Genotype, environment, and management interactions on grain yield and nutrient uptake dynamics in winter wheat

by

Amanda de Oliveira Silva

B.S., Sao Paulo State University, 2011
M.S., Purdue University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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Abstract

Understanding factors underpinning the variation in nitrogen (N) utilization efficiency (NUtE) [i.e., grain yield per unit of N uptake at maturity] is critical to direct future breeding and agronomic management strategies in wheat. However, no study has summarized changes in wheat NUtE across a wide range of environments. Further, the conservative behavior of producers to intensify management practices may have been contributing to the yield stagnation in the US southern Great Plains. Our goals were to: (i) perform a synthesis-analysis using published data to study NUtE in wheat, and (ii) conduct field studies to investigate the influence of genotype, environment, and management on grain yield and nutrient uptake. Results from our synthesis-analysis (*n*=529) showed a positive and curvilinear relationship between grain yield and $\mathrm{Nup_{MAT}}$, indicating that opportunities to enhance yield through improving NUtE would only be possible at greater-than-average yield and N uptake levels. By measuring the effects of other reported variables on the residuals of the relationship between NUtE and N uptake, we observed that the variability in NUtE at particular levels of N uptake was greater for fall- than for winter-sown wheat, but it was similar for all wheat classes. The negative correlation between grain protein concentration and the residuals indicated a challenge to increase yield through improving NUtE with no penalties in grain protein. We conducted two field research experiments at difference sites during the 2015-16 and 2016-17 growing seasons in Kansas. In our experiment 1, we conducted on-farm experiments across three locations and two growing seasons in Kansas using 21 modern winter wheat genotypes grown under either standard (SM) or intensified management (IM) systems. Results showed that across all sites-years and genotypes, the IM increased yield by 0.9 Mg ha^{-1} relative to the SM. Even in the lowest yielding background condition, the IM outyielded SM, and expectedly, the yield response to IM increased with the achievable yield of the environment. The yield response of genotypes to IM was related to the responses of biomass between the two management systems rather than harvest index, strongly driven by improvements in grain number while independent of changes in grain weight, and related to improvements in N uptake. In our experiment 2, we evaluated the partial contribution of 14 management practices on grain yield and the accumulation of N, P, K and S during the growing season using a single bread-wheat genotype grown under four site-years. Fungicide was the main treatment affecting yield and nutrient uptake. Overall, all nutrients were accumulated at a similar proportion at each growth stage relative to their respective accumulation at the end of the season. Shoot concentration for IM seemed to maintain higher concentration of nutrients as compared to the SM control during the growing season. This was emphasized by the significant increase in nutrition indices for N and S from SM to IM control, indicating possible luxury uptake under IM. Hence, crop intensification strategies may alter nutrient uptake at the end of season, but will not affect timing and rate of uptake during the growing season.

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Approved by:

Major Professor
Dr. Romulo Pisa Lollato

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Dedication

For my parents who encouraged me to pursue my dreams. Thank you for the endless love and for teaching me kindness and perseverance.

"If I have seen further it is by standing on the shoulder of giants"
Isaac Newton

Preface

This dissertation provides an overview of the genetic improvement on yield and nutrient utilization efficiency of modern winter wheat genotypes. The main objective of this dissertation was to document the effects of genotype, management, and environment on yield and nutrient uptake dynamics wheat. In Chapter 1, we executed a synthesis analysis using data from published manuscripts to report trends in nitrogen utilization efficiency across several wheat-producing regions in the world. We evaluated the relationship between yield and nitrogen uptake, and the possible factors affecting the variability of this relationship. In Chapter 2, we evaluated the response of genotypes to intensifying management across different background conditions via assessing the relationship between agronomic traits. We estimated that yield could be improved with intensifying management and that knowledge of the agronomic traits controlling the ability of genotypes to respond to management is important for crop intensification strategies. In Chapter 3, we analyzed the impacts of several management practices on yield and uptake of four macronutrients using a single-wheat genotype to assess the partial contribution of crop, nutrient, and disease management practices on improvements in yield and nutrient uptake. We also evaluated changes in plant nutrient utilization efficiency and requirements across standard and intensive management practices and yield levels.

Chapter 1 - Nitrogen utilization efficiency in wheat: A global perspective

Abstract

Understanding factors underpinning the variability in nitrogen (N) utilization efficiency (NUtE) [i.e. grain yield per unit of N uptake at maturity (Nup_{MAT})] is critical to direct future improvements in breeding and agronomic management. To our knowledge, no study has summarized changes in wheat NUtE across a wide range of experimental conditions. We performed a synthesis-analysis using published data to provide a global perspective of NUtE trends in wheat by (i) benchmarking against yield limited by Nup_{MAT}, and (ii) assessing factors contributing to the variation in NUtE. The final database encompassed 55 studies (*n*=529). A nonlinear model explained yield as a function of Nup_{MAT}. The gap between actual yield and Nup_{MAT}-limited yield was negligible at the lowest range of Nup_{MAT} and increased to ~2000 kg ha^{-1} as Nup_{MAT} levels increased. Hence, opportunities to enhance yield through improving NUtE would be more likely at greater-than-average yield and Nup_{MAT} levels. The negative correlation between grain protein concentration and the residuals between NUtE and Nup_{MAT} indicated a challenge to increase yield without penalizing grain protein. Further, there are greater opportunities to increase NUtE in fall- than winter-sown wheat. Identifying the determinants of NUtE will enable to narrow the gap between actual and Nup_{MAT}-limited yields.

Introduction

Wheat (*Triticum aestivum* L.) is the most cultivated crop in the world owing to its adaptability to a wide range of environments (Reynolds et al., 2012a; Snape, 1998). It is an essential component of the human diet: (i) 65% of the global wheat production (760 MT) is used towards human food production and the remainder for livestock feed and food industry (FAO-AMIS, 2018); and (ii) wheat is the primary source of calories for the world population (Braun et al., 2010; Reynolds et al., 2012b). As increases in cultivated wheat area are unlikely (Fischer et al., 2014), maintaining a yield gain rate commensurate with the increased demand is critical for global food security. However, the global rate of yield gain has been decreasing, and yields seem to be plateauing in many major wheat producing regions (Fischer and Edmeades, 2010). Projections suggest that a yield increase of at least 50% (Bruinsma, 2011; Fischer et al., 2014) or more (Hall and Richards, 2013; Ray et al., 2013; Rosegrant et al., 2009) will be needed by 2050.

Advances in plant breeding and agronomic management practices were the major contributors to historical yield gains in wheat (Daberkow and Huang, 2006; Evans, 1999; Slafer and Andrade, 1991; Turner, 2004). The introduction of dwarfing genes (*Rht*) during the Green Revolution period resulted in remarkable wheat yield improvements as it increased yield potential substantially due to an increased number of grains per unit area, improved partitioning towards the juvenile spike before anthesis, and harvest index (HI) (Brooking and Kirby, 1981; Flintham et al., 1997; Miralles et al., 1998; Miralles and Slafer, 1995). Simultaneously, the dwarfing genes improved resistance to lodging making plants able to withstand greater rates of nitrogen (N) fertilizer (Aktar-Uz-Zaman et al., 2017; Borlaug and Dowswell, 2003; Kole, 2013; Pingali, 2012).

Nitrogen is one of the most critical yield-limiting factors in crop production and improving the efficiency with which plants utilize the N supplied to produce yield is essential for the development of sustainable agriculture (Tilman, 2002). However, crop intensification in the past decades with excessive amounts of N application (Alexandratos and Bruinsma, 2012) and the ability of plants to recover only ~50% of the N applied have led to several environmental problems (Gastal et al., 2015; Tilman, 2002). Thus, considering future challenges of food security and exhaustion of natural resources, further increases in wheat production will have to originate from yield improvement in existent cropland with the most efficient use of the available resources (e.g., water, N, and land) (Borlaug and Dowswell, 2003; Daberkow and Huang, 2006; Evans, 1999; Fischer et al., 2014). One way to improve yield limited by N (i.e., reducing the yield gap between actual- and N-limited yields) without disrupting the environment is to enhance crop N use efficiency (NUE) [i.e., grain yield produced per unit of N applied] (Moll et al., 1982).

From a physiological perspective, NUE can be evaluated by the N utilization efficiency (NUtE), i.e., the amount of grain produced per unit of N uptake at maturity ($\mathrm{Nup}_{\mathrm{MAT}}$) (Ciampitti and Vyn, 2014). However; due to the curvilinear relationship between yield and $\mathrm{Nup}_{\mathrm{MAT}}$ (Cassman et al., 2002; Singh, 2001), rate of increases in yield are not always associated with increases in $\mathrm{Nup}_{\mathrm{MAT}}$ resulting in a decline in NUtE (Gastal et al., 2015). In this context, a decrease in NUtE is explained by the nonparallel increases in yield and $\mathrm{Nup}_{\mathrm{MAT}}$, but factors influencing this relationship are not completely understood. For this reason, understanding crop N uptake process that is directly correlated with NUtE is essential to direct agronomic and breeding strategies to concomitantly increase yield and NUE. Multiple studies have investigated ways to increase yield by analyzing yield components (Fischer, 2008; Slafer et al., 1990b), and trends in $\mathrm{Nup}_{\mathrm{MAT}}$ in wheat (Austin et al., 1980; Hamnér et al., 2017; Paccaud et al., 1985; Slafer et al., 1990a). However, there is a need for further investigation of these two factors in an holistic way (Barraclough et al., 2010).

In addition to yield, grain protein concentration is another important trait for wheat. Protein is a result of the ratio between N and carbon (C), and factors impacting yield and $\mathrm{Nup}_{\mathrm{MAT}}$ in general impact protein concentration in the grain (Singh, 2001). The different wheat market classes have different requirements for desired protein levels. For instance, high protein is desired for improved baking quality and food process of hard winter or spring wheat classes (Woolfolk et al., 2002), while low protein is a desirable trait for soft white wheat used in noodle production (Aguirrezábal et al., 2015; Nuttall et al., 2017). Regardless of wheat market classes, selection for high wheat yielding varieties and management practices (i.e. N fertilizer, irrigation, weed control, etc.) frequently resulted in yield increases associated with reductions of protein concentration in the grain (Calderini et al., 1995b; Guarda et al., 2004; Muurinen et al., 2007; Oury et al., 2003; Slafer et al., 1990a; Triboi et al., 2006). Nonetheless, in cases where additional N produced relatively minor yield increases per unit of N fertilized (i.e., late N application or high N doses), increases in protein were measured (Fischer et al., 1993; Woodard and Bly, 1998). Although the inverse relationship between yield and protein has been extensively discussed in the literature (Bogard et al., 2010; Cox et al., 1986), further exploration of this imbalance is still needed.

To better understand the variability in the relationship between yield and $\mathrm{Nup_{MAT}}$ in wheat, it would be beneficial to elucidate whether (and eventually how) other factors may impact the relationship between NUtE at particular levels of N uptake; such as wheat species (e.g., bread, durum or spelt) and class (e.g., hard, soft, and forage) referred to as “wheat class” hereafter, planting season (fall vs. winter) and year of genotype release. As yield potential and protein concentration may vary among different kinds of wheat (e.g. Marti and Slafer, 2014), it is postulated that wheat classes differ on yield responses to N and consequently may be a factor responsible for the variation in NUtE. For geographic reasons, studies usually evaluate yield and N uptake dynamics for either spring or winter wheat types. Thus, the consequences of different planting seasons (fall vs. winter) on yield and $\mathrm{Nup_{MAT}}$ relationship remain largely unknown.

Previous research studies have investigated genetic progresses of yield and NUE using germplasms from US, Europe, and Argentina including broad range of year of genotype release (Acreche and Slafer, 2009; Calderini et al., 1995a; Cormier et al., 2013; Guarda et al., 2004; Guttieri et al., 2017; Miri, 2009; Ortiz-Monasterio et al., 1997; Sayre et al., 1997; Slafer et al., 1990a). Nevertheless, to our knowledge, no studies have executed a synthesis analysis to investigate the breeding effects on NUtE in wheat. Improvements in NUtE have been attributed to increases in yield while Nup_{MAT} has remained unchanged over time (Wang et al., 2017). Thus, it is anticipated that wheat breeding has been indirectly selecting for NUtE over time and have influenced the variability in NUtE at particular Nup_{MAT} levels.

Hence, the aim of this paper was to provide global perspective of the variability on the relationship between yield and Nup_{MAT} (i.e., NUtE) in wheat via (i) benchmarking against yield limited by Nup_{MAT}, and (ii) analyzing physiological and agronomic factors contributing to the variability in NUtE.

Materials and Methods

Building the database

We performed a comprehensive synthesis-analysis using published data to evaluate the relationship between yield and Nup_{MAT} in wheat across a large set of diverse studies carried out across many regions and conditions, similar to the approaches adopted in previous investigations conducted for other crops such as soybean [*Glycine max* (L.) Merr.], sorghum [*Sorghum bicolor* (L.) Moench], maize [*Zea mays* L.], rice [Oriza sativa] and cover crops (Agyin-Birikorang et al., 2017; Balboa et al., 2018; Ciampitti and Prasad, 2016; Ciampitti and Vyn, 2012; Linquist et al., 2013; Mourtzinis et al., 2018; Tonitto et al., 2006), as well as for other traits in wheat (e.g. Hall et al., 2014; Pellegrino et al., 2015; Raseduzzaman and Jensen, 2017; Sadras and Angus, 2006; Slafer et al., 2014; Wang et al., 2017; Wilcox and Makowski, 2014).

To ensure data quality, the data retrieval process restricted manuscripts entered in the database to those obtained from scientific journals included in the top two quartiles of the 2016 Journal Citation Reports® (JCR) (Clarivate Analytics, 2017, accessed on November 2017), pertaining to the categories “Agriculture, Multidisciplinary Sciences”, “Agronomy”, “Plant Science” or “Soil Science”. The keywords “wheat nitrogen uptake anthesis” were used simultaneously to search for manuscripts in the Web of Science database (accessed on November 2017).

The next step was to evaluate every manuscript retrieved from the search above, and only papers meeting a series of minimum requirements were included in the final database. Those requirements were that:

(i) experiments were conducted under field conditions. Thus, we disregarded studies reporting data from controlled conditions (because the performance of isolated plants would hardly represent that of a crop) (Pedró et al., 2012) as well as outcomes from simulation exercises (because the assumptions in the model might have affected the outcomes of simulated results)

(ii) the experimental location and year of study were indicated

(iii) data were reported per unit land area (or information was available that this could be calculated; e.g., when data were reported per plant with plant density information available)

(iv) data were not averaged across wheat classes (whenever more than a single wheat class was compared)

(v) treatment factors did not involve the manipulation of natural ambient conditions (e.g., data of responses to manipulations of CO_2 concentration in the atmosphere or shading were not included).

Variables retrieved or calculated

anuscripts were only included in the database if they reported data, or variables that allowed for the calculation of: grain yield per unit area at maturity, aboveground N uptake per unit area at maturity (Nup_{MAT}), aboveground N uptake per unit area at anthesis (Nup_{ANT}), grain N uptake per unit area at maturity, stover (i.e. leaf + stem + chaff) N uptake per unit area at maturity, nitrogen harvest index (NHI; grain N uptake/Nup_{MAT}), and grain N concentration at maturity. When grain protein concentration at maturity (g kg^{-1}) was not explicitly reported, we calculated it as the grain N concentration multiplied by 5.7 (Sosulski and Imafidon, 1990). Data were retrieved from tables, digitalized figures using the Get Data Graph Digitizer software (http://getdata-graph-digitizer.com) or arithmetically solved based on reported equations. Grain yield was adjusted to 135 g kg^{-1} water basis.

Within the manuscripts matching the criteria above, we also retrieved additional data for further analyses that were not required for inclusion of the paper in the database, whenever the information for these variables was available. These variables included aboveground biomass per unit area at maturity, grain harvest index (HI; grain yield/aboveground biomass at maturity), information of the genotypes tested including mostly cultivars and a few breeding lines [i.e. name, number, year of release (YOR), and wheat class to which they belong to] and methods used (i.e. experimental design, N-fertilization rates, and planting season). When not reported, YOR and wheat class information were collected from online resources including plant variety online databases (e.g., http://genbank.vurv.cz/wheat/pedigree/pedigree.asp, http://wheatatlas.org/varieties) and published manuscripts evaluating the same variety (Brennan and Bialowas, 2001; Hysing et al., 2006; Ormoli et al., 2015; Pugsley, 1983; Zhou et al., 2007).

The final database contained 55 manuscripts retrieving a total of 529 treatment means, satisfying all the minimum requirements described above for manuscript inclusion and a subset of 337 data-points that also reported HI and biomass at physiological maturity. Out of the 55 manuscripts, 26% of the studies were conducted in the US, 19% in UK and France, 14% in China, 10% in Australia, 4% in each country for Argentina, Canada, Syria, and New Zealand, and the remainder were conducted in other European countries (Spain, Denmark, Germany, Italy, Sweden, Greece, Netherlands, Portugal, Netherlands, Switzerland). Thus, our database is broadly representative of experiments conducted in major wheat growing regions in the world.

Data quality and outlier analysis

We performed the Mahalanobis Distance multivariate outlier analysis for the relationship between grain yield and $\mathrm{Nup}_{\mathrm{MAT}}$ or protein concentration (i.e., the most critical traits in this study) using the R software package “stats” (R Core Team, 2017). A Mahalanobis threshold of 13.8 was estimated using a critical alpha value equal to 0.001 and 2 degrees of freedom. Any point above the threshold was considered an outlier, and the corresponding treatment was then discarded from further analyses. If more than 50% of treatments of a particular manuscript produced outliers, we discarded the entire manuscript from the final database. We detected four outliers out of the entire 529 data-points, two resulting from the relationship between grain yield and $\mathrm{Nup}_{\mathrm{MAT}}$ and three from the relationship between grain yield and protein (with one overlapping between both relationships). Two outliers reported grain protein concentration greater than 250 g kg^{-1} and derived from a single manuscript providing a total of three data-points; therefore, we discarded the entire manuscript from the database. The other two outliers were part of a set of 28 data-points from a single manuscript and in this case, we only discarded the two outliers but retained the rest of the data-points in the database. The final database contained a total of 54 manuscripts, 524 treatments means matching our criteria for manuscript inclusion without outliers, and 330 treatment means for the additional reported variables described above (Table 1). We were unable to find information for YOR, wheat class and/or planting season for all data-points within some of the papers; thus, the information regarding these three categories in Table 1 may reflect in few cases only part of the total number of observations within a study. When information was not provided at all, and we could not obtain it from other sources (for YOR and wheat class), we denoted as not available (*NA*).

Table 1-1. Information for the 54 manuscripts included in the database after the data quality control (*n*=524). Author (s) and year of publication, year (s) of the study (s) at harvest, experiment location (s), total number of observations, number of genotype (s) evaluated , year (s) or range of year of genotype release (YOR), planting season, wheat class, N rate or range of N applied in kg ha^{-1} in each experiment.

Authors	**Year (s) study**	**Experiment location**	**N obs.**	**N genotype**	**YOR**	**Planting season**	**Wheat class**	**N rate (kg ha^{-1})**
Acreche and Slafer 2011	2008	Lleida, Spain	12	4	1940-2005	Fall	*NA*	90
Arduini et al. 2006	2003	Pisa, Italy	3	3	1974-1988	Fall	Durum	30
Austin et al. 1976	1974	Cambridge, England	1	Avg 47	*NA*	Fall	*NA*	125
Bacon 1987	1979	NSW, Australia	4	1	1973	Fall	*NA*	0,140
Banziger et al. 1994	1992	Zurich, Switzerland	3	Avg 4	1984-1987	Winter	*NA*	0,50,100
Baresel et al. 2008	2003	Bavaria, Germany	1	Avg 6	*NA*	*NA*	*NA*	NA
Barraclough 2014	2005	Harpenden, England	2	Avg 20	*NA*	Fall	*NA*	0, 200
Bogard et al. 2010	2004-2005	7 sites, France	27	27	1974-2000	Fall	Hard	60-250
Buchi et al. 2016	2006-2007	Nyon, Switzerland	11	11	1981-2005	Fall	Hard, Forage	150
De Abreu et al. 1993	1991	Lisbon, Portugal	3	1	*NA*	Winter	*NA*	190
De Ruiter and Martin 2001	1998	Lincoln, New Zealand	3	4	1985-1994	Winter	Hard	0,150,250
Delin et al. 2008	2002-2003	Lanna, Sweden	4	2	2000	*NA*	*NA*	180
Delogu et al. 1998	1989	Fiorenzuola d'Ard, Italy	3	1	1981	Fall	*NA*	0,140,210
Dhugga and Waines 1989	1985	California, United States	42	14	1932-1977	*NA*	Hard, Durum	0,100,200
Dordas et al. 2009	2005-2006	Thessaloniki, Greece	8	1	1992	Fall	Durum	0,80
Duan et al. 2015	2012	Linzi, China	8	2	2008	Fall	*NA*	150
Ehdaie and Waines 2001	1993	California, United States	84	14	1951-1991	Fall	Hard, Durum	105,170
Fischer 1993	1986	Griffith, Australia	17	1	1984	Fall	*NA*	0-240
Gaju et al. 2011	2008	4 sites, England & France	32	16	1989-2007	Fall	Hard, Soft	30,218
Garabet et al. 1998	1992-1993	Tel Hadya, Syria	4	1	*NA*	Fall	*NA*	0
Giunta et al. 2017	2014-2016	Sardinia, Italy	3	1	1915	Fall	Durum	76
Gooding et al. 2005	2001-2003	Reading, England	18	3	1991-1999	Fall	Hard, Soft	100
Guo et al. 2014	2012-2013	Yanzhou, China	8	1	2010	Fall	*NA*	240
Hamner et al. 2017	2014	Skultuna, Sweden	26	2	2008	Fall	Hard	0-240
Heihold et al. 1990	1986	Oklahoma, United States	2	Avg 7	*NA*	Fall	*NA*	0,50
Hocking et al. 2002	1991-1992	3 sites, Australia	14	2	1985-1987	Fall	Hard, Soft	0-150
Koutrobas et al. 2012	2003-2004	Orestiada, Greece	8	4	1983-2000	Fall	Hard, Spelt	50
Koutrobas et al. 2016	2007 -2009	Himonio, Greece	4	1	1983	Fall	Hard	0,120,240
Lu et al. 2015	2012-2014	Quzhou, China	6	2	*NA*	Fall	*NA*	161
Man et al. 2016	2013-2014	Yanzhou, China	10	1	2010	Fall	*NA*	240

Table 1. Continued

Authors	Year (s) study	Experiment location	N obs.	N genotype	YOR	Planting season	Wheat class	N rate (kg ha^{-1})
Masoni et al. 2007	2001	Pisa, Italy	4	4	1974-1990	Fall	Durum	120
McKendry et al. 1995	1985-1986	Winnipeg, Canada	8	8	1972-1979	*NA*	Hard	*NA*
Meinke et al. 1997	1993	Queensland, Australia	8	1	1982	Winter	*NA*	0-360
Paccaud et al. 1985	1980	4 sites, Switzerland	10	10	1952-1983	Fall	*NA*	100
Palta and Fillery 1995	1990	Beverley, Australia	3	1	1986	Fall	*NA*	15,30,60
Pask et al. 2012	2005-2006	Lincoln, NZ & Norfolk, England	3	1	2004	Fall	Forage	160,183,234
Prew et al. 1983	1979-1981	Harpenden, England	10	1	*NA*	Fall	*NA*	115,140,205
Prew et al. 1986	1982-1984	Harpenden, England	2	1	*NA*	Fall	*NA*	203,220
Rasmussen et al. 2015	2012-2013	Taastrup, Denmark	22	6	2003-2009	Fall	Hard, Soft, Forage	20-350
Recous et al. 1988	1984	Estrees-Mons, France	2	1	1983	Fall	*NA*	160
Salvagiotti et al. 2009	2001	Santa Fe, Argentina	8	1	1998	Winter	Hard	0-104
Smith and Withfield 1990	1986	Tatura, Australia	4	1	1973	Winter	Hard	0,150
Spiertz and Ellen 1978	1975	Flevopolder, Netherlands	4	1	1970	Fall	Hard	50-200
Spiertz and Van De Haar 1978	1977	Wageningen, Netherlands	7	2	1970	Fall	Hard	0,50,100
Stapper and Fisher 1990	1983	Griffith, Australia	4	4	1970-1973	Fall	*NA*	*NA*
Thorne 1981	1979	Harpenden, England	2	2	1971-1977	Fall	Hard	75
Thorne et al. 1988	1979-1982	Bedfordshire, England	6	2	1980	Fall	*NA*	75,180,250
Tian et al. 2016	2008-2009	Nanjing, China	12	Avg 32	1950-2005	Fall	*NA*	113
Van Sanford et al. 1989	1987	Kentucky, United States	3	Avg 9	*NA*	Fall	Soft	100
Wang et al. 2015	2010-2011	Linzi, China	8	2	2010	Fall	*NA*	150
Wang et al. 2016	2010-2012	Wugong, China	4	4	2009	Fall	*NA*	375
Wuest and Cassman 1992	1989	Californina, United States	3	1	1975	Fall	Hard	120,180,240
Xu et al. 2005	2001	Tai'an, China	10	2	1991-1993	Fall	*NA*	210
Ye et al. 2011	2007	Wenxian, China	6	Avg 15	1996-2006	Fall	*NA*	0,150,300

Information not available, NA.

