Nitrogen Uptake, Distribution, and Utilization in Hard Red Spring Wheat Varieties
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Hard Red Spring Wheat (HRSW) is grown on approximately 1.75 million acres in Minnesota and contributed over $700 million to Minnesota's economy in 2008. High grain yields and protein concentrations are essential to sustain HRSW in the crop rotation. Growers measure their production success by grain yield, but the price received for those yields is greatly influenced by grain quality, in particular grain protein concentration. Sometimes price premiums are offered for grain protein over 14%, but more often a price penalty is incurred for grain protein less than 14%. Many factors determine grain yield and protein including the environment; which includes soil characteristics and weather; the variety grown; and N availability, the most critical nutrient determining yield and grain protein content.

Nitrogen is an essential component of the photosynthetic apparatus of the plant by which biomass is accumulated not only for structural components of the plant, but also for grain. Therefore, N status of the whole plant affects grain yield. Nitrogen is also a major component of the grain protein. The difference in N utilization to produce grain yield or protein is the localization of the N within the plant. To produce yield, N must be in the vegetative components of the plant to maintain photosynthetic capacity. To produce protein, N must be in the grain. In most situations, an increase in grain yield after a certain level decreases grain protein concentration.

Spring wheat varieties are categorized by their relative differences in yield and protein. Some varieties have high yield and moderate protein potential while others have moderate yield and high protein potential. This difference suggests that these varieties vary in their N uptake and its utilization within the plant. Can N management vary with variety to optimize fertilizer N use in high yielding wheat production systems? Minnesota research earlier this decade evaluated the response of four HRSW varieties to N fertilizer rates. Maximum grain yield varied among the varieties, but their responses to fertilizer N were quite similar. However, the grain protein responses to N rates among the varieties were less clear. Total amount of protein produced was very similar among the varieties. Therefore, the grain protein differences observed were primarily caused by differences in grain yield. This suggests these varieties were translocating similar amounts of N to the grain (protein), but their ability to maintain biomass production for yield was quite different.

A number of research studies have shown grain protein N is primarily from two sources. Remobilization of vegetative N accumulated prior to anthesis is probably the main source. The second source is N uptake after anthesis which tends to be transported directly to the developing grain. The N status of the vegetative plant components at anthesis and N uptake after anthesis will influence the amount of N remobilized. It is hypothesized that the greater proportion of the vegetative N remobilized, the less capacity the plant has to produce biomass that contributes to grain yield. Several studies have shown variation among wheat varieties in N uptake after anthesis and N remobilization. However, most of these trials were conducted in greenhouse, growth chamber, or irrigated environments. Minnesota wheat production is almost exclusively in rain-fed environments with variable precipitation patterns. Based on available evidence the question still remains; should N fertilizer management vary with HRSW varieties in the dry-land cropping systems of Minnesota? From a grain yield stand point there appears to be little reason to suspect so. However, in recent years, growers excitement over exceptional grain yields has been neutralized by disappointing grain protein. To understand this situation better requires a more in-depth examination of N uptake, distribution, and utilization by HRSW varieties that vary in grain yield and protein potential. Additionally, a wild type allele from Triticum turgidum spp. dicoccoides, designated Gpc-B1 has been identified to effect grain protein. HRSW and durum wheat lines with this allele have significantly higher grain protein and has been introgressed into adapted HRSW backgrounds. This allele tends to accelerate flag leaf senescence indicating a pleiotropic effect on N remobilization. It may be hypothesized that as flag leaf N is remobilized its capacity to produce biomass for grain yield deminishes.

Four HRSW varieties commonly grown in Minnesota were selected based on variety trial results conducted at various locations within the state. Varieties will be screened at some point in the future for the presence or absence of the marker associated with the Gpc-B1 allele. Meanwhile, the likelihood of a variety having the Gpc-B1 allele was deduced based on its pedigree and association with varieties known to have the Gpc-B1 allele. Four N fertilizer rates were included to evaluate varieties under situations of no N application (N1), N stress (N2), N sufficiency (N3), and N excess environments (N4). Specifc objectives were to: 1) Evaluate differences in N uptake efficiency during the growing season of the four varieties; 2) Evaluate vegetative N remobilization characteristics of these varieties during reproductive growth and relate those characteristics to N uptake efficiency; 3) Relate the N uptake and N remobilization efficiencies of these varieties to N utilization to produce grain yield and grain protein.

