Plasticity of wheat yield components in response to N fertilization

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Acronyms

N_{SRW} - residual N in the soil at the end of winter

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Abstract

Nitrogen fertilization is one of the major issues of crop productions in west-Europe. Every year, large quantities of synthetic nitrogen are used to fertilize crops, such as winter wheat (*Triticum aestivum*), and ensure them high yielding conditions. In return, substantial amount of nitrogen ends up in the environment where it generates pollutions. The combination of strategic and tactical approaches has been suggested to improve the accuracy of nitrogen fertilization decisions and their implementation. Combining strategic and tactical approaches requires a detailed understanding of the dynamic of nitrogen use by the plant to both anticipate and adapt fertilization practices.

Inspired by the concept of yield component phenotypic plasticity, this study aimed to analyze the influence of nitrogen fertilization dynamics on yield elaboration and infer strategic decision rules dedicated to wheat yield optimization in a Belgian context. The analysis, implemented from ten-year experiment, was conducted in two different steps. (1) We characterized the effect of nitrogen fertilization on yield and yield components when the crop was submitted to different agro-pedo-climatic contexts, (2) we analyzed the specific influence of nitrogen fertilization dynamic on yield elaboration, normalizing the variables of the experiment to exclude genetic and environmental influences.

The study revealed that highest nitrogen fertilization did not automatically lead to the highest yields. High nitrogen fertilization contributed to increase the negative trade-offs between yield components, which limited the efficiency of nitrogen supplies. Conversely, a balanced dynamic of nitrogen supplies was essential to reach high yields while supplying effective amount of nitrogen. From this Belgian dataset, optimal nitrogen fertilization strategies supposed early nitrogen supplies split in two applications to support the initiation phases of grains per m^2 and another nitrogen supply at flag leaf stage to maintain components of grain per m^2 while still allowing grain filling. This fertilization

structures, adapted to different pedoclimatic contexts would support strategic approach in crop nitrogen nutrition.

Keywords

Nitrogen fertilization, *Triticum aestivum,* Yield components, phenotypic plasticity, N fertilization strategy

1. Introduction

Nitrogen (N) fertilization remains a critical elements to reach high yield levels in wheat productions (Jensen et al., 2011). In European agriculture, large amounts of synthetic N are used despite an unsatisfying N use efficiency (NUE) (Einarsson et al., 2021; Lassaletta et al., 2014). Origins of N losses from fertilization practices and their environmental consequences have been extensively documented over the past decades (Bibi et al., 2016; Erisman et al., 2011; Galloway et al., 2003). Though, N dynamics in the soil-plant continuum is a complex process and a poor synchrony remains between N fertilizer supply and its utilization by crops (Spiertz, 2012; Zhang et al., 2015). Besides environmental concerns, the COVID pandemic and the war in Ukraine have deeply transformed the economic return on wheat due to high N fertilizer's costs (Alexander et al., 2023). This situation urges the development of new fertilization methods.

Wheat yield results from the interaction of genetics (G), environment (E) and agronomic management (M) (Hawkesford and Riche, 2020). Referring to "E", in rain-fed agriculture, interannual climate conditions strongly influence yields (Porter and Semenov, 2005; Asseng, 2012). In west Europe for example, Ray et al. (2015) reported a wheat yield variability of 31 to 51% due to annual weather variations. In this regard, the levers already used to improve NUE came either from breeding programs (G) or from fertilization management (M) (Brancourt-Hulmel et al., 2003; Gaju et al., 2011; Hawkesford and Riche, 2020). Up to now, in this equation, environment has been mainly considered as a static combination of soil and climate conditions. When designing fertilization methods, soil N supply has to be a central concern, as high soil mineral N amount can favor conditions of N pollution through leaching or greenhouse gas emissions (Goulding et al., 2008). Thus, the previous crop, the soil organic matter content, its estimated mineralization rate or residual N in soil at the end of winter are some features commonly integrated in fertilization methods (Gate, 1995; Machet et al., 2017). Usually, soil N assessment is carried out early in the season to balance future N supplies with estimated crop needs. However, this timing faces a major limitation as N fertilization decision is taken based on an expected outcome (yield, potential for postharvest N leaching) occurring months later, independently of the upcoming climatic conditions (Dumont et al., 2016).While important, soil N assessment has yet to be more properly considered in interaction with past and upcoming climatic conditions to better support N fertilization decision (Dumont et al., 2016).

In order to adapt to these variations and avoid overfertilization situations, that might occur from the supply of equal amount of N irrespective of the current yield potential, Basso et al. (2011) suggested to combine strategic and tactical approaches at the subfield scale. Strategic approach is a long-term decision process based on the anticipation of crop needs and foreseen N supply according to crop expected performances. On the opposite, tactical approach is adaptative and aims to adjust N supply to current crop potential. This dual path would lead to structure new fertilization decision rules and optimize NUE. Though, the implementation of both strategic and tactical approaches requires a detailed understanding of the dynamic of crop N requirement and N utilization in the perspective of efficient N use, e.g., relying on the use of crop models as proposed in Dumont et al. (2015a, b). Recent studies reported the possibility to achieve higher N use efficiency by combining prognosis (e.g. crop model or soil-crop balance of N) with diagnosis (e.g. a regular monitoring of crop N status along the fertilization period) rather than solely basing the decision of fertilization on prognosis (Ravier et al. 2016, Lemaire et al. 2021). The concepts of prognosis and diagnosis are respectively concomitant with the strategic and tactical decisions (Basso et al. 2011, Lemaire et al. 2021). The distinction between prognosis and diagnosis is useful as, depending upon the environment and the nutrition status of the crop, N supply will be used differently by the crop. In this regard, an innovative approach has been proposed to pilot N fertilization from its crop N status (Ravier et al., 2018). In this method, crop N status is regularly assessed by farmer from the nitrogen nutrition index (NNI), measured between the stem elongation and anthesis growing stages, with the aim to maintain the crop in an effective nutrition level, i.e., i) below excessive NNI associated to high fertilization level and ii) above a critical NNI that would lead to penalties on yield and protein content (Ravier et al., 2017, 2018). However, in this regard, all methods (from prognosis or the combination prognosis/diagnosis) are lacking to explicitly consider compensations effects in yield composition which could further support N fertilization management adapted to specific environments.

From an ecophysiological perspective, wheat yield is usually expressed as a multiplication of grains per m^2 and average grain weight. Grains per m^2 can further be decomposed into spike per m^2 and grains per spike (Sadras and Slafer, 2012; Gastal et al., 2015). Early on, fertilization methods already suggested to split N fertilizer amount into two or three applications to match the needs of the different yield components and reduce N losses (Boiffin et al., 1981; Gate, 1995; López-Bellido et al., 2005; Barraclough et al., 2010). Grains per m^2 is often considered to be closely related to yield encouraging early N supply to secure yield levels. However, grain per m^2 and average kernel weight are known to have negative relationship (Miralles and Slafer, 1995; Miralles and Slafer, 2007), so as spike per m^2 and grains per spike (Slafer et al., 2014). The negative relationship between yield components affects N use efficiency as high N nutrition positively impact grains per m^2 while reducing the average grain weight (Quintero et al., 2018; Beral et al., 2022). Furthermore, there is an overlap of the impact of N on the two components of grains per m^2, namely spike per m^2 and grains per spike, making difficult to precisely target N applications. Compensation effects in yield elaboration complicate the implementation of efficient N fertilization methods in various environments as yield components responses would influence each other responses (Sadras and Slafer, 2012).

Recently, research conducted in wheat ecophysiology have enlighten the dynamic of yield elaboration and trade-offs between yield component (Sadras et al., 2012; Slafer et al., 2014). Particularly, origin and magnitude of yield components variations induced by genotype or environmental variations were analyzed. Sadras and Slafer (2012) showed the existence of a hierarchy between yield components according to their phenotypic (P) plasticity. Phenotypic plasticity refers to “the amount by which the expressions of individual characteristics of a genotype are changed by different environments” as defined by Bradshaw (1965). Thus, phenotypic plasticity decreases as yield components set-up; tiller number has the largest phenotypic plasticity, followed by grain per spike and spike per m² while grain size has the lowest plasticity among components (Sadras and Slafer, 2012). If phenotypic plasticity represents the variation in grain yield component response to environmental changes, management would be considered as a controlled part of the environment in equation P = G x E x M (Hawkesford and Riche, 2020).

In this study, we have explored the composition of winter wheat yield when submitted to different dynamics of N supplies over multiple cropping systems. From these different dynamics, we aimed to identify the optimal structure of yield composition from which we could infer decision rules aiming to support, through N fertilization, optimal expression of crop in changing environments.

1. Material and method

1.1. General conditions

Field experiments were implemented between 2010 and 2019 in the Hesbaye area, Belgium. Trials were jointly managed by the University of Liège, Gembloux Agro-Bio tech and the Belgian Pilot Center for Cereals, Oilseed and Protein crops (CePiCOP). These trials were implemented on a farmer's field near by the faculty (50.5ºN, 4.7ºE, 165 m). The experimental site has a soil characterized as classic loamy with good to moderate drainage (Fragic, Hypereutric Luvisol (Siltic), FAO-WRB, 2014). The climate is temperate with annual precipitations ranging between 519 and 721 mm, mean annual temperature fluctuating from 8.34°C to 11.4°C and an average solar radiation of 10.3 MJ.m^{-2}.day^{-1}. Interannual variation of weather conditions are presented in Table 1.

Table 1: Weather conditions across the experiment

Year	Temperatures (°C.day^{-1})			Rainfall (mm.day^{-1})		Total annual rainfall (mm)	Average solar radiation (MJ.m^{-2}.day^{-1})
	Min	**Max**	**Avg.**	**Avg.**	**Max**	**Avg.**	**Avg.**
2010	-12.6	33.4	8.34	1.82	34.1	663	10.1
2011	-5.8	33.8	10.6	1.42	35.2	519	10.0
2012	-18.9	34.2	9.59	1.89	21.6	693	9.51
2013	-13.7	33.7	9.44	1.58	32.9	578	10.4
2014	-8.2	33	11.3	1.87	30.8	683	10.4
2015	-3.8	33.3	10.7	1.81	27.1	659	9.68
2016	-8.8	33.8	10.5	1.97	28.6	721	9.11
2017	-7.8	32.8	10.7	1.65	20.5	604	10.6
2018	-9.6	35.7	11.4	1.24	34.7	451	11.7

2019	-6.8	40.9	10.8	1.78	20.9	651	11.1

Trials were located each year on different fields, in a radius of 2.5 km, to benefit from different preceding crops, common in the region (Table 1) and to allow avoiding cumulative effects associated to microplots trials. When two varieties were grown during the same year, they were cultivated on two separated trials. Most frequent preceding crop was sugar beet (8 years). Potato (2 years), winter wheat (2 years), silage maize (1 year) and spinach (1 year) are also regularly cultivated in the area before winter wheat. In 2010, 2011, 2012 and 2019, two varieties were tested while only one variety was sown starting from 2013. In all cases, varieties were modern, high-yielding, cultivars widely used by farmers.

