EFFECT OF TOPDRESS N RATE APPLIED BASED ON GROWING DEGREE DAYS ON WINTER WHEAT (*Triticum aestivum* L.) GRAIN YIELD

By

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Abstract: The majority of in-season nutrient management decisions for numerous crops are based on subjective morphological scales. The objective of this study was to establish whether a numerical scale based on growing degree-days (GDD> 0) utilizing weather science, could be used for nitrogen (N) management in winter wheat. An incomplete factorial within a randomized complete block design was replicated three times, over a period of three growing seasons (2017 to 2019). The locations were Efaw near to Stillwater, OK and Perkins, OK. A total of 15 treatments were included where three treatments received preplant N rates of 0, 90, and 120 kg N ha^{-1}, and remaining treatments received topdress N of 30, 60, and 90 kg N ha^{-1} at 65, 80, 95 and 110 GDD's. Data collection included normalized difference vegetation index (NDVI) sensor readings, grain yield, grain protein concentration, and N uptake.

Peak NDVI values were recorded between 90 and 120 GDD's. Topdress application of N at 80 to 95 GDD's resulted in improved grain yields for three of the site years compared to preplant N applications. Grain protein concentration increased when N was applied topdress (90 kg N ha^{-1}) at 110 GDD's (3 out of 6 sites years).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

EFFECT OF TOPDRESS N RATE APPLIED BASED ON GROWING DEGREE DAYS ON WINTER WHEAT (Triticum aestivum L.) GRAIN YIELD

Abstract

The majority of in-season nutrient management decisions for numerous crops are based on subjective morphological scales. The objective of this study was to establish whether a numerical scale based on growing degree-days (GDD> 0) utilizing weather science, could be used for nitrogen (N) management in winter wheat. An incomplete factorial within a randomized complete block design was replicated three times, over a period of three growing seasons (2017 to 2019). The locations were Efaw near to Stillwater, OK and Perkins, OK. A total of 15 treatments were included where three treatments received preplant N rates of 0, 90, and 120 kg N ha-1, and remaining treatments received topdress N of 30, 60, and 90 kg N ha-1 at 65, 80, 95 and 110 GDD's. Data collection included normalized difference vegetation index (NDVI) sensor readings, grain yield, grain protein concentration, and N uptake. Peak NDVI values were recorded between 90 and 120 GDD's. Topdress application of N at 80 to 95 GDD's resulted in improved grain yields for three of the site years compared to preplant N applications. Grain protein concentration increased when N was applied topdress (90 kg N ha-1) at 110 GDD's (3 out of 6 sites years).

1.1 Introduction

With world population anticipated to be 10 billion, the demand for wheat will rise continuously (Hitz et al., 2017). Wheat grain yields in rain-fed agricultural systems, such as the Central Rolling Red Plains of the United States (i.e., Kansas, Oklahoma, and Texas), are limited due to water availability (Bushong et al., 2014). This region frequently encounters periods of extended drought, irregular rainfall, and variable temperatures (Baath et al., 2018). Additionally, variation in temperature and precipitation have to be considered as a part of the production system to ensure food security (Hatfield et al., 2011). Moreover, N is the most limiting nutrient second to water in cereal crop production (Szumigalski and Van Acker, 2006).

The future of food supply is going to be shaped by climate. Yield variability is comprehensively controlled by fertilizer use, irrigation, and environment (Mueller et al., 2012). Wheat senescence is accelerated with extreme heat, slowing grain-filling rates due to damaged photosynthetic apparatus at higher canopy temperatures (Lobell et al., 2012). Since 1950, global average temperatures have risen by 0.13^{o} C, and over the next two to three decades, an even faster increase of 0.2^{o} C per decade of global warming is expected (Solomon 2007). With each ^{o}C rise in temperature, global wheat production is estimated to fall by 6% (Asseng et al., 2015).

Nutrient use efficiency of winter wheat was noted to be a partial function of weather and nutrient availability (Girma et al., 1997). Nitrogen is one of the most essential nutrients for plant growth, production, and grain quality (Wuest and Cassman 1992; Frink et al., 1999; Kichey et al., 2007). Nonetheless, the reported nitrogen use efficiency (NUE) for cereal crops that include wheat averages only 33% (Raun and Johnson, 1999), and that elucidates the need for improvement

A significant challenge for farmers is to identify ideal management practices such as the optimum fertilizer rate and application timing due to the complexity and randomness of the problem that differs yearly (Lopez-Bellido et al., 2005; Raun et al., 2019). Numerous studies documented wheat yield improvement with N applied (Dhillon et al., 2018; Aula et al., 2019; Omara et al., 2019; Ma et al.,

2019) but a more sustainable approach to a healthy soil is to determine the right amount to apply. Lopez-Bellido et al. (2005) further mentioned that NUE in winter wheat is affected by timing and splitting of N application rather than optimum N rates. Alcoz et al. (1993) noted only 10% N is required before tillering. Strong (1995) in his review showed low fertilizer efficiencies with fall applied N. Besides, Sowers et al. (1994) noted increased N fertilizer recovery with spring top-dress application before stem elongation.

Irrespectively, the prediction of crop stages is essential from a management point of view, for the timing of pesticide application, harvesting (Ritchie and NeSmith, 1991) and nutrient management (Dhillon et al. 2019a). Growing Degree Day (GDD) heat units are a commonly used index to predict dates of flowering, maturity and seasonal variation in harvest index in crops (Lu et al., 2001). Furthermore, it is used to measure the heat units in the areas of crop phenology and development (McMaster and Wilhelm, 1997). The growth of the plant depends on temperature; the specific amount of heat is required by a plant to develop through various growth stages (Miller et al., 2001; Cleland et al., 2007). Precisely, temperature affects the enzymatic activities required for plant development (Bonhomme 2000). Various enzymes are involved in plant development, with particular temperature requirements, and due to this, we have a minimum, maximum and optimum temperature (Bonhomme 2000). Additional uses of GDD includes hybrid maturity descriptor by seed industry (Nielson et al., 2002); to quantify crop yields as affected by planting dates (Bollero et al., 1996); for prediction of N availability and losses from manure (Griffin and Honeycutt, 2000), and grain yield prediction using NDVI/GDD (Dhillon et al. 2019a).

The Oklahoma Mesonet uses a cutoff method to calculate GDD values, based on the following formula:

Degree days = (Maximum Daily Air Temp + Minimum Daily Air Temp)/2 – Base Temp.

For winter wheat GDD calculation, a lower temperature threshold is 0^{o} C; upper-temperature limit of 30^{o}C and base temperature of 4.4^{o} C is used. Likewise, the GDD could be used to conduct climate change research; whereby, it can be used as a climate impact index useful for management decisions (Anandhi 2016). There is a linear relationship between the rate of plant development and GDD (Wang 1960). Considering this direct relationship between crop development and GDD, using GDD's for nutrient management, especially N, would be more convenient for crop nutrient management.

The objective of this study was to identify optimum GDD's for top-dress N rates and its subsequent effect on winter wheat grain yield, protein concentration, and N uptake. Furthermore, the idea is to adopt an easier to use numerical scale compared to the traditional morphological scale.

1.2 Materials and Methods

Winter wheat experiments were established in 2016-17 (2017), 2017-18 (2018), and 2018-19 (2019). These trials were located at Perkins and Efaw just north of Stillwater, Oklahoma. The soil type at Perkins is Teller sandy loam; fine-loamy, mixed, thermic Udic Agriustoll, and at Efaw is Ashport silty clay loam; fine-silty, mixed, superactive, thermic Fluventic Haplustolls.

Soil samples were taken from each site before planting. Fifteen cores per plot were taken to a depth of 15 cm. The soil samples were dried at 60^{o}C overnight and were ground to pass a 2 mm sieve. Further, a 1:1 soil: water suspension and glass electrode were used to measure soil pH and buffer index (Sims, 1996; Sikora, 2006). A 1 M KCl solution was used for extraction of soil NO_3-N and NH_4-N, which were quantified using a Flow Injection Autoanalyzer (LACHAT, 1994). Mehlich 3 solution was used to extract plant-available P and K (Mehlich, 1984), where P and K were determined using a Spectro CirOs ICP spectrometer (Soltanpour et al., 1996). A detailed description of the soil analysis is reported in Table 1.1. To ensure N was the only limiting nutrient, both experiments were fertilized to a 100 percent level based on P and K test following regional fertilizer recommendations (Zhang and Raun 2006).

Herbicides and pesticides were applied as required throughout the season. A vacuum planter was used for planting. Plot dimensions were 6m long by 3m wide. A randomized complete block experimental design (RCBD) with three replications and 15 treatments was used in all trials (Table 1.2), except at Efaw in 2017, where treatment 3 was not included.

Three different topdress N rates were applied at four GDD dates (Table 1.2). Nitrogen as Urea Ammonium Nitrate (UAN) (28-0-0) (N-P-K) was used. Nitrogen was applied when 65, 80, 95, and 110 GDDs from planting had been accumulated where the GDDs were obtained from the Mesonet (www.mesonet.org), computed as follows: (Tmin+Tmax)/2-4.40C). An additional check plot was included where no N was used at any point during the growing season (Treatment 1). For Treatment 2 and 3, 90 and 120 kg N ha-1 pre-plant N was applied. Treatments 4 through 15 all received midseason N at rates of 30, 60, and 90 kg N ha-1 at the 15 GDD interval, starting from 65 days to 110 days. An all-terrain vehicle (ATV) sprayer with a 3m boom using streamer nozzles was used for topdress application.

Greenseeker Normalized Difference Vegetative Index (NDVI) readings were taken throughout the season up to head emergence. Where NDVI = (NIR-red)/NIR+red), NIR reflectance determined at 780 nm and red reflectance at 660 nm.

Grain subsamples from the harvest of each plot were collected for total N and moisture analysis. All grain samples were ground to pass a 60-mesh screen using a Thomas micro-Wiley Laboratory Mill (Thomas Scientific, Swedesboro, New Jersey, USA). Total N analysis for grain samples was performed using LECO Truspec CN dry combustion analyzer (Leco Corp, St Joseph, Michigan, USA).

Data analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, USA), where mean separation employed the least significant difference (LSD) procedure at an alpha level of 0.05. Procedure GLIMMIX was used to explore the treatment differences, where replications were treated as a random variable. Moreover, single-degree-of-freedom contrasts were performed to evaluate specific treatment differences (Mclntosh, 2015). In addition, R statistical software was used for data visualizations.

1.3 Results and Discussion

The growth rates and phenological development of winter wheat are influenced by both temperature and precipitation (Bauer et al., 1984). Total rainfall for all site years were highly variable, where the 2019 growing season received almost 400 mm more rain compared to the 10-year average (Table 1.3). Precipitation increased in May (439 and 404 mm) and June (107 and 119 mm) at both locations in 2019 in comparison to the same months in 2017 and 2018 (Table 1.3). An increase in precipitation during flowering and maturity (May and June) decreases number of late growing season sunshine hours and concurrently could reduce grain yield and grain quality (Song et al., 2019). However, high rainfall in May and June did not result in yield reduction, as mean grain yield at Efaw 2019 was higher than Efaw 2017, whereas Perkins 2019 yielded highest among three years at this location (Figure 1.1).

Typically, GDDs are used to quantify temperature effects and describe different biological processes (McMaster and Wilhelm, 1997; Li et al., 2012). In our study, cumulative GDD's for the entire growing season varied, with Efaw in 2017 having a total of 200 GDDs compared to only 169 GDD's for 2019 at the same location. Furthermore, the average monthly temperature at all site years ranged from 3 to 27^{0}C. The optimal temperature range for improved winter wheat growth is 17 to 23^{0}C with a minimum and maximum of 0 and 37°C (Porter and Gawith, 1999).

At Efaw, environmental mean grain yield ranged from 2.50 (2017) to 3.15 Mg ha^{-1} (2018) (Figure 1.1A). At Perkins mean grain yields were slightly lower and ranged from 2.07 (2017) to 3.08 Mg ha^{-1} (2019) (Figure 1.1B). All these inconsistencies across site years restricted a combined analysis of the data. Furthermore, Raun et al. (2017b) recommended site years to not be combined as environmental differences that change drastically, impact grain yields.

The NDVI data were collected over the entire growing season where the maximum NDVI was noted around 90 to 120 GDD's (Figure 1.2). The NDVI data collected early in the growing season were lower and increased as growing season advanced and decreased following 120 GDD's. Dhillon et al. (2019a) outlined 90 to 120 GDDs as the best window for in-season yield prediction and N recommendation using NDVI data. Furthermore, various researchers have also noted the importance of using NDVI to predict yield potential (Raun et al., 2002; Raun et al., 2005; Dhillon et al. 2019b), which could further be used to determine mid-season fertilizer N rate (Raun et al., 2017a).

Analysis of variance showed that there were significant treatment differences in grain yield in 4 of 6 site-years (Figure 1.3). Growing season 2018 at both locations resulted in similar yields across treatments (Figure 1.3B and 1.3E). At Efaw 2017, grain yield ranged from 1.78 Mg ha^{-1} in the check plot (Treatment 1) to 3.11 Mg ha^{-1} with 90 kg N ha^{-1} preplant (Treatment 2) (Table 1.4). Treatment differences were not noted due to the main effects of either N rate, GDD's or their interaction. Within individual treatment comparison, LSD at an alpha of 0.05 showed similar grain yields 3.11 Mg ha^{-1} (Treatment 2) and 3.00 Mg ha^{-1} (Treatment 12) (Table 1.4), with remaining treatments yielding significantly lower. During 2018 growing season at Efaw grain yield ranged from 2.81 Mg ha^{-1} in check plot (Treatment 1) to 3.49 Mg ha^{-1} in plots receiving 60 kg N ha^{-1} applied at 95 GDD's (Treatment 11). Similar to 2017 none of the main effects had any effect on grain yield in growing season 2018. In addition, single degree of freedom contrasts could not divulge any further information in 2017 and 2018 at the Efaw location. In 2019 at Efaw, significant treatment differences were noted due to N rate, GDDs, and interaction between GDD and N rate. The lowest yield was recorded in check plot at 1.97 Mg ha^{-1} (Treatment 1), and highest return was recorded at 3.99 Mg ha^{-1} when 60 kg N ha^{-1} was applied at 80 GDD's (Treatment 8). Furthermore, single degree of freedom contrasts showed that grain yields were higher with all the N rates applied at 80 GDDs compared to preplant treatments receiving 90 and 120 kg N ha^{-1} (Contrast 2 and 6; Table 1.4). Moreover, within topdress N receiving application, 80 GDD's yielded better in comparison to treatments at 65, 90, and 110 GDD's as per single degree of freedom contrasts (Contrast 9, 12, and 13; Table 1.4).

During the 2017 growing season at Perkins, check plot yield was lowest at 0.80 Mg ha^{-1} (Treatment 1), and 90 kg N ha^{-1} preplant (Treatment 2) yield was highest at 3.20 Mg ha^{-1}. Grain yields were not different due to the main effect of N application, N rate or their interaction (Table 1.5). Additionally, single degree of freedom contrasts revealed that 90 kg N ha^{-1} preplant resulted in significantly better yield compared to other treatments (contrast 1, 2, 3, and 4; Table 1.5). Throughout 2018 growing season, yields ranged from 1.19 Mg ha^{-1} in check (Treatment 1) to 2.58 Mg ha^{-1} with 90 kg N ha^{-1} applied at 80 GDD (Treatment 8). No additional information was gathered with single degree of freedom contrasts at Perkins in 2017 and 2018. In 2019, different N rates used at four different GDD's resulted in yield differences. The lowest yield was recorded in the check plot (Treatment 1) 2.22 Mg ha^{-1}, and highest yield was recorded with 120 kg N ha^{-1} applied preplant (Treatment 3) at 4.15 Mg ha^{-1}.

