showed that increasing N rates is inversely correlated to starch content but positively correlated to  $\beta$ -glucan, soluble fiber, and lysine content of barley kernels (Edney et al., 2012; O'Donovan et al., 2011). Nitrogen availability in excess of barley requirements increases lodging risk and environmental N loss potential (Robertson & Stark, 2003). Lodging increases the risk of disease pressure and reduce yield potential and feed barley grain quality (Lamb et al., 2014). Effective nutrient management techniques involving appropriate fertilizer rate and application timing may be essential to reduce the risk of N losses, maximize yield, minimize nutrient input, and reduce livestock losses. Studies in California, southwestern Oregon, and the US midwest recommended split N application as a common practice to reduce N loss in winter barley production due to the difference in climatic and growing conditions (Sullivan et al., 1999). Barley's response to N fertilizer rates and timing varies with variety, residual soil N levels, and climatic conditions. Like other nutrients, guidelines for N and S rates cannot be seen as static numbers because they are influenced by a variety of changing factors, such as the environment and agronomic techniques (Gutiérrez, 2012; IFA, 2021; Raun & Johnson, 1999; White & Brown, 2010; Yan et al., 2020). Hence, N and S fertilizer should be applied at rates and timing events commensurate to expected yield, nutrient concentration in irrigation water, and residual soil N and S levels (Finkner & Gledhill, 1971; Hellewell et al., 2000; Toews & Soper, 1978). The current University of Idaho fertility guidelines do not credit N from irrigation source and may underestimate barley N requirement resulting in missed yield potential, reduced quality, and

nutrient loss. Additional research is required to provide spring barley growers with accurate diagnosis of nutrient deficiencies, as well as appropriate supplemental N and S fertilizer rates and timing for optimum yield and quality, with considerations of environmental and agronomic factors. Hence, the objective of this study was to determine the effect of N and S fertilizer rates and N application timing on the yield and quality metrics of feed barley.

## 2.2 MATERIALS AND METHODS

### 2.2.1. Site Description

Studies were conducted during the 2021 and 2022 growing seasons at four locations in southern Idaho. In 2021, field sites were located at the University of Idaho Aberdeen Research and Extension Center in Aberdeen, ID (42°57'31.881", 112°49'9.5952") and at the University of Idaho Kimberly Research and Extension Center at Kimberly, ID (42°33'12.6606", 114°20'41.2578"). In 2022, field sites were located at the University of Idaho Aberdeen Research and Extension Center (42°58'28.9776", 112°48'51.249") and Brigham Young University – Idaho in Rexburg, ID (43°48'29.1528", 114°47'42.003"). The Aberdeen field sites for 2021 and 2022 growing seasons were on a Declo loam soil (mixed, coarse-loamy, super-active, mesic Xeric Haplocalcids) (USDA-NRCS & Soil Survey Staff, 2012), the Rexburg field site was on a Pocatello variant silt loam (coarse-silty, mixed, super-active, calcareous, mesic Xeric Torriorthents) (USDA-NRCS & Soil Survey Staff, 2012), and the Kimberly field site was on a Bahem silt loam soil (coarse-silty, mixed, super-active, mesic Xeric Haplocalcids) (USDA-NRCS, 2017) and is characterized by a cold semi-arid climate according to the Köppen-Geiger climate classification system (Kottek et al., 2006). Barley cultivation at Aberdeen followed fallow in 2021 and forage oat (*Avena sativa* L.) in 2022. At Kimberly and Rexburg, barley cultivation followed wheat (*Triticum aestivum* L.). Data on air temperature and precipitation were gathered from the National Weather Service weather stations closest to each field site (Agrimet, 2023).



### 2.2.2. Experimental Design

Treatments were arranged in a randomized complete block design with four replications. Treatments consisted of a factorial of eight N treatments of urea (46-0-0) fertilizer applied at 0, 45, 90, 135 or 180 kg N ha<sup>-1</sup> at planting or a split application of 45 kg N ha<sup>-1</sup> done at planting and top-dressed with 23, 45, or 90 kg N ha<sup>-1</sup> at heading. The second factor was three S rates of potassium sulfate (0-0-53-18) fertilizer applied at 0, 17, or 34 kg S ha<sup>-1</sup> at planting. The fertilizer treatments were banded about 5 cm below the soil surface midway between the rows. The field sites were roller-harrowed one to two weeks before planting to break up soil clods and create the tilth or soil structure necessary for seed sowing. Following the University of Idaho Extension recommendations (Robertson & Stark, 2003), barley was planted at a depth of 3 cm with a seeding rate of 1,980,000 seeds ha<sup>-1</sup> on plot dimensions of 1.5×7.6 m and a row spacing of 18 cm. All field site locations were planted using a JD5075E and Wintersteiger 7-row no-till drill. In 2021, Aberdeen and Kimberly field sites were planted on 21 and 27 April, respectively while Aberdeen and Rexburg were planted on 6 April and 26 April, respectively, in 2022. In the 2021 growing season, harvest was done on 20 August and 3 September, respectively. Harvest was done on 10 August and 17 August at Aberdeen and Rexburg, respectively in the 2022 growing season.

### 2.2.3. Plant Tissue Collection and Sampling

Tissue samples were collected immediately before harvest at physiological maturity and the heads were separated from straw (culms and leaves) and counted. The straw portion was processed similar to the in-season tissue samples while the heads were weighed for dry matter and de-awned and cleaned using SLN Sample Cleaner (Pfeuffer GmbH, Germany). The average number of grain kernels per spike was determined as:

*Average number of kernels per spike*

$$= \frac{\left( \text{mass of deawned grain (g)} \times \frac{200 \text{ seeds}}{\text{mass of 200 seeds (g)}} \right)}{\text{number of heads}}$$

#### **2.2.4. Irrigation and Water Sampling**

Irrigation water was supplied at the Aberdeen and Rexburg field sites using handlines fitted with impact head sprinklers on 60-foot center length with 5/32-inch nozzles. At Kimberly, irrigation was supplied using a solid set Nelson Wind Fighters 2000 Rotator sprinklers with 1/8-inch (with shields on the plot edges) and 9/64-inch (main plots) nozzles mounted on Certa Lok Lateral pvc pipe. The irrigation water was supplied from groundwater wells at all sites except Kimberly that was diverted canal water from the Snake River. Irrigation water samples were collected at an early, mid, and end of season event and analyzed for pH (Rhoades & Miyamoto, 1990), total soluble salts and electrical conductivity (APHA, 1997; Helrich, 1990), carbonate and bicarbonate (Rhoades & Miyamoto, 1990; Robbins & Wiegand, 1990), and chloride (Dahnke, 1975; Gavlak et al., 2003). Irrigation water was further analyzed for calcium, magnesium, sodium, and sodium adsorption ratio (SAR) using AAS/ICP-AES Methods (Rhoades & Miyamoto, 1990; Robbins & Wiegand, 1990; Soltanpour et al., 1996), SO<sub>4</sub>-S (Ajwa & Tabatabai, 1993), NO<sub>3</sub>-N (Dahnke, 1975; Gelderman & Beegle, 2012), boron (Gaines & Mitchell, 1979), and phosphorus using open vessel digestion and dissolution method (Kalra, 1995; Soltanpour et al., 1996). At Aberdeen and Kimberly sites, irrigation was applied every 7 to 10 days with the last irrigation event applied at soft dough to replenish soil moisture reserves to allow the barley plants to reach physiological maturity without experiencing water stress (Neibling et al., 2017). The Rexburg site was managed similar to the other sites except that the irrigation well unexpectedly went dry at Feekes 10.4. 2021 field sites at Aberdeen and Kimberly received 197 and 584 mm total irrigation. Aberdeen and Rexburg field sites in 2022 received 396 and 310 mm total irrigation, respectively.

#### **2.2.5. Planting Material**

The fields were planted with Claymore (WestBred, LLC), a two-row spring barley cultivar grown for feed purposes and cultivated in southern Idaho's irrigation systems. Claymore is characterized by high yield, Fusarium Head Blight resistance, superior standability, and straw strength (Marshall et al., 2022; Nutrien, 2021). In southeastern and southcentral Idaho variety trials, Claymore has a three-year average yield of 8,900 kg ha<sup>-1</sup> yield, plant height of 94 cm, protein concentration of 10.3%, and test weight of 66 kg hL<sup>-1</sup> (Marshall et al., 2022).

### **2.2.6. Soil Sampling**

Before planting, each study location was evaluated for macro- and micronutrient content and soil physical characteristics by collecting a five-core composite soil sample from each replicate at depths of 0 to 30 and 30 to 60 cm using a tractor-mounted hydraulic probe with a 5 cm diameter. The soil samples were immediately sent to a commercial soil testing laboratory for complete nutrient analysis. Prior to analysis, the soil samples were oven-dried at 70 °C for 6-8 hours and ground to pass through a <2 mm sieve (Eckert, 1988). The pre-plant soil samples were then analyzed for pH using a 1:1 (soil : water) ratio (Gavlak et al., 2003; Kalra, 1995), cation exchange capacity (Schollenberger & Simon, 1945), ammonium acetate 1:10 exchangeable potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg) (Doll & Lucas, 1973; Knudsen et al., 1983), NH<sub>4</sub>-N (Bremner, 1996), and NO<sub>3</sub>-N (Gelderman & Beegle, 2012). Chloride (Cl), sulfate (SO<sub>4</sub><sup>-</sup>), and boron (B) were measured using the NH<sub>4</sub>F Kewlona extraction (Gavlak et al., 2003), while zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) were evaluated via the DTPA micronutrient extraction method (Lindsay & Norvell, 1978). Additional soil samples were collected from each treatment plot at depths of 0 to 30 and 30 to 60 cm at Feekes 5-6 (jointing stage) using a tractor-mounted hydraulic probe with a 5-cm diameter, Feekes 10.4 (full emergence stage) by hand using bucket augers, and at post-harvest using a tractor-mounted hydraulic probe with a 5-cm diameter and/or by hand using bucket augers in 2021 and a tractor-mounted hydraulic probe with a 5-cm diameter in 2022. The 0 to 30 cm samples were analyzed like the pre-plant soil samples while 30 to 60 cm samples were analyzed for NO<sub>3</sub><sup>-</sup>-N (Gelderman & Beegle, 2012) and SO<sub>4</sub>-S (Gavlak et al., 2003) content. The pre-plant soil sampling events indicate the soil's potential ability to supply nutrients to the developing crop while the in-season and post-harvest soil sampling events provide information about the soil-crop nutrient balances for macro- and micronutrients and helps to estimate rates of plant nutrient uptake.

### **2.2.7. Data Collection**

#### **2.2.7.1. Plant Height**

Plant height data were collected at physiological maturity and measured as the distance from the soil surface to the plant's apex excluding the awns.

### **2.2.8. Harvest and Post-harvest**

We harvested the plots using a small-plot combine with an attached weighing system (Juniper Systems, Logan, UT). The final grain yield was corrected to a 13.5% moisture content (Isleib, 2012). During harvest, a subsample of ~1000 g was collected from each plot, de-awned and cleaned using a SLN Sample Cleaner (Pfeuffer GmbH, Germany), and analyzed for test weight, plumps and thins (Combs & Smith, 1953), grain protein concentration (Agelet & Hurburgh Jr, 2010), and soluble and insoluble protein concentration (Bradford, 1976).

### **2.2.9. Post-harvest Data Collection**

#### **2.2.9.1. Test Weight, Plumps, and Thins**

In small grains, test weight is an important component of crop quality, and it influences sprouting, seedling growth, and plant performance (Deivasigamani & Swaminathan, 2018; Isleib, 2012). A 0.5 L cylindrical shaped cup was completely filled with the grain samples and a hardwood striker was placed on the cylindrical measure about two times in a zig zag motion to level off the grain with the top edge of the container. The grain samples were then poured into a measuring pan and weight was recorded as outlined in the USDA federal grain inspection handbook (USDA, 2004).

Data was collected on plumps and thins by sieving a dockage-free portion of 250 grams through a mechanical sieve. The barley grains that remain on top of a 6/64" x 3/4" inch slotted-hole sieve after sieving were regarded as the plump kernels while the grains that passed through a 5.5/64" x 3/4" inch screen after shaking were recorded as thins (USDA, 2016). For increased precision, the percent plumps for this study were reported as the combined percentage of grain samples that stayed on top of the 6/64" x 3/4" inch and 5.5/64" x 3/4" inch slotted-hole sieves after shaking.

#### **2.2.9.2. Grain Protein Concentration**

The protein content was analyzed using the Bruker TANGO Near InfraRed (FT-NIR) spectrometer at the Wheat Quality Lab in Aberdeen Research and Extension Station. The FT-NIR spectrometer contains about 10 mm gold-coated integrating sphere with light

transmittance of 800 – 2,500 nm for analysis by diffuse reflection approach and it provided an affordable and time-efficient replacement for the costly wet chemistry technique used in laboratories (Bruker, 2023). Grain samples were placed in a TANGO sample cup and then placed on the TANGO gold-coated integrating sphere after which measurement is started. Light is then shone onto the sample for about a minute in the integrating sphere in a wide, nearly horizontal beam (Bruker, 2023) after which results, including the protein, ash, moisture, fiber, and starch contents are displayed.

### **2.2.9.3. Soluble and Insoluble Protein**

Total grain protein concentration was obtained using a Bruker TANGO Near InfraRed (FT-NIR) spectrometer on whole barley grain. Soluble grain protein was evaluated by grinding ~15.0 g of barley grains using Udy Cyclone Sample Mill 3010-030 (Udy Corporation®, Fort Collins, CO, USA). 0.50 g of ground barley sample was weighed and poured into a 15 mL centrifuge tube. 7.50 mL of 0.5 M NaCl was added to solubilize the protein, and the solution was incubated at room temperature for 1 hour, with brief vortexing using a Vortex Genie 2 (Scientific Industries, Bohemia, NY, USA) at 15-minute intervals. The solution was then centrifuged using the Sorvall® RC5C Plus Refrigerated Centrifuge (Marshall Scientific, Hampton, NH, USA) at 10,000 rpm for 10 minutes and the supernatant was collected into a different 15 ml supernatant tube. The insoluble pellet was resuspended in 7.50 mL of 0.5 M NaCl and incubated at room temperature for 30 minutes, with brief vortexing at 15-minute intervals. The solution was centrifuged at 10,000 rpm for additional 10 minutes and the supernatant was collected and transferred into the supernatant tube. A soluble protein standard curve was prepared by dissolving 2.0 g of OmniPur® Bovine Serum Albumin (BSA) Fraction V Heat Shock Isolation (MilliporeSigma, Burlington, MA, USA) in 2 ml dilute H<sub>2</sub>O (d.H<sub>2</sub>O) to produce 1.0 g/mL BSA solution. A dilutions series was prepared in concentrations: 62.5 µg/mL (125 µL of 1.0 g/mL BSA concentrate + 1.875 mL d.H<sub>2</sub>O), 125 µg/mL (250 µL of 1.0 g/mL BSA concentrate + 1.75 mL d.H<sub>2</sub>O), 250 µg/mL (500 µl of 1.0 g/mL BSA concentrate + 1.50 mL d.H<sub>2</sub>O), 500 µg/mL (1000 µL of 1.0 g/mL BSA concentrate + 1.00 mL d.H<sub>2</sub>O), and 1000 µg/mL (2000 µL of 1.0 g/mL BSA concentrate + 0 mL d.H<sub>2</sub>O). 1:5 dilution (20%) of Bio-Rad protein assay solution was prepared by diluting 6 mL of Bio-Rad Protein Assay Dye (Bio-Rad Laboratories, Inc. Hercules, CA, USA) in 24

mL d.H<sub>2</sub>O. From each sample supernatant container, 40 µL of the supernatant was transferred into a separate 2.0 mL test tube and 1.50 mL diluted protein assay solution was added. The solution was mixed until evenly homogenized, transferred into glass cuvettes, and absorbance was measured at 595 nm using a Du 640 spectrophotometer (Beckman Coulter Life Sciences, Indianapolis, IN, USA). The soluble protein concentration (µg/mL) was calculated using the following equation:

$$Y = mx + b$$

Where:        y = unknown soluble protein concentration  
                   x = Absorbance of unknown concentration (obtained from spectrophotometer)  
                   m = slope of the BSA standard curve  
                   b = intercept of the BSA standard curve

Insoluble protein concentration was calculated by subtracting the soluble protein concentration from the total protein concentration.

### **2.2.10. Data Analysis**

Data was analyzed by individual locations to account for the varying preplant soil N at each location. A linear mixed-effects ANOVA was performed in R statistical language (ver. R 4.2.1) using the lmer function of the lme4 package (ver. 1.1–32) and convenience functions from the lmerTest package (R Core Team, 2021). Linear models were employed for all normally-distributed response variables. Nitrogen and S rates were considered fixed effects and replication was considered a random effect. The distribution of residuals, as well as the normality of the model and assumptions of each dependent variable were visualized to determine if the data fits the assumption of equal variance and normality. The lodging rate was not normally distributed. Hence, we simulated lodging data to create scaled residuals standardized to values between 0 and 1, then analyzed using the generalized linear mixed models. Estimated marginal means were extracted for the significant individual fixed effects or interactions, followed by a pairwise comparison.

To determine the response of grain yield to total N rate (preplant N, fertilizer N, and irrigation N), linear or quadratic regression models were developed only for the means of the single N application rates. Models with the highest correlation coefficients and residuals that were normally distributed were chosen.

### 2.2.11. Nitrogen Use Efficiency Calculations

Nitrogen use efficiency was evaluated by agronomic efficiency, partial factor productivity, and crop recovery efficiency (Snyder & Bruulsema, 2007). Agronomic efficiency was employed to measure the productivity improvement gained by employing one N rate over the other. Partial factor productivity measured the productivity of the cropping system in comparison to N input. Crop recovery efficiency measured the increase in crop uptake of N in above-ground parts of the plant in response to N treatments (Snyder & Bruulsema, 2007). Crop recovery efficiency (CRE), agronomic efficiency (AE), and Partial factor productivity (PFP) were calculated using the following equations.

$$\text{PFP} = \frac{Y}{F} \quad \text{CRE} = \frac{(T_N - T_0)}{N \text{ rate}_N} \quad \text{AE} = \frac{(Y_N - Y_0)}{N \text{ rate}_N}$$

Where Y and T represent grain yield and total aboveground N uptake, respectively, the subscripts "N" and "0" denote the relevant fertilizer N treatment and the unfertilized control treatment, respectively, and Y and F represent yield of harvested portion of crop with applied nutrient and amount of nutrient applied, respectively (Snyder & Bruulsema, 2007).

## 2.3 RESULTS AND DISCUSSIONS

### 2.3.1. Weather

Monthly precipitation was classified as below average if it was at least 25 mm below the 30-year (1981-2010) average and categorized as above average if it was at least 25 mm above the 30-year average. Similarly, monthly mean air temperatures were classified as below average if they were at least 0.55 °C below the 30-year average and above average if they were at least 0.55 °C above the 30-year average.

All field sites, except for Rexburg, were wetter than average. August through September was normal or drier than the 30-year average (Table 2.1). The air temperature was hotter than the 30-year average throughout the growing season at Aberdeen and Kimberly field sites in 2021. In 2022, the air temperature was colder than the 30-year average at Rexburg and Aberdeen field sites in April and May. June through September were warmer than the 30-year average, except Rexburg, which was colder than the 30-year average in June. The elevated temperature conditions, especially in 2021, might have affected seedling growth and grain quality by accelerating germination (Asseng et al., 2015) and spike infertility (Prasad & Djanaguiraman, 2014). The above-average precipitation from May to July in both years may have reduced N availability through processes like denitrification and leaching (Cregger et al., 2014; Robertson & Stark, 2003). The wetter-than-normal conditions corresponded to leaf emergence to booting development stages, typically a sensitive period for barley to excess moisture. Excess moisture may result in increased leaching potential and inadequate N availability and may negatively impact grain yield and quality (Borrego-Benjumea et al., 2018; Robertson & Stark, 2003).

Irrigation and precipitation prevented soil water deficit percent from exceeding 50% of the maximum allowed depletion (Robertson & Stark, 2003) in both years across all field site locations except from mid-July to mid-August 2022 corresponding to the Feekes 10.4 stage of development at the Rexburg field site, where soil water deficit percent increased to levels above maximum allowed depletion (Figure 2.1). The rise in soil water deficit percent during the F.10.4 development stage was due to unforeseen circumstances at the Rexburg field site, where the irrigation well unexpectedly went dry. This soil water deficit percent increase corresponded to the grain filling period, and it may have negatively affected yield, grain protein concentration, single grain weight, and overall grain quality (Samarah et al., 2009; Sánchez-Díaz et al., 2002).

### **2.3.2. Soil Characteristics**

The study was carried out on loam-textured soil with a consistently alkaline pH averaging 8.3 in both years across all field sites (Table 2.2), which aligns with the prevalent characteristics of loam-textured soils with low organic carbon in the study region (Dari et al., 2019). The pH



values at all the field sites are an optimum soil pH for barley production (Council, 2012), although it may potentially increase ammonia volatilization (Rochette et al., 2009, 2013). Except for the 2021 Aberdeen field site where the total inorganic N concentration was notably high at 28.2 mg kg<sup>-1</sup>, all other field sites exhibited total inorganic N concentrations below 16 mg kg<sup>-1</sup> (not shown). For both 2021 field site locations, SO<sub>4</sub>-S concentration was <10 mg kg<sup>-1</sup>. According to University of Idaho guidelines, it is recommended to apply 6 to 12 mg kg<sup>-1</sup> of SO<sub>4</sub>-S (Mahler, 2015; Robertson & Stark, 2003). At Aberdeen in 2022, SO<sub>4</sub>-S concentration was higher than the University of Idaho S recommendation; hence it is unlikely that the crop would respond to S additions (Mahler, 2015). In all site years, the soil P concentration fell within the recommended range (6 – 15 mg kg<sup>-1</sup>) for small grain production in the Western US (Jackson et al., 2006; Mahler, 2015; Reisenauer et al., 1976; Robertson & Stark, 2003), except for the Aberdeen field site in 2022 (Table 2.2). Soil K concentrations for all site years exceeded K fertility recommendation for small grains (0 – 40 mg kg<sup>-1</sup>) (Robertson & Stark, 2003), indicating that an effect on yield response is unlikely (Jackson et al., 2006; Robertson & Stark, 2003). Copper, iron, manganese, molybdenum, and boron deficiencies in Idaho soils are uncommon for small grain production. As a result, response to micronutrients in Idaho has been rare, except for small grains grown on soil that has been severely eroded or on soils with exposed light-colored calcareous soils (Mahler, 2015; Robertson & Stark, 2003), which is not the case in this study.

