plants

Article

Optimizing Nitrogen and Seed Rate Combination for Improving Grain Yield and Nitrogen Uptake Efficiency in Winter Wheat

Hemat Mahmood [1,2], Jian Cai [1,*], Qin Zhou [1], Xiao Wang [1], Allan Samo [1], Mei Huang [1], Tingbo Dai [1], Mohammad Shah Jahan [3] and Dong Jiang [1]

[1] National Technique Innovation Centre for Regional Wheat Production, Key Laboratory of Crop and Ecophysiology in Southern China, Nanjing Agricultural University, Ministry of Agriculture, Nanjing 210095, China; mahmoodhemat@yahoo.com (H.M.); qinzhou@njau.edu.cn (Q.Z.); xiaowang@njau.edu.cn (X.W.); allansamo@njau.edu.cn (A.S.); huangmei@njau.edu.cn (M.H.); tingbod@njau.edu.cn (T.D.); jiangd@njau.edu.cn (D.J.)

[2] Department of Agronomy, Agriculture Faculty, Ghazni University, Ghazni 2301, Afghanistan

[3] Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh; shahjahansau@gmail.com

* Correspondence: caijian@njau.edu.cn

Abstract: Nitrogen (N) supply and seed rate (SR) are two essential factors that affect the accumulation and partitioning of N and dry matter (DM) and, therefore, grain yield (GY) and N use efficiency (NUE). The objective of this experiment was to optimize N application and SR to regulate wheat growth and increase both GY and NU_E. The results revealed that net photosynthetic rate (Pn), stomatal conductance (Gs), chlorophyll content, and activities of metabolic enzymes (NR and GS) significantly increased with increasing of N levels while decreasing SR. Plant tillers, GY, DM before anthesis, and N translocation, N agronomic efficiency (NA_E), N recovery efficiency (NR_E), and N uptake efficiency (NUP_E) were highest in a combined treatment of N_{235} and SR_{180}. However, N levels beyond 235 kg ha^{-1} significantly decreased NA_E, NR_E, and NUP_E. By increasing SR from 135 to 180 kg ha^{-1} an increase of 12.9 % and 9.1% GY and NUPE, respectively, was observed. Based on this result, we estimate that 1 kg N ha^{-1} might be replaced by an increase of approximately 0.6 kg ha^{-1} SR. Our study suggested that using a combination of N and SR (N_{235} + SR_{180}) could attain maximum GY and improve NU_E parameters.

Keywords: high yield; nitrogen application; N use efficiency; seed rate; winter wheat

check for updates

Citation: Mahmood, H.; Cai, J.; Zhou, Q.; Wang, X.; Samo, A.; Huang, M.; Dai, T.; Jahan, M.S.; Jiang, D. Optimizing Nitrogen and Seed Rate Combination for Improving Grain Yield and Nitrogen Uptake Efficiency in Winter Wheat. *Plants* **2022**, *11*, 1745. https://doi.org/10.3390/plants11131745

Academic Editors: Przemysław Barłóg, Jim Moir, Lukáš Hlisnikovský and Xinhua He

Received: 17 May 2022
Accepted: 24 June 2022
Published: 30 June 2022

1. Introduction

Wheat is the main staple crop globally and plays a crucial role in challenging food security, with a total production of 736.1 million tons. Wheat grain yield is not only dependent on genetic potential (variety) and environmental constraints [1] but also depends on management practices [2,3]. Nitrogen is the essential nutrient for wheat growth and production [4,5] which is necessary for maintaining plant growth, biomass, and grain yield [6]. N deficiency in cereal crops reduces fertile tiller numbers [7–9], grain number, and kernel weight [10,11]. However, overuse of N results in environmental problems including N leaching, runoff, and volatilization [12], and reduces overall N use efficiency (NU_E) [13]. In China, a rapid increase in wheat yield was in parallel with the dramatic use of N fertilizer since the 1950s [14]. Importantly, in the last 20 years, N input beyond the threshold level only caused prolonged yield improvement while severe environmental pollution [12,15,16]. Thus, the China government proposed the Double Reduction Plan [15]. A low dose of N with high NU_E must be one of the main research goals in plant nutrition [16].

However, enhancing crop profitability and NU_E simultaneously is necessary for sustainable agriculture [17] and it is a key challenge to improve viable agriculture in the next decades [18]. N fertilizer is often widely overused to obtain ideal productivity while the adjustment of SR is neglected, which usually synchronously improves productivity and NU_E. When the N rate was decreased or cut, the output would be lost by reducing tillering and fertile tillers, grain number, and kernel weight [19]. The increase in SR could partially compensate for the decrease in fertile tillers and spike numbers and final productivity [20]. Increasing plant density from 135 to 405 plants m^{-2} [21] or 75 to 300 plants m^{-2} [11] significantly increased grain yield and other parameters. However, there must be an optimum SR to compensate for the negative effects of decreasing N for balanced high yields and improved NU_E in wheat. Therefore, it is necessary to investigate the compensatory effect of increasing SR on the decreasing N input on wheat productivity and N use efficiency. It is also necessary to clarify their combination effects on the physiological and agronomical performance of wheat to reveal the underlying rules for balanced high grain yield and improved NU_E in winter wheat.

A field experiment with different N and SR levels in two successive years was carried out to determine the optimum combination of N and the seed rate leading to improvement in grain yield and N use efficiency.

2. Results

2.1. Physiological Traits

2.1.1. Photosynthetic Capacity, Chlorophyll Content, and Leaf Area Index

Pn, Gs, SPAD, and LAI were highest at the anthesis stage, followed by the jointing stage, 10 days after anthesis (10 DAA) and 20 DAA, respectively. N application and SR have significant effects on various physiological traits, viz., leaf photosynthetic capacity, chlorophyll content (SPAD value), as well as leaf area index (LAI) at all growth stages (Figures 1–3). Photosynthetic capacity (Pn), stomatal conductance (Gs), and SPAD were significantly increased by increasing the N rate or decreasing SR. A significant increment was observed as the N application rate increased from 0 to 235 kg ha^{-1}. When N increased from 235 to 290 kg ha^{-1}, there was no significant increase for Pn, Gs, and SPAD in both growing seasons. The Pn, Gs, and SPAD values were decreased significantly when SR increased from 135 to 225 kg ha^{-1} in both growing seasons at all sampling stages. Moreover, LAI was significantly increased with an increase in both factors (N + SR). A significant effect was observed when N increased up to N_{235} treatment. There was no significant difference between the N_{235} and N_{290} treatments in LAI in both growing seasons (Figure 3). SR also significantly increased the LAI in both growing seasons from SR_{135} to SR_{180}, beyond this level there was no significant effect.

2.1.2. Enzymatic Activities of NR and GS

Nitrate reductase (NR) and glutamine synthesize (GS) play an essential role in N metabolism assimilation and regulation. NR and GS′s enzyme activities were significantly increased by increasing N and decreasing SR (Figure 4). However, the activities of NR and GS differed at the various levels of N application. Furthermore, there was a significant variation in NR and GS activities between N_0, N_{180}, and N_{235}, whereas there was no significant difference between the N_{235} and N_{290} treatments. The highest value of the NR and GS activities appeared as the combination of N_{290} and SR_{135} treatments (Figure 4).

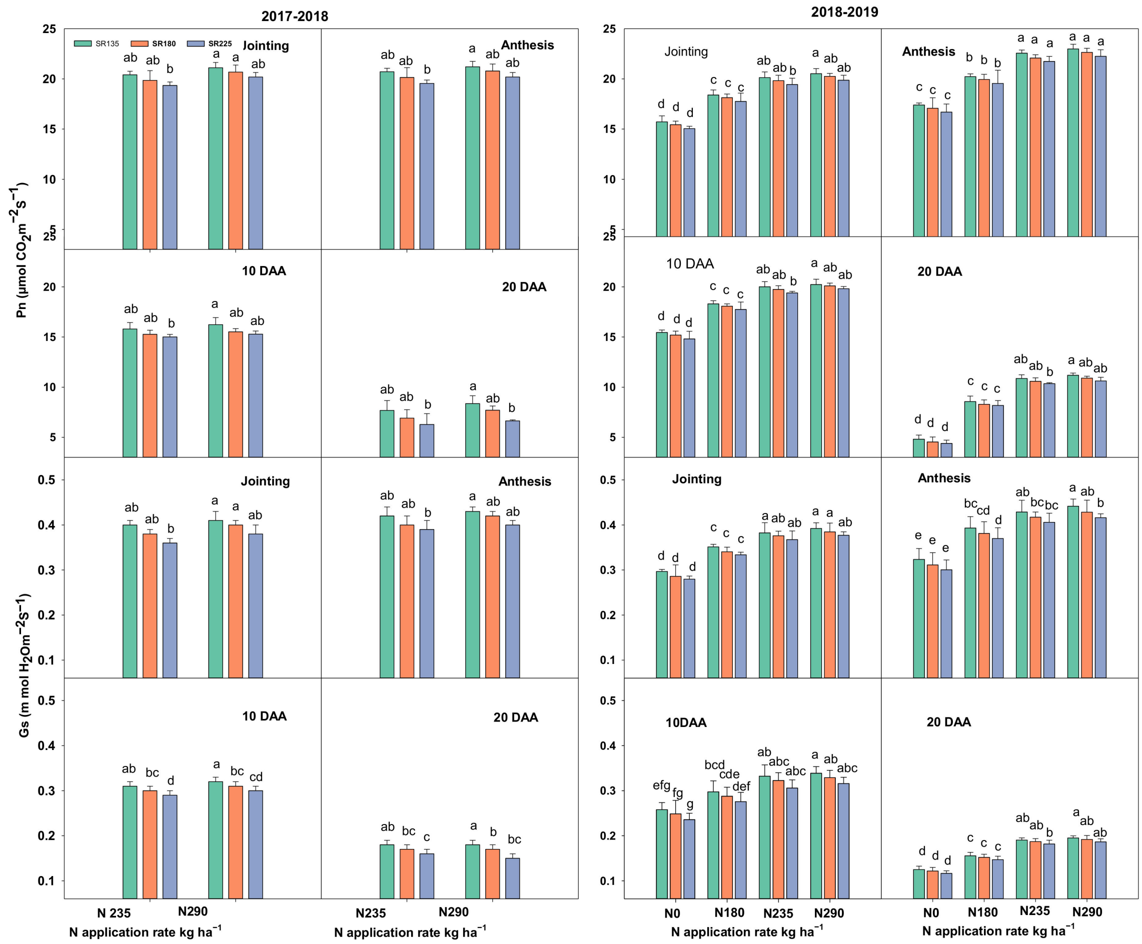

Figure 1. Effects of the combination of N and SR on photosynthesis (Pn) and stomatal conductance (Gs) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

