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Nitrogen management impact on winter wheat grain yield and estimated plant nitrogen loss

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Abstract

Method of N application in winter wheat (*Triticum aestivum* L.) and its impact on estimated plant N loss has not been extensively evaluated. The effects of the pre-plant N application method, topdress N application method, and their interactions on grain yield, grain protein concentration (GPC), nitrogen fertilizer recovery use efficiency (NFUE), and gaseous N loss was investigated. The trials were set up in an incomplete factorial within a randomized complete block design and replicated three times for 5 site-years. Data collection included normalized difference vegetation index (NDVI), grain yield, and forage and grain N concentration. The NDVI before and after 90 growing degree days (GDD) were correlated with final grain yield, grain N uptake, GPC, and NFUE. At Efaw location, NDVI after 90 GDDs accounted for 58% of variation in grain yield and 51% variation in grain N uptake. However, NDVI was found to be a poor indicator of both GPC and NFUE. 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 GPC and NFUE were improved with the topdress applications. Generally, topdress application enhanced GPC and NFUE 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.

1 | INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is an essential crop for national and global food security. It covers approximately 3 million hectares of Central Rolling Red Plains of the United States, including parts of Kansas, Oklahoma, and Texas (Bushong, Arnall, & Raun, 2014). In the state of Oklahoma, winter wheat encompasses 75% of the cropland (Patrignani, Lollato, Ochsner, Godsey, & Edwards, 2014) and is produced under rain-fed conditions (Baath, Northup, Rocateli, Gowda, & Neel, 2018; Vitale, Godsey, Edwards, & Taylor, 2011).

In 2019, a total of 17.8 million hectares of winter wheat were planted, from which 10 million ha were harvested at a rate of 1.8 Mg ha^{-1} (USDA-NASS, 2019). Similar results in Oklahoma were noted by Patrignani et al. (2014), where they noted that winter wheat yield has been stagnant since the 1980s, with a current state yield of 2.0 Mg ha^{-1} compared to

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Abbreviations: F, foliar; GDD, growing degree days; GPC, grain protein concentration; I, injected; NDVI, normalized difference vegetation index; NFUE, nitrogen fertilizer recovery use efficiency; S, surface.

experimental state yields of 6.59 Mg ha^{-1}. Similar yield stagnation issues have also been noticed in other wheat-growing regions of the world, such as the Netherlands, the United Kingdom, India, France, Germany, and Denmark (Grassini, Eskridge, & Cassman, 2013). Irrespective of the yield stagnation, the consumption of fertilizers for wheat production is increasing. Globally, N, P, and K fertilizer consumption per unit area has increased eight times (Lu & Tian, 2017), 3.5 times (Dhillon, Torres, Driver, Figueiredo, & Raun, 2017), and three times (Dhillon, Eickhoff, Mullen, & Raun, 2019), respectively, since the year 1961. Nitrogen remains the most used (Aulakh & Malhi, 2005; Raun et al., 2005) and limiting nutrient in crop production (Fageria & Baligar, 2005; Ladha, Pathak, Krupnik, Six, & Kessel, 2005; Malhi, Grant, Johnston, & Gill, 2001). Without N fertilization, only half of the world's current population would have sufficient food (Dawson and Hilton, 2011). 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 (Moll, Kamprath, & Jackson, 1982). Worldwide, NUE for cereal crops, including wheat, is only 33% (Raun & Johnson, 1999). Lower NUE results in plant N deficiency, which could lead to decreased crop yield and quality (Cassman, Dobermann, & Walters, 2002). Lower NUE could mainly be associated with N losses (Raun & Johnson, 1999), over-fertilization (Sowers, Pan, Miller, & Smith, 1994), odd timing (Macnack, Khim, Mullock, & Raun, 2014), and methods of N application.

One of the predominant N losses from cereal crops is volatilization from crop canopy where N from aboveground biomass is lost in gaseous form. Kanampiu, Raun, and Johnson (1997) reported 4 to 27.9 kg ha^{-1} gaseous N loss from different genotypes of winter wheat. Daigger, Sander, and Peterson (1976) reported 25 to 80 kg N ha^{-1} loss from wheat. Harper, Sharpe, Langdale, and Giddens (1987) found this aerial loss of 8.3 kg N ha^{-1}at 20-d post fertilizer application, and an additional 7.1 kg N ha^{-1} loss from anthesis to harvest. Lees, Raun, and Johnson (2000)) noted a 12 kg N ha^{-1} gain to 42 kg N ha^{-1} loss from flowering to maturity.

Improving crop production, soil health, and the economic and environmental aspects of N fertilizers requires good fertilizer N management practices (Fageria et al., 2003; Singh & Ryan, 2015). 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 & Ryan, 2015).

Historically, yield goals were used for estimating N fertilizer requirement, but their adequacy has been refuted (Raun et al., 2017a). Furthermore, Raun et al. (2017) suggested the use of active sensors using vegetation indices to recommend in-season fertilizer N recommendation. 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, Townshend, & Goff, 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, Monroe, Raun, & Stone, 2012). The crop canopy sensors quantify differences in fertilized and non-fertilized plots via NDVI (Franzen, Kitchen, Holland, Schepers, & Raun, 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).

Core Ideas

- NDVI data collected after 90 GDDs was found to be a good indicator of final grain yield and N uptake
- Timing of N application had a significant impact on grain yield, grain protein concentration, and NUE, and more so than the N application method.
- Topdress application of N enhanced grain protein concentration, NUE and final grain yield.
- Estimated plant N loss was found to be highly variable.

