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Research Article

Evaluating Split Nitrogen Applications and In-Season Tests for Organic Winter Bread Wheat

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Abstract: Achieving high grain yields and crude protein (CP) standards in organic winter wheat (*Triticum aestivum* L.) is challenging because ensuring that adequate nitrogen (N) is available at key periods of wheat growth is difficult in organic systems. Split application regimes and in-season N management tests may improve organic production. In field trials conducted over four site-years in Maine and Vermont, USA, N application regimes were analyzed for their effects on organic winter wheat, N uptake, grain yield, and CP. Tiller density and tissue N tests were evaluated as in-season decision tools. Eight treatments arranged in a non-factorial design differed in terms of N application timing (pre-plant (PP), topdress at tillering (T1), and topdress at pre-stem extension (T2)) and N rate. Treatments were: (1) an untreated check, (2) pre-plant N at a low rate of 78 kg N ha^{-1} (PP$_L$), (3) pre-plant N at a high rate of 117 or 157 kg N ha^{-1} (PP$_H$), (4) T1$_{78}$, (5) PP$_L$ + T1$_{39}$, (6) PP$_L$ + T2$_{39}$, (7) PP$_H$ + T2$_{39}$, and (8) PP$_L$ + T1$_{39}$ +T2$_{39}$. Responses to N treatments were variable among site-years, however some common results were identified. The PP-only treatments increased grain yields more than they increased CP. The T1$_{78}$ and PP$_H$ + T2$_{39}$ treatments were the most effective at increasing yield and CP, compared with the PP-only treatments. Tiller density and tissue N tests were good predictors of grain yield ($r = 0.52$, $p < 0.001$) and CP ($r = 0.75$, $p < 0.001$) respectively. Future work should test in-season decision tools using a wider range of tiller densities, and topdress N rates against tissue N measurements.

Keywords: grain crude protein; grain yield; hard red winter wheat; pre-plant N; plant N uptake

1. Introduction

An expanding market for locally produced bread flour in the northeastern United States has created demand for local, organic bread wheat. Economically, organic bread wheat can be a high-value crop for growers if production targets for grain yield and quality are met. Grain CP is a major indicator of quality as it dictates dough elasticity and workability [1]. On the bread wheat market, a grain CP of generally 120 g kg^{-1} or greater is desired because it gives dough strength and provides loaf volume [2]. Grain with lower CP can be sold as feed but typically receives a lower price [3].

Nitrogen plays a key role in supporting both grain yield and CP in bread wheat [4,5]. Nitrogen not only affects grain yield components such as heads m^{-2}, seeds head^{-1}, and kernel size [6], but is also needed to form the proteins for baking quality [7]. Early in the season, N uptake tends to influence vegetative growth, and therefore grain yield

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more than protein, and these effects shift as the season progresses [8]. This relationship occurs because when N is available early in the season, it determines yield potential and once yield potential is set additional N increases grain protein content [9]. Nitrogen management systems have long been studied to determine the effects of application timing on winter wheat grain yield and CP. Woodward and Bly [4] found 165 kg N ha^{-1} of ammonium nitrate fertilizer applied pre-plant to hard red winter wheat raised yields but not CP, and the inverse effect was true when the application was split between fall and spring. Eilrich and Hageman [8] reported April applications of N, as Ca $(NO_3)_2$, on soft red winter wheat caused a 5% grain yield increase, whereas N applications in May did not increase grain yield but instead increased % grain N. A tradeoff between grain yield and CP can also occur due to factors such as limited moisture [10], cultivar, and environmental conditions [11]. As described by Fowler et al. [9], environmental or genotypic effects that increase grain yield must be met with increased amount of N to create a proportionally positive increase in CP. Brown and Petrie [12] found it possible to produce both high yields and acceptable CP in irrigated hard red winter wheat by providing both early-season and late-season N, and warned of the difficulties in achieving adequate CP without late-season N.

In organic cropping environments, overall N supply tends to be low [13–15] and the availability of N derived from organic sources such as animal manures and plant residues is less predictable than from inorganic sources [16]. Olesen et al. [17] reported that manure was more effective at increasing winter wheat grain yield while a grass-clover pre-crop was more effective at increasing grain protein due to differences in the timing of N availability. Solid animal and green manures are the most cost effective organic N sources but both must be applied before planting, the latter for logistical reasons and the former to reliably comply with the 90-day interval required by the National Organic Program Standards between raw manure applications and crop harvest [18]. Unfortunately wheat uptake of N applied at pre-plant tends to be low. Wuest and Cassman [19], for example, documented N recovery ranging from 30 to 55% for spring wheat. Recovery is likely lower in temperate climates because N in winter crops is susceptible to leaching and denitrification during the plant dormancy period [20,21]. The difficulty of ensuring late-season available N for winter wheat with pre-plant applications makes it challenging to achieve grain CP suitable for the bread flour market [12]. In an organic winter wheat study, Mallory and Darby [3] found that spring applied topdress N, in addition to pre-plant manure, increased grain CP by up to 2 percentage points. While no treatments reached the 12% CP milling standard in this study, had a variety with higher protein potential been used, that 2 percentage point increase might have increased CP to above 12%.

In conventional bread wheat production, split applications of N have been shown to increase grain yield and CP [22]—and to improve N utilization efficiency or grain weight per unit—of N from fertilizer [23,24]. The general concept is to reduce fall pre-plant N and to add spring topdress applications at one or two critical growth periods, such as spring tillering and just prior to stem extension, Zadok growth stages (GS) 25 and 30 [25], respectively. In humid regions of the U.S., in-season diagnostic tests are used successfully to guide topdress decisions for soft winter wheat [6,26]. The application rate of the first split is based on tiller density at GS25 whereas the second split is based on tissue N concentration at GS30. Low tiller density ($<$1000 tillers m^{-2}) indicates some or all fertilizer N application at GS25 is needed immediately to increase tiller numbers to support grain yields [20]. Alternatively, high tiller density ($>$1000 tillers m^{-2}) indicates additional N is not needed until GS30. Next, wheat tissue N at GS30 is used to assess crop fertilizer N requirements just prior to the period of highest N uptake [25] and has been identified as a beneficial indicator of the topdress rates needed to maximize yields in soft wheat systems. In Virginia, for example, Baethgen and Alley [26] identified 39.5 g kg^{-1} as the critical tissue N concentration at GS30 to achieve 90% of the maximum grain yield.

Few studies have analyzed split application regimes for organic winter wheat production [3] and to our knowledge none have used the in-season decision tools under organic conditions. The adoption of these practices by farmers has the potential to reduce N loss to the environment and increase the value of bread wheat through enhancing yield and quality. The objectives of this study were to: 1) evaluate the effects of pre-plant and split application treatments on grain N uptake, yield, and CP, on organic hard red winter wheat; and to 2) assess the potential of in-season tests to optimize grain yield and grain CP.