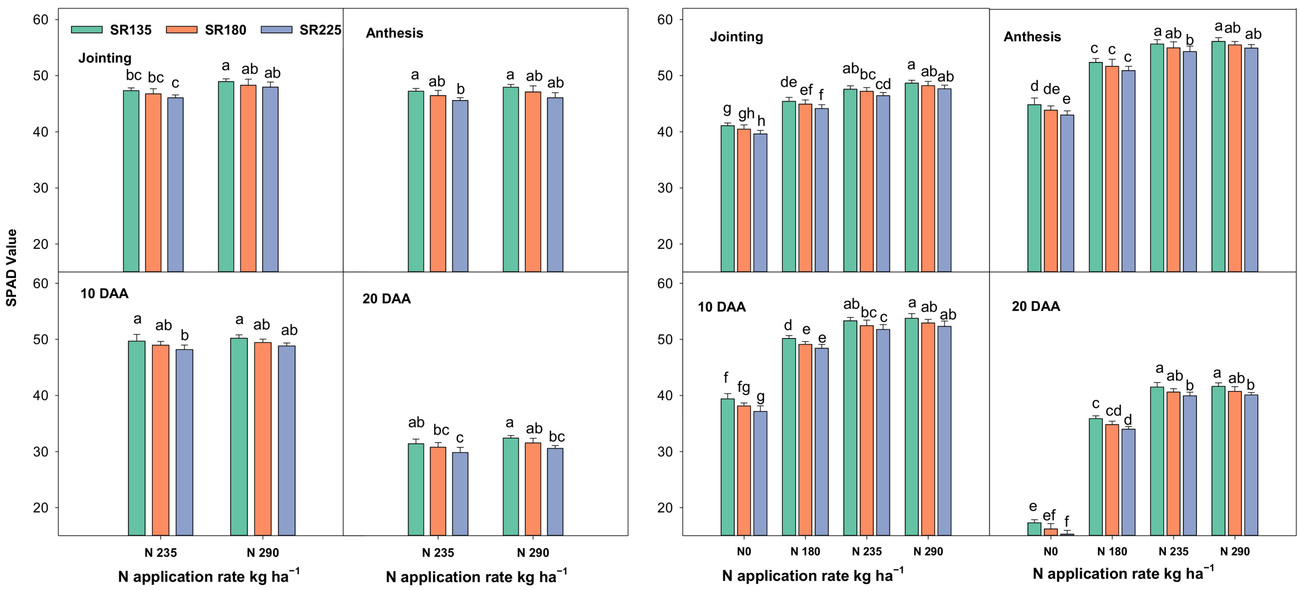

Figure 2. Effects of N and SR on chlorophyll content (SPAD) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

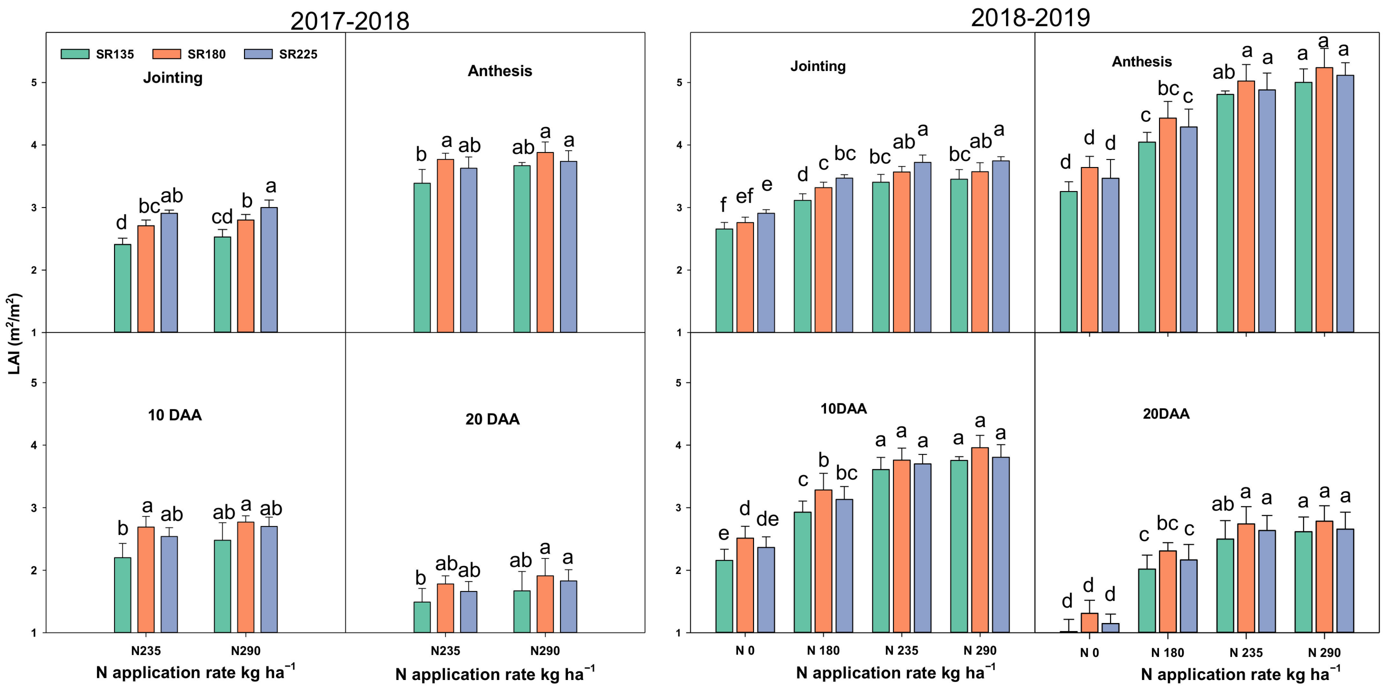

Figure 3. Effects of the combination of N and SR on the values of the leaf area index (LAI) during four differnet growing stages in 2017–2018 and 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test.

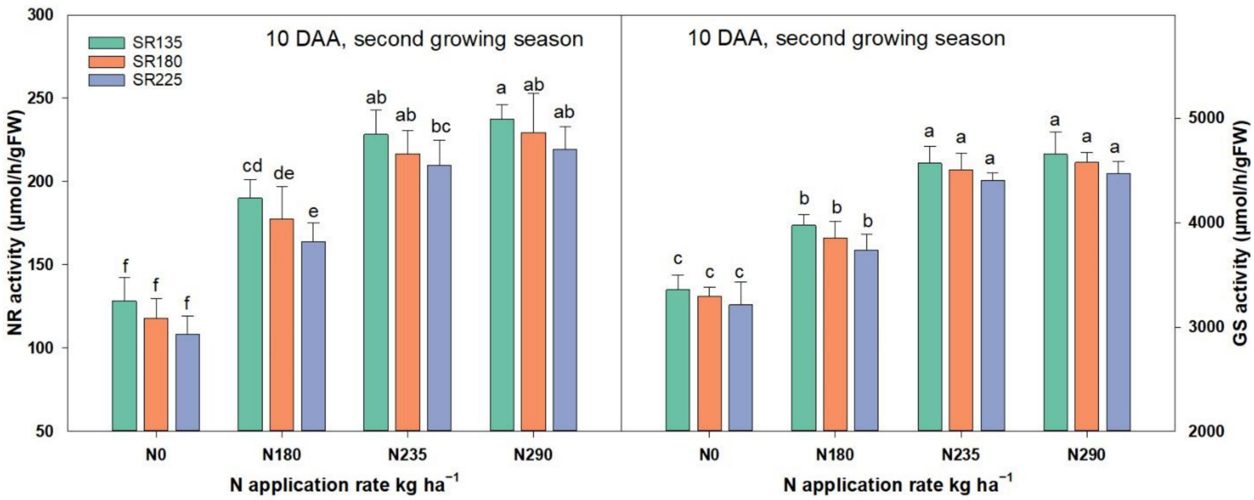

Figure 4. Combination effects of N and SR on nitrate reductase (NR) and glutamine synthesize (GS) activities during four differnet growing stages in 2018–2019. Different letters represent significant differences in mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.2. Grain Yield and Related Agronomic Characteristics

The grain yield (GY) was significantly influenced by N level and seed rate (N and SR), as well as by their interaction in both years (Table 1). With the increase in the N application rate the GY significantly increased. At SR of 180 kg ha^{-1}, GY increased from 6.7 to 8.3 t ha^{-1} when N application increased from N_{180} to N_{235} kg ha^{-1} in 2018–2019. There was no significant difference between N_{290} and N_{235} kg ha^{-1} in both growing seasons. SR significantly affected GY in both growing seasons. Under the same amount of N application (N_{235} kg ha^{-1}), GY increased from 5 to 5.7 t ha^{-1} in the first growing season and from 7.5 to 8.3 t ha^{-1} in the second growing season (Table 1). The highest GY 8.3 t ha^{-1} was obtained with the combination of N_{235} + SR_{180} kg ha^{-1} in 2018–2019. N and SR significantly affected the agronomic parameters viz., number of spikes (NS), 1000-grain weight (TGW), number of grains per spike, harvest index (HI), and plant height (PH). All agronomic parameters (NS, TGW, NGS, HI, and PH) were significantly increased with the increasing N application rate. Compared to the treatment with SR_{180} N_0, NS was significantly increased by 13.2%, 23.8%, and 22.4 % and NGS was increased by 21.8%, 36.8%, and 37.8% in treatments $SR_{180}N_{180}$, $SR_{180}N_{235}$, and $SR_{180}N_{290}$, respectively, in the second growing season. Except for the first growing season in which PH was scarcely increased, TGW and HI were decreased when N application rate increased from N_{235} to N_{290}. At the second growing season, none of the agronomic characteristics were significantly influenced beyond N_{235} kg ha^{-1}.

Table 1. Effects of N and SR on GY and agronomic characteristics in 2017–2018 and 2018–2019.