### **2.3.3. Irrigation Water Properties**

Due to limited annual precipitation in many regions of the western United States, surface and groundwater-fed irrigation play a crucial role in supporting the cropping systems of the region (USDA-ERS, 2019). Depending on the source, irrigation water can supply salts and plant nutrients that must be properly managed to ensure that irrigation nutrient supply is synchronized with crop needs and fertilizer application (Mottman, 2015). At all four field sites, the irrigation water was alkaline (Table 2.3) that can make P and other micronutrients less plant available (Swistock, 2022). The anions- chlorine, boron, NO<sub>3</sub>-N, and carbonate were available at rates favorable for plant growth and development across all years and field sites. Sulfate-S was present in the irrigation water at high levels that likely supplied all S required by the developing barley crop (Table 2.3). The consistently elevated levels of SO<sub>4</sub>-S

across all years and field sites align with the findings reported by Robertson & Stark. (2003), which states that Snake River water can provide sufficient S content for successful small grain production. Elevated  $\text{SO}_4\text{-S}$  concentrations in irrigation water may inhibit crop  $\text{NO}_3\text{-N}$  uptake (Bill, 2018) and potentially increased  $\text{NO}_3\text{-N}$  leaching into groundwater. The bicarbonate levels in the irrigation water may potentially enhance iron and manganese deficiency or calcium and magnesium imbalance (Misaghi et al., 2017; Swistock, 2022). Sodium and potassium concentrations at all sites and years were typically favorable for plant growth, while Ca and Mg were available at high concentrations and may potentially co-limit phosphorus availability for plants (Swistock, 2022).

#### **2.3.4. End of Season Metrics**

Plant height, the number of heads, and grain yield increased with increasing N application rate at Aberdeen and Rexburg 2022 and Kimberly 2021 field sites (Tables 2.5, 2.6). Lodging and grain protein concentration at Aberdeen 2021 field site increased with an increasing N rate. Like the test weight response at Aberdeen and Rexburg 2022 field sites, grain starch concentration, grain protein concentration, and percent plumps responded significantly to the individual fixed effect of N rate (Table 2.4). Sulfur application nor the interaction of N and S did not affect any dependent variable except the number of heads at Kimberly 2021 and test weight at Rexburg 2022 field sites where the individual fixed effect of the S application rate was significant.

##### **2.3.4.1. Plant Height and Lodging**

Plant height increased with N rate at the Kimberly, Aberdeen 2022, and Rexburg 2022 field sites and corroborate previous studies' findings on barley and wheat (Kenbaev & Sade, 2002; Khan et al., 2009; Reddy & Singh, 2018; Shafi et al., 2011). Plant height was similar between the 135 and 180 kg N ha<sup>-1</sup> treatments with average heights of 67, 87, and 62 cm at Kimberly 2021, Aberdeen 2022, and Rexburg 2022 field sites, respectively. Although there was no difference in plant height at the Aberdeen 2021 field site, plant height averaged 75 cm. Single N application at Kimberly 2021 and Aberdeen 2022 field sites produced significantly taller plants than split N applications at equivalent rates. Similar to Hadi et al. (2012), the split N application significantly reduced plant height at Kimberly and Aberdeen in 2022 due

to low initial N at planting when N was split-applied. The average plant height range observed at our Aberdeen 2022, Kimberly, and Rexburg field sites (62-90 cm) is comparable to the plant height range of the Altorado and Oreana feed barley varieties in the 3-year southcentral and southeastern Idaho variety trials (Marshall et al., 2022). Further, this result aligns with the reported plant height range of Claymore in feed barley variety trials conducted in under similar growing conditions in Alberta, Canada (Redel, 2019), as well as in Washington (Neely, 2023) and Oregon (OSU Extension, 2022). There was no lodging at both field sites in 2022 and Kimberly in 2021, while the Aberdeen field site had a significant lodging response to N rates in the 2021 growing season (Table 2.4). Lodging significantly increased with an increasing N rate at the Aberdeen 2021 field site, while Kimberly typically had no lodging (Table 2.5). Similar results of increased lodging with an increasing N rate at Aberdeen in 2021 were reported in Egypt (Ali, 1993; Kheiralla et al., 1993), India, and Mexico (Zuber et al., 1999). High residual total inorganic soil N levels at Aberdeen in 2021 induced taller plants and likely reduced straw strength and consequently increased lodging. Lodging rate was similar at 90, 135, and 180 kg N ha<sup>-1</sup> treatments with average lodging of 59%. There was no difference in lodging between 45/45 and 90 kg N ha<sup>-1</sup> rate. However, single N application significantly increased lodging at 135 kg N ha<sup>-1</sup> compared to split N application, similar to the reports of Fischer & Stapper, (1987), Berry et al. (2000), and Peake et al. (2016) that lodging potential can be decreased by delaying N fertilizer applications. Increased lodging delayed the process of plant drying down (Robertson & Stark, 2003) and harvest for two weeks at Aberdeen in 2021.

#### **2.3.4.2. Number of Heads and Tillers**

There was a significant difference in the number of tillers at 0 and 180 kg N ha<sup>-1</sup> at Kimberly and Rexburg and the highest number of tillers (8) was recorded at the Rexburg field site (Tables 2.5, 2.6). Split and single N application treatments had a similar number of tillers and heads across all years and locations (Tables 2.5, 2.6). A 180 kg N ha<sup>-1</sup> rate produced the highest number of heads at Kimberly in 2021. There was a statistical difference between the number of heads at the maximum N rate (180 kg N ha<sup>-1</sup>) and 0 kg N ha<sup>-1</sup> (check plot) at all the locations except at the Aberdeen 2021 field site (Table 2.6) where tillers and heads were not responsive to N treatments. The statistical difference in the number of tillers and heads

between the 0 and 180 kg N ha<sup>-1</sup> treatments indicates that optimal N availability primarily influences barley growth. However, at the Aberdeen field site in 2021, the low number of tillers and the insignificant response of the number of heads between the N treatments suggest that high doses of N, stemming from both high residual N and fertilizer N treatments, led to a reduction in aboveground vegetative growth (Shafi et al., 2011). Similarly, Ahmad et al. (1986) and Rajput et al. (1993) reported an increase in barley and wheat aboveground vegetative parts due to N application.

#### **2.3.4.3. Grain Yield**

The maximum grain yield across all field sites ranged from 5 – 8.6 Mg ha<sup>-1</sup> (Tables 2.5, 2.6) and was consistent with the average yield reported for Claymore in 2021 and 2022 in the southeastern and south-central Idaho, Oregon and Canada feed barley variety trials (Marshall et al., 2022; OSU Extension, 2022; Redel, 2019). Grain yield increased with increasing N rate at Aberdeen 2022, Kimberly, and Rexburg field sites (Tables 2.5, 2.6). Except at Aberdeen 2021 field site, where there was no observed difference in yield, grain yield was minimized at Kimberly at the check plot (2.6 Mg ha<sup>-1</sup>) and at the check plot and 45 kg N ha<sup>-1</sup> rate with average grain yields of 3.5 and 3.2 Mg ha<sup>-1</sup> at Aberdeen and Rexburg 2022 field sites, respectively. The Rexburg field site produced the lowest grain yield across all equivalent N fertilizer rates, likely due to moisture stress (Bello et al., 2022; Robertson & Stark, 2003) resulting from the abrupt loss of irrigation water source in July (Figure 2.1d) at Feekes 10.4 stage of development. Samarah (2005) and Samarah et al. (2009) also reported the influence of moisture stress at the grain filling stage on yield and yield components. Although mostly planted to winter barley, previous studies in the midwestern US, California, and eastern Oregon have reported split N application as a common practice to increase yield (Sullivan et al., 1999). Alley et al. (2009) and Baethgen & Alley (1989) reported that winter barley utilizes less N during the colder temperatures of the winter and N applied at planting has a higher potential for leaching losses, hence, the split application is necessary to enhance tiller formation when winter barley re-initiates growth in February or March (Alley et al., 2009). However, spring barley develops rapidly from planting through tillering, hence, single N applied at planting has low potential for N loss (Robertson & Stark, 2003). In this study, single N applications produced similar or greater yields, resulting in approximately 6 – 26%

yield advantage over split N applications across all site years (Tables 2.5, 2.6). Split N application produced lower yields, likely due to inadequate N availability at the critical growth stages due to the delayed time of split application in July (after early tillering). Others have reported similar lower yield responses of split N applications than single N applications in Idaho (Robertson & Stark, 2003), Washington (Curry et al., 2019), and Montana (Westcott et al., 1998).

#### **2.3.4.4. Grain Protein and Starch Concentration**

Grain protein concentration exhibited a significant response to N rates across all site years, as indicated in Table 2.4. However, no significant response was observed for S rates. The recorded grain protein concentration average across all site years aligns with the reported grain protein concentration (10.3%) for Claymore in a three-year trial conducted in southeastern and southcentral Idaho (Marshall et al., 2022), as well as study reports in Washington (Curry et al., 2019). There was a significant difference between single and split N applications at Kimberly and Rexburg but not at Aberdeen 2021 and 2022 field sites (Tables 2.5, 2.6). Kimberly and Rexburg field sites had similar grain protein concentration responses to N application timing, and split N application produced kernels with 1–2% higher grain protein concentration than single N application and is similar to previous study reports (Curry et al., 2019; Hackett, 2019; Westcott et al., 1998). This is likely because split N fertilizer was preferentially incorporated into the grain increasing protein concentration whereas more of the fertilizer N is incorporated into vegetative biomass when applied at planting.

There was a significant response to the individual fixed effect of N rate on grain starch concentration at Kimberly and Rexburg, but no response to S rates was observed at Aberdeen (Table 2.4). Grain starch concentration was negatively correlated to N rates and grain yield as there was a pattern of decreasing grain starch concentration with increasing grain yield and N levels across all site years (Tables 2.5, 2.6). The average grain starch concentration reported across all site years was 54%, which corresponds with previous reports of feed barley grain starch concentration (Schulman et al., 2000; Silveira et al., 2007), making the kernels a good source of energy for monogastric animals (Kling et al., 2004).

#### 2.3.4.5. Soluble and Insoluble Protein Concentration

Soluble protein concentration refers to albumin (water soluble) and globulin (salt soluble) while insoluble protein concentration refers to prolamine (alcohol soluble) and glutelin (alkaline soluble) fractions of the total protein (Osborne, 1895). Soluble protein concentration tended to increase with increasing N rate indicating an increase in the albumin and prolamine fractions of the grain protein across all site years except Rexburg 2022 field site. The increase in soluble protein fractions also indicate that more cysteine, lysine, and threonine amino acids are synthesized with increasing N rate suggesting increased catabolism as energy source for the small intestine of animals (Liao et al., 2015). Insoluble protein concentration was similar across all site years and was not affected by N except at the Rexburg 2022 field site (Table 2.7). Insoluble protein concentration averaged 5.8, 6.0, and 5.7% at Aberdeen 2021, Kimberly, and Aberdeen 2022 field sites, respectively. Although not responsive, the insoluble protein fraction was higher than soluble protein fraction across all site years indicating a higher synthesis of isoleucine, phenylamine, and valine amino acids (Liao et al., 2015) which improves animal immune system and growth by increasing animal gut morphology (Fini et al., 2001). Regarding N application timing, soluble protein concentration was similar across all site years except at a 135 kg N ha<sup>-1</sup> rate at Kimberly, where split-applied N had a significantly higher soluble protein concentration (Table 2.7). Like soluble protein concentration, the split-applied N rate had a significantly higher insoluble protein concentration at the Rexburg 2022 field site. The average soluble protein concentration across all N rates and site years was 4% and accounted for 38% of grain protein concentration, while the average insoluble protein concentration was 6.5% and accounted for 62% of total grain protein concentration. In this study, soluble protein concentration was 8% higher while insoluble protein concentration was 8% lower than reports by several authors who reported that two- and six-rowed feed barley had 15-30% soluble protein concentration and 70-85% insoluble protein concentration of total grain protein concentration (Arends et al., 1995; Shewry et al., 2001; Shewry & Halford, 2002; Turulja, 2004). This is likely due to feed barley varietal genetic variability and agronomic management differences.

#### **2.3.4.6. Kernel Plumpness and Test Weight**

Split-applied 45/90 kg N ha<sup>-1</sup> at Kimberly produced plumper kernels (99.0%) than single application (97.6%) (Table 2.6). Averaged across all N rates at each location, kernel plumpness was 98% at Aberdeen 2021 and 2022 and Kimberly field sites except at Rexburg where plumpness averaged 96%. The 2% plumpness reduction was suspected to be an effect of the moisture stress at the Rexburg field site, resulting from irrigation well going dry during grain fill (Feekes 10.4 growth stage), and was similar to reports of Bello et al. (2022) and Samarah et al. (2009), where moisture stress at the anthesis and milk/dough growth stage reduced overall grain quality. Test weight was higher at split-applied N rates at Rexburg in 2022 than single N applications (Table 2.6). Although previous studies (Prystupa et al., 2019; F. J. Zhao et al., 2006) rarely reported test weight response to S, our study at the Rexburg field site showed a statistically significant response of feed barley test weight to S rates. Rates of 17 and 34 kg S ha<sup>-1</sup> were not statistically different but produced statistically higher test weight (67 kg hL<sup>-1</sup>) than 0 kg S ha<sup>-1</sup> (66 kg hL<sup>-1</sup>) (Table 1.7). This reported high test weight (>59 kg hL<sup>-1</sup>) and consistent kernel plumpness (> 94%) from this study represent increased kernel nutrient density and consistent kernel rolling output during milling (Edney et al., 1994; Edney, 2010; Kling et al., 2004). Kernel plumpness had a non-patterned response to N across all site years but only significant individual fixed effects of N and S rates on test weight at the Rexburg field site (Table 2.4).

#### **2.3.5. Nitrogen Use Efficiency and Regression of Yield and Post-harvest Soil Residual N on Total Nitrogen**

Because initial planting conditions are not always uniform year to year and field to field, yield and postharvest soil N were regressed against the total N available in each treatment. Total N is defined as the sum of pre-plant soil N (0-60 cm), irrigation N credit, and the applied fertilizer N. Kimberly 2021 and Aberdeen 2022 field sites had positive linear responses of grain yield to total N yielding 6.9 Mg ha<sup>-1</sup> at Kimberly and 6.6 Mg ha<sup>-1</sup> at Aberdeen 2022 at the highest total N rate (Figure 2.2). The Rexburg field site had a quadratic response with the highest yield of 4.5 Mg ha<sup>-1</sup> at a 178 kg N ha<sup>-1</sup> rate. Aberdeen 2021 had a mean yield of 8.2 Mg ha<sup>-1</sup> but was not responsive to N. These results show that pre-plant soil

N differences, including in-season N supplied through irrigation water, can lead to varying yield responses to fertilizer N rates. The linear responses of grain yield at Kimberly 2021 and Aberdeen 2022 field sites suggest substantial N fertilizer loss (Figure 2.2), potentially increasing the risk of nitrate leaching into groundwater (Lazicki & Geisseler, 2016). From May to July, Kimberly 2021 and Aberdeen 2022 were wetter than average (Table 2.1), and crop water use was low. Hence, N leaching potential was likely high. Rexburg 2022 field site had a quadratic yield response to total N, probably due to the loss of the irrigation water source in July (Figure 2.1d).

Nitrogen use efficiency generally responded to N rates across all site years except crop recovery efficiency at Aberdeen 2021 and 2022 field sites and agronomic efficiency at Aberdeen 2022 field sites (Table 1.10). Similar to reports by Anbessa & Juskiw (2012) and Ali et al. (2022) for spring barley under arid conditions in Canada and Saudi Arabia, as well as reports by Delogu et al. (1998) for winter barley and wheat in Italy, where agronomic efficiency decreased as N input increased. Agronomic efficiency in this study typically decreased with increasing N rates across all site years (Table 2.7). Single N fertilizer treatments had similar agronomic efficiency compared with split N application at Aberdeen 2021 and 2022 field sites. The single N application had higher agronomic efficiency at Kimberly and at 135 kg N ha<sup>-1</sup> rate at Rexburg, contributing a 9-25% agronomic efficiency advantage. The difference in agronomic efficiency between N application timing events indicates poor N utilization when N was split applied in irrigated spring barley production, which is similar to reports by Robertson & Stark (2003), who likewise recommended that a single N application for spring barley production resulted in increased N use efficiency. Like agronomic efficiency, partial factor productivity decreased with increasing N rates (Table 2.10), indicating that the cropping system efficiency reduces with increasing N input. These findings of reduced N use efficiency parameters with increasing N input further indicate increased leaching potential and reduced soil productivity (Snyder & Bruulsema, 2007). Although a significant difference was only observed between N application timing at the Kimberly field site, a single N application had higher partial factor productivity across all site years. Nitrogen rate significantly affected crop recovery efficiency at Kimberly and Rexburg field sites. N application timing did not affect crop recovery efficiency except at a rate of 135 kg N ha<sup>-1</sup> at Rexburg, where single N had a higher crop recovery efficiency than split-applied



N. Crop recovery efficiency averaged  $0.6 \text{ kg kg}^{-1}$  across all site years and corresponds to Dobermann (2007), who reported a  $0.5\text{-}0.8 \text{ kg kg}^{-1}$  crop recovery efficiency values for small grains under best management practices. Except at the Aberdeen 2021 field site where partial factor productivity was minimized ( $47.1 \text{ kg kg}^{-1}$ ) at  $180 \text{ kg N ha}^{-1}$  rate, partial factor productivity at the Kimberly 2021, Rexburg, and Aberdeen 2022 field sites was minimized at  $135 \text{ kg N ha}^{-1}$  and  $180 \text{ kg N ha}^{-1}$  rates with an average partial factor productivity of 34.4, 26.5, and  $38.9 \text{ kg kg}^{-1}$ , respectively. Nitrogen use efficiency was low at the Aberdeen 2021 field site due to N supplied above crop's needs (Lazicki & Geisseler, 2016; Sullivan & Cogger, 2003). Low N use efficiency at Rexburg was likely due to moisture stress from July through August 2022 (Figure 2.1d). The difference in N use efficiency indices in response to fertilizer N application timing indicates that, for southern Idaho soils characteristics and growing conditions (Robertson & Stark, 2003; Stark & Brown, 1987), split N application immediately before the jointing stage may not be an effective practice to increase N use efficiency in feed barley production. In order to improve N use efficiency, it may be necessary to adapt crop genetics further, as well as reduce soil N loss and improve feed barley N uptake.

Aberdeen and Kimberly 2021 field sites had a quadratic response of postharvest soil N to total N, while Rexburg and Aberdeen 2022 field sites were nonresponsive (Figure 2.3). The low soil residual N at Aberdeen and Kimberly 2021 field sites when total N rate was  $<200 \text{ kg ha}^{-1}$  suggest an increased use of N by the developing barley plants. As the total N rate increased  $>200 \text{ kg ha}^{-1}$ , barley plants' N use declined, suggesting an increased potential for N loss at total N rates  $>200 \text{ kg ha}^{-1}$  in spring feed barley production. Aberdeen and Rexburg 2022 field sites had a non-responsive response to postharvest soil N. The non-responsiveness of postharvest residual N at Rexburg may likely result from moisture stress towards the end of the growing season, which resulted in reduced yield (Table 2.6) and lowered N loss to leaching or denitrification. Further, earlier in the growing season, when soil water availability was better, we had warmer-than-average soil temperatures at Aberdeen and Rexburg 2022 field sites (Table 2.1) which may have likely led to higher-than-average N mineralization from soil organic matter. Sullivan & Cogger (2003), in their study, recommended that postharvest soil sampling should be done immediately after harvest before plant residues enhance mineralization and residual N. However, our postharvest soil sampling in Aberdeen

in the 2022 growing season was conducted about 45 days after harvest. The delay in sampling time, plant residue cover, and warmer temperature in August and September (Table 2.1) may have influenced N mineralization by lowering the surface temperature and water evaporation, thereby increasing postharvest residual soil N at the Aberdeen field site.

## **2.4. CONCLUSIONS**

Nitrogen rates and application timing impacted feed barley end-of-season agronomic indices and NUE. Like Idaho spring barley production guidelines, feed barley crop N uptake and end-of-season agronomic indices were non-responsive to S fertilizer application, demonstrating the ineffectiveness of additional S for improving barley production on the Snake River Plain. With respect to the growing conditions in this region, split-applied N done before the jointing stage could be a poor strategy to improve irrigated spring feed barley N use efficiency and economic returns and should be avoided in favor of single N fertilizer applications done at planting. When all N was applied at planting, the feed barley enhanced aboveground vegetative growth, improved yield, and N use efficiency. Single-applied N also has a significantly lower input cost and better agronomic efficiency and crop recovery efficiency than split-applied N, which could result in economic savings and environmental protection from N losses. The University of Idaho N fertility recommendations did not credit irrigation water N, posing the challenge of making specific N recommendations for feed barley production. Hence, to avoid underestimating total soil N under irrigated growing conditions, it would be necessary for growers to combine their knowledge of previous years' weather patterns, soil characteristics, and irrigation water's nutrient content when determining the appropriate N rate for feed barley production.

Table 2.1 Monthly precipitation and mean air temperatures for the four field site locations in Idaho during the 2021 and 2022 growing seasons, with 30-year average (1981–2010) precipitation and temperature in parentheses<sup>a</sup>.

Site-year	Year	Apr	May	Jun	Jul	Aug	Sep
-----Precipitation + Irrigation (mm)-----							
Aberdeen	2021	20 (20)	106 (28)	148 (26)	77 (14)	18 (11)	20 (19)
Kimberly	2021	5 (29)	159 (35)	274 (22)	203 (7)	8 (9)	7 (13)
Aberdeen	2022	20 (20)	141 (28)	176 (26)	126 (14)	27 (11)	20 (19)
Rexburg	2022	16 (30)	34 (46)	212 (41)	119 (19)	17 (18)	29 (22)
-----Temperature avg. (°C)-----							
Aberdeen	2021	8.3 (7.2)	13.3 (12.2)	20.6 (15.6)	23.3 (20.6)	23.9 (19.4)	15.0 (13.9)
Kimberly	2021	9.4 (8.0)	13.9 (13.0)	23.3 (16.4)	25.6 (20.5)	21.6 (20.5)	17.2 (15.0)
Aberdeen	2022	5.0 (7.2)	10.0 (12.2)	16.7 (15.6)	22.2 (20.6)	21.7 (19.4)	16.7 (13.9)
Rexburg	2022	4.4 (7.2)	10.0 (11.7)	15.0 (15.6)	20.6 (20.0)	20.6 (19.4)	16.1 (14.4)

<sup>a</sup> Data on average air temperature and the monthly total precipitation was gathered from the U.S. Climate Data.