2. Materials and Methods

2.1. Study Site and Experimental Design

The field experiment was conducted in 2012 and 2013 in Maine (ME) and Vermont (VT). In ME, the site was a certified organic field (MOFGA Certification Services, LLC) at the University of ME Rogers Farm Forage and Crop Research Facility (44°56' N, 68°42' W) in Old Town. The site was converted to organic production in 2007. The soil was a Melrose fine silt loam (coarse-loamy over clayey, mixed illitic, superactive, frigid Oxyaquic Dystrudepts) with a pH of 6.2, 3.3% organic matter, 11.8 kg ha^{-1} soil test phosphorus (P) by Modified Morgan, 547 kg ha^{-1} soil test potassium (K), and 23 kg ha^{-1} soil test sulfur (S) based on 2,241,702 kg ha^{-1} of soil in a plow layer (16.9 cm deep), as determined per the standard methods of the ME Soil Testing Service. In ME, the 2012 experiment was preceded by a season of tilled fallow to control perennial weeds and was planted to corn silage (*Zea mays* L.) in 2010. Immediately following winter wheat harvest a cover crop of mustard (*Sinapis arvensis* 'Ida Gold') was established and allowed to grow for 4 weeks. It was then incorporated into the soil

two weeks before the 2013 experiment was initiated. The 2013 experiment was initiated in the same field in an area adjacent to the 2012 experiment that, in 2012, was cropped with winter wheat. The VT experiments were located at Borderview Research Farm in Alburgh (45°0' N, 73°18' W). In 2012, the soil was a Benson Rocky silt loam (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts) with a pH of 6.9, 3.8% organic matter, 4.5 kg ha^{-1} soil test P (Modified Morgan), 81.8 kg ha^{-1} soil test K, and 20.2 kg ha^{-1} soil test S, determined as above. The prior crops were winter wheat and no-till sunflowers (*Helianthus annuus* L.) in 2011 and 2010, respectively. In 2013, the soil was a Benson Rocky silt loam (loamy-skeletal, mixed, active, mesic Lithic Eutrudepts) with a pH of 7.5, 5.2% organic matter, 9.6 kg ha^{-1} soil test P (Modified Morgan), 90.7 kg ha^{-1} soil test K, and 22.4 kg ha^{-1} soil test S, determined as above. The prior crop was spring wheat and this site had been in grass-legume sod for 14 to 15 years before being converted to annual cropping of minimum-tilled sunflowers in 2011.

Field plots were 1.8 m by 13.4 m, arranged in a randomized complete block design with four replications. Treatments were designed to evaluate the effectiveness of different N application options that organic farmers in the northeastern region would use to influence grain yield and CP. Table 1 provides a description of the treatments, which differed in terms of N application timing and N rates, but which were not a factorial arrangement of these two factors. Treatments differed in terms of total available N applied depending on pre-plant N application rate and whether topdress applications were made. The different N application timings were pre-plant (PP), topdress at tillering or Zadok 25 (T1), and topdress at stem elongation or Zadok 30 (T2). Dairy manure (*Bos taurus*) was used as the pre-plant N source to reflect the fact that farmers in the northeastern region and elsewhere rely on manure and green manures for pre-plant applications. The target rates for the pre-plant timing were 78 and 117 kg ha^{-1} of available N, with the exception of VT-2013 where a high rate of 157 kg ha^{-1} of available N was used. The PP$_L$ was chosen to represent the standard practice for organic hard red winter wheat in the area. Solid dairy manure was used in ME-2012, ME-2013, and VT-2013, and composted solid dairy manure was used in VT-2012. Estimated available N for dairy manure was calculated as 25% of the total organic N [27] and 40–50% of the total inorganic N [28,29] with a limit of 11.2 kg inorganic N ha^{-1} assuming anything greater was lost over the winter. This limit was based on prior organic winter wheat research conducted over four site-years where the difference in crop N uptake in the early spring at tillering between the pre-plant dairy manure treatment and a no-N check was on average only 7 kg ha^{-1} and never exceeded 10 kg ha^{-1} at any individual site-year. Organic producers in ME and VT have a limited window in the springtime to apply manure due to soil conditions and the National Organic Program 90-day rule [18]. Chilean nitrate (CN) was used because it was the preferred N source for topdressing among regional farmers at the time of trial initiation and it was not feasible to use the same pre-plant materials for topdressing. Chilean nitrate is also the least expensive per unit N of allowable materials that is accessible to farmers in ME and VT. The CN topdress N source is a mined sodium nitrate product (16-0-0) that was approved for use at the time of trial initiation under organic certification in the USA to supply up to 20% of crop N needs [30]. The CN rates in this study exceeded the 20% limit in some plots for experimental purposes.

Table 1. Treatment descriptions for organic winter wheat N management study conducted in Maine (ME) and Vermont (VT) in 2012 and 2013.

Treatment	Topdress N rate† (kg ha^{-1})			
	Pre-plant (PP) manure target total available N rate	GS25‡ Tillering (T1)	GS30‡ Pre-stem extension (T2)	Total estimated available N applied
Check	0	0	0	0
PP$_L$	78	0	0	78
PP$_H$	117§	0	0	117¶
T1$_{78}$	0	78	0	78
PP$_L$ + T1$_{39}$	78	39	0	117
PP$_L$ + T2$_{39}$	78	0	39	117
PP$_H$ + T2$_{39}$	117§	0	39	156¶
PP$_L$ + T1$_{39}$ + T2$_{39}$	78	39	39	156

† Applied as Chilean nitrate.

‡ Zadoks scale for growth staging cereals [25].

§ Pre-plant N rate was 157 kg ha^{-1} in VT-2013.

¶ Total estimated available N in VT-2013 was 157 and 196 kg ha^{-1} for the PP$_H$ and PP$_H$ + T2$_{39}$ treatments, respectively.

2.2. Management Practices

Prior to experiment initiation, one composite soil sample was collected from each trial location to verify adequate P, K, and S levels [31]. Dates of field operations, topdress applications, and sampling are provided by site-year in Table 2. In ME, one day before wheat seeding, manure was applied by hand and incorporated within 4 hours using a Perfecta® II Field Cultivator (Unverferth Manufacturing Co, Inc. Kalida, OH, USA). In VT, manure was applied by hand and immediately incorporated with a Perfecta® II Field Cultivator on the same day as wheat seeding. Manure application rates are presented in Table 3.