	N kg ha^{-1}	**SR kg ha^{-1}**	**TY t ha^{-1}**	**NS × 10^4 ha^{-1}**	**TGW(g)**	**NGS**	**HI**	**PH (cm)**
2017–2018	N_{235}	SR_{135}	5.05 ^{b}	357 ^{c}	39.7 ^{a}	35.63 ^{a}	0.41 ^{c}	73.4 ^{c}
		SR_{180}	5.78 ^{a}	414 ^{b}	39.5 ^{ab}	35.38 ^{a}	0.44 ^{ab}	74.33 ^{bc}
		SR_{225}	5.7 ^{a}	425 ^{ab}	39.2 ^{bc}	34.27 ^{b}	0.45 ^{a}	75.74 ^{ab}
	N_{290}	SR_{135}	5.03 ^{b}	361 ^{c}	39.1 ^{bc}	35.63 ^{a}	0.41 ^{c}	76.43 ^{ab}
		SR_{180}	5.77 ^{a}	420 ^{ab}	39 ^{bc}	35.3 ^{a}	0.43 ^{bc}	77.23 ^{a}
		SR_{225}	5.77 ^{a}	434 ^{a}	38.9 ^{c}	34.18 ^{b}	0.42 ^{bc}	77.57 ^{a}
	F-Value	N	0.113	2.475	13.65 **	0.098	7.478 *	19.88 **
		SR	107.1 **	105.9 **	3.38 *	21.65 **	11.46 **	2.999
		N*S	0.331	0.123	0.63	0.025	1.637	0.426
2018–2019	N_0	SR_{135}	4 ^{g}	391 ^{g}	35.7 ^{bc}	28.8 ^{d}	0.35 ^{e}	62.93 ^{d}
		SR_{180}	4.8 ^{f}	479 ^{ef}	35.6 ^{bc}	28.1 ^{d}	0.4 ^{cde}	63.62 ^{d}
		SR_{225}	4.8 ^{f}	503 ^{de}	35.5 ^{c}	27.2 ^{d}	0.4 ^{cd}	64.07 ^{d}
	N_{180}	SR_{135}	5.6 ^{e}	448 ^{f}	36.4 ^{ab}	34.5 ^{c}	0.38 ^{de}	72.87 ^{c}
		SR_{180}	6.7 ^{d}	543 ^{bc}	36.3 ^{abc}	34.1 ^{c}	0.41 ^{cd}	74.02 ^{bc}
		SR_{225}	6.6 ^{d}	555 ^{b}	36.3 ^{abc}	33.1 ^{c}	0.42 ^{bc}	74.47 ^{b}
	N_{235}	SR_{135}	7.5 ^{c}	518 ^{cd}	36.7 ^{a}	39.6 ^{ab}	0.45 ^{ab}	73.97 ^{bc}
		SR_{180}	8.3 ^{a}	593 ^{a}	36.7 ^{a}	38.3 ^{ab}	0.46 ^{a}	75.15 ^{ab}
		SR_{225}	8.2 ^{a}	597 ^{a}	36.5 ^{ab}	37.5 ^{b}	0.46 ^{a}	75.75 ^{a}
	N_{290}	SR_{135}	7.8 ^{bc}	529 ^{bc}	36.6 ^{a}	40.4 ^{a}	0.45 ^{ab}	73.95 ^{bc}
		SR_{180}	8.2 ^{a}	587 ^{a}	36.4 ^{ab}	38.6 ^{ab}	0.44 ^{ab}	75.22 ^{ab}
		SR_{225}	8.1 ^{ab}	591 ^{a}	36.4 ^{ab}	37.7 ^{ab}	0.45 ^{ab}	75.97 ^{a}
	F-Value	N	690 **	74.4 **	9.66 **	242.5 **	43.55 **	598.3 **
		SR	60.1 **	83.94 **	0.66	12.19 **	4.48 *	17.8 **
		N*SR	2.64 *	1.36	0.027	0.394	1.77	0.25

Note: N, SR, TY, NS, TGW, NGS, HI, and PH indicate nitrogen application, seed rate, theoretical yield number of spikes, thousand-grain weight, the number of grains per spike, harvest index, and plant height, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

SR significantly influenced all of the agronomic parameters. Increasing SR significantly increased NS, HI, and PH during both growing seasons and decreased NGS and TGW. As an example, compared with the $N_{235}SR_{135}$ treatment, NS was significantly increased by 10.8% and 11.6%, while NGS was decreased by 4.5% and 6.7% in treatments $N_{235}SR_{180}$ and $N_{235}SR_{225}$, respectively, in the second growing season (Table 1).

Increasing the application of N increased the fodder part and the yield of wheat. Increasing the seed rate can increase the spike number and compensate for reducing the N application rate. Linear regression was used to assess replacing N with SR for balancing GY and NU_E parameters. According to the equations obtained from linear regression, the increase in grain yield by 1 ton ha^{-1} needs to increase the seed rate by 7669 kg ha^{-1} or increase the fertilization with N by 12,807 kg ha^{-1}. This means that 7669 kg ha^{-1} seed rate is equivalent to 12,807 kg N; therefore, 0.598 kg ha^{-1} seed rate would approximately replace 1 kg of N ha^{-1} (Figure 5). Based on our result, increasing SR from 135 to 180 kg ha^{-1} was observed to increase by 12.9% and 9.1% for GY and NUPE, respectively.

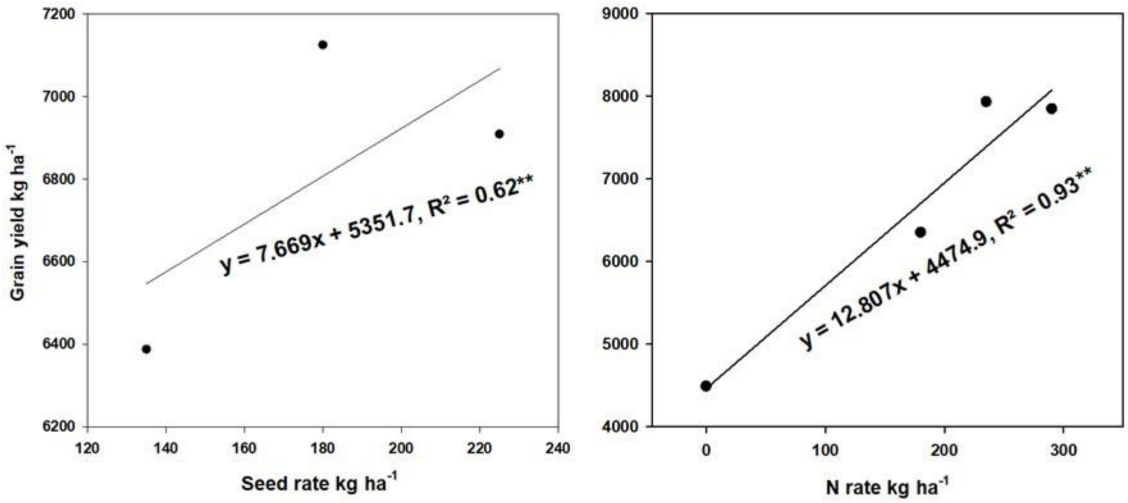

Figure 5. Estimating the regression line to compare N and seed rate for balancing grain yield and improving NU_E. * $p \leq 0.05$ and ** $p \leq 0.001$, respectively. The dots represent the mean value of grain yield under each seed rate (left) or nitrogen rate (right).

2.3. Accumulation, Translation, and Partitioning of DM

DM accumulation (DMA) was significantly affected by the rate of N application and SR (Table 2). Increasing the rate of application of N significantly increased DMA in all growth stages. The highest amount of DMA appeared during the jointing to the anthesis stage. SR significantly increased DMA at all growth stages. The significant effects of SR were up to 180 kg ha^{-1}, and beyond SR_{180} kg ha^{-1} was not further influenced (Table 2). Similarly, when N fertilizer increased beyond N_{235} kg ha^{-1}, the values of DMA were not significantly increased. At maturity, the highest increase in DM was found with $N_{235}SR_{180}$ treatment.

Table 2. Combination effects of N and SR on DM accumulation, translocation in 2018–2019.

Total DM Accumulation kg ha^{-1}						DM Translocation			
N kg ha^{-1}	**SR kg ha^{-1}**	**So-JT**	**JT-An**	**An-M**	**So-M**	**PTA kg ha^{-1}**	**CPT%**	**PAA kg ha^{-1}**	**CPA%**
N_0	SR_{135}	1959 ^{h}	6139 ^{b}	2680 ^{c}	10778 ^{g}	1214.1 ^{f}	30.3 ^{a}	4256 ^{d}	69.7 ^{e}
	SR_{180}	2314 ^{g}	6514 ^{b}	3250 ^{bc}	12079 ^{f}	1322.8 ^{f}	27.6 ^{b}	5072 ^{d}	72.4 ^{d}
	SR_{225}	2401 ^{f}	6682 ^{b}	3025 bc	12108 ^{f}	1439 ^{e}	29.7 ^{a}	5018 ^{d}	70.3 ^{e}
N_{180}	SR_{135}	2395 ^{f}	8585 ^{a}	3708 ^{b}	14689 ^{e}	1529.4 ^{de}	27.2 ^{bc}	6053 ^{c}	72.8 ^{cd}
	SR_{180}	2856 ^{b}	8904 ^{a}	4780 ^{a}	16539 ^{cd}	1633.8 ^{cd}	24.3 ^{d}	7276 ^{b}	75.7 ^{b}
	SR_{225}	2488 ^{e}	8587 ^{a}	4646 ^{a}	15721 ^{d}	1701.4 ^{c}	25.6 ^{cd}	7164 ^{b}	74.4 ^{bc}
N_{235}	SR_{135}	2668 ^{d}	9335 ^{a}	4731 ^{a}	16735 ^{c}	1918.7 ^{ab}	25.5 ^{cd}	7804 ^{ab}	74.5 ^{bc}
	SR_{180}	3019 ^{a}	10027 ^{a}	5130 ^{a}	18177 ^{ab}	1963.7 ^{a}	23.6 ^{de}	8727 ^{a}	76.4 ^{ab}
	SR_{225}	3033 ^{a}	9804 ^{a}	5064 ^{a}	17902 ^{ab}	2028.5 ^{a}	24.9 ^{d}	8490 ^{a}	75.1 ^{b}
N_{290}	SR_{135}	2719 ^{c}	9477 ^{a}	5048 ^{a}	17245 ^{bc}	1905.8 ^{ab}	24.4 ^{d}	8141 ^{ab}	75.6 ^{b}
	SR_{180}	2998 ^{a}	10066 ^{a}	5418 ^{a}	18483 ^{a}	1823.2 ^{b}	22.1 ^{e}	8776 ^{a}	77.9 ^{a}
	SR_{225}	3010 ^{a}	9649 ^{a}	5459 ^{a}	18119 ^{ab}	1906.7 ^{ab}	24 ^{de}	8518 ^{a}	76.4 ^{ab}
F-Value	N	1817 **	28.1 **	32.1 **	258 **	167.9 **	50.4 **	92.454 **	50.4 **
	SR	84 **	0.9	4.3 *	23.8 **	11.1 **	16 **	9.546 **	16 **
	N*SR	67.1 **	0.1	0.3	0.4	1.9	0.2	0.28	0.2