Statistical analyses

To explore worldwide trends in NUtE (i.e. grain yield per unit of $\mathrm{Nup_{MAT}}$) (Moll et al., 1982) over the years, we performed a descriptive statistical analysis using the R packages "doBy" and "dplyr" (Højsgaard and Halekoh, 2016; Wickham et al., 2018) with calculation of the mode, mean, standard error (se), minimum and maximum, 0.25 and 0.75 percentiles for all traits considered in the entire database ($n = 524$).

As the major aim of the work was to understand the effects of some factors (that were treatments in the experiments of the literature searched) on the relationship between yield and $\mathrm{Nup_{MAT}}$, we considered the variation generated only by known factors (rather than due to differences between experiments). For that purpose, we used the deviation of the mean produced by a particular treatment within each experiment to account for the true treatment effects on the considered traits. This was done simply by subtracting the value for each treatment from the mean of all treatments within an experiment. We adopted this procedure to standardize substantial differences in absolute values due to different experimental conditions. The negative and positive deviation values indicate whether treatment means of each trait were smaller or greater than the average of its experiment, respectively. This is also similar to the approach adopted by Kitonyo et al. (2017) and Slafer et al. (2014). Studies with only one data-point per experiment were removed from our database, as it was not possible to calculate the average of the experiment. Thus, the resulting deviation analysis database consisted of 516 treatment means.

Trends in NUtE were estimated by analyzing the relationship between grain yield and $\mathrm{Nup_{MAT}}$ (Ferrante et al., 2012; Sadras, 2006), for which we fitted a non-linear least square model (Archontoulis and Miguez, 2015), exponential rise to the maximum function with 3 parameters (Eq. 1) using the Sigma Plot version 13.0 from Systat Software. We fitted linear, quadratic and power models, but the exponential rise to the maximum produced the best fit with the highest R^2, smallest AIC and Mean Square of the error.

$$Y = Y_{int} + A*[1-exp(-B*X)] \tag{1}$$

where Y is the response variable (i.e., grain yield deviation), Y_{int} is the Y-intercept value, A is the maximum value of Y, B is the rate constant of growth, and X is the explanatory variable (Nup_{MAT} deviation).

Additionally, we evaluated NUtE by benchmarking against yield limited by Nup_{MAT}. For that purpose, we divided the Nup_{MAT} deviation values (that ranged from -114 to 143 kg ha^{-1}) into 12 classes each containing 20 kg ha^{-1} of Nup_{MAT}. Within each Nup_{MAT} class, we performed a regression analysis by plotting the maximum yield value versus its respective Nup_{MAT} (n=12) using the same non-linear model shown in Eq. (1). This resulted in a new equation, and determined the curve of Nup_{MAT}-limited yield (i.e., the maximum attainable yield for each class of Nup_{MAT}). Then, we estimated the Nup_{MAT}-limited yield by plugging each datum of Nup_{MAT} to this new equation (i.e. curve of Nup_{MAT}-limited yield) with the parameters of the adjusted Eq. (1), and the difference between actual and Nup_{MAT}-limited yield for each case estimated the yield gap (shortfall in NUtE) independent of Nup_{MAT}.

To identify factors partially accounting for the variability in NUtE within similar levels of Nup_{MAT}, we studied the effects of other reported independent variables (e.g. planting season, wheat class, YOR) on the residuals of the linear relationship between NUtE and Nup_{MAT} deviation.

In this step, the database (n = 516) was divided into two categorical groups: i) planting season based on calendar dates as “fall”, “winter” and “spring” (sowings in winter are relatively common in major wheat producing regions of the southern hemisphere, where winters are rather mild, but wheat can also occasionally be winter-sown in the northern hemisphere); and ii) wheat species and classes as durum (*Triticum durum* Desf.), bread (*Triticum aestivum* L.) including hard (red and white), soft (red and white), and forage classes, and spelt (*Triticum spelta* L.). There were no trials planted during the "spring" in neither hemisphere in the papers analyzed. We considered "fall" planting season experiments sown between September and November in the N hemisphere or between March and May in the S hemisphere and "winter" planting to experiments sown between December and February or June and August in the N and S hemisphere, respectively. Both categorical grouping schemes resulted in unbalanced sub-datasets, with larger number of treatment means for "fall" (n = 405) relative to "winter" (n = 57) planted trials; and for wheat class hard (n = 189) relative to the other classes durum (n = 51), soft (n = 22), forage (n = 10), and spelt (n = 6).

We constructed boxplots using the R package "ggplot2" (Wickham, 2009), and compared means of residuals from the NUtE and $\mathrm{Nup_{MAT}}$ deviation relationship for each categorical group (i.e. planting season and wheat class) using Tukey test ($p = 0.05$) in the R package "agricolae" (Mendiburu, 2015). Additionally, we performed a regression analysis using the "lm" function in the R package "ExpDes" (Ferreira et al., 2018) to evaluate the impacts of the continuous variable YOR (n = 375) on the residuals of the NUtE and $\mathrm{Nup_{MAT}}$ relationship. Then, to identify the effects of the variability in NUtE within similar levels of $\mathrm{Nup_{MAT}}$ on plant nutrient concentration, we regressed the residuals of this relationship as independent variable against grain protein concentration (n = 516) and stover N concentration (n = 330) deviations. Furthermore, we evaluated the relationships between biomass and N accumulation deviations in the stover and grain to investigate treatment effects across different experiments on biomass and N uptake and partitioning.

Results

Database descriptive statistics

Data gathered showed a broad range of values for each of the critical traits considered in this study (yield, Nup_{MAT}, NUtE, and grain protein concentration; c.a. 14 Mg ha^{-1}, 360 kg N ha^{-1}, 95 kg kg^{-1}, and 145 g kg^{-1}, respectively), as well as a normal distribution (Fig. 1A-D). All other variables considered also exhibited a wealth of variation (Supplementary Table A1). The background conditions of experiments tended to affect all variables quite noticeably (Supplementary Fig. A1). Counting with a broad range of variation for the traits considered is relevant to reach conclusions that are not focused on anecdotic cases. However, as usually occurs when re-analyzing data from the literature after extensive searching, the background environmental conditions in which each particular experiment was carried out contributed relevantly to the magnitude of these ranges and to the shape of the relationships (Supplementary Fig. A1, A-E). In this case, even though the treatments imposed within several experiments produced a substantial variation in the critical variables considered, the scope of differences across experiments was broader than within them. The normalized data corrects for the variation of background condition caused by factors other than treatment effects, and thus, it is a better representation of the treatment impacts on the variables of interest. For this reason, using the raw data in this synthesis analysis was not appropriate, the main reason being that with the raw data the differences between experiments became a dominant factor driving the relationship and then conclusions would be related to comparing overall background conditions. Using raw data, most relationships would be positive and linear as when the background condition is better (e.g. high yielding environments with water availability and fertile soils with high water holding capacity) all variables are higher than when the background condition is poorer. Thus, we calculated the deviation of the mean constrained to each experiment (as explained above). Although removing the effects of the experimental backgrounds expectedly diminished considerably the range of variation, the resulting database reflecting the effects of treatments on the variables within experiments did still evidence a rather wide range of variation of the considered variables (e.g. yield varied c.a., 11 Mg ha^{-1}, Nup$_{MAT}$ c.a., 250 kg N ha^{-1}, NUtE c.a., 57 kg kg^{-1}, and grain protein concentration c.a., 85 g kg^{-1}) and maintained the normal distribution (Fig. 1E-H).

Trends in NUtE – Benchmarking against yield limited by N uptake

Changes in yield due to the treatments imposed across the different experiments were positively and curvilinear associated with changes in Nup$_{MAT}$ ($p<0.001$) (Fig. 2A). Our analysis showed that a non-linear model better represented this relationship as compared to a linear model (*$p<0.05$, data not shown*). The exponential rise to a maximum model indicates that increases in Nup$_{MAT}$ had the most significant impact on yield at the lowest N uptake levels and diminished consistently as N uptake increased. Consequently, there was a negative linear relationship between NUtE and Nup$_{MAT}$ ($p<0.001$) (Fig. 2B).

The gap between the actual yield and Nup$_{MAT}$-limited yield curves was virtually inexistent at the lowest range of Nup$_{MAT}$, then increased to c.a. 2,000 kg ha^{-1} and remained constant as Nup$_{MAT}$ increased further (Fig. 2A and Supplementary Fig. A2). Thus, opportunities to further enhance yield through improving NUtE would be only possible at conditions of relatively higher-than-average yields at medium to high levels of Nup$_{MAT}$, while increasing Nup$_{MAT}$ would be required when the levels of uptake are modest. Still, half of the actual yields observed in the database are below the line representing the overall adjustment between yield and Nup$_{MAT}$, and therefore opportunities to improve yields by reducing the gap between actual yields to those that could be achieved for that level of Nup_{MAT} (the Nup_{MAT}-limited yield) are even more substantial in many cases.

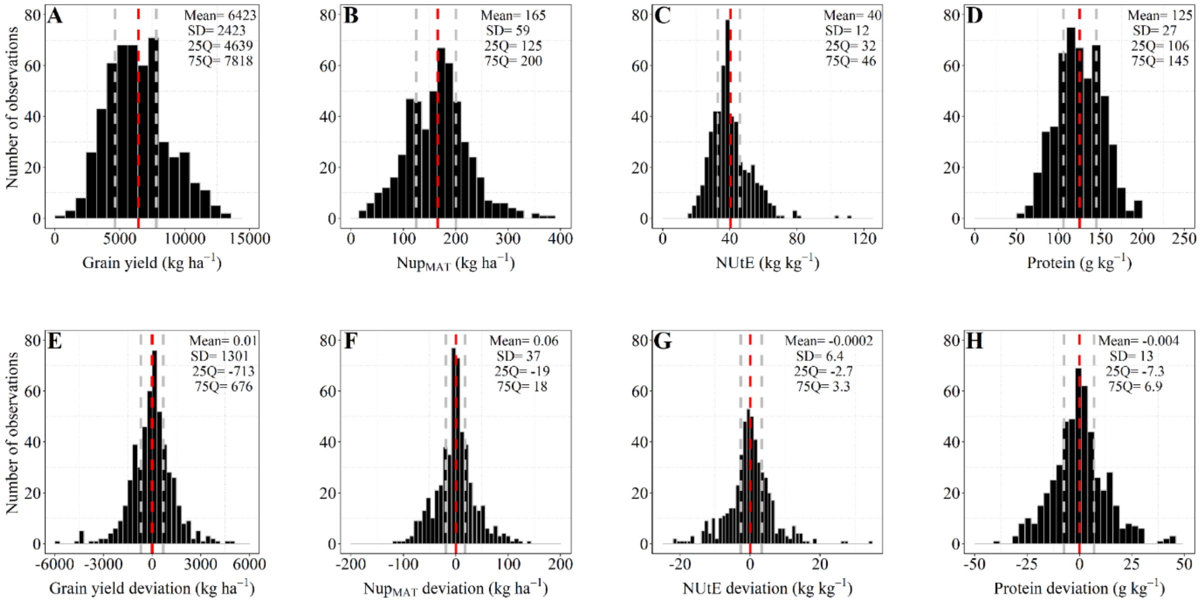

Figure 1.1. Distribution of the raw-data (A-D) (*n*=524) and standardized-data (deviation) (E-H) (*n*=516) for grain yield (A and E), aboveground N uptake at physiological maturity (Nup_{MAT}, B and F), N utilization efficiency (NUtE, C and G), and grain protein concentration (D and H). Gray dashed lines represent 0.25 and 0.75 quantiles, and the red dashed line represents the mean for each trait. Standard deviation (SD) is also shown.

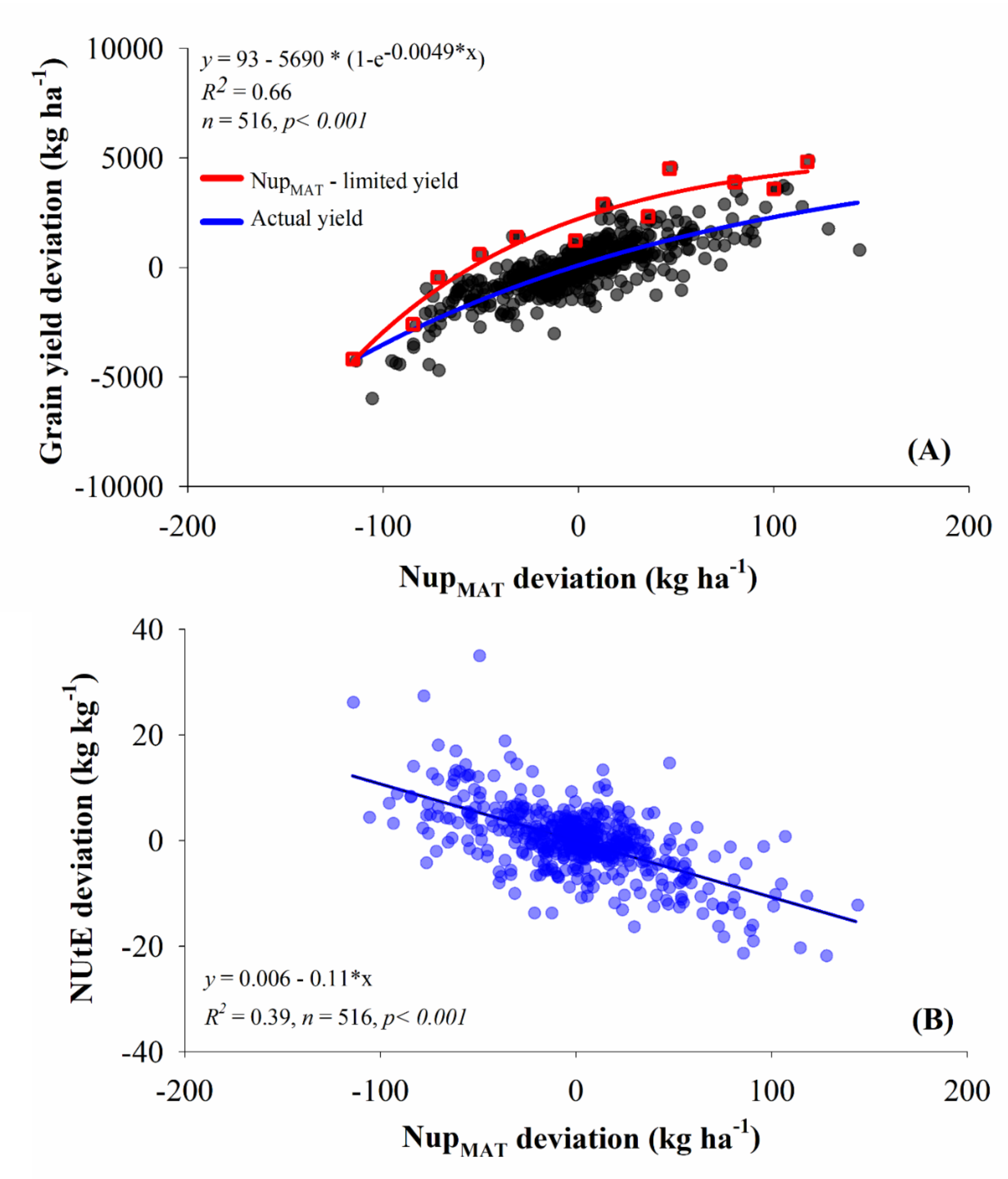

Figure 1.2. Relationship between grain yield per unit area and aboveground N uptake per unit area at maturity (Nup$_{MAT}$) from published studies around the world (A). The blue line represents the N utilization efficiency (grain yield to aboveground N uptake per unit area at maturity ratio, NUtE) calculated from actual grain yield reported for each experiment. The red line illustrates the NUtE calculated from Nup$_{MAT}$-limited yield at particular Nup$_{MAT}$ levels (for every 20 kg N uptake ha^{-1}). Equation for the red line: [$y= 2239 + 3271 * (1-e^{-0.0097*x})$, $R^2= 0.94$, $n= 12$,

$p<0.001$]. Relationship between NUtE and Nup_{MAT} deviations (n=516) (B). Data are reported as the treatment mean deviation from its experimental mean.

Impact of factors on NUtE at particular N uptake levels

The variability in NUtE for particular levels of Nup_{MAT} was greater for fall- than for winter-sown wheat, with average residual values slightly positive in fall and slightly negative in winter ($p<0.05$) (Fig. 3A). This significant difference in variability among planting season suggests that delaying sowing from fall to winter would reduce NUtE, but it may also reflect the fact that number of data points was much higher (almost eight-fold more) in fall than in winter-sown wheat. There was no significant variability in NUtE for particular levels of Nup_{MAT} among all wheat classes (Fig. 3B). Although there was a trend for forage wheat to be responsible for more variation in NUtE than the other classes, mean differences were not significant and opportunities to improve NUtE seem to be similar for all wheat classes (Fig. 3B).

Breeding new cultivars seemed to have contributed to increasing NUtE at particular levels of Nup_{MAT} in the first decades considered in the present study (Fig. 3C), i.e., from the 1930's to late 1980's (n=98, $p<0.05$); but there was no evidence to support that cultivars released in the post-Green Revolution era continued to consistently increase their average NUtE (n=277, $p>0.05$, from 1980 onwards). Not surprisingly, the main reason for improved NUtE resultant from breeding before the 1980's seemed to have been the improvement of HI, as the relationship described for breeding effects on NUtE (Fig. 3C) seemed a mirror image of that with HI (i.e., both quadratic equations peaked at late 1980's) (Supplementary Fig. A3). Although, our database contains much smaller number of datapoints to represent trends in NUtE prior 1980's as compared to after that period.

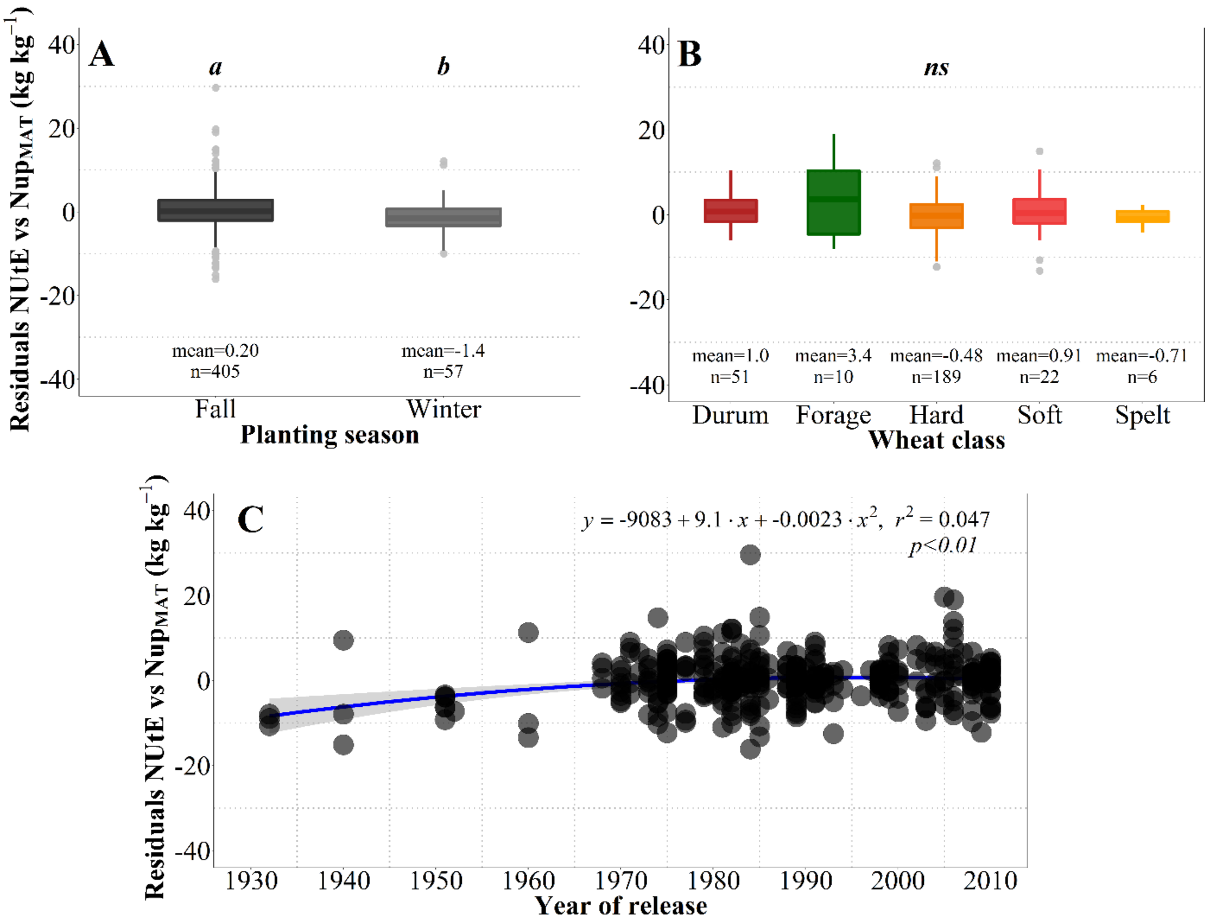

Figure 1.3. Residuals from the relationship between N utilization efficiency (grain yield to aboveground N uptake per unit area at maturity ratio) (NUtE) and aboveground N uptake per unit area at maturity ($\mathrm{Nup_{MAT}}$) deviations as affected by planting season (A), wheat class (B), and year of genotype release from 1930 to 2010 (*n*=375) (C). When not reported in published manuscripts, wheat class and year of genotype release information were collected from online databases (e.g., European wheat database, http://genbank.vurv.cz/wheat/pedigree/pedigree.asp; wheat atlas, http://wheatatlas.org/varieties). Experiments with non-identifiable information for wheat class and year of genotype release were not included in its respective analysis.