Plots that did not receive pre-plant manure were not amended with P and K because soils had adequate nutrient levels, based on pre-plant soil testing. In ME, hard red winter wheat (variety AC Morley) was seeded at a density of 350 viable seeds m^{-2} and row spacing of 17.7 cm using an Almaco cone seeder with double-disk openers (Almaco Inc., Nevada, IA, USA) after which plots were packed with a Brillion 1.5 m Sure Stand grass seeder (Landoll Co., Marysville, KS, USA). In VT, hard red winter wheat (variety Harvard) was seeded at a rate of 335 viable seeds m^{-2} in 2012 and 306 viable seeds m^{-2} in 2013 with a Sunflower 9412 3.0 m grain drill (Sunflower Manufacturing, Beloit, KS, USA) double disc opener outfitted with a row spacing of 17.8 cm. Topdress N applications were applied by hand at wheat developmental stages on dates outlined in Table 2 and at rates indicated in Table 3.

Table 2. Summary of field operations, topdress applications, and biomass sampling in the organic winter wheat N management study conducted in Maine (ME) and Vermont (VT) in 2012 and 2013.

Operation	Wheat growth stage†	ME-2012	ME-2013	VT-2012	VT-2013
Manure application, PP‡	-	19 Sept 2011	14 Sept 2012	27 Sept 2011	24 Sept 2012
Wheat seeding	-	20 Sept 2011	15 Sept 2012	27 Sept 2011	24 Sept 2012
Wheat biomass sampling no. 1	Tillering, GS25	19 Apr	30 Apr	12 Apr	19 Apr
Topdress N application, T1	Tillering, GS25	20 Apr	30 Apr	12 Apr	19 Apr
Wheat biomass sampling no. 2	Pre-stem extension, GS30	30 Apr	13 May	26 Apr	03 May
Topdress N application, T2	Pre-stem extension, GS30	02 May	13 May	26 Apr	03 May
Wheat biomass sampling no. 3	Soft dough, GS85	06 Jul	03 Jul	02 Jul	09 Jul
Wheat harvest	Maturity, GS93	25 Jul	1 Aug	11 Jul	19 Jul

† Zadoks scale for growth staging cereals [25].

‡ PP, pre-plant; T1, topdress at tillering; and T2, topdress at pre-stem extension.

Table 3. Material and nutrient application rates for N sources applied as pre-plant and topdress to winter wheat in Maine (ME) and Vermont (VT) in 2012 and 2013.

Material and nutrient application rates	Pre-plant manure target N rate†								Topdress Chilean nitrate target N rate†	
	ME-2012		ME-2013		VT-2012		VT-2013			
	78	117	78	117	78	117	78	157	39	78
Dry matter (%)	28.3		26.6		19.8		20.2			
Material (Mg ha^{-1})	72	108	56	84	45	67	40	74	0.25	0.49
Organic N (kg ha^{-1})	260	390	193	290	275	412	307	563	0	0
Inorganic N (kg ha^{-1})	46	68	25	38	32	48	23	42	39	78
Estimated available N‡ (kg ha^{-1})	76	109	59	84	80	114	86	152	39	78
Total P (kg ha^{-1})	150	224	173	259	91	137	73	134	0	0
Total K (kg ha^{-1})	286	429	325	488	154	232	101	184	0	0

† Estimated available N (kg ha^{-1}).

‡ Estimated available N was calculated as 25% of the total organic-N [27] and 40-50% of the total inorganic N for dairy manure [27–29] with a limit of 11.2 kg inorganic N ha^{-1}.

2.3. Measurements and Analytical Procedures

Tiller density was determined at tillering for the PP-only treatments by counting wheat shoots with three or more leaves in eight 0.3-m sections of row per plot. These treatments were sampled to measure pre-plant N effects on tillering because all other N applications came at or after tillering. Leaf tissue N concentration at pre-stem extension was measured via destructive sampling that took place in one half of each plot. Plants were clipped at 2 cm from the soil surface from three 0.3-m sections of rows (avoiding border rows). On consecutive sampling dates, sample areas were positioned 0.3 m away from the preceding sample area. Samples were bulked to represent a total sample area of 0.9 m of row per plot. Plants were dried at 60° C, weighed, and ground through a 2-mm mesh. Total N concentration was determined by combustion for a 250-mg subsample using a Leco CN2000 analyzer (Leco Corp., St. Joseph, MI, USA) in ME, whereas in VT, a 100-gram sample was submitted to Cumberland Valley Analytical Services (Hagerstown, MD, USA), for Near Infrared Reflectance spectroscopy. Plant N uptake by wheat and weed biomass was determined at three wheat developmental stages: tillering, pre-stem extension, and soft dough (GS85, or "peak biomass")—all using the same methods as for leaf tissue N sampling. Weed pressure was very low so no weed control measures were taken. Weed samples were collected from the sample area and included in plant N calculations when weed biomass composed $>$2% of the total plant biomass. Plant N uptake was calculated by multiplying plant above ground biomass by % N. At soft dough, the number of spikes per bulk sample was counted and recorded.

Grain was harvested between 25 July and 1 August from a 1.5 m by 9.1 m harvest area with a Wintersteiger small-plot combine (Ried, AT) in ME, and between 11 and 19 July, from a 1.4 m by 5.5 m harvest area with a Almaco SPC50 plot combine (Almaco, Inc., Nevada, IA, USA) in VT. Grain was cleaned with a small Clipper (Clipper, A.T. Ferrell Co., Bluffton, IN, USA) to remove weed seeds and inert material. Grain samples were weighed. Moisture was measured (GAC 2100, DICKEY-john Corp., Auburn, IL, USA) and adjusted to 135 g kg^{-1} on cleaned samples to determine grain yield. Grain was subsampled (100 g) and ground (2 mm mesh). In ME, grain CP was determined on a 250-mg sub-subsample by multiplying Leco N by 5.7 N, according to American Association of Cereal Chemists (AACC) method 46–30.01 [32], and adjusted to 120 g kg^{-1} grain moisture. In VT, grain CP was determined on a 250-mg sub-subsample using a Perten Inframatic 8600 Flour Analyzer (PertenElmer Co., Hägersten, SWE). Combustion and NIR techniques are both accepted methods for CP determination [33]. Thousand kernels weights (TKW) were collected in ME. One thousand seeds per plot were counted using a seed counter (Count-A-Pak Seed Totalizer, Seedburo Equipment Co., Des Plaines, IL, USA), weighed, and adjusted to 135 g kg^{-1} moisture. Weather data were collected at these research sites unless otherwise noted.