Materials and Methods:

Field experiments were established in the spring of 2010, 2011, and 2012 at the Northwest Research and Outreach Center near Crookston, Minnesota. Experiments were set up as randomized complete block design with split-plot arrangement of treatments and four replications. Whole plot treatments were nitrogen (N) rates of 0, 60, 120, and 180 lb N ac^{-1}, which will be referred in subsequent sections as N1, N2, N3, and N4, respectively. Split-plot treatments were four HRSW varieties that vary in grain yield and protein potential (Table 1). Experimental split-plots were 13 ft wide and 30 ft long. Each split-plot consisted of two 5 ft wide strips of planted wheat with approximately 45 cm between each strip. Each strip consisted of ten planted rows spaced 15 cm apart. There was a 10 ft alley between each replication. In 2010 and 2011, the experiments were placed where soybean was the previous crop. In 2012, wheat was the previous crop. Spring soil tests on samples collected within each alley immediately after planting indicated about 35 lbs. residual nitrate-N ac^{-1} in the top 60 cm of the soil profile.

All fertilizer used in the experiment was applied in the spring and incorporated with a field cultivator just prior to planting. Phosphorus and potassium fertilizers were hand-broadcasted and applied over the entire experimental area at rates based on soil test results and University of Minnesota recommendations. Phosphorus was applied as 0-46-0 and potassium as 0-0-60. Nitrogen was applied as urea (46-0-0) and broadcasted by hand. The N rate broadcasted was based on the whole plot treatment assignment, but the urea was weighed and applied on a split-plot basis to ensure a more uniform distribution of the N fertilizer across the whole plot. All the wheat varieties were planted at a seeding rate of 1.6 million seeds per acre.

Table 1. Genetic characteristics of selected HRSW cultivars.

Cultivar	Grain yield potential	Grain protein concentration potential	Probability of Gpc-B1 allele
Faller	High	Low	Likely
Samson	High	Low	Likely not
Glenn	Low	High	Likely
Vantage	Low	High	Likely not

Plant sampling was initiated at the emergence of the first node. Subsequent samplings occurred at anthesis, anthesis + 10 days, anthesis + 20 days, and physiological maturity. Plant sampling was done by clipping all the plants as close to the soil surface as possible within a 9 ft^2 area (middles 6 rows, 3 ft long) of each planted strip. Though each planted strip was sampled and bagged separately in the field, once transported to the laboratory the two bags corresponding to a split-plot were combined for processing. All the varieties were sampled at the same time in the first sampling, but in subsequent samplings each variety was sampled based on their growth development. At the anthesis sampling in 2011 and 2012, Faller and Samson were the first varieties to be sampled followed by Glenn and Vantage spaced about 4 days apart.

In the first sampling (node), the entire plant was kept intact for processing to determine dry matter and N concentration. The entire sample was weighed then subsampled. The subsample was weighed, dried and reweighed for dry matter and calculated back to the whole sample.

In subsequent samplings the entire sample was weighed then spread out over a flat surface. Multiple random samples from throughout the spread out pile were selected and processed into four plant parts. Plants were separated into heads, uppermost node (including stem, flag leaf and sheath), second uppermost node (included stem and penultimate leaf and sheath), and the remaining nodes (leaves and sheath, and stem). Stem separation continued until at least 140 gms of the smallest plant part had been accumulated. Every plant that contributed to this plant part was completely separated so there were no partial plants included in the four separated plant parts. Each plant part was weighed, dried, and reweighed for dry matter accumulation. The plant part ratio in the subsample was assumed to be the same as that of the whole sample and used to estimate the whole sample dry matter.

At the final sampling, physiological maturity, the head was separated from the stem, but no further plant separation occurred because all the leaves were gone by this time and only brown stems and leaf sheaths remained. The head was dried and weighed before thrashing. Thrashed grain was collected and weighed and the difference was considered chaff, which was not saved.