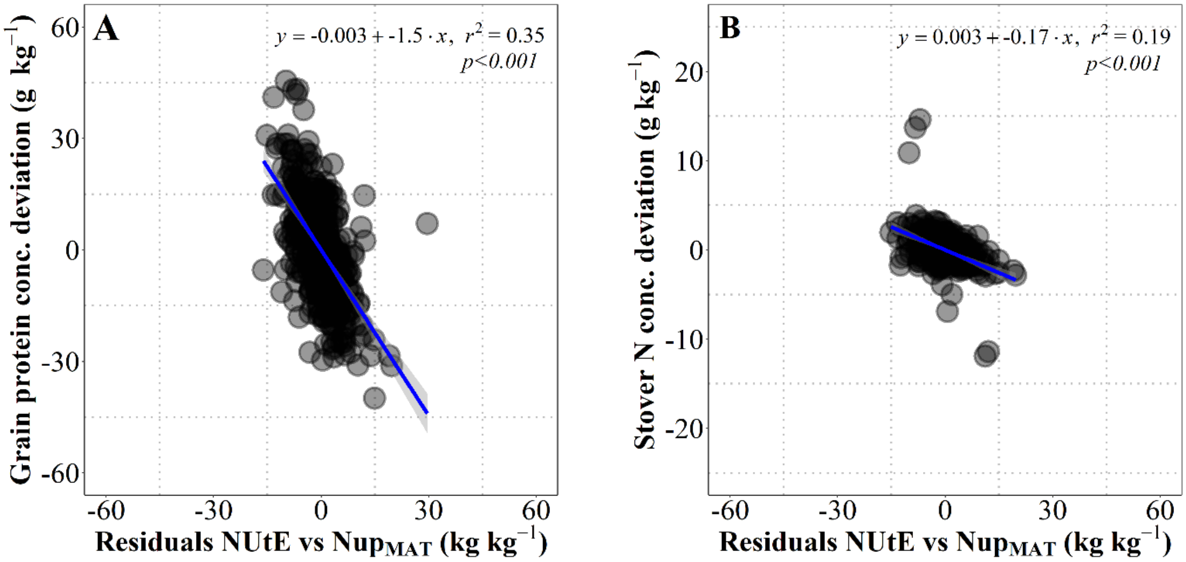

Figure 1.4. Relationship between grain protein concentration deviation at maturity (A) (*n*=516) and stover N concentration deviation at maturity (B) (*n*=330) vs. the residuals of the relationship between N utilization efficiency (grain yield to aboveground N uptake per unit area at maturity ratio, NUtE) and aboveground N uptake per unit area at maturity ($\mathrm{Nup}_{\mathrm{MAT}}$) on a deviation basis.

Consequences of variation in NUtE at particular N uptake levels on grain protein and stover N concentration

A highly significant negative correlation was observed between grain protein or stover N concentration and the residuals of the NUtE and $\mathrm{Nup}_{\mathrm{MAT}}$ relationship ($p<0.001$) (Fig. 4A, B), implying that increases in yield through improved NUtE will often penalize grain protein concentration. Data also indicated a declining tendency in average grain protein concentration for groups with higher average residuals. For instance, the average grain protein concentration was numerically smaller for fall-sown (-0.53 g kg^{-1}) relative to winter-sown wheat (3.70 g kg^{-1}), although not significant ($p>0.05$) (*data not shown*). Likewise, wheat classes did not show any statistically significant difference in this plant trait even though a trend was observed ($p>0.05$, *data not shown*). Thus, impacts of planting season and wheat class on the variation in NUtE at particular levels of N uptake is likely to reflect on changes in grain protein concentration.

Impacts of stover to grain N ratios on NUtE

As treatments impacting $\mathrm{Nup}_{\mathrm{MAT}}$ affected the final N content in both stover and grain, changes in stover N uptake were positively and linearly associated with changes in grain N uptake. However, grain N uptake showed larger magnitude of change than stover N yield (Fig. 5A). The greater changes in grain N uptake than in stover N uptake resulted from a higher plasticity in the grain as compared to the stover biomass (Fig. 5B). Changes in yield followed changes in stover biomass though with a slightly lower plasticity, indicating that treatments that increased yield did in general so through mainly improving total crop growth, though also producing a subsidiary increase in biomass partitioning (Fig. 5B). Furthermore, changes in stover N concentration were not accompanied by changes in grain N concentration at maturity (Fig. 5C), stover N concentration had a much larger magnitude of change than grain N concentration with a slope of almost 3 g kg^{-1}.

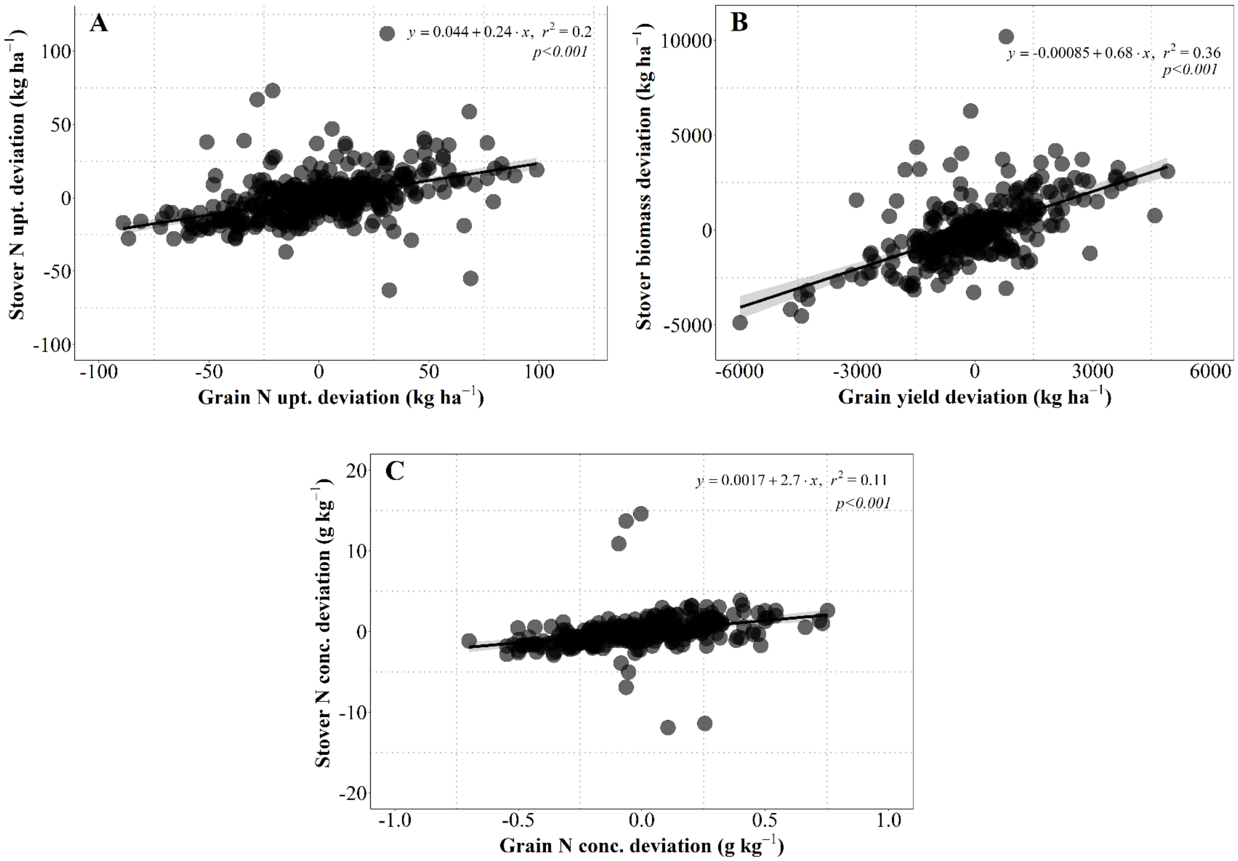

Figure 1.5. Relationship between stover N uptake per unit area at maturity and grain N uptake per unit area at maturity (A). Relationship between stover biomass per unit area and grain yield per unit area (B). Relationship between stover N concentration and grain N concentration (C). Data are reported as the treatment mean deviation from its experimental mean.

Discussion

Trends in NUtE

Nitrogen is vital for growth and reproduction (firstly grain formation then grain filling). Therefore, any limitation to N uptake and/or NUtE imposed by environmental, physiological, and agronomic processes results in yield penalties (Debaeke et al., 1996). Grain yield accounts for most of the variability in NUtE (Gaju et al., 2011). However, trends in NUtE are also significantly explained by plant N content. Barraclough et al. (2010) demonstrated that at lower

$\mathrm{Nup_{MAT}}$ levels (57 kg $\mathrm{ha^{-1}}$) plant N content accounted for 50% of the variability in NUtE, while yield explained 77% of the variability in NUtE as $\mathrm{Nup_{MAT}}$ levels increased (229 kg $\mathrm{ha^{-1}}$).

Our results support previous findings where a high rate of yield gain occurs at initial increments of plant N uptake, and yield gain is minimal or null as $\mathrm{Nup_{MAT}}$ levels increases (Desai and Bhatia, 1978; Gauer et al., 1992; Ye et al., 2011). Moreover, our benchmark analysis provided direct quantification of $\mathrm{Nup_{MAT}}$-limited yield and may help future yield-enhancing strategies to develop goals for particular levels of $\mathrm{Nup_{MAT}}$. Thus, identifying and correcting for factors other than $\mathrm{Nup_{MAT}}$ potentially influencing NUtE (e.g., radiation, temperature, water, pests, management, and other nutrients) would potentially help to increase NUtE further. In that respect, breeding influence on improving NUtE until the Green Revolution has been decisive as a tool for increasing yield (e.g. Calderini et al., 1995a). However, as our global analysis showed for NUtE, and individual studies evidenced for particular regions on yield itself (e.g., Acreche et al., 2008; Flohr et al., 2018a) there has been a stagnation in progress during the last decades.

Impacts of factors on NUtE at particular N uptake levels.

Our analysis suggests that wheat-planting seasons vary on their contribution to NUtE (Fig. 3A) and further investigation may be useful. The lower contribution of winter planting season to the variability on NUtE at particular N uptake levels relative to fall indicates potential for increasing NUtE by switching from winter to fall planting. This possibility is geographically restricted to regions with mild winter (i.e., Argentina, Australia, or southern Texas, USA) where wheat can be sown either in fall or winter seasons, and varieties with either winter or spring winter growth habit can be sown. One of the few studies comparing planting seasons attributed changes on the variability in NUtE to different NHI among planting seasons (Hernandez-Ramirez et al., 2011). They observed that wheat planted during (our pre-established) winter season tended to have greater NHI than the fall-sown while there were no differences in yield and Nup_{MAT} across planting seasons, suggesting a different trend as compared to our findings. Although we found that the mean residuals were slightly positive for fall and slightly negative for winter, our results indicated no significant differences in NHI, yield, and Nup_{MAT} among planting seasons (*$p>0.05$, data not shown*), except for grain protein concentration (*$p<0.05$*). An opposite trend for yield and protein concentration was found among planting seasons with greater yield and lower protein values for fall as compared to the winter season. Possible influences of planting season on NUtE found in our study may be related to the different flowering stability of genotypes across environments. Flohr et al. (2018b) proposed that utilizing a novel winter wheat genotype with fast-developmental rate and larger window for optimal flowering period would allow for planting wheat earlier than its typical time, and result in yield improvements of up to 20% in several regions in Australia.

In agreement with our findings, other studies have shown the consequences of variability in NUtE on the trade-off mechanism between yield and grain protein concentration regardless of wheat class (Clarke et al., 1990; Foulkes et al., 1998). Gaju et al. (2011) compared 16 UK and French genotypes, including forage and bread wheat types, and credited differences in NUtE to low protein and high yield potential of forage wheat with average NUtE of 55 and 36 kg kg^{-1} respectively for N rates of 0 and 240 kg N ha^{-1}, as opposed to the high protein and lower yield of the bread wheat genotypes with average NUtE of 49 and 32 kg kg^{-1}, respectively for the low and high N rates. On the other hand, Marti and Slafer (2014) compared bread versus durum wheat and found neither differences in the relationship between yield and Nup_{MAT} nor grain N concentration among wheat classes. In their study, NUtE values ranged from 15 to 55 kg kg^{-1} for both wheat types. They also found that hexaploid wheat tended to have greater N uptake at low-yielding environments as compared to durum wheat, indicating resilience and larger ability of hard wheat to produce well in regions with limited input.

Donald (1968) suggested that breeding for ideotypes with the minimum possible allocation of resources to stems and roots to the preferential distribution for grain production would be the best strategy to produce plants able to utilize resources more efficiently. Accordingly, wheat breeders have improved yield by increasing partitioning of biomass to the grain, mainly as a consequence of the greater production of grain per unit area (Miri, 2009; Reynolds et al., 2012a), and indirectly reducing root length due to the improved efficiency in N uptake per unit root length (Aziz et al., 2017). Meanwhile, grain weight, biomass, and aboveground N uptake at anthesis and maturity remained basically unchanged (Austin et al., 1980; Calderini et al., 1995a; Hay, 1995; Reynolds et al., 2012a; Royo et al., 2009; Slafer et al., 1994). Our database shows that up to late 1980's, wheat breeding improved NUtE via increases in yield resultant from greater HI. Later improvements in NUtE occurred at the expense of grain protein concentration as grain yield increased over time (Calderini et al., 1995b; Ortiz-Monasterio et al., 1997) and Nup_{MAT} remained unchanged (Paccaud et al., 1985; Wang et al., 2017). Thus, the proportionally lower plant N uptake relative to the higher yield may explain the reduction of grain protein over time due to dilution process (Calderini et al., 1995b; Slafer et al., 1990a). Dubois and Fossati (1981) indicated that yield improvement was a result of the greater grain and N HIs, while Barraclough et al. (2010) and Li et al. (2012) observed that grain yield increase was due to greater biomass. The latter is in line with our findings. Our data also suggest that breeding has indirectly improved NHI in parallel to HI (Slafer et al., 1994), but the higher rate of improvement in HI relative to NHI over the years (Calderini et al., 1995b) resulted in the decline in grain N concentration with yield increases. Nevertheless, HI may have reached its theoretical maximum of ~50%, or with extrapolation ~60% and NHI is already very high in modern cultivars (80-90%) (Austin et al., 1980; Calderini et al., 1999; Miralles and Slafer, 2007; Reynolds et al., 2012a). Thus, increasing biomass and N content when N uptake levels are moderate may be the best strategy to achieve both higher yield and protein through improving NUtE. Hence, improving knowledge on traits and potential tradeoffs of physiological mechanisms involved with yield gain is critical to direct future breeding strategies at the crop field level (Evans, 1999; Slafer, 2003). Recent reviews describing such mechanisms (e.g. adaptation patterns and resources use efficiency) include Araus et al., 2008; Cormier et al., 2016; and Slafer et al., 2015.

Consequences of variation in NUtE at particular N uptake levels on grain protein and stover N concentration

The opposite relationship between grain yield and protein has been extensively discussed in the literature (Clarke et al., 1990; Cox et al., 1986; Desai and Bhatia, 1978), presenting multiple hypotheses: (i) dilution of protein in higher yield (Acreche and Slafer, 2009; Barneix, 2007; Guarda et al., 2004; Heitholt et al., 1990; Martre, 2003); (ii) higher rate of improvement in HI relative to NHI over the years (Slafer et al., 1994); (iii) competition for energy and assimilates between biomass and N during the grain formation as some genotypes will continue to direct their energy and assimilates for vegetative growth after anthesis, and consequently less amount of assimilates for grain formation (Bogard et al., 2010; Cox et al., 1985; Dhugga and Waines, 1989); and (iv) the different accumulation rates between grain protein and carbohydrates during the grain filling period (Jenner et al., 1991).

The negative correlation between grain protein or stover N concentration with NUtE at particular $\mathrm{Nup}_{\mathrm{MAT}}$ levels found in our study is explained by the previously established dilution processes that outer limit the relationship between yield and Nup_{MAT} (Janssen et al., 1990). Our database supports prior research showing that increases in yield usually reflects a decline in grain protein concentration and that when yield gain began to stabilize at higher N uptake levels, grain protein concentration increase (Barraclough et al., 2010; McMullan et al., 1988). This is possibly explained by the fact that grain protein concentration is usually source-limited (Borghi et al., 1986; Martre, 2003), particularly towards the end of grain filling period, as opposed to grain yield being sink-limited (Jenner et al., 1991). Controversially, Cox et al. (1985) were able to select lines with both high yield and protein under different N inputs. Therefore, our results show that regardless of the causes on the variation in NUtE at particular N uptake levels, the variability in NUtE will reflect in the partial trade-off between yield and protein.

The NUtE can be examined as a function of NHI and grain N concentration (protein x 5.7) (Sadras, 2006). Although NHI and grain N concentration are poorly associated (Heitholt et al., 1990), these components are essential for investigating changes in NUE and grain quality attributes (Masclaux-Daubresse et al., 2010). Similar to our findings, numerous studies have shown that NUtE and grain N concentration are strongly and negatively correlated (Clarke et al., 1990; Yue et al., 2012). The NHI contributions to NUtE is inconsistent across the literature, and when existent, NHI impacted NUtE at much lesser extent as compared to grain N concentration (Barneix, 2007; Gaju et al., 2011; Sadras, 2006). These differences in contribution to NUtE can be explained by the influence of genotype, environmental conditions, and management on NHI and grain N concentration, and further by the stronger influence of variations in NUtE at particular N uptake levels on grain N concentration demonstrated in our data.

Our results support the framework proposed by Sinclair (1998), suggesting that NHI can oscillate as a function of plant N concentration (i.e., grain and stover N concentration) and HI. In our database, we observed a proportional partitioning of N and biomass to the grain. However, in contrast with previous findings (Austin et al., 1977; Dhugga and Waines, 1989), our study shows that the substantial variation in plant N concentration had more impact on plant N uptake than biomass. Furthermore, our data suggest that stover N concentration was the main component responsible for changes in plant N uptake relative to grain N concentration, which is explained by grain N concentration being a more conservative trait relative to stover N concentration (Barneix, 2007). Thus, the positive influence of NHI on the variability of NUtE at particular levels of $\mathrm{Nup}_{\mathrm{MAT}}$ (R^2=0.25, *data not shown*) can be explained by increases in stover N concentration as $\mathrm{Nup}_{\mathrm{MAT}}$ levels increased and to a lesser extent by changes in grain N concentration and HI. For oilseeds and legumes, stover N concentration variation mostly explained the contribution of NHI to NUtE (Sadras, 2006; Tamagno et al., 2017). Moreover, the larger magnitude of change for stover N concentration relative to grain N concentration concurs with the idea that stover is a strong plant N reservoir (Martre, 2003), and amounts of N retained in the stover corresponding to structural N compounds, can be easily remobilized to the grain and therefore must have very high plasticity in response to treatments (Kichey et al., 2007). Although N accumulation in the grains seem to be clearly source-limited, grain N concentration would likely have a limited response to treatments affecting N availability to growing grains (Borghi et al., 1986; Martre, 2003).

The main challenges of selecting for either grain yield or NUtE are their multi-trait characteristics and the tradeoffs between mechanisms involved with these traits. Selecting for genotypes with the ability to accumulate higher amounts of $\mathrm{Nup}_{\mathrm{MAT}}$ while increasing or maintaining yield high N uptake levels (i.e., higher NUtE) could alleviate the negative relationship between yield and protein (Slafer et al., 1994). To increase rates of yield gain for future global food security, it is necessary to develop agronomic management and breeding strategies that are based on a foundational knowledge of crop physiological factors governing yield and resource use efficiency (Jackson et al., 1996; Reynolds and Langridge, 2016; Slafer, 2003). Hence, future research should focus on sources of N to the grain (i.e., source: sink ratio) to identify genotypes with high protein while sustaining grain yield (Debaeke et al., 1996; Gauer et al., 1992). Further, as the accumulation of N in the plant is influenced by soil N availability and plant development (Gastal et al., 2015), investigating genotypes with improved plant N uptake efficiency under low N input levels may also provide insights for future yield-enhancing strategies for improving overall NUE in wheat farming systems.

This manuscript provides a worldwide assessment on trends in NUtE in wheat, which has not been performed before in a global scale. In general, information on this topic has been reported from experiments in specific regions or using a small set of genotypes with rather narrow genetic variation. In our work, we used information from experiments conducted across the world while conducting a strict data quality control. We used only data from papers published in journals that were indexed in the top two quartiles of the JCR that likely passed through rigorous peer review and conducted appropriate data analysis (i.e. normalization) for drawing conclusions across different experiments. Therefore, this research improves the scientific knowledge on global trends in NUtE and provides insights on the importance of identifying the determinants of NUtE to improve yield in a sustainable manner (i.e. to increase yield and narrow the gap between actual and N uptake-limited yields).