Table 2.2 Initial soil physical and chemical properties in the top 0-60 cm for four Idaho field site years.

Property	2021				2022			
	Aberdeen		Kimberly		Aberdeen		Rexburg	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
pH, water	8.2	8.3	8.0	8.3	8.3	8.2	7.8	7.9
CEC, cmolc kg <sup>-1</sup>	12.3	13.2	16.5	15.2	13.8	14.0	10.7	11.1
SOM, g kg <sup>-1</sup>	11.0	10.0	18.0	11.0	9.5	8.0	13.0	9.0
P, mg kg <sup>-1a</sup>	16.0	11.3	12.0	3.0	24.0	7.5	22.5	20.5
K, mg kg <sup>-1 a</sup>	214.3	160.0	138.5	61.8	194.8	94.8	188.0	143.5
Ca, mg kg <sup>-1 a</sup>	9.0	9.4	11.2	12.5	10.7	11.0	7.9	8.2
Mg, mg kg <sup>-1 a</sup>	2.5	3.0	3.4	3.6	2.2	2.4	2.2	2.3
NO <sub>3</sub> -N, mg kg <sup>-1</sup>	40.8	10.3	14.5	10.3	4.8	13.8	4.0	3.8
NH <sub>4</sub> -N mg kg <sup>-1</sup>	3.1	2.0	3.2	2.0	3.8	2.8	4.4	3.4
SO <sub>4</sub> -S, mg kg <sup>-1</sup>	4.5	6.8	11.3	7.0	21.0	44.8	3.8	3.5
Mn, mg kg <sup>-1</sup>	2.5	3.0	4.2	1.6	2.9	1.7	2.8	2.0
Cu, mg kg <sup>-1</sup>	0.9	0.9	1.0	0.4	0.7	0.5	0.6	0.6
B, mg kg <sup>-1</sup>	0.6	0.5	0.7	0.4	0.7	0.5	0.5	0.4
Zn, mg kg <sup>-1</sup>	0.7	0.4	3.1	0.4	1.4	0.5	3.2	1.5
Fe, mg kg <sup>-1</sup>	8.3	7.2	4.2	3.2	4.7	4.9	7.7	9.1
Bulk density, g cm <sup>-3</sup>	1.7	1.7	1.5	1.5	1.7	1.7	1.6	1.7

<sup>a</sup> Olsen P (pH > 7.2). Potassium, calcium, and magnesium are extracted with ammonia acetate.

Table 2.3 Season average of physical and chemical properties and nutrient content of irrigation water.

Property	2021		2022	
	Aberdeen	Kimberly	Aberdeen	Rexburg
pH, water	8.1	8.2	7.9	7.7
Carbonate, mg L <sup>-1</sup>	0	5	0	0
Bicarbonate, mg L <sup>-1</sup>	144.9	151.8	238	285.5
Hardness, mg L <sup>-1</sup>	12.1	9.4	11.9	13.4
EC, mmhos cm <sup>-1</sup>	629.8	384.3	471.5	532.9
Total Soluble Salts, mg L <sup>-1</sup>	338.3	281.6	384.6	419.1
Cl, mg L <sup>-1</sup>	47.9	25.7	37.3	21.3
SO <sub>4</sub> -S, mg L <sup>-1</sup>	25.7	16.7	16.9	12.1
Ca, mg L <sup>-1</sup>	43.9	33.7	53	58.7
Mg, mg L <sup>-1</sup>	23.6	20	17.1	20
Na, mg L <sup>-1</sup>	36.3	32.8	48.4	15.8
SAR	1.1	0.8	0.5	0.5
NO <sub>3</sub> -N, mg L <sup>-1</sup>	5.7	1.6	1	2.7
K, mg L <sup>-1</sup>	5.9	5	3.4	3
P, mg L <sup>-1</sup>	0.02	0.2	0.04	0.03
B, mg L <sup>-1</sup>	0.1	0.1	0.1	0.1

Table 2.4 Test of fixed effects for feed barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test weight (TW) in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, and Brigham Young University-Idaho in Rexburg, Idaho, USA.

Source of variation	Test of Fixed Effects								
	-----P>F value-----								
	PH	Tillers	Heads	Lodging	GY	GPC	GSC	Plumps	TW
<b>Aberdeen 2021</b>									
Nitrogen rate (N)	0.235	0.964	0.925	<0.001	0.199	0.003	0.421	0.023	0.843
Sulfur rate (S)	0.452	0.327	0.908	0.5	0.747	0.264	0.647	0.509	0.299
N x S	0.416	0.683	0.27	0.744	0.089	0.46	0.167	0.169	0.575
<b>Kimberly 2021</b>									
Nitrogen rate (N)	<0.001	<.001	<0.001	NA	<0.001	<.001	0.008	<0.001	0.923
Sulfur rate (S)	0.673	0.928	0.013	NA	0.638	0.481	0.328	0.056	0.256
N x S	0.234	0.029	0.798	NA	0.999	0.19	0.833	0.715	0.617
<b>Aberdeen 2022</b>									
Nitrogen Rate (N)	<0.001	<0.001	<0.001	NA	<0.001	0.017	0.095	<0.001	0.188
Sulfur Rate (S)	0.653	0.378	0.542	NA	0.142	0.093	0.231	0.181	0.876
N x S	0.922	0.613	0.763	NA	0.99	0.254	0.072	0.923	0.749
<b>Rexburg 2022</b>									
Nitrogen Rate (N)	<0.001	<0.001	0.006	NA	<0.001	<0.001	0.034	0.044	0.012
Sulfur Rate (S)	0.529	0.795	0.687	NA	0.772	0.208	0.343	0.063	0.001
N x S	0.782	0.887	0.515	NA	0.979	0.184	0.911	0.742	0.888

NA denotes not applicable.

Table 2.5 Treatment means for feed barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, Idaho, USA.

	PH cm	Tillers ----- # -----	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC ----- % -----	GSC -----	Plumps -----	TW kg hL <sup>-1</sup>
<b>Aberdeen 2021</b>									
N rate (kg ha <sup>-1</sup> )									
0	74 <sup>b</sup>	5	135	14e	7.9	9.4c	55.2	98.7a	66
45	73	5	135	32cde	7.8	9.9bc	55.1	98.7a	66
45/23	78	5	137	20de	8.3	10.1abc	55.0	99.1a	66
45/45	77	5	137	39bcd	8.5	10.8a	54.8	97.7c	66
90	73	5	136	47abc	8.1	10.5ab	54.8	98.4abc	67
45/90	75	5	123	27cde	8.5	10.7a	55.2	98.6ab	66
135	76	5	132	60ab	8.6	10.8a	54.8	98.4abc	66
180	76	5	138	69a	8.5	10.7a	54.6	97.8bc	65
SE	2	0.4	9.6	9.9	0.3	0.3	0.3	0.3	0.7
<b>Kimberly 2021</b>									
N rate (kg ha <sup>-1</sup> )									
0	41d	3d	72d	NA	2.6d	10.1c	53.8ab	98.2bcd	66
45	52c	4c	93c	NA	3.8c	9.3d	54.0a	98.0cd	66
45/23	50c	5b	110c	NA	3.7c	10.8b	53.9ab	98.7abc	66
45/45	51c	5b	101c	NA	4.2c	11.1b	54.0ab	98.9ab	66
90	61b	5b	131b	NA	5.1b	9.9c	54.0ab	98.2bcd	66
45/90	51c	4c	101c	NA	4.1c	11.8a	54.1ab	99.0a	66
135	65a	5b	142b	NA	5.6b	9.9c	53.5bc	97.6de	65
180	69a	6a	169a	NA	6.9a	10.7b	53.2c	97.0e	66
SE	1.4	0.5	6.5	NA	0.3	0.2	0.2	0.3	0.5
SO <sub>4</sub> -S rate (kg ha <sup>-1</sup> )									
0	55	5	114ab	NA	4.4	10.4	53.9	97.9	64

Table 2.5 cont'd

17	55	5	125a	NA	4.5	10.5	53.7	98.2	65
34	54	5	108b	NA	4.6	10.4	53.8	98.5	65
SE	1.1	0.4	4	NA	0.2	0.1	0.1	0.2	0.4

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level. NA denotes not applicable.



Table 2.6 Treatment means for feed barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2022 growing season at the University of Idaho Aberdeen Research and Extension Center and Brigham Young University-Idaho in Rexburg, Idaho, USA.

	PH cm	Tillers -----#----- --	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC ----- % -----	GSC	Plumps -----	TW kg hL <sup>-1</sup>
<b>Aberdeen 2022</b>									
N rate (kg ha <sup>-1</sup> )									
0	62df <sup>b</sup>	3c	40d	NA	3.3e	9.8bc	53.9a	98.3a	67ab
45	69c	3c	48cd	NA	3.7de	9.2c	53.2abc	98.6a	66b
45/23	73c	5b	52bc	NA	4.5cd	10.4ab	52.5c	97.5a	66b
45/45	73c	4bc	50bcd	NA	4.9bc	10.8ab	53.2abc	97.6a	71a
90	80b	5b	60abc	NA	5.2bc	9.9bc	53.6ab	98.7a	68ab
45/90	74c	6a	61ab	NA	4.9bc	10.8ab	52.4c	95.8b	67b
135	84ab	5b	60abc	NA	5.7ab	10.1bc	53.2abc	98.6a	67b
180	89a	4bc	69a	NA	6.6a	11.3a	52.6bc	98.2a	67b
SE	2.7	0.6	4.9	NA	0.5	0.5	0.5	0.4	1.2
<b>Rexburg 2022</b>									
N rate (kg ha <sup>-1</sup> )									
0	48c	6c	45c	NA	2.8d	9.6e	52.3a	96.3abc	66b
45	55b	7b	59abc	NA	3.6cd	9.1e	52.1a	95.7abc	66b
45/23	59ab	7b	58bc	NA	4.2bc	10.5cd	51.8ab	96.9ab	66b
45/45	55b	7b	68ab	NA	4.0bc	11.3bc	52.0ab	97.2ab	67a
90	58ab	7b	64ab	NA	4.5ab	9.5e	51.8ab	94.0c	66b
45/90	58ab	7b	54bc	NA	4.0bc	12.5a	51.1bc	97.4a	67a
135	61a	8a	73a	NA	5.0a	10.4d	51.7abc	94.0c	65c
180	62a	8a	68ab	NA	4.2bc	11.4b	50.9c	94.8bc	65c
SE	3.4	0.2	5.9	NA	0.3	0.4	0.3	1.1	0.4
SO <sub>4</sub> -S rate (kg ha <sup>-1</sup> ) <sup>1)</sup>									
0	58	7	63	NA	4.0	10.7	51.5	94.7	66b

Table 2.6 cont'd

17	58	7	60	NA	4.0	10.5	51.8	96.4	67a
34	56	7	60	NA	4.1	10.3	51.9	96.3	67a
SE	3.0	0.2	4.3	NA	0.2	0.3	0.2	0.8	0.3

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level.  
NA denotes not applicable.

Table 2.7 Treatment means for feed barley soluble protein concentration (SPC), insoluble protein concentration (ISPC), agronomic efficiency (AE), crop recovery efficiency (CRE), and partial factor productivity (PFP) of applied nutrients with standard errors (SE) with response to N fertilizer rates in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University in Rexburg, Idaho, USA.

	SPC	ISPC	AE <sup>a</sup>	CRE	PFP	SPC	ISPC	AE	CRE	PFP
	-----%-----		-----kg kg <sup>-1</sup> -----			-----%-----		-----kg kg <sup>-1</sup> -----		
N rate (kg ha <sup>-1</sup> )	----- <b>Aberdeen 2021</b> -----					----- <b>Kimberly 2021</b> -----				
0	3.1d <sup>b</sup>	6.3	-	-	-	4.1bc	6.0	-	-	-
45	4.5bc	5.4	31.4a $\phi$	0.5	174.5a	3.9c	5.4	49.5a	0.9b	85.2a
45/23	4.1c	5.9	27.8ab	1.1	122.3b	4.6abc	6.2	30.7c	1.5a	54.3bc
45/45	5.0abc	6.2	22.2bc	1.1	93.8c	4.4bc	6.7	29.1c	1.1ab	47.0cd
90	4.9abc	5.6	19.0c	0.2	90.5c	4.4bc	5.5	39.1b	1.6a	57.0b
45/90	5.2ab	5.5	15.4cd	0.2	63.1d	5.5a	6.3	18.7d	0.8b	30.6f
135	4.7abc	6.1	16.0cd	0.6	63.7d	3.7c	6.2	29.5c	1.3ab	41.4de
180	5.6a	5.1	11.5d	0.5	47.1e	5.0ab	5.7	29.3c	1.3ab	38.2ef
SE	0.4	0.4	2.7	0.4	2.7	0.4	0.4	3.0	0.2	3.0
<i>Pr(&gt;F)</i>	<0.001	0.501	<0.001	0.216	<0.001	0.017	0.104	<0.001	<0.001	<0.001
N rate (kg ha <sup>-1</sup> )	----- <b>Aberdeen 2022</b> -----					----- <b>Rexburg 2022</b> -----				
0	3.5c	6.3	-	-	-	2.9	6.7cd	-	-	-
45	3.6c	5.6	22.6	0.1	81.4a	3.4	5.7d	27.4a	0.1b	79.9a
45/23	4.5abc	5.9	27.3	0.4	66.2b	2.9	7.7bc	26.5a	0.1b	61.2b
45/45	5.3ab	5.5	26.3	0.3	55.7c	2.7	8.6b	18.8bc	0.3a	45.1c
90	4.6abc	5.3	28.5	0.5	57.9bc	3.2	6.3d	23.5ab	0.2ab	49.7c
45/90	5.2ab	5.6	17.6	0.6	37.2d	2.5	10.0a	12.1cd	0.1b	29.6e
135	4.0bc	6.1	22.9	0.5	42.5d	2.4	8.0b	19.9ab	0.3a	37.4d
180	5.8a	5.5	22.3	0.6	37.0d	2.8	8.7b	10.3d	0.2ab	23.4e
SE	0.6	0.3	5.0	0.2	5.0	0.3	0.5	3.4	0.1	3.4

Table 2.7 cont'd

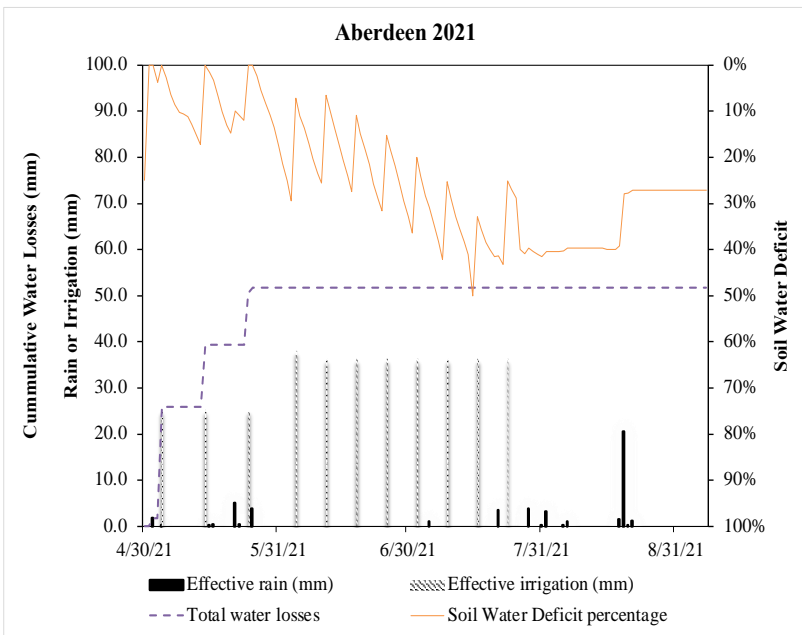
<i>Pr(&gt;F)</i>	0.014	0.484	0.447	0.073	<0.001	0.328	<0.001	<0.001	0.015	<0.001
------------------	-------	-------	-------	-------	--------	-------	--------	--------	-------	--------

<sup>a</sup> The difference in yield for feed barley N uptake between the treatment of interest and 0N is divided by the applied N rate to determine AE and CRE.

<sup>b</sup> Within site-year and dependent variable, same lower-case letters within the column are not significantly different at 0.05 probability level.

Figure 2.1 Daily soil water deficit percent, cumulative water loss, precipitation, and irrigation applied from planting to harvest for irrigated feed barley in Aberdeen 2021 (a), Kimberly (b), Aberdeen 2022 (c), and Rexburg (d) Idaho for 2021 and 2022 planting

(a)



(b)

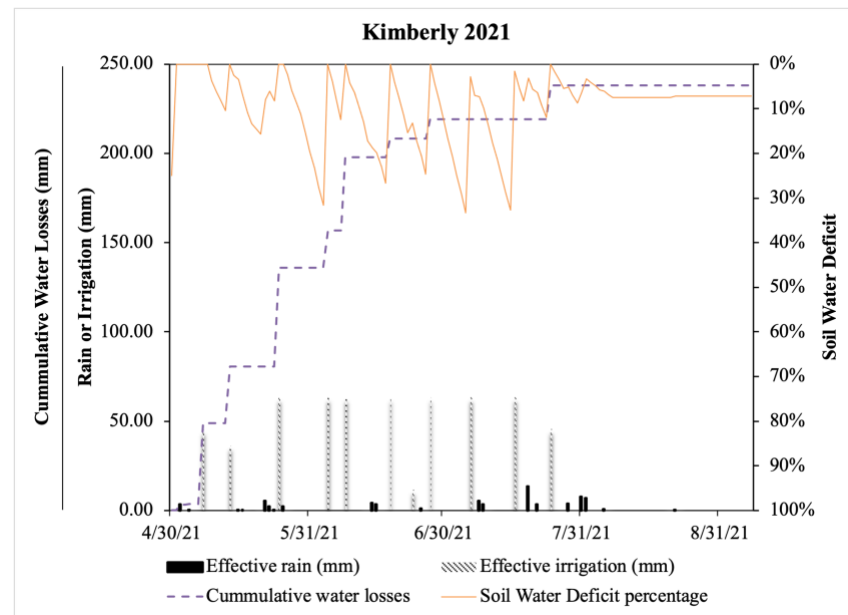
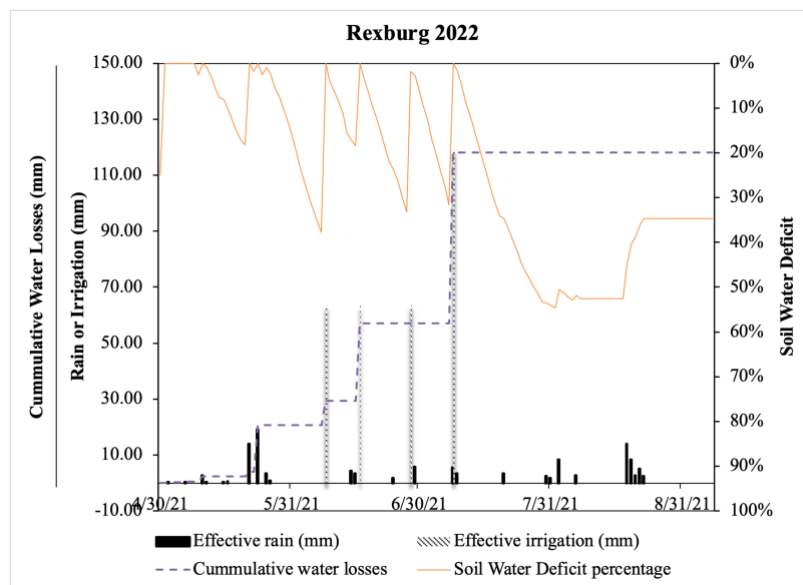
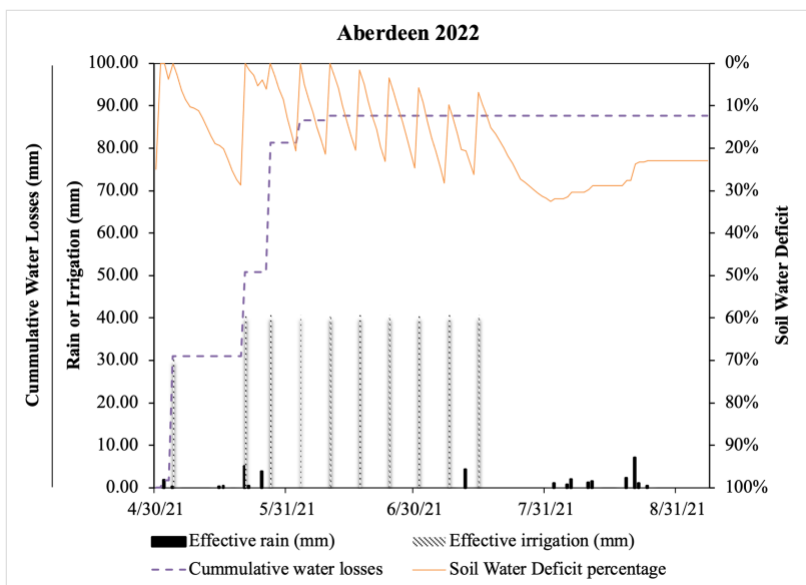


Figure 2.1 cont'd. Daily soil water deficit percent, cumulative water loss, precipitation, and irrigation applied from planting to harvest for irrigated feed barley in Aberdeen 2021 (a), Kimberly (b), Aberdeen 2022 (c), and Rexburg (d) Idaho for 2021 and 2022 planting season.