Overall at all site years topdress application at 80, and 95 GDDs resulted in the highest grain yield for 3 site-years (Efaw 2018 and 2019, Perkins 2018). Preplant applications resulted in higher yields at 2 of the six sites (Perkins 2017 and 2019). Whereas similar yield with preplant and topdress at 95 GDD was obtained at one site (Efaw 2017). Results in our study are in agreement with many other researchers who have noted a yield increase with topdress application of N in winter wheat (Knowles et al., 1994; Mohammed et al., 2013; Liu et al., 2019), however, all of these management decisions were based on a subjective morphological scale (Large, 1954).

Grain protein is an essential quality for determining the market premiums and end-use purposes of winter wheat. Globally a 12.5% protein content for hard red winter wheat is required; market price falls as protein content falls below this percentage (Wang et al., 2019). Mean grain protein was lower at Perkins as compared to Efaw (Figure 1.4). Protein levels were highly disperse in Perkins ranging from 9.48 % in 2017 to 12.59 % in 2019 (Figure 1.4B), whereas at Efaw the protein range was 10.96 % (2019) to 12.56 % (2018) (Figure 1.4A).

At all site years, grain protein was different due to treatment (Figure 1.5). The grain protein content increased as N rate increased for each topdress application date. This trend was most evident in Efaw 2018 (Figure 1.5B), Efaw 2019 (Figure 1.5C), and Perkins 2018 (Figure 1.5E). Grain protein content was not affected by the main effects of GDD, N rate, and interaction between GDD and N rate during 2017 growing season at Efaw (Table 1.4). However, single degree of freedom contrasts indicated higher grain protein recovery when 90 kg N ha^{-1} was applied pre-plant compared to topdress applications at 65 GDD (Contrast 1; Table 1.4), whereas no such deviations were noted in comparison to other application timings. During 2018 growing season, N rates had a significant influence on protein accumulation (Table 1.4). Protein content increased with increment in N rate at each successive topdress N application scheduling (Table 1.4; Figure 1.5B). During 2019 growing season at Efaw, both GDD and N rate had a significant effect on grain protein concentration (Table 1.4; Figure 1.5C). Single degree of freedom contrasts revealed that N application at 110 GDDs increased protein content compared to preplant N (Contrast 4, 8; Table 1.4), and other topdress applications (Contrast 11, 13, and 14, Table 1.4). At Perkins in 2017, both timings of N application (GDD) and N rate had a notable impact on grain protein concentration. According to single degree of freedom contrasts, the protein accumulation in grain improved with N application at 110 GDD's compared to preplant applications of 90 kg N ha^{-1} (Contrast 4; Table 1.5) and 120 kg N ha^{-1} (Contrast 8, Table 1.5). Furthermore, 110 GDD's timing of application was better than other topdress application days, 65 GDD's (Contrast 11, Table 1.5), 80 GDD's (Contrast 13, Table 1.5), and 95 GDD's (Contrast 14, Table 1.5). During 2018, only N rates affected protein accumulation. Protein content ranged from 11.42% in check plot (Treatment 1) to 15.08% with 90 kg N ha^{-1} applied as topdress at 110 GDD's (Treatment 15) (Table 1.5). An increasing trend in protein content was also noted with an increase in N rate within timing of N application (Figure 1.5E). In 2019 season at Perkins location, protein concentration was altered by GDD's schedule and N rate application rates (Table 1.5). Furthermore, application timing at 110 GDD was better compared to preplant application of 90 kg N ha^{-1} as per single degree of freedom contrasts (Contrast 4, Table 1.5). Overall 90 kg N ha^{-1} application at 110 GDDs resulted in highest protein content in 3 of 6 site years. Whereas application of 60 kg N ha^{-1} at 110 GDDs and 90 kg N ha^{-1} at 80 GDDs were better at 1 site year each. Results in this study are in agreement with several researchers where they noted a protein content increase with topdress N application (Wuest and Cassman, 1992; Bänziger et al., 1994; Mohammed et al., 2013, Lollato et al., 2019).

Nitrogen uptake was different at each site year, where mean N uptake at Efaw ranged from 51 kg N ha^{-1} (2017) to 69 kg N ha^{-1} (2018), whereas these values were lower at Perkins and extended from 34 kg N ha^{-1} (2017) to 58 kg N ha^{-1} (2019) (Figure 1.6). Analysis of variance revealed no treatment differences at Efaw during the 2017 season (Table 1.4; Figure 1.7A). During 2018 growing season at Efaw, N rate made an impact on N uptake (Table 1.4). Furthermore, improvement in N uptake was noted with an increase in N rate at each specific application timing (Figure 1.7B). However, limited differences were present when single degree of freedom contrasts was performed. During 2019 in Efaw, the main effect of GDD, N rate, and interaction between GDD and N rate was significant (Table 1.4). A trend in N uptake upsurge with increase in N rate within timing of N application was noted (Figure 1.7C). Additionally, single degree of freedom contrasts showed that topdress application was better compared to preplant applications in terms of N uptake (Contrast 2, 3, 4, and 7; Table 1.4). At Perkins in 2017, preplant application of 90 kg N ha^{-1} resulted in highest N uptake of 59 kg N ha^{-1}. In addition, single degree of freedom contrasts showed that preplant application of 90 kg N ha^{-1} was significantly better compared to other treatments (Contrast 1, 2, 3, and 4; Table 1.5). During 2018 at Perkins, N rate resulted in significant difference in N uptake. Nitrogen uptake increased as N rate increased at different application schedules (Figure 1.5E). Similar results were noted by Lollato et al. (2019) where a linear increase in N uptake was found with an increase in N application rate. At

Perkins during 2019, interaction of timing and N rates were significant for N uptake. As per single degree of freedom contrasts preplant application of 120 kg N ha^{-1} resulted in better N recovery compared to other treatments (Contrasts 5,6,7, and 8; Table 1.5). Overall site years, N uptake followed a similar trend as yield, where treatments with high yields resulted in higher N uptake.

In this study, we have documented that GDDs could be efficiently used for topdress application management where N timing between 80 and 95 GDDs is ideal for improved winter wheat grain yield. Furthermore, topdress application resulted in higher protein and N recovery compared to preplant application. This might be due to various factors affecting treatments receiving high preplant N applications. Such as excess fall tillering and biomass production resulting in late-season drought stress (van Herwaarden et al., 1998), weakening of vegetative organs (Borghi, 1999), increased lodging potential (Lollato and Edwards, 2015), parasite vulnerability (Howard et al., 1994), and late spring freeze induced stress (Dhillon et al. 2019c). However, all these adverse conditions could be avoided with topdress applications, where a GDD based numerical scale could be easily used for making N management decisions.

1.4 Conclusions

This work shows that an easier-to-use-numerical-scale based on GDD's could be effectively utilized for N management strategies in winter wheat. The findings also showed that the highest NDVI values occurred between 90 and 120 GDD's. We deduced that topdress application of N applied between 85 and 95 GDD's resulted in increased yields and N uptake. Furthermore, we also concluded that topdress application of 90 kg N ha^{-1} at 110 GDD was best for improving grain protein content.

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Table 1.1: Initial chemical properties of soils (0-15 cm) collected for 2017, 2018, and 2019 growing season, Efaw and Perkins, OK.

Site	Year	pH	NH_4-N	NO_3-N	P	K
				mg kg $^{-1}$		
Efaw	2017	5.77	9	12	17	109
	2018	6.11	36	10	21	190
	2019	5.51	15	11	19	191
Perkins	2017	6.96	14	1	13	132
	2018	6.91	6	2	13	134
	2019	6.80	5	3	14	162

pH-1:1 soil:water; NH_4-N and NO_3-N – 2 M KCl; K and P- Mehlich III

Table 1.2. Treatment structure employed to evaluate different N rates and application times of topdress fertilizer N, using the number days from planting to sensing where growth was possible, or growing degree days (GDD) that were > 0, where GDD was determined as (Tmin+Tmax)/2 – 4.4°C, Efaw, and Perkins, OK

Treatment no.	The timing of fertilizer application	GDD>0	N rate kg N ha^{-1}
1			0
2	Pre-plant		90
3	Pre-plant		120
4	Top-dress		30
5	Top-dress	65	60
6	Top-dress		90
7	Top-dress		30
8	Top-dress	80	60
9	Top-dress		90
10	Top-dress		30
11	Top-dress	95	60
12	Top-dress		90
13	Top-dress		30
14	Top-dress	110	60
15	Top-dress		90

Table 1.3: Monthly cumulative precipitation (PT., mm), average temperature (TAVG, °C), and monthly growing degree days (GDD) for 2016-17, 2017-18, and 2018-19 growing season, Efaw and Perkins, OK.

Location		2017			2018			2019			10-yr avg	
Efaw	Planting date	Oct 14, 2016			Oct 20, 2017			Oct 23, 2018				
	Month	PT.	TAVG	GDD	PT.	TAVG	GDD	PT.	TAVG	GDD	PT.	TAVG
	Oct	98	20	18	161	16	10	119	15	9	81	16
	Nov	22	13	29	8	11	29	23	6	20	47	10
	Dec	10	3	14	24	4	15	93	4	20	32	4
	Jan	65	5	18	6	2	15	67	3	12	23	3
	Feb	56	10	21	63	4	12	50	3	13	49	5
	Mar	48	13	28	30	11	31	58	8	23	51	11
	Apr	252	16	30	52	12	27	134	16	30	115	16
	May	66	20	31	99	24	31	439	20	31	134	20
	June	73	6	11	152	27	15	107	24	11	81	24
	Total	690	12	200	595	12	185	1090	11	169	613	12
Perkins	Planting date	Oct 1, 2016			Oct 12, 2017			Oct 11, 2018				
	Oct	54	20	31	144	16	19	123	15	21	87	16
	Nov	55	13	29	7	11	29	20	6	20	55	10
	Dec	12	3	18	16	4	15	97	4	20	38	4
	Jan	67	5	18	4	2	15	72	3	12	26	3
	Feb	50	10	22	83	4	14	36	3	13	46	5
	Mar	60	-	-	20	11	31	54	8	23	49	9
	Apr	230	-	-	66	12	26	134	16	30	114	16
	May	100	20	-	100	24	31	404	19	31	136	20
	June	53	25	-	145	26	11	119	24	11	82	26
	Total	681	11	-	596	12	191	1059	11	181	633	12

Table 1.4: Treatment structure, treatment means, main effect model, and single-degree-of-freedom contrasts for grain yield, protein, and N uptake for 2017, 2018, and 2019 growing season, Efaw, OK

		2017				2018			2019		
Treatments	GDD>0	N rate kg N ha^{-1}	Grain yield Mg ha^{-1}	@Grain protein	N uptake kg ha^{-1}	Grain yield Mg ha^{-1}	Grain protein	N uptake kg ha^{-1}	Grain yield Mg ha^{-1}	Grain protein	N uptake kg ha^{-1}
1		0	1.78	10.15	33	2.81	10.48	52	1.97	10.06	34
2	Pre-plant	90	3.11	12.99	71	3.21	12.77	72	2.44	10.35	44
3		120	-	-	-	2.95	12.39	64	2.88	10.60	53
4		30	2.78	10.57	52	3.26	11.61	66	1.50	10.67	28
5	65	60	2.63	11.67	54	3.24	12.05	68	1.91	11.90	37
6		90	2.48	10.95	48	3.34	13.18	77	3.52	11.18	69
7		30	2.37	11.99	50	3.32	10.97	64	3.90	10.69	73
8	80	60	2.21	12.53	48	3.38	12.48	74	3.99	10.93	77
9		90	2.63	11.40	52	3.12	14.31	78	3.07	10.32	55
10		30	1.75	11.18	34	2.98	12.67	67	1.94	10.48	36
11	95	60	2.85	12.08	60	3.49	12.73	77	3.31	11.48	67
12		90	3.00	12.29	65	3.06	13.58	73	3.43	11.16	67
13		30	2.21	11.32	44	2.85	13.18	66	2.35	10.76	44
14	110	60	2.53	11.08	49	2.88	13.04	66	2.71	12.09	57
15		90	2.54	12.38	54	3.36	13.10	77	3.20	12.61	71
SED			0.42	0.53	8.7	0.24	0.44	5.4	0.24	0.34	4.4
Main Effects											
GDD			ns	ns	ns	ns	ns	ns	*	*	*
Nrate			ns	ns	ns	ns	*	*	*	*	*
GDD*Nrate			ns	ns	ns	ns	ns	ns	*	ns	*
Contrasts (Treatments)											
1. Pre-plant 90 (2) vs GDD-65 (4,5,6)			ns	*	ns	ns	ns	ns	ns	ns	ns
2. Pre-plant 90 (2) vs GDD-80 (7,8,9)			ns	ns	ns	ns	ns	ns	*	ns	*
3. Pre-plant 90 (2) vs GDD-95(10,11,12)			ns	ns	ns	ns	ns	ns	ns	ns	**
4. Pre-plant 90 (2) vs GDD-110 (13, 14, 15)			ns	ns	*	ns	ns	ns	ns	*	**

5. Pre-plant 120 (3) vs GDD-65 (4,5,6)	-	-	-	ns	ns	ns	**	ns	ns
6. Pre-plant 120 (3) vs GDD-80 (7,8,9)	-	-	-	ns	ns	ns	*	ns	*
7. Pre-plant 120 (3) vs GDD-95 (10,11,12)	-	-	-	ns	ns	ns	ns	ns	ns
8. Pre-plant 120 (3) vs GDD-110 (13, 14, 15)	-	-	-	ns	ns	ns	ns	*	ns
9. GDD-65 (4,5,6) vs GDD-80 (7,8,9)	ns	**	ns	ns	ns	ns	*	ns	*
10. GDD-65 (4,5,6) vs GDD-95 (10,11,12)	ns	ns	ns	ns	ns	ns	*	ns	*
11. GDD-65 (4,5,6) vs GDD-110 (13,14,15)	ns	ns	ns	ns	**	ns	**	*	*
12. GDD-80 (7,8,9) vs GDD-95 (10,11,12)	ns	ns	ns	ns	ns	ns	*	ns	*
13. GDD-80 (7,8,9) vs GDD-110 (13,14,15)	ns	ns	ns	ns	ns	ns	*	*	*
14. GDD-95 (10,11,12) vs GDD-110 (13,14,15)	ns	ns	ns	ns	ns	ns	ns	*	ns

SED – standard error of the difference between two equally replicated means, Main effect excludes treatments 1, 2, and 3 where N was pre-plant applied; ns, *, and ** not significant, and significant at 0.01 and 0.05 probability levels; @- Grain protein = % N in grain *5.7

Table 1.5: Treatment structure, treatment means, main effect model, and single-degree-of-freedom contrasts for grain yield, protein, and N uptake for 2017, 2018, and 2019 growing season, Perkins, OK