Acknowledgments

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References

Acreche, M.M., Briceño-Félix, G., Sánchez, J.A.M., Slafer, G.A., 2008. Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. European Journal of Agronomy 28, 162–170. https://doi.org/10.1016/J.EJA.2007.07.001

Acreche, M.M., Slafer, G.A., 2009. Variation of grain nitrogen content in relation with grain yield in old and modern Spanish wheats grown under a wide range of agronomic conditions in a Mediterranean region. Journal of Agricultural Science 147, 657–667. https://doi.org/10.1017/S0021859609990190

Aguirrezábal, L., Martre, P., Pereyra-Irujo, G., Echarte, M.M., Izquierdo, N., 2015. Improving grain quality: Ecophysiological and modeling tools to develop management and breeding strategies, in: Sadras, V.O., Calderini, D.. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. Elsevier Inc., pp. 423–465. https://doi.org/10.1016/B978-0-12-417104-6.00017-0

Agyin-Birikorang, S., Fugice, J., Singh, U., Sanabria, J., Choudhuri, S., 2017. Nitrogen uptake kinetics of key staple cereal crops in different agro-ecological regions of the world. Journal of Plant Nutrition 40, 995–1023. https://doi.org/10.1080/01904167.2016.1262408

Aktar-Uz-Zaman, M., Tuhina-Khatun, M., Hanafi, M.M., Sahebi, M., 2017. Genetic analysis of rust resistance genes in global wheat cultivars: an overview. Biotechnology & Biotechnological Equipment 31, 431–445. https://doi.org/10.1080/13102818.2017.1304180

Alexandratos, N., Bruinsma, J., 2012. World agriculture towards 2030/2050: the 2012 revision, World agriculture towards 2030/2050: the 2012 revision. Rome, FAO. https://doi.org/10.1016/S0264-8377(03)00047-4

Araus, J.L., Slafer, G.A., Royo, C., Serret, M.D., 2008. Breeding for yield potential and stress adaptation in cereals. Critical Reviews in Plant Science 27, 377–412. https://doi.org/10.1080/07352680802467736

Archontoulis, S.V., Miguez, F.E., 2015. Nonlinear regression models and applications in agricultural research. Agronomy Journal 107, 786–798. https://doi.org/10.2134/agronj2012.0506

Austin, R.B., Bingham, J., Blackwell, R.D., Evans, L.T., Ford, M., Morgan, C.L., Taylor, M., 1980. Genetic improvement in winter wheat yields since 1900 and associated physiological changes. Journal of Agricultural Science 94, 675–689.

Austin, R.B., Ford, M.A., Edrich, J.A., Blackwell, R.D., 1977. The nitrogen economy of winter wheat. The Journal of Agricultural Science 88, 159–167. https://doi.org/10.1017/S002185960003389X

Aziz, M.M., Palta, J.A., Siddique, K.H.M., Sadras, V.O., 2017. Five decades of selection for yield reduced root length density and increased nitrogen uptake per unit root length in Australian wheat varieties. Plant and Soil 413, 181–192. https://doi.org/10.1007/s11104-016-3059-y

Balboa, G.R., Sadras, V.O., Ciampitti, I.A., 2018. Shifts in soybean yield, nutrient uptake, and nutrient stoichiometry: A historical synthesis-analysis. Crop Science 58, 43–54. https://doi.org/10.2135/cropsci2017.06.0349

Barneix, A.J., 2007. Physiology and biochemistry of source-regulated protein accumulation in the wheat grain. Journal of Plant Physiology 164, 581–590. https://doi.org/10.1016/j.jplph.2006.03.009

Barraclough, P.B., Howarth, J.R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C.E., Hawkesford, M.J., 2010. Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. European Journal of Agronomy 33, 1–11. https://doi.org/10.1016/j.eja.2010.01.005

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., MacHet, J.M., Jeuffroy, M.H., Gate, P., Martre, P., Le Gouis, J., 2010. Deviation from the grain protein concentration-grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. Journal of Experimental Botany 61, 4303–4312. https://doi.org/10.1093/jxb/erq238

Borghi, B., Corbellini, M., Cattaneo, M., Fornasari, M.E., Zucchelli, L., 1986. Modification of the sink/source relationships in bread wheat and its influence on grain yield and grain protein content. Journal Agronomy & Crop Science 157, 245–254.

Borlaug, N.E., Dowswell, C.R., 2003. Feeding a world of ten billion people: A 21st century challenge., in: Tuberosa R., Phillips R.L., Gale M. (Eds.), Proceedings of the International Congress “In the Wake of the Double Helix: From the Green Revolution to the Gene Revolution”. Bologna, Italy, pp. 3–23.

Braun, H.-J., Atlin, G., Payne, T., 2010. Multi-location testing as a tool to identify plant response to global climate change, in: Reynolds, M.P. (Ed.), Climate Change and Crop Production. CABI, Wallingford, UK, pp. 115–138.

Brennan, J.P., Bialowas, A., 2001. Changes in Characteristics of NSW Wheat Varieties, 1965-1997, Economic Research Report No. 8. NSW Agriculture, Wagga Wagga.

Brooking, I.R., Kirby, E.J.M., 1981. Interrelationships between stem and ear development in winter wheat: the effects of a Norin 10 dwarfing gene, Gai/Rht2. The Journal of Agricultural Science, Cambridge 97, 373–381. https://doi.org/10.1017/S0021859600040806

Bruinsma, J., 2011. The resources outlook: by how much do land, water and crop yields need to increase by 2050?, in: Conforti, P. (Ed.), 'Looking Ahead in World Food and Agriculture: Perspectives to 2050.' Food and Agriculture Organization of the United Nations, Rome, pp. 233–278.

Calderini, D.F., Dreccer, M.F., Slafer, G.A., 1995a. Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. Plant Breeding 114, 108–112. https://doi.org/10.1111/j.1439-0523.1995.tb00772.x

Calderini, D.F., Reynolds, M.P., Slafer, G.A., 1999. Genetic gains in wheat yield and main physiological changes associated with them during the 20th century, in: Satorre, E.H., Slafer, G.A. (Eds.), Wheat: Ecology and Physiology of Yield Determination. Food Product Press, New York, pp. 351–377.

Calderini, D.F., Torres-Leon, S., Slafer, G.A., 1995b. Consequences of wheat breeding on nitrogen and phosphorus yield, grain nitrogen and phosphorus concentration and associated traits. Annals of Botany 76, 315–322.

Cassman, K.G., Dobermann, A.R., Walters, D.T., 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. Ambio 31.

Ciampitti, I.A., Prasad, P.V.V., 2016. Historical Synthesis-Analysis of Changes in Grain Nitrogen Dynamics in Sorghum. Frontiers in Plant Science 7, 275. https://doi.org/10.3389/fpls.2016.00275

Ciampitti, I.A., Vyn, T.J., 2014. Understanding global and historical nutrient use efficiencies for closing maize yield gaps. Agronomy Journal 106, 1–11.

https://doi.org/10.2134/agronj14.0025

Ciampitti, I.A., Vyn, T.J., 2012. Physiological perspectives of changes over time in maize yield dependency on nitrogen uptake and associated nitrogen efficiencies: A review. Field Crops Research 133, 48–67. https://doi.org/10.1016/j.fcr.2012.03.008

Clarivate Analytics, 2017. 2016 Journal Citation Reports [WWW Document]. URL https://jcr.incites.thomsonreuters.com (accessed 11.1.17).

Clarke, J.M., Campbell, C.A., Cutforth, H.W., Depauw, R.M., Winkleman, G.E., 1990. Nitrogen and phosphorus uptake, translocation, and Utilization Efficiency of Wheat in Relation To Environment and Cultivar Yield and Protein Levels. Canadian Journal of Plant Science 70, 965–977. https://doi.org/10.4141/cjps90-119

Cormier, F., Faure, S., Dubreuil, P., Heumez, E., Beauchêne, K., Lafarge, S., Praud, S., Le Gouis, J., 2013. A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (Triticum aestivum L.). Theoretical and Applied Genetics 126, 3035–3048. https://doi.org/10.1007/s00122-013-2191-9

Cormier, F., Foulkes, J., Hirel, B., Gouache, D., Moënne-Loccoz, Y., Le Gouis, J., 2016. Breeding for increased nitrogen-use efficiency: A review for wheat (T. aestivum L.). Plant Breeding 135, 255–278. https://doi.org/10.1111/pbr.12371

Cox, M.C., Qualset, C.O., Rains, D.W., 1986. Genetic variation for nitrogen assimilation and translocation in wheat. II. Nitrogen translocation in relation to grain yield and protein. Crop Science 26, 737–740. https://doi.org/10.2135/cropsci1985.0011183X002500030003x

Cox, M.C., Qualset, C.O., Rains, D.W., 1985. Genetic variation for nitrogen assimilation and translocation in wheat. I. Dry matter and nitrogen accumulation. Crop Science 25, 430–435.

Daberkow, S., Huang, W., 2006. Nutrient Management, in: Wiebe, K., Gollehon, N. (Eds.), Agricultural Resources and Environmental Indicators. U.S. Department of Agriculture, Economic Research Service, EIB-16. July., pp. 117–123.

Debaeke, P., Aussenac, T., Fabre, J.L., Hilaire, A., Pujol, B., Thuries, L., 1996. Grain nitrogen content of winter bread wheat (Triticum aestivum L.) as related to crop management and to the previous crop. European Journal of Agronomy 5, 273–286. https://doi.org/10.1016/S1161-0301(96)02038-2

Desai, R.M., Bhatia, C.R., 1978. Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica 27, 561–566. https://doi.org/10.1007/BF00043182

Dhugga, K.S., Waines, J.G., 1989. Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Science 29, 1232. https://doi.org/10.2135/cropsci1989.0011183X002900050029x

Dubois, J.B., Fossati, A., 1981. Influence of nitrogen uptake and nitrogen partitioning efficiency on grain yield and grain protein concentration of twelve winter wheat genotypes (Triticum aestivum L.). Z. Pflanzenzuchtg 86, 41–49.

Evans, L.T., 1999. Steps towards feeding the ten billion - A crop physiologists view. Plant Production Science 2, 3–9.

FAO-AMIS, 2018. Food and Agriculture Organization of the United Nations - Agricultural market information system [WWW Document]. URL http://statistics.amis-outlook.org/data/index.html#DOWNLOAD. (accessed 2.20.18).

Ferrante, A., Savin, R., Slafer, G.A., 2012. Differences in yield physiology between modern, well adapted durum wheat cultivars grown under contrasting conditions. Field Crops Research 136, 52–64. https://doi.org/10.1016/j.fcr.2012.07.015

Ferreira, E.B., Cavalcanti, P.P., Nogueira, D.A., 2018. ExpDes: Experimental Designs. R package version 1.2.0.

Fischer, R.A., 2008. The importance of grain or kernel number in wheat: A reply to Sinclair and Jamieson. Field Crops Research 105, 15–21. https://doi.org/10.1016/j.fcr.2007.04.002

Fischer, R.A., Byerlee, D., Edmeades, G.O., 2014. Crop yields and global food security: will yield increase continue to feed the world?, ACIAR Monograph No. 158. Australian Centre for International Agricultural Research: Canberra.

Fischer, R.A., Edmeades, G.O., 2010. Breeding and cereal yield progress. Crop Science 50, S-85-S-98. https://doi.org/10.2135/cropsci2009.10.0564

Fischer, R.A., Howe, G.N., Ibrahim, Z., 1993. Irrigated spring wheat and timing and amount of nitrogen fertilizer . I. Grain-yield and protein content. Field Crops Research 33, 37–56. https://doi.org/10.1016/0378-4290(93)90093-3

Flintham, J.E., Borner, A., Worland, A.J., Gale, M.D., 1997. Optimizing wheat grain yield: effects of Rht (gibberellin-insensitive) dwarfing genes. Journal of Agricultural Science, Cambridge 128, 11–25.

Flohr, B.M., Hunt, J.R., Kirkegaard, J.A., Evans, J.R., Swan, A., Rheinheimer, B., 2018a. Genetic gains in NSW wheat cultivars from 1901 to 2014 as revealed from synchronous flowering during the optimum period. European Journal of Agronomy 98, 1–13. https://doi.org/10.1016/J.EJA.2018.03.009

Flohr, B.M., Hunt, J.R., Kirkegaard, J.A., Evans, J.R., Trevaskis, B., Zwart, A., Swan, A., Fletcher, A.L., Rheinheimer, B., 2018b. Fast winter wheat phenology can stabilise flowering date and maximise grain yield in semi-arid Mediterranean and temperate environments. Field Crops Research 223, 12–25.

https://doi.org/10.1016/J.FCR.2018.03.021

Foulkes, M.J., Sylvester-Bradley, R., Scott, R.K., 1998. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertiliser nitrogen. J. Agric. Sci. (Camb.) 10, 29–44.

Gaju, O., Allard, V., Martre, P., Snape, J.W., Heumez, E., LeGouis, J., Moreau, D., Bogard, M., Griffiths, S., Orford, S., Hubbart, S., Foulkes, M.J., 2011. Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. Field Crops Research 123, 139–152. https://doi.org/10.1016/j.fcr.2011.05.010

Gastal, F., Lemaire, G., Durand, J., Louarn, G., 2015. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency, in: Sadras, V.O., Calderini, D.F. (Eds.), Crop Physiology: Applications for Genetic Improvement and Agronomy. Elsevier, pp. 161–205.

Gauer, L.E., Grant, C.A., Gehl, D.T., Bailey, L.D., 1992. Effects of nitrogen fertilization on grain protein content, nitrogen uptake, and nitrogen use efficiency of six spring wheat (Triticum aestivum L.) cultivars, in relation to estimated moisture supply. Canadian journal of plant science = Revue canadienne de phytotechnie 72, 235–241.

Guarda, G., Padovan, S., Delogu, G., 2004. Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. European Journal of Agronomy 21, 181–192. https://doi.org/10.1016/j.eja.2003.08.001

Guttieri, M.J., Frels, K., Regassa, T., Waters, B.M., Baenziger, P.S., 2017. Variation for nitrogen use efficiency traits in current and historical great plains hard winter wheat. Euphytica 213, 1–18. https://doi.org/10.1007/s10681-017-1869-5

Hall, A.J., Richards, R.A., 2013. Prognosis for genetic improvement of yield potential and water-limited yield of major grain crops. Field Crops Research 143, 18–33.

https://doi.org/10.1016/J.FCR.2012.05.014

Hamnér, K., Weih, M., Eriksson, J., Kirchmann, H., 2017. Influence of nitrogen supply on macro- and micronutrient accumulation during growth of winter wheat. Field Crops Research 213, 118–129. https://doi.org/10.1016/j.fcr.2017.08.002

Hay, R.K.M., 1995. Harvest index: A review of its use in plant breeding and crop physiology. Ann. Appl. Biol. 126, 197–216. https://doi.org/10.1111/j.1744-7348.1995.tb05015.x

Heitholt, J.J., Croy, L.I., Maness, N.O., Nguyen, H.T., 1990. Nitrogen partitioning in genotypes of winter wheat differing in grain N concentration. Field Crops Research 23, 133–144. https://doi.org/10.1016/0378-4290(90)90108-N

Hernandez-Ramirez, G., Fletcher, A.L., Jamieson, P.D., 2011. Nitrogen partitioning in spring winter wheat. Agronomy New Zealand 41, 125–132.

Højsgaard, S., Halekoh, U., 2016. doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. R package version 4.5-15.

Hysing, S.-C., Singh, R.P., Huerta-Espino, J., Merker, A., Liljeroth, E., Diaz, O., 2006. Leaf rust (Puccinia triticina) resistance in wheat (Triticum aestivum) cultivars grown in Northern Europe 1992-2002. Hereditas 143, 1–14. https://doi.org/10.1111/j.2005.0018-0661.01917.x

Jackson, P., Robertson, M., Cooper, M., Hammer, G., 1996. The role of physiological understanding in plant breeding; from a breeding perspective. Field Crops Research 49, 11–37. https://doi.org/10.1016/S0378-4290(96)01012-X

Janssen, B.H., Guiking, F.C.T., Van Der Eijk, D., Smaling, E.M.A., Wolf, J., Van Reuler, H., 1990. A system for quantitative evaluation of the fertility of tropical soils (QUEFTS). Geoderma 46, 299–318.

Jenner, C., Ugalde, T., Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. Australian Journal of Plant Physiology 18, 211. https://doi.org/10.1071/PP9910211

Kichey, T., Hirel, B., Heumez, E., Dubois, F., Le Gouis, J., 2007. In winter wheat (Triticum aestivum L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Research 102, 22–32. https://doi.org/10.1016/j.fcr.2007.01.002

Kitonyo, O.M., Sadras, V.O., Zhou, Y., Denton, M.D., 2017. Evaluation of historic Australian wheat varieties reveals increased grain yield and changes in senescence patterns but limited adaptation to tillage systems. Field Crops Research 206, 65–73. https://doi.org/10.1016/j.fcr.2017.02.017

Kole, C., 2013. Genomics and breeding for climate-resilient Crops: concepts and strategies. Springer. https://doi.org/10.1007/978-3-642-37045-8

Li, F., Mistele, B., Hu, Y., Yue, X., Yue, S., Miao, Y., Chen, X., Cui, Z., Meng, Q., Schmidhalter, U., 2012. Remotely estimating aerial N status of phenologically differing winter wheat cultivars grown in contrasting climatic and geographic zones in China and Germany. Field Crops Research 138, 21–32. https://doi.org/10.1016/j.fcr.2012.09.002

Linquist, B.A., Liu, L., van Kessel, C., van Groenigen, K.J., 2013. Enhanced efficiency nitrogen fertilizers for rice systems: Meta-analysis of yield and nitrogen uptake. Field Crops Research 154, 246–254. https://doi.org/10.1016/J.FCR.2013.08.014

Marti, J., Slafer, G.A., 2014. Bread and durum wheat yields under a wide range of environmental conditions. Field Crops Research 156, 258–271. https://doi.org/10.1016/J.FCR.2013.10.008

Martre, P., 2003. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. Plant Physiology 133, 1959–1967. https://doi.org/10.1104/pp.103.030585

McMullan, P.M., McVetty, P.B.E., Urquhart, A.A., 1988. Dry matter and nitrogen accumulation and redistribution and their relationship to grain yield and grain protein in oats. Canadian Journal of Plant Science 68, 311–322.

Mendiburu, F.D., 2015. Agricolae. R package version 1.2-3. https://doi.org/10.2307/2411227

Miralles, D.J., Katz, S.D., Colloca, A., Slafer, G.A., 1998. Floret development in near isogenic wheat lines differing in plant height. Field Crops Research 59, 21–30. https://doi.org/10.1016/S0378-4290(98)00103-8

Miralles, D.J., Slafer, G.A., 2007. Sink limitations to yield in wheat: How could it be reduced? Journal of Agricultural Science 145, 139–149. https://doi.org/10.1017/S0021859607006752

Miralles, D.J., Slafer, G.A., 1995. Yield, biomass and yield components in dwarf, semi-dwarf and tall isogenic lines of spring wheat under recommended and late sowing dates. Plant Breeding 114, 392–396. https://doi.org/10.1111/j.1439-0523.1995.tb00818.x

Miri, H.R., 2009. Grain yield and morpho-physiological changes from 60 years of genetic improvement of wheat in Iran. Experimental Agriculture 45, 149–163. https://doi.org/10.1017/S001447970800745X

Moll, R.H., Kamprath, E.J., Jackson, W.A., 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal 74, 562–564. https://doi.org/10.2134/agronj1982.00021962007400030037x

Mourtzinis, S., Kaur, G., Orlowski, J.M., Shapiro, C.A., Lee, C.D., Wortmann, C., Holshouser, D., Nafziger, E.D., Kandel, H., Niekamp, J., Ross, W.J., Lofton, J., Vonk, J., Roozeboom, K.L., Thelen, K.D., Lindsey, L.E., Staton, M., Naeve, S.L., Casteel, S.N., Wiebold, W.J., Conley, S.P., 2018. Soybean response to nitrogen application across the United States: A synthesis-analysis. Field Crops Research 215, 74–82. https://doi.org/10.1016/j.fcr.2017.09.035

Muurinen, S., Kleemola, J., Peltonen-Sainio, P., 2007. Accumulation and translocation of nitrogen in spring cereal cultivars differing in nitrogen use efficiency. Agronomy Journal 99, 441–449. https://doi.org/10.2134/agronj2006.0107

Nuttall, J.G., O'Leary, G.J., Panozzo, J.F., Walker, C.K., Barlow, K.M., Fitzgerald, G.J., 2017. Models of grain quality in wheat—A review. Field Crops Research 202, 136–145. https://doi.org/10.1016/j.fcr.2015.12.011

Ormoli, L., Costa, C., Negri, S., Perenzin, M., Vaccino, P., 2015. Diversity trends in bread wheat in Italy during the 20th century assessed by traditional and multivariate approaches. Scientific Reports 5. https://doi.org/10.1038/srep08574

Ortiz-Monasterio, J.I.R., Sayre, K.D., Rajaram, S., McMahon, M., 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Science 37, 898–904. https://doi.org/10.2135/cropsci1997.0011183X003700030033x

Oury, F.X., Brancourt-Hulmel, M., Depatureaux, C., Doussinault, G., Galic, N., Giraud, A., Heumez, E., Lecomte, C., Pluchard, P., Rolland, B., Rousset, M., Trottet, M., 2003. Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. Journal of genetics and breeding 57, 59–68.

Paccaud, F.X., Fossati, A., Cao, H.S., 1985. Breeding for Yield and Quality in Winter Wheat: Consequences for Nitrogen Uptake and Partitioning Efficiency. Zeitschrift fur Pflanzenzuchtung= Journal of Plant Breeding 94, 89–100.

Pedró, A., Savin, R., Slafer, G.A., 2012. Crop productivity as related to single-plant traits at key phenological stages in durum wheat. Field Crops Research 138, 42–51.

https://doi.org/10.1016/J.FCR.2012.09.016

Pingali, P.L., 2012. Green revolution: impacts, limits, and the path ahead. Proceedings of the National Academy of Sciences of the United States of America 109, 12302–12308. https://doi.org/10.1073/pnas.0912953109

Pugsley, A.T., 1983. The impact of plant physiology on australian wheat breeding. Euphytica 32, 743–748.

R Core Team, 2017. R Development Core Team. R: A Language and Environment for Statistical Computing.

Ray, D.K., Mueller, N.D., West, P.C., Foley, J.A., 2013. Yield trends are insufficient to double global crop production by 2050. PLoS ONE 8, e66428. https://doi.org/10.1371/journal.pone.0066428

Reynolds, M.P., Foulkes, J., Furbank, R., Griffiths, S., King, J., Murchie, E., Parry, M., Slafer, G.A., 2012a. Achieving yield gains in wheat. Plant, Cell and Environment 35, 1799–1823. https://doi.org/10.1111/j.1365-3040.2012.02588.x

Reynolds, M.P., Langridge, P., 2016. Physiological breeding. Current Opinion in Plant Biology 31, 162–171. https://doi.org/10.1016/J.PBI.2016.04.005

Reynolds, M.P., Pask, A.J.D., Mullan, D.M., 2012b. Physiological breeding I: interdisciplinary approaches to improve crop adaptation. Mexico, D.F.: CIMMYT. https://doi.org/10.1017/CBO9781107415324.004

Rosegrant, M.W., Fernandez, M., Sinha, A., Alder, J., Ahammad, H., de Fraiture, C., Eickhour, B., Fonseca, J., Huang, J., Koyama, O., Omezzine, A.M., Pingali, P., Ramirez, R., Ringler, C., Robinson, S., Thornton, P., van Vuuren, D., Yana-Shapiro, H., 2009. Looking into the future for agriculture and AKST, in: McIntyre, B.D., Herren, H.R., Wakhungu, J., Watson, R.T. (Eds.), International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD): Agriculture at a Crossroads, Global Report. Island Press, Washington, DC, USA, pp. 307–376.