(c)

(d)



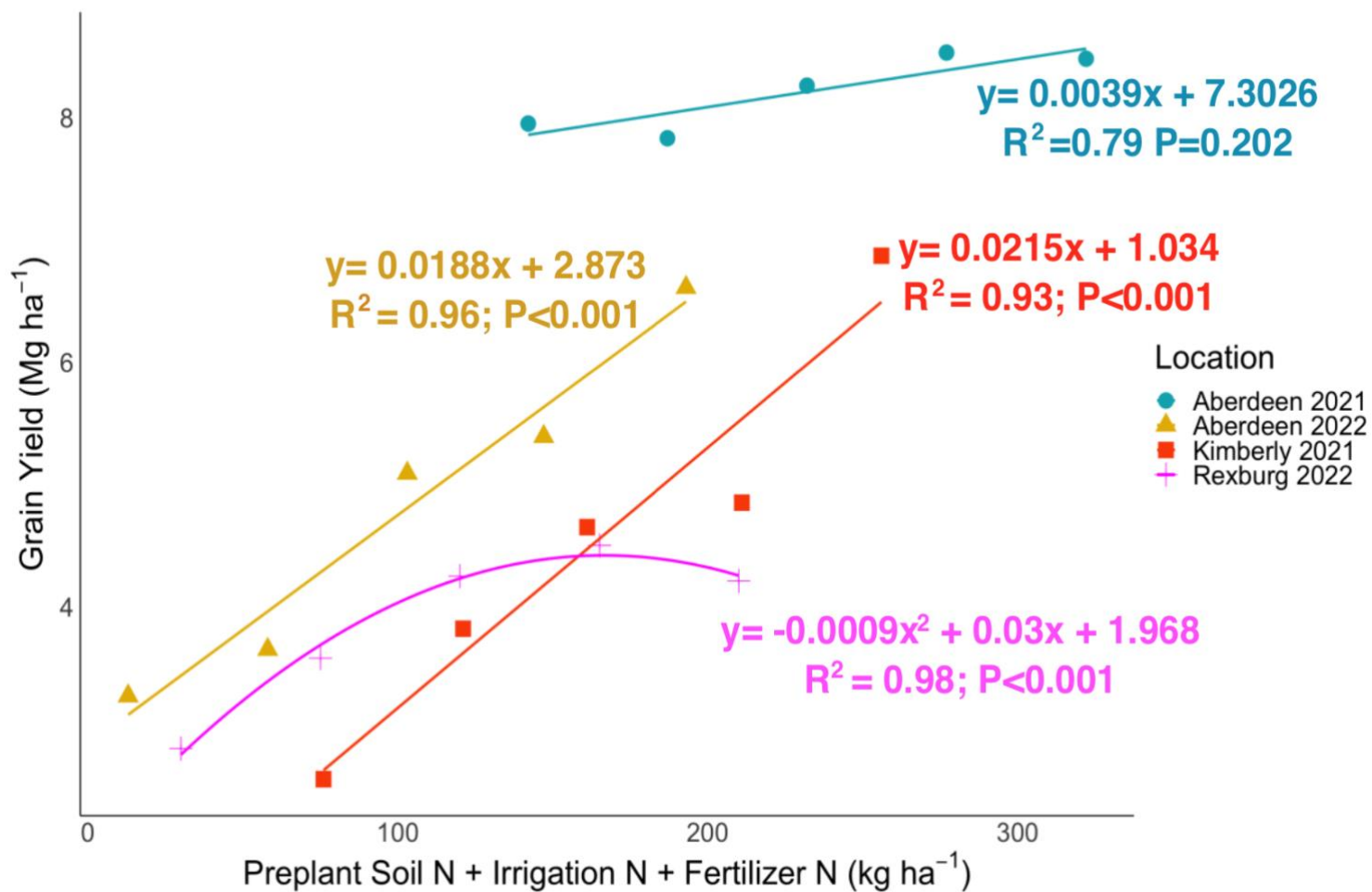


Figure 2.2 Regression analysis for feed barley grain yield means of single N application rates with response to total N (preplant N, irrigation N, and fertilizer N) in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

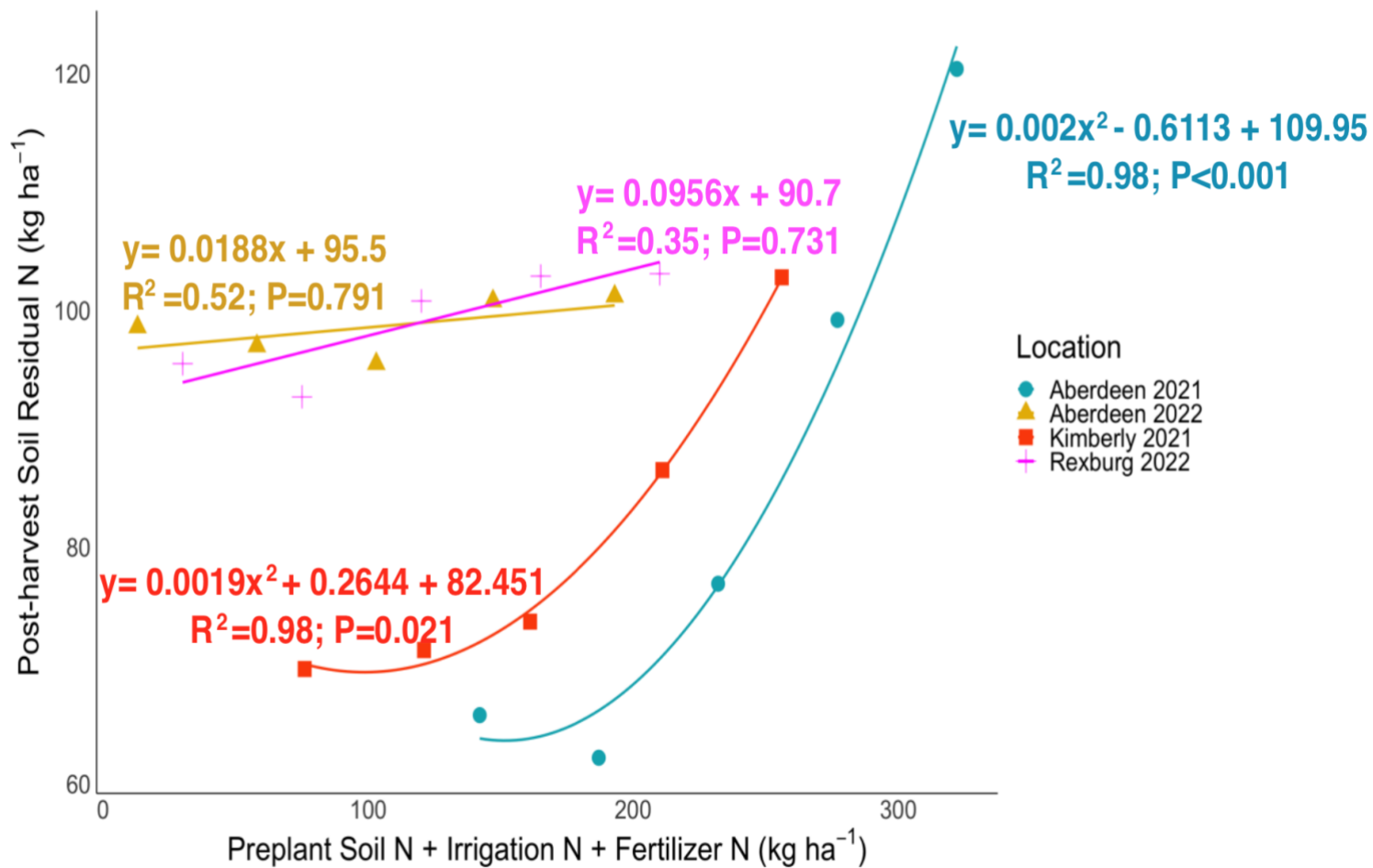


Figure 2.3 Regression analysis for feed barley post-harvest soil residual N means of single N rates with response to total N (preplant N, irrigation N, and fertilizer N) at 0 – 60 cm depth in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.



## **CHAPTER 3: MALT BARLEY YIELD, PROTEIN RESPONSE, AND MALT QUALITY PARAMETERS AS INFLUENCED BY NITROGEN AND SULFUR FERTILIZER RATES AND APPLICATION TIMING**

### **3.1. INTRODUCTION**

United States' barley production is primarily targeted towards the malting and brewing industries (Garstang et al., 2011). The brewing industry contributes more than 424,000 jobs and an estimated \$101.5 billion to the United States' economy (USDA NASS, 2022). Idaho accounts for 36% of total malt barley production in the United States, with grain yields approximately 45% higher than the national average (USDA NASS, 2022).

To ensure maltsters and brewers produce a uniform, marketable product, malt barley grain must meet a very modest and clearly defined set of malt barley quality traits (AMBA, 2021; Wilder, 2022). Barley grain parameters include grain protein concentration, malt extract, moisture content, germination rate, disease presence, test weight, kernels plumpness and uniformity, and adequate levels of amylase enzyme activity (AMBA, 2021; Burger & LaBerge, 1985) and malt parameters include malt extract, diastatic power (DP), and free amino N (FAN) (Rogers et al., 2022; D. Kumar et al., 2013). Malt barley growers often reduce their yield potential to avoid producing grain that falls outside these parameters resulting in the malt barley grain being rejected at the elevator and being sold instead for livestock feed at about half the original contracted price (Wilder, 2022). Because maltsters and brewers require malt with consistent brewing results and flavor profiles, malt barley cultivars tend to persist for many seasons unlike other grains like corn (*Zea mays* L) (AMBA, 2021).

Malt barley varieties are differentiated from feed and food barley varieties because they are better able to modify grain starch producing a more fermentable extract in the brewing process. Grain starch concentration is negatively correlated with grain protein concentration requiring maltsters to primarily only accept grain with a protein concentration between 10% and 13% (dry basis) (AMBA, 2021; Anheuser Busch, 2022; Brewers Association, 2022). Grain protein concentration is negatively correlated to starch, malt extract, nonuniform modification, and low water absorption resulting in a slow malting process and the

production of hazy beer (Lake et al., 2008). Protein concentrations <10% can hinder the development of malt color in the kiln, reduce FAN concentration, and result in insufficient enzyme activity leading to difficulties with starch conversion during brewing (Anheuser Busch, 2022; Bamforth, 2006; Chen et al., 2016). Like maltsters, brewers require malt with consistent quality characteristics to achieve an acceptable end-use product. When malt with heterogeneous properties is brewed, it results in hazy and off-flavored beer (Brophy, 2022).

Malt barley quality and production are significantly influenced by environmental conditions and agronomic management, with N rates and application timing having a noticeable impact (Izydorczyk & Edney, 2017). In previous studies conducted in western US, increased N fertility was positively correlated to yield (Castro et al., 2008; McKenzie et al., 2004; Sainju et al., 2013; Stevens et al., 2015), kernel size (Lazor, 2013; Stevens et al., 2015), and test weight (Castro et al., 2008), but negatively correlated to N use efficiency (Delogu et al., 1998; Sainju et al., 2013; Stevens et al., 2015) and malt quality in terms of higher protein (Castro et al., 2008; Edney et al., 2012; McKenzie et al., 2004) and decreased kernel plumpness (Sainju et al., 2013). O'Donovan et al., (2011) found that increasing N rate above 135 kg ha<sup>-1</sup> increased grain protein concentration beyond the protein specification and reduced kernel plumpness, demonstrating a direct effect of N rate on malting barley quality. Shrestha & Lindsey, (2019) also reported a negative correlation between N rate above 100 kg ha<sup>-1</sup> and malt quality parameters in Colorado and Arizona. Studies conducted by Edney et al., (2012) and O'Donovan et al., (2011) reported that increasing N rate above 120 kg ha<sup>-1</sup> resulted in inadequate water absorption during malting, decreased carbohydrate content, uneven kernel plumpness, and reduced malt extract. Studies in the Midwest reported that N applied at planting is susceptible to leaching due to low uptake and excessive precipitation (Alley et al., 2009; Jones & Jacobsen, 2005). Split N application (at planting and tillering) has been reported as an option to maximize malt barley yield while meeting malt quality specifications in Montana and North Dakota (Franzen & Goos, 2019; G. D. Jackson, 2008; Walsh & Christiaens, 2016). However, applying excess N levels close to flowering stage may increase grain protein concentrations beyond malt quality requirements (Walsh & Christiaens, 2016).

Sulfur (S) is a vital component of plant metabolism, and its deficiency has adverse effects on crop quality (Zhao et al. 1999). Sulfur exists in barley grains as S amino acids. Essentially, S performs a biochemical function in the stabilization of barley protein structures (Hawkesford & De Kok, 2006) and has been demonstrated to stabilize grain protein concentration in small grains such as wheat (Teboh et al., 2018). In a study conducted by Shewry et al., (1983), S was demonstrated to be directly correlated to malt barley protein concentration. Sulfur deficiency resulted in a decrease in S-rich B- and D-hordein fractions, which form the major component of gel protein, and may limit endosperm modification during malting. This suggests that S availability to malt barley may have an impact on the quality of the malting process (Shewry et al., 2001). Previous studies suggested that if best agronomic management practices, as well as N and S fertilizer interaction are employed in malt barley production, an increase in yield could be positively correlated to grain protein concentration and malt quality (Brophy, 2022; Legzdina et al., 2022; Perrott et al., 2018a; Thompson et al., 2018). Current University of Idaho guidelines for irrigated spring barley suggest that 22 to 45 kg sulfate-S ha<sup>-1</sup> should be applied if the sulfate-S concentration (0-60 cm) in the soil is < 10 mg kg<sup>-1</sup>. However, relatively little attention has been devoted to the impact of interaction of N and S fertilizer rates and application timing on malt quality.

Many studies have explored the effect of pre-plant and at-planting N fertilizer rates on the end of season metrics of barley, such as yield and protein concentration, but due to the significant cost and time requirements associated with malt quality analysis (Edney et al. 2012), previous barley agronomic management studies in the western US have rarely assessed malt quality characteristics other than protein concentration or kernel plumpness. Malt barley requirements for N and S can fluctuate significantly from year to year depending on climate and weather patterns, crop rotation, soil physical and chemical properties, and variety. Further, although barley variety turnover is slower than in other crops, new malt barley varieties have been released that may have different nutrient requirements and nutrient use efficiencies than historic varieties. Hence, the objectives of this study were to re-evaluate the optimal N and S fertilizer rates and application timings for irrigated malt barley on a) yield and grain protein concentration and b) malt extract and other malt quality parameters of malt barley.

## **3.2. MATERIALS AND METHODS**

### **3.2.1. Planting Material**

Moravian 179 is a two-row malt barley line developed by Molson Coors Beverage Company adapted to the high production requirements of southern Idaho, and is characterized by high percent plumps, low test weight, and average lodging potential (Marshall et al., 2022). The cultivar was released for adjunct brewing (AMBA, 2021). Moravian 179 is one of Idaho's most cultivated malt barley cultivars and represents 14% of total malt barley acreage in Idaho, behind ABI Voyager (37%) and CDC Copeland (16%) (AMBA, 2022). In variety trials conducted in southeastern and southcentral Idaho, Moravian 179 had a three-year average yield of over 9,500 kg ha<sup>-1</sup>, protein content of 10.7%, and plant height of 80 cm (Marshall et al., 2022).

### **3.2.2. Malt Quality Analysis**

In 2021, grain was assessed for malt extract, diastatic power, time to filter, free amino N, alpha-amylase, soluble/total protein ratio, and beta-glucan at two N fertilizer rates (0 and 90 kg ha<sup>-1</sup>) at Aberdeen and three N rates (0, 90, and 180 kg ha<sup>-1</sup>) at Kimberly. In addition to the parameters analyzed in 2021, 2022 malt quality analysis included barley color, wort color, wort clarity, adjunct quality score, and all malt quality score for all N rates. All analyses were conducted following the American Society of Brewing Chemists recommended procedures (ASBC, 1992).

Site description, soil sampling, plant tissue collection and sampling, experimental design, irrigation and water sampling, in season data collection, and harvest and post-harvest data and analysis were conducted similar to the methods described in chapter 2.

## **3.3. RESULTS**

### **3.3.1. Plant Height and Lodging**

Plant height responded significantly to N rates across all site years except at the Aberdeen 2021 field site (Table 3.1). No significant response was observed for S rates across field sites. Plant height increased with a pattern of increasing N at Kimberly, Rexburg, and Aberdeen

2022 field sites. The 180 kg N ha<sup>-1</sup> rate had the greatest plant height of 57 cm at Kimberly (Table 3.2). The 135 and 180 kg N ha<sup>-1</sup> rates at Aberdeen 2022 field site had similar heights with an average height of 67 cm (Table 3.3). The single N application produced greater plant height at Kimberly and Aberdeen 2022 than the split applications. However, at Rexburg, of the three single vs split N applications done at equivalent N rates, only the 135 kg N ha<sup>-1</sup> rate increased plant height over the equivalent 45/90 split application (Tables 3.2, 3.3). Generally, plant height averaged across N rates across all site years was 54 cm. Although the plant height average was lower than the 3-year average reported for Moravian 179 in southern and southeastern Idaho malt barley variety trials (Marshall et al., 2022), it was similar to the plant height reported for Moravian 69, CDC Copeland, and LCS Odyssey in Idaho, Oregon, Montana, and Wyoming under similar growing conditions (Killen & Frost, 2007; McVay, 2017; OSU Extension, 2022; Rogers et al., 2022). Lodging was defined as the percentage of the plot where the stems were at a 45° angles from vertical or contained broken straw or heads. Lodging was only observed at the Aberdeen 2021 field site (Table 3.2), and it responded significantly to the individual fixed effect of N rate (Table 3.1). Lodging increased with increasing N rate (Table 3.3). The lodging average (41%) across all N rates in this study was lower than the three-year lodging average for Moravian 69 (49%), CDC Copeland (59%), and ABI Voyager (44%) in a southern and southeastern Idaho malt barley variety trial (Marshall et al., 2022).

### **3.3.2. Number of Tillers and Heads**

The number of tillers and heads per plant increased with increasing N rate with a maximum of 5 to 7 tillers per plant at the highest N rates across all locations. Sulfur rates did not have an effect on the number of heads and tillers across all site years. Generally, there was no difference in the number of tillers produced between the single and split applications at equivalent N rates except at Kimberly 2021 when the 135 kg N ha<sup>-1</sup> single application produced 1 more tiller than a split N application.

### **3.3.3. Grain Yield**

Grain yield generally increased with N rate across all sites except Aberdeen 2021 which was non-responsive to N fertilizer and averaged 6.8 Mg ha<sup>-1</sup> across all treatments. There was no

response of grain yield to S rate and no interactions between N and S rates across all site years. Grain yield was minimized (1.4, 2.7, and 2.5 Mg ha<sup>-1</sup>) at the check plot at the Kimberly, Aberdeen 2022, and Rexburg field sites, respectively. Grain yield was similar at 135 and 180 kg N ha<sup>-1</sup> rates at Kimberly, Aberdeen 2022, and Rexburg 2022 field sites with average yields of 5.9, 5.2, and 4.5 Mg ha<sup>-1</sup>, respectively. There was no difference in yield due to the timing of fertilization for the 135 kg N ha<sup>-1</sup> rate at Aberdeen 2021 and the 90 and 135 kg N ha<sup>-1</sup> treatments at Rexburg 2022. The highest yield across all site years averaged 6.0 Mg ha<sup>-1</sup> and was similar to average yields reported for Moravian 69, ABI Voyager, CDC Copeland, and AAC Connect in the southeastern and southcentral Idaho malt barley variety trials (Marshall et al., 2022). Further, the yield in this study corresponds to AAC Synergy, AAC Connect, and Explorer yields in similar malt barley variety trials conducted in North Dakota, Montana, Washington, and Maine (Cash et al., 2004; Mallory & Molloy, 2020; Neely, 2023; Ransom et al., 2020).

#### **3.3.4. Grain Protein and Starch Concentration**

Grain protein concentration increased with N rates at the Aberdeen 2021 field site while the response at Kimberly, Aberdeen 2022, and Rexburg were non-patterned with average of 12.1, 11.1, and 11.2% at Kimberly, Aberdeen 2022, and Rexburg field sites, respectively. (Table 3.2). Split N applications raised grain protein concentration beyond levels of acceptability for malt barley at 90 kg N ha<sup>-1</sup> at Kimberly (13.6%) and Rexburg (13.4%) and at 135 kg N ha<sup>-1</sup> at Kimberly (14.4%) (Tables 3.2, 3.3). The grain protein concentration across all site years ranged between 10.9 – 12.5%, and it corresponds to the reported grain protein concentration for Moravian malt barley lines, CDC Copeland, and ABI Voyager under similar growing conditions in Montana, North Dakota, and southern and southeastern Idaho malt barley variety trials (Jackson & Miller, 2006; Marshall et al., 2022; Ransom et al., 2020). Except when N was split applied, grain protein concentration across all site years was within levels of acceptability for malt barley. Grain starch concentration was similar across all timing events across all site years, indicating that N application timing did not significantly impact grain starch concentration. The highest grain starch concentration was observed at the check plot at Aberdeen 2021 (56%) and 45 kg N ha<sup>-1</sup> at Kimberly field sites (Table 3.3). The average grain starch concentration from this study (54%) falls within the

previously reported grain starch concentrations for malt barley (Bamforth, 2006; Greenwood & Thomson, 1959), and may result in higher malt extract, except at split applied N rates when GPC was above malting quality standards.

### **3.3.5. Soluble and Insoluble Protein Concentration**

Soluble and insoluble protein concentration responses were non-patterned in response to N rate or timing across all site years, and no effect of S was recorded. The soluble protein concentration at all N single rates across all locations averaged 5.3%, indicating a solid basis for yeast nutrition and foam retention during malting (Sherman et al., 2021), enhancing malting quality. At Aberdeen and Kimberly, in the 2021 growing season, split-applied N significantly increased soluble protein concentration to above or near levels of the maximum acceptable soluble protein concentration recommended by the American Malting Barley Association (AMBA, 2023). Similarly, the increased insoluble protein concentration at Kimberly and Rexburg corresponded to the split N application events at a rate that would go into suspension during malting, resulting in diminished malt or beer quality (AMBA, 2023). Averaged across all site years, soluble protein concentration accounted for 50% of grain protein concentration average. In a similar study under similar growing conditions in North Dakota, Explorer and LCS Genie had similar soluble protein concentration values (Schwarz & Horsley, 2001).

### **3.3.6. Kernel Plumpness and Test Weight**

The recorded plumps ranged from 97 – 99%, and the test weight average was 63 kg hL<sup>-1</sup>. The average test weight result surpasses the minimum test weight for the top-grade malting quality barley (62 kg hL<sup>-1</sup>) (CMH, 2020). The plumps and test weight averages are similar to the averages reported for Moravian 69 and 179, AAC Synergy, and ABI Eagle in the malt barley variety trial in southern and southeastern Idaho (Marshall et al., 2022). Malt variety trials in Montana, Washington, and North Dakota also reported similar plumps and TW results for CDC Copeland and ABI Voyager (G. D. Jackson & Miller, 2006; Neely, 2023; Ransom et al., 2020).

### **3.3.7. Malt Quality Characteristics**

#### **3.3.7.1. Malt Extract**

Analysis of variance indicated that except at Aberdeen 2022 field site, the main effect of N rates affected malt extract (Tables 3.5, 3.6). The main effect of S rate affected malt extract at the Aberdeen 2021 field site, but no interaction of N and S rates was recorded (Tables 3.5, 3.6). The 180 kg N ha<sup>-1</sup> rate had 1 to 2% higher malt extract than the check plot at Aberdeen 2022 and Kimberly field sites (Tables 3.8, 3.9). Rates of 17 and 34 kg S ha<sup>-1</sup> were not statistically different. However, they produced 1% higher malt extract than 0 Kg S ha<sup>-1</sup> at the Aberdeen 2021 field site (Table 3.8). The malt extract across all site years, and N and S rates averaged 81.5% and correspond to the American Malting Barley Association standards of ≥80 (AMBA, 2023). Similar average malt extract was reported for AAC synergy, Klages, LCS Genie, and CDC Copeland varieties in North Dakota, Vermont, and Michigan (Li, 2019). In general, the malt extract consistency from this study is likely due to the consistent kernel plumpness and high TW, which was reported to improve malt barley extract (Bishop, 1930).