All plant parts were ground separately and analyzed for N concentration. The N concentration of the chaff was assumed to be similar to the stem N concentration at maturity. Estimated chaff dry matter was combined with the stem dry matter. Nitrogen concentration was determined through dry combustion methods at Agvise commercial laboratories in Northwood, North Dakota. Total plant N accumulation (or uptake) was calculated as the sum of the

product of dry matter and N concentration of the various plant parts. Other considered parameters and their formulas are given below:

1. Harvest index (HI) = Grain dry matter/ dry matter accumulation (whole plant) at maturity.
2. Nitrogen harvest index (NHI) = Grain N content/ N accumulation (whole plant) at maturity.
3. Pre-anthesis dry matter and N accumulation: dry matter and N accumulation (whole plant) at anthesis.
4. Post-anthesis dry matter and N accumulation: difference between dry matter and N accumulation at the anthesis and at the maturity.
5. Dry matter remobilization efficiency: (DMRME, %) = [Dry matter accumulation (whole plant) at anthesis - [(leaf + culm) + chaff] dry matter accumulation at maturity] X 100/dry matter accumulation (total) at anthesis.
6. Nitrogen remobilization efficiency (NRME, %) = [Nitrogen accumulation (whole plant) at the anthesis - [(leaf + culm) + chaff] nitrogen accumulation at maturity] X 100/ Nitrogen accumulation (total) at the anthesis.

In the above estimates, we assumed the remobilization of photo-assimilates from vegetative tissues to only developing grain without counting the plant respiration or any N losses, thus the calculated estimate of the variable will have its overestimation.

Statistical analyses and plotting were done using GLIMMIX, NLMIXED, GPLOT, GTL (graph template language), and SGPLOT procedures (SAS Institute, 2011). The normality assumption was checked using Shapiro–Wilk test and residual plots (residuals vs. fitted values) were used to check the assumption of variance homogeneity. Site-year and blocks nested within site-year were considered random. For generalized linear models, N fertilizer rate was considered as a continuous factor. Its linear and quadratic effects and their interactions with wheat cultivar were tested. Significant fixed effects of N rate and wheat cultivar and their interactions were further analyzed to perform mean separations. Least square mean differences were adjusted using the Holm (1979) technique included in MULTTEST procedure of SAS (SAS Institute, 2011) to control the family wise error rate at 5%.

For non-linear data analysis, the interest was to find the distribution of dry matter and nitrogen accumulation in various plant parts and in total among wheat cultivars in the growing season under various N supplying environments but not to study response of wheat cultivars to applied N. Thus, data were divided based on N applied as 0 lb ac^{-1} (N1; Control), 60 lb ac^{-1} (N2; Low N), 120 lb ac^{-1} (N3; Optimum N), and 180 lb ac^{-1} (N4; High N). Initial analyses of non-linear models suggested using natural logarithm transformation of response variables to obtain homogeneity or errors. Results are reported on anti-logarithm back transformation and plotted as growth curves subjected to spline smooth curve fitting between response variables and time with GPLOT procedure. In this study, following nonlinear models were tested (Draper & Smith, 1998) along with linear and quadratic models:

1) Gompertz Model, $Y = \alpha e^{-e^{\beta - kx}}$,

2) Logistic Model $Y = \frac{\alpha}{(1+\beta e^{-kx})}$,

3) Richards Model $Y = \frac{\alpha}{(1+e^{\beta - kx})^{1/\delta}}$, and

4) Three $-$ parameter exponential function (Ratkowsky, 1990), $Y = e(a + bx + cx^2)$

Where, Y; response variable, α; the value of asymptote, β; value of plants growth beginning stage, k; net growth ratio, δ; parameter at inflexion point, x; time, and a, b, and c are unknown constants. The best model was selected on the basis of lower AIC, BIC, and AICC values.

Non-linear models were fit for N concentration and dry matter- and N- accumulation in head, flag leaf, penultimate leaf, lower stem, and in whole stem and plant.

In this report, we are presenting data collected during all three funded years (2010-2012). However, the analysis is still going on, therefore the given section of Results and Discussion is not complete. Our objective is to thoroughly investigate all the collected data and publish as *peer-review* article(s) in journal(s). Complete data analysis and writing is expected to finish in coming months. Upon its completion, we will prepare another report that will be submitted in March, 2014.