Note: S_O-JT, JT-An, An-M, S_O–M, PTA, CPT, PAA, and CPA represent sowing to jointing, jointing to anthesis, anthesis to maturity, sowing to maturity, pre-anthesis DM translocation amount, contribution of pre-anthesis translocation to grain, post-anthesis accumulation amount, and contribution of post-anthesis DM accumulation to grain, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

The translocation and contribution of DM were significantly affected by the application rate of N and SR (Table 2). With increasing N rate, pre-anthesis translocation (PTA), post-anthesis accumulation (PAA), and contribution of post-anthesis to grain (CPA) were significantly increased, while the contribution of pre-anthesis translocation to grain (CPT) was significantly decreased. The maximum value of PTA appeared in the N_{235} treatment, which was significantly higher than the N_{290} treatment. Furthermore, the value of PTA increased with increasing SR, while PAA and CPA significantly increased up to SR_{180} kg ha^{-1}. The CPT values first decreased and then increased as the SR increased.

The partition of DM into different parts of the plant differed between the N and SR treatments (Table 3). At the anthesis stage, the DM of culm + sheath was higher than the DM of rachis + glumes and the DM of rachis + glumes was higher than the DM of the leaves. However, at the harvesting stage, the grain DM was higher than the DM of the culm + sheath, the DM of culms + sheaths was higher than the DM of rachis + glumes, and the DM of the rachis + glumes was higher than the DM of leaves. The proportion of distribution of DM of grains, rachis + glumes, culms + sheathes, and leaves ranged from 37.1% to 45.8%, 12.7% to 14.1%, 33% to 41.4%, and 7.8% to 8.5%, respectively, at harvesting.

Table 3. Effects of N and SR on DM accumulation and partitioning at the anthesis and maturity stages in 2018–2019.

N kg ha^{-1}	SR kg ha^{-1}	Grain		Rachis + Glumes		Culms + Sheaths		Leaves	
		Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity
N_0	SR_{135}		4005 ^{g}	1576 ^{c}	1465 ^{f}	5442 ^{g}	4462 ^{c}	1080 ^{f}	846 ^{f}
	SR_{180}		4788 ^{f}	1821 ^{c}	1606 ^{e}	5631 ^{f}	4741 ^{bc}	1376 ^{e}	944 ^{e}
	SR_{225}		4851 ^{f}	1994 ^{c}	1606 ^{e}	5701 ^{f}	4682 ^{bc}	1389 ^{e}	968 ^{e}
N_{180}	SR_{135}		5623 ^{e}	2344 ^{bc}	1959 ^{d}	7217 ^{d}	5932 ^{a}	1420 ^{e}	1175 ^{d}
	SR_{180}		6712 ^{d}	2496 ^{bc}	2198 ^{bc}	7426 ^{c}	6299 ^{a}	1837 ^{c}	1330 ^{c}
	SR_{225}		6647 ^{d}	2517 ^{bc}	2218 ^{bc}	6716 ^{e}	5525 ^{ab}	1841 ^{c}	1330 ^{c}
N_{235}	SR_{135}		7535 ^{c}	3072 ^{ab}	2187 ^{c}	7156 ^{d}	5642 ^{a}	1775 ^{d}	1370 ^{b}
	SR_{180}		8324 ^{a}	3597 ^{a}	2366 ^{a}	7375 ^{c}	6017 ^{a}	2075 ^{a}	1468 ^{a}
	SR_{225}		8152 ^{ab}	3426 ^{ab}	2366 ^{a}	7341 ^{c}	5919 ^{a}	2070 ^{a}	1464 ^{a}
N_{290}	SR_{135}		7814 ^{bc}	3092 ^{ab}	2232 ^{b}	7199 ^{d}	5807 ^{a}	1906 ^{b}	1391 ^{b}
	SR_{180}		8244 ^{a}	3358 ^{ab}	2355 ^{a}	7641 ^{a}	6425 ^{a}	2066 ^{a}	1458 ^{a}
	SR_{225}		8087 ^{ab}	3058 ^{ab}	2337 ^{a}	7545 ^{b}	6241 ^{a}	2057 ^{a}	1453 ^{a}
F-Value	N		690 **	14.8 **	2002 **	2858.3 **	18.4 **	955.9 **	1001 **
	SR		60.1 **	0.92	207 **	96.3 **	2.3	324.9 **	85.5 **
	N*SR		2.64 *	0.17	6.29 **	61.7 **	0.58	11.1 **	2.92 *

Note: N and SR represent nitrogen application and seed rate, respectively. Different letters in the same column represent significant differences of mean values of three replicate plots at $p \leq 0.05$ levels according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.4. Accumulation, Translocation, and Partitioning of N

Accumulation of N (NA) at all parts of the plant was significantly increased by increasing N application and SR up to certain levels (N_{235}, SR_{180}) at both anthesis and maturity stages. At the maturity stage, the total content of N compared to control (N_0) treatment increased by 76.9%, 134%, and 139% in the treatments N_{180}, N_{235}, and N_{290}. The N content compared to SR_{135} was increased by 8.8% and 5% in the SR_{180} and SR_{225} treatments (Table 4). As well, N translocation before anthesis to grain (NTA), N accumulation after anthesis (NAA), and the contribution rate of NA after anthesis to grain (CAG) were significantly increased with the increase in N application. NTA increased 32.18% and 2.9% when N increased from N_{180} to N_{235} from N_{235} to N_{290}, respectively (average of three SR treatments). Furthermore, the contribution rate of pre-N translocation to grain (CTG) had the same trend as CPT. Furthermore, by increase in SR up to 180 kg ha^{-1}, NTA, NAA, and CAG were significantly increased, while CTG was at first significantly decreased then increased (Table 4).

Table 4. Combination effects of N and SR on total N accumulation and translocation in 2018–2019.

		Total N Accumulation kg ha^{-1}				N Translocation			
N kg ha^{-1}	**SR kg ha^{-1}**	**So-JT**	**JT-An**	**An -M**	**So-M**	**NTA kg ha^{-1}**	**CTG%**	**NAA kg ha^{-1}**	**CAG%**
N_0	SR_{135}	25.6 ^{h}	53.4 ^{i}	12.4 ^{g}	91.4 k	48.3 ^{j}	70.1 ^{ab}	27.4 j	29.9 ^{de}
	SR_{180}	28.5 ^{g}	60.4 ^{h}	14.5 ^{f}	103.4 ^{i}	55.5 ^{i}	69.4 ^{bc}	31.5 ^{h}	30.6 ^{cd}
	SR_{225}	28.8 ^{g}	60.4 ^{h}	11 ^{g}	100.2 ^{j}	55.3 ^{i}	71 ^{a}	29.2 ^{i}	29 ^{e}
N_{180}	SR_{135}	41.3 ^{f}	97.2 ^{g}	23.2 ^{e}	161.6 ^{h}	84.3 ^{h}	68.4 ^{cd}	48.9 ^{g}	31.6 ^{bc}
	SR_{180}	45.6 ^{e}	108.5 ^{e}	30.2 ^{cd}	184.3 ^{f}	97.8 ^{f}	68.2 ^{d}	56.5 ^{e}	31.8 ^{b}
	SR_{225}	46.1 ^{e}	100.6 ^{f}	29.1 ^{d}	175.8 ^{g}	94.7 ^{g}	68.3 ^{cd}	54.2 ^{f}	31.7 ^{bc}
N_{235}	SR_{135}	57.4 ^{d}	135.1 ^{d}	29.7 ^{cd}	222.2 ^{e}	118.1 ^{e}	67.5 ^{de}	70 ^{d}	32.5 ^{ab}
	$SR1_{80}$	61.7 ^{ab}	145.3 ^{a}	31.5 ^{c}	238.6 ^{ab}	125.9 ^{ab}	66.7 ^{e}	76.9 ^{a}	33.3 ^{a}
	SR_{225}	61.6 ^{ab}	136.3 ^{d}	31.5 ^{c}	229.4 ^{d}	122.1 ^{d}	67.3 ^{de}	72.7 ^{bc}	32.7 ^{ab}
N_{290}	SR_{135}	59 ^{c}	140.1 ^{b}	31.3 ^{c}	230.4 ^{d}	123.2 ^{cd}	67.8 ^{de}	72.1 ^{c}	32.2 ^{ab}
	SR_{180}	61.9 ^{a}	145.8 ^{a}	34.2 ^{b}	241.8 ^{a}	126.9 ^{a}	66.6 ^{e}	77.7 ^{a}	33.4 ^{a}
	SR_{225}	61 ^{b}	138 ^{c}	36.1 ^{a}	235 ^{c}	124.6 ^{bc}	67.4 ^{de}	74.2 ^{b}	32.6 ^{ab}
F-Value	N	1159 **	2229 **	628.1 **	6418 **	9584 **	44.2 **	436.6 **	44.2 **
	SR	270.4 **	377.9 **	31.8 **	1283 **	195.7 **	5.7 *	115.6 **	5.7 *
	N*SR	7.1 **	31.7 **	8.72 **	44.3 **	15.81 **	1	3.03 *	1

Note: SO-JT, JT-An, An-M, SO–M, NTA, CTG, NAA, and CAG represent jointing, jointing to anthesis, anthesis to maturity, sowing to maturity, pre-anthesis N translocation, contribution rate of N translocation to grain, nitrogen accumulation amount, and contribution rate of N accumulation to grain, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

Plant N partitioning was also influenced by N and SR. Compared with the control treatment (N_0), the N_{180}, N_{235}, and N_{290} treatments increased the N content by 53.8%, 153%, and 144% in the rachis + glumes, by 84.2%, 149.2%, and 157.6% in the culms + sheathes, and by 65.8%, 107.7%, and 109.4% in the part of leaves, respectively, at anthesis stage. However, this increase in N at the harvesting stage was by 78.7%, 140.3%, and 145.6% for grains, by 52.9%, 98.5%, and 103% for rachis + glumes, by 61.5%, 88.1%, and 99% for culms + sheathes, and by 113.7%, 182%, and 186% for leaves in treatments N_{180}, N_{235}, and N_{290} compared to N0 treatment, respectively. Additionally, compared to the SR_{135} treatment, the SR_{180} and SR_{225} treatments increased N content by 8.6% and 1% at the rachis + glumes, by 1.7% and 2% at the culms + sheathes, and by 16% and 3.7% at the parts of leaves, respectively, at anthesis stage. Compared to SR_{135} treatment, the N content of the SR_{180} and SR_{225} treatments increased by 9.8% and 6.2% in grains, by 5.5% and 1.8% in the rachis + glumes, by 5.1% and −2% in the culms + sheathes, and by 5.5% and 2.7% in the leaves, respectively, at the maturity stage (Table 5). The range of the N distribution ratio of different parts, i.e., rachis + glumes, culms + sheathes, and leaves were from 17% to 21.9%, from 39.1% to 47.1%, and from 33.5% to 41.6% at anthesis, respectively. The maturity stage range of N distribution range was 75.4% to 79.2%, from 5.8% to 7.4%, 8.8% to 11.9%, and from 5% to 6.4% for grains, rachis + glumes, culms + sheathes, and leaves, respectively (Table 5).