			2017			2018			2019		
Treatments	GDD>0	N rate kg N ha^{-1}	Grain yield Mg ha^{-1}	@Grain protein	N uptake kg ha^{-1}	Grain yield Mg ha^{-1}	Grain protein	N uptake kg ha^{-1}	Grain yield Mg ha^{-1}	Grain protein	N uptake kg ha^{-1}
1		0	0.80	9.70	14	1.19	11.42	24	2.22	10.92	43
2	Pre-plant	90	3.20	10.62	59	2.14	12.61	47	3.23	10.03	57
3		120	1.64	8.69	25	2.17	12.87	49	4.15	10.87	79
4		30	2.29	9.09	37	2.00	11.10	39	2.78	10.63	52
5	65	60	2.27	9.02	36	2.08	12.68	46	3.16	10.58	59
6		90	2.12	9.02	33	2.02	13.61	47	3.37	10.93	65
7		30	2.57	9.37	43	1.86	11.70	38	2.50	10.32	45
8	80	60	2.38	9.30	38	2.33	12.37	51	3.03	10.80	57
9		90	1.76	9.00	28	2.58	13.59	61	3.52	11.05	68
10		30	1.79	9.46	30	2.08	11.76	43	2.76	10.24	50
11	95	60	2.19	9.63	37	2.43	13.07	56	3.10	10.49	57
12		90	1.78	9.21	29	2.57	12.67	57	3.32	10.52	61
13		30	1.98	10.05	35	2.16	11.61	44	2.71	10.44	49
14	110	60	2.17	10.75	41	2.23	12.78	50	3.44	11.19	67
15		90	2.22	9.22	36	2.02	15.08	53	2.95	11.64	60
SED			0.29	0.25	4.8	0.26	0.58	5.7	0.18	0.23	3.8
Main Effects											
GDD			ns	*	ns	ns	ns	ns	ns	*	ns
Nrate			ns	**	ns	ns	*	*	*	*	ns
GDD*Nrate			ns	ns	ns	ns	ns	ns	ns	ns	*
Contrasts (Treatments)											
1. Pre-plant 90 (2) vs GDD-65 (4,5,6)			*	*	*	ns	ns	ns	ns	**	ns
2. Pre-plant 90 (2) vs GDD-80 (7,8,9)			*	*	*	ns	ns	ns	ns	**	ns
3. Pre-plant 90 (2) vs GDD-95(10,11,12)			*	*	*	ns	ns	ns	ns	ns	ns
4. Pre-plant 90 (2) vs GDD-110 (13, 14, 15)			*	**	*	ns	ns	ns	ns	*	ns
5. Pre-plant 120 (3) vs GDD-65 (4,5,6)			ns	ns	ns	ns	ns	ns	*	ns	ns
6. Pre-plant 120 (3) vs GDD-80 (7,8,9)			ns	ns	ns	ns	ns	ns	*	ns	*
7. Pre-plant 120 (3) vs GDD-95 (10,11,12)			ns	**	ns	ns	ns	ns	*	ns	*
8. Pre-plant 120 (3) vs GDD-110 (13, 14, 15)			ns	*	**	ns	ns	ns	*	ns	*

9. GDD-65 (4,5,6) vs GDD-80 (7,8,9)	ns	ns	ns	ns	ns	ns	ns	ns	*
10. GDD-65 (4,5,6) vs GDD-95 (10,11,12)	ns	ns	ns	ns	ns	ns	ns	ns	ns
11. GDD-65 (4,5,6) vs GDD-110 (13,14,15)	ns	*	ns	ns	ns	ns	ns	ns	ns
12. GDD-80 (7,8,9) vs GDD-95 (10,11,12)	ns	ns	ns	ns	ns	ns	ns	ns	ns
13. GDD-80 (7,8,9) vs GDD-110 (13,14,15)	ns	*	ns	ns	ns	ns	ns	ns	ns
14. GDD-95 (10,11,12) vs GDD-110 (13,14,15)	ns	**	ns	ns	ns	ns	ns	*	ns

SED – standard error of the difference between two equally replicated means, Main effect excludes treatments 1, 2, and 3 where N was pre-plant applied; ns, *, and ** not significant, and significant at 0.01 and 0.05 probability levels; @- Grain protein = % N in grain *5.7

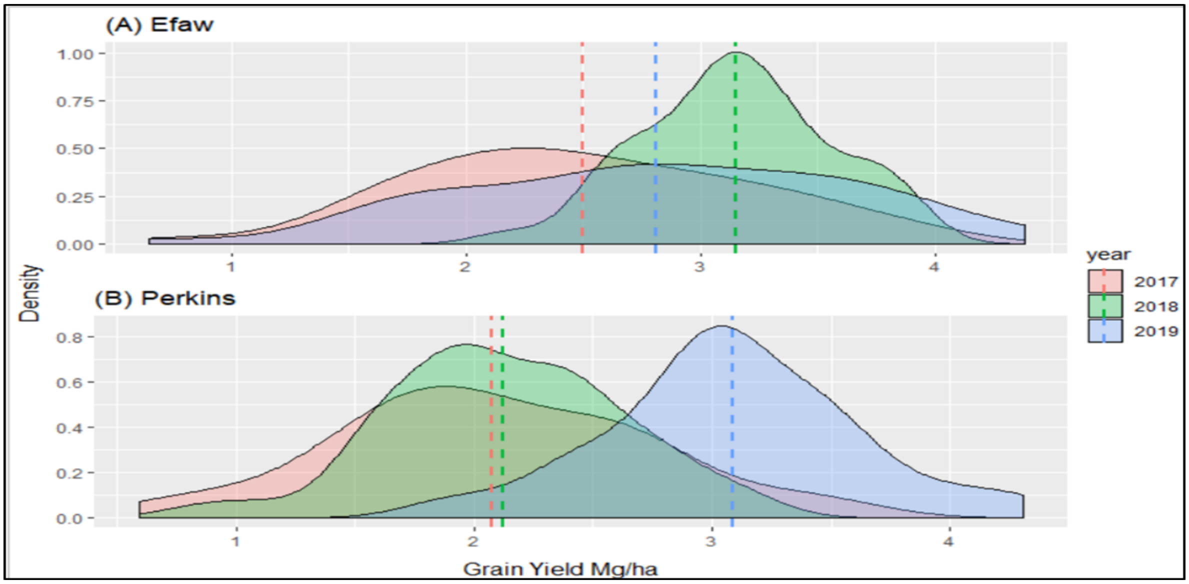

Figure 1.1: Distribution of grain yield across years with vertical dashed lines representing environmental mean for each year at Efaw (A) and Perkins (B).

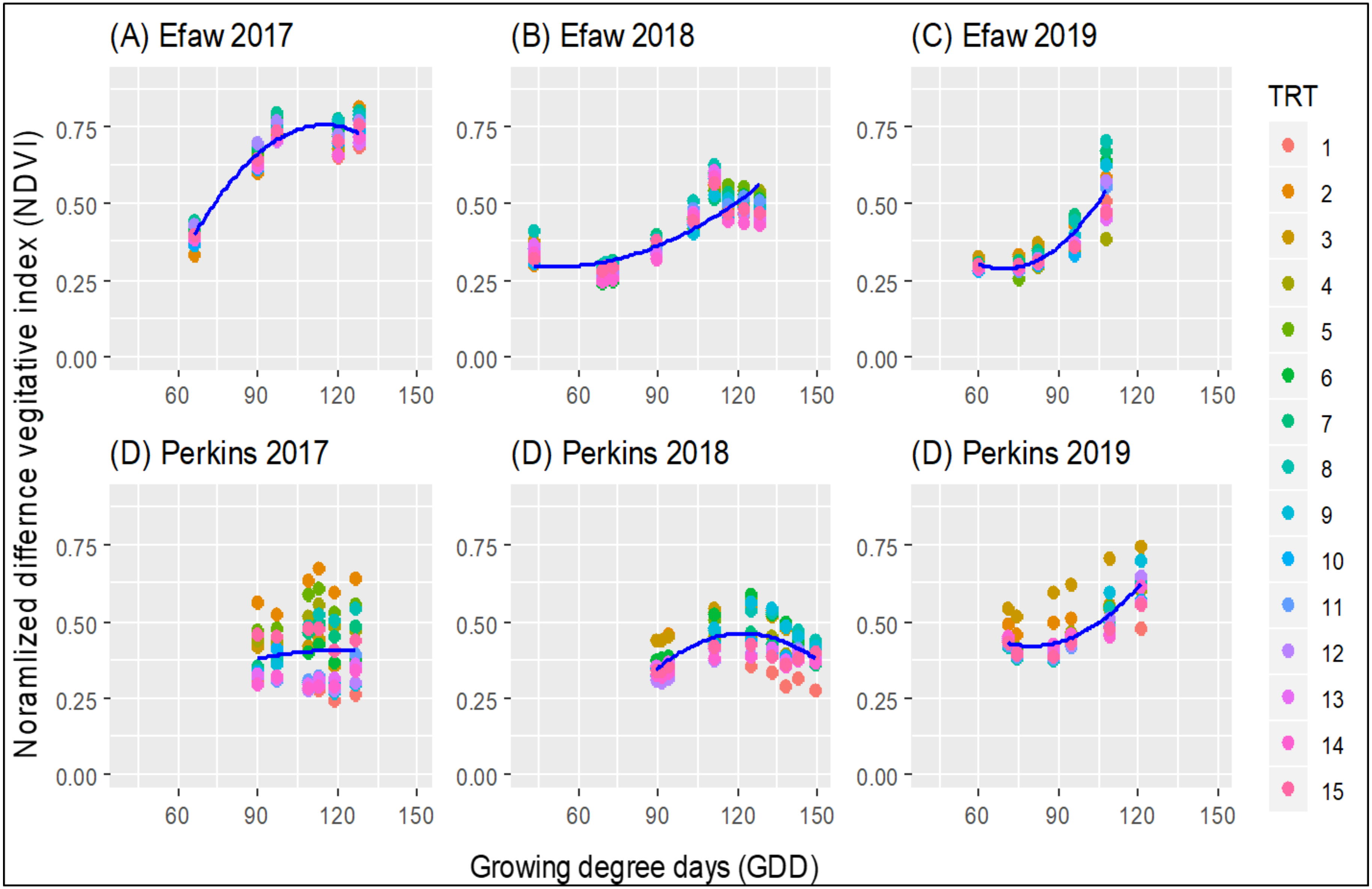

Figure 1.2: Change in NDVI values across the growing season in different treatments.

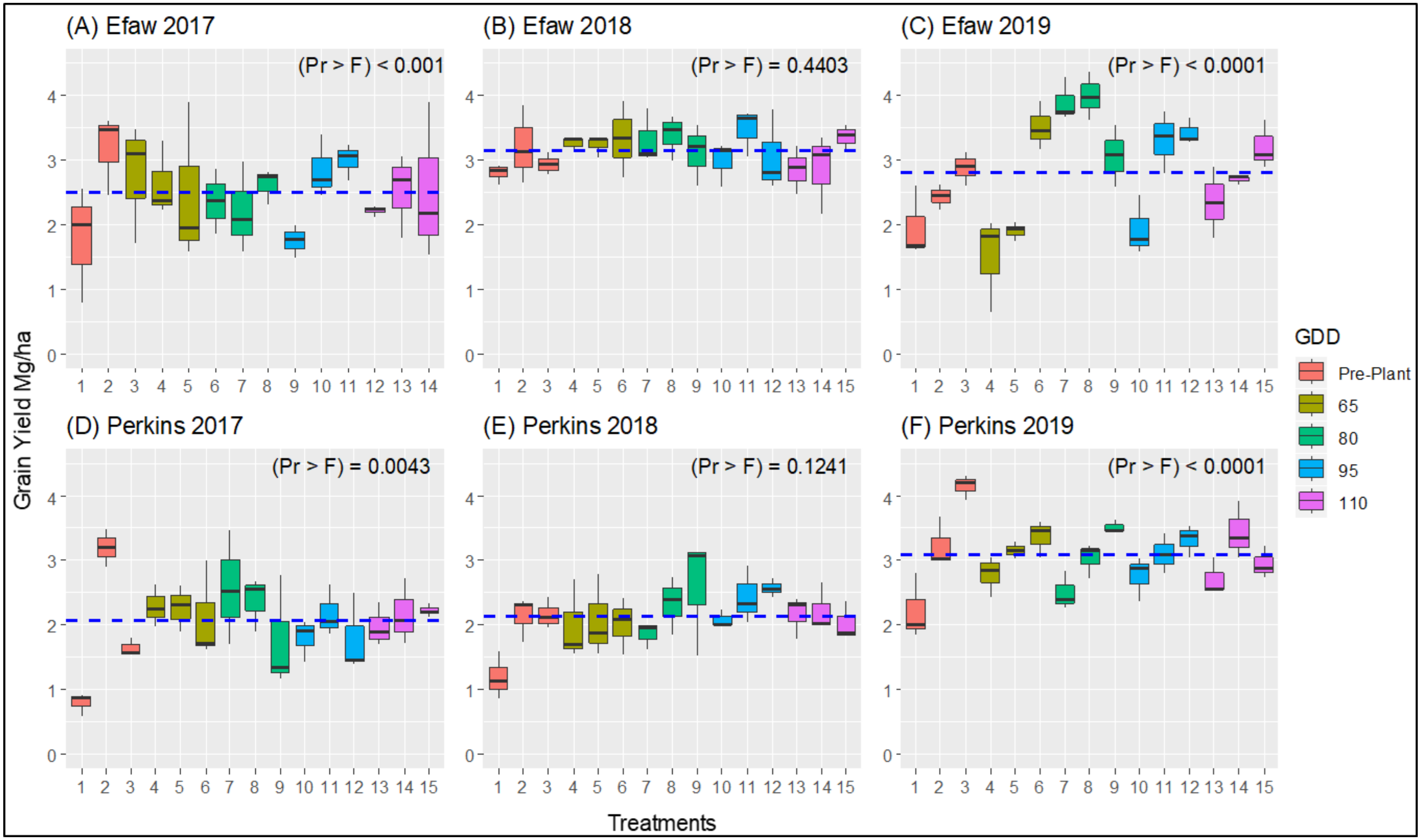

Figure 1.3: Dispersal of grain yield by treatment, color coded by N application timing for each site year with horizontal dashed line representing environmental mean grain yield for Efaw 2017 (A), Efaw 2018 (B), Efaw 2019 (C), Perkins 2017 (D), Perkins 2018 (E), and Perkins 2019 (F) growing seasons.

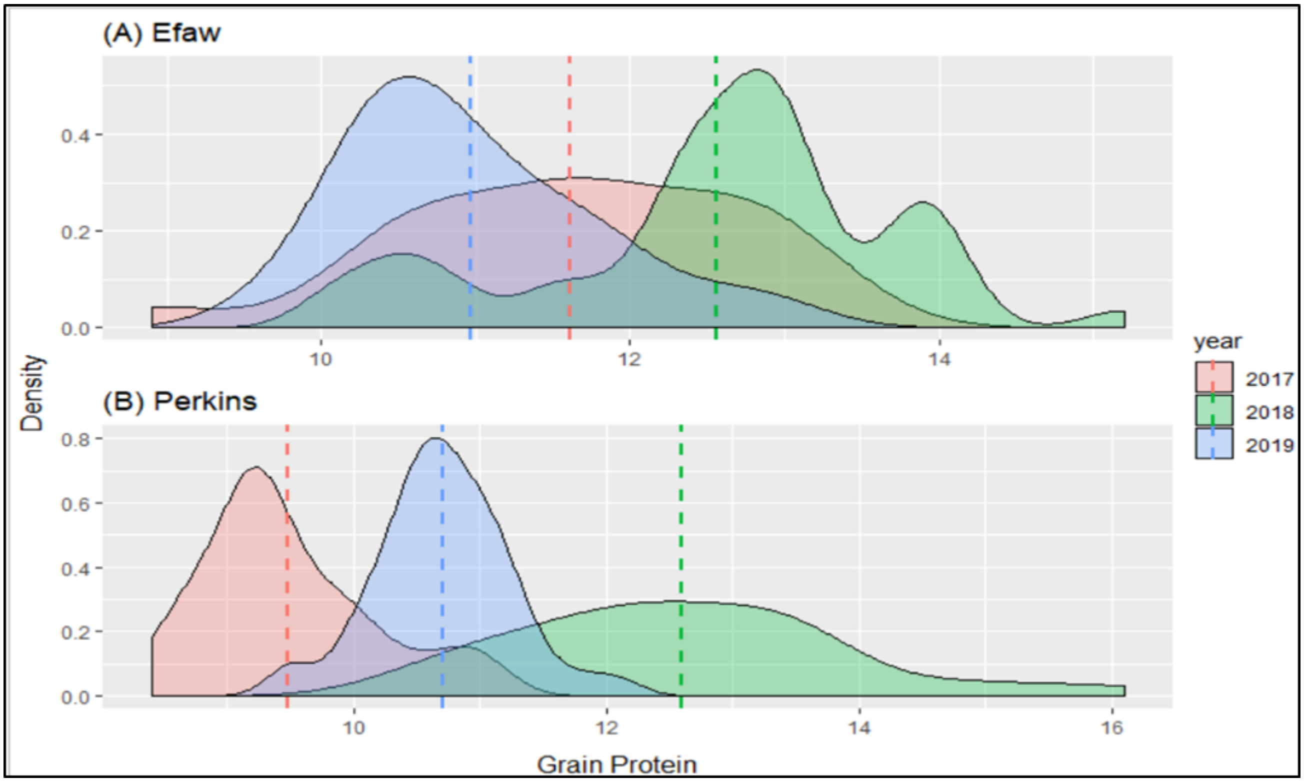

Figure 1.4: Dispersal of grain protein across years with vertical dashed lines representing mean protein concentration for each year at Efaw (A) and Perkins (B).