Results:

Grain Dry Matter, Grain N Concentration, Harvest Index, and Nitrogen Harvest Index

Grain dry matter, grain N concentration, and nitrogen harvest index were significantly different among wheat cultivars and increased linearly with increasing N fertilizer rate (Table 2). Two-way interaction of cultivar and N rate was non-significant for these variables. Glenn and Vantage had greater grain N concentration and lower dry matter than Faller and Samson, yet all cultivars accumulated similar amounts of grain N (Fig. 1). The difference in grain dry matter of Glenn and Vantage from Samson was non-significant. The differences in grain dry matter and grain N concentration among selected cultivars can be attributed to their genetic characteristics (Table 1). Differences in grain dry matter and grain N concentration but similar grain N content among cultivars is consistent with previous observations and reinforces that grain protein concentration variation is caused by the dilution effects of dry matter.

Harvest index (HI) and N harvest index (NHI) was significantly different among the four wheat cultivars and increased with increasing N rate (Table 2). Vantage had lower HI than other cultivars. Glenn produced similar amounts of grain dry matter as Vantage but produced significantly greater HI than Vantage and greater NHI than all cultivars suggesting that Glenn was more efficient in utilizing total plant dry matter for grain dry matter and grain N comcentration (Fig. 1).

Table 2. Significance of F values of fixed effects on HRSW grain dry matter, grain N concentration, harvesting index (HI), and nitrogen harvesting index (NHI).

Source of variation	Grain dry matter	Grain N concentration	Grain N content	HI	NHI
			P value		
Cultivar	0.0122	<.0001	0.6821	<.0001	0.0063
Nitrogen fertilizer rate (Nrate)	<.0001	<.0001	<.0001	0.3675	<.0001
Nrate × C	0.8946	0.4228	0.5997	0.0387	0.4317

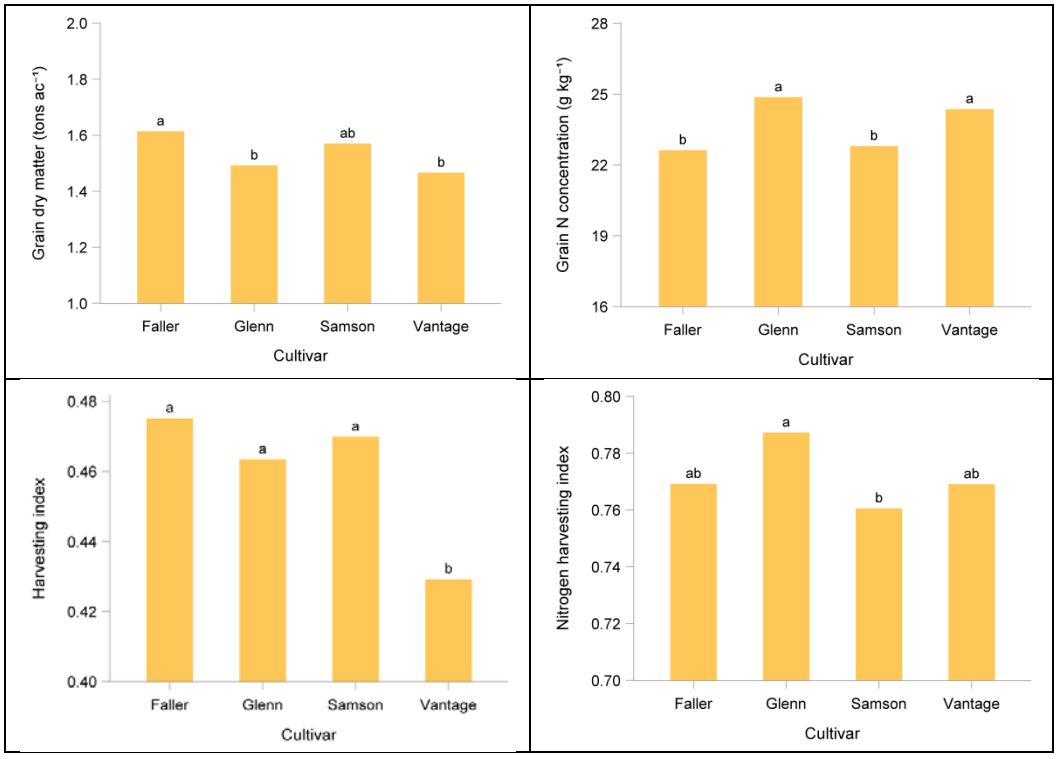

Fig. 1. Grain dry matter, grain N concentration, harvesting index, and nitrogen harvesting index of four HRSW cultivars averaged over N rates and three-years (2010-2012).