Table 2: Winter wheat management

Years	Variety	Sowing date	Sowing density (seeds.m^{-2})	Preceding crop	Residual soil N at the end of winter (kg ha^{-1})
2010	Istabraq	Oct. 22	220	Winter wheat	23
	Julius	Oct. 21	220	Sugar beet	37
2011	Istabracq	Oct. 22	250	Winter wheat	25
	KWS Ozon	Oct. 25	250	Sugar beet	29
2012	Istabracq	Oct. 14	225	Winter wheat	42
	KWS Ozon	Oct. 20	250	Sugar beet	61
2013	KWS Ozon	Oct. 19	250	Sugar beet	51
2014	KWS Ozon	Oct. 21	250	Sugar beet	64
2015	Mentor	Oct. 19	250	Sugar beet	28.5
2016	Mentor	Oct. 12	250	Sugar beet	28
2017	Mentor	Oct. 26	300	Sugar beet	73
2018	Edgar	Oct. 13	250	Potato	45
2019	Mentor	Oct. 23	250	Spinach	165
	Safari	Oct. 17	250	Potato	100

Winter wheat was always sown within the optimal sowing dates in the region, between mid- and late-October, with an optimal plant density (ranging from 220 to 300 seed m^{-2}; Table 1), as advised in Wallonia. Integrated pest management practices were deployed to limit the disturbances of biotic factors.

1.2. Treatments and design

Treatments were structured to support the process of advising Belgian farmers on the management of N fertilization. The reference N fertilization scheme in southern Belgium is a total N amount of 180 kg ha^{-1} divided in three supplies of 60 $kg.ha^{-1}$, respectively brought to the crop (i) after winter during tillering (decimal code DC 23-25 of Zadoks et al., 1974), (ii) at the onset of stem elongation (DC 30), and (iii) at flag leaf (DC 39) stages. Farmers are usually advised to adapt this reference.

Table 3: N fertilization protocol applied in 2015

Treatment	**N splitting**			**Total N supply**
	DC_{23-25}	**DC_{30}**	**DC_{39}**	
1	0	0	0	**0**
2	50	-	-	**50**
3	-	50	-	**50**
4	-	-	50	**50**
5	50	50	-	**100**
6	50	-	50	**100**
7	-	50	50	**100**
8	50	50	50	**150**
9	50	50	75	**175**
10	50	50	100	**200**
11	50	50	125	**225**
12	50	75	50	**175**
13	50	75	75	**200**
14	50	75	100	**225**

15	75	75	-	**150**
16	75	-	75	**150**
17	-	75	75	**150**
18	75	75	75	**225**
19	75	75	50	**200**
20	75	75	100	**250**
21	-	75	50	**125**
22	75	-	50	**125**
23	-	75	100	**175**
24	75	-	100	**175**
25	100	-	-	**100**
26	-	100	-	**100**
27	-	-	100	**100**
28	100	-	50	**150**
29	-	100	50	**150**
30	100	-	75	**175**
31	-	100	75	**175**
32	100	100	-	**200**
33	100	-	100	**200**
34	-	100	100	**200**
35	100	100	100	**300**

This reference is usually adapted to consider the residual N in the soil at the end of winter (Table 2) (residual N in the soil at the end of winter - N_{SRW}), the preceding crop, and the carry-over effect of organic matter. The experimental treatments aim at exploring the crop response to different N strategies, considering the preceding crop and associated N_{SRW}. An example of N fertilization protocol is provided in Table 3 where the effects of individual N fractions on yields are tested as well as the consequences of N omission during early or late phenological stages. This protocol was adapted over years to answer different assumptions about the dynamic of crop N needs. Thus, in 2010, 2011 and

2013, only treatments 1, 8, 14 and 18 were tested. From 2016 to 2019, the minimal N supply of 50 kg ha^{-1} is replaced by 60 kg ha^{-1}, 75 kg ha^{-1} is replaced by 90 kg ha^{-1} and 100 kg ha^{-1} is replaced by 120 kg^{-1}. The maximum total N supply over the period 2016-2019 is 360 kg ha^{-1} instead of 300 kg ha^{-1}.

1.3. Sampling and measurements

The final grain yield can be decomposed into its different components, according to Eq.1 and 2 :

$$Yield = KW \times Grain\ dens. \quad (1)$$

$$Yield = KW \times Spike.dens \times Spike.ferti \quad (2)$$

where *Yield* is the final grain yield in [$g.m^{-2}$], *KW* is the weight of one kernel in [g], *Grain dens.* is the grain density in [$Kernels.m^{-2}$], *Spike.dens* is the spike density in [$Kernels.m^{-2}$] and *Spike.ferti* is the spike fertility in [$Kernels.spike^{-1}$].

Later in the manuscript, grain density will refer to the number of grains per m² ; both terms will be indistinctively used. Similarly, spike density will refer to the number of spikes per m² and spike fertility will refer to the number of grains per spikes.

Trials were conducted on 12 m^2 plots: 14 rows sown, 0.146 m apart over 6m long. Plots were arranged in a randomized complete block design, with four replicates to ensure independence of measurements and prevent bias due to soil heterogeneity. In all experiments, grain yield, Thousand Kernel Weight (TKW) and spike densities were measured while grain density and spike fertility were mathematically derived using equation 1 and 2. Grain yield was measured over the whole plots using an experimental combine harvester and expressed at 15% humidity. TKW were measured on each treatment and replication, weighing four times hundred kernels from harvest samples. Weights of samples were then averaged by plot (replication) and re-expressed as weight of thousand kernels at 15% humidity. Spike densities were measured on each treatment and replication by counting the number of spikes of four different raw sections of 0.5 m long, randomly determined on the plot.

1.4. Data analyses

The analysis was conducted in two steps. Firstly, analysis was conducted on raw values of the different trials. Descriptive statistics were used to reveal interannual variability in the response of yield and yield components to different regimes of N fertilization. Pearson's correlation analyses were performed to (1) identify the main relationships existing between yield components and (2) to characterize the influence of individual and total N supplies on yield and yield components.

Secondly, we propose to deepen the analysis of the relationships between the expressions of yield components under ranges of N fertilization. Seasonal effect, including interannual weather variability, variety potential and N_{SRW} would influence yield and the relationships between its components, as illustrated on Supplementary Figure 1. Therefore, we propose to dissociate the influence of N fertilization on yield components from varietal and seasonal effects, to further characterize the isolated effect of N on yield components plasticities. To do so, we decided to implement different normalization procedures according to the following subsection's assumptions.

1.4.1. Maximum grain density as a potential target

Grain density is considered as the major component explaining yield elaboration. Following this principle, highest yields were expected where maximum grain density was observed. To analyze the influence of N fertilizer on grain density and its consequence on yield, we standardized both grain density and yield, for each trial (year and variety), with maximum grain density as a target. Within each trial, grain density values were thus divided by the maximum grain density observed (Eq. 3). Yields were divided by the yield recorded where the maximum grain densities were observed on the related trials (Eq. 4). This procedure was used to identify a potential lag between behaviors of grain density and yield.

$$FracGDmax_{i,j} = \frac{Grain.dens_{i,j}}{\max_i(Grain.dens_{i,j})} \quad (3)$$

$$FracY\_GDmax_{i,j} = \frac{Yield_{i,j}}{Yield_{i_{max},j}} \quad (4)$$

where $FracGDmax_{i,j}$ is the normalized grain density for each treatment *i* and trial (year and variety) *j*. $Grain.dens._{i,j}$ is a value of grain density in each treatment and trial and $\max_i(Grain.dens_{i,j})$ is the maximal value of grain density in each trial and treatment. $FracY\_GDmax_{i,j}$ is the normalized yield for each treatment and trial, $Yield_{i,j}$ is a yield value in each treatment and trial and i_{max} is such that $Grain\ dens._{i_{max},j} = \max_i(Grain\ dens._{i,j})$.

1.4.2. Grain density (de)composition:

To further understand grain density elaboration and how it was influenced by N fertilization, we normalized spike density, spike fertility and grain density and yield by year and variety using a conventional "min-max" procedure following eq. 5. This normalization procedure was considered to express "the potential" of each variable by year and variety. At this step, each variable was normalized independently to preserve the shape of each distribution.

$$SX_{i,j} = \frac{X_{i,j} - \min_i(X_{i,j})}{\max_i(X_{i,j}) - \min_i(X_{i,j})} \quad (5)$$

where $SX_{i,j}$ is the normalized variable, $X_{i,j}$ is the actual value of that variable.

Spike density and spike fertility are not completely independent variables. Therefore, to analyze the relative contribution of each variable on normalized grain density, we adjusted a generalized additive model with two explanatory variables (Eq. 6) :

$$g\big(E(y_i)\big) = \beta 0 + f1(x_i) + f2(z_i) + f3(x_i, z_i) + \epsilon_i \quad \epsilon \sim N(0, \sigma^2) \quad (6)$$

where g is a link function, y is the response variable, here the estimated grain density, $\beta 0$ is the intercept, *f1, f2, f3* are smooth functions, x_i and z_i are the covariates, namely spike fertility and spike density, ϵ is a random effect.

Following the same assumptions of dependance, and after having proven the interactive effect of the two variables (*cf.* Results section), it was decided to deepen the analysis of the interrelationship between them. To do so, (1) we discretized normalized values of spike density (and spike fertility) by intervals of 0.1 in the range zero-one and (2) linearly regressed grain density over spike density (alternatively spike fertility) for each level of discretized spike fertility (respectively spike density). Linear regressions were also performed between total N fertilizer supplies and spike density (alternatively spike fertility) for each level of spike fertility (respectively spike density). These regressions aimed to evaluate the impact of N supply on grain density through its components.

1.4.3. Trade-off between grain density and thousand kernel weight

Finally, grain density and TKW were also independently normalized by their respective maximum values (Eq. 7). As yield closely relates to both components, we explored the influence of different N fertilization strategies on a best trade-off between both grain density and TKW.

$$SY_{i,j} = \frac{S_{i,j}}{\max_i(S_{i,j})} \qquad (7)$$

where $SY_{i,j}$ is the normalized variable and $Y_{i,j}$ is the actual value of that variable, corresponding to grain density or TKW.

Normalized grain density and normalized TKW were classified by the N fertilization strategies they resulted from. N Fertilizations were categorized from the values of their individual N supplies compared to the standard N fertilization observed in Wallonia (Be), namely 60 – 60 – 60 kg ha^{-1}.

Supplies higher or lower than the standard were marked with a “+” and a “-“, respectively. As an example, “N1+ N2+ N3+” is a strategy where each N supply equal or exceed 60 kg ha^{-1}.

These categories were used to compare the variability and performance of both normalized grain density and normalized TKW. Variability was estimated computing coefficient of variations of each combination of individual yield component (normalized grain density or normalized TKW) and N strategy. Then, we computed the median value of both the normalized grain density and the normalized TKW, within each N fertilization strategy. Among N strategies, we performed a double classification of components according their coefficients of variation and their medians. Strategies having the lowest coefficient of variation and the highest median were considered as having the optimal performance. At the contrary, strategies with the highest coefficient of variation and the lowest median were considered bad performers.

As the N strategies were defined *a posteriori*, samples were heterogeneous and variances between samples were not equivalent. A Games-Howell test was performed to compare the different groups and test the significance of their differences.

While normalization encompasses the seasonal variations, including the N_{SRW} values recorded each year on each field, yield components expressions would still be influenced by the N supply and should respond differently, according to various N_{SRW} situations. To assess the potential impact of N_{SRW} on the subsequent optimal N strategies, Games-Howell test was further performed by categories of N_{SRW}, with the aim to evaluate the consistency of the strategies selection according to the N_{SRW} . A N_{SRW} threshold was defined at 46 kgN ha^{-1} . This value corresponds to the average N_{SRW} recorded in the region where the experiments were conducted (Protecteau, 2023), and allows to cluster situations that are known to lead to high N_{SRW} (potatoes, legumes, flax, etc.) from low N_{SRW} (Sugarbeets, maize, cereals, etc.) (Nysten et al. 2023). The Games-Howell test was then performed to compare N fertilization strategies belonging to trials under and above this threshold.