#### **3.3.7.2. Diastatic Power**

Diastatic power was minimized in the check plot at Aberdeen 2021 (119 °L) and Rexburg (105 °L). Average diastatic power (130 °L) across all N rates and site years (Tables 3.7, 3.8) corresponds to the 110-140 °L diastatic power quality recommendation (AMBA, 2023).

#### **3.3.7.3. Time to Filter 160 mL and Free Amino Nitrogen**

Time to filter 160 mL was recorded only in the 2021 growing season. Time to filter was maximized (73 min) at 90 kg N ha<sup>-1</sup> rate but minimized (42 min) in the check plot at the Kimberly field site, suggesting that an increased N rate may likely increase time to filter. Free amino N responded to N in an unpronounced pattern, and it averaged 243 mg L<sup>-1</sup> across all N rates and locations, corresponding to the malt barley quality standard (>210 mg L<sup>-1</sup>) (AMBA, 2023). However, when N was split-applied, free amino N was in excess of malt quality standards. Similar free amino N values were reported for Champion, CDC Copeland, AAC Synergy, and AC Metacalfe across the Western and Eastern US (USDA-ARS, 2018).



#### **3.3.7.4. Alpha-Amylase, Soluble to Total (S/T) Protein, and $\beta$ -glucan**

In the 2021 growing season, there was no significant response of alpha-amylase, ratio of soluble to total protein, and  $\beta$ -glucan concentration to the individual fixed effect of N rate at Aberdeen. Alpha amylase and ratio of soluble to total protein decreased with increasing N rate at Kimberly (Table 3.5). In the 2022 growing season, alpha-amylase and ratio of soluble to total protein had varying differences but non-patterned responses to N rate at Aberdeen and Rexburg, respectively with an average alpha-amylase of 88 DU and ratio of soluble to total protein of 54% across all N rates at both locations in 2022. Aberdeen 2021 and Rexburg 2022 field sites had numerically higher  $\beta$ -glucan concentrations (Tables 3.8, 3.9). This high  $\beta$ -glucan concentration was likely due to the elevated N rate (Güler, 2003) above crop needs at the Aberdeen 2021 field site and slightly droughty conditions during the grain-filling stage at the Rexburg field site. Previous studies also reported that high temperature and moisture stress might increase or decrease  $\beta$ -glucan concentration (Choi et al., 2020; Güler, 2003; Meints & Hayes, 2019; Wu et al., 2017). Alpha amylase, ratio of soluble to total protein, and  $\beta$ -glucan concentration across all site years and N rates correspond to the quality standards for malt (AMBA, 2023). Single and split-applied N had similar alpha-amylase, ratio of soluble to total protein, and  $\beta$ -glucan concentration across all site years. Check plot and highest N rate had similar values for alpha-amylase, ratio of soluble to total protein, and  $\beta$ -glucan concentration across all site years.

#### **3.3.7.5. Wort Color, Wort Clarity, and Quality Score**

The wort quality parameters and malt quality score were evaluated for the 2022 growing season. Wort clarity was graded on a scale of 1-3 (1= clear, 2= slightly hazy, 3= hazy) based on USDA-ARS (2022) specifications. Wort clarity was 1 (clear) across all site years, and wort color ranged between 1.8-2.5 (Table 3.10), suggesting the production of desired beer and spirit flavor (AMBA, 2023) and a potential ability for long-term storage (AHBA, 2020). Wort clarity and color results correspond to the US malt barley quality standards (AMBA, 2023). Similar wort clarity and color were reported for the other two-rowed malt barley varieties in western and eastern US malt barley quality reports (USDA-ARS, 2018).

At the Rexburg site, split-applied N rates had lower adjunct quality score compared to single N applications, resulting in a 2-10% decrease in adjunct quality score. Although not statistically different, single N application rates produced a 7-13% all-malt quality score advantage over split-applied N rates across both site years, concurrent with the adjunct quality score (Table 3.10). Although not in a specific order, adjunct quality score and all-malt quality score had some varying differences within N rates in 2022 with a recorded average of 54 and 31, respectively. This was likely due to the elevated grain protein concentration above malt quality standards at the Rexburg field site. Craine et al. (2023), in their study in eastern Washington and northern Idaho, reported similar adjunct quality score for CDC Copeland, LCS Genie, and LCS Odyssey. Further, these results correspond to the malt barley adjunct quality score and all-malt quality score standards (USDA-ARS, 2022) as well as values reported for other two-rowed malt barley varieties in the US (USDA-ARS, 2018).

### **3.3.8. Nitrogen Use Efficiency and Regression of Yield and Post-harvest Soil Residual N on Total Nitrogen**

Total N was calculated as the sum of pre-plant soil N, irrigation N credit, and fertilizer N rate. Kimberly, Aberdeen 2022, and Rexburg field sites responded linearly to total N rates and yielded 6.0 Mg ha<sup>-1</sup>, 5.6 Mg ha<sup>-1</sup>, and 4.5 Mg ha<sup>-1</sup>, respectively, at the highest total N rate (Figure 3.1). At Aberdeen 2021 field site, grain yield had a quadratic response, which showed that yield increased, on average, from around 6.3 Mg ha<sup>-1</sup> at 142 kg ha<sup>-1</sup> of total N and plateaued at about 7.0 Mg ha<sup>-1</sup> at 277 kg ha<sup>-1</sup> total N rate (Figure 3.1), beyond which further increases in total N were unlikely to have an impact on grain yield. These findings demonstrate that pre-plant soil N variations, including in-season irrigation water N content, can influence how fertilizer N rates affect yields. The linear response of grain yield to total N at Kimberly 2021 and Aberdeen and Rexburg 2022 field sites suggest significant N fertilizer loss (Figure 3.1), which may potentially increase the risk of nitrate leaching into groundwater (Lazicki & Geisseler, 2016). The possibility for N leaching was likely high at Kimberly and Aberdeen 2022 field sites because May to July was wetter than usual (Table 2.1), and agricultural water use was low.

According to findings by Anbessa & Juskiw (2012) and Delogu et al. (1998), and following general trends across all site years, agronomic efficiency decreased with increasing N rates at the expense of grain yield. At Aberdeen 2021 and 2022 field sites, single N fertilizer and split N applications showed comparable agronomic efficiency. At Kimberly, single N application rates of 90 and 135 kg N ha<sup>-1</sup> contributed an average of 57% agronomic efficiency benefit over split-applied N. The difference in agronomic efficiency between N application timing events indicates reduced N availability, poor N utilization, and increased N loss when N was split applied and is in line with the Idaho spring barley production guidelines (Robertson & Stark, 2003). The average crop recovery efficiency was lower at Aberdeen 2021 field site (0.3 kg kg<sup>-1</sup>) than at other sites where crop recovery efficiency averaged 0.6 kg kg<sup>-1</sup>. This is likely due to the total N at the location being greater than crop N demand, suggesting an increased aboveground N uptake when N is available in moderate amount. Like agronomic efficiency, partial factor productivity decreased with increasing N rates (Table 3.10). Single and split N application had similar partial factor productivity across all site years, although a statistical difference was found between N application timing events at Kimberly and the 90 kg N ha<sup>-1</sup> rate at Aberdeen 2021. The partial factor productivity was maximized across all site years at 45 kg N ha<sup>-1</sup> rate. The low N use efficiency in Rexburg was probably due to the moisture stress in July and August 2022 (Figure 2.1d) limiting N availability and uptake due to reduced N transport from the soil to the root surfaces (Bender et al., 2013). In similar studies reported by Robertson & Stark (2003) and Stark & Brown (1987), split N application right before the jointing stage may not be a practical strategy to increase N use efficiency in malt barley production due to differences in the response of N use efficiency indices to fertilizer N application timing in southern Idaho. To increase N use efficiency, it might be necessary to further modify N application timing and method, crop genetics, as well as decrease soil N loss and enhance malt barley N uptake.

In contrast to Rexburg and Aberdeen 2021 and 2022 field sites, which showed a quadratic response, Kimberly 2021 field site had a linear response of postharvest soil N to total N (Figure 3.2). Other research (Lazicki & Geisseler, 2016; Sullivan & Cogger, 2003) described similar reactions of end-of-season soil N to varied rates of N fertilizer as we had at Aberdeen and Kimberly 2021 field sites. Despite having low pre-plant soil N at these locations, Aberdeen and Rexburg 2022 field sites showed high residual postharvest soil N. Rexburg's

high levels of postharvest residual N may be due to moisture stress toward the end of the growing season, which reduced yield output and may also have decreased N loss. At the Aberdeen and Rexburg 2022 field locations, we also saw warmer-than-average soil temperatures earlier in the growing season, when there was better soil water availability (Table 2.1), which may have contributed to high N mineralization from soil organic matter. According to a study by Sullivan & Cogger (2003), postharvest soil sampling should be done immediately after harvest before plant residues enhance mineralization and residual N. However, our postharvest soil sampling in Aberdeen in 2022 was carried out about 45 days following harvest. The delayed sampling period, plant residue cover, and warmer-than-average temperatures in August and September (Table 2.1) may have influenced N mineralization by lowering surface temperatures and water evaporation, increasing postharvest residual soil N.

### 3.4. DISCUSSION

#### 3.4.1. Nitrogen Effects

A sufficient amount of N is necessary to increase malt barley yields and yield components (Franzen & Goos, 2019; Robertson & Stark, 2003). Plant height increased with a pattern of increasing N rate across all site years except at Aberdeen 2021. Similarly, the number of tillers and heads increased with increasing N rate. Like the number of tillers, the 0 kg N ha<sup>-1</sup> rate produced the least number of heads across all site years. Concurrent with the pattern of increasing plant height and number of tillers and heads to increasing N rate, grain yield increased at Kimberly, Aberdeen 2022, and Rexburg field sites. This indicates that N fertilizer enhanced malt barley vegetative growth on the grain yield. The difference in the number of tillers and heads between the check plot and the highest N rate applied, as well as the increase in yield and yield components with increasing N rate indicate the importance of N availability for barley vegetative growth and development and yield output. This corresponds to findings by [Dofing & Knight \(1992\)](#), [Hunduma \(2020\)](#), and [Nerson \(1980\)](#) who reported that increased tiller number and plant height increased grain yield in wheat and malt barley. Similarly, the findings from Kimberly, Aberdeen 2022, and Rexburg field sites correspond to previous studies conducted in the western US and Canada under similar

growing conditions where malt barley grain yield increased with N fertility (Castro et al., 2008; McKenzie et al., 2004; Sainju et al., 2013; Stevens et al., 2015). Lodging at the Aberdeen 2021 field site increased with increasing N rate due to excess N above crop needs, which is consistent with previous research by Franzen & Goos (2019), Lazicki & Geisseler (2016), Sullivan & Cogger (2003), and Zubriski et al. (1970) who reported an increase in wheat and barley lodging as an adverse effect of excess N. The elevated lodging rate at this field site may likely increase disease pressure and induce the germination process (Berry et al., 2004; Edney et al., 2012; O'Donovan et al., 2011), representing a significant quality and economic loss from crop rejection, based on malt quality specifications.

Several studies have reported an effect of N on malt barley grain quality in terms of kernel plumpness, starch concentration, test weight, and grain protein concentration (Conry, 1994; Therrien et al., 1994; Sørensen and Truelsen, 1985). They reported that increased N application may enhance the overall quality of the grain, although excess N and application timing may reduce kernel plumpness and increase the grain protein concentration above malt quality requirements. In this study, the grain protein concentration at the Aberdeen 2021 field site responded linearly with increased N rate, which is consistent with previous studies by Conry (1994) and Sørensen & Truelsen (1985). Although the pre-plant N was in excess at the Aberdeen 2021 field site, grain protein concentration was within the malting industries' protein quality standard (10-13%) and N application timing did not significantly affect the grain protein concentration at this location. Although there was a significant effect of N rate, the grain protein concentration at Kimberly and Rexburg did not respond to N rate in a pronounced pattern (Tables 3.2, 3.3). The grain starch concentration, test weight, and percent plump kernels were within acceptable levels for malting quality. At these rates, there will be an increased malt extract because the grain has a greater proportion of starch-rich endosperm and less bran and hull. However, the increased grain protein concentration and soluble protein concentration when N was split-applied will make it more difficult for water and enzymes to interact with the starch. Thus, it leads to brewing issues such as too much color development during wort boiling, water absorption and filtration issues, and risk of haze formation.

Malt extract represents the degree of enzymatic breakdown and the solubility of grain components after mashing and grinding (Swanston et al., 2014). Diastatic power describes the enzymatic power of malt to break down starches into simpler fermentable sugars during the mashing process (Charmier et al., 2021). Time to filter 160 mL represents the time to filter out the sediment-free wort from grain sediments after mashing. Free amino N typically indicates the amount of free amino groups available to yeast during fermentation. Nitrogen rates significantly affected many of the malt quality parameters across all site years. The effects of the malt quality parameters within N treatments, however, did not follow any specific pattern (Tables 3.7, 3.8, 3.9). Wort color and clarity,  $\beta$ -glucan, free amino N, alpha-amylase, and S/T protein ration were within AMBA recommendations and when affected by the main effect of N rate, they did not follow any specific pattern. Despite their varying response to N fertilizer application, the malt quality parameters in this study were within acceptable levels for malting except in some instance of too high protein content above quality specifications.

### **3.4.2. Sulfur Effects**

Sulfur fertilizer did not affect the agronomic response of malt barley in this study. Previous studies conducted in dryland production system under S deficient soil conditions have reported an effect of S fertilizer application on malt barley yield and quality parameters (Prystupa et al., 2019; Zhao et al., 2006). The non-responsiveness of agronomic parameters to S in this study are similar to what was observed in studies conducted under similar growing conditions with this study, where agronomic and malt quality parameters were not affected by S fertilizer rate due to using elemental S source (Dari et al., 2019) and high residual soil S at the time of planting (Jackson, 2008; McKenzie et al., 2005). These results show that no additional S should be applied to improve the agronomic yield response of malt barley in this region because the irrigation S supply is adequate to meet the malt barley crop demand. This finding supports recommendations for Idaho barley production, where additional S fertilizer treatments are not advised to maximize yield on field sites irrigated with Snake River water (Robertson & Stark, 2003). Although we did not see a positive or negative response of agronomic parameters to S rates across all site locations, fertilizer S application may have stabilized the grain protein concentration by controlling the gene

expression of prolamin storage proteins (Halford & Shewry, 2007; Shewry et al., 2001), contributing to the stability of grain protein concentration across all site years to within levels of industry protein quality standards. Interestingly, there were some effects of S on malt extract ( $P=0.045$ ) at Aberdeen 2021, free amino N ( $P=0.031$ ) at Kimberly and adjunct quality score ( $P=0.022$ ) at the Aberdeen 2022 field sites despite the high S level of the irrigation water. Sulfur fertilizer increased malt extract by 1%, indicating that S addition increased starch-water-enzyme interaction, thereby enhancing starch conversion to sugar in the mashing process (Sherman et al., 2021). In a study under moderate S deficiency conditions in the Mediterranean climate of Argentina, Prystupa et al. (2019) reported that S fertilizer application increased malt extract. A previous study by Dari et al. (2019) in Idaho did not report a significant effect of elemental S on malt quality characteristics. As opposed to the elemental sulfur source employed in the prior study by Dari et al. (2019), this study utilized a  $SO_4$ -S source, which is immediately available for plant use. The variation indicates that under various conditions where irrigation water S content is low or moderate,  $SO_4$ -S fertilizer may be a promising source to consider to enhance malt barley quality.

### **3.4.3. Nitrogen Application Timing**

Split N application produced similar or significantly lower yield and yield components across all site years. At equivalent total N rates, a single N application produced a 6-46% yield advantage over split N application across all site years. These findings contradict the expected cereals (e.g., corn, *Zea mays* L.) and winter seeded small grains yield response in areas of the US Midwest where the growing season is longer and growing conditions are different than this region. In a previous corn production study conducted in Iowa on a soil with  $>7.5$  pH, Kyveryga et al. (2004) reported that single N application at planting increased loss of anhydrous ammonia to nitrification. Similarly, (Randall & Mulla, 2001) in their study on the effect of application timing on corn yield in Minnesota, reported that single N application produced lower yields than split N application. Alley et al. (2009), in their study on winter barley production in the US Northeast, also concluded that due to the longer growing season, a split N application is necessary to enhance tiller formation when winter barley re-initiates growth in February or March. In southern Idaho, spring barley uses more N during the early growth phases and grows quickly from planting through tillering, indicating

a reduced possibility and risk for N loss. Hence, the preference of single- over split-applied N in this region. The split application event in this study occurred after early tillering in July, hence, the reduced yield from split-applying N relative to single N application was likely as a result of insufficient N availability at the crucial growth stages at tillering due to low initial N application. As previously reported by Al-Kanani et al. (1991) and Holcomb et al., (2011) in their studies under irrigated conditions in Montana and Oregon where irrigation events may have increased surface moisture during split application, enhancing volatilization losses and reducing N availability for plant uptake. Previous studies under irrigated conditions in Montana and Oregon similarly reported reduced N uptake due to high volatilization losses. The difference in yield relative to N application timing may also have been as a result of insufficient N availability at the crucial growth stages due to the delayed split application after early tillering in July. Further, these findings correspond to guidelines for regions with irregular rainfall patterns and medium-textured loam and silt loam soils, where split N applications are not recommended because split N application contributes more to protein concentration than grain yield (Franzen & Goos, 2019; Robertson & Stark, 2003).

The split-applied N rates at Kimberly and split-applied 45/90 kg N ha<sup>-1</sup> rate at Rexburg had high grain protein concentration concentrations above malt quality standards (Tables 3.2, 3.3). This is consistent with previous studies conducted by [Hills & Paynter \(2009\)](#) under medium to high rainfall areas in Western Australia and [Taalab et al. \(2015\)](#) on a sandy loam soil in Egypt. Similarly, Curry (2019) and Westcott et al. (1998) reported that split N application after tillering stage resulted in a 0.6 - 2.0% increase in barley and wheat grain protein concentration. These findings corroborate the suggestions by Franzen & Goos (2019) and Robertson & Stark (2003) in the spring barley production guidelines for North Dakota and Idaho production systems that N application after tillering stage enhance N translocation to the grain and contributes more to grain protein concentration than yield. The downside of excess grain protein concentration under split-applied N rates is that the grains are rejected for malting purposes and sold at about half the price as animal feed ([Rogers et al., 2022](#); [Wilder, 2022](#)), representing an economic loss for the growers. Soluble and insoluble protein concentrations are important considerations for malt barley. Soluble protein concentration influence beer foam, mouthfeel, beer color and flavor, and yeast metabolism (Koller & Perkins, 2022) and should be available at rates commensurate to malting quality



specifications of 4.8-6.0% (Sherman et al., 2021). Unless extracted, insoluble protein concentration at high levels >7.0% may go into suspension during malting and lead to hazy beer production (Wang & Ye, 2021). Similar to grain protein response, soluble protein concentration at split-applied 45/90 kg N ha<sup>-1</sup> rates at Kimberly and Aberdeen 2022 field sites, and insoluble protein concentration at both split N rates at Kimberly and 135 kg ha<sup>-1</sup> at Rexburg were above AMBA specifications (Table 3.10) (AMBA, 2023). Free amino N was high at both sites in 2021 and when N was split-applied, free amino N was higher than required for malt, favoring utilization by micro-organisms to produce undesirable flavors (White, 2023).

Split N application reduced malt extract by 1-2% at the Rexburg field site and at split applied 90 kg ha<sup>-1</sup> at Aberdeen 2022 field site, likely due to the elevated grain protein concentration at this location (Table 3.4), which was above malt quality standards. Previous studies reported reduced malt extract due to high grain protein concentration (Bishop, 1930; Eagles et al., 1995) and split N application (Chen et al., 2006). Generally, single N application contributed approximately 2.5% malt extract advantage over split N application across all locations. Similarly split-applied N significantly reduced adjunct quality score, which is the criteria used to quantify the quality of malt barley grains, by 2-10% compared to single N application. According to this study, diastatic power increased in relation to application timing because of the strong correlation to protein concentration and N application after the tillering stage is transported to the grain for protein synthesis. According to previous studies by Arends et al. (1995), Dahiya et al. (2019), and Singh et al. (2013), who reported that high grain protein concentration is typically associated with low malt extract and DP, the corresponding reduction in malt extract, soluble and insoluble protein concentration increase above AMBA quality specifications in this study further shows the potential economic loss that is associated with split N application for spring malt barley production in this region.

### 3.5. CONCLUSIONS

The data on malt barley indicates that grain yield increased linearly in response to N rates except when pre-plant soil N exceeds crop needs. Nitrogen rates and application timing significantly affected malt barley agronomic and malt quality parameters. As observed in this

study, single N application is an economically viable option over split N application for spring malt barley production in southern Idaho. Split N application reduced grain yield and malt extract and increased grain protein concentration beyond malting standards, representing a potential economic loss as grains will be sold at lower prices as feed. These findings indicate that a large portion of the split-applied N is transferred into the grain for protein synthesis, especially when applied after the tillering stage. Unlike single N applications, split-applied N significantly reduced N use efficiency, indicating a less-productive system when N was split-applied. Temperature and drought substantially impact the potential production and grain quality of malt barley, as evidenced by moisture stress and the subsequently decreased yield at one of the site years. While there is undoubtedly still much to learn about the effect of S on malt barley production in the PNW, this study demonstrates that for conditions under which this study was conducted, additional S fertilizer is not required to optimize malt barley yield or improve grain quality or malting quality parameters.

Table 3.1 Test of fixed effects for malt barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test Weight (TW) in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, and Brigham Young University-Idaho in Rexburg, Idaho, USA.