TABLE 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[a]	NH_4-N mg kg^{-1}	NO_3-N	P	K
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

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

TABLE 2 Treatment structure employed at Efaw, 2017, 2018, and 2019 and Perkins, 2017, and 2018

Treatment	Pre-plant N rate kg N ha^{-1}	Pre-plant 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	Injected (I)	0	-	90	Pre -90 (I)
4	45	Surface (S)	45	Surface (S)	90	Pre-45 (S)+Top-45 (S)
5	45	Surface (S)	45	Injected (I)	90	Pre-45 (S)+Top-45 (I)
6	45	Surface (S)	45	Foliar (F)	90	Pre-45 (S)+Top-45 (F)
7	45	Injected (I)	45	Surface (S)	90	Pre-45 (I)+Top-45 (S)
8	45	Injected (I)	45	Injected (I)	90	Pre-45 (I)+Top-45 (I)
9	45	Injected (I)	45	Foliar (F)	90	Pre-45 (I)+Top-45 (F)
10	0	-	90	Surface (S)	90	Top-90 (S)
11	0	-	90	Injected (I)	90	Top-90 (I)
12	0	-	90	Foliar (F)	90	Top-90 (F)

TABLE 3 Monthly cumulative precipitation (PT., mm), and average temperature (TAVG,°C) for Efaw, 2017, 2018, 2019, and Perkins, 2017, and 2018, OK

		2017		2018		2019		10-yr avg.	
Location	Month	PT.	TAVG	PT.	TAVG	PT.	TAVG	PT.	TAVG
Efaw									
	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

There is an absence 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 | MATERIALS AND METHODS

For this study, wheat experiments were established in 2016–2017 (2017), 2017–2018 (2018), and 2018–2019 (2019). These experiments were located in Perkins and Efaw, just

TABLE 4 Correlation between normalized difference vegetation index (NDVI) with grain yield, grain protein concentration (GPC), N uptake, and nitrogen fertilizer recovery use efficiency (NFUE) across site-years

Correlation	Efaw		Perkins	
NDVI vs	GDD less than 90	GDD more than 90	GDD less than 90	GDD more than 90
Grain yield	.35	.58	.01	.01
GPC	.12	.01	.08	.11
Nitrogen uptake	.20	.51	.01	.06
NFUE	.04	.01	.12	.07

North of Stillwater, OK. 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 from 0–15-cm depth composed of 15 cores were taken from each plot before planting. The soil samples were dried at 60°C overnight and ground to pass a 2-mm sieve. A 1:1 soil/water suspension and glass electrode were used to measure soil pH and buffer index (Sikora, 2006; Sims, 1996). Soil NO_3–N and NH_4–N were extracted with a 1 M KCl solution and quantified using a Flow Injection Autoanalyzer. Plant-available P and K were extracted with Mehlich 3 solution (Mehlich, 1984) and quantified using a Spectro CirOs ICP spectrometer (Soltanpour, Johnson, Workman, Jones, & Miller, 1996). A comprehensive description of the soil analysis is reported in Table 1.

Herbicide and pesticide applications were applied as required throughout the season. Experimental plots were 6 m long and 3 m wide. An incomplete factorial treatment structure with 12 treatments in a randomized complete block design with three replications. 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 (Table 2). All other treatments had 90 kg N ha^{-1} applied, which differed having 0, 50, or 100% of N applied pre-plant with remaining applied mid-season. The pre-plant was applied on the surface or injected to 10 cm using a coulter applicator. The mid-season application was a surface application, injected at 10-cm soil depth with coulter, or foliar applied with streamer nozzles. The coulter applicator used had six single disc wavy coulters each spaced at 0.50 m apart on a 3 m 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 (Treatments 6, 9, and 12), where nozzles where placed at a spacing of 0.15 m.

TABLE 5 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 (NFUE) by site-year

	Site-year														
	Efaw 2017			Efaw 2018 Efaw 2019						Perkins 2017			Perkins 2018		
Source	GY	GP	NFUE	GY	GP	NFUE	GY	GP	NFUE	GY	GP	NFUE	GY	GP	NFUE
Pre-plant method	ns[a]	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
Pre-plant×Topdress	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatments	**	***	*	ns	*	ns	***	***	ns	***	***	**	***	***	**
Contrasts (Treatments)															
Pre-plant (2,3) vs Split (4-9)	ns	*	ns	*	ns	ns	ns	ns	ns	*	*	**	ns	**	ns
Split (4-9) vs Topdress (10-12)	ns	***	***	ns	ns	ns	ns	***	**	ns	***	**	***	ns	***
Pre-plant (2,3) vs Topdress (10-12)	ns	***	**	ns	ns	ns	ns	***	ns	*	***	***	**	*	**
Pre-plant: S (2) vs I (3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Topdress: S (10) vs I (11)	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	*	ns	ns	ns
Topdress: S (10) vs F (12)	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	***	**	*	***	ns
Topdress: I (11) vs F (12)	ns	*	ns	ns	ns	ns	ns	**	ns	ns	***	ns	ns	**	ns
Split: S (4-6) vs I (7-9)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	ns
Split: S+S (4) vs I+S (7)	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Split: S+I (5) vs I+I (8)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	ns
Split: S+F (6) vs I+F (9)	ns	ns		ns	ns		ns	ns		ns	**		ns	*	

[a]ns: not significant at the .05 level.

*Significant at the .05, .01, and .001 level.