Dry Matter and N accumulation

Total dry matter and N accumulation measured at the time of maturity was similar among four wheat cultivars and increased with increasing N fertilizer rate (Table 3) which is opposite to the grain dry matter differences measured at the time of maturity. It means that differences observed in grain dry matter and grain N concentration at the time of maturity should have been translated from earlier differences occurring in total plant dry matter and N accumulation. Our data suggests that differences in total plant dry matter and N accumulation (P=0.0641) among the four wheat cultivars were already apparent during the pre-anthesis period which is consistent with earlier reports. It suggests that the pre-anthesis period is most critical for spring wheat to develop grain dry matter (or grain yield) and N accumulation. In the post-anthesis period, Glenn accumulated significantly greater total plant N than Faller (P=0.0755) (Fig. 2). Greater grain dry matter in Faller than Glenn can be associated with greater pre-anthesis total plant dry matter accumulation. Vantage also had significantly greater pre-anthesis total plant dry matter accumulation than Glenn and Samson but this cultivar seems not efficient enough to translate greater plant dry matter in to greater grain dry matter. Lower plant N accumulation in pre-anthesis and greater in post-anthesis period of Glenn than other cultivar suggests that Glenn had greater capacity to accumulate N in the plant over the growing season and also has greater capacity to transfer that N from vegetative parts to grain N.

Table 3. Significance of F values of fixed effects on dry matter and nitrogen accumulation measured at the time of pre-anthesis, post-anthesis and at maturity.

Source of variation	Dry matter accumulation			Nitrogen accumulation		
	Pre-anthesis	Post-anthesis	At maturity	Pre-anthesis	Post-anthesis	At maturity
	P value					
Cultivar	<.0001	0.7727	0.7901	0.0641	0.0755	0.5873
Nitrogen fertilizer rate (Nrate, lin)	<.0001	0.1882	<.0001	<.0001	0.0394	<.0001
Nrate, quad	<.0001		<.0001	<.0001		<.0001
Nrate, lin × C	0.4245	0.9938	0.8663	0.6878	0.9735	0.7744
Nrate, quad × C	0.5581		0.6158	0.8633		0.4685