2.4. Statistical Analysis and Calculations

Data were analyzed with the statistical program R [34] using a mixed model Analysis of Variance (ANOVA) with block as a random effect and treatment and site-year as fixed effects. The "nlme" package [35] was used to test the significance of site-year, treatment, and site-year by treatment interactions. The ANOVA assumption of equal variance was verified with Levene's test using the 'car' package [36]. Residual values were used to assess normal distribution with the Shapiro-Wilk Normality test. When residuals did not conform to equal variances and normality, a Box-Cox power transformation was used using the 'MASS' package [37]. The treatments were arranged in an incomplete factorial to test only treatments of specific interest to farmers in the region. The data were analyzed with a means separation using Fisher's Protected Least Significant Difference (LSD) using the 'multcomp' package [38]. Plant N uptake effects were analyzed by date as not all treatments were measured at every date thereby precluding a repeated measures analysis. Grain N yield was determined by multiplying grain N (%) by grain yield (kg ha^{-1}). The difference method was used to calculate apparent nitrogen recovery (ANR) for PP-only treatments by subtracting the plant N uptake of the check treatment from the plant N uptake of the PP-only treatments divided by the estimated amount of plant available N applied pre-plant [39]. Apparent nitrogen recovery was similarly calculated for topdress treatments by subtracting the plant N uptake of the PP-only treatments from the plant N uptake of the topdress treatment divided by the estimated amount of plant available N applied at topdress. Coefficient of variation (CV) was calculated as a function of square root of error mean square divided by the site-year mean for each response variable. In-season test data were analyzed with linear regression using treatment means over site-years because the tests should show relationships between variables over a range of sites, seeding rates, and varieties. These analyses were used to determine the correlations between: 1) grain yield and tiller density at GS25, 2) grain yield and tissue N concentration at GS30, and 3) CP and tissue N concentration at GS30.

3. Results

3.1. Weather

Monthly mean temperature and precipitation amounts for the four site-years are presented in Table 4. During seeding and pre-plant applications in September, all site-years except ME-2012 experienced greater than the 30-year normal precipitation. In VT-2012 approximately 24 mm of rainfall occurred 2 days after the pre-plant application and could have caused N leaching. For all site-years, March was warmer than average and there was a period of drier than average weather beginning in March and extending through April. The VT-2013 site-year experienced wetter than normal precipitation during the months of May and June but

the majority of rainfall occurred at least a week after the T2 treatment application. In July, weather conditions turned dry, especially in ME-2012 and VT-2013 when rainfall was 65 and 59 mm less than the 30-year average, respectively.

Table 4. Monthly mean air temperature measured at 1.5 m from the ground, rainfall from September through November of the seeding year and from March through July of the harvest year at the experiment sites in Maine (ME) and Vermont (VT) compared with average climate data for 1981 to 2010.

	Maine			Vermont		
	2012	2013	30-year aver.	2012	2013	30-year aver.
Month	Mean temperature (°C)					
September†	16.1	13.5	13.9	17.1	16	16.1
October†	9.4	9.8	7.8	10.1	11.3	8.9
November†	5.0	0.7	2.2	6.3	3.1	3.9
March	2.3	0.3	-1.4	4.3	0.1	-0.6
April	6.8	5.1	5.3	7.2	6.4	7.2
May	12.7	11.9	11.4	15.8	15.1	13.3
June	15.9	16.9	16.4	19.4	17.8	18.9
July	20.0	20.8	19.7	21.9	22.1	21.7
	Rainfall (mm)					
September†	48	204	96	141	136	91
October†	109	179	101	89	105	91
November†	66	40	112	36	17	79
March	50	66	104	38	26	56
April	93‡	36	96	67	54	71
May	109	107	99	99	122	89
June	153	152	103	82	234§	94
July	25	112	90	96	48	107

† Seeding year.

‡ Precipitation data was not available for 26 April 2012 in ME.

§ June 2013 precipitation data for the VT site was taken from the National Weather Service, South Hero, VT (44.65° N 73.31° W).

3.2. Plant Nitrogen Uptake

Plant N uptake data were analyzed over site-years (Table 5). In ME-2013, weeds comprised 6% of aboveground biomass at the soft dough stage and 11% of total plant N uptake, and thus weed N uptake was included in plant N uptake (Table 5). However, there were no significant differences among treatments in either weed biomass or weed N uptake ($p = 0.157$ and 0.132, respectively; data not shown). In all other site-years, weed biomass never exceeded 2% of the aboveground biomass, thus plant N uptake reported in the results directly represents wheat N uptake.

The PP-only treatments (PP_L and PP_H) generally did not increase N uptake compared with the untreated check. The exception was in ME-2012 at tillering when the PP_L treatment increased N uptake by 7.1 and 4.6 kg N ha^{-1} compared with the check and PP_H treatments, respectively. Delaying all N applications until tillering ($T1_{78}$) increased plant N uptake at soft dough by 48.4 kg N ha^{-1} compared with applying the equivalent amount of N at pre-plant (PP_L).

The PP_L + $T1_{39}$ treatment consistently increased N uptake compared with the PP-only treatments, and uptake was on average 20% and 28% greater at pre-stem extension and at soft dough, respectively. Topdressing supplemental N at pre-stem extension (PP_L + $T2_{39}$ and PP_H + $T2_{39}$) increased plant accumulated N compared with their respective PP-only treatments, by an average of 30%. Topdressing twice (PP_L + $T1_{39}$ + $T2_{39}$) resulted in N uptake at soft dough that was similar to the PP_L + $T1_{39}$ and PP_L + $T2_{39}$ treatments but greater than PP_H + $T2_{39}$ by 27.5 kg N ha^{-1}. Apparent N recovery rates of the PP-only treatments were the lowest among all treatments and were 15% on average (data not shown). The ANR of topdress treatments ranged from 60 to 89%. Both the $T1_{78}$ and PP_L + $T2_{39}$ treatments had ANR values greater than 80%.

3.3. Tiller and Spike Densities

Due to significant treatment by site-year interactions for tiller density, spike density, and the other response variables listed in Table 6, the data were analyzed and are presented by site-year (Table 7). Tiller densities averaged 1371, 738, 906, and 890 tillers m^{-2} in ME-2012, ME-2013, VT-2012, and VT-2013, respectively, and were not influenced by PP-only treatments (data not shown).

The PP-only treatments also did not influence spike density except in ME-2013, where the PP_L treatment produced 38% more spikes than the check. The addition of topdress N increased spike density in most cases in ME-2012; $T1_{78}$ vs. PP_L, and PP_L + $T1_{39}$ vs. PP_L, and PP_L + $T1_{39}$ + $T2_{39}$ vs. PP_L + $T2_{39}$ treatments increased spike density by 48, 47, and 34%, respectively. In ME-2013 and VT-2013, the PP_L + $T1_{39}$ + $T2_{39}$ treatment also increased spike density relative to the PP_L + $T1_{39}$ treatment by 25 and 43%, respectively. Spike densities were unaffected by treatments in VT-2012, which had higher %CV than the other site years (Table 7).