**Significant at the .05, .01, and .001 level.

***Significant at the .05, .01, and .001 level.

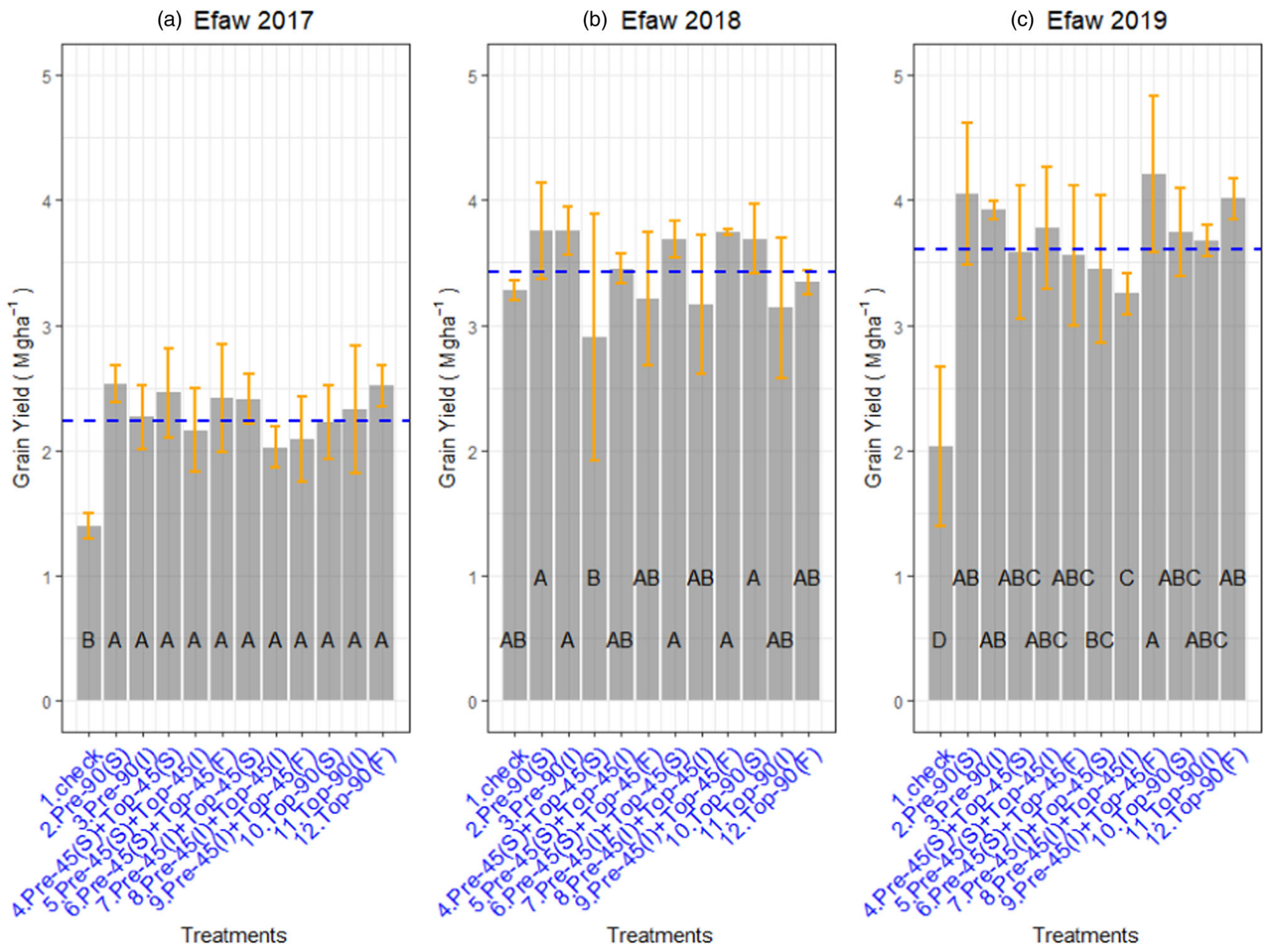

FIGURE 1 Grain yield as affected by treatments with the horizontal dashed line representing the environmental mean grain yield at (A) Efaw, 2017; (B) Efaw, 2018; and (C) Efaw, 2019. Columns with the same letters were not significantly different, LSD ($\alpha \leq .05$)

The treatment structure used at both locations is noted in Table 2.

Greenseeker sensor readings were taken throughout the season using Trimble's handheld green seeker. The NDVI readings were started at 50 growing degree days (GDDs) (Feekes 2) up to 137 GDDs (head emergence). Sufficient area was available in each plot to accommodate forage and grain yield harvest. Biomass samples were collected from 0.55 m^2 in 2017 by hand clipping at 2 cm above the ground at anthesis, 14-d post-anthesis, and physiological maturity to determine N uptake. In the season 2018 and 2019, the sampling area was 0.09 m^2. Grain was harvested for yield determination with a Massey Ferguson 8XP self-propelled combine. Subsamples from each plot were collected and analyzed for total N. All the forage and grain samples were ground properly to obtain subsamples using Thomas Wiley Laboratory mill (Thomas Scientific, Swedesboro, NJ). 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, MI).

Grain protein concentration, grain N uptake, and nitrogen fertilizer recovery use efficiency (NFUE) were calculated based on percentage N in grain using Equation (1), (2), (3):

$$\text{Grain protein conventration (GNC)} = \%\text{N} \times 5.7 \quad \text{(Tkachuk, 1969)}. \tag{1}$$

$$\text{Grain N uptake} = \%\text{N in grain} \times \text{grain yield} \tag{2}$$

$$NFUE = \frac{Grain\ N\ uptake\ (x) - Grain\ N\ uptake\ (check)}{Total\ N\ applied\ (x)} \times 100 \tag{3}$$

where x in Equation (3) stands for the treatment for which NFUE 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