Royo, C., Elias, E.M., Manthey, F.A., 2009. Durum wheat breeding, in: Carena M. (Eds) Cereals. Handbook of Plant Breeding. Springer, New York, NY, pp. 199–226. https://doi.org/10.1007/978/-0-387-72297-9

Sadras, V.O., 2006. The N:P stoichiometry of cereal, grain legume and oilseed crops. Field Crops Research 95, 13–29. https://doi.org/10.1016/j.fcr.2005.01.020

Sayre, K.D., Rajaram, S., Fischer, R.A., 1997. Yield potential progress in short bread wheats in northwest Mexico. Crop Science 37, 36–42. https://doi.org/10.2135/cropsci1997.0011183X003700010006x

Singh, M., 2001. Nitrogen uptake and distribution in wheat (Triticum aestivum L.). M.S. Thesis. The University of Guelph, Ontario, Canada.

Slafer, G.A., 2003. Genetic basis of yield as viewed from a crop physiologist's perspective. Annals of Applied Biology 142, 117–128. https://doi.org/10.1111/j.1744-7348.2003.tb00237.x

Slafer, G.A., Andrade, F.H., 1991. Changes in physiological attributes of the dry matter economy of bread wheat (Triticum aestivum) through genetic improvement of grain yield potential at different regions of the world. Euphytica 58, 37–49. https://doi.org/10.1007/BF00035338

Slafer, G.A., Andrade, F.H., Feingold, S.E., 1990a. Genetic improvement of bread wheat (Triticum aestivum L.) in Argentina: relationships between nitrogen and dry matter. Euphytica 50, 63–71. https://doi.org/10.1007/BF00023162

Slafer, G.A., Andrade, F.H., Satorre, E.H., 1990b. Genetic-improvement effects on pre-anthesis physiological attributes related to wheat grain-yield. Field Crops Research 23, 255–263. https://doi.org/10.1016/0378-4290(90)90058-J

Slafer, G.A., Elia, M., Savin, R., García, G.A., Terrile, I.I., Ferrante, A., Miralles, D.J., González, F.G., 2015. Fruiting efficiency: An alternative trait to further rise wheat yield. Food and Energy Security 4, 92–109. https://doi.org/10.1002/fes3.59

Slafer, G.A., Satorre, E.H., Andrade, F.H., 1994. Increases in grain yield in bread wheat from breeding and associated physiological changes, in: Slafer, G.A. (Ed.), Genetic Improvements of Field Crops. Marcel Dekker, New York, NY, pp. 1–68.

Slafer, G.A., Savin, R., Sadras, V.O., 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crops Research 157, 71–83. https://doi.org/10.1016/j.fcr.2013.12.004

Snape, J.W., 1998. Golden calves or white elephants? Biotechnologies for wheat improvement. Euphytica 100, 207–217. https://doi.org/10.1023/A:1018343906495

Sosulski, F.W., Imafidon, G.I., 1990. Amino Acid Composition and Nitrogen-to-Protein Conversion Factors for Animal and Plant Foods. Food Chem 38, 1351–1356.

Tamagno, S., Balboa, G.R., Assefa, Y., Kovács, P., Casteel, S.N., Salvagiotti, F., García, F.O., Stewart, W.M., Ciampitti, I.A., 2017. Nutrient partitioning and stoichiometry in soybean: A synthesis-analysis. Field Crops Research 200, 18–27. https://doi.org/10.1016/j.fcr.2016.09.019

Tilman, 2002. Agriculture sustainability and intensive production practices. Nature 418, 671–677.

Tonitto, C., David, M.B., Drinkwater, L.E., 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics.

Agriculture, Ecosystems & Environment 112, 58–72. https://doi.org/10.1016/J.AGEE.2005.07.003

Triboi, E., Martre, P., Girousse, C., Ravel, C., Triboi-Blondel, A.M., 2006. Unravelling environmental and genetic relationships between grain yield and nitrogen concentration for wheat. European Journal of Agronomy 25, 108–118. https://doi.org/10.1016/j.eja.2006.04.004

Turner, N.C., 2004. Sustainable production of crops and pastures under drought in a Mediterranean environment. Annals of Applied Biology 144, 139–147. https://doi.org/10.1111/j.1744-7348.2004.tb00327.x

Wang, Z., Sadras, V.O., Hoogmoed, M., Yang, X., Huang, F., Han, X., Zhang, S., 2017. Shifts in nitrogen and phosphorus uptake and allocation in response to selection for yield in Chinese winter wheat. Crop and Pasture Science 68, 807–816. https://doi.org/10.1071/CP17220

Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis, in: Springer-Verlag New York. p. 211. https://doi.org/10.1007/978-0-387-98141-3

Wickham, H., François, R., Henry, L., Müller, K., 2018. dplyr: A Grammar of Data Manipulation.

Woodard, H.J., Bly, A., 1998. Relationship of nitrogen management to winter wheat yield and grain protein in South Dakota. Journal of Plant Nutrition 21, 217–233. https://doi.org/10.1080/01904169809365397

Woolfolk, C.W., Raun, W.R., Johnson, G. V., Thomason, W.E., Mullen, R.W., Wynn, K.J., Freeman, K.W., 2002. Influence of late-season foliar nitrogen applications on yield and grain nitrogen in winter wheat. Agronomy Journal 94, 429–434. https://doi.org/10.2134/agronj2002.0429

Ye, Y., Wang, G., Huang, Y., Zhu, Y., Meng, Q., Chen, X., Zhang, F., Cui, Z., 2011. Understanding physiological processes associated with yield-trait relationships in modern wheat varieties. Field Crops Research 124, 316–322. https://doi.org/10.1016/j.fcr.2011.06.023

Zhou, Y., He, Z.H., Chen, X.M., Wang, D.S., Yan, J., Xia, X.C., Zhang, Y., 2007. Genetic improvement of wheat yield potential in north China, in: Buck, H.T., Nisi, J.E., Salomon, N. (Eds.), Wheat Production in Stressed Environments. Springer, pp. 583–589.

Chapter 2 - Physiological basis of genotypic response to management in dryland wheat

Abstract

A great majority of dryland wheat producers are reluctant to intensify management due to the assumption that lack of water availability is the most critical factor limiting yield and thus, the response to management intensification would be limited. We conducted on-farm field experiments across three locations and two growing seasons in Kansas using 21 modern winter wheat genotypes grown under either standard (SM) or intensified management (IM) systems. The goals of this study were to (*i*) determine whether the SM adopted is adequate to reach achievable yields by farmers in the region and (*ii*) identify differences in responsiveness to IM among a range of modern genotypes. Across all sites-years and genotypes, the IM increased yield by 0.9 Mg ha^{-1}, outyielding the SM system even in the lowest yielding conditions. As expected, the yield response to IM increased with the achievable yield of the environment and genotype. Across all sources of variation, the yield responsiveness to IM was related to increased biomass rather than harvest index, strongly driven by improvements in grain number (and independent of changes in grain weight), and by improvements in N uptake which resulted from greater biomass and shoot N concentration. The IM system generally also increased grain N concentration and decreased the grain N dilution effect from increased yield. Genotypes varied in their response to IM, with major response patterns resulting from the combination of response magnitude (large *vs* small) and consistency (variable *vs* consistent). Genotypes with high mean response and high variability in the response to IM across years could offer greater opportunities for producers to maximize yield as those genotypes showed greater yield gain from IM when conditions favored their response. For the background conditions evaluated, intensifying

management could improve wheat yield in between c. 0.2 and 1.5 Mg ha^{-1} depending on genotype.

Introduction

Wheat (*Triticum aestivum* L.) is critical for food security as it provides c. 20% of calories and protein of human daily nutrition requirements (Shewry and Hey, 2015). It is the crop cultivated across the largest acreage in the world (more than 200 million hectares year^{-1}; FAO-AMIS, 2018), and is mostly (80%) grown under rainfed conditions (FAO, 2003). Many of these regions produce rather variable, though overall relatively low, yields mainly due to the exposure to water stress. Rainfall in these regions is characteristically variable from season to season and is generally insufficient to maximize yield (FAO, 2003; Reynolds, 2010).

Farmers in most of these dryland regions are reluctant to intensify agronomic management. One major reason is the assumption that lack of water availability will limit yield potential and intensified management will provide no benefit, as expected from the Liebig's '*law of the minimum*'. However, this reluctance may be unjustified as several empiric and theoretical frameworks show the inadequacy of this '*law*' (De Wit, 1992; Sinclair and Park, 1993). In fact, crop yields could be enhanced when there is co-limitation of different factors [i.e. when different resources are similarly limiting rather than when growth is severely limited by a single factor (Cossani et al., 2010; Cossani and Sadras, 2018; Sadras, 2004)]. The proven inadequacy of Liebig's '*law of the minimum*' implies that the most limiting factor could be used more efficiently when increasing the availability of other factors through intensifying management (Sadras, 2005). Thus, conservative behavior of farmers regarding intensification of management in dryland wheat regions may prevent them from achieving higher yields, even in the lowest yielding environments. Good empirical evidence of this is that Australian wheat yields have increased consistently due to reducing biotic stresses (nematodes) and increasing N fertilization (Passioura, 2002), even though water availability has not improved in Australia (Hochman et al., 2017).

Kansas is the largest winter wheat producing state in the US (c. 15% of the total US production, growing wheat in c. 3.4 Mha; USDA-NASS, 2018a), and experiences constraints to production which are typical of dryland wheat producing regions of the globe. Average farm yields have been relatively low (c. 3 Mg ha^{-1} during the past 30 years; FAO-AMIS, 2018) mainly due to highly variable, and overall scarce level of, rainfall (Araya et al., 2019; Lollato et al., 2017). Farmers in Kansas tend to be conservatively averse to risk, limiting the use of inputs due to the expectation on inconsistent yield responses. However, similar to other wheat regions (e.g. Cossani et al., 2011), there is empirical evidence in Kansas (Jaenisch et al., 2019) that wheat yields may improve by intensifying rainfed management practices.

The two major inputs that might be inadequately managed in standard management systems in Kansas are nitrogen (N) fertilization and chemical protection against foliar fungal diseases (Lollato et al., 2019a). Nitrogen fertilization rates in Kansas average c. 60 kgN ha^{-1} (USDA-ERS, 2017), which is considerably lower than the estimated long-term agronomic optimum rate of the region (c. 90 kgN ha^{-1}; Lollato et al., 2019b). Nitrogen limitation early in the growing season can reduce wheat tiller formation and survival, consequently reducing the number of spikes produced per unit area (Borghi, 1999; Montemurro et al., 2007) and the floret survival, resulting in reductions in grains per spike (Albrizio et al., 2010; Ferrante et al., 2013). Fertile tiller and grains per spike are major regulators of wheat yield (Slafer et al., 2014), thus lack of adequate N fertilization may limit water use and water use-efficiency (Asseng et al., 2001; Cossani et al., 2012; Sadras and Roget, 2004), even in dryland wheat production.

Moreover, inadequate N availability during grain filling can reduce grain N concentration (Lollato et al., 2019a; Oury and Godin, 2007), which is a critical determinant of wheat end-use quality. Likewise, only about 25% of the wheat grown in Kansas is typically protected with foliar fungicides (USDA-NASS, 2018a). Severe incidence of foliar diseases can reduce wheat yield by lowering the source-sink ratio (Serrago et al., 2019). Moreover, even though the types and severity of fungal diseases (e.g. stripe rust [*Puccinia striiformis* f.sp. *tritici*] and leaf rust [*Puccinia triticina*]) vary depending on weather and genotypes, yield penalties due to diseases are common, as empirically evidenced by Jaenisch et al. (2019) and Lollato et al. (2019b). Furthermore, there has been an increase in stripe rust disease pressure and evolution of new pathogen races in recent years (DeWolf et al., 2017), which has challenged breeding programs to identify new sources of genetic resistance quickly. Thus, we believe that rainfed wheat in Kansas, and in dryland wheat growing regions in general, is likely grown under conditions that are chemically under-protected against foliar diseases that frequently reduce yield (USDA-NASS, 2018a) and where soil N availability is noticeably lower than the demand of the crop. Therefore, we hypothesize that current yields in this region are below those achievable under more intensive management in the form of higher N availability and chemical protection against diseases.

Although this hypothesis is proposed in general for modern wheat genotypes, different magnitudes of responsiveness to management intensification would be expected for specific genotypes. Thus, the hypothesis was tested considering a wide range of genotypes available to farmers in the region, allowing recognition of the level of genotypic variation and concurrently providing insight for breeding genotypes more responsive to intensive management. Future yield improvement in this (and any other) dryland region requires recognition of genetics characteristics underlying responsiveness to intensified management. Understanding agronomic traits associated with genotypic responses to management and yield determination can help breeding programs develop better adapted genotypes and enable producers to maximize yield while maintaining environmental quality.

We carried out field experiments with 21 modern winter wheat genotypes grown across three locations and two growing seasons in Kansas under either standard (SM) or intensified management (IM) systems to

(*i*) determine whether the SM used in Kansas is adequate to reach achievable yields by farmers in the region by (*i.a*) quantifying the response to an IM system of improved N availability and protection against diseases, as well as, (*i.b*) ascertaining crop-physiological traits associated with yield responsiveness to IM across environments and genotypes; and

(*ii*) recognize genotypic differences in responsiveness to IM among a range of modern cultivars, identifying degrees of overall responsiveness (expectedly from very responsive to mostly unresponsive) together with consistency in responsiveness to IM.

Materials and Methods

General experiment information

Five rainfed field experiments were established in actual farmers' fields (i.e. the background conditions were those of real farms, not experimental fields) of three locations in Kansas (Conway Springs, Ellsworth, and McPherson) during two growing seasons: 2015-16 and 2016-17 (Table 1). The soil type was Bethany silt loam (fine, mixed, superactive, thermic pachic paleustoll) for Conway and Crete silt loam (fine, smectitic, mesic pachic udertic argiustolls) for Ellsworth and McPherson. The average yield recorded by farmers for the past 3-5 years before the establishment of the field trials in these fields was 3.3 Mg ha^{-1} for Ellsworth and 4.0 Mg ha^{-1} for Conway and McPherson.

Table 2-1. Experiment information. Site-years, plot coordinates, sowing and harvesting dates, previous crop, and total N rate (kg ha^{-1}) for standard management (SM) at each location during the 2015-16 and 2016-17 growing seasons

Year	**Location**	**Coordinates**	**Planting date**	**Harvesting date**	**Previous crop**	**N rate SM (kg ha^{-1})**
2015-2016	Conway	37°27'34.94"N 97°37'43.33"W	10/13/2015	6/7/2016	soybean	157
	McPherson	38°15'56.99"N 97°35'34.04"W	10/7/2015	6/28/2016	wheat	106
2016-2017	Ellsworth	38°35'37.99"N 98°19'58.18"W	10/7/2016	6/27/2017	wheat	67
	Conway	37°27'36.7"N 97°37'48.3"W	10/11/2016	6/22/2017	corn	101
	McPherson	38°15'50.83"N 97°35'33.36"W	10/11/2016	6/20/2017	wheat	101

Conventional tillage was performed in the fall prior to wheat sowing in Ellsworth and McPherson, while a no-till system was used in Conway. Sowing and harvesting dates were within the optimal ranges in all cases (Table 1). Field trials were sown with a 6-row Hege small plot cone drill. Plots were 4.6 m long and 1.5 m wide, comprised by six rows 0.25 m apart. At all sites, the seeding rate was 101 kg ha^{-1} (a weight-basis seeding rate being the usual recommendation for the region (Shroyer et al., 1997), due to the relative small variability in seed size among the most common cultivars). Insect and weed occurrence was minimal and controlled with commercially available chemical products as needed. Weather data (Table 2) was collected daily (from sowing to harvest) from the Kansas Mesonet (http://mesonet.k-state.edu/) climate monitoring network from stations located near (c. 500 m) to the experimental sites. Soil fertility was evaluated within 2 weeks after sowing in all locations (Table 3). Soil samples were collected between plots to avoid plant and soil disturbance within plots, using hand-probes at 0-15 and 15-

60 cm depth. At each depth, 15 soil cores were combined to represent the soil characteristics of each field experiment.

Table 2-2 Weather information. Cumulative precipitation (Cum PPT) in millimeters, maximum, minimum, and average daily temperature (T) in Celsius during the growing season and average of 30 years (1981-2011), cumulative growing degree-days (Cum GDD) in Celsius, and cumulative evapotranspiration (Cum ET) in millimeters per day at each location during the 2015-16 and 2016-17 growing seasons.

Year	**Site**	**Season**	**Cum PPT (mm)**	**30-yr avg Cum PPT (mm)**	**T max °C**	**T min °C**	***T avg °C***	**30-yr avg T max °C**	**30-yr avg T min °C**	**Cum GDD (°C)**	**Cum ET (mm day^{-1})**
2015-2016	Conway	Fall	189	148	15	2	*8*	14	1	699	148
		Winter	80	133	13	-2	*5*	10	-3	658	198
		Spring	494	324	26	13	*20*	25	13	1919	447
	McPherson	Fall	117	125	15	2	*8*	14	1	963	159
		Winter	39	119	11	-2	*4*	12	-1	772	187
		Spring	351	325	25	12	*19*	24	11	1982	426
2016-2017	Ellsworth	Fall	30	108	15	-1	*7*	13	-1	783	*NA*
		Winter	135	102	12	-2	*5*	9	-6	615	172
		Spring	239	276	25	11	*18*	24	11	1573	383
	Conway	Fall	36	148	15	1	*8*	14	1	566	202
		Winter	187	133	13	0	*7*	10	-3	443	244
		Spring	332	324	25	12	*19*	25	13	1284	391
	McPherson	Fall	43	125	14	1	*8*	14	1	524	151
		Winter	132	119	12	-1	*5*	12	-1	357	221
		Spring	217	325	24	11	*18*	24	11	1170	405

Note: There were no solar radiation data available for the fall period at the Ellsworth site, therefore cum ET in this location was calculated from January to June (harvesting). Fall; October to December, Winter; January to March, Spring; April to Harvest.

Table 2-3 Soil fertility information two weeks after sowing at each location during the 2015-16 and 2016-17 growing seasons. Soil test includes soil pH, nitrate- (NO_3-N) and ammonium- (NH_4-N) nitrogen, Mehlich-3 extractable phosphorus (P), potassium (K), calcium (Ca), sulfate-sulfur (SO_4-S), chloride (Cl), cation exchange capacity (CEC), organic matter (OM), and percentage sand, silt and clay in the soil at sampling depths from 0 to 15 cm and 15 to 45 cm.

	2015-2016				**2016-2017**					
	Conway		**McPherson**		**Ellsworth**		**Conway**		**McPherson**	
Depth (cm)	**15cm**	**45cm**	**15cm**	**45cm**	**15cm**	**45cm**	**15cm**	**45cm**	**15cm**	**45cm**
pH	6	5	6	6	6	7	6	6	6	6
NO_3-N (ppm)	7	6	31	36	33	23	13	8	49	41
NH_4-N (ppm)	13	6	27	13	16	15	8	6	16	14
P_Mehlich (ppm)	62	15	92	33	36	32	56	25	79	68
K (ppm)	239	231	383	243	365	301	226	251	370	309
Ca (ppm)	2271	2528	2567	2811	2182	2450	1709	2503	2464	2498
SO_4-S (ppm)	19	15	14	10	16	7	6	6	11	10
Cl (ppm)	8	4	11	12	9	8	9	6	12	16
CEC (meq $100g^{-1}$)	22	25	19	21	21	16	22	24	22	20
OM (%)	3	2	3	2	3	3	3	2	3	3
sand %	25	21	15	12	18	13				
silt %	48	42	58	56	57	56				
clay %	27	37	27	32	25	31				

Treatments and Experimental Design

Twenty-one winter wheat genotypes, commercially available to farmers in the region (Table 4), were tested under two management practices at each location. The management systems tested were common farmer's practice (actual management made by the specific farmer in whose field the experiments were conducted) hereafter referred to standard management (SM) versus intensive management (IM). In the SM treatment, there was no fungicide application, and the N management (source, rate, and timing of application) varied slightly across fields depending on each farmer's practice (Table 1). In general, farmers applied N at planting and at early tillering stage (stage Z26 in the scale of Zadoks et al., 1974) in the spring with a total rate sufficient to achieve a yield goal of approximately 5 Mg ha^{-1}, according to the recommendation guide from Kansas State University (Leikam et al., 2003). This rate considered soil N availability prior sowing in the topsoil layer (0 –15 cm), soil NO_3 in the profile (0 – 60 cm) (both shown in Table 2), previous crop credits, and tillage practice (Leikam et al., 2003). The IM treatment consisted of the SM treatment in each particular field with (i) an additional N rate of 45 kg ha^{-1} of N broadcasted as urea (46-0-0) at the onset of stem elongation stage (Z30), and (ii) two fungicide applications. The first fungicide application was made when the first node was detectable (Z31) to protect leaves and stems using a two mode of action product (24 g a.i. of fluxapyroxad ha^{-1} and 49 g a.i. of pyraclostrobin ha^{-1}). The second fungicide was a three mode of action product (20 g a.i. of fluxapyroxad ha^{-1}, 139 g a.i. of pyraclostrobin ha^{-1}, and 82 g a.i. of propiconazole ha^{-1}) applied at the heading stage (Z58) to protect upper leaves and spikes. The average yield produced under the IM treatment represents the water-limited achievable yield of site-years and genotypes, as defined by Evans and Fischer (1999).

Treatments within each of the experiments were arranged in a split-plot design with genotypes assigned to the main plots and management to the sub-plots. Main plots were arranged in a randomized complete block design with three replications.

Measurements

Aboveground biomass was sampled at physiological maturity from 0.5 m of a middle plot-row and the number of spikes counted before the material was fractioned into stover (leaves and stems), and spike (chaff and grains). Samples were dried at 60ºC for one week, and then dry weights recorded. Spikes were counted and threshed; grains were weighed and counted to estimate yield and its numerical components: grain number per unit area and 1000-grain weight on a dry weight basis. Samples were then ground (sieve 2 mm), and plant N concentration in stover and grains was determined via the LECO TruSpec CN combustion analyzer. The nutrient concentration of the chaff was estimated from that of the stover. Aboveground N uptake was estimated as the product between the weighted average of N concentration among organs by biomass and reported on a dry weight basis. Harvest index (HI) was determined as the ratio of grain yield by aboveground biomass at maturity. Nitrogen utilization efficiency was estimated as the ratio of grain yield by aboveground N uptake at maturity (Moll et al., 1982).