Source of variation	Test of Fixed Effects								
	-----P>F value-----								
	PH	Tillers	Heads	Lodging	GY	GPC	GSC	Plumps	TW
<b>Aberdeen 2021</b>									
Nitrogen rate (N)	0.572	0.742	0.728	<0.001	0.065	<0.001	0.008	0.401	0.129
Sulfur rate (S)	0.418	0.684	0.462	0.167	0.435	0.425	0.137	0.409	0.935
N x S	0.909	0.641	0.074	0.351	0.594	0.482	0.889	0.194	0.169
<b>Kimberly 2021</b>									
Nitrogen rate (N)	<0.001	<0.001	<0.001	NA	<0.001	<0.001	0.002	<0.001	0.046
Sulfur rate (S)	0.439	0.723	0.871	NA	0.825	0.516	0.217	0.275	0.616
N x S	0.653	0.954	0.545	NA	0.715	0.596	0.374	0.006	0.042
<b>Aberdeen 2022</b>									
Nitrogen rate (N)	<0.001	0.087	<0.001	NA	<0.001	0.073	0.159	0.029	0.157
Sulfur rate (S)	0.123	0.819	0.613	NA	0.846	0.978	0.368	0.904	0.019
N x S	0.749	0.919	0.769	NA	0.807	0.686	0.964	0.394	0.035
<b>Rexburg 2022</b>									
Nitrogen rate (N)	<0.001	<0.001	0.002	NA	<0.001	<0.001	0.289	<0.001	0.001
Sulfur rate (S)	0.229	0.067	0.718	NA	0.115	0.35	0.331	0.895	0.489
N x S	0.692	0.905	0.308	NA	0.447	0.441	0.449	0.665	0.832

NA denotes not applicable

Table 3.2 Treatment means for malt barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, Idaho, USA.

	PH cm	Tillers ----- # -----	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC ----- % -----	GSC	Plumps	TW kg hL <sup>-1</sup>
<b>Aberdeen 2021</b>									
N rate (kg N ha <sup>-1</sup> )									
0	58 <sup>b</sup>	5	105	16d	6.3	10e	56.0a	98.9	65
45	62	5	119	38bc	6.7	10.8d	55.4bc	99.0	64
45/23	63	5	113	32cd	6.7	11.2cd	55.5bc	99.1	65
45/45	61	5	118	17cd	6.5	11.3bc	55.3bc	99.3	64
90	64	4	128	57ab	7.2	11.3bc	55.5bc	99.0	65
45/90	62	5	122	23cd	6.8	11.9a	55.1c	99.1	65
135	63	4	104	76a	7.2	11.7ab	55.3bc	98.9	65
180	62	5	122	72a	6.9	11.9a	55.3bc	98.7	65
SE	2.2	0.4	11.8	10.3	0.2	0.3	0.3	0.2	0.3
<b>Kimberly 2021</b>									
N rate (kg N ha <sup>-1</sup> )									
0	37d	3e	51d	NA	1.4d	12.9c	53.5d	96.6d	62ab
45	45c	4d	90c	NA	2.9c	11.2de	54.6a	97.5c	61bc
45/23	45c	4d	93c	NA	2.9c	12.7c	54.0bc	97.6bc	63a
45/45	45c	5bc	93c	NA	3.0c	13.6b	54.1b	97.8bc	62ab
90	52b	5bc	122b	NA	4.4b	10.9e	54.0bc	98.0bc	62ab
45/90	44c	5bc	89c	NA	3.1c	14.4a	54.0bc	97.6bc	62ab
135	53b	6a	135ab	NA	5.7a	11.5d	53.7bcd	98.7a	63a
180	57a	6a	149a	NA	6.0a	12.3c	53.6cd	98.3ab	63a
SE	1.2	0.4	6.8	NA	0.2	0.2	0.2	0.3	0.4

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level. NA denotes not applicable.

Table 3.3 Treatment means for malt barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2022 growing season at the University of Idaho Aberdeen Research and Extension Center and Brigham Young University-Idaho in Rexburg, Idaho, USA.

	PH cm	Tillers ----- # -----	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC ----- % -----	GSC -----	Plumps -----	TW kg hL <sup>-1</sup>
<b>Aberdeen 2022</b>									
N rate (kg N ha <sup>-1</sup> )									
0	51e <sup>b</sup>	3	33d	NA	2.7d	11.1	54.4	98.3abc	67
45	55de	4	43cd	NA	3.6c	10.7	54.6	98.5ab	68
45/23	60cd	5	53bc	NA	4.3bc	11.0	54.3	98.4ab	65
45/45	57d	5	62ab	NA	4.7b	11.5	54.0	98.1bc	66
90	62bc	4	55bc	NA	4.4bc	10.1	54.8	98.2abc	66
45/90	57d	6	70a	NA	5.1ab	11.8	53.7	97.7c	70
135	65ab	5	62ab	NA	5.0ab	11.2	54.1	98.6ab	64
180	68a	5	60ab	NA	5.6a	11.0	54.2	98.7a	66
SE	1.9	0.6	5.2	NA	0.5	0.4	0.3	0.2	1.9
<b>Rexburg 2022</b>									
N rate (kg N ha <sup>-1</sup> )									
0	43e	6d	47d	NA	2.5d	9.3c	53.7	97.3e	65b
45	45de	7abc	56bcd	NA	3.3c	10.0c	53.2	97.9cd	65b
45/23	49bcd	7abc	51cd	NA	3.4c	11.5b	53.1	98.5ab	67a
45/45	51abc	7abc	65ab	NA	3.9bc	11.5b	48.1	98.7a	67a
90	50abcd	7abc	65ab	NA	4.0bc	10.6bc	52.7	98.1bc	66ab
45/90	47cde	7abc	67ab	NA	3.6c	13.4a	52.1	98.5ab	66ab
135	52ab	8a	66ab	NA	4.4ab	11.7b	52.4	97.6de	66ab
180	54a	7abc	76a	NA	4.6a	11.7b	52.4	97.9cd	66ab
SE	1.8	0.2	5	NA	0.3	0.5	1.6	0.2	0.5

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level. NA denotes not applicable.

Table 3.4 Test of fixed effects for malt extract, diastatic power (DP), time to filter 160 mL, free amino nitrogen (FAN), alpha amylase (AA), ratio of soluble/total (S/T) protein, and beta ( $\beta$ )- glucan in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, Idaho, USA.

Source of variation	Test of Fixed Effects						
	-----P>F value-----						
	Extract	DP	Time to filter 160 mL	FAN	AA	S/T	$\beta$ -glucan
<b>Aberdeen 2021</b>							
Nitrogen rate (N)	0.041	0.008	0.398	0.221	0.181	0.557	0.399
Sulfur rate (S)	0.045	0.662	0.058	0.978	0.364	0.921	0.797
N x S	0.609	0.365	0.381	0.887	0.432	0.526	0.861
<b>Kimberly 2021</b>							
Nitrogen rate (N)	<0.001	<0.001	0.009	<0.001	<0.001	0.002	0.133
Sulfur rate (S)	0.166	0.301	0.763	0.031	0.495	0.046	0.064
N x S	0.127	0.148	0.981	0.023	0.051	0.332	0.175

Table 3.5 Test of fixed effects for malt extract, diastatic power (DP), barley color, free amino nitrogen (FAN), alpha amylase (AA), ratio of soluble/total (S/T) protein, and beta ( $\beta$ )- glucan in the 2022 growing season at the University of Idaho Aberdeen Research and Extension center and Brigham Young University-Idaho in Rexburg, Idaho, USA, Idaho, USA.

Source of variation	Test of Fixed Effects						
	-----P>F value-----						
	Extract	DP	Barley color	FAN	AA	S/T	$\beta$ -glucan
<b>Aberdeen 2022</b>							
Nitrogen rate (N)	0.016	0.700	0.026	0.033	<0.001	0.547	0.585
Sulfur rate (S)	0.589	0.734	0.549	0.931	0.857	0.711	0.452
N x S	0.879	0.613	0.796	0.504	0.942	0.825	0.684
<b>Rexburg 2022</b>							
Nitrogen rate (N)	<0.001	<0.001	<0.001	<0.001	0.175	<0.001	0.422
Sulfur rate (S)	0.405	0.751	0.266	0.399	0.158	0.190	0.819

Table 3.5 cont'd

N x S	0.086	0.103	0.299	0.064	0.096	0.152	0.185
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Table 3.6 Test of fixed effects for wort color, wort clarity, adjunct quality score (AQS), adjunct overall rank (AOR), all malt quality score (AMQS), and all malt overall rank (AMOR) in the 2022 growing season at the University of Idaho Aberdeen Research and Extension center and Brigham Young University-Idaho in Rexburg, Idaho, USA, Idaho, USA.

Source of variation	Test of Fixed Effects			
	-----P>F value-----			
	Wort color	Wort clarity	AQS	AMQS
<b>Aberdeen 2022</b>				
Nitrogen rate (N)	0.802	0.571	0.123	0.025
Sulfur rate (S)	0.896	0.138	0.022	0.734
N x S	0.816	0.502	0.453	0.503
<b>Rexburg 2022</b>				
Nitrogen rate (N)	0.096	0.667	0.022	<0.001
Sulfur rate (S)	0.108	0.129	0.135	0.899
N x S	0.188	0.446	0.226	0.621

Table 3.7 Treatment means for malt extract, diastatic power (DP), time to filter 160 mL, free amino nitrogen (FAN), alpha amylase (AA), ratio of soluble/total (S/T) protein, and beta ( $\beta$ )-glucan with standard errors (SE) with response to N and S fertilizer rates in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, Idaho, USA.

	Extract %	DP °L	Time to filter 160 mL min	FAN mg L <sup>-1</sup>	AA DU	S/T %	$\beta$ -glucan mg L <sup>-1</sup>
<b>Aberdeen 2021</b>							
N rate (kg ha <sup>-1</sup> )							
0	82a <sup>b</sup>	119b	72	219	57	44	86
90	81b	129a	68	224	61	43	93
SE	0.2	6.1	9.5	7.2	6.7	1.0	7.6
SO4-S rate (kg S ha <sup>-1</sup> )							
0	81b	124	69	221	58	43	90
17	82a	122	62	221	61	44	86
34	82a	125	78	222	57	44	92
SE	0.2	6.3	9.8	7.5	6.8	1.2	8.5
<b>Kimberly 2021</b>							
N rate (kg ha <sup>-1</sup> )							
0	78b	168a	42b	316a	113a	49a	31
90	80a	136c	73a	271b	97b	49a	39
180	80a	153b	52b	267b	96b	46b	37
SE	0.3	4.1	6.5	3.4	2.6	0.7	2.5
SO4-S rate (kg S ha <sup>-1</sup> )							
0	79	157	52	292a	104	49a	32
17	80	150	59	283ab	101	47b	34
34	79	150	56	279b	101	48ab	41
SE	0.3	4.1	6.5	3.4	2.6	0.7	2.5

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level.



Table 3.8 Treatment means for malt extract, diastatic power (DP), barley color, free amino nitrogen (FAN), alpha amylase (AA), ratio of soluble/total (S/T) protein, and beta ( $\beta$ )-glucan with standard errors (SE) with response to N and S fertilizer rates in the 2022 growing season at the University of Idaho Aberdeen Research and Extension center and Brigham Young University-Idaho in Rexburg, Idaho, USA, Idaho, USA.

	Extract %	DP °L	Barley color Agtron	FAN mg L <sup>-1</sup>	AA DU	S/T %	$\beta$ -glucan mg L <sup>-1</sup>
N rate (kg ha <sup>-1</sup> )	<b>Aberdeen 2022</b>						
0	81b <sup>b</sup>	122	56a	281ab	86ab	56	37
45	81b	116	55a	266bc	88ab	56	44
45/23	81b	120	55a	272bc	87ab	55	41
45/45	81b	124	55a	275abc	85b	54	37
90	82a	113	55a	260c	88ab	57	39
45/90	81b	115	52b	290a	79c	54	29
135	81b	127	54ab	276abc	88ab	54	39
180	82a	121	55a	268bc	89a	57	29
SE	0.3	6.5	1.0	7.6	2.0	1.7	6.6
N rate (kg ha <sup>-1</sup> )	<b>Rexburg 2022</b>						
0	82a	105f	76a	238d	87	55a	90
45	82a	113ef	75ab	241d	90	57a	69
45/23	82a	134cd	73cd	263b	90	52b	82
45/45	81b	142bc	71d	279a	89	51b	84
90	82a	123de	74bc	249cd	88	52b	66
45/90	80c	156a	69e	281a	89	46c	72
135	82a	131d	73c	257bc	86	52b	70
180	82a	145b	72cd	259bc	88	51b	77
SE	0.5	4.8	0.8	6.3	2.1	1.5	10.8

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level.

Table 3.9 Treatment means for wort color, wort clarity, adjunct quality score (AQS), and all malt quality score (AMQS) with standard errors (SE) with response to N and S fertilizer rates in the 2022 growing season at the University of Idaho Aberdeen Research and Extension center and Brigham Young University-Idaho in Rexburg, Idaho, USA, Idaho, USA.

	Wort color	Wort clarity	AQS	AMQS
<b>Aberdeen 2022</b>				
N rate (kg ha <sup>-1</sup> )				
0	2.5 <sup>b</sup>	1	51	29b
45	2.4	1	51	32ab
45/23	2.3	1	51	35a
45/45	2.4	1	51	31ab
90	2.4	1	52	35a
45/90	2.5	1	50	28b
135	2.4	1	53	30ab
180	2.3	1	55	35a
SE	0.2	0.1	1.3	2
SO <sub>4</sub> -S rate (kg S ha <sup>-1</sup> )				
0	2.4	1	53a	31
17	2.4	1	51b	33
34	2.4	1	52ab	32
SE	0.1	0.1	0.8	1.5
<b>Rexburg 2022</b>				
N rate (kg ha <sup>-1</sup> )				
0	2	1	48c	34ab
45	2	1	53ab	37a
45/23	2	1	53ab	28cd
45/45	2	1	50bc	27cd
90	1.8	1	54a	31bc
45/90	2	1	47c	25d
135	2	1	52ab	27cd

Table 3.9 cont'd

180	1.8	1	52ab	27cd
SE	0.1	0.1	2.1	2.3

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level.

Table 3.10 Treatment means for malt barley soluble protein concentration (SPC), insoluble protein concentration (ISPC), agronomic efficiency (AE), crop recovery efficiency (CRE), and partial factor productivity (PFP) with standard errors (SE) with response to N fertilizer rates in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

N rate (kg ha <sup>-1</sup> )	SPC	ISPC	AE <sup>a</sup>	CRE	PFP	SPC	ISPC	AE	CRE	PFP
	----- % -----	----- % -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- % -----	----- % -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----
	<b>Aberdeen 2021</b>					<b>Kimberly 2021</b>				
0	6.2a <sup>b</sup>	3.8c	-	-	-	3.1d	9.8a	-	-	-
45	5.2abc	5.6b	5.2	0.2	148.3a	3.8cd	7.4cd	29.7ab	0.6.ab	65.4a
45/23	5.5ab	5.7b	4.2	0.1	102.8b	3.8cd	8.9ab	19.7cd	0.7ab	43.3bc
45/45	6.0a	5.3b	0.6	0.1	72.1d	4.2bc	9.4ab	15.7de	0.8ab	33.6d
90	4.2c	7.1a	9.4	0.5	80.9c	5.0b	6.0e	31.1a	0.8ab	48.9b
45/90	5.5ab	6.5ab	2.3	0.6	51.8e	6.3a	8.1bc	11.5e	0.5b	23.4e
135	4.3c	7.4a	5.5	0.1	53.2e	5.2ab	6.3de	30.8a	0.9a	42.7c
180	4.7bc	7.2a	2.6	0.3	38.3f	5.2ab	7.1cde	24.6bc	0.8ab	33.5d
SE	0.4	0.6	2.2	0.2	2.5	0.4	0.5	2.6	0.2	2.6
<i>Pr(&gt;F)</i>	0.004	<0.001	0.101	0.239	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	<b>Aberdeen 2022</b>					<b>Rexburg 2022</b>				
0	5.9	5.1	-	-	-	4.4b	5.0de	-	-	-
45	5.8	5	23.1	0.5	77.5a	5.4a	4.6e	21.4a	0.7ab	73.9a
45/23	5.9	5.2	25.1	0.5	63.9b	5.6a	6.0c	17.7ab	0.8ab	49.6b
45/45	6	5.5	23.5	0.8	52.9bc	5.9a	6.7b	14.9abc	1.1a	43.6b
90	5.6	4.5	19.7	0.6	49.1cd	5.2a	5.4cd	17.7ab	0.7ab	44.0b
45/90	6.3	5.5	18.4	0.6	38.0de	5.8a	7.7a	9.2c	0.8ab	26.7c
135	5.7	5.5	17.3	0.7	36.9de	5.9a	5.9c	15.3abc	0.4b	32.8c
180	6.0	4.8	16.6	0.5	31.2e	5.6a	6.1bc	12.7bc	0.5b	25.8c
SE	0.2	0.3	6.1	0.2	6.8	0.2	0.4	3.2	0.2	3.2

Table 3.10 cont'd

<i>Pr(&gt;F)</i>	0.223	0.112	0.06	0.092	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
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<sup>a</sup> The difference in yield for malt barley N uptake between the treatment of interest and 0N is divided by the applied N rate to determine AE and CRE.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level.

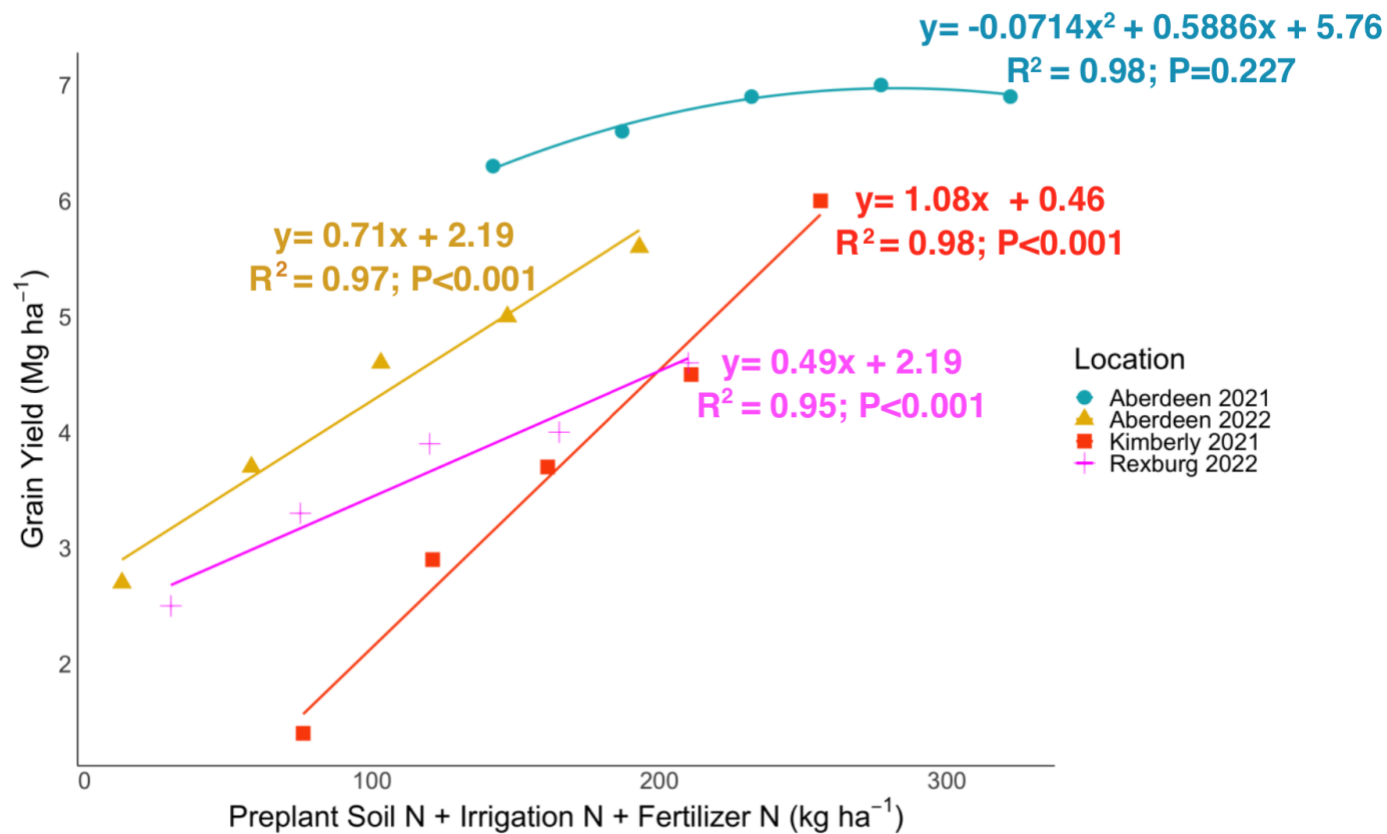


Figure 3.1 Regression analysis for the means of malt barley grain yield at single N rates to total N (preplant N, irrigation N, and fertilizer N) in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

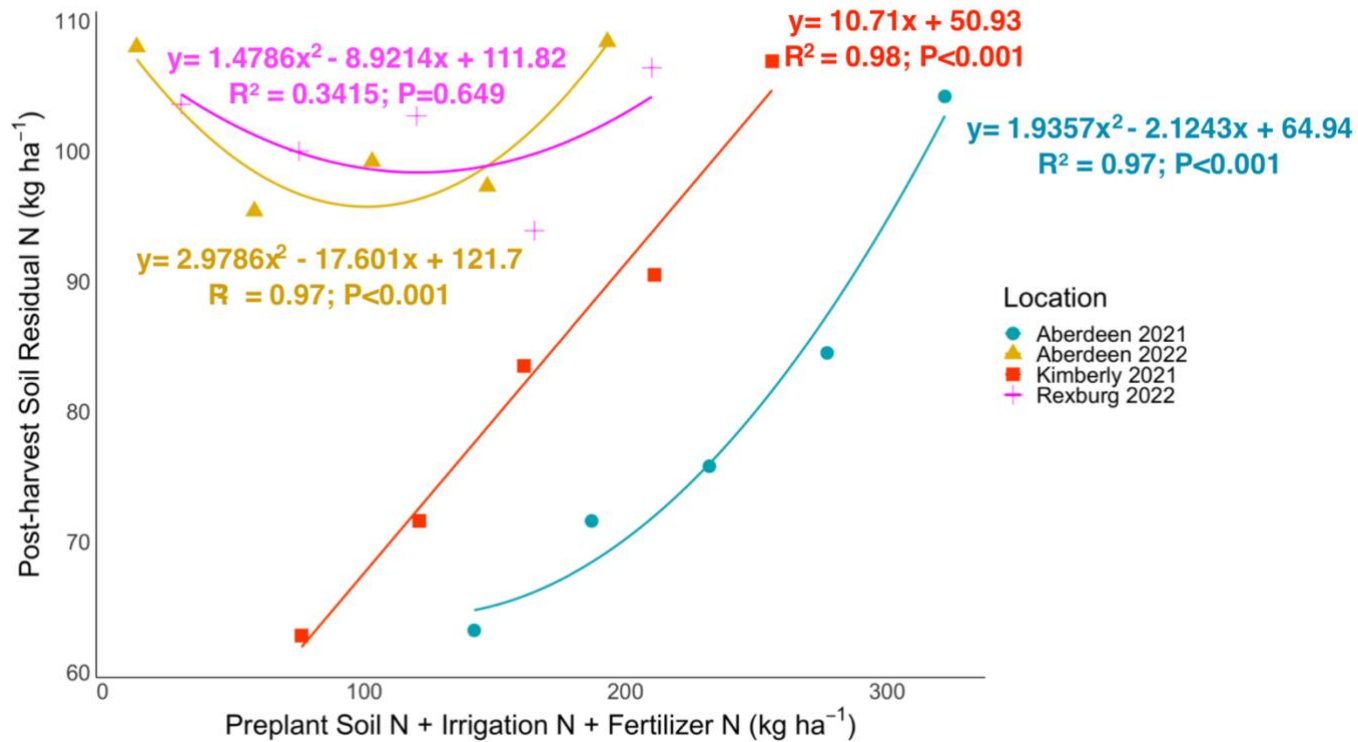


Figure 3.2 Regression analysis for the means of malt barley postharvest soil residual N at single N rates to total N (sum of preplant N, irrigation N, and fertilizer N) at 0-60 cm depth in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

## **CHAPTER 4: YIELD AND QUALITY RESPONSE OF HULLESS FOOD BARLEY TO NITROGEN AND SULFUR RATES AND APPLICATION TIMING**

### **4.1. INTRODUCTION**

Food barley can be classified as hulled or hulless by presence or absence of a hull tightly adhering to the grain. Hulled barley contains kernels with adhering hulls (Taketa et al., 2008) which undergoes an abrasion process to remove the hull and bran (Darby et al., 2020) while in contrast, hulless barley contains kernels with non-adhering hulls that are eliminated easily during threshing (Xue et al., 1997), producing whole grain barley for human consumption (Darby et al., 2020). Hulled barley used for food must be pearled, which eliminates the hull and a sizable percentage of the pericarp and bran, where phytonutrients and minerals are primarily concentrated (Bleidere et al., 2017; Grando & Macpherson, 2005; Moreau et al., 2007). Over the years, hulled barley has gained more prominence than hulless barley because of its high yielding characteristics and the possibility for use for malting purposes (Darby et al., 2020). Previous studies reported that hulless barley yields 10% - 30% lower than hulled barley (Choo et al., 2001; Liu & Harder, 1996). However, hulless barley is preferable to hulled barley for use as food because it has better flavor and nutritional content (Darby et al., 2020) and increased digestible energy (Griffey et al., 2010; Ingledew et al., 1995) due to its high starch content (57 – 75% of grain on dry matter basis) (Bhatty, 1999; Griffey et al., 2010; Liu & Harder, 1996). Further, hulless barley production reduces input cost as it saves producers the expense of pearling (Meints & Hayes, 2019). In the context of this study, food barley is hulless barley.