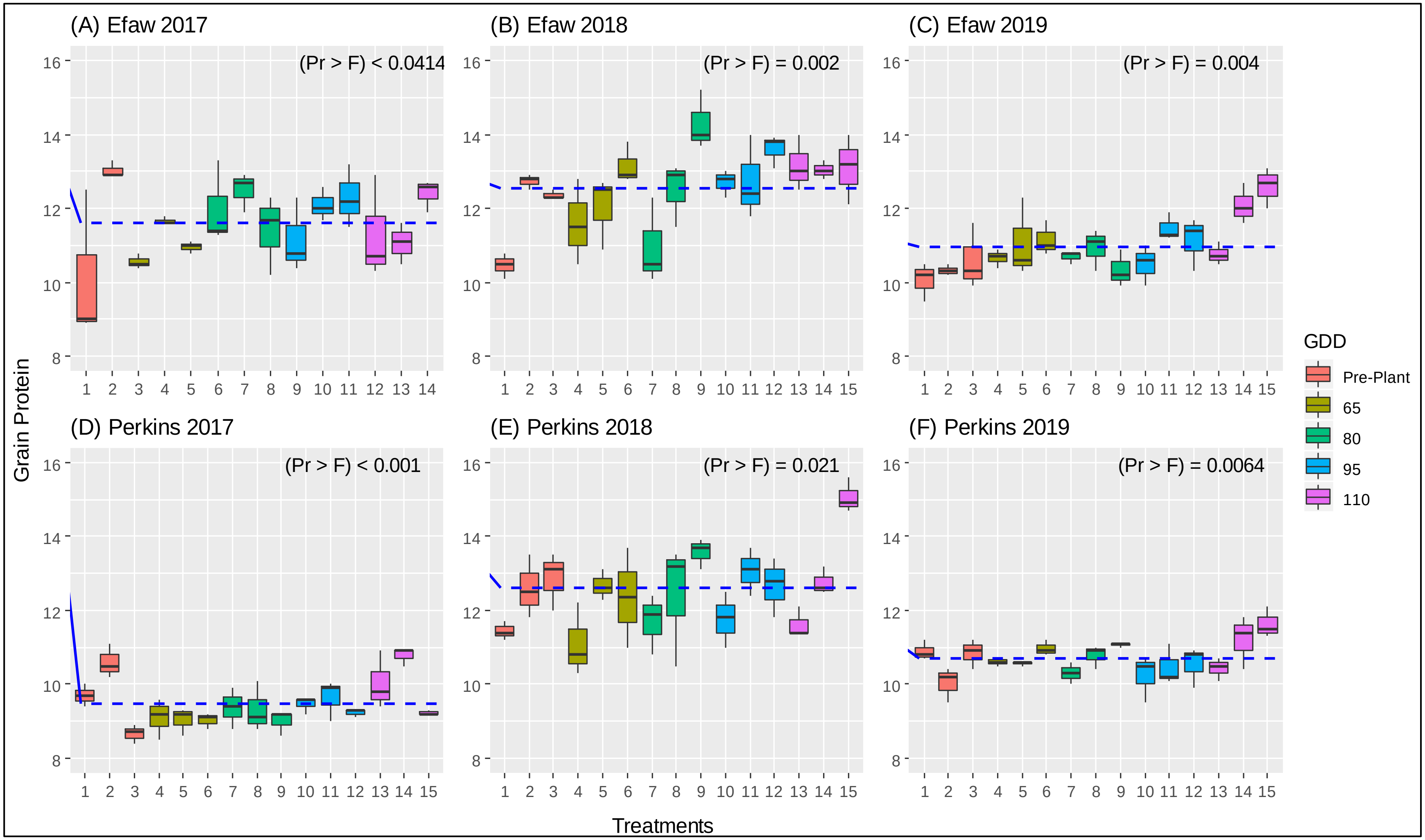

Figure 1.5: Distribution of grain protein by treatment grouped by N application timing for each site year with horizontal dashed line representing environmental mean grain protein for Efaw 2016-17 (A), Efaw 2017-18 (B), Efaw 2018-19 (C), Perkins 2016-17 (D), Perkins 2017-18 (E), and Perkins 2018-19 (F) growing seasons.

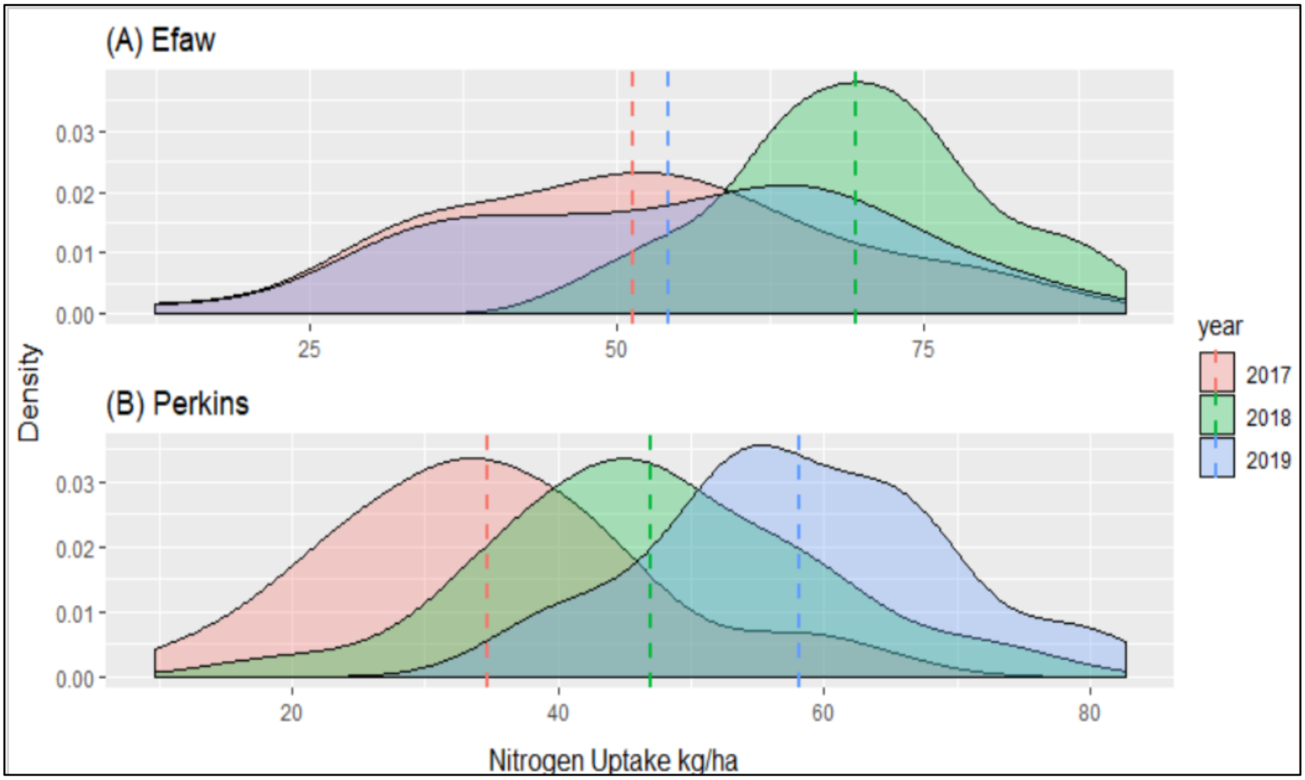

Figure 1.6: Dispersal of grain N uptake across site years with vertical dashed lines representing mean protein concentration for each year at Efaw (A) and Perkins (B).

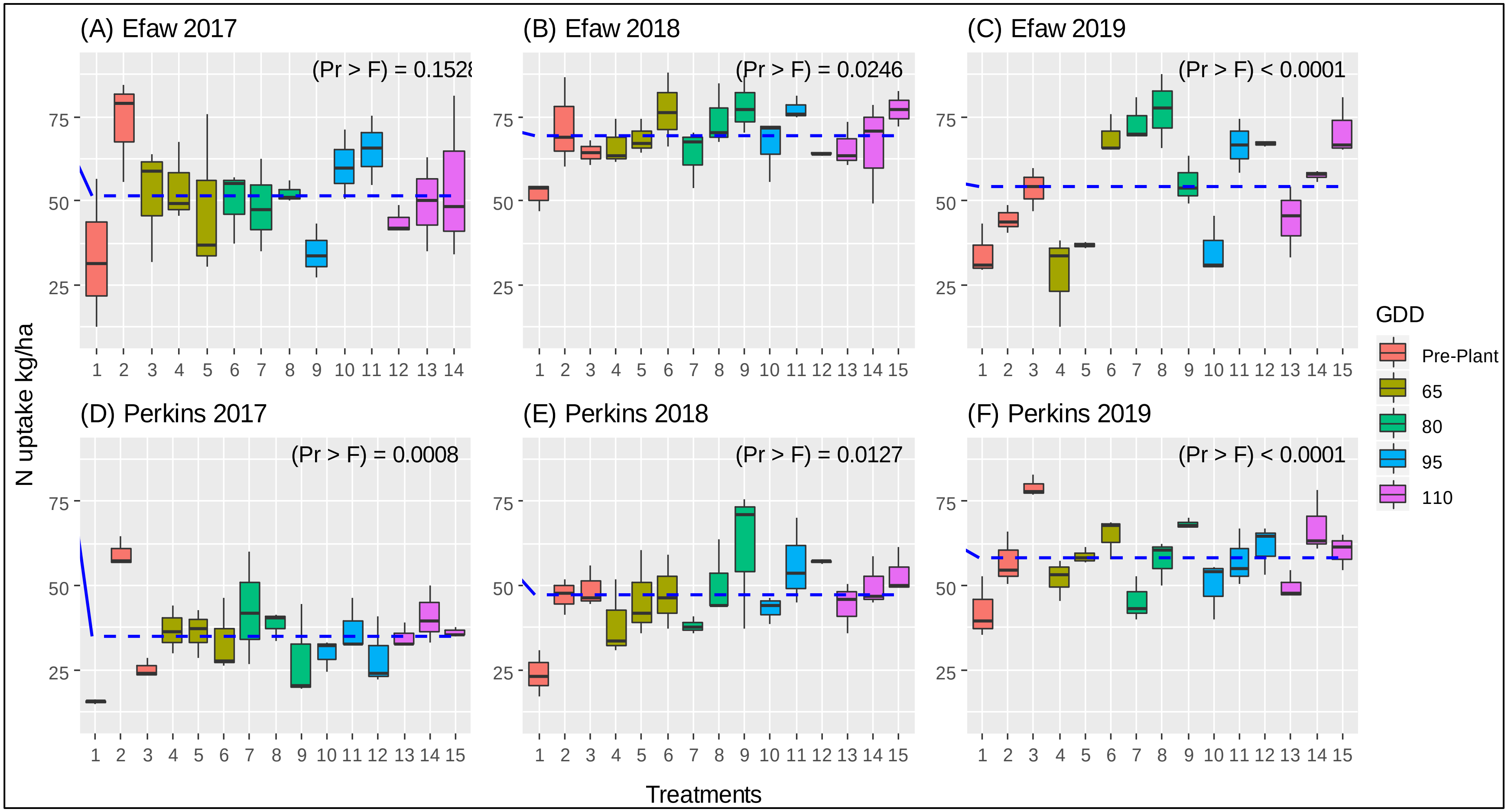

Figure 1.7: Nitrogen uptake by treatment grouped by N application timing for each site year with horizontal dashed line representing mean N uptake for Efaw 2016-17 (A), Efaw 2017-18 (B), Efaw 2018-19 (C), Perkins 2016-17 (D), Perkins 2017-18 (E), and Perkins 2018-19 (F) growing seasons.

CHAPTER II

INFLUENCE OF METHOD, TIMING, AND N RATE ON WINTER WHEAT (Triticum aestivum L.) GRAIN YIELD AND ESTIMATED PLANT NITROGEN LOSS

Abstract

Method of nitrogen (N) application in winter wheat (*Triticum aestivum* L.) and its impact on estimated plant N loss has not been extensively evaluated. The effects of preplant and topdress N application methods together with their interactions on grain yield, grain protein, nitrogen use efficiency (NUE), and gaseous N loss were investigated. The trials were set up in an incomplete factorial within a randomized complete block design and replicated three times for five site years. Data collection included normalized difference vegetation index (NDVI), grain yield, and forage and grain N content.

Sensor NDVI data were divided into two groups based on GDDs (before and after 90 GDDs) and were correlated with final grain yield, grain N uptake, grain protein, and NUE. The best results were achieved at Efaw, where NDVI collected after 90 GDDs was able to explain 58% and 51% variation in grain yield and grain N uptake, respectively. Nonetheless, NDVI was found to be a poor indicator for both grain protein and NUE.

Grain yield was not affected by the method and timing of N application at Efaw. Alternatively, at Perkins, topdress applications resulted in higher yields. The grain protein content and NUE were improved with the topdress applications. Generally, topdress application enhanced grain protein and NUE without decreasing the final grain yield. The difference method used in calculating gaseous N loss did not always reveal similar results, and estimated plant N loss was variable by site year, and depended on daily fluctuations in the environment.

2.1. Introduction

Winter Wheat (*Triticum aestivum* L.) is an essential crop for national and global food security. It covers approximately three million hectares of Central Rolling Red Plains of the United States, including parts of Kansas, Oklahoma, and Texas (Bushong et al. 2014). In the state of Oklahoma, winter wheat encompasses 75% of the cropland (Patrignani et al., 2014) and is produced under rain-fed conditions (Vitale et al., 2011). Wheat grain yields in Oklahoma have been stagnant since the 1980s, with a current state yield average of 2.0 Mg ha^{-1} compared to experimental state yields of 6.59 Mg ha^{-1} (Patrignani et al., 2014).

Irrespective of the yield stagnation, the consumption of fertilizers for wheat production is increasing. Globally, nitrogen (N), phosphorus (P), and potassium (K) fertilizer consumption per unit area has increased 8 times (Lu and Tian, 2017), 3.5 times (Dhillon et al., 2017), and 3 times (Dhillon et al., 2019c), respectively, since the year 1961. Nitrogen remains the most used (Raun et al., 2005; Aulakh and Malhi, 2005) and limiting nutrient in crop production (Malhi et al., 2001; Ladha et al., 2005; Fageria and Baligar, 2005). Without N fertilization, only half of the world's current population would have sufficient food (Dawson and Hilton, 2010). In Oklahoma, N fertilizer accounts for approximately 15-25% of production costs (Biermacher et al., 2006).

Nitrogen use efficiency (NUE) is an indicator used to compute and communicate the utilization efficiency of N (Brentrup and Lammel, 2016). Nitrogen use efficiency is defined as the ratio between N removed by the crop to the N supplied by the soil and fertilizer (Brentrup and Lammel, 2016; Moll et al., 1982). Worldwide, NUE for cereal crops, including wheat, is only 33% (Raun and Johnson, 1999). Lower NUE results in plant N deficiency, which could lead to decreased crop yield and quality (Cassman et al., 2002). Lower NUE could mainly be associated with N losses (Raun and Johnson 1999), over-fertilization (Sower et al., 1994), odd timing (Macnack et al., 2014), and method of N application.

One of the predominant N losses from cereal crops is the release of N from above-ground biomass, mainly as NH_3. Kanampiu et al. (1997) reported 4 to 27.9 kg ha^{-1} gaseous N loss from different genotypes of winter wheat. Daigger et al. (1976) reported 25 to 80 kg N ha^{-1} loss from wheat. Harper et al. (1987) found the aerial loss of 8.3 kg N ha^{-1}, 20-day post fertilizer application, and 7.1 kg N ha^{-1} additional losses from anthesis to harvest. Nitrogen losses from above-ground biomass ranged from 45 to 81 kg N ha^{-1} in maize (Zea mays L.) in a ^{15}N balance study (Francis et al., 1993). Furthermore, Francis et al. (1993) mentioned higher plant losses after anthesis, and at higher levels of soil N.