Table 2-4 Information of agronomic traits (drought tolerance, maturity range [heading date], straw strength) and genetic resistance to most occurring fungal diseases in KS (leaf rust [Puccinia triticina], stem rust [Puccinia gramini], stripe rust [Puccinia striiformis], powdery mildew [Blumeria graminis], tan spot [Pyrenophora tritici-repentis], and Septoria tritici blotch [Mycosphaerella graminicola]) for the 21 genotypes tested in 2016 and 2017 growing seasons

	Genotypes	**Drought**	**Maturity**	**Straw strength**	**Leaf rst**	**Stem rst**	**Stripe rst**	**P. Mildew**	**Tan spot**	**Septoria**
1	1863	6	5	7	7	1	3	6	6	6
2	AGRob	*NA*	3	2	4	5	2	*NA*	8	6
3	Bentley	*NA*	4	6	5	2	5	6	6	5
4	Doub.CL+	7	7	4	3	2	5	5	6	6
5	Everest	7	2	2	3	3	8	3	7	5
6	HotRod	7	3	2	3	5	4	4	6	5
7	KanM	5	5	1	2	3	6	7	6	6
8	Larry	6	6	3	7	2	2	5	5	6
9	LCSChro	5	8	3	2	2	3	6	4	4
10	LCSMi	4	7	6	7	4	5	6	5	5
11	LCSPis	5	4	7	6	8	7	3	7	*NA*
12	SYFlint	5	4	4	6	3	4	7	7	7
13	SYMo	6	7	5	2	2	2	5	5	4
14	T158	4	3	4	8	8	2	3	7	7
15	Tatanka	5	6	7	6	2	2	7	7	7
16	WB4303	*NA*	4	1	3	1	4	5	6	6
17	WB4458	6	4	2	7	1	4	7	5	7
18	WBCe	7	2	1	5	3	4	2	5	4
19	WBGra	5	6	3	4	2	6	6	6	6
20	Winterh.	4	6	3	7	8	6	5	6	7
21	Zenda	7	4	2	3	2	3	5	5	4

Note: NA; not available due to insufficient information. Legend for agronomic traits. Drought tolerance: 1 = excellent; 5 = good; 9 = poor. Maturity: 1 = early; 5 = medium; 9 = late. Straw strength: 1 = excellent; 5 = good; 9 = poor (high lodging risk). Legend for disease resistance levels: 1 = highly resistant, 3 = moderately resistance, 5 = intermediate, 7 = moderately susceptible, 9 = highly susceptible (DeWolf et al., 2017).

Statistical Analyses

Sources of variation in ANOVA comprised of genotype, management, site-year, and their interactions as fixed factors; and block nested within site and genotype nested within block as random effects, the latter to account for the split-plot design. Analysis of variance was conducted using the "lmerTest" package (Kuznetsova et al., 2017) in R software version 3.4.0. Descriptive statistics were calculated using the R package "doBy" (Højsgaard and Halekoh, 2016) and included mean, standard deviation (sd), and 0.25 and 0.75 percentiles for grain yield. To evaluate the impact of management on yield across genotypes and site-years, we built boxplots using the R package "ggplot2" (Wickham, 2009).

A biplot GGE model was used with yield, aboveground biomass, and HI as dependent variables to evaluate the genotypes performance and genotype and environment interactions across management and site-years (Romagosa et al., 2013).

We evaluated the relationships among measured variables by regression analyses using the "lm" function in the R package "ExpDes" (Ferreira et al., 2018). To estimate the impacts of agronomic traits on yield differences among environments and genotypes (i.e. the global responses), results are shown for all site-years and genotypes (*n*=210), but also on average of genotypes for each site-year (*n*=5), and on average of site-years across genotypes (*n*=21).

Trait response to management within each particular background condition was estimated by subtracting the mean under IM by mean under SM. Likewise, the magnitude of genotypic yield responsiveness to management was evaluated as the difference between yield at IM and SM, averaged across background environments. The variability (i.e. lack of consistency) of genotypic response to management was assessed by the standard deviation of the mean yield response to management. The relationship between mean yield at IM and SM versus mean yield response to management was evaluated by regression analyses using the "lm" function in the R package "ExpDes" (Ferreira et al., 2018).

To investigate the causes of differences in N uptake due management we built a critical N dilution curve for each management system across all environments and genotypes by fitting the negative power function (Eq.1) suggested by (Justes et al., 1994).

$$\text{Shoot N concentration} = \mathrm{a} * \mathrm{biomass}^{-\mathrm{b}} \qquad \text{(Eq.1)}$$

where a is the shoot N concentration when biomass is equal to 1 Mg ha^{-1} and b is the dilution coefficient (i.e. rate of decrease in shoot N concentration as the biomass increases). We compared the intercepts and slopes of the relationship between grain N concentration and yield between IM and SM using the standardized major axis (SMA) analysis in the R package "smatr" (Warton et al., 2012).

General conditions

For both 2016 and 2017 growing seasons, the average daily temperature was similar to the 30 year-normal (1981-2000) of each region (NCDC-NOAA, 2019), except for winter season which was warmer than expected by approximately 3°C (Table 1). Precipitation during the fall of the 2015-16 growing season was similar to the long-term in McPherson and slightly above average in Conway. Moderate drought and few freeze events were observed in the winter and early spring (around flag leaf emergence [mid-April]), which was then followed by greater than normal precipitation and below-normal temperatures. During the 2016-17 growing season, fall months were drier and winter months were wetter than expected from an average year. The drier fall resulted in crops with less tiller formation (visually observed), which was then followed by a period of greater than average water availability and warm temperatures. In the spring (from flag leaf emergence and afterwards) weather was similar to those of an average year.

Results

Overall effect of management system on crop yield

Across all sources of variation (five background environments given by the combination of sites and years and 21 cultivars grown in each of them), IM outyielded SM by an average of 0.9 Mg ha^{-1} (Fig. 1A). Across the study, yields were normally distributed for both management systems and showed a larger standard deviation for the IM as compared to the SM (c. 0.97 and 0.67 Mg ha^{-1}, respectively; Fig. 1A). Usually, the lowest yields achieved in both systems tended to be similar while yields under IM were clearly larger than under SM in higher yielding conditions (Fig. 1A). Therefore, the yield advantage of IM over SM was neither uniform across background environments (the interaction between management and site-year was significant at $p<0.05$), nor across genotypes (although the interaction between genotype and management was significant only at a p=0.14). The three-way (site-year x genotype x management) interaction was not significant (p=0.81). However, the magnitude of the management effect was much larger than its interaction with the background environment (the mean square for management effect was more than 10-fold higher than that of the site-year × management interaction), and therefore, that interaction was not cross-over. That is, the IM always outyielded SM, though the magnitude of the difference was not uniform across sites-years (Fig. 1B). Indeed, the response of wheat yield to the IM tended to increase with achievable yield (i.e. yield under IM) of the background environment (Fig. 1B, inset). Regarding the overall differential response of the genotypes, we observed a consistent trend for IM outyielding SM in all genotypes, though that difference was not statistically significant in five out of the 21 genotypes (Fig. 1C).

All these elements are clearly illustrated in the GGE biplot analysis (Fig. 1D). In general, varieties under SM tended to have lower yields as compared to IM. The IM system seemed to have been better adapted, in terms of increased yield, than the SM across all site years (Fig. 1D); although specific varieties were better adapted to certain particular background conditions.

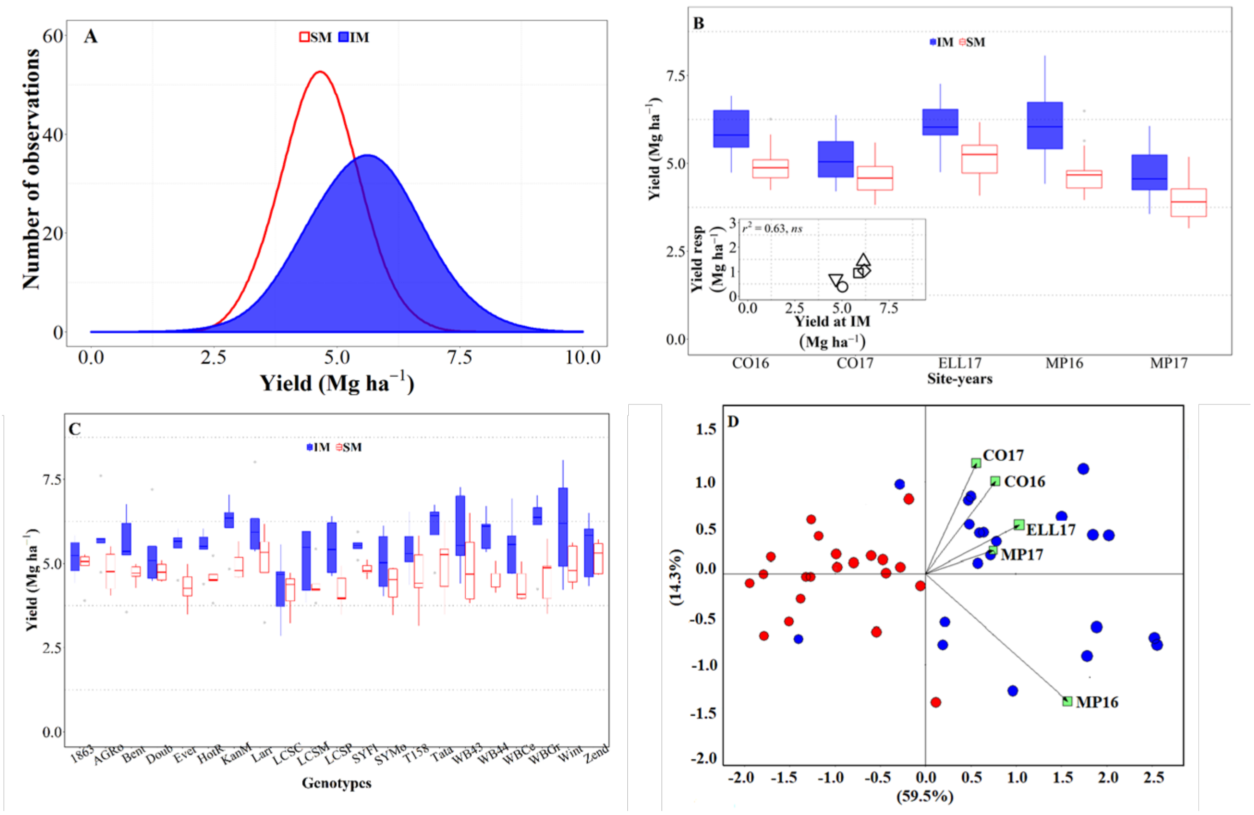

Figure 2.1 Distribution of the mean yield across environments and genotypes for intensive (IM) and standard (SM) management systems (A). Mean yield for IM and SM systems on average of genotypes for each site-year (B), with an inset showing the relationship between the average yield response to IM and achievable yield (yield in IM). Mean yield for IM and SM systems on average of site-years for each genotype (C). Genotype and genotype × environment (GGE) biplot analysis for yield of 21 genotypes grown in five site-years at SM and IM systems (D).

Traits associated with yield responsiveness to intensive management

There was an overall positive relationship between yield and aboveground biomass at maturity, with 45% of the variation in yield due to the combination of background environments, genotypes, and management systems explained by differences in biomass accumulation at maturity (Fig. 2A), even though there was a clear penalty in harvest index in Ellsworth 2017 (Rhombs in Fig. 2A, B, D, E, supplementary Fig. B1). On the other hand, across all sources of variation considered, there was no relationship between yield and biomass partitioning towards the grains (Fig. 2D), although this relationship was positive and significant within location-management combination (ranging from $r^2 = 0.14$ in Conway 2016 to $r^2 = 0.60$ in McPherson 2017) mainly driven by genotypic differences within each growing condition (supplementary Fig. B2). Focusing on the background environmental conditions, the overall positive trend between yield and biomass demonstrates that differences in yield between site-years were in general due to differences in biomass accumulation (Fig. 2B), and rather independent of site-year differences in harvest index across management systems (Fig. 2E). Neither the relationship between yield and biomass, nor that between yield and harvest index, were significant within each management system ($p>0.05$). It was clear, however, that biomass was more relevant than harvest index in explaining the differences in yield across sites-years, even within management systems (Figs. 2B and 2E). Thus, the yield response to IM across sites-years was related differences between the two management systems for biomass rather than harvest index (Figs. 2B, inset and 2E, inset). On the other hand, the yield differences between genotypes were significantly related to both biomass and harvest index across management systems, though the degree of association was substantially higher for biomass (cf. Figs. 2C and 2F). Overall, it was clear that biomass responses to IM were the primary driver of the yield response of the genotypes. Evidence for this includes not only that coefficients of determination were more highly significant for biomass than for harvest index but also that while responses to IM of yield and biomass were always positive (Fig. 2C, inset) in several cases, IM did not improve, and sometimes decreased, harvest index (Fig. 2F, inset).

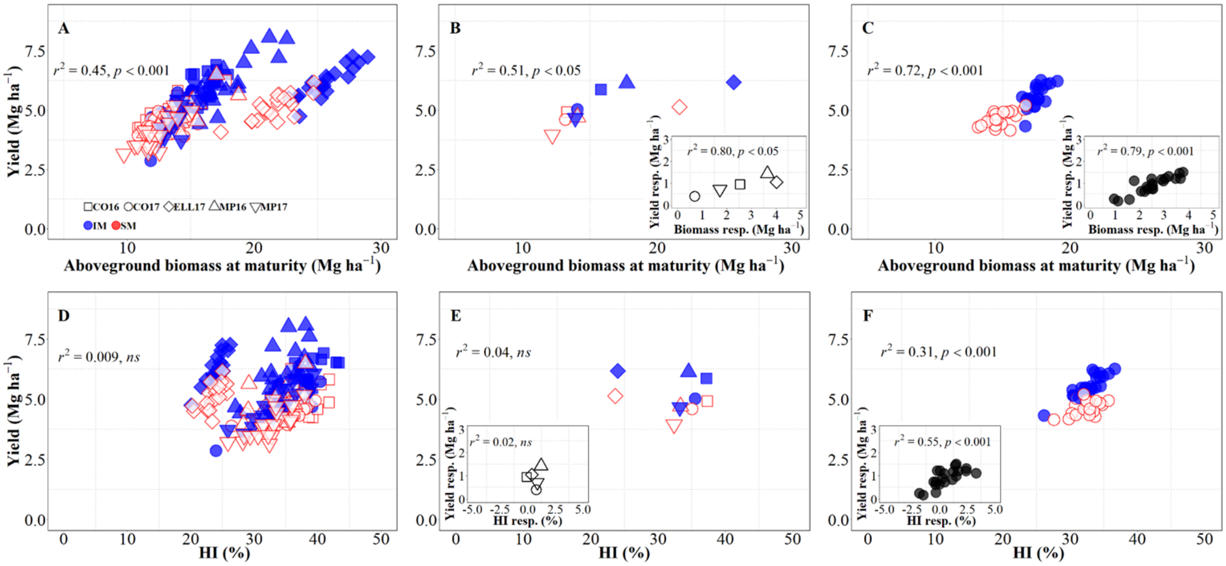

Figure 2.2 Relationship between yield and either aboveground biomass at maturity (A, B, C) or harvest index (D, E, F) across environments, genotypes and management systems (intensive management (IM) and standard management (SM) (*n*=210) (A, D), on average of genotypes for each site-year (B, E) (*n*=10), and on average of site-years for each genotype (C, F) (*n*=42). Insets are the relationships between the responses of the variables to intensive management (difference in the variable between IM and SM) averaged across either genotypes for each site-year (B, E insets) (*n*=5) or site-years for each genotype (C, F insets) (*n*=21).

Changes in grain number per unit area explained 61% of the overall variation in grain yield, i.e. when accounting for environments, genotypes, and management systems together (Fig. 3A). Although grain weight also significantly associated with differences in yield, the proportion explained was much lower (c. 6%, Fig. 3D). Yield differences across environments were well explained by differences in grain number (Fig. 3B), not only due to their high association across site-years (Fig. 3B), but also because yield responses to IM within each of the site-years were strongly driven by improvements in grain number (Fig. 3B, inset). On the other hand, differences in yield among environments were not explained by differences in grain weight within or across management systems (Fig. 3E). Yield responses to IM of the different background environments were rather independent of those in grain weight (Fig. 3E, inset). Indeed, there was almost no difference in grain weight between IM and SM within each of the site-years (Fig. 3E), and therefore neither in the response of grain weight to IM (Fig. 3E, inset). Similarly, differences in yield among genotypes across management systems were exclusively brought about by differences in grain number (Fig. 3C), as the relationship with grain weight was negligible (Fig. 3F). The relationship between yield and grain number across genotypes was strong within each of the management systems, but also the yield response to IM of the genotypes was associated with increases in grain number (Fig. 3C, inset). The lack of relationship between yield and grain weight across genotypes and management was also true within each of the two management systems (Fig. 3F). Even though the yield response of genotypes to IM was related to their grain weight response (Fig. 3F, inset), the relationship could hardly be mechanistic as IM always improved yields even in situations where it decreased grain weight of several genotypes (Fig. 3F, inset).

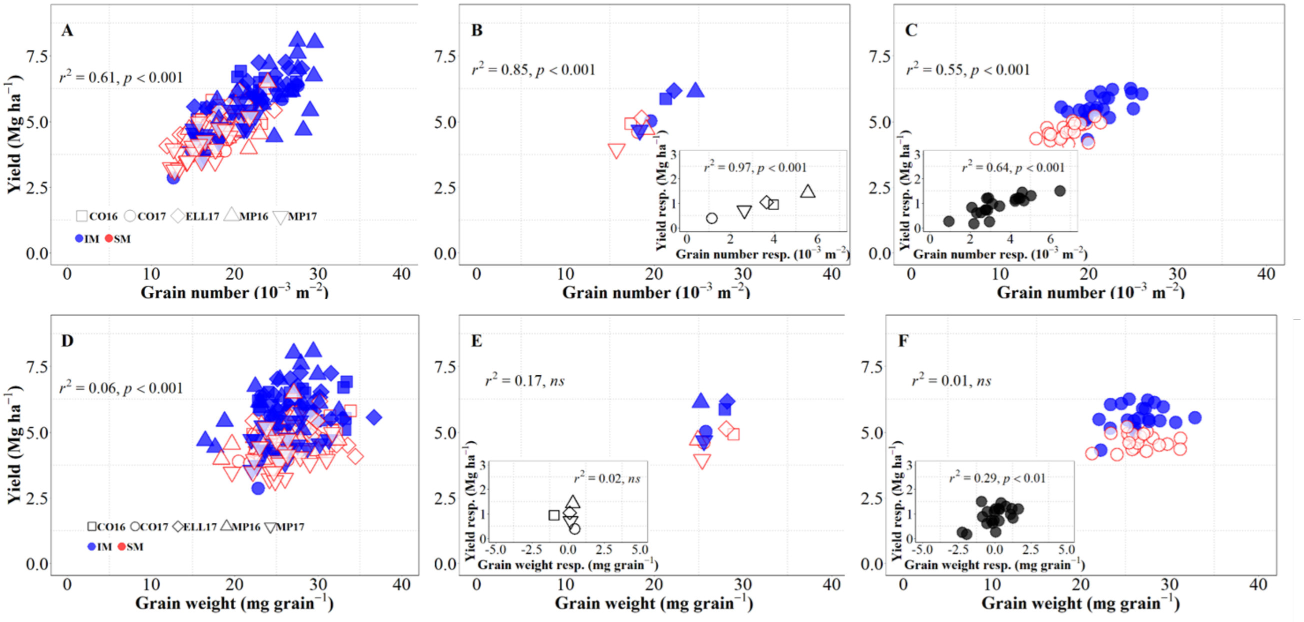

Figure 2.3 Relationship between yield versus grain number and grain weight at maturity across environments, genotypes and management systems intensive management (IM) and standard management (SM) (*n*=210) (A, D), on average of genotypes for each site-year (B, E) (*n*=10), and on average of site-years for each genotype (C, F) (*n*=42). Insets are the relationships between the responses of the variables to intensive management (difference in the variable between IM and

SM) averaged across either genotypes for each site-year (B, E insets) (*n*=5) or site-years for each genotype (C, F insets) (*n*=21).

There was an overall positive relationship between yield and N uptake at maturity. Differences in N uptake explained 64% of the variation in yield across background environments, genotypes, and management systems (Fig. 4A). By dissecting the N uptake into shoot N concentration and biomass, we observed that differences in N uptake due to IM across site-years and genotypes were due to greater shoot N concentration under IM as compared to SM as biomass levels increased (supplementary Fig. B3). Conversely, changes in NUtE did not explain overall differences in yield across the entire dataset (Fig. 4D). Considering only the differences between environments, there was a strong positive relationship reflecting that differences in yield among site-years were largely due to differences in N uptake across and within management systems (Fig. 4B). Differences between sites-years in yield response to IM were related to their differences in N uptake response to IM (Fig. 4B, inset). On the other hand, differences in yield between environments were not explained by their differences in NUtE (Fig. 4E). In fact, there was a trend ($p=0.06$) for site-years with higher yields to exhibit lower levels of NUtE (Fig. 4E) and yield responses to IM of the different site-years was not mediated through NUtE response (Fig. 4E, inset). Considering the differences between genotypes across management systems, there was also a positive relationship between yield and N uptake (Fig. 4C), and differences among genotypes in yield response to IM were preceded by their differences in responses of N uptake (Fig. 4C, inset). Yield differences between genotypes across management systems were not related to differences in NUtE (Fig. 4F), but genotypic differences in yield within each management system were well explained by NUtE (Fig. 4F) ($p<0.05$, $R^2= 0.78$ for IM and $R^2=0.26$ for SM). Although genotypic differences in yield response to IM were significantly related to their response in terms of both N uptake and NUtE, the former was the determinant of yield response, as NUtE was actually reduced (with most of values of NUtE response near or below zero) by intensifying management, partly compensating for the larger effect of management on N uptake relative to yield (Fig. 4F, inset)

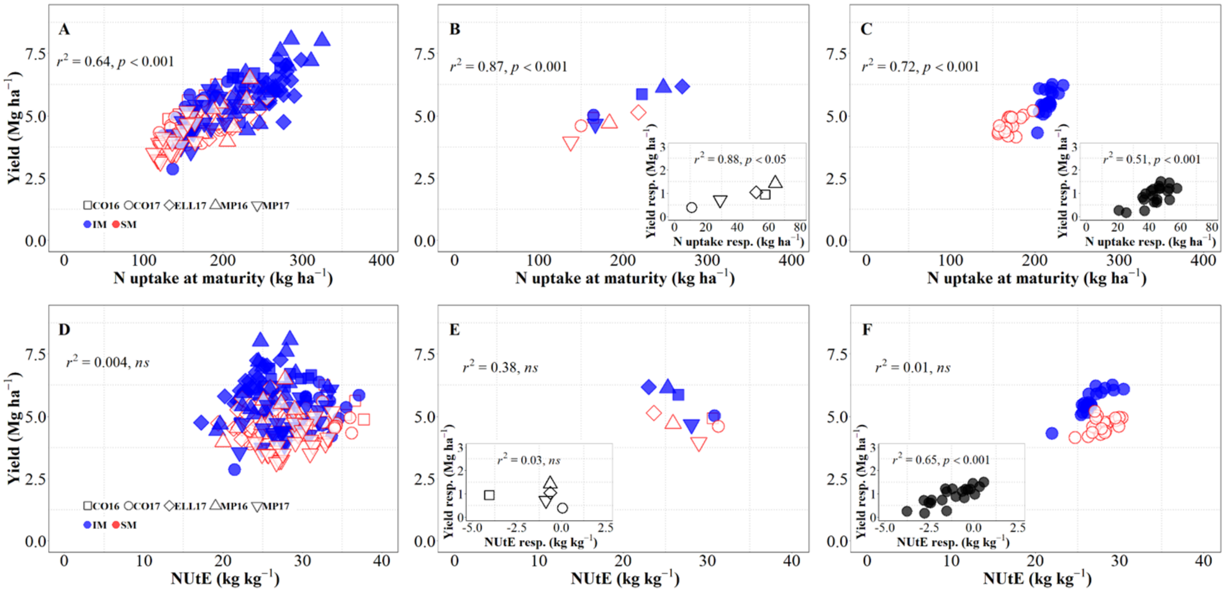

Figure 2.4 Relationship between yield versus nitrogen uptake and utilization efficiency at maturity across environments, genotypes and management systems intensive management (IM) and standard management (SM) (*n*=210) (A, D), on average of genotypes for each site-year (B, E) (*n*=10), and on average of site-years for each genotype (C, F) (*n*=42). Insets are the relationships between the responses of the variables to intensive management (difference in the variable between IM and SM) averaged across either genotypes for each site-year (B, E insets) (*n*=5) or site-years for each genotype (C, F insets) (*n*=21).