The data treatment was performed with R software version 4.2.2, using packages “tidyr”, (Wickham et al., 2023) “rstatix” (Kassambra et al., 2022), “mgcv” (Wood, 2017), “plotly” (Plotly Technologies Inc, 2015) and “ggplot2” (Wickham, 2016).

2. Results

2.1. Annual records of yield and yield components:

Yields obtained on the different trials (years and varieties) differed significantly from one year to the other. Although, variations among varieties remained limited. Detailed results are presented in Supplementary Table 1 and summarized in Figure 1.

Over the different trials, minimum yields were always obtained under non-fertilized treatments and ranged from 4.2 Mg ha^{-1} in 2012 to 10 Mg ha^{-1} in 2019 (Fig. 1). At the opposite, the highest yields were achieved with highest N supplies. The lowest maximum yield, 7.2 Mg ha^{-1}, was achieved in 2012 with 100-100-100 kg ha^{-1}. The highest maximum yield, 12.3 Mg ha^{-1}, was achieved in 2017 with 120-120-120 kg ha^{-1}. However, maximum N supply did not always outperform trial yields. On our experiments, only 3 trials out of 14 achieved their maximum yields with highest N fertilization schemes. On 8 trials out of 14, yields were maximized with a total N supply lower than the maximum rate applied {2010, 2012, 2013, 2014, 2016, 2018, 2019}. On the last 3 trials, in 2012, 2015 and 2017, a much lower total N supply (at least 90 kg ha^{-1}) got significantly similar yields than maximum N supply (300 – 360 kg ha^{-1}).

Grain densities greatly differed between trials and N fertilization schemes. Minimum grain densities range from 9002 kernels m^{-2} in 2011 to 23017 grains m^{-2} in 2019 and were always recorded where no N was applied. The maximum grain densities ranged from 19589 kernels m^{-2} in 2011 to 34891 kernels m^{-2} in 2019 (Fig. 1). These were achieved with N fertilizations schemes of 100-100-100 kg ha^{-1} and 90-90-90 kg ha^{-1}, respectively. Highest grain densities were not reached every year with the maximum

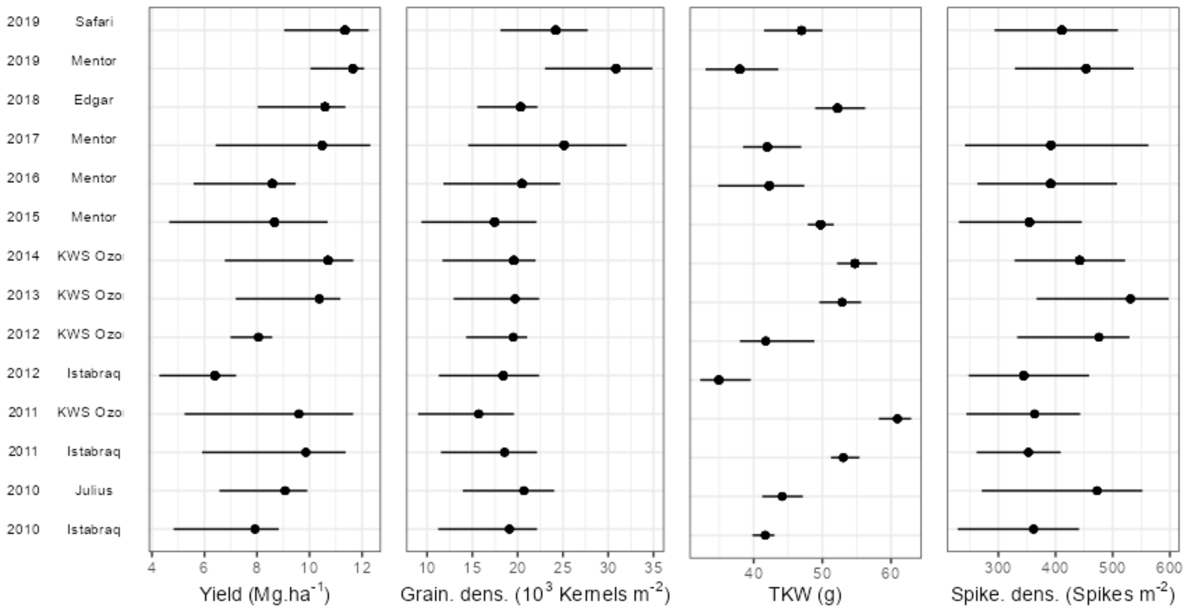

Figure 1: Annual variations of yield and yield components recorded on the different trials (years and varieties)

total fertilizer N supply. In 2012, 2013, 2018 and 2019, significantly lower total N supplies than the highest rate used led to maximum grain densities. Though, these high grain density levels did not always achieve maximum yields within the corresponding trials.

In almost all seasons, Thousand Kernel Weight (TKW) followed an opposite response to N supply compared to yield: low N supply led to high TKW, while high N supply resulted in reduced TKW. Thus, minimum TKW were achieved under high N supply that had increased grain density (those ranging from 180 to 300 kg ha^{-1}). Maximum TKW were observed under N supplies that produced lower grain densities, *i.e.* those ranging from 0 to 150 kg ha^{-1}. The exception occurred in 2011 when the unfertilized control achieved the minimum TKW.

Unfertilized plots always resulted in lowest spike densities. Minimum values ranged from 229 to 367 spikes m^{-2}, respectively in 2010 and 2013. Maximum spike densities ranged from 409 spikes m^{-2} in 2011 to 598 spikes m^{-2} in 2013. Both were obtained under high fertilization strategies of 100-100-100 kg ha^{-1}. Globally, highest spike densities were achieved with total N supply ranging from 225 to 315 kg ha^{-1}.

2.1.1. Correlation analysis among yield components

Table 4 presents the Pearson's correlations computed between the yield and the different yield components. Grain density had the strongest influence on the final yield whereas a rather small proportion of yield variability was explained by thousand kernel weight (TKW) (Table 4). Grain density was in turn similarly explained by spike density and spike fertility (Table 4). Both components had a significant effect on yield, but the effect was found to be better for spike density than spike fertility (Table 4).

Table 4 : Pearson's correlations among yield components and their relation to yield

	Yield	Spike density	Spike fertility*	Grain density*	TKW
Yield	1.00	0.66	0.34	0.76	0.25
Spike density		1.00	-0.17	0.62	-0.01
Spike fertility*			1.00	0.65	-0.50
Grain density*				1.00	-0.42
TKW					1.00

* *Estimated yield components*

A significantly negative correlation was observed between spike fertility and TKW, while spike density and TKW were not related (Table 4). Correlation between grain density and TKW reflected the dual influence of spike fertility and spike density on grain density elaboration.

2.1.2. Correlation analysis of N supply influence on yield and yield component:

Correlation analysis between each N supply and yield (Table 5) revealed a stronger impact of early N supply compared to N supplied at flag leaf (DC 39). The impact of total fertilizer N supply on yield remained lower than the apparent effect of N_{SRW} (Table 5). It should be noticed that N_{SRW} were measured once a year. N_{SRW} variation occurred at the interannual scale whereas N fertilization varied on annual scale and remained constant over years.

Table 5 : Pearson (r) correlation analysis between N applications and yield components

	Yield	**Spike density**	**Spike fertility**	**Grain density**	**TKW**
$\mathbf{N_{DC25}}$	0.31	0.58	-0.14	0.35	-0.10
$\mathbf{N_{DC30}}$	0.28	0.41	0.03	0.33	-0.12
$\mathbf{N_{DC39}}$	0.12	0.11	0.14	0.16	-0.10
$\mathbf{N_{Tot}}$	**0.39**	**0.60**	**0.02**	**0.47**	**-0.18**
$\mathbf{N_{SRW}}$	0.60	0.34	0.59	0.75	-0.24
$\mathbf{N_{Tot} + N_{SRW}}$	0.64	0.71	0.31	0.78	-0.28

N_{DC25} is N supply provided after winter, during tillering stage (DC 23-25) ; N_{DC30} is N provided at the beginning of stem elongation (DC 30); N_{DC39} is N supply provided at flag leaf stage (DC 39), N_{Tot} is the sum of N supplied at each phenological stage; N_{SRW} is the residual soil N measured at the end of winter.

Among yield components, total fertilizer N supply had a stronger influence on both spike density and grain density compared to spike fertility and TKW (Table 5). The first two N applications (N_{DC25} and N_{DC30}) had the highest impact on spike density and grain density. First N supply had a stronger influence on spike density than N supply at stem elongation (N_{DC30} – Table 5). However, the impact of this first N supply on spike density was offset by a negative influence on spike fertility. It resulted in similar impact of both first and second N supply on grain density. Only the N supplied at flag leaf (N_{DC39}) was positively, although poorly, correlated with spike fertility.

Except TKW, each yield component was positively influenced by N_{SRW}. However, if N_{SRW} seemed to shift positively yield levels, the effect was not constant over years. For example, N_{SRW} was higher in 2012 than in 2011 (Table 2). At the opposite, yield levels were higher in 2011 compared to 2012 (Figure 1). Also, effect of N_{SRW} on yield components was not equivalent to the effect of N fertilization. Spike fertility seemed more influence by N_{SRW} than N fertilizer whereas spike density was more influence by N fertilization (Table 5).

2.2. Analysis of N influence on yield and yield components through normalization of the variables

The analysis of results conducted in the previous section relied on absolute values of yield and yield components and involved the variation produced not only by the N treatments but also the environmental differences among the trials. In an attempt to isolate the N-driven variations determining yield response, we normalized the data within the experiments (see equations 3, 4, 5 and 7 in Materials and Methods).

2.2.1. Disentangling the influence of grain density on yield

The normalization procedure implemented from eq 3 and 4 are graphically illustrated at Fig. 2. From these equations, normalized grain density varied from 0.42 to 1, the latter being the maximum grain density of each treatment. Normalized yield values ranged between 0.44 and 1.10 while normalized yields equal to 1.0 correspond to yield values when maximum grain density was reached. Fig. 2 revealed three main features of the relationship between grain density and yield.

Firstly, grain density always contributes to yield improvement. On Fig. 2, the bisector indicates that for each unit of normalized grain density, a unit of yield is obtained. Almost no points (4%) were observed under the bisector (Fig. 2 - zone 1). Not only these cases were very few but also the magnitude of the yield reduction in all these exceptional cases was negligible. The presence of normalized observations under the bisector (Fig. 2 - zone 1) would imply a deterioration of yield following an increase in grain density.

Secondly, we found out a 30 % positive yield variation at each grain density level (Fig. 2 – zone 2). Yield resulted of the combination of grain density and kernel weight. Kernel weight could vary from very low yield impact to a 30% variation above the grain density contribution to yield.

Thirdly, the maximum grain density did not always contribute to achieve the maximum yield. Then, 25% of yield values were situated above the yield value corresponding to maximum grain density (Fig. 2 – zone 3). Among these 25% of yield values, the corresponding grain density ranged between 0.81 and 1.