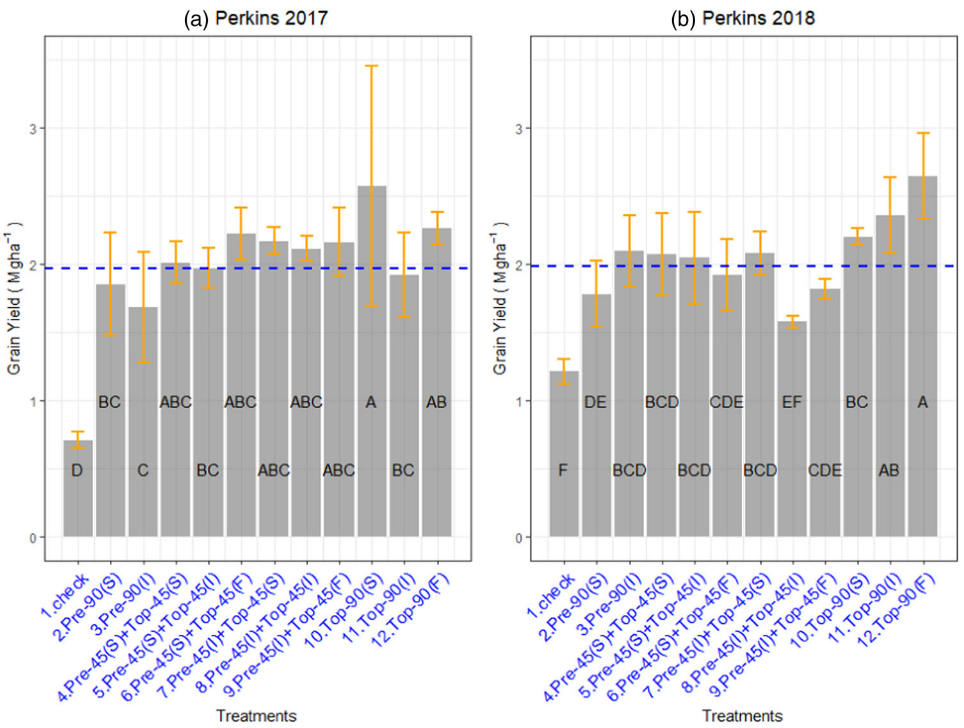

FIGURE 2 Grain yield as affected by treatments with a horizontal dashed line representing the environmental mean grain yield at (A) Perkins, 2017 ; and (B) Perkins, 2018. Columns with the same letters were not significantly different, LSD ($\alpha \leq .05$)

obtain plant N loss (Kanampiu et al., 1997). Total forage N at different stages were calculated using Equation (4)

$$\text{Forage N uptake} = \%\text{N in grain} \times \text{forage yield} \tag{4}$$

Data analysis was performed by keeping the treatments as random using the GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC), and treatment means were compared using the LSD mean separation procedure at the 5% significance level. In addition, a single degree of freedom contrasts was conducted to identify specific treatment differences. Additionally, R statistical software (R core team) was used for data visualization.

3 | RESULTS AND DISCUSSION

The study period encountered highly variable weather conditions, where 2019 received 1090 mm of rainfall, in comparison to 613 mm of 10-yr average rain (Table 3). At Perkins's location, total precipitation was consistent with the 10-yr average during both years and received 681 and 596 mm rainfall in 2017 and 2018, respectively. Considering these differences and following Raun et al. (2017b) reference where they mentioned that environmental effects are ignored upon combining yield data from experimental fields, as such data over the years was not integrated. We split NDVI reading based on GDD's for its comparison with grain yield, grain protein concentration (GPC), N uptake, and NFUE. The relationship between NDVI with grain yield at Efaw had an R^2 of 0.35 before 90 GDD and 0.58 after 90 GDD, but R^2 was <.01 at Perkins (Table 4). Recently, Dhillon, Figueiredo, Eickhoff, and Raun (2019) used a GDD based numerical scale and established that NDVI collected between 90 and 120 GDDs was ideal for N recommendation in winter wheat.

We further associated NDVI with GPC, where no correlation was found between these two variables (Table 4). 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 normalized difference red edge index (NDRE) was noted by Wang, Huggins, and Tao (2019).

The accuracy of site-specific in-season fertilizer management decisions is dependent on accurate prediction of N uptake (Ali, Ibrahim, & Singh, 2020) and grain yield potential (Dhillon et al., 2020). Numerous researchers have deduced that NDVI is a good indicator of N uptake (Raun et al., 2001, 2002; Solie, Raun, Whitney, Stone, & Ringer, 1996; Stone et al., 1996). 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 (Table 4). Alternatively, no correlation was noticed at Perkins