3.4. Grain Yield

Average grain yields by site-year were 5.22, 2.41, 3.09, and 4.44 Mg ha^{-1} for ME-2012, ME-2013, VT-2012, and VT-2013, respectively (Table 7). Yields in ME-2013 and VT-2012 were approximately 1.08 and 1.63 Mg ha^{-1} lower, respectively, than average yields from trials conducted with the same varieties and locations in those years whereas VT-2013 average grain yields were 0.57 Mg ha^{-1} higher than the local equivalent [40].

Table 5. Mixed model ANOVA and LSD results of mean plant N uptake for wheat at different growth stages as affected by pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013. Treatment means presented are the means of the 4 site-years.

	Plant N Uptake (kg N ha^{-1})		
Effects and sources of variation	Tillering	Pre-stem extension	Soft dough
Site-year			
ME2012	41.5†	37.1	73.4
ME2013	10.5	26.8	91.1
VT2012	31.9	47.4	125
VT2013	18	51.8	231
Treatment			
Check	22.3^{a} ‡	34.6^{a}	93.7^{a}
PP$_L$†	29.4^{b}	39.2^{ab}	111.7^{ab}
PP$_H$	24.8^{a}	39.5^{ab}	102.2^{a}
T1$_{78}$	-	43.3^{bc}	160.1^{e}
PP$_L$ + T1$_{39}$	-	47.3^{c}	136.7^{cd}
PP$_L$ + T2$_{39}$	-	-	146.6^{ce}
PP$_H$ + T2$_{39}$	-	-	131.2^{bc}
PP$_L$ + T1$_{39}$ + T2$_{39}$	-	-	158.7^{de}

Sources of variation	df	F-value	df	F-value	df	F-value
Site-year (S)	3	35.9***	3	21.6***	3	32.2***
Treatment (T)	2	6.8**	4	5.8***	7	9.1***
S $\times$ T	6	1.62	12	1.01	21	1.03
CV, %		19.2		16.9		24.0

* Significant at P $<$0.05; ** Significant at P $<$0.01; *** Significant at P $<$0.001.

† PP$_L$, 78 kg N ha^{-1} manure at pre-plant; PP$_H$, 117 or 157 kg N ha^{-1} manure at pre-plant; T1$_{78}$, 78 kg N ha^{-1} topdress at tillering; T1$_{39}$, 39 kg N ha^{-1} topdress at tillering; T2$_{39}$, 39 kg N ha^{-1} topdress at pre-stem extension.

‡ Within column and site-year, treatment means with the same lower case letter are not significantly different at P$<$0.05.

Table 6. Mixed model ANOVA results of mean spike density, grain yield, GS30 tissue N, grain crude protein, and grain N yield for wheat as affected by pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013.

Sources of variation	Spike density		Grain yield		GS30 tissue N		Grain crude protein		Grain N yield	
	df	F-value	df	F-value	df	F-value	df	F-value	df	F-value
Site-year (S)	3	4.7*	3	67.5***	3	33.6***	3	86.2***	3	69.3***
Treatment (T)	7	6.0***	7	8.7***	4	31.5***	7	13.0***	7	14.8***
S x T	21	2.1**	21	2.8***	21	2.7**	21	1.8*	21	3.2***
CV, %		17.0		12.7		7.2		4.2		12.7

* Significant at P $<$0.05; ** Significant at P $<$0.01; *** Significant at P $<$0.001.

Table 7. LSD and ANOVA results for spike density, grain yield, GS30 tissue N, grain crude protein (at 120 g kg^{-1} grain moisture), and grain N yield for wheat grown with different pre-plant and topdress N treatments in Maine (ME) and Vermont (VT) in 2012 and 2013. VT-2012 GS30 tissue N and grain CP data were transformed (λ= -2 and -4, respectively). Back transformed values are in parentheses.

Site-year	Treatment	Spike density (spike m^{-2})	Grain yield (Mg ha^{-1})	GS30 tissue N (g kg^{-1})	Grain crude protein (g kg^{-1})	Grain N yield (kg ha^{-1})
ME-2012	Check	476^{c}†	3.57^{d}	24.5^{b}	99^{b}	61^{d}
	PP$_L$‡	445^{c}	4.75^{bc}	24.3^{b}	96^{b}	79^{cd}
	PP$_H$	544^{bc}	4.55^{c}	24.6^{b}	99^{b}	78^{cd}
	T1$_{78}$	657^{ab}	5.71^{a}	34.2^{a}	115^{a}	113^{a}
	PP$_L$ + T1$_{39}$	656^{ab}	5.82^{a}	32.8^{a}	104^{b}	104^{ab}
	PP$_L$ + T2$_{39}$	553^{bc}	5.35^{ab}	-	98^{b}	91^{bc}
	PP$_H$ + T2$_{39}$	629^{ab}	5.96^{a}	-	101^{b}	105^{ab}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	739^{a}	6.01^{a}	-	103^{b}	107^{ab}
	Source of variation			ANOVA		
	Treatment	**	***	***	*	***
	CV, %	14.6	10.3	4.6	6.8	13.9
ME-2013	Check	333^{d}	1.79^{c}	32.5^{c}	118^{cd}	36^{b}
	PP$_L$	459^{bc}	1.96^{bc}	30.4^{c}	119^{bcd}	40^{b}
	PP$_H$	432^{cd}	2.01^{bc}	31.8^{c}	117^{d}	41^{b}
	T1$_{78}$	569^{ab}	2.77^{a}	44.9^{a}	130^{a}	62^{a}
	PP$_L$ + T1$_{39}$	484^{bc}	2.87^{a}	40.3^{b}	116^{d}	58^{a}
	PP$_L$ + T2$_{39}$	505^{abc}	2.58^{ab}	-	127^{ab}	56^{a}
	PP$_H$ + T2$_{39}$	535^{abc}	2.50^{ab}	-	125^{abc}	54^{a}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	606^{a}	2.83^{a}	-	130^{a}	63^{a}
	Source of variation			ANOVA		
	Treatment	**	**	***	**	***
	CV, %	16.1	18.3	6.5	4.3	16.3
VT-2012	Check	431	2.52^{b}	0.101 (32.0)	8.04 (106)^{d}	46^{c}
	PP$_L$	558	2.96^{b}	0.106 (31.3)	7.88 (106)^{cd}	54^{bc}
	PP$_H$	336	2.89^{b}	0.098 (32.3)	7.45 (108)^{bcd}	49^{bc}
	T1$_{78}$	552	4.31^{a}	0.084 (36.0)	5.32 (118)^{a}	88^{a}
	PP$_L$ + T1$_{39}$	696	3.12^{b}	0.072 (38.0)	6.32 (112)^{abc}	60^{bc}
	PP$_L$ + T2$_{39}$	629	3.01^{b}	-	6.14 (113)^{ab}	59^{bc}
	PP$_H$ + T2$_{39}$	473	2.87^{b}	-	5.40 (118)^{a}	63^{b}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	535	3.07^{b}	-	4.75 (121)^{a}	63^{b}
	Source of variation			ANOVA		
	Treatment	ns	**	ns	**	***
	CV, %	30.3	16.2	21.7	17	16.9
VT-2013	Check	612^{ab}	4.41^{bc}	40.5	114^{d}	87^{b}
	PP$_L$	604 ab	5.20^{a}	41.6	129^{abc}	116^{a}
	PP$_H$	623^{a}	4.19^{c}	42.8	125^{c}	90^{b}
	T1$_{78}$	670^{a}	4.22^{c}	45.8	130^{abc}	95^{b}
	PP$_L$ + T1$_{39}$	495^{b}	4.27^{c}	43.2	127^{bc}	94^{b}
	PP$_L$ + T2$_{39}$	725^{a}	4.26^{c}	-	129^{abc}	95^{b}
	PP$_H$ + T2$_{39}$	689^{a}	5.01^{ab}	-	132 ^{ab}	114^{a}
	PP$_L$ + T1$_{39}$ + T2$_{39}$	709^{a}	4.02^{c}	-	135^{a}	93^{b}
	Source of variation			ANOVA		
	Treatment	*	*	ns	***	**
	CV, %	12.9	10.6	6.2	3.7	11.8
df		7	7	4	7	7