Improving crop production, soil health, and the economic and environmental aspects of N fertilizers requires the utilization of best management practices (Singh and Ryan, 2015). Nitrogen requirement for optimum crop production while reducing N losses could be accomplished through these best management practices (Fageria et al., 2003). The best management practices include applying the right nutrient source at the right time, at the right rate, and in the right place, collectively known as the 4R's Principle (Roberts 2006; 2007; Singh and Ryan, 2015).

There are a limited number of convincing studies for the appropriate method and timing of N application in winter wheat in rain-fed regions. This study investigated whether different timing and methods of N application could increase winter wheat grain yields and reduce gaseous plant N loss.

2.2. Materials and Methods

For this study, wheat experiments were established in 2016-17 (2017), 2017-18 (2018), and 2018-19 (2019) growing seasons. These experiments were located in Perkins and Efaw, just north of Stillwater, Oklahoma. The soil type at Perkins is a Teller sandy loam; fine-loamy, mixed, thermic Udic Agriustoll and at Efaw, Ashport silty clay loam; fine-silty, mixed, superactive, thermic Fluventic Haplustolls.

Soil samples were taken from each plot before planting. Fifteen cores per plot were taken to a depth of 15 cm, and soil samples were dried at 60°C overnight and ground to pass a 2 mm sieve. Further, a 1:1 soil: water suspension and glass electrode were used to measure soil pH and buffer index (Sims, 1996; Sikora, 2006). A 1M KCl solution was used for the extraction of soil NO_3-N and NH_4-N, which were quantified using a Flow Injection Autoanalyzer (LACHAT, 1994). Mehlich 3 solution was used to extract plant-available P and K (Mehlich, 1984), where P and K were quantified using a Spectro CirOs ICP spectrometer (Soltanpour et al., 1996). A comprehensive description of the soil analysis is reported in Table 2.1.

Herbicide and pesticide applications were applied as per the requirement throughout the season. Experimental plots were 6m long and 3m wide and were set up as an incomplete factorial treatment structure with 12 treatments in a randomized complete block design with three replications. The blocks were separated from each other with an alley of 3m. Various combinations of N application timings, application methods, with two N rates were assessed to determine the best management practice for rain-fed winter wheat (Table 2.2). Nitrogen was applied as urea ammonium nitrate (UAN) (28-0-0) (N-P-K). Treatment 1 was kept as check where no N was used over the entire season. Treatment 2 through 9, all received preplant N at rates of 45 and 90 kg N ha^{-1}. Two methods of preplant N application were used where N was placed on the soil surface and 10 cm below the surface using a coulter applicator. Treatment 4 through treatment 12, all received topdress N at rates of 45 and 90 kg N ha^{-1}. Topdress N application comprised of three different methods, where one application was made on the surface, another one at a depth of 10 cm, and the last one was foliar using the streamer nozzles. The coulter applicator used had six single disc wavy coulters each spaced at 0.50 m apart on a 3m wide frame. An all-terrain vehicle (ATV) sprayer with a 3 m boom fitted with streamer nozzles was used for the top-dress N application (Treatment 6, 9, and 12), where nozzles were placed at a spacing of 0.15 m. The treatment structure used at both locations is noted in table 2.2.

Greenseeker sensor readings were taken throughout the season, up to head emergence. Sufficient area was available in each plot to accommodate forage and grain yield harvest. Each plot was harvested at anthesis, post-anthesis, and harvest for forage N uptake. This was accomplished by hand clipping 0.55 m^2 area 2 cm above the ground. Similarly, the same area from another part was harvested 14-days post-anthesis. The third time forage collection was done at the physical maturity of the crop when wheat was ready for harvest. However, in season 2018 and 2019, only 0.09 m^2 area was hand clipped at anthesis, post-anthesis, and harvest. Massey Ferguson 8XP self-propelled combine was used for collecting grain yield. Subsamples from each plot were collected for total N and moisture analysis. All the forage and grain samples were ground properly to obtain subsamples using Thomas Wiley Laboratory mill (Thomas Scientific, Swedesboro, New Jersey, USA). For forage samples, whole plant samples were grounded without separations, for N analysis. Total N analysis for forage and grain samples was done using a LECO Truspec CN dry combustion analyzer (Leco Corp, St Joseph, Michigan, USA).

Grain protein, grain N uptake, and NUE were calculated based on % N in grain using equation (1), (2), (3):

Grain protein = %N * 5.7 (Tkachuk, 1969) (1)

Grain N uptake = %N in grain * grain yield (2)

NUE = (Grain N uptake (x)-Grain N uptake (check))/(Total N applied(x))*100 (3)

Where x in equation (3) stands for the treatment for which NUE was calculated, divided by N applied in that particular treatment.

Additionally, the difference in total forage N accumulated at anthesis, post-anthesis, and the harvest was calculated to obtain plant N loss (Kanampiu et al., 1997). Total forage N at different stages were calculated using equation (4)

Forage N uptake = %N in grain * forage yield (4)

Data analysis was performed using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC, USA), and treatment means were compared using the LSD mean separation procedure at the 5% significance level. Additionally, R statistical software (R core team) was used for data visualization.

2.3 Results and Discussion

The study period embraced highly variable weather conditions, where the year 2019 received 1090 mm of rainfall, in comparison to 613 mm of 10-year average rain (Table 2. 3). Total precipitation was consistent with the 10-year average during both years at Perkins and received 681 and 596 mm rainfall in 2017 and 2018, respectively. Considering these differences and following Raun et al. (2017) reference, combining data over the years was not attempted. A commonly used vegetation index in crop nutrient management is the Normalized Difference Vegetation Index (NDVI) (Piedallu et al., 2019). Values for NDVI are based on red and near-infrared spectral band absorption (Tucker et al., 1985), and is strongly correlated to the photosynthetically active radiation signifying vegetation productivity (Piedallu et al., 2019). Plant biomass estimate via NDVI collected using optical sensors have improved fertilizer N recommendations (Solie et al., 2012). The crop canopy sensors quantify differences in fertilized and non-fertilized plots via NDVI (Franzen et al., 2016), which are used for proper N recommendations. Early season NDVI readings are used for yield prediction, which is further used to refine in-season fertilizer rates (Raun et al., 2001). Recently, Dhillon et al. (2019a) used a GDD based numerical scale and established that NDVI collected between 90 and 120 GDDs was ideal for N recommendation in winter wheat. In this study, the final grain yield was plotted with NDVI data collected before and after 90 GDDs. The relationship between NDVI and grain yield at Efaw were improved with NDVI readings collected after 90 GDD's (R^2 = 0.58) (Figure 2.1B) when compared to before 90 GDD's (R^2 = 0.35) (Figure 2.1A). However, no relationship was recognized between NDVI and grain yield at Perkins (Figure 2.1C and 2.1D).

We further associated NDVI with grain protein, where no correlation was found between these two variables (Figure 2.1 E to H). Consistent with our results, Freeman et al. (2003) found no relationship between NDVI data collected at various growth stages and grain protein. They inferred the insufficiency of NDVI in monitoring N translocated into the grain. Likewise, a weak relationship between grain protein and two vegetation indices, NDVI, and NDRE (Normalized difference red edge index) was noted by Wang et al. (2019). Magney et al. (2016) also indicated a poor prediction of protein with NDVI values collected throughout the growing season.

The accuracy of site-specific in-season fertilizer management decisions is dependent on accurate prediction of N uptake (Ali et al., 2019) and grain yield potential (Dhillon et al., 2019b). Numerous researchers have deduced that NDVI is a good indicator of N uptake (Stone et al., 1996; Solie et al., 1996; Raun et al., 2001; 2002). In our study, a comparison between NDVI and N uptake at Efaw revealed a 51% variation in N uptake when NDVI data were collected after 90 GDDs (Figure 2.2 B). Alternatively, no correlation was noticed at Perkins between NDVI and grain N uptake (Figure 2.2 C; 2.2 D). Finally, we tried to explore whether NDVI could be a good indicator of NUE. Nonetheless, no association was found between these two factors at any location (Figure 2.2 E; 2.2 F; 2.2 G; 2.2 H). Similar results were found by Macnack et al. (2014), where they cited the inadequacy of NDVI in distinguishing the quantity of N uptake of applied N and N lost through the soil-plant system.

Analysis of variance at Efaw 2017 showed that treatments had a significant effect on grain yield (Table 2.4; Figure 2.3A). However, the main effect of the preplant application method, the topdress application method, and their interaction were not significant (Table 2.4). Furthermore, single-degree-of-freedom-contrasts showed no treatment differences. However, treatment differences due to the lower yield in check plot treatment were seen (Figure 2.3A). At Efaw 2018, treatments did not have any influence on grain yield (Table 2.4; Figure 2.3B). The LSD at alpha = 0.05 showed that preplant (treatments 2,3), split (treatments 7,8), and topdress application (treatment 10) all resulted in similar and higher grain yields than other treatments (Figure 2.3B). Comparing preplant (treatment 2,3) with all the split applied applications (treatment 4 to 9) via the single degree of freedom contrast indicated better grain yield with preplant N application (Contrast 1; Table 2.4). Similarly, a single degree of freedom contrasts showed that within split applications, the combination of surface applied (treatment 4) yielded better than split between preplant and top-dressed at the surface (Treatment 7) (Contrast 9; Table 2.4). For Efaw 2019, only differences in grain yield were noted due to treatments (Table 2.4), with lower yields in the check plot compared to other treatments (Figure 2.3C).

At Perkins during 2017, treatments resulted in different grain yields (Table 2.4). Exploration of a single degree of freedom contrasts specified that split applications yielded better than preplant N (Contrast 1; Table 2.4). Alternatively, topdress applications were better yielding than preplant applications (Contrast 3; Table 2.4, Figure 2.4A). Moreover, the surface applied topdress application was better than N applied at depth (Contrast 5; Table 2.4, Figure 2.4A). During the 2018 season in Perkins highest grain yield was recorded when topdress was applied using streamer nozzles (Figure 2.4B). As per contrasts, topdress applied N was better compared to preplant and split applications in terms of improving grain yield (Contrast 2 and 3; Table 2.4). Within topdress, an application using streamers (Treatment 12) improved yield over surface application (Treatment 10) (Contrast 6; Table 2.4). The mean grain protein content at Efaw 2017 was 12% (Figure 2.5A). Grain protein within treatments was affected due to the topdress N application method (Table 2.4).

Single degree of freedom contrasts revealed high grain protein content with split applications in comparison to preplant treatments (Contrast 1, Table 2.4). Nevertheless, the grain protein was considerably improved with topdressing application compared to preplant (Contrasts 2: Split [4-9] vs. Topdress [10-12]) and split (Contrast 3: Preplant (1,2)) vs topdress (10-12) Table 2.4, Figure 2.5A). The mean grain protein content was slightly higher at Efaw 2018 at 12.5% (Figure 2.5B). Additionally, no information could be gathered through single degree of freedom contrasts (Table 2.4). According to LSD at alpha 0.05, suggestively better grain protein was obtained with split application of 45 kg N ha-1 preplant and topdress both applied at a depth of 10 cm, and topdress application at the surface (Figure 2.5B). The lowest mean grain protein content of 11% was noted in 2019 at Efaw (Figure 2.5C). During the 2019 growing season highest grain protein was accumulated with the topdress application of 90 kg N ha-1 applied 10 cm underneath soil surface (Figure 2.5C). However, nothing was revealed by single degree of freedom contrasts and analysis of main effects (Table 2.4).

Overall mean grain protein content at Perkins in 2017 was 10.7% (Figure 2.6A). Analysis of variance suggested noteworthy differences in grain protein with treatment and topdress method at Perkins in 2017 (Table 2.4). With a single degree of freedom contrasts, it was noted that protein content improved with split application compared to preplant application (Contrast 1; Table 2.4). Topdress application developed this trend further compared to preplant and split applications (Contrast 2, 3; Table 2.4). Within topdress applications, both surface and depth applications were better than streamer applications (Contrast 5 and 6; Table 2.4). The treatment difference found using LSD revealed considerably better grain protein with topdress application on the surface (Treatment 10) and depth (Treatment 11) compared to the rest of the treatments (Figure 2.6A). During the 2018 growing season at Perkins the mean protein content was 14% (Figure 2.6B), where the preplant method had higher grain protein accumulation (Table 2.4). As per a single degree of freedom contrasts, preplant application at depth was better compared to the surface application (Contrast 1; Table 2.4). In preplant vs topdress application, comparison in preplant application resulted in higher protein content (Contrast 3: Table 2.4). However, LSD showed similar protein content with preplant application at depth (Treatment 3) and topdress N application on the surface (Treatment 10) (Figure 2.6B). Among split applications, preplant applied at depth resulted in higher protein content than surface application (Contrast 8; Table 2.4).

Nitrogen use efficiency was calculated using equation 3. At Efaw in 2017, the differences in NUE was only noted due to treatments, whereas none of the main effects made any difference. As per LSD, topdress application resulted in higher NUE compared to the rest of the treatments (Figure 2.7A). The same information was noted with a single degree of freedom contrast where NUE with topdress applications were better than split (Contrast 2; Table 2.4) and preplant application (Contrast 3; Table 2.4). In the 2018 growing season at Efaw, the average NUE was lower compared to 2017. Some negative values were noted due to higher N uptake in check treatment (Figure 2.7B). As per LSD, topdress application at the surface (Treatment 10) and split application with preplant at depth and topdress with a streamer (Treatment 9) both resulted in high NUE compared to the rest of the treatments (Figure 2.7B). No differences in NUE were noted at Efaw 2019, whereas, single degree of freedom contrast noted higher NUE in topdress applications compared to split applications (Contrast 2; Table 2.4). Analysis of variance revealed that only treatments were significant at altering NUE at Perkins in 2017. Single degree of freedom contrasts showed higher NUE with split application compared to preplant applications (Contrast 1; Table 2.4). However, it was further noticed with the contrasts that topdress applications were better than both split (Contrast 2; Table 2.4) and preplant applications (Contrast 3; Table 2.4). Relying on treatment differences with LSD, topdress application, and surface resulted in higher NUE compared to the rest of the treatments (Figure 2.8A). Similarly, during 2018 at Perkins topdress applications were better compared to the rest of the treatments (Figure 2.8B). Similar results were revealed with a single degree of freedom contrasts (Table 2.4).

Many researchers have noted the significance of split application in the optimization of yield and grain protein (Mascagni and Sabbe 1991; Cassman et al., 1992; Boman et al., 1995; Ottoman and Pope, 2000; Woolfolk et al., 2001) which is somewhat different than what was found in our study. Wuest and Cassman (1992) suggested early-season N fertilization for increasing grain yields and late-season N fertilization for increasing grain protein. Incidentally, with our study, we noted that a single topdress application was sufficient at improving both grain yield and grain protein. The timing of topdress applications was variable over five site years of our study, and based on the GDDs scale; it was between 92 and 127. Similarly, Dhillon and Raun (2019) found improved grain yield and grain protein with topdress application compared to preplant N application.

Furthermore, our results indicated higher NUE with topdress application, which is also in disagreement with various researchers who pointed out enhanced NUE with split N application (Alcoz et al., 1993; Sowers et al., 1994; Ayoub et al., 1995). Generally, different application methods did not make any significant impact in altering grain yields, but grain protein was improved with topdress application made at the surface at 4 of 5 site-years of study. In terms of NUE, topdressed application at 10 cm depth was better compared to the rest, and these results are in agreement with Sowers et al. (1994). Sowers et al. (1994) also noted in season point injection or topdressing to be efficient for increasing NUE compared to preplant N.