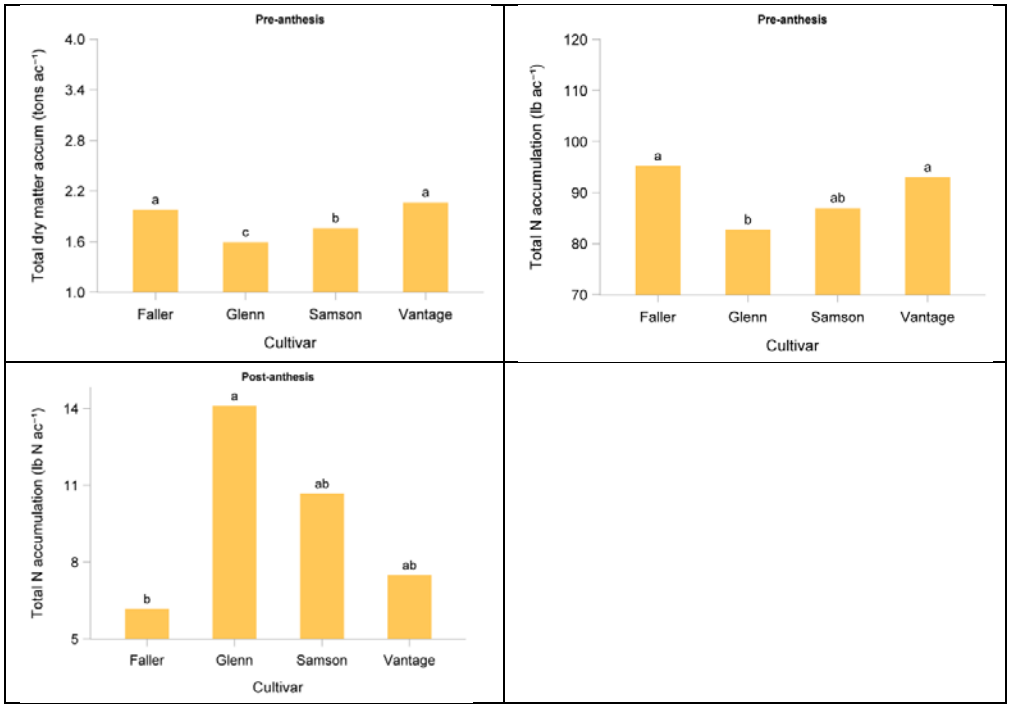

Fig. 2. Dry matter and nitrogen accumulation measured at the time of pre-anthesis, post-anthesis and at maturity of four HRSW cultivars averaged over N rates and three-years (2010-2012).

Total (whole plant) dry matter accumulation measured at different times of the growing season was modeled with the quadratic response function (Fig. 3). This figure shows that differences in total dry matter accumulation among the four wheat cultivars were prevalent under low N treatments (N2) and at the end of the growing season. Faller and Samson accumulated greater dry matter than other cultivars. Greater accumulation of dry matter of Faller and Samson also resulted in greater dry matter measured in the grain (Fig. 2).