* Significant at P <0.05; ** Significant at P <0.01; *** Significant at P <0.001; ns: not significant at P <0.05.

† Within column and site-year, treatment means with the same lower case letter are not significantly different at P<0.05.

‡ PP$_L$, 78 kg N ha^{-1} manure at pre-plant; PP$_H$, 117 or 157 kg N ha^{-1} manure at pre-plant; T1$_{78}$, 78 kg N ha^{-1} topdress at tillering; T1$_{39}$, 39 kg N ha^{-1} topdress at tillering; T2$_{39}$, 39 kg N ha^{-1} topdress at pre-stem extension.

Impacts of the PP-only treatments on grain yield varied by site-year. Significant increases were observed in ME-2012 and VT-2013 when site-years were analyzed individually. In ME-2012, both PP-only treatments increased yields relative to the check by an average of 30%. In VT-2013, only the PP_L treatment increased yields by 18%. The $T1_{78}$ treatment increased grain yields by 20% in ME-2012, 41% in ME-2013, and 46% in VT-2012, but reduced yields by 23% in VT-2013. The PP_L + $T1_{39}$ treatment increased grain yields in ME-2012 and 2013 versus the PP_L treatment by 23 and 46%, respectively, but reduced grain yields by 22% in VT-2013. The PP_H + $T2_{39}$ treatment increased grain yields by 31 and 20% in ME-2012 and VT-2013, respectively, over the PP_H treatment. The PP_L + $T1_{39}$ + $T2_{39}$ treatment had no influence on grain yield in any site-year compared with PP_L + $T1_{39}$ or PP_L + $T2_{39}$ treatments.

Thousand kernel weights were measured for the ME site-years but are not presented because there were no significant treatment effects. Thousand kernel weights averaged 39.5 g in 2012 and 29.5 g in 2013, and were significantly correlated with grain yields (r = 0.48, $p < 0.01$ and r= 0.59, $p < 0.001$ for 2012 and 2013, respectively).

3.5. GS30 Tissue N, Grain Crude Protein, and Grain N Yield

Treatment effects on GS30 wheat tissue N concentrations were evident only in ME and were restricted to tillering N additions; N applied at pre-plant had no significant effects (Table 7). Compared with PP_L, the $T1_{78}$ treatment increased tissue N by 44% on average and the PP_L + $T1_{39}$ produced a 34% average increase.

Grain CP averaged 102, 123, 113, and 128 g kg^{-1} in ME-2012, ME-2013, VT-2012, and VT-2013, respectively (Table 7). The PP-only treatments had no significant effect on CP except in VT-2013 where the 78 kg N ha^{-1} rate increased CP by 13% as compared with the check. The $T1_{78}$ treatment increased CP compared with the PP_L and PP_H treatments by an average of 14% at the ME sites and by 11% in VT-2012, but had no effect in VT-2013. The PP_L + $T1_{39}$ treatment produced no measurable increases in CP and the PP_L + $T2_{39}$ treatment increased CP in VT-2012 by 7% compared with the PP_L treatment. The PP_H + $T2_{39}$ treatment increased CP in three of four site-years compared with the PP_H treatment by 7, 9, and 6% in ME-2013, VT-2012, and VT-2013, respectively. The PP_L + $T1_{39}$ + $T2_{39}$ treatment increased CP only in ME-2013 by 12% compared with the PP_L + $T1_{39}$ treatment.

Grain N yield results were similar to grain yield results with two exceptions (Table 7). In ME-2012, the PP_H treatment did not increase grain N yield compared with the check, and in ME-2013, the PP_L + $T2_{39}$ treatment increased grain N yield by 40% compared with the PP_L treatment.

3.6. In-season Tests

Tiller density was a better predictor of grain yield (r = 0.52, $p < 0.001$; Figure 1) than tissue-N at GS30 (r = 0.09, p = 0.426; data not show) when compared across site-years. The residuals from the regression line in Figure 1 were not influenced by treatment (p = 0.175).

Correlations were weak when analyzed by site-year (data not shown) likely due to limited tiller range and variability within site-year. Tiller densities in ME-2012, for example, ranged from 1184 to 1668 tillers m^{-2} with a standard deviation of 149 tillers m^{-2}. Tissue N at GS30 was a good predictor of CP (r = 0.75, $p < 0.001$; Figure 2). The residuals from the regression line in Figure 2 were influenced by treatment (p = 0.005), indicating that additional variance in the model was explained by the treatments.