Gaseous N losses were based on forage N uptake calculated using equation 4. Higher anthesis and/or post-anthesis N uptake compared to forage N uptake at harvest implied gaseous N loss. At Efaw 2017, there was, on average, 10 kg N ha^{-1} loss from post-anthesis to harvest (Figure 2.9A). Preplant applied treatments resulted in most N loss since anthesis (Figure 2.9A). Whereas split and topdress applications did not indicate any N loss between samples collected at anthesis and harvest. Alternatively, Efaw 2018 showed no N loss, as the highest forage N uptake was registered at harvest compared to other stages. Incidentally, this site showed very high forage N uptake at all the stages compared to 2017 (Figure 2.9B). Similar to 2018, during the 2019 growing season at Efaw, no N loss was found. Winter wheat was accumulating N until harvest at this location (Figure 2.9C). Perkins 2017, was consistent with Efaw 2018 and 2019 and showed no N loss (Figure 2.10A). Forage N uptake was similar at post-anthesis and harvest stages. Alternatively, at Perkins in 2018, there was low gaseous N loss. This work showed that N uptake continued prior to and beyond anthesis (Figure 2.10B). It should be noted that differences in estimated plant N loss are highly dependent on the environment from flowering to grain fill (Kanampiu et al., 1997). In general, losses are expected to be greater when moisture stress occurs during this period. Nonetheless, climatic differences from one day to the next can alter these estimates even when using isotopic difference methods (Lees et al., 2000).

2.4 Conclusions

This work aimed to evaluate methods, time and rates of N application and their impact on winter wheat grain yield, protein, NUE, and gaseous N loss under rainfed conditions. This work indicated that the timing of N application had a significant impact on grain yield, grain protein, and NUE, and more so than the N application method. Likewise, we noted similar grain yield, improved grain protein concentration, and higher NUE with topdressing in comparison to preplant and split N application. The difference method used in this work for calculating gaseous N loss did no always reveal similar results from one year or location to the next. Estimated plant N loss was variable from site to site and year to year and that depended on daily fluctuations in the environment.

2.5 References

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Table 2.1: Initial chemical properties of soils (0-15 cm) collected for 2017, 2018, and 2019 growing season, Efaw and 2017 and 2018 Perkins, OK.

Site	Year	pH	NH_4-N	NO_3-N	P	K
				mg kg $^{-1}$		
Efaw	2017	5.7	3	8	20	232
	2018	5.9	35	12	22	187
	2019	5.9	3	2	20	194
Perkins	2017	6.9	27	2	27	225
	2018	6.9	10	1	13	170

pH-1:1 soil: water; NH_4-N and NO_3-N – 2 M KCl; K and P- Mehlich 3

Table 2.2: Treatment structure employed at Efaw, 2017, 2018, and 2019 and Perkins, 2017, and 2018.

Treatment	Preplant N rate kg N ha^{-1}	Preplant application method	Midseason N rate kg N ha^{-1}	Midseason application method	Total N applied kg N ha^{-1}	Acronym
1	0	-	0	-	0	Check
2	90	Surface (S)	0	-	90	Pre-90 (S)
3	90	Depth (D)	0	-	90	Pre -90 (D)
4	45	Surface (S)	45	Surface (S)	90	Pre-45 (S)+Top-45 (S)
5	45	Surface (S)	45	Depth (D)	90	Pre-45 (S)+Top-45 (D)
6	45	Surface (S)	45	Streamer (R)	90	Pre-45 (S)+Top-45 (R)
7	45	Depth (D)	45	Surface (S)	90	Pre-45 (D)+Top-45 (S)
8	45	Depth (D)	45	Depth (D)	90	Pre-45 (D)+Top-45 (D)
9	45	Depth (D)	45	Streamer (R)	90	Pre-45 (D)+Top-45 (R)
10	0	-	90	Surface (S)	90	Top-90 (S)
11	0	-	90	Depth (D)	90	Top-90 (D)
12	0	-	90	Streamer (R)	90	Top-90 (R)

Table 2.3: Monthly cumulative precipitation (PT., mm), and average temperature (TAVG, °C) for Efaw, 2017, 2018, 2019, and Perkins, 2017, and 2018, OK.

Location		2017		2018		2019		10-yr avg	
Efaw	Month	PT.	TAVG	PT.	TAVG	PT.	TAVG	PT.	TAVG
	Oct	98	20	161	16	119	15	81	16
	Nov	22	13	8	11	23	6	47	10
	Dec	10	3	24	4	93	4	32	4
	Jan	65	5	6	2	67	3	23	3
	Feb	56	10	63	4	50	3	49	5
	Mar	48	13	30	11	58	8	51	11
	Apr	252	16	52	12	134	16	115	16
	May	66	20	99	24	439	20	134	20
	June	73	6	152	27	107	24	81	24
Total		690	12	595	12	1090	11	613	12
Perkins	Oct	54	20	144	16			87	16
	Nov	55	13	7	11			55	10
	Dec	12	3	16	4			38	4
	Jan	67	5	4	2			26	3
	Feb	50	10	83	4			46	5
	Mar	60	-	20	11			49	9
	Apr	230	-	66	12			114	16
	May	100	20	100	24			136	20
	June	53	25	145	26			82	26
Total		681	11	596	12			633	12

Table 2.4: Analysis of variance for main effects and interaction effects of factors and single degree of freedom contrasts for grain yield (GY), Grain Protein (GP), and nitrogen use efficiency (NUE) by site year.

	Site year														
	Efaw 2017			Efaw 2018			Efaw 2019			Perkins 2017			Perkins 2018		
Source	GY	GP	NUE	GY	GP	NUE	GY	GP	NUE	GY	GP	NUE	GY	GP	NUE
Preplant method	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns
Topdress method	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	***	ns	ns	ns	ns
Preplant*Topdress	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatments	**	***	*	ns	*	ns	***	***	ns	***	***	**	***	***	**
Contrasts (Treatments)															
1. Preplant (2,3) vs Split (4-9)	ns	*	ns	*	ns	ns	ns	ns	ns	*	*	**	ns	**	ns
2. Split (4-9) vs Topdress (10-12)	ns	***	***	ns	ns	ns	ns	***	**	ns	***	**	***	ns	***
3. Preplant (2,3) vs Topdress (10-12)	ns	***	**	ns	ns	ns	ns	***	ns	*	***	***	**	*	**
4. Preplant: S (2) vs D (3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
5. Topdress: S (10) vs D (11)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns
6. Topdress: S (10) vs R (12)	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	***	**	*	***	ns
7. Topdress: D (11) vs R (12)	ns	*	ns	ns	ns	ns	ns	**	ns	ns	***	ns	ns	**	ns
8. Split: S (4-6) vs D (7-9)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns
9. Split: S+S (4) vs D+S (7)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10. Split: S+D (5) vs D+D (8)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns
11. Split: S+R (6) vs D+R (9)	ns	ns		ns	ns		ns	ns		ns	**		ns	*	

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels, respectively. ns: not significant at the 0.05 level.

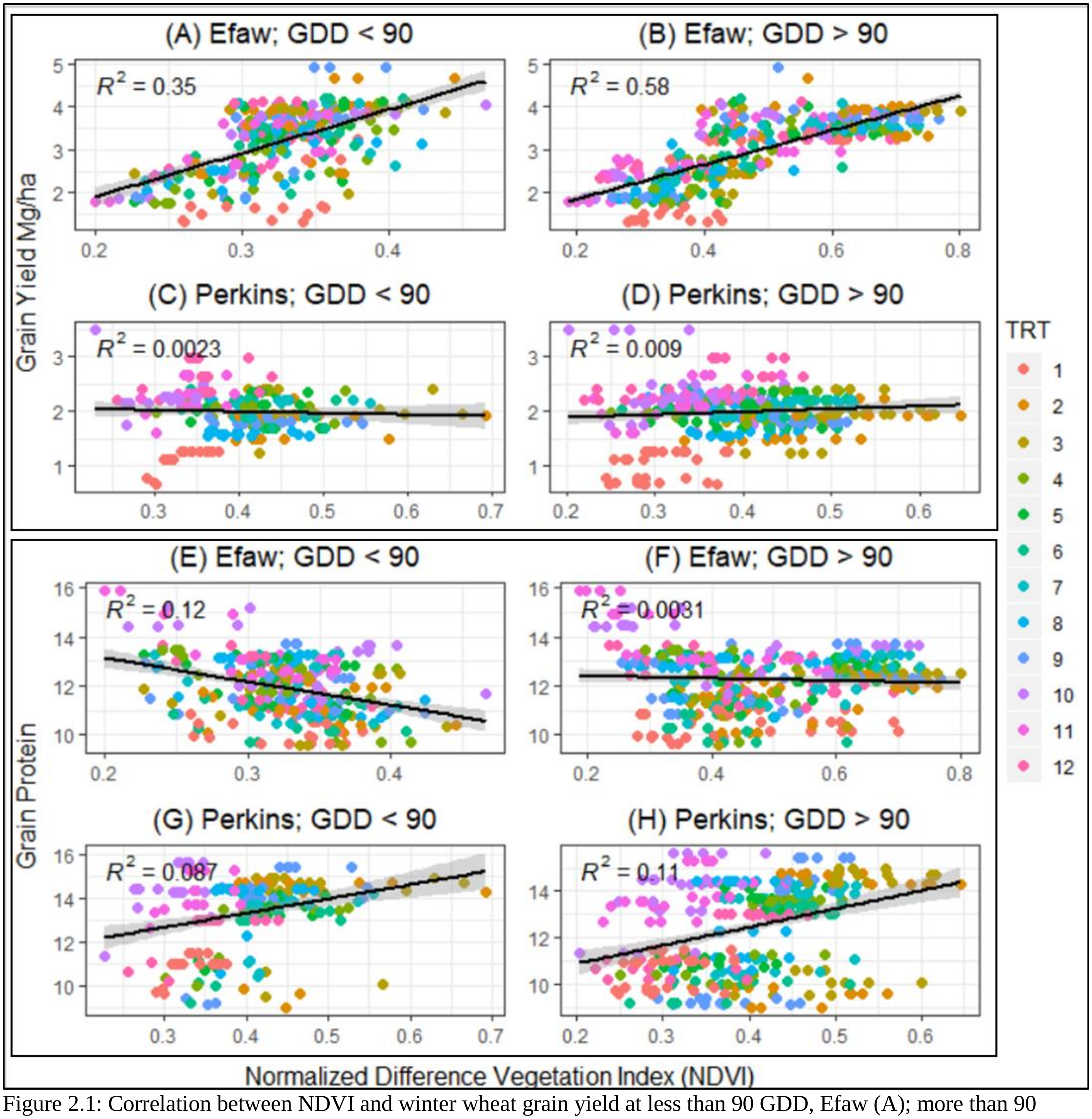

Figure 2.1: Correlation between NDVI and winter wheat grain yield at less than 90 GDD, Efaw (A); more than 90 GDD, Efaw (B); less than 90 GDD, Perkins (C); more than 90 GDD, Perkins (D). Grain protein and NDVI correlated at less than 90 GDD, Efaw (E); more than 90 GDD, Efaw (F); less than 90 GDD, Perkins (G); more than 90 GDD, Perkins (H).

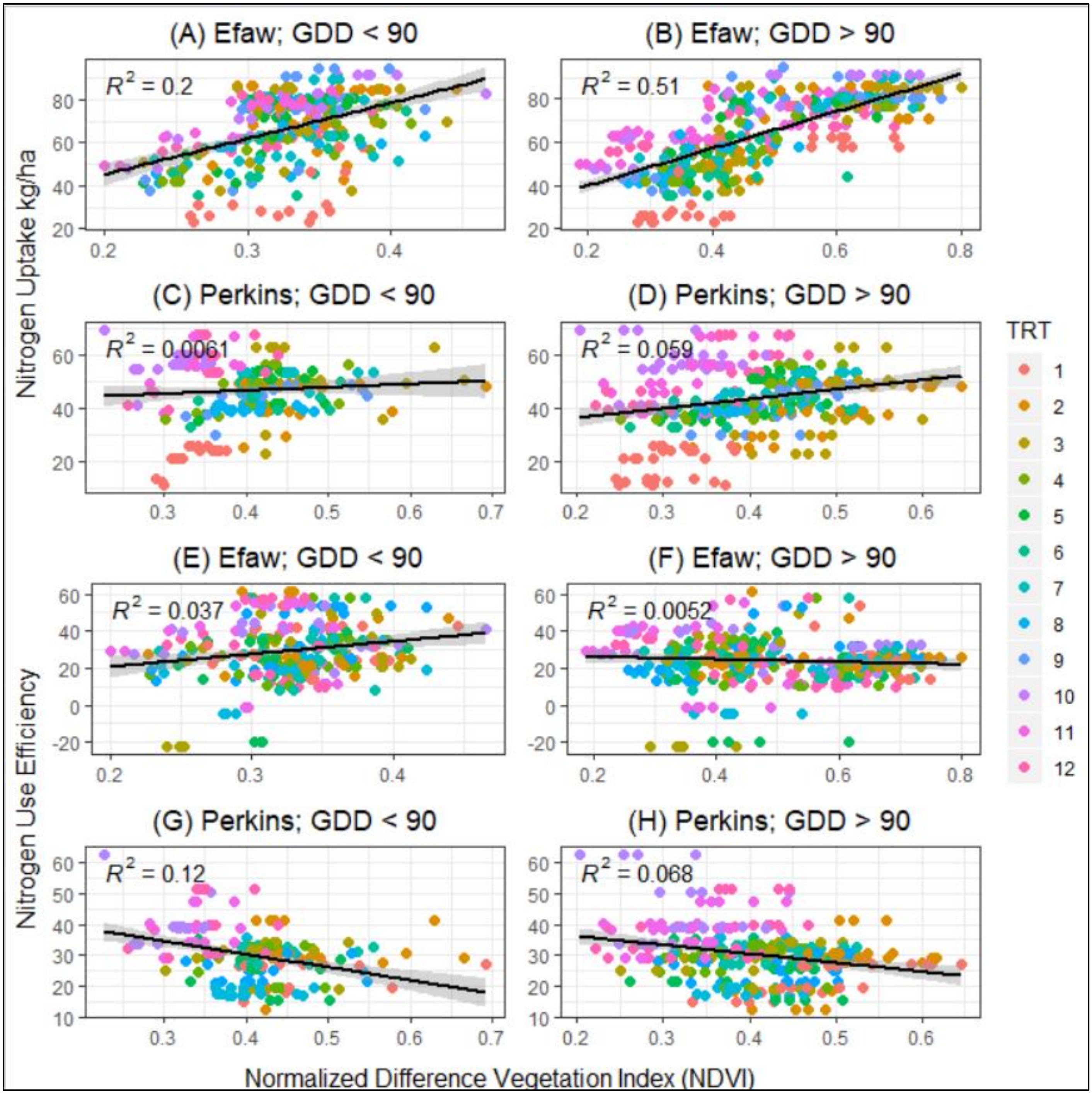

Figure 2.2: Correlation between NDVI and N uptake at less than 90 GDD, Efaw (A); more than 90 GDD, Efaw (B); less than 90 GDD, Perkins (C); more than 90 GDD, Perkins (D). Nitrogen use efficiency and NDVI correlated at less than 90 GDD, Efaw (E); more than 90 GDD, Efaw (F); less than 90 GDD, Perkins (G); more than 90 GDD, Perkins (H).