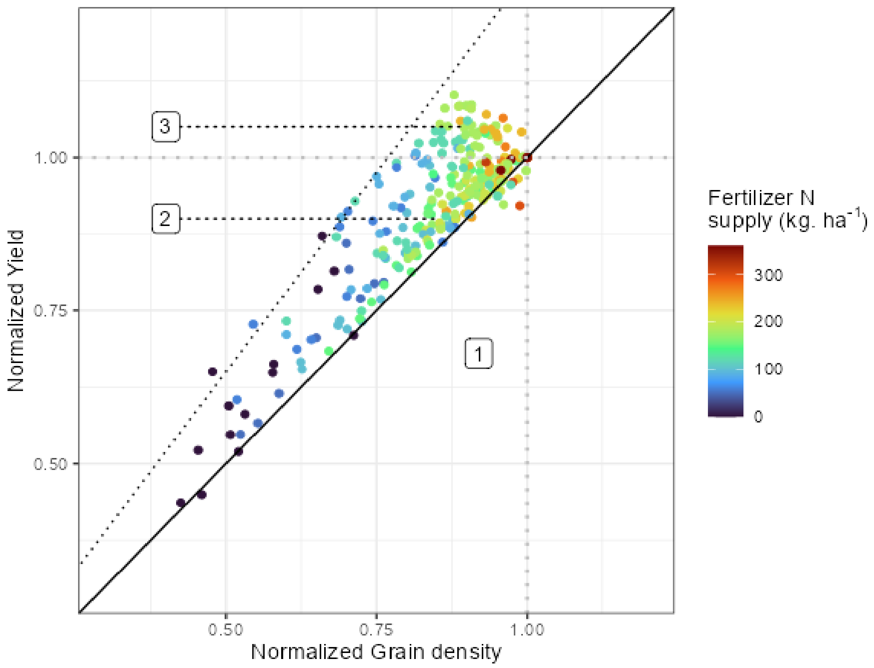

Figure 2: Normalized yield as a function of normalized grain density. Grain density is normalized by its maximum in each trial. Yield is normalized by yield value corresponding to

Finally, it should be noticed that N fertilizer supply was more related to grain density than yield. According to Spearman rank method, correlation between N fertilization and normalized grain density was 0.80 when the correlation between N fertilization and yield was 0.54.

2.2.2. Splitting N application allows to improve spike density without negatively affecting spike fertility

A generalized additive model (GAM) was fitted on grain density, spike density and spike fertility according to eq. 6 (see Supplementary Panel 1 and Table 2). The best fit was found to exhibit an R^2 = 0.90 with smooth functions applied individually on spike density and spike fertility and a tensor product interaction applied on both variables (see Supplementary Table 2). The model was compared to bivariate linear model to evaluate the interest of non-linear procedure. In the linear model (R^2 = 0.86), both spike density and spike fertility had an individual significant influence on grain density, however the linear interaction of the two variables was not significant. Also, the linear model over-represented the behavior of grain density in high grain density level. At the opposite, the tensor product interaction between spike density and spike fertility was significant in the selected GAM and improved the fitting of the model. A 3-Dimensions representation of the model is presented Fig. 3 and different displays including observation values are provided in Supplementary Figure 2.

In low grain density, each increase of spike density and spike fertility contributed to a higher grain density (Fig. 3). The individual contribution of spike density on grain density was stronger than the individual contribution of spike fertility. At low spike fertility level (<0.1), grain density followed an “exponential” trend over spike density. However, the maximum grain density could not be reached when spike fertility was lower than 0.35. At high spike fertility level (>0.8), increase of grain density followed a “logarithmic” trend over spike density. This trend was due to a change in the slope of the curve starting at spike density near 0.46 (resp. spike fertility = 1). The whole grain density surface reached a flattening shape at upper spike fertility level (>0.6), starting from the change in slope at spike density 0.46. This flattening part of the grain density surface occurred above a normalized grain density of 0.80. Above this threshold, gain in grain densities could only be reached at the prices of large increase of spike density and/or spike fertility (larger than what was required when considering lower grain density levels). Let’s also notice that around maximum spike density (~1), changes in slope were reported and grain density tended to be lower when spike fertility was below 0.35 or superior to 0.77. (Fig. 3).

Finally, the maximum grain density was reached with the maximum spike density and an average range of spike fertility. For example, grain densities above 0.99 correspond to spike densities in the range 0.98 – 1 and spike fertilities in the range 0.33-0.87 (close to the changes in slope).

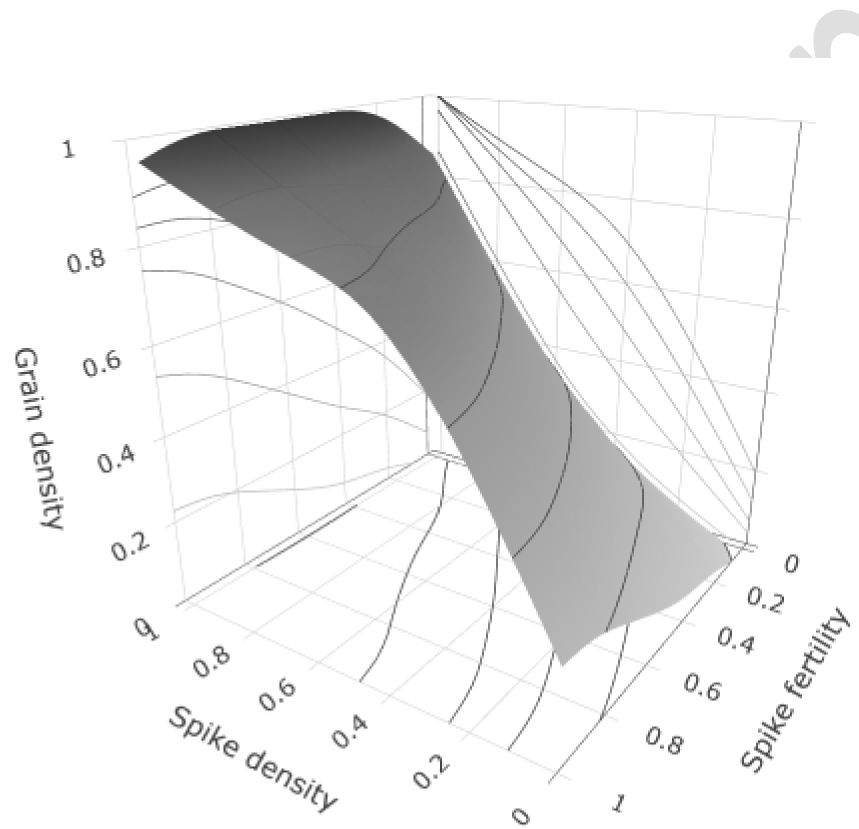

Figure 3: Generalized additive model of grain density regressed on spike density and spike fertility

As both spike density and spike fertility were dependent, impact of N fertilization on each component was analyzed at different levels of the dependent variable and under total fertilizer N supply (Fig. 4). Total fertilizer N supplied had a global positive effect on both components (Fig. 4 – left panels). Regressions between spike density and N supplies have R^2 ranging from 0.37 to 0.91. All regressions were significant among the different levels of spike fertility (Fig. 4a | Supplementary Table 3).

The isolated effect of N supply at tillering on spike density was found to be significant ($R^2 \in 0.18$ and 0.81) whatever the spike fertility level, while the isolated effect of N supply at the onset of stem elongation (DC 30) was overall not significant. The sum of both these N supplies showed stronger linear regression with spike density than the sole N supply at tillering (Fig. 4a | Supplementary Table 4) ($R^2 \in$ 0.5 – 0.9).

The influence of N fertilization on spike fertility was more complex. Spike fertility reacted to fertilization in middle ranges of spike density only (0.3-0.7). Under spike density of 0.3, lack of data penalized the analysis. Thus, in the range of spike density 0.3 - 0.7, slopes of regressions between spike fertility and total N supplies were significantly positive ($R^2 \in 0.42 – 0.66$). Above spike density 0.7, relations were not significant and even not always positive (Fig. 4c). Individually, N supplied at DC 25, DC 30 and DC 39 did not have the same effect on spike fertility. While relation between spike fertility and N supplied at DC 25 and DC 30 were globally not significant, N supplied at DC 39 showed significant positive effect on spike fertility again solely for spike densities in the range 0.3 – 0.7 ($R^2 \in 0.16 – 0.45$) (Fig. 4 d).

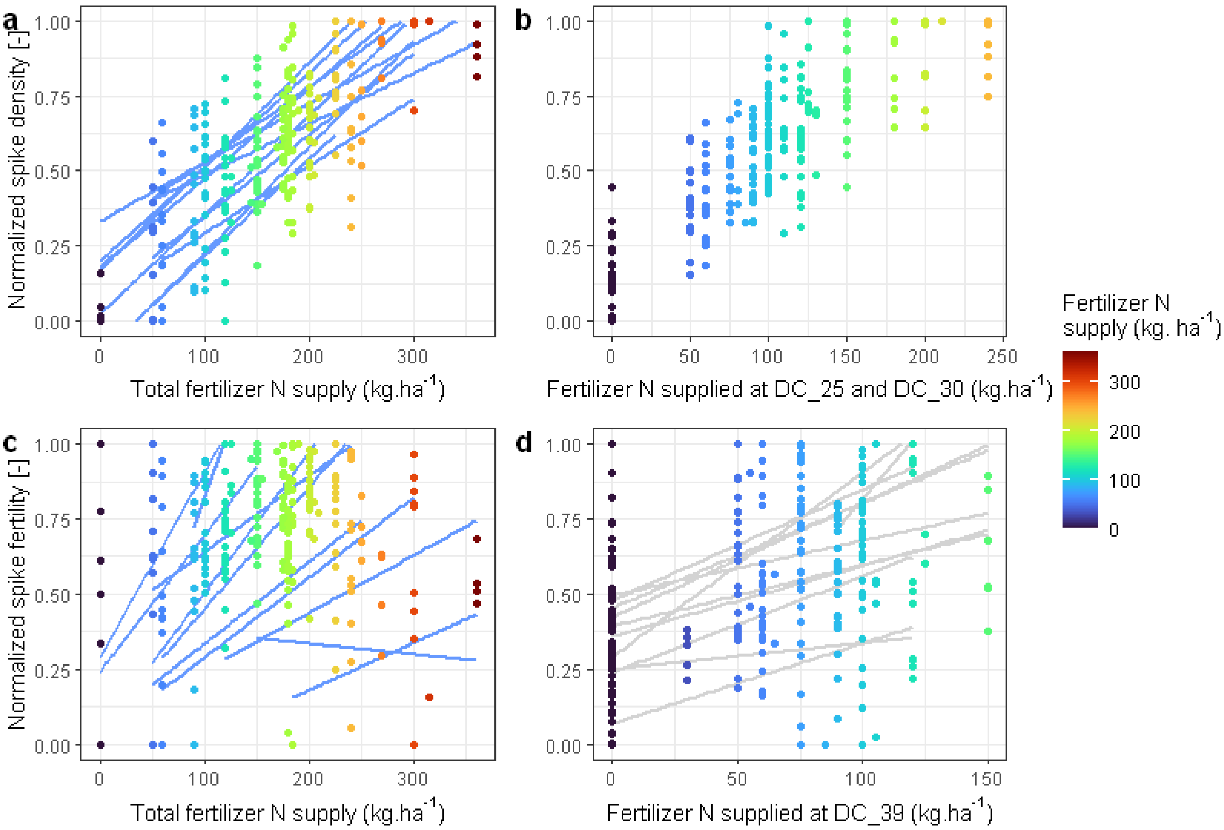

*Figure 4: Co-dependance of normalized spike density and normalized spike fertility under fertilizer N supplies. (**a**) Spike density was regressed by level of spike fertility under total fertilizer N supply. (**b**) Effect of cumulated fertilizer N supplied at DC 25 and DC 30 on spike fertility. (**c**) Spike fertility was regressed by level of spike density under total fertilizer N supply. (**d**) Effect of fertilizer N supplied at DC 39 on spike fertility compared to total fertilizer N supply.*

Above all, we observed a differentiated effect of early N supplied on spike density and late N supplied on spike fertility.