The relationship between grain N concentration and yield was weak when considering all sources of variation together and IM improved both yield and grain N concentration as compared to SM, reducing the dilution of N in the grain (Fig. 5A). This lack of relationship is actually hiding two contrary relationships, depending on whether the source of variation was site-years or genotypes. When considering the differences in site-years and management systems, the relationship was significantly positive, with changes in yield explaining 64% of the variation in grain N concentration across site-years and management systems (Fig. 5B), mainly because IM improved both yield and grain N concentration in all five site-years (Fig. 5B, inset). Conversely, changes in grain N concentration were not explained by differences in yield of genotypes considering both management systems together, though there was a significant negative relationship within management systems (Fig. 5C) ($p<0.05$; $R^2=0.33$ for IM and $R^2=0.20$ for SM). This implies that within management systems there was a general trend for higher-yielding cultivars to dilute the N in the grain and *vice-versa*. The fact that the relationship was not maintained when considering genotypes × management together reflects the positive effect of the IM system on both yield and grain N concentration. This may seem at odds with the fact that grain N concentration response to IM was negatively related to yield response of genotypes to management (Fig. 5C, inset). However, the data were all in the positive quadrant: IM increased yields and grain N concentration of all genotypes; although there was a general trend for cultivars more responsive in yield to be less responsive in grain N concentration (Fig. 5C, inset). Within each management system encompassing all sources of variation, the IM increased yield and maintained similar levels of grain N concentration while for SM there was a clear penalty in grain N concentration as yield increased (supplementary Fig. B4 and Table B1).

Yield (in terms of grain dry matter) was consequently a strong determinant of the total amount of N harvested (grain N uptake). Considering the overall variation due to background environments, genotypes and management systems, changes in yield explained 86% of the variation in grain N uptake (Fig. 6A). This relationship was also very strong when focusing on either environment, both across and within management systems (Fig. 6B), or genotypes (Fig. 6C). The differences in grain N uptake response to IM, both between site-years (Fig. 6B, inset) and between genotypes (Fig. 6C, inset), mimicked the corresponding differences in yield responses.

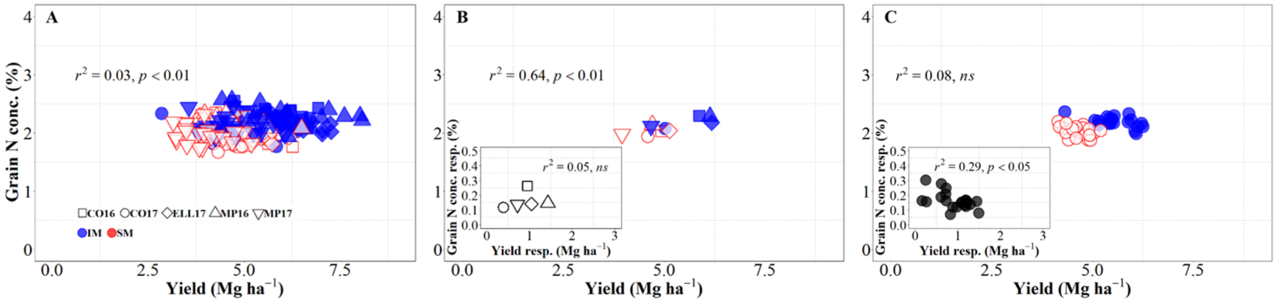

Figure 2.5 Relationship between grain nitrogen (N) concentration and yield across environments, genotypes and management systems [intensive management (IM) and standard management (SM)] (*n*=210) (A), on average of genotypes for each site-year (B) (*n*=5), and on average of site-years for each genotype (C) (*n*=42). Relationship between grain N concentration and yield responses to IM on average of genotypes for each site-year (B inset) (*n*=5) and on average of site-years for each genotype (C inset) (*n*=21).

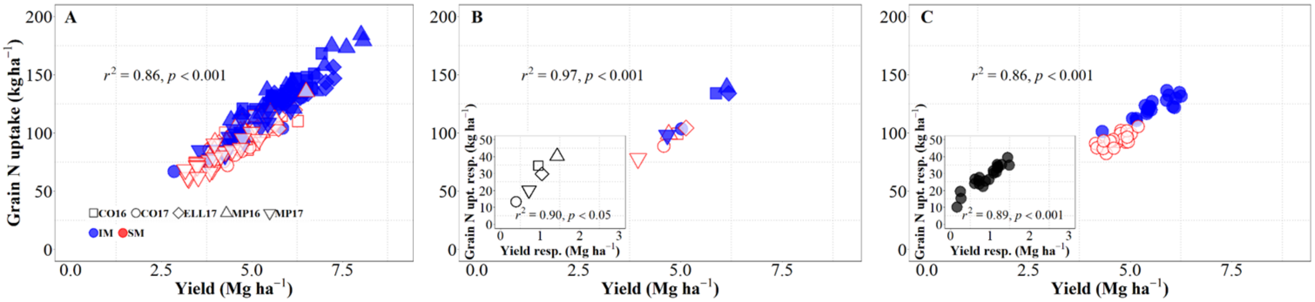

Figure 2.6 Relationship between grain nitrogen (N) uptake and yield across environments, genotypes and management systems [intensive management (IM) and standard management (SM)] (*n*=210) (A), on average of genotypes for each site-year (B) (*n*=10), and on average of site-years for each genotype (C) (*n*=42). Relationship between grain N concentration and yield responses to IM on average of genotypes for each site-year (B inset) (*n*=5) and on average of site-years for each genotype (C inset) (*n*=21).

Genotypic differences in yield response consistency

We restricted the analysis of the data so far to recognize differences and relationships across all sources of variation together or focusing on general responses to IM across sites-years (with averages across genotypes for each background condition) or across genotypes (with averages across background conditions for each genotype). This was done in order to determine whether an intensification of rainfed wheat management in Kansas would generally result in increased achievable yields and to assess the consistency of the outcomes (the first aim of the study). Nevertheless, genotypes varied specifically in their adaptation and responsiveness to IM. Examining overall responsiveness to IM was critical to draw general conclusions but also masked specific responses of particular genotypes. In this section we dissected these genotype-specific responses to IM, considering not only their responsiveness to IM but also their response consistency.

As mentioned above, we observed a generalized increase in yield due to IM in all genotypes, but with noticeable differences in magnitude and significance of the response (i.e. across all site-years yield increased between c. 0.2 and 1.5 Mg ha^{-1}; this overall increase was statistically significant in 16 genotypes whilst only a trend in 5 genotypes; Figs. 1C). This is reinforced by analyzing the yield of each of the 21 genotypes averaged across sites-years under both management systems (Fig. 7A). As expected from overall results previously presented (Fig. 1C), there was a considerable diversity in performance within each of the management systems, all data-points were above the 1:1 ratio (implying that all cultivars exhibited higher average yield under IM than under SM), and the performance of cultivars under IM depended largely on their responsiveness to intensification of the management (Fig. 7B). It is relevant that performance of cultivars under IM was generally consistent with their performance under SM (in general, low- and high-yielding cultivars under IM were also low- and high-yielding cultivars under SM; Fig. 7A). Even though the coefficient of determination was statistically highly significant, diversity in achievable yield and responsiveness to IM was still agronomically very significant, as evidenced by the 67% of the variation in IM not explained by that in SM. Thus, the overall response to IM across site-years included genotypes with relatively low responsiveness having either low (e.g., LCS Chrome), intermediate (e.g., 1863) or relatively high yield (e.g., Zenda) under SM; as well as genotypes with high responsiveness with either of the yield scenarios in SM (e.g. LCS Pistol, WB4458, Larry) (Fig. 7A). Thus, the yield responsiveness to IM of the genotypes was largely unrelated to their performance under SM (Fig. 7C), indicating that overall responsiveness to IM was mostly independent of adaptation to current management practices and thus achievable yield was strongly dependent upon the inherent genotypic responsiveness to IM (Fig. 7B; please note that not only was the coefficient of determination highly significant but also that the slope was very close to one). Not only did genotypes vary in overall responsiveness to IM across site-years but also their differences in responsiveness were largely unrelated to their consistency in response to IM (inversely assessed by the standard deviation of their average response; Fig. 7D). Although instability in response of the genotype did not contribute to the average yield in IM, it was naturally relevant to achieve the maximum yields that were equally related to the average response across sites-years and the instability in the response (Supplementary Fig. B5). Being the variability in response (measured by the standard deviation of yield response to management) independent of the mean yield response (Fig. 7D), maximum yields shall be obtained by genotypes combining a high average response and a high variability in response (Fig. B5).

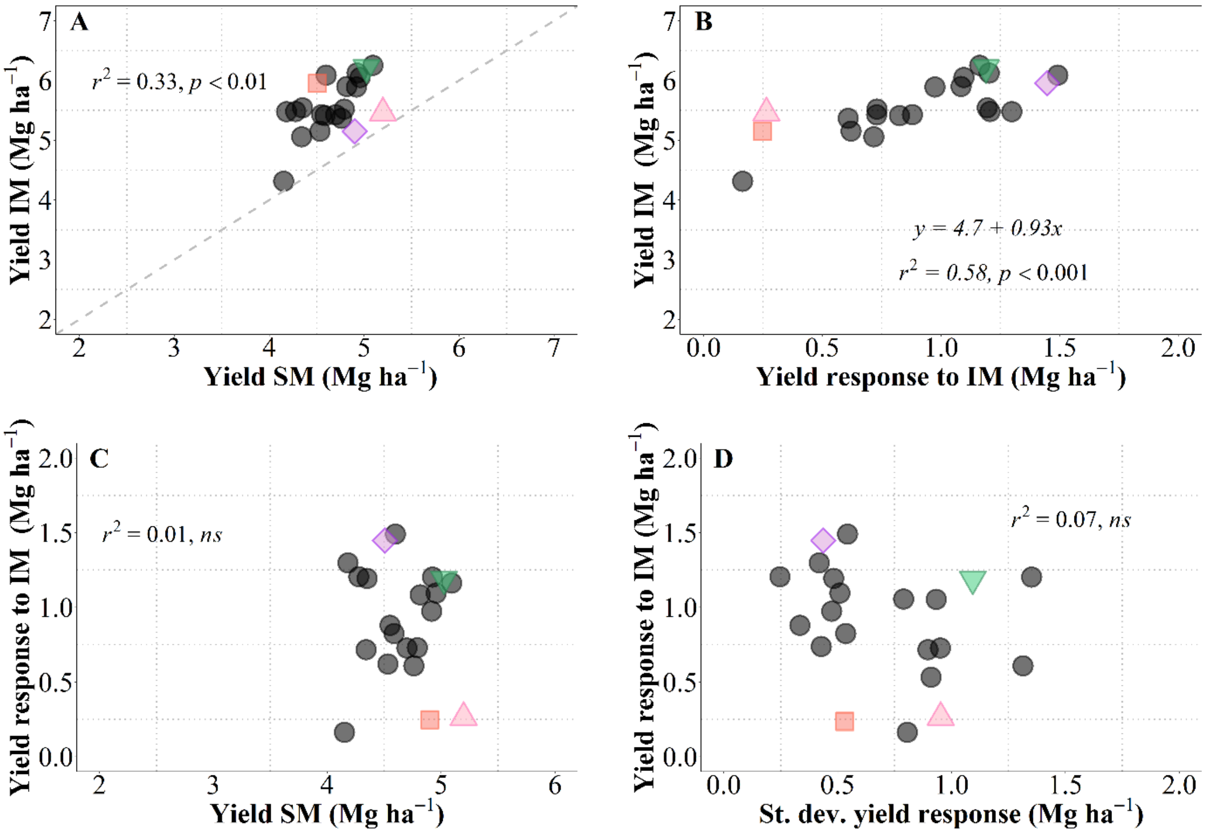

Figure 2.7 Relationship between mean yield under intensive (IM) versus standard management (SM) for the 21 genotypes tested averaged across site-years (A). Relationship between mean yield under IM and yield response to IM (i.e. yield IM minus yield SM) (B). Relationships between yield response to IM and either mean yield of SM (C), or standard deviation of the yield response to IM (D). The different symbols shows four genotypes selected to represent contrasting behaviors in terms of average responsiveness to intensive management (IM) and in stability of that responsiveness across all site-years selected genotypes, Zenda (triangle), Larry (inverted triangle), 1863 (square) and WB4458 (rhombus).

To illustrate the issue in more detail, we selected four cultivars representing contrasting average response to IM and contrasting stability in the response (Fig. 7D). Cultivars 1863 and Zenda had both a small overall responsiveness but contrasted noticeably in consistency. Cultivar 1863 showed positive responses in four out of the five site-years, although with relatively small increases (from 0.18 to 0.87 Mg ha^{-1}) and, in an exceptional case, showed a yield penalty though the magnitude was small (c. 0.52 Mg ha^{-1}; Fig. 8). On the other hand, due to its instability in response Zenda had c. 1 Mg ha^{-1} decrease in yield in Conway 2017 but also more than 1 Mg ha^{-1}

yield gain in both Conway 2016 and McPherson 2016, and marginal responses in the other two environments; Fig. 8). The same sort of lack of uniformity in consistency across sites-years was evident for genotypes with larger average responsiveness. For instance, cultivars such as WB4458 had simultaneously high and stable responsiveness to IM (Fig. 7D), therefore responding with noticeable improvements in yield across all five site-years (ranging in response from 1 to 2 Mg ha^{-1}; Fig. 8). Meanwhile, genotypes such as Larry were highly responsive to management on average, but their response was not stable across site-years, with a very large response in some environments (>2 Mg ha^{-1} yield gain in McPherson 2016 and 17), a high response in other environments (>1 Mg ha^{-1} gain in Conway 2016), but mostly unresponsive in the other two site-years (Fig. 8).

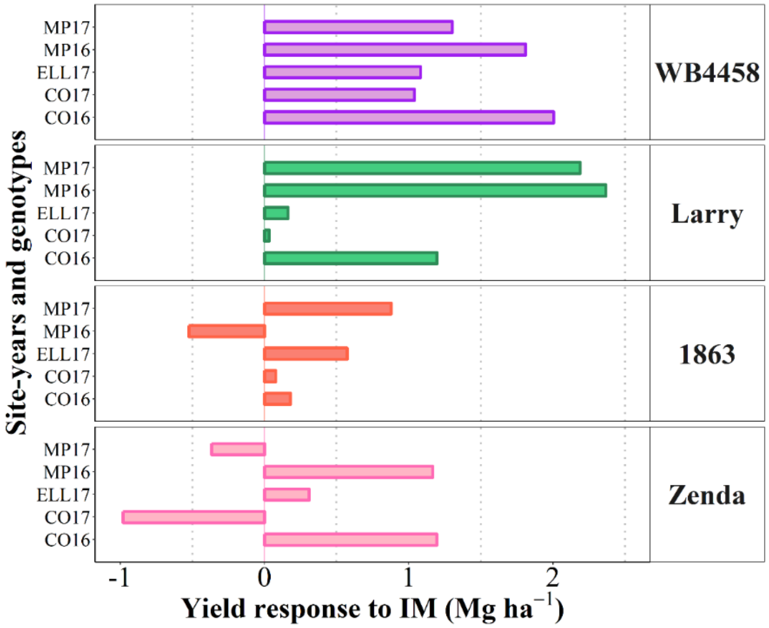

Figure 2.8 Yield response to intensive management (IM) for the selected contrasting genotypes at each individual site-year.

Discussion

Results reported in this paper come from a study carried out in real farmers' fields. Working in realistic farming systems instead of carrying out experiments in experimental stations implies accepting restrictions in experimental procedures and produce 'noisier' datasets, but has a clear advantage when conclusions are expected to be pertinent (Rzewnicki et al., 1988). Moreover, conclusions were reached based on a very simplistic approach of applying a single intensification measure against what farmers were actually doing regardless of the particular situation. The aim was to test yield responses to management across different site-years to determine whether farmers are too conservative and thus missing opportunities of achieving greater yields. Naturally, an optimal level of intensification would likely be different for particular fields. Therefore, this paper does not contribute a tool to define the level of intensification required but only to uncover whether or not the current level of intensification is too conservative, evidencing whether or not there are opportunities to increase yield from the baseline. Similar to our data, several studies have registered average achievable yield for the region of c. 5.5 Mg ha^{-1} in field experiments (Jaenisch et al., 2019; Lollato and Edwards, 2015) , simulation studies (Lollato et al., 2017), and survey of yield contest fields (Lollato et al., 2019b).

Intensifying management to increase rainfed wheat yield

Intensification of management practices and adoption of genotypes highly responsive to management can contribute to increasing wheat yields required for achieving food security, while improving the relatively low N use efficiency of production systems (Raun and Johnson, 1999). However, following a more conservative approach, dryland-wheat producers have been traditionally reluctant to intensify crop management and frequently prefer growing "stable" genotypes that are expected to perform relatively well under conservative conditions but are less responsive when under better growing conditions (i.e. intensified management, fertile soils, etc.).

Climate variability affects the performance of genotypes and their response to management, challenging an effective implementation of management practices across seasons. Changes in precipitation (e.g. amount, intensity, and timing) and temperature patterns may interfere with crop adaptation (Reynolds and Ortiz, 2010), availability of resources (Chloupek et al., 2004), and enable conditions for pests to develop (Agrios, 2005; Legrève and Duveiller, 2010). Although the factors above may explain the variation in yield response to management across site-years, there was no single background condition in our study in which wheat yield, averaged across the 21 cultivars considered, decreased in response to IM. This suggests that, for the background environments evaluated, an excessively conservative attitude regarding the intensification of agronomic management is restricting farmers-yield in the region. Similar results were shown for rainfed wheat in other dryland regions (Connor et al., 2011; McDonald, 1989) as well as in other studies in the same region (Dorsey, 2014; Jaenisch et al., 2019; Lollato et al., 2019b). While we characterized the physiological basis of yield response to IM, future studies could focus on yield comparisons between IM and SM on a large number of fields to determine the most often probability of yield response and perhaps the magnitude of the yield gap.

Adequate N availability during the growing season is critical for wheat grain yield and quality (Entz and Fowler, 1989). There is usually a curvilinear relationship between yield and N rate (Simpson et al., 2016), but this relationship depends on yield potential (Savin et al., 2019) and might be linear or non-existent (Lollato et al., 2019b). In the present study, yield was improved due to N rate and positively associated with higher N uptake and grain number, similar to previous reports which also suggested an increase in water use-efficiency (Entz and Fowler, 1989). Determining the agronomic optimum N rate is challenging in rainfed cereal production due to the variability in growing season precipitation and yield potential (Lollato et al., 2017), and leads to a dominant producer-mindset based on Liebig's '*law of the minimum*' that induces to under-fertilize (Connor et al., 2011). Thus assuming (correctly) that water is commonly the most stressful factor limiting yield, it is overlooked that N availability may well improve water use and water use efficiency (Cossani et al., 2012; Sadras, 2004). The other factor supporting reluctance to fertilize rainfed wheat is the idea that it may bring about 'haying-off' (i.e. an expected negative yield response to N fertilization of dryland wheat; van Herwaarden et al., 1998). However, it seems that this effect has been consistently reported only in Eastern Australia; as in other dryland regions this yield penalty is not evidenced beyond exceptional cases, and yield gains are frequently reported (Asseng and van Herwaarden, 2003; Cossani et al., 2011; Palta and Fillery, 1995) in line with results reported herein, with the exception of the cultivars with low overall responsiveness that may eventually exhibit a yield penalty (once again the 'conservative' attitude of selecting "stable" cultivars induced to the very few cases of 'haying-off' reported in the present study.

Moreover, the appearance of new populations of fungal diseases able to break genetic resistance of modern wheat genotypes (Chen, 2005) can result in need of fungicide application, in some cases even for relatively new cultivars that are expected to be resistant. The magnitude of yield loss from lack of fungicide varies according to the disease pressure, weather, fungicide management (i.e. timing and source), and genetic resistance (Benin et al., 2017; Lopez et al., 2015; Thompson et al., 2014). Naturally, years with considerable disease pressure will result in greater yield response to fungicide (Cruppe et al., 2017; Jaenisch et al., 2019) on cultivars susceptible to the most prevalent disease in the season (Thompson et al., 2014). However, we showed that yield advantages of a management intensification, including fungicide protection, produced yield gains across a range of sites-years and modern cultivars. This indicates that in most conditions of this dryland region, the penalty imposed by foliar diseases would be significant (at least within the site-years evaluated in this study and other years with similar growing conditions). Furthermore, we found a positive relationship between the yield response to IM and the achievable yield under IM, which agrees with literature suggesting that the magnitude of responses to N and fungicide applications depend on the environmental yield potential of the growing season (Cruppe et al., 2017; Lollato et al., 2019b). Thus, it seems that the consequences of the aversion to risk are worse in conditions of higher achievable yield, which can be detrimental for further yield progress.

Relevance of yield determining traits in the response of wheat to intensive management

The magnitude and consistency of yield response to agronomic management can vary due to physiological aspects (e.g. ability to produce greater yields per unit of N supplied [NUE]) (Russell et al., 2017) and adaptation patterns of genotypes across different environmental conditions (Barraclough et al., 2010; Chloupek et al., 2004). In line with our results, other studies have found that genotypes more responsive to N management have greater biomass accumulation and N uptake at maturity (Kanampiu et al., 1997), and that their differences in yield are associated with differences in HI through differences in grain number produced per unit area (Calderini et al., 1995). The response of genotypes to N can be associated with their high yield potential and N use efficiencies (Ortiz-Monasterio et al., 1997). Grain yield improvements due to N management was achieved by increasing N uptake at maturity (López-Bellido et al., 2005), through improving N uptake efficiency (Barraclough et al., 2010) or utilization efficiency of genotypes (Cossani et al., 2012). However, reduction in NUtE are expected when improvements in N uptake from management occur at larger magnitude relative to yield (Gaju et al., 2011). In our data, yield increases due to IM occurred through improvements in N uptake, and the greater increase in N uptake from IM relative to yield reflected a reduction in NUtE. Although IM improved both yield and grain N concentration, genotypes with large yield gain from IM showed a reduction in grain N concentration (Giunta et al., 2019; Lollato et al., 2019a). Overall, our experiments were conducted during two growing seasons resulting in overall low grain protein concentration under SM and improved grain protein under IM, suggesting an opportunity to increase yield and maintain quality with IM. Previous research has proposed a critical value for grain protein concentration of 11.5% above which yield is not limited by N for hard red winter wheat in the region (Goos et al., 1982). In our study, average grain protein concentration for SM and IM were 11.5% and 12.5%, respectively. Thus, considering the narrower range of yield values (from 0.7 to 4 Mg ha^{-1}) in the latter study as compared to our data (from 3 to 8 Mg ha^{-1}) and the N dilution process in larger grain dry matter (Justes et al., 1994), we could postulate that yield was somewhat limited by N under SM in our study. A broader range of N rates would have to be tested to definitively make such conclusions.