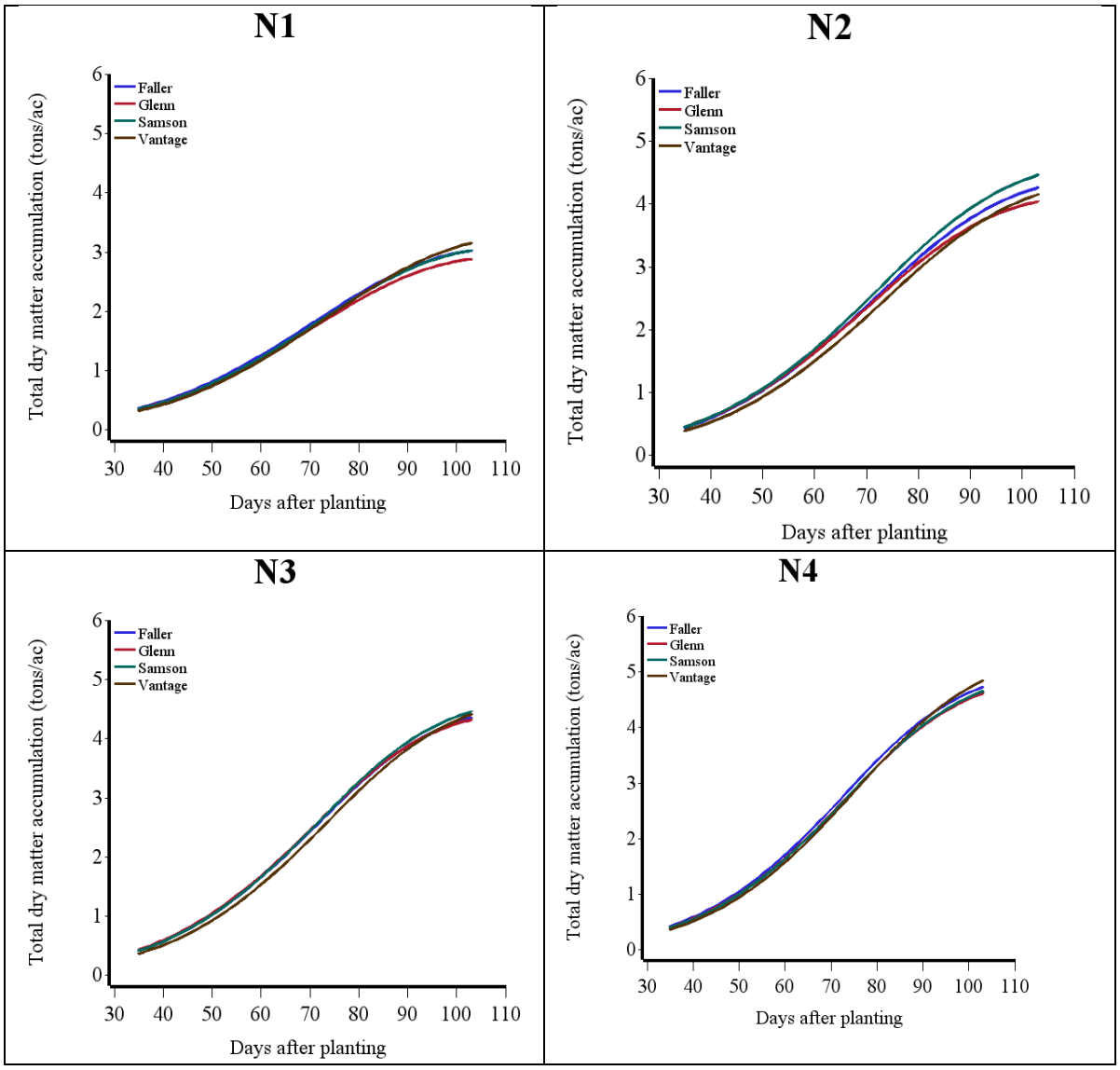

Fig. 3. Total dry matter accumulation of four wheat cultivars in the growing season over three-years (2010-2012) and in different N supplying environments

Total (whole plant) N accumulation measured at different times of the growing season was modeled with the three-parameter exponential function (Fig. 4). This figure shows that differences in total N accumulation among four wheat cultivars were prevalent under low N (N2) and optimum N (N3) treatments. In both of these N treatments, Vantage accumulated lower N than other cultivars while Samson accumulated greater N in N2 treatment and Glenn accumulated greater N in N3 treatment than other cultivars. Greater N accumulation by Glenn and Samson resulted in greater N measured in the grain than other cultivars (Fig. 2).