Table 5. Combination effects of N and SR on N partitioning during anthesis and maturity stages in 2018–2019.

N kg ha^{-1}	**SR kg ha^{-1}**	**N at Grain**		**N at Rachis + Glumes**		**NA at Culms + Sheaths**		**N at Leaves**	
		Anthesis	**Maturity**	**Anthesis**	**Maturity**	**Anthesis**	**Maturity**	**Anthesis**	**Maturity**
N_0	SR_{135}		68.9 ^{f}	15 ^{g}	6.8 ^{c}	34.9 ^{g}	10.9 ^{e}	29.2 ^{i}	4.9 ^{d}
	SR_{180}		80 ^{e}	17.1 ^{f}	7.1 ^{c}	35.1 ^{g}	11.2 ^{e}	36.8 ^{g}	5.2 ^{d}
	SR_{225}		77.8 ^{ef}	18.2 ^{f}	6.6 ^{c}	34.9 ^{g}	10.6 ^{e}	36.1 ^{h}	5.1 ^{d}
N_{180}	SR_{135}		123.2 ^{d}	25.7 ^{e}	10 ^{b}	65.2 ^{e}	18.1 ^{cd}	47.6 ^{f}	10.3 ^{c}
	SR_{180}		143.4 ^{c}	26.2 ^{e}	10.8 ^{b}	67.1 ^{d}	18.8 ^{bc}	60.8 ^{e}	11.3 ^{b}
	SR_{225}		138.5 ^{c}	25.1 ^{e}	10.3 ^{b}	60.6 ^{f}	15.9 ^{d}	60.9 ^{e}	11 ^{bc}
N_{235}	SR_{135}		174.9 ^{b}	40.3 ^{c}	13.2 ^{a}	87.7 ^{b}	20.1 ^{abc}	64.5 ^{d}	14 ^{a}
	SR_{180}		188.9 ^{a}	45.4 ^{a}	13.9 ^{a}	88.4 ^{b}	21 ^{abc}	73.3 ^{a}	14.7 ^{a}
	SR_{225}		181.4 ^{ab}	41.2 ^{c}	13.4 ^{a}	85.7 ^{c}	20.2 ^{abc}	70.9 ^{b}	14.4 ^{a}

Table 5. *Cont.*

N kg ha^{-1}	SR kg ha^{-1}	N at Grain		N at Rachis + Glumes		NA at Culms + Sheaths		N at Leaves	
		Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity	Anthesis	Maturity
N_{290}	SR_{135}		181.7 ^{ab}	40.8 ^{c}	13.5 ^{a}	89.1 ^{b}	20.7 ^{abc}	69.2 ^{c}	14.4 ^{a}
	SR_{180}		190.4 ^{a}	43.5 ^{b}	14.1 ^{a}	91.2 ^{a}	22.5 ^{a}	73 ^{a}	14.8 ^{a}
	SR_{225}		184.9 ^{a}	38.1 ^{d}	13.9 ^{a}	89.5 ^{ab}	21.7 ^{ab}	71.4 ^{b}	14.5 ^{a}
F-Value	N		845.1 **	2010.4 **	322.7 **	5132.3 **	83.3 **	24379 **	595 **
	SR		19.80 **	36.8 **	4.35 *	19.9 **	2.02	2256 **	3.63 *
	N*SR		0.98	13.9 **	0.2	5.6 *	0.66	172.9 **	0.3

Note: N, SR, and NA represent nitrogen application, seed rate, and N accumulation, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively. Revision as above.

2.5. N Use Efficiency (NU_E) Parameters

N rates, SR, and their interaction had a significant effect on N agronomy efficiency (NA_E), N uptake efficiency (NUP_E), and N partial factor productivity (NPFP). Increasing N level up to N_{235} kg ha^{-1}, NA_E, NUP_E, N recovery efficiency (NR_E), and N harvest index (NHI) were significantly increased. However, increasing N levels beyond N_{235} kg ha^{-1}, NA_E, NR_E, and NUP_E were significantly decreased. The NPFP values were decreased at all N levels. Furthermore, the NA_E, NR_E, NUP_E, NPFP, and NHI values decreased by 12.8%, 10.4%, 17.3%, 13.6%, and 0.4%, respectively, at N_{290} treatment compared to N_{235} treatment. Similarly, SR had a considerable effect on the NU_E parameters, but the effect of SR was less compared to the N treatment. Maximum values for the parameters NA_E, NR_E, and NUP_E were observed from the combination of treatment with N_{235} and SR_{180} (Table 6).

Table 6. Combination effects of N and SR on N use efficiency parameters in 2018–2019.

N Rate kg ha^{-1}	SR kg ha^{-1}	NA_E	NR_E	NUP_E	NPFP	NHI
		kg kg^{-1}	%	kg kg^{-1}	kg kg^{-1}	%
N_0	SR_{135}					0.75 ^{f}
	SR_{180}					0.77 ^{de}
	SR_{225}					0.77 ^{de}
N_{180}	SR_{135}	9.0 ^{f}	39 ^{e}	0.9 ^{c}	31.2 ^{c}	0.76 ^{ef}
	SR_{180}	10.7 ^{de}	44.9 ^{cd}	1.02 ^{a}	37.3 ^{a}	0.78 ^{bc}
	SR_{225}	10 ^{e}	42 ^{de}	0.98 ^{a}	36.9 ^{a}	0.79 ^{ab}
N_{235}	SR_{135}	14.4 ^{a}	53.4 ^{a}	0.95 ^{b}	30.8 ^{c}	0.79 ^{ab}
	SR_{180}	14.4 ^{a}	55.2 ^{a}	1.02 ^{a}	34 ^{b}	0.79 ^{ab}
	SR_{225}	13.5 ^{b}	52.7 ^{a}	0.98 ^{b}	33.3 ^{b}	0.8 ^{a}
N_{290}	SR_{135}	13.4 ^{b}	48.8 ^{b}	0.8 ^{d}	27.4 ^{e}	0.79 ^{ab}
	SR_{180}	12.1 ^{c}	48.6 ^{b}	0.83 ^{d}	28.9 ^{d}	0.79 ^{ab}
	SR_{225}	11.4 ^{cd}	47.3 ^{bc}	0.81 ^{d}	28.4 ^{de}	0.79 ^{ab}
F-value	N	156.4 **	82.6 **	3774.8 **	180.8 **	17.6 **
	SR	6.4 **	4.4 *	20.04 **	55.4 **	7.1 **
	N*SR	8.76 **	2.16	4.27 **	9.12 **	1.88

Note: N, SR, NA_E, NR_E, NUP_E, NPFP, and NHI represent nitrogen rate, seed rate, nitrogen agronomy efficiency, N recovery efficiency, N uptake efficiency, N partial factor productivity, and N harvest index, respectively. Different letters in the same column represent significant differences in mean values of three replicate plots according to Duncan's test; * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.6. Correlation Analysis

2.6.1. Correlation of GY with Agronomic and Photosynthesis Traits

The key relationships between the GY-related parameter variables are shown in Table 7. There was a significant positive correlation between GY and NS, NGS, TGW, PH, HI, Pn, Gs, SPAD value, and LAI. However, there was no significant relationship between GY and PH (Table 7).

Table 7. Correlation analysis of grain yield with agronomic and photosynthesis traits.

	GY	NS	NGS	TGW	PH	HI	Pn	Gs	SPAD	LAI
GY	**1**	0.854 **	0.893 **	0.626 **	0.116	0.879 **	0.917 **	0.788 **	0.889 **	0.961 **
NS		1	0.535 **	0.418 *	0.346*	0.733 **	0.641 **	0.492 **	0.638 **	0.777 **
NGS			1	0.613 **	−0.08	0.804 **	0.942 **	0.860 **	0.909 **	0.900 **
TGW				1	0.212	0.432 **	0.676 **	0.566 **	0.699 **	0.633 **
PH					1	−0.102	0.031	−0.138	0.22	0.117
HI						1	0.747 **	0.660 **	0.673 **	0.821 **
Pn							1	0.852 **	0.951 **	0.923 **
Gs								1	0.813 **	0.814 **
SPAD									1	0.894 **
LAI										1

Note: GY: grain yield; NS: number of spikes; NGS: number of grains per spike; TGW: 1000 grain weight per gram; PH: plant height (cm); HI: harvest index; Pn: net photosynthesis; Gs: stomatal conductance; SPAD: leaf greenness; LAI: leaf area index. * and ** mean significantly correlation at * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

2.6.2. Relationship between GY and Enzyme Activities and NUE Parameters

Grain yield had a significant and positive relationship with nitrogen reductase (NR) and glutamine synthesis (GS) enzymes, N agronomic efficiency (NA_E), N recovery efficiency (NR_E), and N uptake efficiency (NUP_E). However, GY had a significantly negative relationship with N translocation's contribution before anthesis to grain (CTG) (Figure 6).