2.2.3. Could yield be the best trade-off between grain density and TKW ?

The relationships between normalized grain density, normalized TKW and normalized yield is reported Fig. 5. Grain density and TKW were normalized according to Eq. 6 while yield was normalized with Eq. 5 to highlight a "potential" between a minimum and a maximum. The variability of the normalized grain density was found to range between 0.4 and 1, exhibiting similar range as the one reported at Fig. 2 (Equation 3 and 7 are mathematically similar for grain density). For what concerns the variability of the normalized TKW, it was found to range between 0.725 and 1. This variation was similar to the one reported between the bisector and the upper threshold described at Fig. 2.

Highest yield values were found when both grain density and TKW tended to their respective maximum values (Fig. 5b). Each fertilization strategy tended to constrain differently the variation of the two yield components (Fig. 5a).

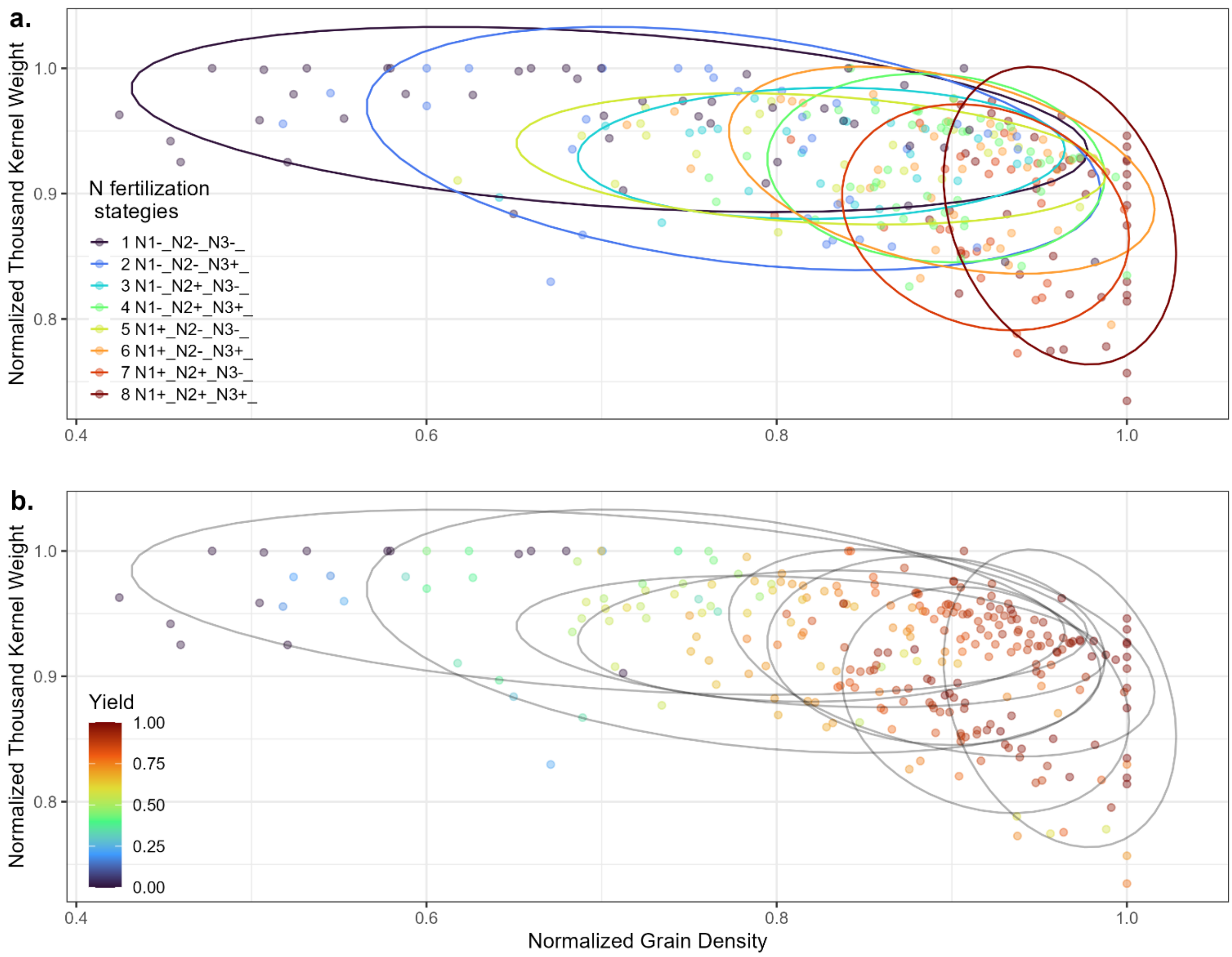

Figure 5: (a) Relationship between normalized grain density and normalized TKW as impacted by N fertilization strategies. (b) Impact of both variables on normalized yield.

N fertilization strategies minimizing the variation of grain density and optimizing its performances (greater median values) were found to be structured around at least two high individual N supplies out of the three, that is two N supplies equal or above 60 kg ha^{-1} (Table 6). Impact of strategy “8” on grain density was significantly different from every other strategies. Strategies 4, 6 and 7 had no significant diffences reported on grain density but were significantly different from other strategies (Table 6| Supplementary Table 5a).

Strategies minimizing the variation of TKW and maximizing its performance ranged between low N supplies at each of the three phenological stages (i.e “1 N1- N2- N3-“) to strategies with two higher N supplies. The impact of strategy “1” on TKW was the only to be significantly different from each other strategies, except for strategy “2” (Table 6 | Supplementary Table 5b). Strategies 7 and 8 were not statistically different from each other, neither from strategies 6, but were significantly different from other strategies.

Strategies were also quantified as their ability to achieve 90% of the maximum yield. This threshold was achieved by strategies 8 (frequency : 68%), 6 (55%), 4 (38%) and 7 (25%). At the opposite, strategies 1, 2, 3, and 5 only succeeded few times (less than 9%) to reach the threshold. High yield levels were achieved under broad range of fertilizer N supply (120 to 360 kg ha^{-1}). Considering median values per strategy, strategies 8 and 7 provided more N (200 -250 kg) than strategies 4 and 6 which provided respectively 185 and 180 kg ha^{-1} of N. These results are illustrated with Supplementary Figure 3.

Results showed that the 4th and 6th strategies allowed to optimize both yield components distributions (Table 6), while often achieving higher yields than strategies 7 and 8. Globally, strategies 4 and 6 led to apply less N than strategies 7 and 8 which making them more efficicent. According to Eq. 5 which express the annual potential of yield components, strategies 4 and 6 reached a median spike density of 0.56 and 0.62 respectively while spike fertility ranged between 0.66 and 0.54. These variations in both sub-components resulted in a very similar grain density 0.78 and 0.77 for strategies 4 and 6. TKW were also very similar and ranged between 0.53 and 0.51 for the latter strategies respectively (Supplementary Table 7).

Table 6 : Classification of N fertilization strategies by median and coefficient of variation of Grain density or TKW and significance difference among strategies. "ns" stands for non-significant and "" stand for at least significantly different. Detailed results of the Games-Howell test are reported in Supplementary Table 3.*

	Classification of N strategies according to the median and coefficient of variation, from high to low performance		Significant difference between N strategies (Games-Howell test)	
			ns	*
Grain density	8	N1+ N2+ N3+		4, 3, 6, 7
	7	N1+ N2+ N3-	4, 6	3, 8
	4	**N1- N2+ N3+**	6, 7	3, 8
	6	**N1+ N2- N3+**	4, 7	3, 8
	3	N1- N2+ N3-		4, 6, 7, 8
TKW	3	N1- N2+ N3-	4, 5, 6	1
	5	N1+ N2- N3-	3, 4, 6	1
	1	N1- N2- N3-		3, 4, 5, 6
	6	**N1+ N2- N3+**	3, 4, 5	1
	4	**N1- N2+ N3+**	3, 5, 6	1

Selecting strategies 4 and 6 questioned an underlying influence of N_{SRW} despite the normalization procedures. Games-Howell test performed on subsets of strategies classified according to the initial N_{SRW} level shown an overall decrease in the strength of the significance (Supplementary Tables 5 and 6). The non-significant difference between strategies 4, 6 and 7 remained for both levels of N_{SRW}, on grain density and TKW. However, strategies that were initially significantly different on grain density, e.g., 4 and 5 or 5 and 6 (Supplementary Tables 5 a,b), were found to lose their significant difference once split in each group (Supplementary Tables 6 a,b).

3. Discussion

Based on ten years experiment, we aimed to analyze the elaboration of yield when the crop is submitted to a wide range of N fertilizations. Inspired by the concept of phenotypic plasticity, we explored the relationships existing between the different yield components as well as the specific influence of N supplies on their variations. From the responses plasticities of yield components to N management, one side goal of this study was to infer the global structure of a fertilization scheme able to optimize the elaboration of yield through its components with the minimum supply of N. Such a structure could further be used to develop N fertilization strategies and tactics in response to specific crop contexts.

3.1. Fertilization methods must adapt to environmental variations.

We observed strong interannual yield variations over the different trials (Figure 1). Part of theses variations were due to residual soil N at the end of winter (N_{SRW}) over years and/or trials (Table 2). In 2011-2012, yield records followed an opposite trend as N_{SRW}, revealing different sources of influence (*i.e.* climate, radiation). Though, in this first part of the analysis conducted with raw values, yield components relationships respected the trends of phenotypic plasticity hierarchies described by Slafer *et al.* (2014). Yield was more influenced by grain density than TKW while grain density was equally explained by spike density and spike fertility (Table 4). The impact of N splitting and N_{SRW} on yield components was also analyzed (Table 5). While spike fertility was not influenced by fertilization, the influence from N_{SRW} on this component was high. Oppositely, spike density was more influenced by N fertilization than by N_{SRW}. These differences in the impact of each source of N would be due to the timing at which they occur. N fertilization only varied across trials and remained constant over the different trials when N_{SRW} was considered constant within each trial.

However, considering the differential effect of N_{SRW} on spike density and spike fertility, we can hypothesize about the availability of N_{RSW} along the crop growth period, in our Belgian context where soils are deep. N_{SRW} was measured on the whole soil profile and a unique value was obtained from the soil N content analysis. At the time N_{SRW} was measured (end of winter), the crop root system was poorly developed and could not catch N from the deeper part of the soil profile. As the crop grow, the root system was able to access to this deeper N pool, even more as the crop uptake capacity increased (Limaux et al., 1999). Even if both components elaboration overlap, the initiation of spike fertility occurs later than the initiation of spike density (Miralles and Slafer, 2007). This time laps would explain the stronger influence of N_{SRW} on spike fertility than on spike density (Table 5), as a larger part of N_{SRW} would be available during the period of spike fertility elaboration compared to spike density. Though, further analyses should be considered to quantify across the soil deep, the availability of N_{SRW} along the crop growth period.