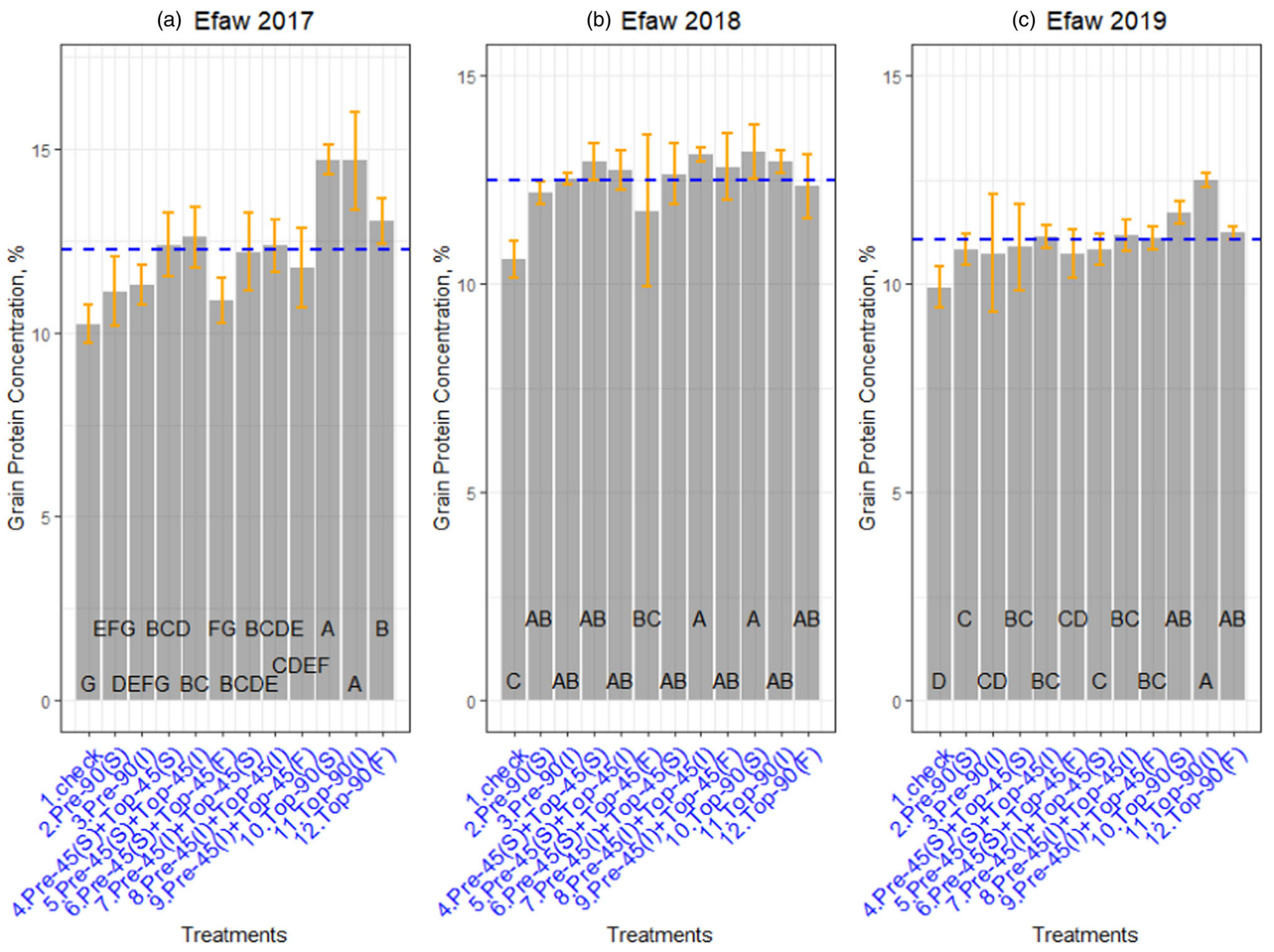

FIGURE 3 Grain protein as affected by treatments with the horizontal dashed line representing the mean protein level at (A) Efaw, 2017; (B) Efaw (2018); and Efaw, 2019. Columns with the same letters were not significantly different, LSD (α ≤ .05)

between NDVI and grain N uptake. Finally, we tried to explore whether NDVI could be a good indicator of NFUE. Nonetheless, no association was found between these two factors at any location (Table 4). 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.

3.1 | Grain yield

Analysis of variance at Efaw 2017 showed that treatments had a significant effect on grain yield (Table 5; Figure 1a). Where these differences were only noted due to a lower yield in the check plot (Figure 1a). However, no additional information was revealed via a single degree of freedom contrasts and mean separation using LSD (Table 5).

At Efaw 2018, LSD at alpha = .05 showed that pre-plant (Treatments 2 and 3), split (Treatments 7 and 9), and topdress application (Treatment 10) all resulted in similar and higher grain yields than other treatments (Figure 1b). Pre-plant (Treatments 2 and 3) resulted in higher yield when compared with all the split applied applications (Treatments 4–9) via the single degree of freedom contrasts(Contrast 1; Table 5). Similarly, a single degree of freedom contrasts showed that within split applications, the combination of surface applications (Treatment 4) yielded better than split between injected pre-plant and top-dressed at the surface (Treatment 7) (Contrast 9; Table 5). During 2019 at Efaw, only differences in grain yield were noted due to main treatment effect (Table 5), with lower yields in the check plot followed by the combination of injected split applications compared to other treatments (Figure 1c). Overall, in 3 site-years at this location, grain yields were improved with 90 kg N ha^{-1} regardless of method or timing of N application.

At Perkins during 2017, the main effect of treatments resulted in different grain yields (Table 5). Exploration of a single degree of freedom contrasts specified that split applications yielded better than pre-plant (Contrast 1; Table 5). Alternatively, topdress applications were better yielding than split applications (Contrast 2; Table 5; Figure 2a). Moreover, the surface-applied topdress application was better than

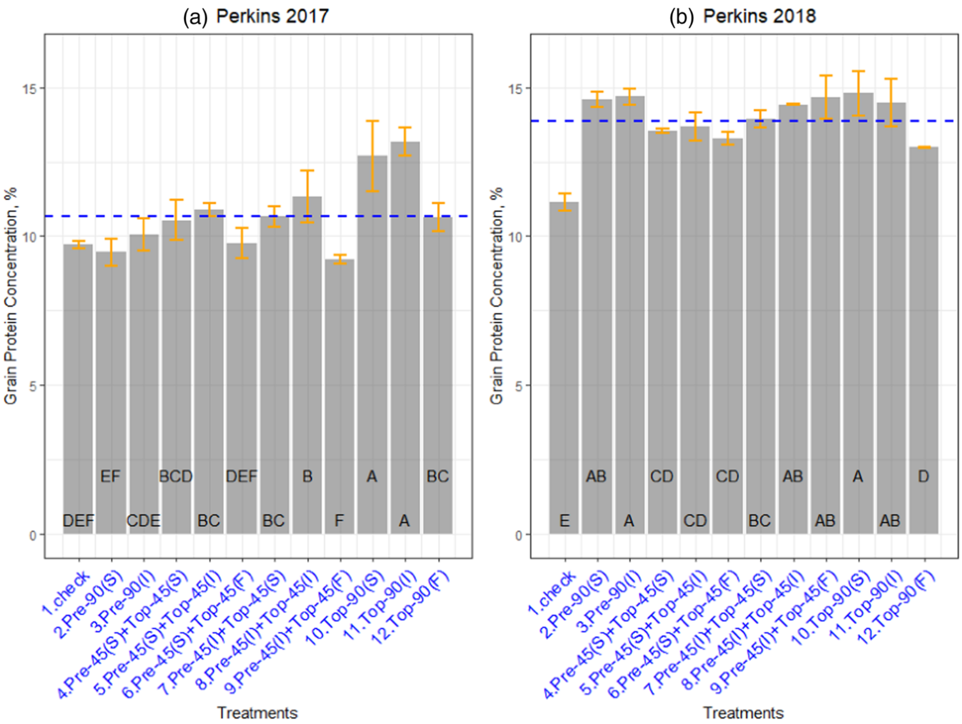

FIGURE 4 Grain protein as affected by treatments with a horizontal dashed line representing the mean protein level at (A) Perkins, 2017; and (B) Perkins, 2018. Columns with the same letters were not significantly different, LSD ($\alpha \leq .05$)

injected N (Contrast 5; Table 5; Figure 2a). During the 2018 season in Perkins highest grain yield was recorded with foliar topdress application (Figure 2b). As per contrasts, topdress applied N was better compared to pre-plant and split applications in terms of improving grain yield (Contrasts 2 and 3; Table 5). Within topdress, foliar application (Treatment 12) improved yield over surface application (Treatment 10) (Contrast 6; Table 5). Over 2 site-years at Perkins, topdress application resulted in higher yields compared to the rest of treatments with no clear distinction among methods used for N application. Westfall, Havlin, Hergert, and Raun (1996) noted N rate to be the dominant factor in obtaining optimum yields in dryland cropping systems in comparison to N placement, which is similar to our findings.