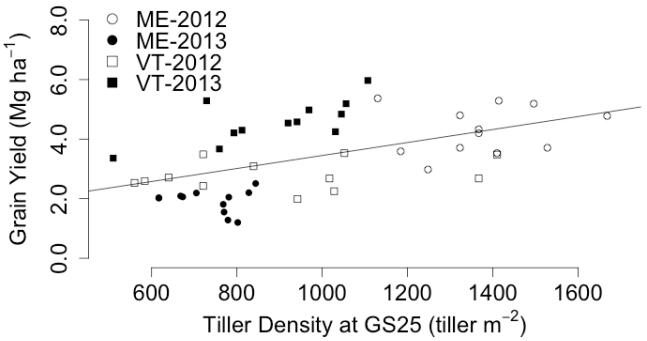

Figure 1. Correlations between tiller density at GS25 and grain yield in Maine (ME) and Vermont (VT) in 2012 and 2013 across different pre-plant N treatments (y = 0.0021x + 1.2668; r = 0.52; $p < 0.001$). Data are treatment means from each site year. The standard error of the regression coefficients was 0.540 and 0.001 for β_0 and β_1, respectively.

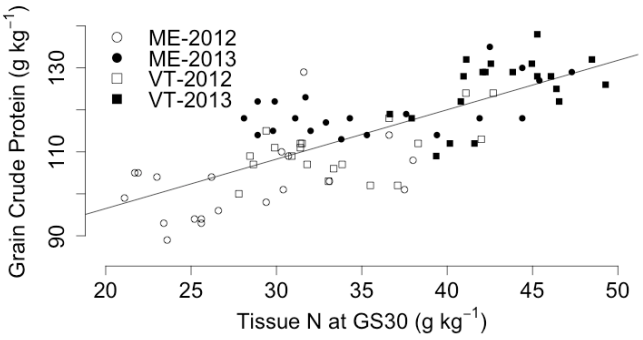

Figure 2. Correlation between tissue N and crude protein in Maine (ME) and Vermont (VT) in 2012 and 2013 across different pre-plant and topdress N treatments (y = 1.176x + 72.963; r = 0.75; $p < 0.001$). Data are treatment means from each site year. The standard error of the regression coefficients was 4.164 and 0.116 for β_0 and β_1, respectively.

4. Discussion

4.1. Site-year Effects

Differences in growing conditions among the four site-years made it difficult to draw general conclusions and recom-

mendations based on the effects of N treatment. In both ME-2012 and VT-2013, favorable growing conditions supported high yield potentials, as evidenced by the high yields in the check, although varying levels of N availability from soil %OM may have caused different treatment responses between these two site-years. Where %OM was low (ME-2012), all N treatments produced positive and substantial increases in grain yield. The only treatment to increase CP was the highest single topdress application. When an increase in yield occurs, it is often accompanied by an increase in grain N yield but not necessarily CP as the increase in carbohydrates dilutes the N [2,41]. In contrast, where %OM was high (VT-2013), N treatments produced fewer positive yield responses but more increases in CP. While soil nitrate was not measured in this study, the relatively high %OM measured at this site suggests greater soil N supply [42,43]. This site-year also was the only one to demonstrate an increase in CP from the PP-only treatments. These results are congruent with a study by Woodward and Bly [4] showing that N must be of sufficient amount and appropriately timed to support positive responses in both yield and CP. Terman et al. [10] found that under high soil nitrate conditions ($>$67 kg ha^{-1} NO_3-N), additional N applied to hard red winter wheat produced an increase in grain protein content but low or absent responses in grain yield. Similarly, Frederick and Marshall [44] found early spring topdressing to soft red winter wheat on soils with high N reserves decreased grain yields by reducing kernel weight or productive tillers below the level necessary for optimal yield.

In ME-2013 and VT-2012, the check treatment yields suggest reduced yield potentials. In ME-2013, yield potential was likely limited by observed weed and disease pressure resulting from a lack of rotation. It was less likely the preceding mustard affected wheat yield because the mustard crop accumulated relatively little biomass before incorporation and stand counts show no effect as compared with prior years when wheat followed fallow (data not shown). Nonetheless, these factors did not limit the responsiveness of this site to N treatments. Treatment effects were observed for both grain yield and CP. As a consequence of the low yields, CP potential was relatively high, which was consistent with the tradeoff between yield and protein reported by others [9,11]. All treatments exceeded 110 g kg^{-1} CP and nearly all those receiving topdress N had CP levels above 120 g kg^{-1}. In VT-2012, it was possible that heavy rainfall, occurring 2 days after the pre-plant applications, may have been a contributing factor to the relatively low grain yield potential and limited yield response to N. Only the highest topdress N rate (T1$_{78}$) produced a yield response, whereas more treatment responses were observed for CP.

4.2. Nitrogen Treatment Effects

The PP-only treatments improved yields at three of four site-years (when ME site-years were analyzed together) but were less likely to produce an increase in CP. These results are congruent with others who have found that applications at the pre-plant timing alone do not supply an adequate amount of late-season available N to enhance CP in winter wheat [3,45]. At the majority of site-years, it was possible that the amount or timing of mineralization from the organic N fraction of manure was insufficient to support protein production. The VT-2013 site was the exception because high %OM may have impacted yield-CP dynamics as previously described. These results support findings that matching the N availability of organic N sources with the periods of high crop N demand presents a major challenge for organic bread wheat producers [14].

The T1$_{78}$ treatment produced increases in both yield and CP in the majority of site-years suggesting that the springtime application was better matched with crop N demand than the pre-plant application timing. This enhanced plant N uptake at soft dough and ANR over the PP-only treatment. Increases in both grain yield and CP also suggest available N was in excess of yield requirements and was sufficient to increase CP [11,46]. It should be noted that N application timing and source are confounded in these comparisons and the difference in N source (manure vs. CN) could also be a factor in the observed effects.

Treatments receiving a topdress application often showed a yield and CP advantage over the PP-only treatments. The PP$_L$ + T1$_{39}$ treatment produced some measurable increases in yields and plant N uptake compared with the PP-only treatments but never produced a measurable increase on CP. More frequent effects on yield and CP were found with the PP$_H$ + T2$_{39}$ treatment versus the PP$_H$ comparison possibly because greater mineralization of N from the PP$_H$ treatment may have been adequate to support CP. The timing of supplemental N applied in the PP$_L$ + T1$_{39}$ and PP$_L$ + T2$_{39}$ treatments had no influence on yield and CP results likely because the applications were too close in time to cause differences. The application at T2 was relatively early compared to other studies that showed topdress applied later, at flag leaf (GS39) and boot (GS45), were more effective at increasing CP than the T1 application [3].

Nitrogen applied at both T1 and T2 did not increase yields relative to supplemental N applied at T1 or T2. The fact that N applied at both timings produced among the highest yields in ME, indicates two topdress applications were in excess of that required to reach a yield plateau. Interestingly, the opposite effect occurred in VT; treatment yields with two topdress applications were equivalent to the check and among the lowest of all treatments. The VT data suggests this response was attributed to the aforementioned site factors. Application timing may have also been a factor in the observed effects on CP. Two N applications increased CP relative to the T1 application at two of four site-years but never increased CP relative to the T2 application. Greater differences in CP may have occurred if the second application of topdress were delayed to GS45 or later [41].