Also, the interactions between N fertilization, N_{SRW} and other environmental factors hidden specific behaviors of the crop when adapting to N resource availability. To further analyze yield and yield component variations specifically related to N fertilization, we used different normalization procedures on the response variables.

3.2. Favoring balanced N fertilization along the dedicated period

In the following paragraph spike $density_n$, spike $fertility_n$, grain $density_n$, TKW_n and $yield_n$ refer to normalized variables, regardless of the normalization equation used. Variable normalization was proposed to reduce environmental and genetic influences.

Among the yield components, we observed different types of responses to N fertilization. Densities components, namely spike $density_n$ and grain $density_n$ strongly reacted to increasing amounts of N (Fig. 2 & Fig. 4). Spike $density_n$ was highly sensitive to N supplied at tillering and to the sum of the two first N supplies (DC 25 and DC 30) (Fig. 4 a-b), independently of the spike fertility level (Supplementary

Table 3). From dedicated experiments, Masle (1985) found the development of tillers and the setting of spikes on tillers to be closely related to the availability of N. If N supplied at DC 25 had a specific effect on tillering, N supplied at DC 30 rather contributed to avoid tiller senescence (Masle 1985), explaining its individual non-significant effect on spike density. Despite these differences, the sum of the two N supplies was more strongly related to spike density than each individual N supplies (Fig. 4 | Supplementary Table 3).

In our dataset, grain density was overall similarly influenced by spike density and spike fertility (Table 4). This result is consistent with Slafer *et al.* (2014) who indicate different sensitivities of the components; spike density is mainly influenced by the environment when spike fertility is mostly, but not only, influenced by genetic determinism.

To better catch the effect of N fertilization on spike fertility$_n$, we expressed spike fertility$_n$ by level of spike density$_n$, discretizing the latter component. In low and high spike densities$_n$ (resp. spike density$_n$ <0.3 and spike density$_n$ > 0.7), no relation of N fertilization on spike fertility$_n$ were found (Fig. 4 c-d |Supplementary Table 3). The relatively low amount of data available under low spike density$_n$ would explain a non-significant relation. Also, under high spike density$_n$, we observed a negative relation between spike density$_n$ and spike fertility$_n$ (Fig. 3). This negative trade-off intiated from the change of slope above a grain density$_n$ = 0.8 (i.e., 80% of the potential grain density$_n$) corresponded to the area above spike density$_n$ = 0.46 (at maximum spike fertility) and above spike fertility$_n$ = 0.77 (at maximum spike density), *i.e*. the upper left corner of the model surface (Fig. 3).

Therefore, early N supplies leading to higher spike density$_n$ than 0.7 should be avoided as the risk of negative trade-off between spike density$_n$ and spike fertility$_n$ increases and cannot be managed with N fertilization anymore. At high spike density levels, Xu *et al.* (2015) reported differences in the number of grains per spikes among tillers. Secondary tillers spikes include less grains per spike than spikes from the main tillers (Xu et al., 2015). This would explain this apparent negative relation, due to a dilution effect in the proportion of grains per spikes at the plant level and blur the effect of N on the component.

In middle range of spike density$_n$ (0.3 > spike density$_n$ < 0.7), however, the effect of N supplied at DC 39 on spike fertility$_n$ was significantly positive (Fig. 4 |Supplementary Table 3). Ferrante *et al.* (2017) indicate that N availability during the development of spikes contributed more to florets survival than to their initiation. In our experiment, N supplied at DC 39 was the closest in time to this second phase of spike growth which could explain the greater contribution of the last N supply to spike fertility$_n$, maintaining N nutrition. Though, individually, N supplied at DC 39 had a low effect on spike density$_n$ and an inconstant effect on spike fertility$_n$.

However, the cumulative effect of N supply at DC 39 with earlier supplies on yield components, added to the importance of spike fertility$_n$ in grain density$_n$ variations, sustained the systematic selection of fertilization strategies containing a high N supply (> 60 kg ha^{-1}) at DC 39 (see section 5 of the "Results"). Also, the two N fertilization strategies selected as optimal to concomitantly optimize TKWn and grain densityn relied on a cumulated relatively high N from early supplies, which would lead to satisfactory plant N status that would partly alleviate the sole specific effect of N supply at DC 39 on the components. Hence, grain density$_n$ would be as much influenced by the total amount of N provided than by the dynamic of N supplies. In that respect, Jeuffroy & Bouchard (1999) reported the possibility to monitor N nutrition with different N splitting to compensate deficiency affecting either spike density or spike fertility, thus maintaining grain density. In our dataset, achieving at least 80% of the potential grain density$_n$ relied on large variations in spike density$_n$ [0.31 – 1] and spike fertility$_n$ [0 – 1] (Fig. 3).

Finally, TKW was apparently not positively influenced by any of individual N supplies (Table 5). The lowest N fertilizations were associated to high TKW$_n$ while the highest N schemes were associated to low values of this component (Figure 5 | Supplementary Table 7). This negative relationship arise from the dependence of TKW$_n$ on both grain density (sink) - through the above mentioned "dilution" effect

- and the plant N status impacting photosynthesis potential as well as remobilization of N (source) (Gaju et al. 2014). Indeed, high grain density involves a higher proportion of smaller grains per spikes (individual sinks) which result in a higher proportion of smaller grains (global sink) at the canopy level (Mirales and Slafer, 1995). The effect of N fertilization on this component can be considered as indirect since N from plant vegetative tissues is primarily stored or used in photosynthetic activity and then remobilized to the grain after anthesis (Barbottin et al. 2005, Martre, 2003). This was nuanced by Pask et al. in 2012 who reported highly variable influence of Post Anthesis N Uptake (PANU), which is a direct effect of N on spike N concentration and contribute to TKW. Kichey et al. (2007) and Martre et al. (2003) both reported that remobilization was not only governed by the crop genetic but also depended upon N fertilization. In this study, only extremely opposed strategies 1 (lower N levels), 7 and 8 (higher N levels) were significantly different from other strategies. This would find an explanation in the limited difference of sink (grain size) allowed by each strategy. Strategy 1 led to low grain density (Figure 5) with a higher proportion of bigger grains while strategies 7 and 8 produced high levels of grain density with higher proportion of smaller grains, having lower sink size. The absence of signal to dissociate other strategies would be related to the large overlying ranges of total N supplies included within assessed strategies (Supplementary Table 3). Still, Sadras and Slafer (2012) reported TKW to have the lowest phenotypic plasticity among yield components, which would amplify the difficulties to dissociate the effects of N strategies on TKW. Consequently, the variation of TKW that can be directly managed with N fertilization remained relatively low.

Ultimately, the N fertilization strategies able to optimize the trade-off between grain density and TKW were articulated around two N supplies above 60 kgN ha^{-1} and one N supply under 60 kgN ha^{-1}. Both strategies relied on high N supply at DC 39, when growth rate was high (Limaux et al. 1999). Concerning the two first supplies, strategy “6 N1+ N2- N3+” could be considered as a “spike density promoting” strategy while strategy “4 N1- N2+ N3+” as a “spike fertility maintaining” strategy. Indeed, while the median “strategy 4” achieved 56% of spike density$_n$ and 66% of spike fertility$_n$ potentials, the “strategy

6” achieved 62% of the spike density$_n$ and 54% of spike fertility$_n$. With these strategies, the trade-off highlighted on Fig. 3 could be avoided, leading to more efficient N supplies (Supplementary Table 7). Both N fertilizations leading to similar grain density$_n$ and yield$_n$ levels revealed the compensation potential of spike fertility over spike density.

These close performances between the two strategies questioned the influence of N_{SRW}, despite normalization procedures applied to the different variables. However, the distribution of N_{SRW} values within trials belonging to each strategy was similar as strategies were artificially determined *a posteriori* for the data analysis. Performing the Games-Howell test between strategies classified according to N_{SRW} threshold revealed no influence of N_{SRW} on strategies 4 and 6. Yet, it seemed to have a significant effect on the expression of e.g. strategies 1 and 8 (Supplementary Tables 6), relatively to its absolute value (below or above 46 kgN.ha^{-1}). As the size of the subsets on which the test was performed better explained these differences compare to the initial test (Supplementary Table 5 and 6), we cannot completely exclude an influence of N_{SRW} on the yield components, following the assumption of the first discussion section.

3.3. Towards an adaptable planification of N supply ?

The N fertilization structure highlighted in the previous section, allowing to reach an optimal yield level while reducing the total N supply, were identified by optimizing the trade-offs between (1) spike density and spike fertility, and (2) grain density and TKW. The selected strategies 4 and 6 matched an “ideal” situation of yield composition, where the influence of the environment was reduced (through the normalization procedures). If the 4th and 6th strategies were best suited to reach optimal ranges of normalized yield components and their trade-offs (Fig. 3 | Supplementary table 7), the strategies did not ambition to link quantities of N supplied with specific absolute values of yield components. Indeed, it’s important to acknowledge that a large literature reported the pitfall of “response curves” approaches, as a large gap between N supplies and plant N status exists, either due to processes at play with plant and the plant environment (Hawkesford 2014, Lemaire and Ciampitti, 2020). Rather, one of the objectives of this study was to highlight how the structure of dynamic supplies of N would allow to build optimal paths to elaborate yield through its components.

Unfortunately, the experiments mobilized in this study were not originally designed to evaluate or monitor the dynamic of the crop N status. However, we believe that adding this new information layer would be highly beneficial as it would allow to explore more in depth the relationships between N supply and yield components the plasticities. Building upon the results gained in this study and the recent literature, here after, we propose a conceptual framework to reach such an objective.

We believe that the N Nutrition Index (NNI), which provides a “continuous” information about the dynamic of plant N status, could be further related to the plasticity of yield components and therefore help to adjust the quantities and the timing of N supplies (Lemaire et al. 2008). The underlying idea is to take advantage of the “continuous” character of the variable, both in the temporal dimension but also as a metric of the intensity of N-related stress, to pilot optimally the elaboration of yields through its components. In this regard, Jeuffroy and Bouchard (1999) pointed out the extents of tolerable N deficiency levels, allowing to achieve sufficient grain densities, through the compensation effect of spike density and spike fertility. More recently, where NNI was previously restricted to aerial dry matter prior anthesis, Zhao et al. (2020, 2021) determined a critical N dilution curve for the spikes on winter wheat. Practically, linking the dynamics of plant and spike N status to individual yield components would help adjusting N supplies (Yao et al. 2023). However, this yet require the prior exploration of the concomittant impacts of timing and quantities of N supplies on plant N nutrition dynamics and the components of yield. Expending the concept of tolerable N deficiencies of Jeuffroy and Bouchard (1999), Ravier et al. (2017, 2018) determined the boundaries within which NNI could navigate, with a minimal NNI trajectory allowing to avoid yield losses, and considering that NNI > 1 would lead to environmental N losses. We believe that the optimal NNI envelop identified by Ravier et al. (2017) conceptually corresponds to the plasticity hierarchy observed between yield components (Sadras and Slafer 2012). The greater NNI variation allowed earlier in the season (DC 30 - 32) are clearly linked to the plasticity tolerated on the first and coarser yield component put in place, namely spike density. As we progress along the season (DC 32 -39 - 65), while the finer tuner of yield expresses (spike fertility and ultimately TKW) and as the NNI envelopes tend to narrow, so does the compensation potential. Therefore, we hypothesizes that redesigning the NNI trajectory envelopes to target the dynamic expression of yield components (rather than the final yield) might help define a more optimal and adaptable structure of yield elaboration. The knowledge of such relationships would help to support the practical structuration of N fertilization strategies and further improve tactical adaptation allowing to benefit from theyield components compensations effects. Future researches are however required to link an optimal plant N status with optimal trade-offs among yield components to improve the efficiency of N fertilization.