Increasing N application from 235 to 290 kg ha^{-1}, GY did not increase significantly (0.4%) while NA_E, NR_E, and NUP_E were decreased 12.8%, 10.4%, and 17.3%, respectively. Maximum GY and highest values of NU_E parameters, particularly NA_E, NR_E, and NUP_E, were observed from the combination of treatment with N_{235} and SR_{180} (Tables 1 and 6).

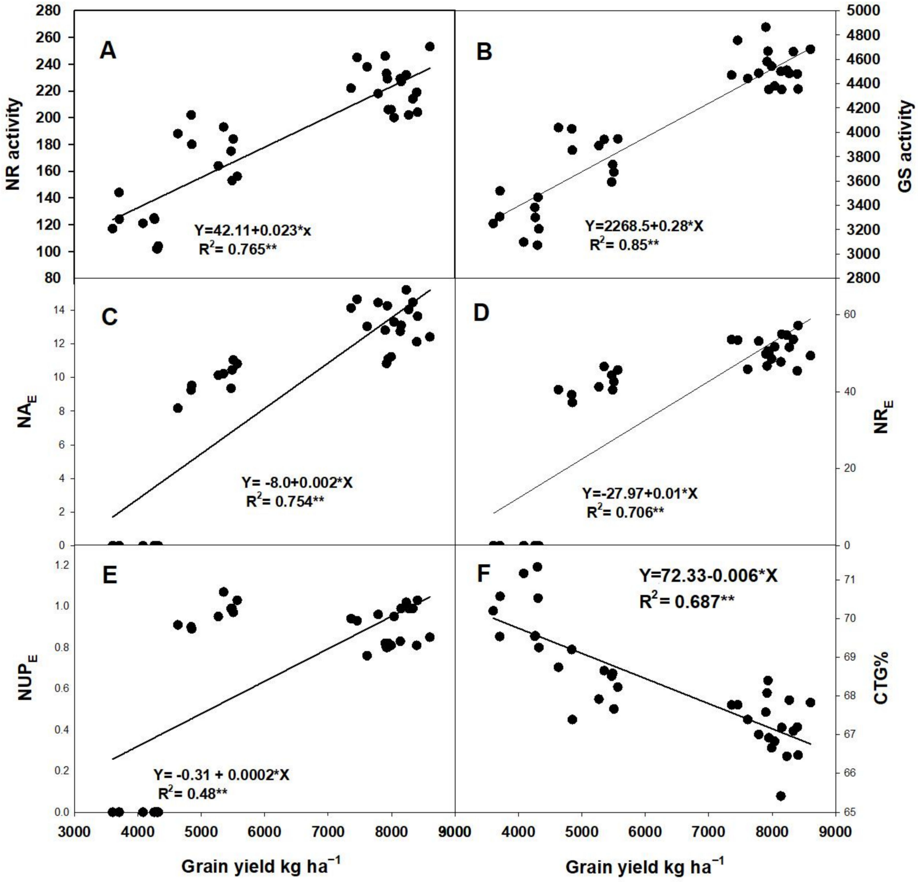

Figure 6. Regression analyses among GY and nitrate reductase (NR)/(**A**) glutamine synthesis (GS)/(P**B**), N agronomy efficiency (NAE)/(**C**), N recovery efficiency (NR_E)/(**D**), N uptake efficiency (NUP_E)/(**E**), and contribution of N translocation to the grain after the anthesis stage (CTG)/(**F**), respectively. * $p \leq 0.05$ and ** $p \leq 0.001$, respectively.

3. Discussion

DM and N translocation are well-known to greatly contribute to the final GY [22,23]. However, there is a lack of research on the combined effects of N and SR on the DM and N translocation, NU_E parameters, growth physiological parameters, and their relationship with GY in winter wheat. The effects of the excessive rate of N and N′s compensation by increasing SR on final GY and NU_E parameters were unknown.

3.1. Physiological Characteristics

The plant leaf′s photosynthesis capacity plays a crucial role in plant growth and grain yield [24], and approximately 70% of productivity is derived from post-anthesis photosynthesis. In the present study, increasing N application and decreasing SR, resulted in a significantly increased Pn, Gs, and SPAD values in both growing seasons. Furthermore, LAI was significantly increased by increasing N application and SR (Figures 1–3). However, it has shown that the maximum values for Pn, Gs, and SPAD parameters were exhibited from the combination of N_{290} + SR_{135} treatment but there were no significant differences between the mentioned and N_{235} + SR_{180} treatment. The main reason for decreasing the Pn, Gs, and SPAD values by increasing SR might be due to more competition and the over-crowded shading effect as reported by [25]. In addition, N reductase (NR) and glutamine synthesize (GS) increased significantly with the N application rate and the declaration of SR. Notably, there was no significant difference for the value of both mentioned enzyme activities when N fertilizer amount increased from N_{235} to N_{290}. These findings suggest N and SR′s optimization benefits for maintaining strong photosynthesis capacity and N assimilation ability in wheat plants.

3.2. Grain Yield (GY) and N Use Efficiency (NU_E)

Simultaneous improvement in GY and NU_E of wheat is an important objective in modern agriculture management. Here, we investigated the suitable combinations of N and SR to obtain higher GY and NUE. The maximum value was observed with the treatment of N_{235} + SR_{180} kg ha^{-1} in both growing seasons. The previous finding can explain that too high plant density had no significant effect on wheat grain yield [11,26]. According to our results, yield loss caused by reducing the N rate can be compensated by increasing SR (Figure 2). It was estimated by a linear regression that every decrement of 1 kg N ha^{-1} can be replaced by adding 0.6 kg ha^{-1} SR. The GY obtained by adding SR is clearly attributed to the increasing spike number (SN) (Table 1). Moreover, the yield in 2017–2018 (Y1) was much lower than that in 2018–2019 (Y2), which may be due to the adverse weather conditions. There was excessive rainfall and less sunshine during grain filling in the first growing season (Figure 1), leading to lower yield components and GY [1].

NU_E results from the incorporation of N-uptake efficiency (NUP_E) and N-utilization efficiency (NUT_E) [27]. In detail, NUP_E the plant′s capacity to extract N from the soil, and depending on the root structure and the relation of N transporters [28]. In this study, increasing N application from N_{235} to N_{290} resulted in a decrease in NAE, NR_E, NUP_E, and NPFP by 12.8%, 10.4%, 17.3%, and 13.6%, respectively. Similarly, a result was obtained by [29] that treatment with N_{240} and N_{300} compared to treatment with N180, NPFP were decreased by 24.5% and 37.4% and NA_E were decreased by 23.5% and 31.9%, respectively. The decrease in NU_E after the optimal rate might be due to more losses by increasing N application according to the previous finding of [11]. Here, our results further confirmed that NU_E components were significantly increased up to a certain amount of SR (180 kg ha^{-1}), which is in good agreement with the previous study [30]. This increase in the NU_E response to the high SR could be due to an increase in the density of the roots in the soil, which enhanced N from deeper parts of the soil. Therefore, it is not surprising that maximum values of NA_E, NR_E, and NUP_E were also observed from the optimal combined treatment (N_{235} + SR_{180}), which was also the case for grain yield (Table 2). Our study thereby provides a practical management method approaching higher GY and NUP_E by the optimization of N and SR.

3.3. Accumulation and Translocation of DM and N

Total dry matter accumulation and partitioning into separate parts of the plant were significantly affected by combined N and SR′s combined treatments. The maximum value of total dry matter and individual parts especially grains, rachis + glumes, and leaves, was also obtained from the combination of N_{235} + SR_{180} treatment. Recent studies reported that the contribution of pre-anthesis translocation to grain was significantly decreased when the N application rate increased [31,32]. Similarly, the result of the current experiment showed that the contribution of pre-anthesis DM translocation was significantly decreased with increasing N rate, while pre-anthesis DM translocation, post-anthesis DM accumulation, and contribution of post-anthesis DM accumulation to grain were significantly increased. The main reason for the decreasing contribution of pre-anthesis DM translocation to grain might be that early senescence occurred due to N deficiency, which would speed up the pre-translocation from leaf and stem to spike. Furthermore, increasing seed rate significantly increased pre-anthesis dry matter translocation, post-anthesis DM accumulation, and contribution of post-anthesis DM accumulation to the grain. Parallel to our finding, it was reported that post-heading DM and N accumulation was significantly increased with increasing SR [33]. It should be noted that an excessive amount of N application (N_{290} kg ha^{-1}) as well as SR (SR_{225} kg ha^{-1}) did not increase the amount of DM translocation.

Total N accumulation, partitioning, and translocation showed the same trend with the above part of DM. Both N and SR up to optimal levels (N_{235}, SR_{180}) significantly increased the total N content of individual parts. The same finding reported that no further increase was observed in the uptake of N at N fertilizer and the density of the plant beyond 240 kg N ha^{-1} and 405 plants m^{-2} [21,34]. N translocation, postanthesis N accumulation, and postanthesis N accumulation contribution of post-anthesis N accumulation to the grain were higher in the high N treatments compared to control and low N treatment. A similar result was found that N translocation and post-anthesis N accumulation were enhanced with increasing N application rate [23]. In the current experiment, N translocation, N accumulation after anthesis, and contribution of post-anthesis to grain response to seed rate were significantly increased from SR $_{135}$ to SR $_{180}$ kg ha^{-1}.

3.4. Relationship of GY with Related Parameters

Our results showed that GY has a significant and positive correlation with Pn, Gs, SPAD value, LAI, and other GY components (Table 7). This is similar to the results from the study by Jiang et al. [24]. Furthermore, the regression analyses also revealed that GY had a positive correlation with NR, GS, NA_E, NR_E, and NUP_E while showing a negative correlation with CTG (Figure 6). It was also observed that NR and GS activities were highly positively correlated with photosynthesis capacity, which is consistent with previous studies [35,36]. Here, we found that GY had a significant positive correlation with NU_E parameters such as NA_E, NRE, and NUP_E. This was not in agreement with the previous finding that GY showed a negative correlation with NU_E [37,38]. The reason might be due to a certain amount of N + SR (N_{235} + SR_{180}), in which both NU_E and GY were significantly higher up to the previous partnership. In conclusion, we found that N and SR′s improper rate cannot increase GY, but significantly decreased NU_E. In this regard, to achieve the maximum GY and NU_E, it would be better to use the optimal amount of both N and SR, which is the result of the current experiment, and the suitable combined treatment was N_{235} + SR_{180}. Furthermore, by using a suitable combination of N and SR (SR_{180} N_{235}), replacing N to SR especially for balancing GY and NU_E would be the best method for sustainable agriculture. According to our findings, we infer that, based on low SR (SR_{135}), 1 kg ha^{-1} N could be saved by increasing approximately 0.6 kg ha^{-1} SR.