Barley has long been a dependable source of human food (Newman & Newman, 2006), especially in some regions of North Africa, and Central Asia (Grando & Macpherson, 2005). Food barley is typically grown in arid areas where other cereals do not thrive because of insufficient precipitation (<300 mm), high elevations, or saline soils (Grando & Macpherson, 2005). Further, given barley's relatively short growing season and its use as a substitute for wheat and other cereals when cereals market prices are too high, food barley also acts as a relief crop during times of food scarcity, and thus, plays an important role in food security in many parts of the world (Grando & Macpherson, 2005; Mohammed et al., 2016; Zhou, 2009b). Food barley has high  $\beta$ -glucan concentration (>7%) (Baik & Ullrich, 2008;



Thorwarth et al., 2017), and its consumption has been linked to reduced blood plasma cholesterol, enhanced lipid metabolism, and low glycemic index (Behall et al., 2004, 2005, 2006; Delaney et al., 2003; Garcia-Mazcorro et al., 2018; Keenan et al., 2007; Li et al., 2003). Whole grain barley and products containing barley were permitted to claim that they lower the risk of coronary heart disease by the US Food and Drug Administration (FDA) in 2005 (Food and Drug Administration, 2006). Barley grain is also a source of both soluble and insoluble protein, as well as other bioactive ingredients like vitamin E, B-complex vitamins, minerals, and phenolic compounds (Madhujith et al., 2006; Slavin et al., 2000). Further, the existence of components in barley believed to prevent or treat certain ailments such as coronary heart diseases is another factor driving greater interest in it as a dietary crop (Arndt, 2006; Baidoo et al., 2019; De Angelis et al., 2015; Habiyaremye et al., 2021; Madhujith et al., 2006; Slavin et al., 2000).

Due to the nutritional and health benefits of food barley, it is predicted that there will be an increased application of barley as food (Newton et al., 2011b). The PNW is a high-yielding barley production region with a reputation for establishing dietary and nutritional trends (Meints et al., 2015). Hence, the PNW is a good region for improving the food barley market. Fertilizer N is an important input with potentially detrimental environmental effects if lost to the environment. Existing management practices for N, S, and other nutrients and agronomic practices for barley production in Idaho, Montana, North Dakota, and Washington provide information on malt and feed barley, but not food barley (Franzen & Goos, 2019; McVay, 2017; Robertson & Stark, 2003; Turner et al., 2000). Hence, food barley growers use fertilizer recommendations based on the available feed and malt barley (McVay, 2017). Given the distinct genotypic and phenotypic characteristics of the malt, feed, and food barley varieties, it is expected that each barley class will respond differently to varying levels of available N, S, and other agronomic management practices. Studies in Montana reported that food barley is an effective cholesterol-lowering food (MSU Extension, 2021). Food barley  $\beta$ -glucan concentration is correlated to genotypic properties and agronomic and environmental factors (Güler, 2003). Habiyaremye (2019) and Güler (2003) in a study conducted in Washington and Idaho, reported a positive correlation between  $\beta$ -glucan and N rate. A few studies in the PNW have explored benefits of hullless barley and agronomic strategies to

optimize hulless barley production effect of N fertilizer on hulless food barley (Habiyaemye, 2019; Meints et al., 2015). However, little to no research has been conducted to report the effects N and S fertilizer rates and application timing on hulless food barley to achieve optimum yields and quality in the PNW. For better fertility management practices for hulless food barley growers, it is essential to have a better understanding of N and S fertilizer requirements. Hence, the objectives of this study were to (a) evaluate grain yield and quality response of spring two-rowed hulless food barley to varying N and S fertilizer rates (b) assess the effect of application timing on the yield and quality response of hulless food barley.

## **4.2. MATERIALS AND METHODS**

### **4.2.1. Planting Material**

Julie is a two-rowed hulless barley line released by the USDA-ARS, Aberdeen, Idaho and the University of Idaho Agricultural and Experimental Station in 2010. Julie was developed from the cross of 10, Azhul, and CDC Alamo (10/‘Azhul’//‘CDC Alamo’) (Obert et al., 2011) and is characterized by high  $\beta$ -glucan, high test weight, protein, and Fusarium Head Blight resistance (Marshall et al., 2022; Obert et al., 2011). In variety trials conducted in southeastern and southcentral Idaho over a three years period, Julie had an average yield of 6,900 kg ha<sup>-1</sup>, protein content of 13.5%, and seed  $\beta$ -glucan content of 7% (Marshall et al., 2022).

### **4.2.2. $\beta$ -glucan Concentration**

$\beta$ -Glucan concentration analysis was performed with a commercially available  $\beta$ -Glucan Mixed Linkage K-BGLU kit (Megazyme Ltd., Ireland) which utilizes the American Association of Cereal Chemists (AACC) approved method 32–23 or the McCleary method (McCleary & Codd, 1991). Prior to  $\beta$ -Glucan concentration analysis, 5.0 g of barley grains was milled through a 0.55 mm screen using a laboratory cyclone mill (Udy Corporation, Fort Collins, CO, USA). 80.0 mg of the ground barley flour was mixed with 200  $\mu$ L 50% ethanol and 4.0 mL sodium phosphate (Na<sub>3</sub>PO<sub>4</sub>) buffer. The suspension was then incubated for 1 hour with 50 $\mu$ L lichenase enzyme, and the enzymatic reaction was halted with 5.0 mL of

sodium acetate buffer. 1.0 mL of each reaction suspension was transferred into a 1.2 mL cluster tube in a 96-well format (G. Hu & Burton, 2008) using a pipet and centrifuged. The suspension was incubated for an additional 15 to 30 minutes after which 10.0  $\mu\text{L}$  of each test well was transferred into a second assay plate containing 150  $\mu\text{L}$  of glucose oxidase peroxidase developing reagent (GOPOD). Absorbance readings were determined at 510 nm using a plate reader and the formula from the original McCleary approach was used to calculate the  $\beta$ -Glucan concentration.

Site description, soil sampling, plant tissue collection and sampling, experimental design, irrigation and water sampling, in season data collection, and harvest and post-harvest data and analysis were conducted similar to the methods described in chapter 2.

## **4.3. RESULTS**

### **4.3.1. Plant Height and Lodging**

Lodging had a significant response to the main effect of N rate at the Aberdeen 2021 field site, while there was no lodging at other field sites (Table 4.1). The plant height recorded at the check plot was significantly lower than the plant height recorded at the highest N rate of 180 kg N ha<sup>-1</sup> across all site years except at Aberdeen 2021 field site where plant height was not responsive to N (Tables 4.2, 4.3). Like plant height, lodging increased with a pattern of increasing N rate (Table 4.2). Plant height did not differ between N timing events except at Kimberly 2021 field site, where a single N application produced significantly taller plants. The plant height from this study was lower than the plant height results for Julie, Goldenhart, and Transit in the southern and southeastern Idaho food barley variety trials (Marshall et al., 2022). Habiyaemye et al. (2021), in their study conducted in Washington and northern Idaho, also reported greater plant heights for Julie and Havener. There was not a significant S or N by S rate interaction effect on plant height (Table 4.1).

### **4.3.2. Tillers and Heads**

Like plant height, number of heads and tillers also increased with increasing N rate across all site years except at Aberdeen 2021 field site. Single and split-applied N applications produced a similar number of tillers and heads across all site years. The Aberdeen 2021 field

site demonstrated a statistically significant response of number of heads to S rates (Table 4.1). Rates of 0 and 17 kg S ha<sup>-1</sup> did not differ statistically but produced a numerically greater number of heads (112 and 109) than 34 kg S ha<sup>-1</sup> (96) (Table 4.2).

#### **4.3.3. Grain Yield**

Grain yield was maximized at 180 kg N ha<sup>-1</sup> rate at Kimberly (3.8 Mg ha<sup>-1</sup>), while 135 and 180 kg N ha<sup>-1</sup> rates at Aberdeen 2022 had similar grain yield with an average yield of 3.3 Mg ha<sup>-1</sup>. Grain yield at Aberdeen 2021 and Rexburg field sites was similar at 45 to 180 kg N ha<sup>-1</sup> rates. Single and split-applied N rates had similar grain yield across all site years. Grain yield across all site years and N rates in this study are lower than the average grain yield reported for Julie, Transit, Goldenhart, and Havener at optimal N rates in the southern and southeastern Idaho trials (Marshall et al., 2022), as well as in a study conducted in Washington and northern Idaho (Habiyaemye et al., 2021). Sulfur rates did not affect grain yield across all site years.

#### **4.3.4. Grain Protein Concentration**

Grain protein concentration increased with increasing N rates at Aberdeen 2021 field site but differed in no specific pattern within N rates across other field sites (Tables 4.2, 4.3). Grain protein concentration was maximized at Kimberly and Rexburg at a split-applied rate of 45/90 kg N ha<sup>-1</sup> (23.6 and 19.7%) while 135 and 180 kg N ha<sup>-1</sup> rates at Aberdeen 2021 and 2022 field sites were similar with average grain protein concentration of 17.9 and 15.3%, respectively. Split-applied N rates of 45/45 and 45/90 kg N ha<sup>-1</sup> at Kimberly and Rexburg and 135 kg N ha<sup>-1</sup> at Aberdeen 2022 field sites had 0.5-5% higher grain protein concentration values than a single N application. Although not statistically different, 17 and 34 kg S ha<sup>-1</sup> rates produced statistically significant grain protein concentration compared to 0 kg S ha<sup>-1</sup>. The grain protein concentration result from this study is higher than Julie's grain protein concentration in a 3-year study in southern and southeastern Idaho (Marshall et al., 2022) and the Palouse region of the PNW (Habiyaemye et al., 2021).

#### 4.3.5. Soluble and Insoluble Protein Concentration

Soluble and insoluble protein concentration responded significantly to N rates across all site years except at the Aberdeen 2021 field site, where the N rate did not affect insoluble protein concentration (Table 4.7). Soluble protein concentration and insoluble protein concentration differed within N levels but not in a pronounced pattern. Previous studies found that soluble protein concentration and insoluble protein concentration accounted for 15-30% and 70-85% of total grain protein concentration, respectively (Baxter, 1981; Howard et al., 1996; Qi et al., 2006). Fox & Fastnaught. (2022) also reported an 8-10% range for both soluble protein concentration and insoluble protein concentration in hulless food barley grain. However, across site years and N levels, soluble protein concentration from this study averaged 6.6%, accounting for 40% of total grain protein concentration, while the insoluble protein concentration average was 9.9%, accounting for 60% of total grain protein concentration.

#### 4.3.6. $\beta$ -glucan concentration

Nitrogen rate did not affect  $\beta$ -glucan concentration across all site years (Table 4.1). Rexburg field site had the lowest  $\beta$ -glucan concentration averaged across all site years (Tables 4.2, 4.3). Single and split N timing events produced similar  $\beta$ -glucan concentrations across all site years. Averaged across site years and N rates,  $\beta$ -glucan concentration was 6.7%, similar to mean  $\beta$ -glucan concentration of 6.6% for Havener food barley reported by Habiyaemye et al. (2021) but lower than the mean  $\beta$ -glucan concentration reported for Julie (8.2%) in the same study.

#### 4.3.7. Plumps and Test Weight

Generally, percent plumps ranged between 90-98% across all site years (Tables 4.2, 4.3). Sulfur did not affect percent plumps across all site years (Table 4.1). The average of percent plumps at the Rexburg field site (91%) was lower compared to Aberdeen 2021 (98%), Kimberly (97%), and Aberdeen 2022 (95%). Plumps did not differ with N application timing events across all site years. Test weight was significantly affected by N rate at Kimberly but not across other site years (Table 4.1). Although not statistically significant, the check plot had a numerically lower test weight (72 kg hL<sup>-1</sup>) than the 180 kg N ha<sup>-1</sup> rate (75 kg hL<sup>-1</sup>)

(Table 4.3). Single and split-applied N produced similar test weight, and S rate was insignificant across all site years (Table 4.1, 4.2, 4.3). Similar test weight values were reported for Julie, Transit, and Goldenhart in the southern and southeastern Idaho trials (Marshall et al., 2022). Habiyaremye et al. (2021), in a study conducted in the Palouse region of the PNW, also reported similar test weight results for Julie and Havener.

#### **4.3.8. Nitrogen Use Efficiency and Regression of Yield and Post-harvest Soil Residual N on Total Nitrogen**

Total N available to the developing crop was considered as pre-plant soil N (0-60 cm), irrigation N credit, and the applied fertilizer N rate. Kimberly 2021 and Aberdeen and Rexburg 2022 field sites had a linear response of grain yield to total N (Figure 4.1). The lowest grain yield across each site was recorded at the lowest total N rate, while the highest grain yield (3.8, 3.5, 2.4 Mg ha<sup>-1</sup>) was recorded at the highest total N rate. Aberdeen 2021 field site had a quadratic grain yield response to total N (Figure 4.1). An increase in grain yield from the lowest total N at Aberdeen was followed by a plateau at a total N range of 187-277 kg ha<sup>-1</sup>, followed by a grain yield decline starting from a total N rate of 277 kg ha<sup>-1</sup>. The highest grain yield produced at the highest total N rate at Kimberly (256 kg N ha<sup>-1</sup>) and Rexburg (210 kg N ha<sup>-1</sup>) corresponds to the grain yield plateau response at Aberdeen 2021 field site. The linear grain yield responses at Kimberly 2021 and Aberdeen and Rexburg 2022 field sites may indicate substantial N fertilizer loss (Figure 4.1), potentially resulting in nitrate leaching into groundwater (Lazicki & Geisseler, 2016). The quadratic yield response at Aberdeen 2021 field site was likely due to total N supplied more than crop needs (Lazicki & Geisseler, 2016; Sullivan & Cogger, 2003).

All site years had a quadratic response of postharvest residual soil N to total N except the Rexburg field site (Figure 4.2). The elevated levels of postharvest soil residual N at Aberdeen and Rexburg 2022 field sites likely stemmed from the increased N mineralization, as reported in Chapter 2.

Agronomic efficiency decreased with increasing N rate at Aberdeen 2021 field site. The 45 kg N ha<sup>-1</sup> rate, the lowest N rate considered for N use efficiency, had the highest agronomic efficiency across all site years except at Kimberly, where agronomic efficiency was highest

at a single-applied rate of 90 kg N ha<sup>-1</sup>. Partial factor productivity decreased with increasing N rates across all site years (Table 4.7). Partial factor productivity was minimized at a rate of 180 kg N ha<sup>-1</sup> at Aberdeen 2021 (29.6 kg kg<sup>-1</sup>) and Rexburg (13.5 kg kg<sup>-1</sup>) field sites while minimized at a split-applied rate of 45/90 kg N ha<sup>-1</sup> at Kimberly (15.8 kg kg<sup>-1</sup>) and single application rate of 135 kg N ha<sup>-1</sup> at Aberdeen 2022 (18.3 kg kg<sup>-1</sup>) field sites. When comparing N application timing events, a single N application had significantly higher agronomic efficiency and partial factor productivity at Kimberly 2021 field site, contributing 7.5-9.0 kg kg<sup>-1</sup> agronomic efficiency and partial factor productivity over split N application. These findings indicate that a single N application provided a 21-32% and 37-45% increase in partial factor productivity and agronomic efficiency, respectively, further indicating that a single N application increased total economic output relative to nutrient uptake and utilization (Cassman et al., 1996; Yadav, 2003).

## 4.4. DISCUSSION

### 4.4.1. Nitrogen Effects

Plant height, number of tillers and heads,  $\beta$ -glucan concentration, and test weight were typically not responsive ( $P \geq 0.05$ ) at the Aberdeen 2021 field site. At the Rexburg 2022 field site, grain yield,  $\beta$ -glucan concentration, test weight, and percent plumps were not responsive ( $P \geq 0.05$ ) to N application. The non-responsiveness of some of the dependent variables to N application was likely due to high pre-plant soil N at the Aberdeen 2021 field site and moisture stress resulting from the loss of irrigation water source at the Rexburg 2022 field site. In contrast to N rate response at Aberdeen 2021 and Rexburg 2022 field sites, all the dependent variables were responsive to N rate except  $\beta$ -glucan ( $P \geq 0.05$ ) at Kimberly and  $\beta$ -glucan and test weight ( $P \geq 0.05$ ) at Aberdeen 2022 field sites.

Nitrogen typically increases yield by enhancing barley's ability to produce effective tillers and increase plant height, number of heads, and grains per spike (Ahmad et al., 1986). However, at N rates above crop needs, there is an increased risk of lodging (Zuber et al., 1999). Like plant height, number of tillers, and number of heads, grain yield increased with a pattern of increasing N rate at Kimberly, Aberdeen 2022, and Rexburg field sites. Further, the highest N rate of 180 kg ha<sup>-1</sup> produced numerically greater yield metrics than the check plot, indicating the importance of N availability for food barley vegetative growth and development and a potential for increased yield. Similarly, the highest N rate of 180 kg ha<sup>-1</sup> produced statistically greater yield than the check plot except at Aberdeen 2021 field site. Similarly, lodging at Aberdeen 2021 field site increased similarly with an increasing N rate. This is in line with Ahmad et al. (1986), Habiyaemye (2019), Habiyaemye et al. (2021), Roth et al. (1987), Tehulie (2021), and Tehulie & Eskezia (2021) who reported a significant response of lodging, yield output, and yield metrics to different levels of N. They reported that N fertilizer application enhances food barley vegetative growth, although excessive use may result in lodging and yield loss. A single N application produced similar or greater yield and yield metrics than the split N application. These findings contrast with what can be observed for corn, barley, and other small grains production in the US Midwest, Northeast, California, and eastern Oregon (Alley et al., 2009; Baethgen & Alley, 1989; D. M. Sullivan



et al., 1999), where growing season tends to be longer. For example, Clark et al. (2020), in a corn production study conducted across eight Midwestern states, reported that a single N application at planting led to a higher potential for leaching losses due to high rainfall events and low N uptake during the early growth stages. Similarly, Alley et al. (2009), in a winter barley production study in the US Northeast, reported that due to the longer growing season, a split N application is necessary to enhance tiller formation when winter barley re-initiates growth in February or March. However, spring barley, the prevalent barley type in this region, utilizes more N at early growth stages and develops rapidly from planting through tillering, suggesting a lower potential for N loss. Hence, the observed difference in yield output and yield metrics relative to N application timing in this study corroborates the existing guidelines for spring barley production in this region, where a single N application is recommended to optimize yield (Robertson & Stark, 2003). Compared to other site years, the average grain yield ( $2.1 \text{ Mg ha}^{-1}$ ) and percent plump kernels (91%) at the Rexburg field site were the lowest, likely due to moisture stress during the grain filling period. These findings are similar to previous reports by Bello et al. (2022), Samarah (2005), and Samarah et al. (2009), who reported that moisture stress during the grain filling period reduced grain yield by reducing the number of tillers, spikes, and grains per plant. The grain yield and percent plump kernels at the Rexburg field sites indicate that, in addition to N, environmental conditions have an impact on the yield and grain quality of barley (Conry, 1994b; Zhang et al., 2001).