3.2 | Grain protein concentration

The mean grain protein concentration at Efaw 2017 was noted at 12% (Figure 3a). Grain protein concentration within treatments was affected due to the topdress N application method (Table 5). Single degree contrasts revealed high GPC with split applications related to pre-plant treatments (Table 5). Nevertheless, the GPC was considerably improved with topdressing compared to pre-plant [(Contrasts 2: Split (4–9) vs. Topdress (10–12)] and split [(Contrast 3: Pre-plant (1,2)) and topdress (10–12) Table 5, Figure 3a)]. The mean GPC was slightly higher at Efaw 2018 at 12.5% (Figure 3b). Additionally, no information could be gathered through single degree of freedom contrasts (Table 5). The lowest mean grain protein concentration of 11% was noted in 2019 at Efaw (Figure 3c). During the 2019 growing season highest GPC was accumulated with the topdress application of 90 kg N ha^{-1} injected 10 cm underneath soil surface (Figure 3c). At Efaw, over 3 site-year topdress applications improved GPC regardless of the method of N application.

Overall mean GPC at Pekins in 2017 was 10.7% (Figure 4a). Analysis of variance suggested noteworthy differences in GPC with the topdress method used at Perkins in 2017 (Table 5). With single degree of freedom contrasts, it was noted that GPC improved with split application compared to pre-plant application (Contrast 2; Table 5). Topdress application developed this trend further compared to split applications (Contrast 3; Table 5). Within topdress applications, both surface and injected applications were better than foliar applications (Contrasts 5 and 6; Table 5). The treatment difference found using LSD revealed considerably better GPC with topdress application on the surface (Treatment 10) and injected (Treatment 11) compared to the rest of the treatments (Figure 4a). During the 2018 growing season at Perkins, the mean GPC was 14% (Figure 4b), where the pre-plant and topdress applications resulted in higher GPC (Table 4). As per a single degree of freedom contrasts, pre-plant application at depth was better-compared to the surface application (Contrast 1; Table 5). However, LSD showed similar GPC with injected pre-plant application (Treatment 3) and topdress

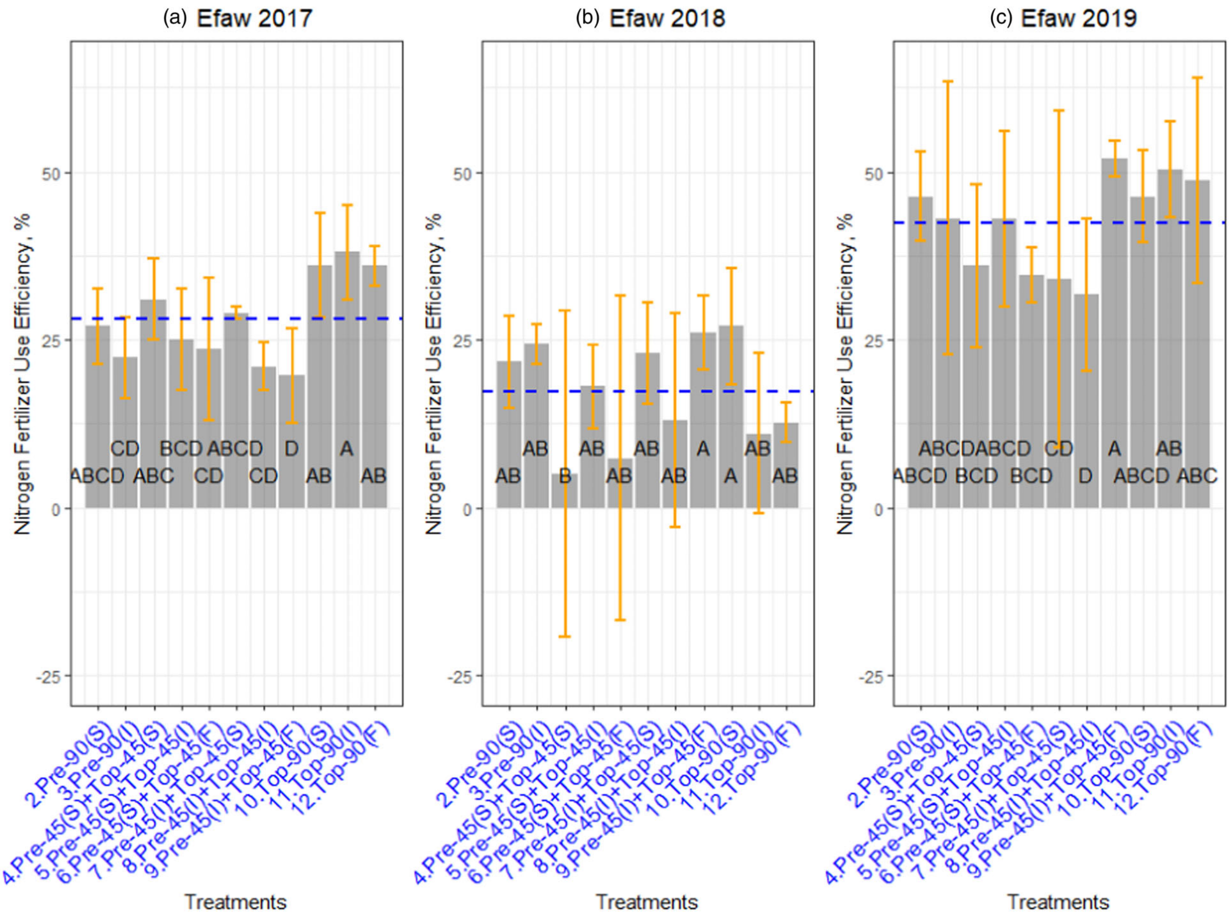

FIGURE 5 Nitrogen use efficiency as affected by treatments with the horizontal dashed line representing the mean NFUE at (A) Efaw, 2017; (B) Efaw (2018); and Efaw, 2019. Columns with the same letters were not significantly different, LSD ($\alpha \leq .05$)