4. Materials and Methods

4.1. Plant Material and Experimental Site

The field experiment was conducted during two successive growing seasons (2017–18 and 2018–19) at the XuYi Rice and Wheat demonstration center (118°43′ N and latitude 32°59′ E) in Jiangsu province, China. The winter wheat cultivar Ningmai 13 was used in both growing seasons. The soil type was clay loam and the pH was 6.8. It contained 31.07 g kg^{-1} of organic matter, 2449 g kg^{-1} of available N, 27.3 mg kg^{-1} of available phosphate, and 240 mg kg^{-1} of available potassium. Seeds were sown on 31 October 2017, and 1 November 2018, and the crop was harvested on 3 June 2018, and 6 June 2019, respectively.

4.2. Experiment Design

The experiment was carried out according to the split-plot design with three replicates. The main plot consisted of three seed rates (SR_{135}, SR_{180}, and SR_{225} kg ha^{-1}) and the subplot comprised two levels of doses of N in the first year (N_{235} and N_{290} kg ha^{-1}) and three N levels in second year of the experiment (N_{180}, N_{235}, and N_{290} kg ha^{-1}). At the first growing season, there was no significant effect between N_{235} and N_{290} for GY, because of that we added N_{180} kg ha^{-1} treatment at the second growing season to determine the N effect as well to determine the optimum rate of N for GY. An N-control plot (N_0) was also used at the second growing season per replication for the calculation of NU_E parameters. N fertilizer was applied as urea (46%), phosphorus (P) and potassium (K) fertilizer as calcium superphosphate (15%), and potassium chloride (60%) at the rates of 120 (P_2O_5) and 120 (K_2O) kg ha^{-1}. All of the phosphorus and potassium fertilizer and 70% of the total amount of N fertilizer were spread by hand before plowing at the time of sowing. The remaining N fertilizer (30%) was applied at the first node (31) according to BBCH. Each plot size was 4 × 3 m and consisted of 12 rows with a row-to-row distance of 25 cm.

In this experiment, the rice–wheat rotation system was undertaken for the long term. Rice cultivation techniques such as puddling, transplanting, and flooding, and the whole amount of straw returned to the field almost in the last decade. Only 5 cm of rice straw remains above the ground. Moldboard plough was followed by rotary plough as primary and secondly tillage. The depth of moldboard tillage was 20 cm and that of the rotary plough was 10 cm. Wheat seeds were sown with a seed drill precise machine with surface stubble plowing and roll compaction. For high-yield production, insects, diseases, and weeds were controlled two times by spraying insecticide (Biscaya), fungicide (Capalo), and herbicide (sulfosulfuron) during both growing seasons.

4.3. Grain Yield and Yield Components

Uniform plants at the flowering stage were tagged with labels and were sampled at a later stage. At the maturity stage, ears/spikes f rom the area of 0.5 m^2 (without taking a sample) of each plot were collected to determine GY and yield components.

4.4. Photosynthesis, SPAD, LAI, N, and Enzyme Activities in Leaves

Photosynthesis (Pn), stomatal conductance (Gs), SPAD, and LAI were measured at the jointing stage, anthesis, 10 days after anthesis (DAA), and 20 DAA. Pn and GS were measured by a portable gas exchange analyzer (LI-6400XT;LI-COR-Inc., Lincoln NE, USA) at 9:00–11:00 a.m. on a sunny day. The concentration of CO_2 in the leaf chamber, light intensity, and relative humidity were set as 380 µmol mol^{-1}, 1000–1100 µmol $m^{-2}s^{-1}$, and 500 mL min $^{-1}$, respectively. The SPAD value was determined by Minolta 502 chlorophyll meter (Minolta, Japan). LAI was measured with using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). N concentration was determined by the micro Kjeldahl method [39]. Nitrate reductase (NR) and glutamine synthesis (GS) were determined according to the method previously described by [36] and [40].

4.5. Dry Matter (DM) and N Translocation

DM and N accumulation (DMA and NA), translocation, and their contribution were estimated according to [31] and [41] by using the following equations (Table 8).

Table 8. Equations for estimating dry matter and nitrogen translocation.

Parameters		Equation	Unit
Abbreviation	**Denotation**		
PTA	Pre-anthesis DM translocation	DM of vegetative parts at anthesis—at maturity	kg ha^{-1}
CPT	Contribution of pre-anthesis DM translocation to grain	PTA ÷ GY at maturity × 100	%
PAA	Post-anthesis DM accumulation	Biomass at maturity- biomass at anthesis	kg ha^{-1}
CPA	Contribution of post-anthesis DM accumulation to grain	PAA ÷ GY at maturity × 100	%
NTA	Pre-anthesis N translocation	N of vegetative parts at anthesis—at maturity	kg ha^{-1}
CTG	Contribution of pre-anthesis N translocation to grain	NTA ÷ grain N×100	%
NAA	Post-anthesis N accumulation	Plant N accumulation at maturity—N accumulation at anthesis	kg ha^{-1}
CAG	Contribution rate of post-anthesis N accumulation to grain	NAA ÷ grain N ×100	%

4.6. Use Efficiency (NU_E) Parameters

NU_E parameters were determined using the following equations described by [17], and [42] (Table 9).

Table 9. Equations for estimating NU_E parameters.

Parameters		Equation	Unit
Abbreviation	**Denotation**		
NA_E	N agronomy efficiency	(GY with N—GY without N) ÷ N application rate	kg kg^{-1}
NR_E	N recovery efficiency	(total N uptake with N- total N uptake without N) ÷ N application rate	%
NUP_E	N uptake efficiency	Above-ground N at harvesting ÷ N application rate	%
NPFP	N partial factor productivity	GY ÷ N application rate	kg kg^{-1}
NHI	N harvest index	Grain N accumulation at maturity/plant N accumulation at maturity	mg mg^{-1}

4.7. Weather Condition

Monthly average temperature, rainfall, and sunshine at the experimental site over two successive years (2017–2018 and 2018–2019) are presented in Figure 7. There was considerable variation between the two growing seasons. At the active tiller stage (from late January to the end of the first week of February), the minimum temperature in 2017–2018 was lower (−4.5 °C) compared to that of the second growing season (−0.5 °C). At the anthesis stage, the average rainfall in the first growing season was 538 mm, which was 64.02% higher than that in the second growing season (328 mm).

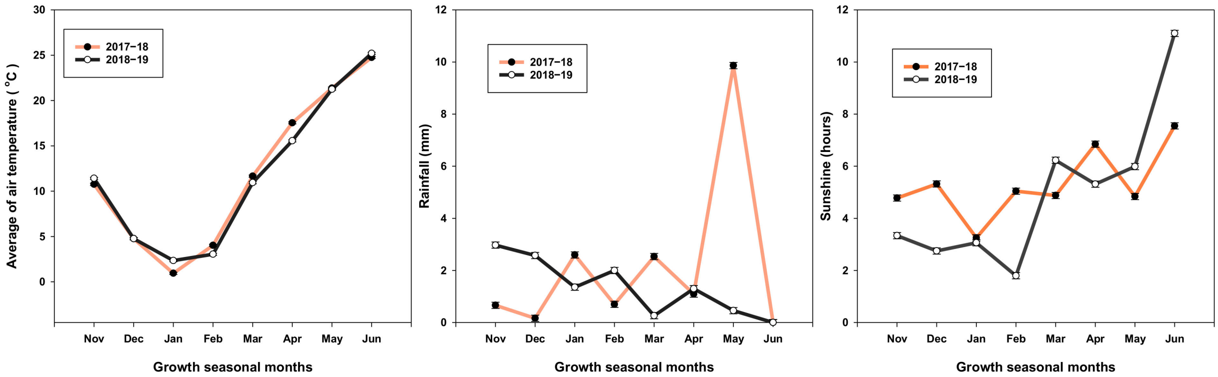

Figure 7. Metrological data: (monthly average temperature, rainfall (mm), and sunshine per hour) in two successive growing season.

4.8. Statistical Analysis

Two-way ANOVA (SPSS version 17.1) was used for analyzing the variance among different treatments. The means were tested with the least significant difference at the 0.05 probability level ($p \leq 0.05$ by Duncan's). Pearson's correlation between grain yield and related parameters were calculated through the SPSS version17.1. All graphs and linear regression analyses was done by sigmaplot 14.0 software (Chicago, IL, USA).

5. Conclusions

In summary, the GY, DMA, NAC, NU_E parameters and physiological parameters increased significantly with the combination of N_{235} and SR_{180}. However, the excessive rate of N application cannot increase GY and other parts of plant DM but it decreased NA_E, NR_E, and NUP_E. Our result confirmed that maximum GY and higher NU_E components could be achieved via avoiding excessive use of N, and optimizing the compensation effect of increasing SR for reducing N application.