Nitrogen fertilizer is essential to enhance barley grain quality. High protein and  $\beta$ -glucan concentration, plump kernels, and high test weight are components of hulless food barley quality (Choi et al., 2020). Given its health and nutritional benefits,  $\beta$ -glucan is an important fiber in food barley (Darby et al., 2020; Griffey et al., 2010). High grain protein concentration is necessary to enhance human growth and development, and test weight and kernel plumpness represent increased kernel nutrient density (Edney, 2010). Soluble protein controls blood glucose and cholesterol levels, while insoluble protein aids digestion and relieves constipation (Food and Drug Administration, 2006). The findings by Conry (1994) and Sørensen & Truelsen (1985), who reported that grain protein concentration increased with increasing N application, corroborate the grain protein concentration response at

Aberdeen 2021 field site but contrast response at Kimberly, Aberdeen 2022, and Rexburg field sites where grain protein concentration responded to N rate in no specific pattern. Single and split N applications typically had similar percent plumps and test weights except at Kimberly, where single N had significantly higher percent plump kernels (Table 4.2). The grain protein concentration, insoluble protein concentration, and soluble protein concentration generally had higher values at split-applied N than single N applications. Like previous authors in the western US who reported that split N application after tillering increased grain protein concentration (Franzen & Goos, 2019; Jackson & Miller, 2006; Robertson & Stark, 2003), split N applications for this study were done as the crop transitioned to the reproductive development stage favoring the translocation of N away from vegetative tissues and into the grain. The effect of N fertilizer application on  $\beta$ -glucan has been inconsistent. For example, some studies reported an increase in  $\beta$ -glucan concentration with increasing N fertilizer rates (Henry, 1986; Oscarsson et al., 1998; Sørensen & Truelsen, 1985), while some studies claimed that environmental factors and genotype have a more significant effect on  $\beta$ -glucan concentration than N (Aastrup, 1979; Güler, 2003; Hesselman et al., 1981; Zhang et al., 2001). The  $\beta$ -glucan concentration in our study was not responsive to N rate, but when averaged across N rates at each location, the Rexburg field site had a 1.5% lower  $\beta$ -glucan concentration than other site years. This was likely due to moisture stress stemming from the loss of the irrigation water source. This is in line with reports by Rakszegi et al. (2014), Wu et al. (2017), and Ye & Zhang (2020) who reported that moisture stress, heat, and temperature after anthesis and during the grain filling period reduced  $\beta$ -glucan concentration. Generally, N rates did not have an adverse effect on the quality of hulless spring food barley in this study.

#### **4.4.2. Sulfur effects**

The field sites used for this study and other field sites on the Snake River plain where irrigation water is obtained directly or indirectly from the Snake River, have available S content in sufficient quantities to maximize barley productivity (Robertson & Stark, 2003). Therefore, this is probably why there was no noticeable response of dependent variables to additional S fertilizer. The previous work of Dari et al. (2019) under similar growing conditions reported no significant effect of elemental S fertilizer on barley end-of-season

metrics. Interestingly, despite the high irrigation water S content, we observed some effects of S on the number of heads and grain protein concentration at the Aberdeen 2021 and Kimberly field sites, respectively. The slight difference in response to S between this study and the study by Dari et al. (2019) is likely related to the difference in the S fertilizer source, where we used sulfate S rather than the elemental sulfur source. Hence, under various conditions where irrigation water S content is low or moderate, such as in high-rainfall mountain valleys and foothill areas of this region,  $\text{SO}_4\text{-S}$  fertilizer may be a promising source to consider to maximize barley yield.

#### 4.5. CONCLUSIONS

Nitrogen significantly affected hulless spring food barley yield and end-of-season metrics but did not affect  $\beta$ -glucan concentration. Sulfur fertilizer application significantly affected the number of harvest heads and grain protein concentration. On average, S application yielded a 3% grain protein concentration increase compared with no S application. These findings on S suggest that under favorable growing conditions, S fertilizer application may improve grain protein concentration, but more research is required to assess S fertilizer effects further. Regarding N application timing, split-applied N had similar or lesser end-of-season metrics (except grain protein concentration) and N use efficiency compared to a single N application. Under the conditions for which this study was conducted, split N application may not be a reliable approach to increase hulless spring food barley yield and N use efficiency and should be avoided in favor of a single application. Irrespective of the pre-plant soil N, higher mean grain yield was achieved across all site years with increased N application up to a total N rate range of 187-277 kg ha<sup>-1</sup>. Although further studies are required to evaluate optimal N for hulless food barley yield, our findings suggest a total N of 187-277 kg ha<sup>-1</sup> to growers planting hulless spring food barley in this region.

Table 4.1 Test of fixed effects for hulless food barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test Weight (TW) in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, and Brigham Young University-Idaho in Rexburg, Idaho, USA.

Source of variation	Test of Fixed Effects								
	P>F value								
	PH	Tillers	Heads	Lodging	GY	GPC	β-glucan	Plumps	TW
<b>Aberdeen 2021</b>									
Nitrogen Rate (N)	0.255	0.103	0.462	0.025	0.027	<.001	0.844	0.015	0.324
Sulfur Rate (S)	0.871	0.667	0.007	0.799	0.447	0.324	0.515	0.96	0.367
N x S	0.635	0.263	0.134	0.198	0.858	0.309	0.771	0.979	0.297
<b>Kimberly 2021</b>									
Nitrogen Rate (N)	<0.001	<0.001	<0.001	NA	<0.001	<0.001	0.911	<0.001	0.021
Sulfur Rate (S)	0.779	0.116	0.264	NA	0.788	0.003	0.643	0.202	0.097
N x S	0.627	0.485	0.305	NA	0.394	0.348	0.309	0.312	0.094
<b>Aberdeen 2022</b>									
Nitrogen Rate (N)	<0.001	0.013	0.027	NA	<0.001	<0.001	0.288	0.024	0.622
Sulfur Rate (S)	0.989	0.459	0.204	NA	0.881	0.346	0.745	0.809	0.996
N x S	0.733	0.999	0.638	NA	0.343	0.262	0.453	0.602	0.799
<b>Rexburg 2022</b>									
Nitrogen Rate (N)	0.007	0.032	0.045	NA	0.317	<0.001	0.326	0.666	0.321
Sulfur Rate (S)	0.647	0.682	0.651	NA	0.709	0.877	0.526	0.86	0.569
N x S	0.815	0.206	0.675	NA	0.91	0.495	0.184	0.49	0.93

NA denotes not applicable.

Table 4.2 Treatment means for hulless food barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test Weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2021 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension centers, Idaho, USA.

	PH cm	Tillers ----- # -----	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC -----	β-glucan % -----	Plumps -----	TW kg hL <sup>-1</sup>
<b>Aberdeen 2021</b>									
N rate (kg N ha <sup>-1</sup> )									
0	72 <sup>b</sup>	4	94	3b	4.7b	13.1e	7.5	98.3a	75
45	74	4	107	3b	5.5a	14.1e	7.6	98.1a	76
45/23	77	5	104	8b	5.7a	15.8d	7.5	97.9ab	76
45/45	78	5	111	7b	5.9a	16.6bcd	7.6	98.2a	76
90	80	5	107	12ab	5.5a	16.0cd	7.4	98.2a	75
45/90	76	5	113	2b	5.7a	17.5abc	7.6	98.0ab	77
135	77	5	104	22a	5.6a	17.6ab	7.4	97.2bc	75
180	75	4	104	12ab	5.3ab	18.2a	7.4	96.8c	75
SE	2.4	0.4	6.6	4.6	0.2	0.8	0.1	0.5	0.7
SO4-S rate (kg S ha <sup>-1</sup> )									
0	77	5	112a	10	5.6	15.7	7.5	97.8	76
17	76	5	109a	7	5.4	16.3	7.5	97.8	75
34	76	5	96b	8	5.5	16.3	7.6	97.9	76
SE	1.7	0.3	4.3	3.2	0.1	0.6	0.1	0.4	0.4
<b>Kimberly 2021</b>									
N rate (kg N ha <sup>-1</sup> )									
0	43d	3d	49e	NA	0.8d	20.8b	7.1	96.2bc	72c
45	49c	4bc	74d	NA	1.9c	17.5d	7.3	97.3ab	75ab
45/23	51c	5abc	69d	NA	1.9c	21.1b	7.1	97.1ab	75ab
45/45	51c	5abc	80bcd	NA	2.2c	21.2b	7.1	95.8c	74abc
90	56b	5abc	97a	NA	3.0b	16.4e	7.1	97.5a	75ab
45/90	51c	4bc	77cd	NA	2.1c	23.6a	7.0	94.3c	73bc
135	64a	5abc	90abc	NA	3.1b	18.2cd	7.1	97.5a	76a

Table 4.2 cont'd

180	64a	6a	92ab	NA	3.8a	19.3c	7.2	97.0ab	75ab
SE	1.7	0.4	5.2	NA	0.1	0.5	0.1	0.4	0.8
SO <sub>4</sub> -S rate (kg S ha <sup>-1</sup> )									
0	54	4	78	NA	2.4	16.8c	7.1	96.9	74
17	54	5	82	NA	2.4	20.4a	7.2	96.3	75
34	53	4	75	NA	2.3	19.2ab	7.1	96.5	75
SE	1.1	0.3	3.6	NA	0.1	0.4	0.1	0.3	0.5

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level. NA denotes not applicable.

Table 4.3 Treatment means for hulless food barley plant height (PH), tillers, heads, lodging, grain yield (GY), grain protein concentration (GPC), grain starch concentration (GSC), plumps, and test Weight (TW) with standard errors (SE) with response to N fertilizer rates in the 2021 growing season at the University of Idaho Aberdeen Research and Extension Center and Brigham Young University-Idaho, Idaho, USA.

	PH cm	Tillers ----- # -----	Heads <sup>a</sup> -----	Lodging %	GY Mg ha <sup>-1</sup>	GPC ----- % -----	β-glucan %	Plumps -----	TW kg hL <sup>-1</sup>
<b>Aberdeen 2022</b>									
N rate (kg N ha <sup>-1</sup> )									
0	55c <sup>b</sup>	3c	39c	NA	2.0d	14.3bc	6.5	96.2a	70
45	62b	4bc	38abc	NA	2.5bc	12.4cd	6.6	96.0a	67
45/23	63b	5abc	35bc	NA	2.5bc	13.7bcd	6.4	94.0bc	67
45/45	68b	5abc	46a	NA	2.9bc	13.9bcd	6.6	95.7ab	69
90	65b	4abc	44ab	NA	2.7bc	11.8d	6.8	96.3a	69
45/90	68b	7a	46a	NA	3.0ab	17.1a	6.6	93.6c	69
135	65b	6ab	43ab	NA	2.4cd	13.4bcd	6.3	95.3abc	67
180	76a	6ab	41abc	NA	3.5a	15.3ab	6.5	96.4a	69
SE	3.6	0.6	3.4	NA	0.3	1.1	0.1	0.7	0.7
<b>Rexburg 2022</b>									
0	43c	6c	35d	NA	1.7b	13.6e	6.0	92.4	77
45	50bc	7ab	40bcd	NA	2.1ab	13.3e	6.0	93.5	71
45/23	50bc	6c	38cd	NA	1.8ab	16.2cd	5.8	91.4	74
45/45	53ab	7ab	45abcd	NA	2.1ab	17.6b	6.1	92.0	74
90	54ab	7ab	45abcd	NA	2.2ab	15.2d	5.9	92.1	71
45/90	52ab	7ab	50ab	NA	2.0ab	19.7a	5.9	91.7	70
135	55ab	7ab	50ab	NA	2.5a	16.4bc	5.5	90.0	74
180	58a	7ab	45abcd	NA	2.4a	16.5bc	5.9	90.5	75
SE	2.7	0.2	3.7	NA	0.3	0.5	0.2	1.8	2

<sup>a</sup> Data collected as the number of heads per meter of row.

<sup>b</sup> Within site-year and dependent variables, same lower-case letters within the column are not significantly different at 0.05 probability level. NA denotes not applicable.

Table 4.4 Treatment means for hulless food barley soluble protein concentration (SPC), insoluble protein concentration (ISPC), agronomic efficiency (AE), crop recovery efficiency (CRE), and partial factor productivity (PFP) with standard errors (SE) with response to N fertilizer rates in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho, Idaho, USA.

N rate (kg ha <sup>-1</sup> )	SPC	ISPC	AE <sup>a</sup>	CRE	PFP	SPC	ISPC	AE	CRE	PFP
	----- % -----	----- % -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- % -----	----- % -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----	----- kg kg <sup>-1</sup> -----
	----- <b>Aberdeen 2021</b> -----					----- <b>Kimberly 2021</b> -----				
0	3.0d <sup>b</sup>	10.1	-	-	-	9.3a	11.5bcd	-	-	-
45	5.2bc	8.9	18.0a <sup>φ</sup>	2.3a	123.6a	7.1cde	10.3d	23.4a	2.6ab	41.4a
45/23	4.7c	11.2	13.4ab	2.5a	83.2b	8.4abc	12.7b	15.9b	2.0bcd	27.8c
45/45	4.9c	11.7	13.4ab	1.9ab	66.1c	8.9ab	12.3bc	15.1b	2.3abc	24.1cd
90	6.6ab	9.4	8.2bc	1.8ab	61.0c	5.4f	11.0cd	24.2a	2.7a	33.2b
45/90	5.9abc	11.5	7.5bc	1.5ab	42.7d	7.7bcd	15.9a	9.8c	1.9cd	15.8e
135	6.9a	10.8	6.3bcd	1.2b	41.5d	6.2ef	12.1bc	17.4b	1.9cd	23.4cd
180	6.7a	11.5	3.3cd	0.9b	29.6e	6.5def	12.8bc	16.5b	1.5d	20.9d
SE	0.5	1.0	2.5	0.4	2.5	0.5	0.7	1.6	0.2	1.6
<i>Pr(&gt;F)</i>	<0.001	0.071	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	----- <b>Aberdeen 2022</b> -----					----- <b>Rexburg 2022</b> -----				
0	4.7c	9.6ab	-	-	-	8.9ab	4.7c	-	-	-
45	5.2bc	7.3bcd	13.2a	1.2a	56.4a	7.4bcd	5.9c	8.5	0.7ab	46.9a
45/23	6.8a	6.9cd	8.6ab	0.6b	37.1b	8.2abc	8.1b	2.1	0.7ab	27.5b
45/45	4.8c	9.1abc	10.2ab	1.1ab	31.8bc	9.1a	8.5b	4.2	0.9a	23.4bc
90	6.2abc	5.6d	8.1ab	0.9ab	29.7c	6.3d	8.9b	4.8	0.8ab	24.0bc
45/90	6.1abc	11.0a	8.2ab	1.1ab	22.6d	6.7cd	13.0a	1.8	0.9a	14.6d
135	6.7ab	6.7cd	3.9bc	0.6b	18.3d	6.3d	10.1b	5.8	0.9a	18.6cd
180	6.6ab	8.7abc	8.4ab	0.8ab	19.2d	6.8cd	9.7b	4.0	0.5b	13.5d
SE	0.6	1.1	2.9	0.2	2.9	0.6	0.7	2.9	0.2	2.8
<i>Pr(&gt;F)</i>	0.404	0.002	0.004	0.001	<0.001	0.001	<0.001	0.390	0.043	<0.001



Table 4.4 cont'd

<sup>a</sup> The difference in yield or food barley N uptake between the treatment of interest and 0N is divided by the applied N rate to determine AE and CRE.

<sup>b</sup> Within site-year and dependent variable, same lower-case letters within column are not significantly different at 0.05 probability level.

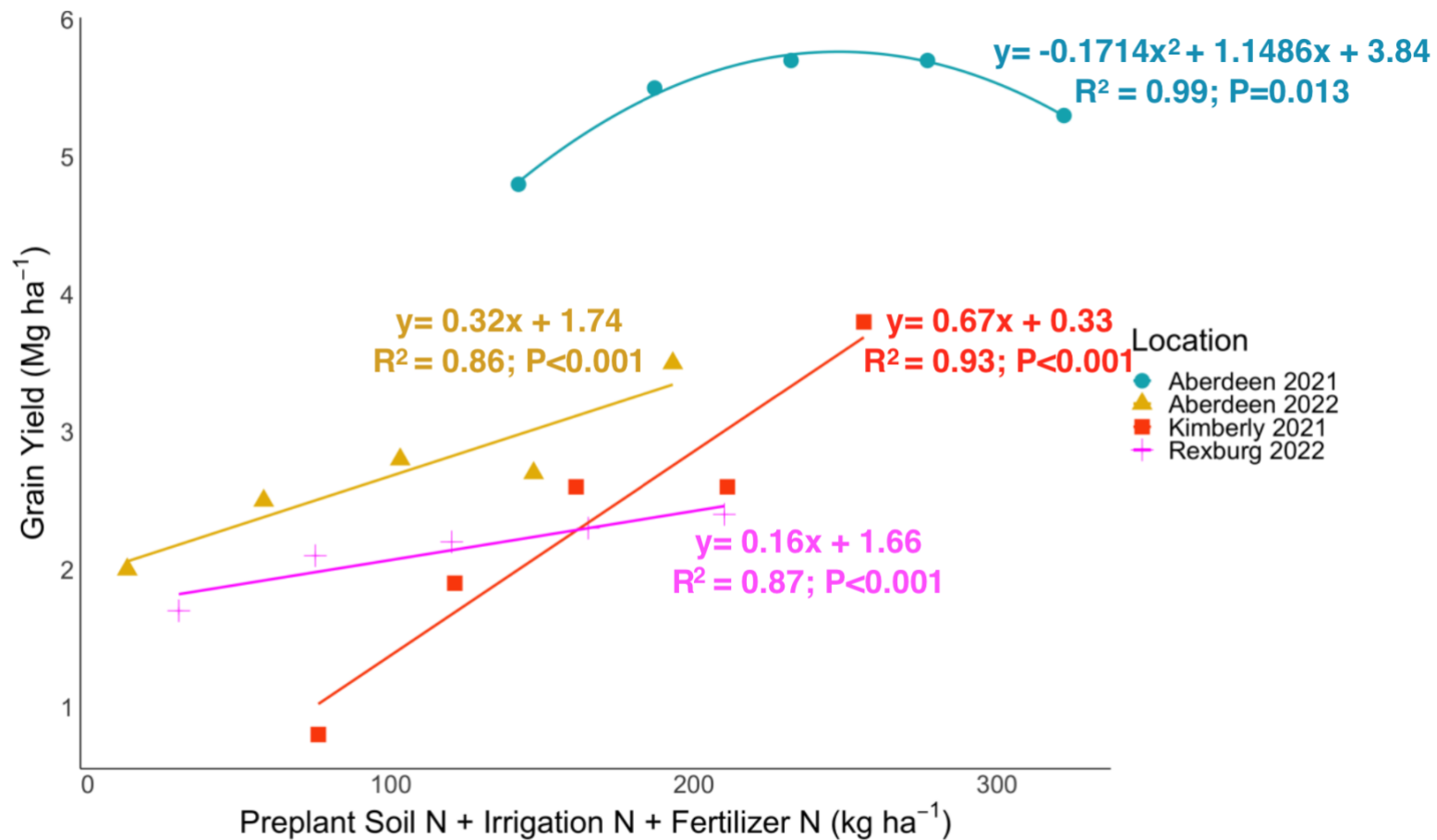


Figure 4.1 Regression analysis for means of hulless food barley grain yield at single N rates to total N (sum of preplant N, irrigation N, and fertilizer N) in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

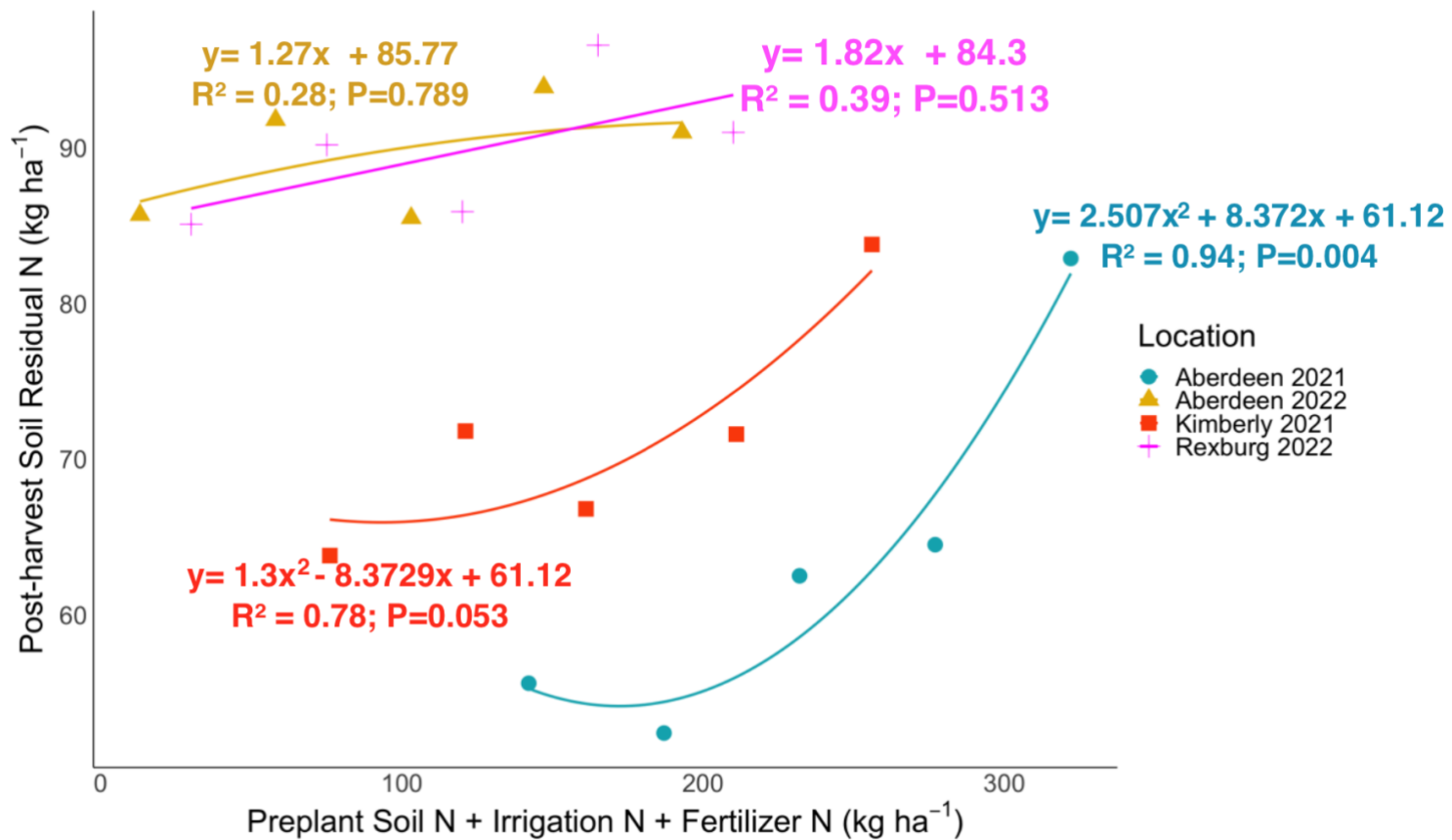


Figure 4.2 Regression analysis for means of hulless food barley postharvest soil residual N at single N rates to total N (sum of preplant N, irrigation N, and fertilizer N) at 0-60 cm depth in the 2021 and 2022 growing season at the University of Idaho Aberdeen and Kimberly Research and Extension Centers and Brigham Young University-Idaho in Rexburg, Idaho, USA.

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