N application on the surface (Treatment 10) (Figure 4b). Among split applications, injected pre-plant application resulted in higher GPC than surface application (Contrast 8; Table 5). Generally, over the study period, the GPC was predominantly influenced by timing rather than the method of N application, where the highest GPC was noted with topdressing followed by split and pre-plant N application. Similar results have been found by various other researchers (Mohammed et al. 2013); Bänziger, Feil, Schmid, & Stamp, 1994; Wuest & Cassman, 1992), where they noted increased GPC with topdressing N application. Lollato, Figueiredo, Dhillon, Arnall, and Raun (2019), in the synthesis analysis of three long term trials, concluded an increase in grain N concentration with an increase in N rates, but N concentration reduction with an increase in phosphorus (P) and potassium (K).

3.3 | Nitrogen fertilizer recovery use efficiency

At Efaw in 2017, the differences in NFUE were only noted due to the main effect of treatments. In contrast, the method and timing of N application did not result in any differences. As per LSD, topdress application resulted in higher NFUE compared to the rest of the treatments (Figure 5a). The same information was noted with a single degree of freedom contrast where NFUE with topdress applications were better than split (Contrast 2; Table 5) and pre-plant application (Contrast 3; Table 5). In the 2018 growing season at Efaw, the average NFUE was lower compared to 2017. Some negative values were noted due to higher grain yield and grain protein concentration in check treatment (Figure 5b). As per LSD, topdress application at the surface (Treatment 10) and split application with pre-plant at depth and foliar topdress (Treatment 9) both resulted in high NFUE compared to the rest of the treatments (Figure 5b). During 2019 at Efaw, analysis of variance revealed that only treatments were significant at altering NFUE. Single degree of freedom contrasts showed higher NFUE with split application compared to pre-plant applications (Contrast 1; Table 5). However, it was further noticed with the contrasts that topdress applications were better than both split (Contrast 2; Table 5) and pre-plant applications (Contrast 3; Table 5). Relying on treatment differences with LSD, topdress application resulted in higher NFUE compared to the rest of the treatments (Figure 6a). Similarly, during

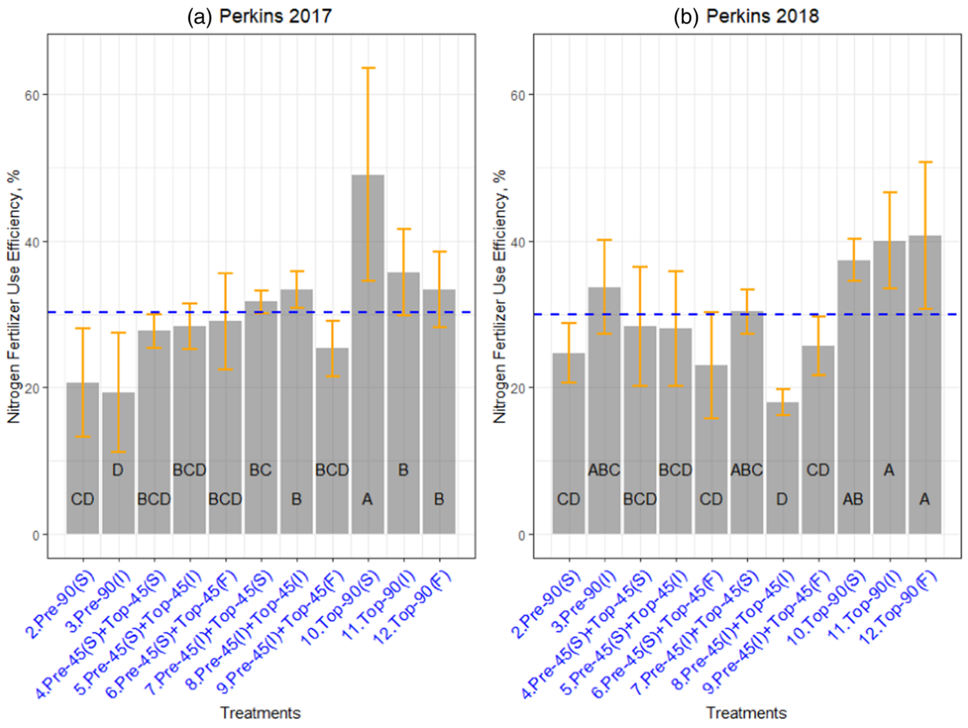

FIGURE 6 Nitrogen use efficiency as affected by treatments with a horizontal dashed line representing the mean NFUE at (A) Perkins, 2017; and (B)Perkins, 2018. Columns with the same letters were not significantly different, LSD (α ≤ .05)