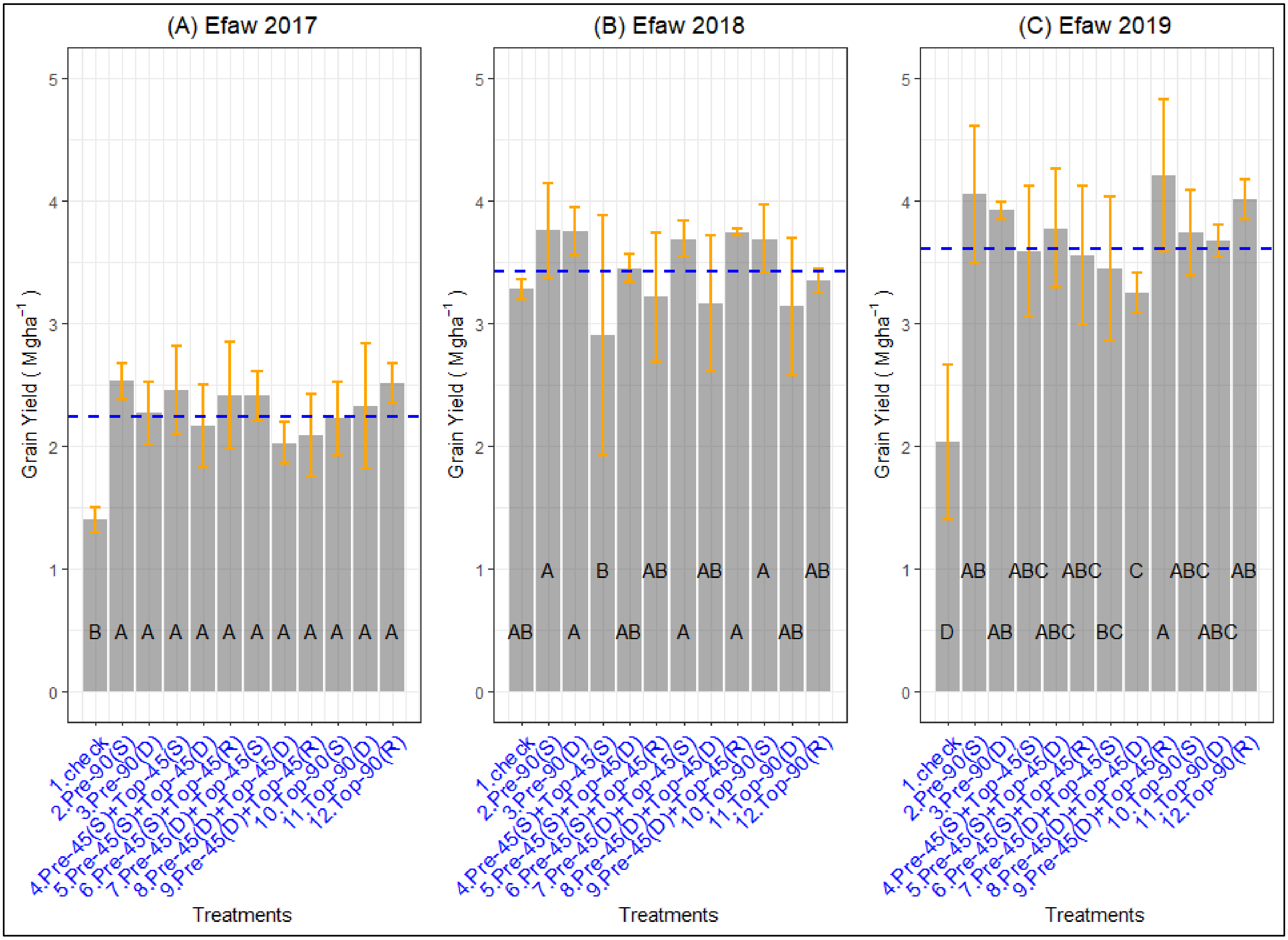

Figure 2.3: Grain yield as affected by treatments with horizontal dashed line representing the environmental mean grain yield at Efaw, 2017 (A); Efaw, 2018 (B); and Efaw, 2019 (C). Columns with same letters were not significantly different, LSD (α ≤ 0.05).

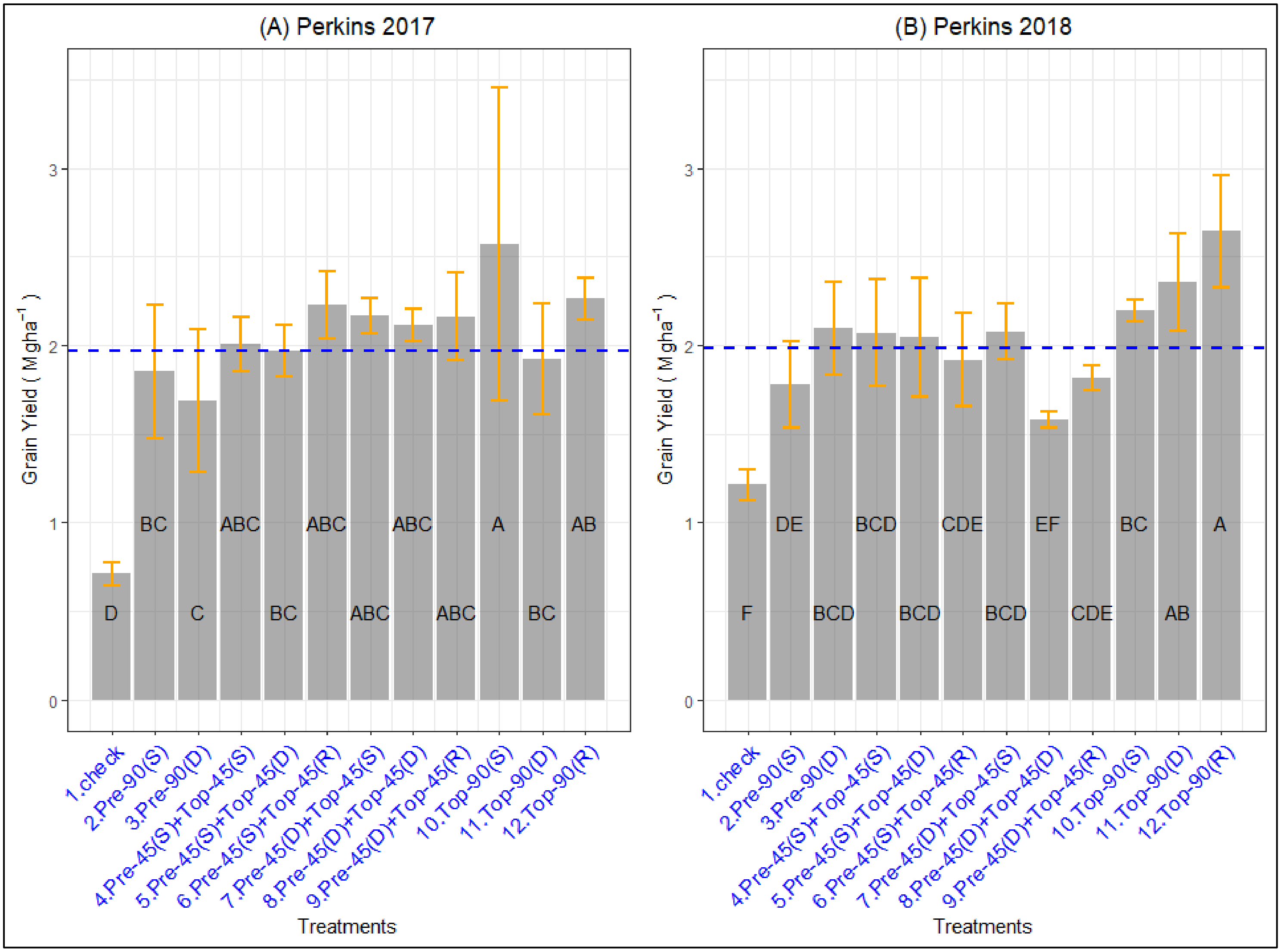

Figure 2.4: Grain yield as affected by treatments with horizontal dashed line representing the environmental mean grain yield at Perkins, 2017 (A); and Perkins, 2018 (B). Columns with same letters were not significantly different, LSD ($\alpha \leq 0.05$).

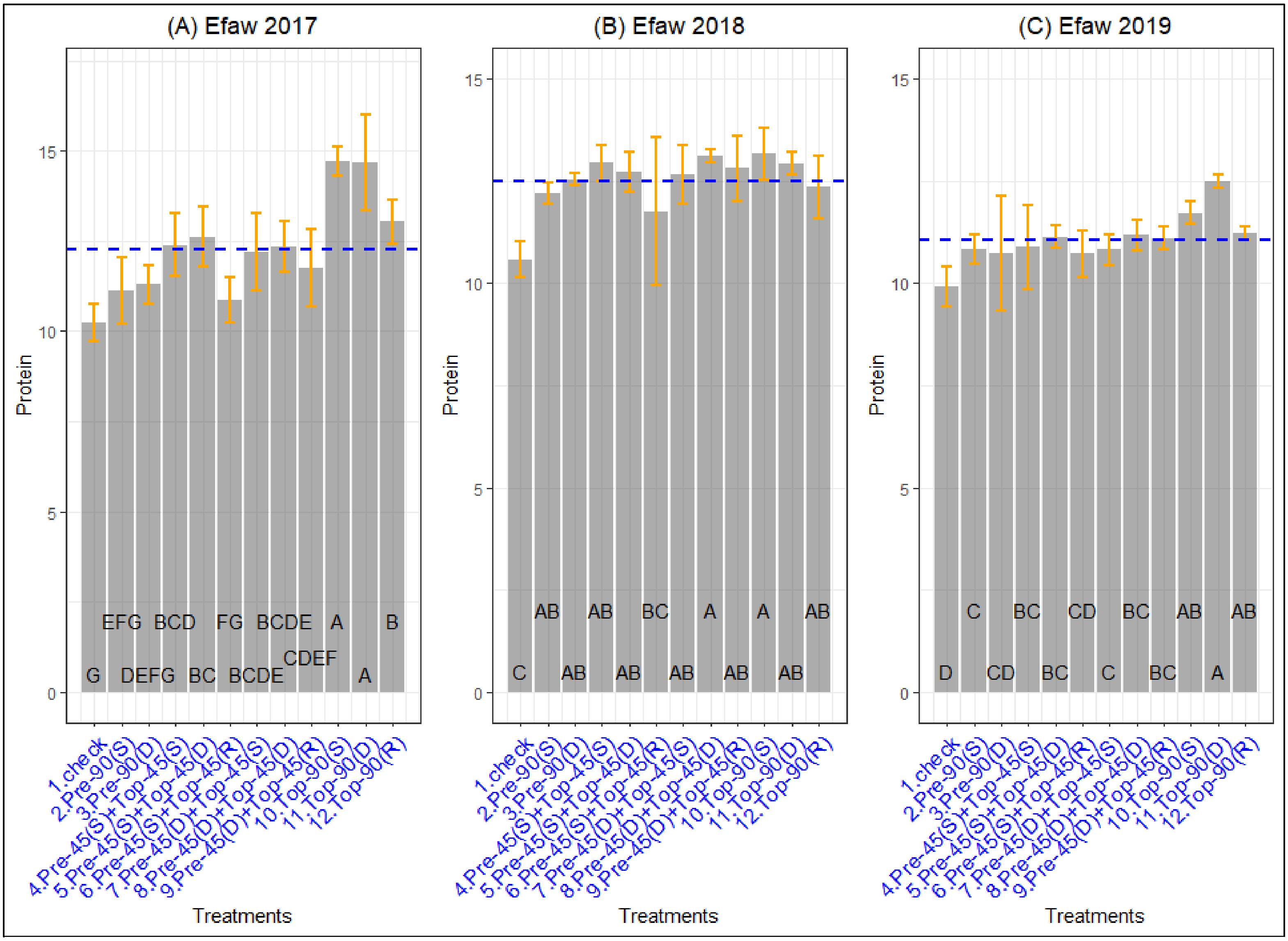

Figure 2.5: Grain protein (%) as affected by treatments with horizontal dashed line representing the mean protein level at Efaw, 2017 (A); Efaw (2018); and Efaw, 2019 (B). Columns with same letters were not significantly different, LSD (α ≤ 0.05).

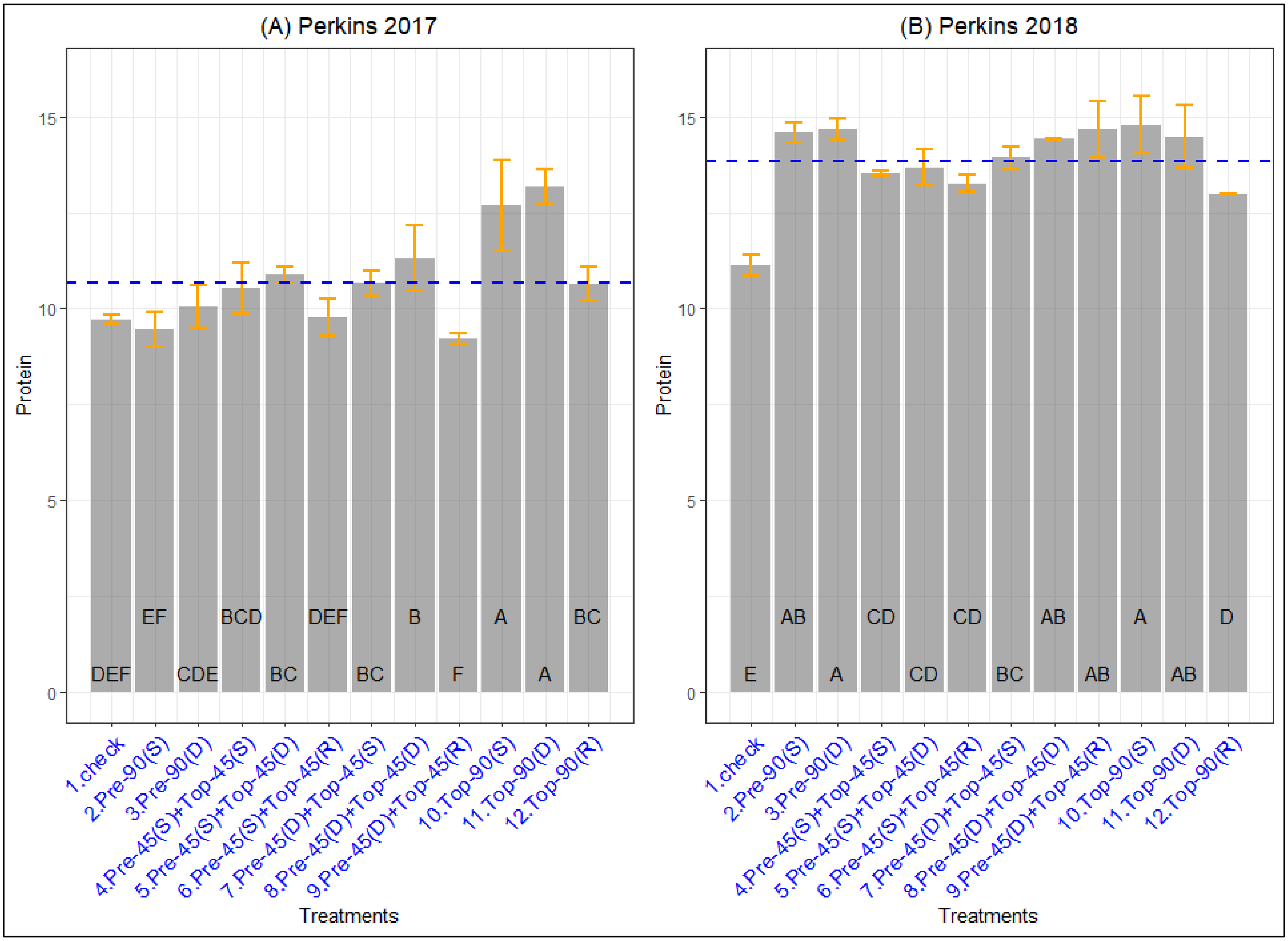

Figure 2.6: Grain protein as affected by treatments with horizontal dashed line representing the mean protein level at Perkins, 2017 (A); and Perkins, 2018 (B). Columns with same letters were not significantly different, LSD (α ≤ 0.05).