Author Contributions: M.H., Q.Z. and D.J. designed the experiments. M.H. analysed data, interpreted data, and wrote the original draft of the manuscript. M.H., A.S. and M.S.J. carried out the experiments, curated and analyzed data. M.H., J.C., X.W., Q.Z. and T.D. were involved in the management of the experiment. D.J. participated in supervision of the project. M.S.J., Q.Z. and D.J. participated in the critical reading and discussion of the manuscript, H.M. did formal analysis and methodology. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the projects of National Key Research and Development Program of China (2021YFF1000204, 2020YFE0202900), National Natural Science Foundation of China (32030076, U1803235, 31901458, 32172116), Jiangsu Agriculture Science and Technology Innovation Fund (JASTIF, CX(20)3086), Natural Science Foundation of Jiangsu Province (BK20190509), the China Agriculture Research System (CARS-03), Jiangsu Collaborative Innovation Center for Modern Crop Production (JCIC-MCP), Jiangsu 333 program, and the 111 Project (B16026). The first author is thankful to Chinese Government Scholarship Council for providing scholarship to pursue an Msc at Nanjing Agricultural University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Slafer, G.A.; Savin, R.; Sadras, V.O. Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Res.* **2014**, *157*, 71–83. [CrossRef]

2. Lu, D.; Lu, F.; Yan, P.; Cui, Z.; Chen, X. Elucidating population establishment associated with N management and cultivars for wheat production in China. *Field Crops Res.* **2014**, *163*, 81–89. [CrossRef]
3. Efretuei, A.; Gooding, M.; White, E.; Spink, J.; Hackett, R. Effect of nitrogen fertilizer application timing on nitrogen use efficiency and grain yield of winter wheat in Ireland. *Ir. J. Agric. Food Res.* **2016**, *55*, 63–73. [CrossRef]
4. Bly, A.G.; Woodard, H.J. Foliar nitrogen application timing influence on grain yield and protein concentration of hard red winter and spring wheat. *Agron. J.* **2003**, *95*, 335–338. [CrossRef]
5. Fageria, N.; Baligar, V. Enhancing nitrogen use efficiency in crop plants. *Adv. Agron.* **2005**, *88*, 97–185.
6. Lawlor, D.W.; Lemaire, G.; Gastal, F. Nitrogen, plant growth and crop yield. In *Plant Nitrogen*; Springer: Cham, Switzerland, 2001; pp. 343–367.
7. Wang, Y.; Ren, T.; Lu, J.; Ming, R.; Li, P.; Hussain, S.; Cong, R.; Li, X. Heterogeneity in rice tillers yield associated with tillers formation and nitrogen fertilizer. *Agron. J.* **2016**, *108*, 1717–1725. [CrossRef]
8. Huang, M.; Yang, C.; Ji, Q.; Jiang, L.; Tan, J.; Li, Y. Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern China. *Field Crops Res.* **2013**, *149*, 187–192. [CrossRef]
9. Balkcom, K.; Burmester, C. Nitrogen applications for wheat production across tillage systems in Alabama. *Agron. J.* **2015**, *107*, 425–434. [CrossRef]
10. Terrile, I.I.; Miralles, D.J.; González, F.G. Fruiting efficiency in wheat (*Triticum aestivum* L): Trait response to different growing conditions and its relation to spike dry weight at anthesis and grain weight at harvest. *Field Crops Res.* **2017**, *201*, 86–96. [CrossRef]
11. Yang, D.; Cai, T.; Luo, Y.; Wang, Z. Optimizing plant density and nitrogen application to manipulate tiller growth and increase grain yield and nitrogen-use efficiency in winter wheat. *PeerJ* **2019**, *7*, e6484. [CrossRef]
12. Ju, X.-T.; Xing, G.-X.; Chen, X.-P.; Zhang, S.-L.; Zhang, L.-J.; Liu, X.-J.; Cui, Z.-L.; Yin, B.; Christie, P.; Zhu, Z.-L. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 3041–3046. [CrossRef]
13. Mandic, V.; Krnjaja, V.; Tomic, Z.; Bijelic, Z.; Simic, A.; Ruzic Muslic, D.; Gogic, M. Nitrogen fertilizer influence on wheat yield and use efficiency under different environmental conditions. *Chil. J. Agric. Res.* **2015**, *75*, 92–97. [CrossRef]
14. Fan, M.; Christie, P.; Zhang, W.; Zhang, F. *Crop Productivity, Fertilizer Use, and Soil Quality in China*; CRC Press: Boca Raton, FL, USA, 2010; pp. 87–107.
15. Ma, G.; Liu, W.; Li, S.; Zhang, P.; Wang, C.; Lu, H.; Xie, Y.; Ma, D.; Kang, G. Determining the optimal N input to improve grain yield and quality in winter wheat with reduced apparent N loss in the North China Plain. *Front. Plant Sci.* **2019**, *10*, 181. [CrossRef]
16. Qingfeng, M.; Shanchao, Y.; Peng, H.; Zhenling, C.; Xinping, C. Improving yield and nitrogen use efficiency simultaneously for maize and wheat in China: A review. *Pedosphere* **2016**, *26*, 137–147.
17. Wu, Y.; Xi, X.; Tang, X.; Luo, D.; Gu, B.; Lam, S.K.; Vitousek, P.M.; Chen, D. Policy distortions, farm size, and the overuse of agricultural chemicals in China. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 7010–7015. [CrossRef]
18. Hirel, B.; Le Gouis, J.; Ney, B.; Gallais, A. The challenge of improving nitrogen use efficiency in crop plants: Towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J. Exp. Bot.* **2007**, *58*, 2369–2387. [CrossRef]
19. López-Bellido, L.; López-Bellido, R.J.; Redondo, R. Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. *Field Crops Res.* **2005**, *94*, 86–97. [CrossRef]
20. Zhai, B.; Li, S. Response to nitrogen deficiency and compensation on growth and yield of winter wheat. *Plant Nutr. Fertitizer Sci.* **2005**, *11*, 308–313.
21. Tian, G.; Gao, L.; Kong, Y.; Hu, X.; Xie, K.; Zhang, R.; Ling, N.; Shen, Q.; Guo, S. Improving rice population productivity by reducing nitrogen rate and increasing plant density. *PLoS ONE* **2017**, *12*, e0182310. [CrossRef]
22. Dai, X.; Xiao, L.; Jia, D.; Kong, H.; Wang, Y.; Li, C.; Zhang, Y.; He, M. Increased plant density of winter wheat can enhance nitrogen–uptake from deep soil. *Plant Soil* **2014**, *384*, 141–152. [CrossRef]
23. Meng, Q.; Yue, S.; Chen, X.; Cui, Z.; Ye, Y.; Ma, W.; Tong, Y.; Zhang, F. Understanding dry matter and nitrogen accumulation with time-course for high-yielding wheat production in China. *PLoS ONE* **2013**, *8*, e68783. [CrossRef]
24. Dordas, C. Dry matter, nitrogen and phosphorus accumulation, partitioning and remobilization as affected by N and P fertilization and source–sink relations. *Eur. J. Agron.* **2009**, *30*, 129–139. [CrossRef]
25. Jiang, D.; Dai, T.; Jing, Q.; Cao, W.; Zhou, Q.; Zhao, H.; Fan, X. Effects of long-term fertilization on leaf photosynthetic characteristics and grain yield in winter wheat. *Photosynthetica* **2004**, *42*, 439–446. [CrossRef]
26. Bhatta, M.; Eskridge, K.M.; Rose, D.J.; Santra, D.K.; Baenziger, P.S.; Regassa, T. Seeding rate, genotype, and topdressed nitrogen effects on yield and agronomic characteristics of winter wheat. *Crop Sci.* **2017**, *57*, 951–963. [CrossRef]
27. Geleta, B.; Atak, M.; Baenziger, P.; Nelson, L.; Baltenesperger, D.; Eskridge, K.M.; Shipman, M.; Shelton, D. Seeding rate and genotype effect on agronomic performance and end-use quality of winter wheat. *Crop Sci.* **2002**, *42*, 827–832.
28. Xu, G.; Fan, X.; Miller, A.J. Plant nitrogen assimilation and use efficiency. *Annu. Rev. Plant Biol.* **2012**, *63*, 153–182. [CrossRef]
29. Masclaux-Daubresse, C.; Daniel-Vedele, F.; Dechorgnat, J.; Chardon, F.; Gaufichon, L.; Suzuki, A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.* **2010**, *105*, 1141–1157. [CrossRef]
30. Zhang, J.; Dong, S.; Dai, X.; Wu, T.; Wang, X.; Bai, H.; Wang, L.; He, M. Combined effect of plant density and nitrogen input on grain yield, nitrogen uptake and utilization of winter wheat. *Vegetos-Int. J. Plant Res.* **2016**, *29*, 63–73. [CrossRef]

31. Zhang, J.H.; Jian-Li, L.; Zhang, J.-B.; Fu-Tao, Z.; Cheng, Y.-N.; Wei-Peng, W. Effects of Nitrogen Application rates on translocation of dry matter and Nitrogen utilization in Rice and Wheat. *Acta Agron. Sin.* **2010**, *36*, 1736–1742. [CrossRef]
32. Ma, D.-H.; Wang, Y.F.; Zhou, H.; Sun, H.J. Effect of Postanthesis Soil Water Status and Nitrogen on Grain Yield and Canopy Biomass Accumulation and Transportation of Winter Wheat. *J. Triticeae Crops* **2007**, *5*, 847–851.
33. Arduini, I.; Masoni, A.; Ercoli, L.; Mariotti, M. Grain yield, and dry matter and nitrogen accumulation and remobilization in durum wheat as affected by variety and seeding rate. *Eur. J. Agron.* **2006**, *25*, 309–318. [CrossRef]
34. Liu, W.; Wang, J.; Wang, C.; Ma, G.; Wei, Q.; Lu, H.; Xie, Y.; Ma, D.; Kang, G. Root growth, water and nitrogen use efficiencies in winter wheat under different irrigation and nitrogen regimes in North China Plain. *Front. Plant Sci.* **2018**, *9*, 1798. [CrossRef] [PubMed]
35. Xu, Z.Z.; Zhou, G.S. Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass Leymus chinensis. *Planta* **2006**, *224*, 1080–1090. [CrossRef] [PubMed]
36. Ferrario-Méry, S.; Valadier, M.-H.; Foyer, C.H. Overexpression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. *Plant Physiol.* **1998**, *117*, 293–302. [CrossRef]
37. Ma, B.-L.; Biswas, D.K. Precision nitrogen management for sustainable corn production. In *Sustainable Agriculture Reviews*; Springer: Cham, Switzerland, 2015; pp. 33–62.
38. Biswas, D.K.; Ma, B.-L. Effect of nitrogen rate and fertilizer nitrogen source on physiology, yield, grain quality, and nitrogen use efficiency in corn. *Can. J. Plant Sci.* **2016**, *96*, 392–403. [CrossRef]
39. Bremner, J.M.; Mulvaney, C. Nitrogen—Total. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, A.L., Miller, R.H., Keeney, D.R., Eds.; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; pp. 595–624.
40. Zhang, C.; Peng, S.; Bennett, J. Glutamine synthetase and its isoforms in rice spikelets and rachis during grain development. *J. Plant Physiol.* **2000**, *156*, 230–233. [CrossRef]
41. Wu, H.; Xiang, J.; Zhang, Y.; Zhang, Y.; Peng, S.; Chen, H.; Zhu, D. Effects of post-anthesis nitrogen uptake and translocation on photosynthetic production and rice yield. *Sci. Rep.* **2018**, *8*, 12891. [CrossRef]
42. Pask, A.J.D.; Sylvester-Bradley, R.; Jamieson, P.D.; Foulkes, M.J. Quantifying how winter wheat crops accumulate and use nitrogen reserves during growth. *Field Crops Res.* **2012**, *126*, 104–118. [CrossRef]