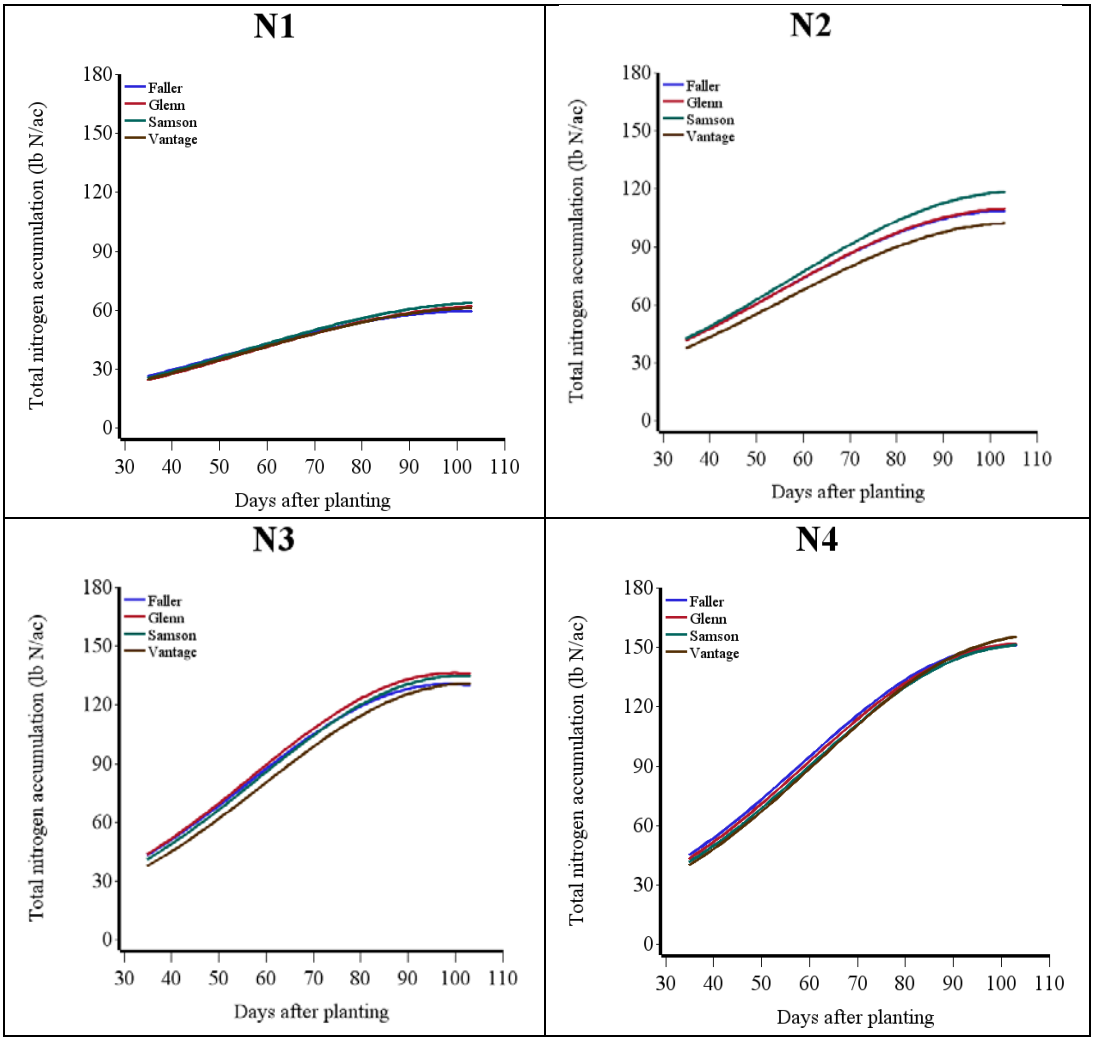

Fig. 4. Total nitrogen accumulation of four wheat cultivars in the growing season over three-years (2010-2012) and in different N supplying environments.

Dry Matter and N Remobilization Efficiency

Dry matter and N remobilization efficiency was significantly affected by the main effect of N rate and cultivar (Table 4). Dry matter and N remobilization efficiency were found greater for Faller, lower for Glenn and other two cultivars, Sampson and Vantage, in between (Fig. 3). Dry matter and N remobilization efficiency followed the pattern of dry and N accumulated at the time of anthesis (Fig. 2). Glenn despite the fact it had lower N remobilization efficiency, still produced greater grain N and NHI than other cultivars showing that Glenn has better ability to accumulate N in post-anthesis period and transfers this N to the grain. These findings were verified through greater post-anthesis plant N accumulation in Glenn than other cultivars (Fig. 2).

Table 4. Significance of F values of fixed effects on dry matter remobilization efficiency (DMRME, %), and nitrogen remobilization efficiency (NRME, %).

Source of variation	DMRME	NRME
	——— *P* value ———	
Cultivar	<.0001	0.0456
Nitrogen fertilizer rate (Nrate)	0.0060	<.0001
Nrate × C	0.8295	0.9821

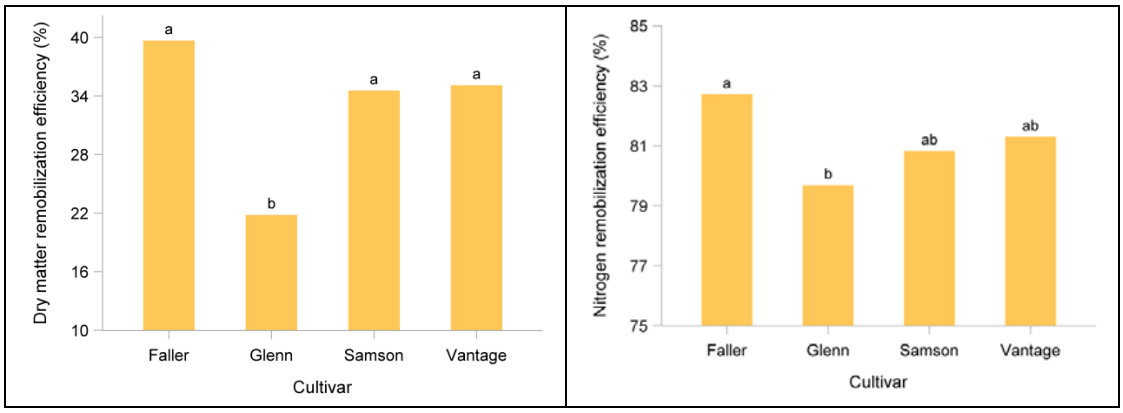

Fig. 5. Dry matter and nitrogen remobilization efficienciey of four HRSW cultivars averaged over N rates and three-years (2010-2012).