4. Conclusion

Nitrogen fertilization is a critical issue in the economy of European farms and even more considering its detrimental effects on the environment. In a context where N fertilization is often overestimated or misadjusted due to the uncertainty on near future climate conditions, this study argue for parsimonious and balanced N fertilization strategies. We showed that highest yields were not always achieved with the maximum N supply but were conversely achieved when the different yield components can express their individual potential. N fertilization exclusively promoting specific yield components, e.g., the components with the highest phenotypic plasticity, can reach high yield levels but at the expense of heavy N supplies. Therefore, managing an optimal balance between yield components, through N fertilization, appeared to be the most efficient strategy. In this study, the N fertilization strategies identified as the most effective had a similar structure. Early N supplies are formed of one larger and one lighter N supply indistinctly positioned at tillering or stem elongation while the last N supply was always large and supplied at flag leaf. This N fertilization scheme must be adapted each year according to the changing climate conditions.

This study paves the way to future researches on dynamically (through NNI relationship) and spatially explicit N fertilization methods where N fertilization is adapted to the subfield potential.

Resulting from a ten-year experiment located in a high yielding context, the identified N fertilization strategies should further be tested in this specific context but also in different contexts to evaluate the genericity of this N fertilization schemes. Additional work is also needed to define tactical adaptations to be implemented from these N fertilization schemes when the plant is submitted to stresses during its growth cycle and is consequently not able to efficiently use N. This evaluation could be performed with a crop model to integrate both these new knowledges on the shape of N fertilization strategies, NNI and changing climate conditions.

CRediT authorship contribution statement

Arthur Lenoir: Conceptualization, Methodology, Data analysis, Visualization, Writing – original draft. **Gustavo Slafer:** Conceptualization, Writing - Review & Editing. **Ali Siah**: Supervision. **Benjamin Dumont**: Conceptualization, Experimental design, Methodology, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material:

Supplementary figure 1: Relationship between Yield and Grain density on raw data

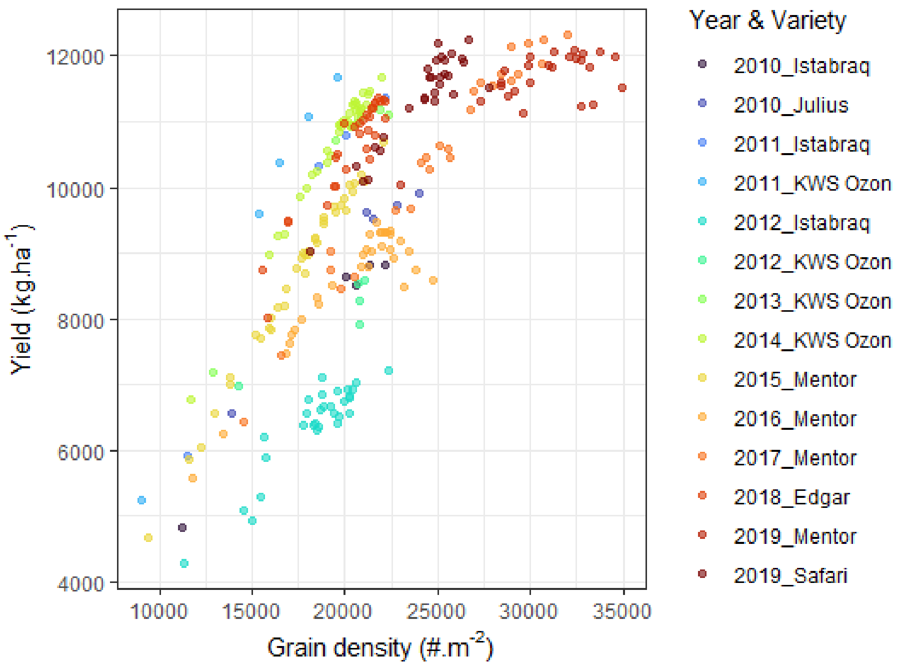


Figure 1: Yield as a function of grain density for each trial (year and variety).

“Year and variety” classification encompass experiment variability due to annual weather, differences in residual soil N available at the end of winter and genotypes.

Supplementary Table 1 : Raw results of observed yield and yield components under the different N fertilization regimes tested each year between 2010 and 2019

N Management					Yield (kg ha^{-1})		Grain.dens. (# m^{-2})		TKW (g)		Spike.dens. (# m^{-2})	
Years	DC_{23-25}	DC_{31}	DC_{39}	Total N fertilizer supplied	Avg.	Std.dev	Avg.	Std.dev	Avg.	Std.dev	Avg.	Std.dev
2010 -> 2019	0	0	0	0	6539.5	1661	13467.9	3687	49.0	5.8	285.3	43.8
2012, 2014 -> 2019	0	0	50-70	50-70	7974.8	2176	17226.4	5634	47.1	7.9	308.9	70.8
2012, 2014 -> 2019	0	0	100+	100-120	8901.1	2178	19376.4	4708	46.2	7.4	309.0	52.2
2012, 2014 -> 2019	0	50-70	0	50-70	8737.7	1904	19031.5	4668	46.4	6.3	358.7	68.1
2012, 2014 -> 2019	0	50-70	50-70	100-140	9612.5	1930	21014.5	4859	46.4	7.1	363.6	56.0
2012	0	50-70	100+	150-190	6363.2	12	18453.5	226	34.5	0.5	328.0	1.9
2012, 2014 -> 2019	0	100+	0	100-120	9980.8	1533	22284.8	4153	45.3	5.6	396.3	46.7
2014, 2015	0	100+	50-70	150-190	9869.3	1079	18754.3	1137	52.5	3.0	392.7	75.8
2012, 2014 -> 2019	0	100+	100+	200	9963.4	1864	22122.6	4181	45.5	7.6	384.2	43.5
2019	30-40	30-40	30-40	90-120	10079.3	-	20954.9	-	48.1	-	350.0	-
2012 -> 2018	30-40	50-70	100+	180-230	10768.1	983	24521.8	3352	44.3	5.5	388.3	19.2
2016, 2018	30-40	100+	50-70	180-230	10179.2	1625	21750.4	432	46.7	6.5	407.5	-
2012, 2014 -> 2019	50-70	0	0	50-70	8655.7	1937	18719.1	4539	46.6	5.4	369.3	46.0
2012, 2014 -> 2019	50-70	0	50-70	100-140	9601.7	2170	20771.3	5166	46.8	6.7	377.8	53.8
2012	50-70	0	100+	150-190	6486.5	259	18681.8	210	34.7	1.0	336.9	12.0
2016	50-70	30-40	100+	180-230	9473.3	-	21702.9	-	43.7	-	400.8	-
2012, 2014 -> 2019	50-70	50-70	0	100-140	9847.8	1775	22217.6	4901	44.9	6.2	413.2	38.5
2010 -> 2019	50-70	50-70	50-70	150-210	10301.3	1440	21956.0	4813	48.1	8.3	419.0	61.9
2010, 2012 -> 2019	50-70	50-70	100+	200-260	9782.7	1523	21290.8	3534	46.4	7.5	426.6	60.4
2016	50-70	100+	30-40	180-230	9044.8	-	22421.4	-	40.3	-	463.3	-
2011, 2014, 2015, 2019	50-70	100+	50-70	200-260	10918.5	902	23115.7	5485	48.5	7.0	413.2	57.5

2010, 2012, 2014,2015	50-70	100+	100+	250-310	9637.6	1741	20669.7	562	46.5	7.8	415.1	64.5
2016 -> 2019	70-90	0	0	70-90	10024.2	1229	22376.4	4138	45.3	4.9	429.9	31.8
2014, 2015	70-90	0	50-70	120-160	9466.1	2063	17907.1	2689	52.6	3.6	369.6	31.2
2012, 2014 -> 2019	70-90	0	70-90	140-180	10191.9	1733	21726.0	4533	47.5	6.7	401.6	47.2
2016, 2018, 2019	70-90	30-40	50-70	150-200	10560.3	1307	22895.2	2211	46.1	3.3	444.4	34.6
2016 -> 2019	70-90	50-70	30-40	150-200	10806.7	1316	24679.7	3562	44.1	5.5	462.5	40.5
2012, 2014 -> 2019	70-90	70-90	0	140-180	10202.7	1736	23928.6	5025	43.3	7.2	434.9	43.8
2014, 2015, 2018, 2019	70-90	70-90	50-70	190-250	10887.5	867	21737.0	2417	50.3	3.3	417.8	53.1
2010 -> 2019	70-90	70-90	70-90	210-270	10398.2	1561	22902.5	4199	46.2	8.1	469.1	64.5
2012, 2014 -> 2019	100+	0	0	100-120	9806.8	1755	22230.9	4757	44.7	6.2	416.5	50.4
2014, 2015	100+	0	50-70	150-190	9409.3	2173	18093.6	3040	51.7	3.3	405.8	55.4
2012 -> 2019	100+	0	100+	200-240	10173.8	1980	22824.2	4938	45.3	8.3	439.6	71.1
2012, 2014 -> 2019	100+	100+	0	200-240	10351.5	1786	24479.2	4880	43.0	7.8	477.4	59.2
2010 -> 2019	100+	100+	100+	300-360	10416.7	1550	24154.9	4031	43.8	8.0	490.9	54.7

Supplementary panel 1: Model fitting procedure for the prediction of grain density with spike density and spike fertility

The following models were compared to evaluate the fit improvement using generalized additive model (GAM) over linear model.

Linear : $E(y_i) = \beta 0 + \beta 1 x_i + \beta 2 z_i + \epsilon$

GAM_1 : $g(E(y_i)) = \beta 0 + \beta 1 x_i + f2(z_i) + \epsilon$

GAM_2 : $g\left(E(y_i)\right) = \beta 0 + f1(x_i) + f2(z_i) + \epsilon$

GAM_3 : $g\left(E(y_i)\right) = \beta 0 + f1(x_i) + f2(z_i) + f3(x_i, z_i) + \epsilon$

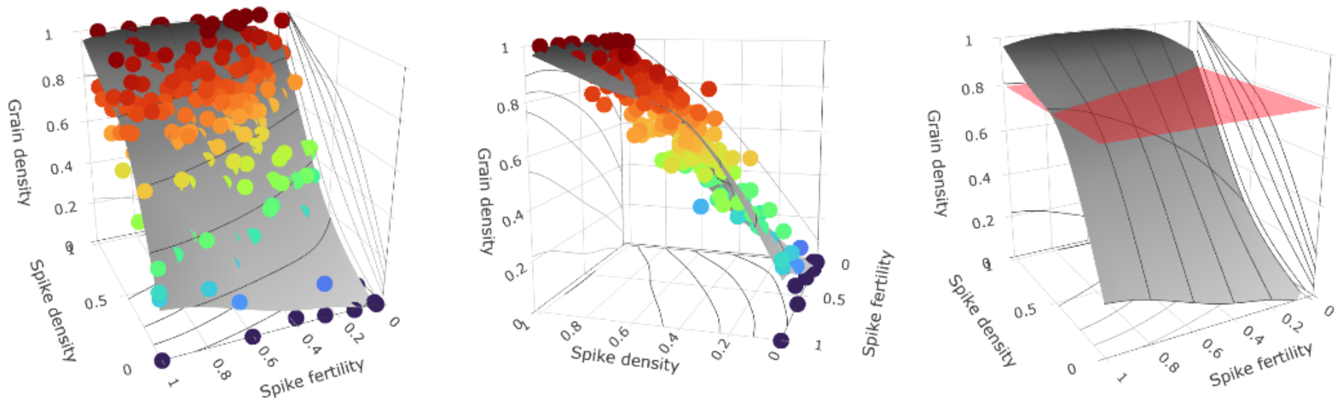
where *y* is the response variable, here the normalized grain density, *β0* is the intercept, *β1* and *β2* are the parameters of x and y in linear mode. X is the normalized spike density and z is the normalize spike fertility. The functions *f1*, *f2* refer to a smooth function when *f3* refers to a tensor product interaction.