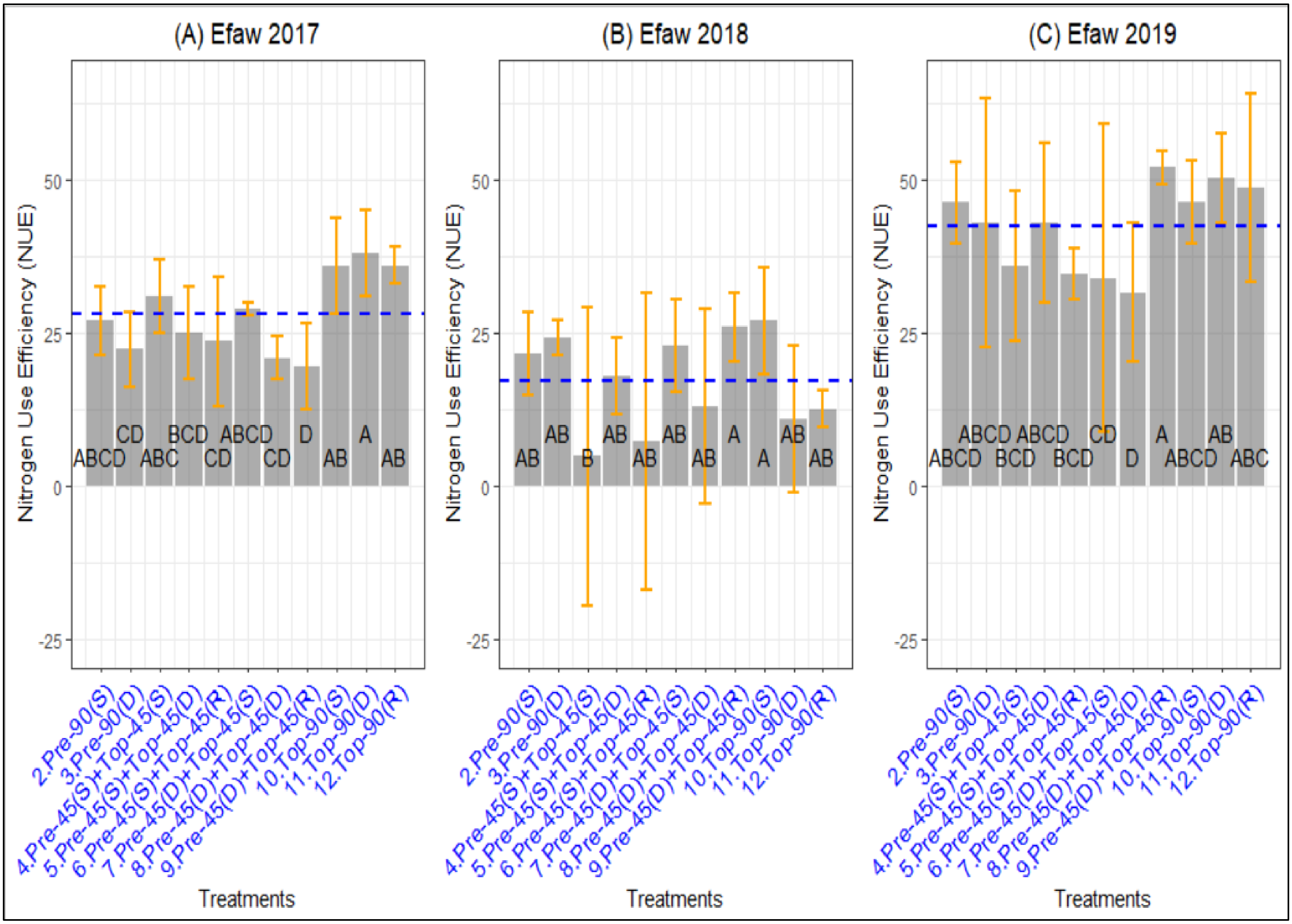

Figure 2.7: Nitrogen use efficiency as affected by treatments with horizontal dashed line representing the mean NUE at Efaw, 2017 (A); Efaw (2018) (B); and Efaw, 2019 (C). Columns with same letters were not significantly different, LSD (α ≤ 0.05).

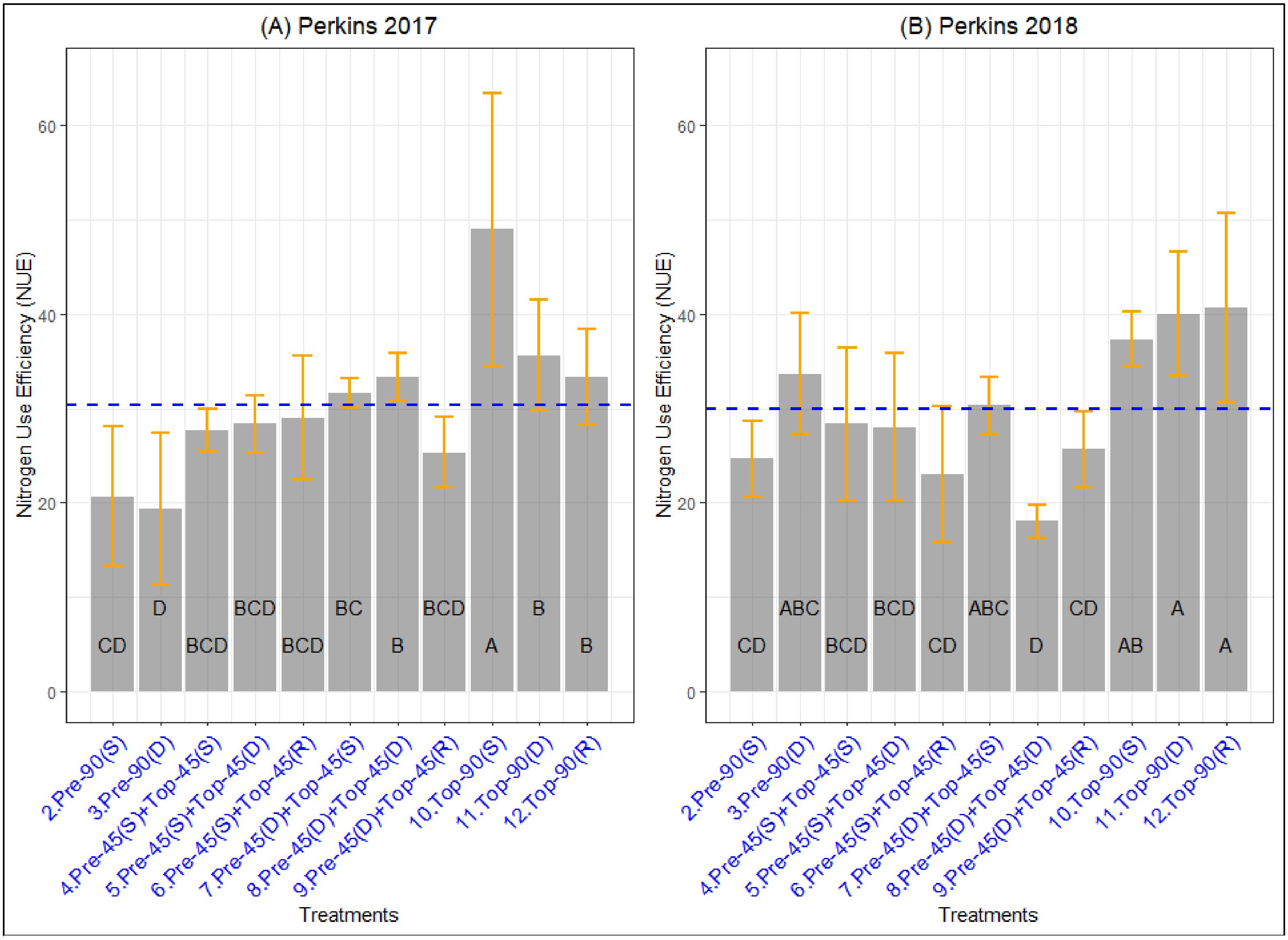

Figure 2.8: Nitrogen use efficiency as affected by treatments with horizontal dashed line representing the mean NUE at Perkins, 2017 (A); and Perkins, 2018 (B). Columns with same letters were not significantly different, LSD ($\alpha \leq 0.05$).

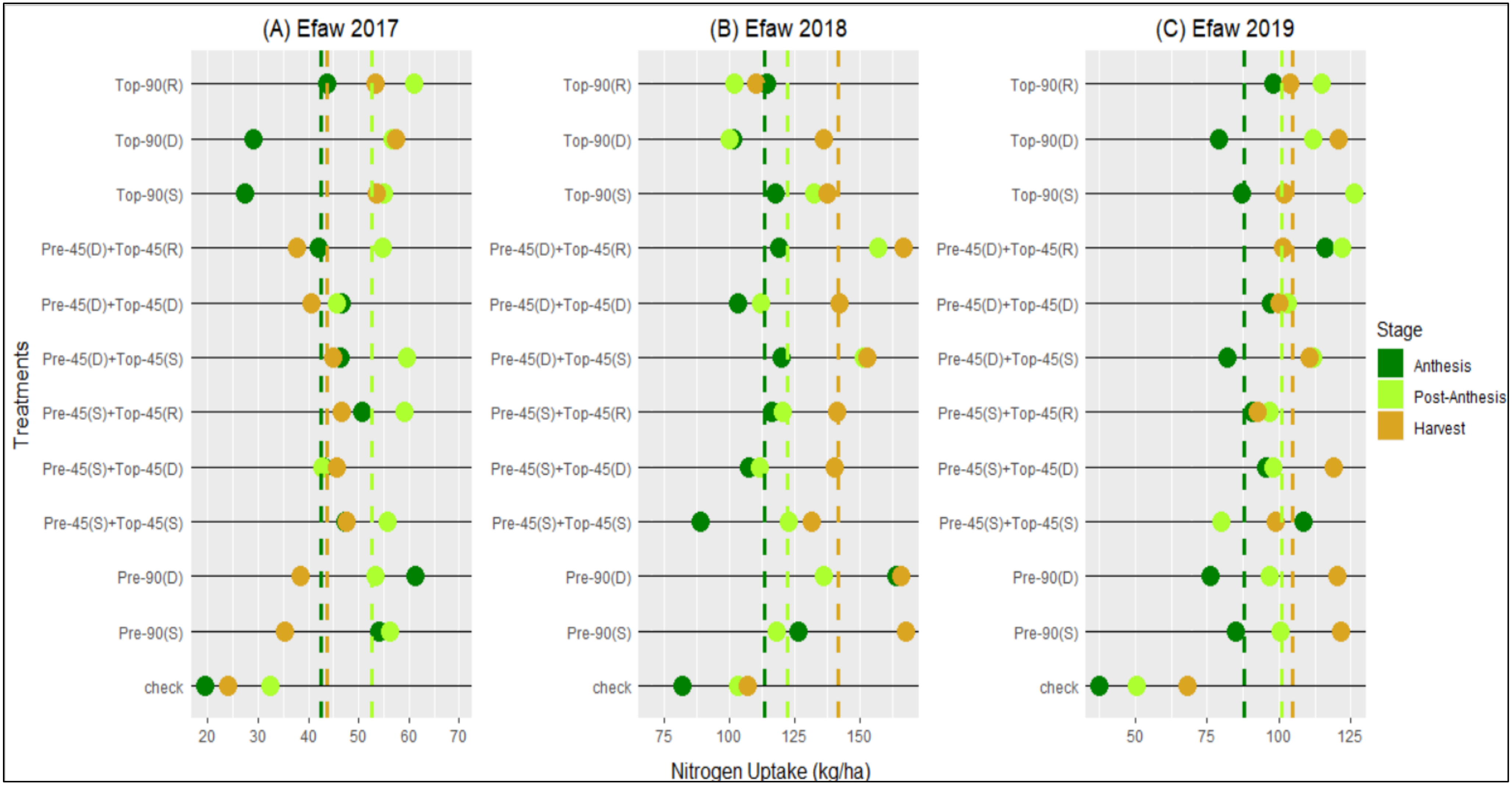

Figure 2.9: Forage N uptake by treatments at anthesis, post-anthesis and harvest. Vertical dashed lines represent mean N uptake at anthesis (green), post-anthesis (light green), and harvest (brown) at Efaw, 2017 (A); Efaw, (2018) (B) and Efaw, 2019 (C).

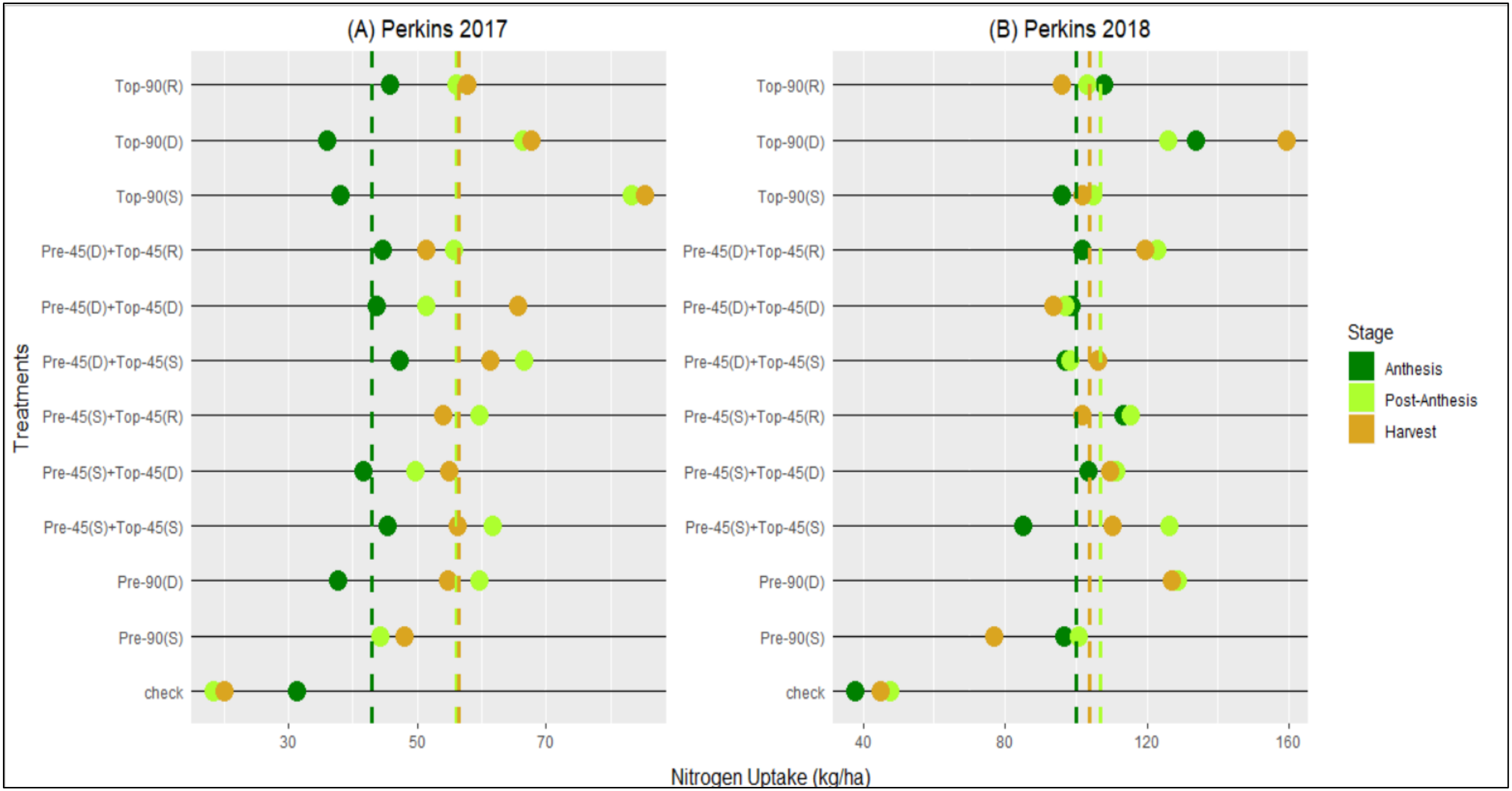

Figure 2.10: Forage N uptake by treatments at anthesis, post-anthesis and harvest. Vertical dashed lines represent mean N uptake at anthesis (green), post-anthesis (light green), and harvest (brown) Perkins, 2017 (A); and Perkins, 2018 (B).

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