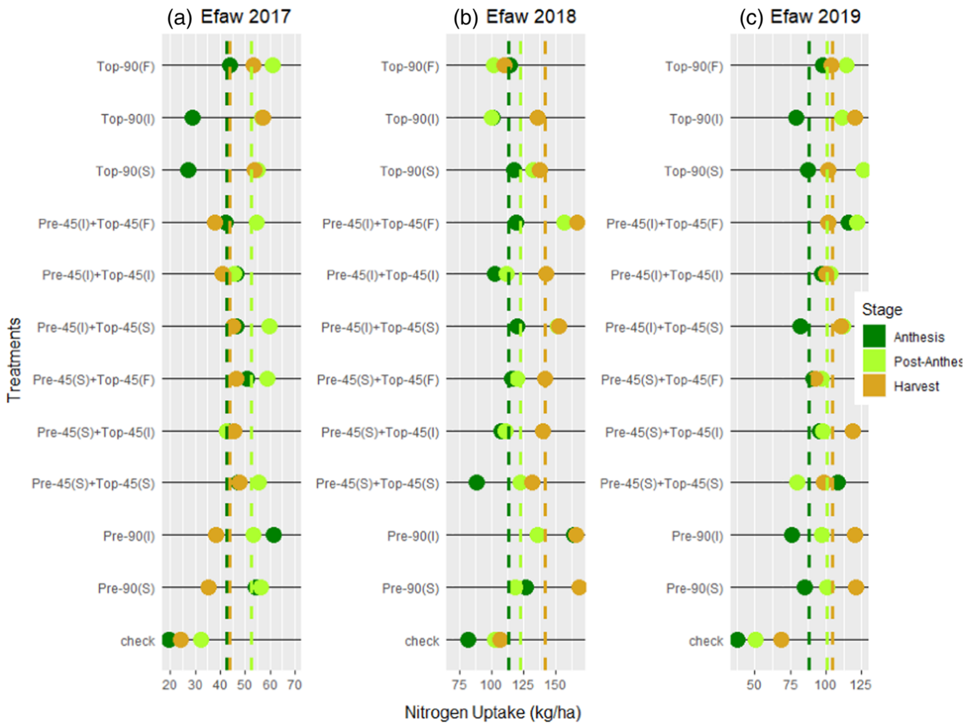

FIGURE 7 Forage N uptake by treatments at anthesis, post-anthesis and harvest. Vertical dashed lines represent mean N uptake at anthesis (green), post-anthesis (light green), and harvest (brown) at (A) Efaw, 2017; (B) Efaw, (2018); and(C) Efaw, 2019

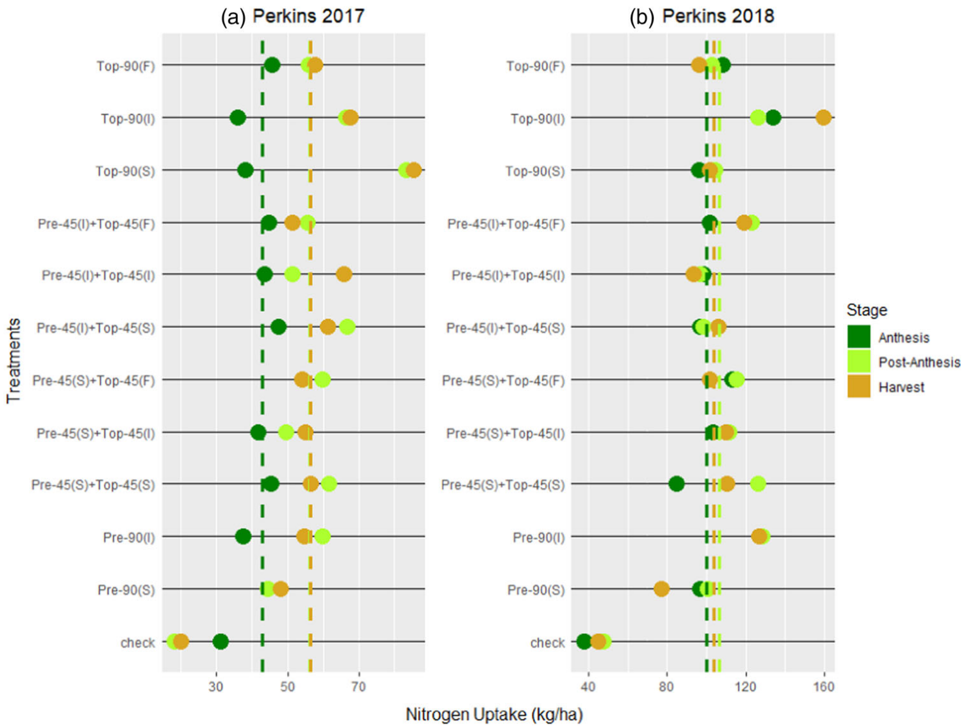

FIGURE 8 Forage N uptake by treatments at anthesis, post-anthesis and harvest. Vertical dashed lines represent mean N uptake at anthesis (green), post-anthesis (light green), and harvest (brown) (A) Perkins, 2017; and (B) Perkins, 2018

2018 at Perkins, topdress applications were better compared to the rest of the treatments (Figure 6b). Similar results were revealed with a single degree of freedom contrasts (Table 5).

Our results indicated higher NFUE with the topdressing application, which is also in disagreement with various researchers who pointed out enhanced NFUE with split N application (Alcoz, Hons, & Haby, 1993; Ayoub, MacKenzie, & Smith, 1995; Sowers et al., 1994). In terms of NFUE, topdressed application injected at 10 cm 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 NFUE compared to pre-plant N.

Many researchers have noted the significance of split application in the optimization of yield and grain protein (Boman, Westerman, Raun, & Jojola, 1995; Cassman, Bryant, Fulton, & Jackson, 1992; Mascagni & Sabbe, 1991; Ottman and Pope, 2000; Woolfolk et al., 2002) which is somewhat different than what we found. 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. Generally, different application methods did not make any significant impact in altering grain yields. Still, grain protein was improved with the topdressing N at the surface in 4 of 5 site-years of study.

3.4 | Estimated gaseous nitrogen loss

Gaseous N losses were determined 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 7a). Pre-plant applied treatments resulted in most N loss since anthesis (Figure 7a). 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 uptake at all the stages compared to 2017 (Figure 7b). 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 7c). Perkins 2017, was consistent with Efaw 2018 and 2019 and showed no N loss (Figure 8a). Forage N uptake was similar at post-anthesis and harvest stages. Alternatively, at Perkins in 2019, there was low gaseous N loss. This work showed that N uptake continued before and beyond anthesis (Figure 8b). 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 higher 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).

4 | CONCLUSIONS

This work aimed to evaluate methods and timing of N application and their impact on winter wheat grain yield, GPC, NFUE, and gaseous N loss under rainfed conditions. This work indicated that the timing of N application had a significant impact on grain yield, GPC, and NFUE, and more so than the N application method. Likewise, we noted similar grain yield, improved GPC, and higher NFUE with topdressing in comparison to pre-plant and split N application. The difference method used in this work for calculating gaseous N loss did not 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 relied on daily fluctuations in the environment.

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