Restricted maximum likelihood (REML) was used to fit models individually to the data.

Supplementary Table 2: Performance parameters of the tested models during the fitting procedure

	Adjusted R^2	REML	AIC
Linear	0.860	-230.4	-472.8
GAM_1	0.864	-231.2	-476.9
GAM_2	0.872	-235.8	-489.9
GAM_3	0.904	-260.2	-548.9

Supplementary Figure 2: Visualization of the fitted Generalized Additive Model



3D representation of the generalized additive model and the normalized values of grain density over spike fertility and spike density. Points colors represent the level of grain density, blue being the lowest values while red being the highest grain densities. The red surface on the 3rd panel represents the grain $density_n = 0.8$.

Supplementary Table 3 : Linear regressions of normalized grain density, spike density and spike fertility and the influence of total N fertilizer. Normalization procedures correspond to equation 5 in Material & Method.

Linear regression of grain density and spike density by level of spike fertility			Linear regression of grain density and spike fertility by level of spike density			Linear regression of spike density and N supply (DC 25 & DC 30) by level of spike fertility			Linear regression of spike fertility and N supply (DC 39) by level of spike density		
Spike fertility	R²	p-value	Spike density	R²	p-value	Spike fertility	R²	p-value	Spike density	R²	p-value
0	0.883	0.00005	0	0.584	0.0770	0	0.905	0.00002	0	0.205	0.367
0.1	0.869	0.00676	0.1	0.724	0.0674	0.1	0.841	0.01	0.1	0.771	0.0502
0.2	0.917	0	0.2	0.581	0.0464	0.2	0.708	0.00001	0.2	0.0669	0.575
0.3	0.828	0	0.3	0.687	0.00013	0.3	0.622	0	0.3	0.427	0.00824
0.4	0.826	0	0.4	0.678	0	0.4	0.651	0	0.4	0.664	0
0.5	0.675	0	0.5	0.753	0	0.5	0.374	0.0002	0.5	0.608	0
0.6	0.769	0	0.6	0.556	0	0.6	0.875	0	0.6	0.448	0
0.7	0.821	0	0.7	0.407	0.00003	0.7	0.593	0.00001	0.7	0.416	0.00002
0.8	0.893	0	0.8	0.394	0.00303	0.8	0.77	0	0.8	0.173	0.0681
0.9	0.651	0.00003	0.9	0.519	0.0285	0.9	0.457	0.00148	0.9	0.0104	0.794
1	0.728	0.00001	1	0.294	0.0200	1	0.679	0.00005	1	0.00646	0.751

Supplementary Table 4 : Linear regressions of normalized spike density and different fertilizer N supplies. Normalization procedures correspond to Eq. 5 in Material & Method.

Spike fertility levels	Linear regression of spike density and N supply at DC 25		Linear regression of spike density and N supply at DC 30		Linear regression of spike density and total N supply	
	R²	p-value	R²	p-value	R²	p-value
0	0.805	0.0000	0.508	0.0009	0.822	0.0000
0.1	0.744	0.0125	0.498	0.0765	0.879	0.0018
0.2	0.554	0.0004	0.701	0.0000	0.817	0.0000
0.3	0.326	0.0029	0.170	0.0403	0.597	0.0000
0.4	0.185	0.0112	0.237	0.0035	0.617	0.0000
0.5	0.228	0.0058	0.082	0.1124	0.493	0.0000
0.6	0.490	0.0001	0.203	0.0183	0.656	0.0000
0.7	0.351	0.0018	0.128	0.0791	0.618	0.0000
0.8	0.519	0.0002	0.423	0.0011	0.781	0.0000
0.9	0.390	0.0043	0.066	0.2888	0.609	0.0001
1	0.406	0.0025	0.556	0.0002	0.903	0.0000

Supplementary Table 5 : Games - Howell test. Results of the Games-Howel post-hoc test on (**a**) grain density and (**b**) TKW. both variables being normalized between their trial's minimal and maximal values

a — Normalized Grain density

strategies	2 N1-_N2-_N3+	3 N1-_N2+_N3-	4 N1-_N2+_N3+	5 N1+_N2-_N3-	6 N1+_N2-_N3+	7 N1+_N2+_N3-	8 N1+_N2+_N3+
1 N1-_N2-_N3-	ns	***	****	**	****	****	****
2 N1-_N2-_N3+		ns	****	ns	***	****	****
3 N1-_N2+_N3-			*	ns	*	***	****
4 N1-_N2+_N3+				*	ns	ns	****
5 N1+_N2-_N3-					*	***	****
6 N1+_N2-_N3+						ns	****
7 N1+_N2+_N3-							**

b — Normalized TKW

strategies	2 N1-_N2-_N3+	3 N1-_N2+_N3-	4 N1-_N2+_N3+	5 N1+_N2-_N3-	6 N1+_N2-_N3+	7 N1+_N2+_N3-	8 N1+_N2+_N3+
1 N1-_N2-_N3-	ns	*	***	**	***	****	****
2 N1-_N2-_N3+		ns	ns	ns	ns	**	**
3 N1-_N2+_N3-			ns	ns	ns	***	**
4 N1-_N2+_N3+				ns	ns	*	*
5 N1+_N2-_N3-					ns	**	**
6 N1+_N2-_N3+						*	ns
7 N1+_N2+_N3-							ns

Supplementary Table 6 : Games - Howell test including the impact of N_{SRW}. Results of the Games-Howel post-hoc test on (**a**) grain density and (**b**) TKW, both variables being normalized between their trial’s minimal and maximal values

a. Normalized grain density

N_{SRW}	Strategies	2 N1-_N2-_N3+_	3 N1-_N2+_N3-_	4 N1-_N2+_N3+_	5 N1+_N2-_N3-_	6 N1+_N2-_N3+_	7 N1+_N2+_N3-_	8 N1+_N2+_N3+_
Under 46 kg ha^{-1}	1 N1-_N2-_N3-	ns	**	****	*	****	****	****
	2 N1-_N2-_N3+		ns	**	ns	**	***	****
	3 N1-_N2+_N3-			ns	ns	ns	*	***
	4 N1-_N2+_N3+				ns	ns	ns	****
	5 N1+_N2-_N3-					ns	ns	**
	6 N1+_N2-_N3+						ns	**
	7 N1+_N2+_N3-							ns
Above 46 kg ha^{-1}	1 N1-_N2-_N3-	ns	ns	*	ns	*	**	***
	2 N1-_N2-_N3+		ns	ns	ns	ns	*	**
	3 N1-_N2+_N3-			ns	ns	ns	*	***
	4 N1-_N2+_N3+				ns	ns	ns	**
	5 N1+_N2-_N3-					ns	*	**
	6 N1+_N2-_N3+						ns	*
	7 N1+_N2+_N3-							*

b. Normalized TKW

N_{SRW}	Strategies	2 N1-_N2-_N3+_	3 N1-_N2+_N3-_	4 N1-_N2+_N3+_	5 N1+_N2-_N3-_	6 N1+_N2-_N3+_	7 N1+_N2+_N3-_	8 N1+_N2+_N3+_
Under 46 kg ha^{-1}	1 N1-_N2-_N3-	ns	ns	*	ns	ns	**	*
	2 N1-_N2-_N3+		ns	ns	ns	ns	ns	ns
	3 N1-_N2+_N3-			ns	ns	ns	*	ns
	4 N1-_N2+_N3+				ns	ns	ns	ns

	5 N1+_N2-_N3-					ns	ns	ns
	6 N1+_N2-_N3+						ns	ns
	7 N1+_N2+_N3-							ns
Above 46 kg ha^{-1}	1 N1-_N2-_N3-	ns	ns	ns	ns	ns	***	****
	2 N1-_N2-_N3+		ns	ns	ns	ns	**	***
	3 N1-_N2+_N3-			ns	ns	ns	ns	**
	4 N1-_N2+_N3+				ns	ns	ns	*
	5 N1+_N2-_N3-					ns	ns	**
	6 N1+_N2-_N3+						ns	*
	7 N1+_N2+_N3-							ns

Supplementary Table 7 : Median, 1st and 3rd quantiles of normalized yield components values per strategies of N fertilization. Normalization procedure corresponds to Eq. 5 of the material & method.

N fertilization strategies	Normalized spike density	Normalized spike fertility	Normalized grain density	Normalized thousand Kernel Weight	Yield
1 N1-_N2-_N3-	0.36 [0.01 ; 0.54]	0.39 [0.24 ; 0.72]	0.41 [0.00 ; 0.61]	0.71 [0.49 ; 0.95]	0.51 [0.00 ; 0.73]
2 N1-_N2-_N3+	0.29 [0.13 ; 0.46]	0.77 [0.65 ; 0.89]	0.51 [0.32 ; 0.68]	0.62 [0.38 ; 0.86]	0.67 [0.43 ; 0.79]
3 N1-_N2+_N3-	0.48 [0.44 ; 0.66]	0.40 [0.29 ; 0.63]	0.62 [0.52 ; 0.70]	0.57 [0.42 ; 0.68]	0.71 [0.65 ; 0.81]
4 N1-_N2+_N3+	0.56 [0.44 ; 0.65]	0.66 [0.51 ; 0.84]	0.78 [0.71 ; 0.82]	0.53 [0.35 ; 0.58]	0.88 [0.83 ; 0.93]
5 N1+_N2-_N3-	0.58 [0.49 ; 0.68]	0.34 [0.19 ; 0.46]	0.55 [0.50 ; 0.71]	0.51 [0.39 ; 0.61]	0.68 [0.54 ; 0.85]
6 N1+_N2-_N3+	0.62 [0.52 ; 0.69]	0.54 [0.36 ; 0.64]	0.77 [0.69 ; 0.87]	0.51 [0.41 ; 0.63]	0.91 [0.79 ; 0.95]
7 N1+_N2+_N3-	0.73 [0.63 ; 0.82]	0.41 [0.21 ; 0.61]	0.84 [0.79 ; 0.86]	0.30 [0.20 ; 0.41]	0.87 [082 ; 0.90]
8 N1+_N2+_N3+	0.90 [0.75 ; 1.00]	0.45 [0.25 ; 0.71]	0.92 [0.85 ; 1.00]	0.22 [0.02 ; 0.35]	0.92 [0.84 ; 1.00]

Supplementary figure 3: Distribution of normalized yield within each N strategy. Each yield level is colored with the total amount of N provided. The vertical dotted line corresponds to a normalized yield value of 0.9.