These findings indicate that when yield potential was high, treatments that included topdress N generally produced CP greater than the 100 g kg^{-1} threshold considered

sufficient by local artisan bakers in our region. With this in mind, the costs of applying organic-approved sources of N must be compared against the crop value. Chilean nitrate is cheaper (US$ 229 ha^{-1}) than other organic-approved sources of N though it is not allowed in Canada and Europe and may be prohibited in the future under the US National Organic Standards Board. Other topdress sources, such as dehydrated poultry litter, are more expensive (US$ 459 ha^{-1}) and may have lower N availability compared with the soluble CN [3], which may reduce its efficacy.

4.3. In-season Test: Tiller Density

Results indicated that tiller densities can be a predictor of grain yield but a wider range of densities is needed to better understand the utility of this measurement as a decision tool. When tiller densities were below the 1000 tillers m^{-2} threshold established by Scharf and Alley [47], there was not a yield penalty for delaying supplemental topdressing from GS25 to GS30 in ME-2013, VT-2012, and VT-2013. Similarly, when average tiller densities were $<$1000 tillers m^{-2}, there was no penalty for supplying N earlier at GS25 [6]. In fact, only the PP$_L$ + T1$_{39}$ and PP$_L$ + T1$_{39}$ + T2$_{39}$ treatments in the ME site-years enhanced yields over the PP$_L$ whereas the PP$_L$ + T2$_{39}$ treatment did not. Nitrogen topdress rates of 39 and 78 total kg N ha^{-1} applied in this study were slightly above the range recommended of approximately 30 to 56 kg N ha^{-1} for densities $<$1000 tillers m^{-2} [6]. It is possible that tiller densities in this study were not low enough to produce the measurable yield differences between the PP$_L$ + T1$_{39}$ and PP$_L$ + T2$_{39}$ applications that others have found. For instance, in a study with non-organically managed no-till winter wheat, Weisz et al. [48] showed that when tiller densities were below 550 tillers m^{-2}, treatments with supplemental N applied at GS25 and split applied between GS25 and GS30 produced greater yields than the treatment with supplemental N at GS30. Therefore, fully evaluating topdressing timing effects at the threshold established by Scharf and Alley [47] was limited by the fact that tiller densities at most site-years were adequate but never well below the threshold (738, 906, and 890 tillers m^{-2} in ME-2013, VT-2012, and VT-2013, respectively) and exceeded 1000 tillers m^{-2} in just ME-2012 (1371 tillers m^{-2}). The PP-only treatments did not produce statistically different tiller densities and an effort to capture a wider range through seeding rates and dates may be needed. For instance, Weisz et al. [48] found that different seeding rates and dates produced a range of 162 to 1774 tillers m^{-2} in soft red winter wheat.

4.4. In-season Test: Tissue Nitrogen

Tissue N values in this study ranged from 24.3 to 45.8 g kg^{-1} and were similar to the $>$20.0 to $<$50.0 g kg^{-1} values reported by Baethgen and Alley [26] for soft winter wheat. A stronger correlation between tissue N and CP than between tissue N and yield suggests this test may be useful to guide N management for CP even though other studies do not explore this purpose. Using the slope of the regression line (Figure 2) the critical level for achieving CP of 120 g kg^{-1} was a tissue N concentration of 40.0 g N kg^{-1}. This value aligns with the critical value of 39.5 g N kg^{-1} reported by Baethgen and Alley [26] for achieving 90% relative yield without further fertilization. The critical level was met at the site-years with the highest overall CPs. Specifically, delaying topdress N until GS25 in ME-2013 and all N treatments in VT-2013 met the critical level (Table 7). In site-years with low overall CPs such as ME-2012 and VT-2012, the critical level was never met but individual cases suggest the tissue N test has the predictive power to obtain the desired CP response.

In ME-2012, low tissue N concentrations (24.4 g kg^{-1} for the PP-only treatments; 32.8 g kg^{-1} for PP$_L$ + T1$_{39}$) implied the need for approximately 120 and 78 kg N ha^{-1}, respectively, at GS30 according to Alley et al. [6]. The rate of 39 kg N ha^{-1} applied at GS30 was possibly inadequate because the desired CP was never met. Conversely, in VT-2012, the 39 kg N ha^{-1} applied at GS30 for the same treatment (PP$_L$ + T2$_{39}$) aligned more with the rate recommended by the tissue test (47 kg N ha^{-1}) and was adequate to meet the desired CP.

These results suggest that testing various N application rates at GS30 against the measured tissue N values would broaden understanding of the rates need to maximize CP. Beyond applying a sufficient N rate, N application timing may have been an influential factor in the aforementioned results such that the N applied at GS30 may have been too early to increase CP. Others have found later applications of N at the boot stage (GS45) were more effective at increasing CP in hard red winter wheat than applications at or prior to GS30 [3,22,49]. However, Gooding et al. [50] noted foliar urea applied at or soon after anthesis increased CP but post anthesis applications pose higher risk of N loss. For an organic producer, the threshold at which tissue N testing is relevant should be based on the producer's means to apply an N source later in the season as well as their access to an organic approved N source with rapid N availability. As discussed by Mallory and Darby [3], while topdressing could be a good strategy for organic winter bread wheat producers, further evaluation of topdress N sources is needed. Lastly, measuring tissue N concentrations beyond GS30 may reveal that late-season mineralization from organic N attributes to CP, but studies addressing this area are lacking. Brown et al. [41] and Brown and Petrie [45] reported that flag leaf total N taken at early heading or anthesis (GS50-60) was better related to CP at harvest than samples collected earlier because the majority of plant N uptake occurs by flag leaf emergence.

5. Conclusions

The primary objective of this study was to analyze split N application regimes and in-season tests to guide N applications for organic production. The PP-only treatments were

unreliable for producing market quality bread wheat. The $T1_{78}$ treatment produced the highest yield and CP, except for one case, but delaying all N application until spring is challenging in terms of the feasibility of applying a cost-effective fresh animal or green manure N or the cost of easily applied pelletized organic N sources. Topdressing supplemental N was effective at increasing yield and CP when preceded by the PP_H application. The PP_L + $T1_{39}$ + $T2_{39}$ treatment generally did not enhance results compared with single topdress application at T1 or T2. Responses to added N were variable among site-years and influenced by yield potential and soil %OM. In-season tests hold promise as decision tools for organic winter bread wheat production but additional evaluation and calibration is needed. Future studies should include a variety of organic-approved and locally available pre-plant and topdress sources, a wider range of background tiller densities and topdress N rates, and perform tissue testing at growth stages beyond GS30, but prior to GS60.

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