Top-dress N application at late tillering stages improves yield by increasing grain number per unit area (Ercoli et al., 2013). Therefore, yield differences among genotypes are usually explained by differences in grain number as compared to grain weight at maturity (Arduini et al., 2006). The larger plasticity of grain number relative to grain weight (Sadras and Slafer, 2012; Wang et al., 2017) likely plays a role in this observation and may clarify our findings where grain number was the main yield component contributing to the response of genotypes to management (Slafer et al., 2014). Furthermore, the possible increase in late-season tiller production and survival from the N and fungicide applications may have resulted in additional formation of smaller spikes with smaller grains. Thus, the overall decrease in grain weight due to IM could be attributed to the larger number of smaller grains resulting from the late tillers, consequently decreasing the overall average grain weight in the IM relative to the SM (see Acreche and Slafer, 2006).

In general, the impacts of management on the performance of genotypes are evaluated for a small set of genotypes (Russell et al., 2017), and information about the scope of physiological determinants of genotypic responsiveness to management is usually limited. Our study utilized a large set of modern wheat genotypes differing in agronomic traits and genetic origin and characteristics, and thus, it provides insights on physiological mechanisms associated with response to the management of modern winter wheat genotypes.

Producers could consider approaches regarding the risks of intensifying management. The more risky approach is to grow genotypes with high average responsiveness to management and high variability on the response (i.e. unstable, as the standard deviation of the response was positively related to yield under IM, Fig. B5) while the less risky approach is to grow genotypes with high mean response but stable yields in response to management. The former indicates that farmers who are willing to accept some risks to maximize productivity should select genotypes with unstable response, as those are the ones that maximize yield when the conditions favor response. In general, high yielding genotypes tended to be more unstable although with greater chance to maximize yield than low-yielding genotypes (the concept of stability can be also seen as lack of responsiveness to improvements in growing conditions; Calderini and Slafer, 1999). This is similar to the findings of Grogan et al. (2016) in which phenotypic plasticity (or the opposite of stability) of grain yield was a positive trait for 299 hard red winter wheat genotypes evaluated in the Great Plains. Indeed, breeding programs tend to select under more favorable conditions than those representing the average of the target population of environments in which the released cultivars are to be grown (Box1). This is because cultivars of higher yield potential tend to outyield low-yield potential cultivars under a rather wide range of conditions (Slafer et al., 2005 and references quoted therein). Understanding the physiological bases at the crop level of organization determining yield can help guide breeding to select prospective parents to produce strategic crosses aiming to increase the genetic gains in yield (Box 2), which would in turn require higher levels of intensification of management to reach the achievable yield of the newer cultivars produced. Thus, through understanding performance and responsiveness capacity of new genotypes, breeding programs would be more likely to identify genotypes with relatively good yield under standard conditions, but highly responsive when resources are available.

Box 1. Relevance of high-yielding selection environment

Data collected in the current study allowed us to discuss on the convenience for breeding programs to select in growing conditions that are as close as possible to those of the target environments in which released cultivars are to be grown or otherwise under better growing conditions (i.e. within the best yielding conditions that can be expected in the region). For this purpose we related the overall performance in the region for each individual cultivar with the yield of each cultivar in one particular condition. To take into account the overall performance of each genotype we calculated their average yield across nine growing conditions (all locations × years × management systems, but the particular condition that was used to predict the overall performance). These particular conditions were (i) the lowest-yielding environment, in which the most resilient genotypes would perform best; (ii) the growing condition producing an average yield closest to the overall average yield of the 10 environments; or (iii) the highest-yielding environment, in which the cultivars with the highest achievable yield would perform best (Fig. B1, from left to right, respectively).

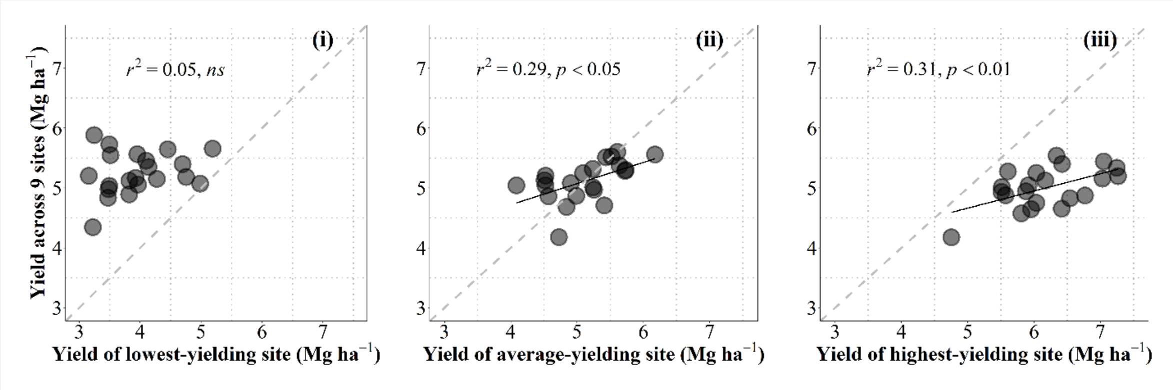

Figure B1. Relationship between the overall average yield across all environments but the one being used as independent variable and yield under the lowest-yielding, mean- and highest-yielding conditions across the study (from leaf to right) for the 21 cultivars grown in 10 environments of Kansas produced by the combination of locations, growing seasons and management systems. The dashed line stands for the line representing Y=X (i.e. the 1:1 ratio) and solid lines represent the linear regression (when significant).

Naturally data-points fell above and below the line representing the 1-to-1 ratio in the left and right panels, respectively; and around that line when the environment used to predict the overall performance across all other environments was the growing condition with an average yield closest to the overall average yield (Fig. B1).

Overall performance in the region was totally unrelated to the yield in the lowest-yielding condition (Fig. B1, left panel). This implies that the specific characteristics making cultivars particularly adapted (or unadapted) to the most stressful condition did not contribute to the overall performance across the region (in fact the cultivars with the overall highest and lowest yielding were both rather low-yielding in this particular low-yielding condition (Fig. B1 left panel). Prediction of the overall performance from a single condition improved considerably (and became statistically significant) when using yield of an environment closest to the average-yielding growing condition as independent variable (Fig. B1, middle panel). However, prediction of the overall performance from yield of the cultivars in the highest-yielding condition was even better than that from the average-yielding condition (Fig. B1, right panel). Although each of the other environments were more stressful (with different levels of severity), it seemed that some attributes conferring water-limited yield potential somehow also produced a constitutive improved performance under lower-yielding environments. This result justifies that breeding programs select promising lines under field conditions that are frequently higher-yielding than those targeted population of environments in which released cultivars are to be grown. This is in agreement with previous evidence advocating that the selection would be best if performed in high-yielding environments (Cooper et al., 1997). Using an environmental yielding condition representing higher than average yield of those targeted population of environments would likely increase the predictive performance (cf. middle and right panels in Fig. B1).

This result also concurs with the idea that an improved yield potential (that can only be selected for in high-yielding conditions) would bring about improved performance under a range of environments with different degrees of stressful conditions; even though they would be less stable (as high yield potential implies strong responsiveness to better growing conditions; Calderini and Slafer, 1999) they might also perform better than lower-yield potential cultivars (Araus et al., 2008; Cattivelli et al., 2008; Ferrante et al., 2017; Richards, 2000). Indeed, wheats selected in CIMMYT for their high yield potential were released in drought environments (van Ginkel et al., 1998). Furthermore, selecting in higher-yielding conditions would also improve the efficacy of the program through increasing the achieved genetic gains. This is because the expected differences in performance are in line with the average yield of the environment and therefore increase the confidence in the selection process (van Ginkel et al., 1998) and explains why selection for yield in low-yielding conditions slows the progress achieved by the program (Blum, 1988; Richards et al., 2002). An empirical quantitative evidence of this is the reported positive relationship between the genetic gains achieved and the environmental average yield (Calderini et al., 1999; Sadras et al., 2016).

Box 2. Difficulties for achieving significant genetic gains in yield

We analyzed the performance of commercial cultivars. That means that in a traditional historic analysis of yield gains (i.e. considering several decades of breeding), all of them would be uniformly grouped as "modern cultivars" which is relevant when comparing the breeding effect over long periods. However, analyzing the performance of cultivars released over a much shorter period may be relevant to determine the needs for maintaining/changing breeding strategies. Although far less common, analyses of short-term breeding effects (Chairi et al., 2018) are also done for this reason. Cultivars of the current study were released in the US southern Great Plains from 2007 to 2016.

Although a decade may be a rather short period to confidently analyze the performance of breeding programs, it was worrying to see no gains in yield over the whole decade, regardless of the condition in which we estimated these gains (Fig. B2).

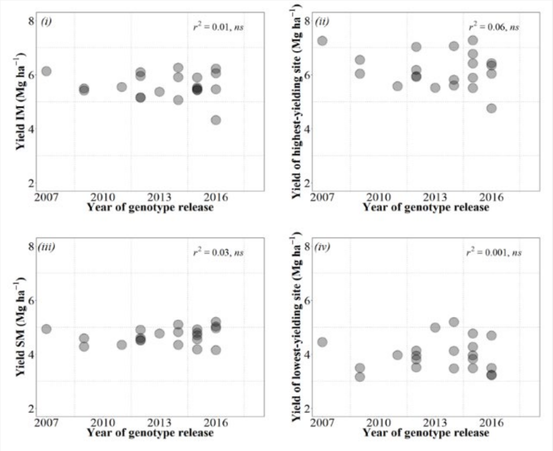

Figure B2. Relationships between yield of the cultivars and their year of release considering yield under IM (top left panel), SM (bottom left panel), averaged across site-years for each management system, as well as under the highest- (top right panel) and lowest-yielding condition (bottom right panel) out of the 20 combinations of site × years × management systems.

This evidence that recent breeding in the US southern Great Plains failed to consistently increase wheat yield is actually further supported by a previous independent study carried out in Kansas recently in which it was shown that there were virtually no yield gains since 1992 (Maeoka, 2019). Furthermore, this does not seem to be a particular case for Kansas. Conclusions derived from some studies considering in particular the most recent yield gains from long-term breeding gains (e.g. Acreche et al., 2008; Flohr et al., 2018; Lo Valvo et al., 2018; M. Sanchez-Garcia et al., 2012) or from studies exclusively focused in the recent past (e.g. Chairi et al., 2018) indicate that recent gains in yield have been much lower than in previous decades and in some cases rather marginal or inexistent. Although part of the failure in actually increasing yields could be attributed to the fact that genetic gains in environments like Kansas, characterized by low and variable yields, are more difficult to achieve (see discussion in Box 1), this may not be the unique cause. The studies analyzing long periods of breeding in other low and variable yield environments (Acreche et al., 2008; Flohr et al., 2018; Lo Valvo et al., 2018; Sanchez-Garcia et al., 2012) all showed clear gains in yield from mid to late 20th century, and the environments then were at least as low-yielding and as variable as they are nowadays (and for that reason they normally exhibited lower genetic gains than in high-yielding environments, but gains were clear; Calderini et al., 1999; Sadras et al., 2016). Thus, the lack of current genetic gains may well mean that a change of strategy may be required to recover the genetic gains, which are clearly needed. Identifying germplasm possessing physiological traits that may contribute to improve yield would be ideal for strategic crosses with increased likelihood of delivering the necessary transgressive segregation required to improve yield. Thus, a physiological approach, where the physiological attributes limiting yield are recognized, complementing empirical breeding might enhance the expected gains in yield (e.g. Richards et al., 2002; Slafer, 2003).

Conclusion

A major conclusion from this study is that the standard management of rainfed wheat in dryland Kansas does consistently fall short of achievable yields, should the management be more intensive through increasing the levels of fertilization and protecting the crop against fungal diseases. In general, yield improvement due to IM was related to a greater N uptake by the crop that brought about increases in biomass accumulation with no major changes in partitioning (and in grains per m^2 with no compensation in average grain weight) determining a simultaneous increase in yield and protein concentration consistently across sites-years analyzed. Identifying crop physiological mechanisms associated with the ability of genotypes to respond to management across different environmental conditions will help to develop efficient production systems, and assist breeding programs on the selection of genotypes with high yield potential and resource use efficiency. Hence, additional N fertilization and foliar fungicide application can help wheat producers to attain achievable yields in dryland systems via improving aboveground biomass and N uptake at maturity while maintaining HI.

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References

Acreche, M. M., Briceño-Félix, G., Sánchez, J. A. M., and Slafer, G. A. (2008). Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. *European Journal of Agronomy* 28, 162–170. doi:10.1016/J.EJA.2007.07.001.

Acreche, M. M., and Slafer, G. A. (2006). Grain weight response to increases in number of grains in wheat in a Mediterranean area. *Field Crops Research* 98, 52–59. doi:10.1016/J.FCR.2005.12.005.

Agrios, G. N. (2005). "Plant disease epidemiology," in *Plant Pathology* (Elsevier Academic Press), 265–291. doi:10.1016/B978-0-08-047378-9.50014-2.

Albrizio, R., Todorovic, M., Matic, T., and Stellacci, A. M. (2010). Comparing the interactive effects of water and nitrogen on durum wheat and barley grown in a Mediterranean environment. *Field Crops Research* 115, 179–190. doi:10.1016/j.fcr.2009.11.003.

Araus, J. L., Slafer, G. A., Royo, C., and Serret, M. D. (2008). Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science* 27, 377–412. doi:10.1080/07352680802467736.

Araya, A., Prasad, P. V. V., Gowda, P. H., Kisekka, I., and Foster, A. J. (2019). Yield and Water Productivity of Winter Wheat under Various Irrigation Capacities. *Journal of the American Water Resources Association* 55, 24–37. doi:10.1111/1752-1688.12721.

Arduini, I., Masoni, A., Ercoli, L., and Mariotti, M. (2006). Grain yield, and dry matter and nitrogen accumulation and remobilization in durum wheat as affected by variety and seeding rate. *European Journal of Agronomy* 25, 309–318. doi:10.1016/j.eja.2006.06.009.

Asseng, S., Turner, N. C., and Keating, B. A. (2001). Analysis of water-and nitrogen-use efficiency of wheat in a Mediterranean climate. *Plant and Soil* 233, 127–143. Available at: http://www.springerlink.com/index/Q240561J12764H82.pdf.

Asseng, S., and van Herwaarden, A. F. (2003). Analysis of the benefits to wheat yield from assimilates stored prior to grain filling in a range of environments. *Plant and Soil* 256, 217–229.

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C. E., et al. (2010). Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy* 33, 1–11. doi:10.1016/j.eja.2010.01.005.

Benin, G., Storck, L., Marchioro, V. S., Bornhofen, E., Woyann, L. G., Trevizan, D. M., et al. (2017). Environment-specific selection to identify high yielding wheat genotypes and response to fungicide application. *Revista Ceres* 64, 167–175. doi:10.1590/0034-737x201764020009.

Blum, A. (1988). Plant breeding for stress environments.

Borghi, B. (1999). “Nitrogen as determinant of wheat growth and yield.,” in *Wheat Ecology and Physiology of Yield Determination*, eds. E. Satorre and G. A. Slafer (Binghamton, NY: Food Products Press), 503.

Calderini, D. F., Dreccer, M. F., and Slafer, G. A. (1995). Genetic improvement in wheat yield and associated traits. A re-examination of previous results and the latest trends. *Plant Breeding* 114, 108–112. doi:10.1111/j.1439-0523.1995.tb00772.x.

Calderini, D. F., Reynolds, M. P., and Slafer, G. A. (1999). “Genetic gains in wheat yield and main physiological changes associated with them during the 20th century,” in *Wheat: Ecology and Physiology of Yield Determination*, eds. E. H. Satorre and G. A. Slafer (New York: Food Product Press), 351–377.

Calderini, D. F., and Slafer, G. A. (1999). Has yield stability changed with genetic improvement of wheat yield? *Euphytica* 107, 51–59. doi:10.1023/A:1003579715714.

Cattivelli, L., Rizza, F., Badeck, F.-W., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., et al. (2008). Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field Crops Research* 105, 1–14. doi:10.1016/J.FCR.2007.07.004.

Chairi, F., Vergara-Diaz, O., Vatter, T., Aparicio, N., Nieto-Taladriz, M. T., Kefauver, S. C., et al. (2018). Post-green revolution genetic advance in durum wheat: The case of Spain. *Field Crops Research* 228, 158–169. doi:10.1016/J.FCR.2018.09.003.

Chen, X. M. (2005). Epidemiology and control of stripe rust [Puccinia striiformis f . sp . tritici] on wheat. *Canadian Journal of Plant Pathology* 27, 314–337. doi:10.1071/ar07045.

Chloupek, O., Hrstkova, P., and Schweigert, P. (2004). Yield and its stability, crop diversity, adaptability and response to climate change, weather and fertilisation over 75 years in the Czech Republic in comparison to some European countries. *Field Crops Research* 85, 167–190. doi:10.1016/S0378-4290(03)00162-X.

Connor, D. J., Loomis, R. S., and Cassman, K. . (2011). *Crop Ecology: productivity and management in agricultural systems*. Ed.2. New York: Cambridge University Press.

Cooper, M., Stucker, R. E., DeLacy, I. H., and Harch, B. D. (1997). Wheat Breeding Nurseries, Target Environments, and Indirect Selection for Grain Yield. *Crop Science* 37, 1168. doi:10.2135/cropsci1997.0011183X003700040024x.

Cossani, C. M., and Sadras, V. O. (2018). *Water–Nitrogen Colimitation in Grain Crops*. 1st ed. Elsevier Inc. doi:10.1016/bs.agron.2018.02.004.

Cossani, C. M., Slafer, G. A., and Savin, R. (2010). Co-limitation of nitrogen and water, and yield and resource-use efficiencies of wheat and barley. *Crop and Pasture Science* 61, 844. doi:10.1071/CP10018.

Cossani, C. M., Slafer, G. A., and Savin, R. (2012). Nitrogen and water use efficiencies of wheat and barley under a Mediterranean environment in Catalonia. *Field Crops Research* 128, 109–118. doi:10.1016/j.fcr.2012.01.001.

Cossani, C. M., Thabet, C., Mellouli, H. J., and Slafer, G. A. (2011). Improving wheat yields through N fertilization in Mediterranean Tunisia. *Experimental Agriculture* 47, 459–475. doi:10.1017/S0014479711000044.

Cruppe, G., Edwards, J. T., and Lollato, R. P. (2017). In-season canopy reflectance can aid fungicide and late-season nitrogen decisions on winter wheat. *Agronomy Journal* 109, 2072–2086. doi:10.2134/agronj2016.12.0720.

De Wit, C. T. (1992). Resource Use Efficiency in Agriculture. *Agricultural Systems* 40, 125–151. Available at: https://library.wur.nl/WebQuery/wurpubs/fulltext/197660 [Accessed April 11, 2019].

DeWolf, E., Lollato, R. P., and Whitworth, R. J. (2017). Wheat Variety Disease and Insect Ratings 2017. MF991. Available at: https://www.bookstore.ksre.ksu.edu/pubs/MF991.pdf.

Dorsey, N. D. (2014). Nitrogen use efficiency and nitrogen response of wheat varieties commonly grown in the Great Plains,USA.

Entz, M. H., and Fowler, D. B. (1989). Response of winter wheat to N and water: Growth, water use, yield and grain protein. *Canadian Journal of Plant Science* 69, 1135–1147.

Ercoli, L., Masoni, A., Pampana, S., Mariotti, M., and Arduini, I. (2013). As durum wheat productivity is affected by nitrogen fertilisation management in central Italy. *European Journal of Agronomy* 44, 38–45. doi:10.1016/j.eja.2012.08.005.

Evans, L. T., and Fischer, R. A. (1999). Yield Potential: Its Definition, Measurement, and Significance. *Crop Science* 39, 1544–1551.

FAO-AMIS (2018). Food and Agriculture Organization of the United Nations - Agricultural market information system. Available at: http://statistics.amis-outlook.org/data/index.html#DOWNLOAD. [Accessed February 20, 2018].

FAO (2003). Food and Agriculture Organization of the United Nations . Agriculture, food and water. 61. Available at: http://www.fao.org/3/Y4683E/y4683e00.htm#P-1_0 [Accessed April 10, 2019].

Ferrante, A., Cartelle, J., Savin, R., and Slafer, G. A. (2017). Yield determination, interplay between major components and yield stability in a traditional and a contemporary wheat across a wide range of environments. *Field Crops Research* 203, 114–127. doi:10.1016/J.FCR.2016.12.028.

Ferrante, A., Savin, R., and Slafer, G. A. (2013). Floret development and grain setting differences between modern durum wheats under contrasting nitrogen availability. *Journal of Experimental Botany* 64, 169–184. doi:10.1093/jxb/ers320.

Ferreira, E. B., Cavalcanti, P. P., and Nogueira, D. A. (2018). ExpDes: Experimental Designs. R package version 1.2.0. Available at: https://cran.r-project.org/package=ExpDes.

Flohr, B. M., Hunt, J. R., Kirkegaard, J. A., Evans, J. R., Swan, A., and Rheinheimer, B. (2018).

Genetic gains in NSW wheat cultivars from 1901 to 2014 as revealed from synchronous flowering during the optimum period. *European Journal of Agronomy* 98, 1–13. doi:10.1016/J.EJA.2018.03.009.

Gaju, O., Allard, V., Martre, P., Snape, J. W., Heumez, E., LeGouis, J., et al. (2011). Identification of traits to improve the nitrogen-use efficiency of wheat genotypes. *Field Crops Research* 123, 139–152. doi:10.1016/j.fcr.2011.05.010.

Giunta, F., Pruneddu, G., and Motzo, R. (2019). Grain yield and grain protein of old and modern durum wheat cultivars grown under different cropping systems. *Field Crops Research* 230, 107–120. doi:10.1016/J.FCR.2018.10.012.

Goos, R. J., Westfall, D. G., Ludwick, A. E., and Goris, J. E. (1982). Grain Protein Content as an Indicator of N Sufficiency for Winter Wheat. *Agronomy Journal* 74, 130–133. doi:10.2134/agronj1982.00021962007400010033x.

Grogan, S. M., Anderson, J., Baenziger, P. S., Frels, K., Guttieri, M. J., Haley, S. D., et al. (2016). Phenotypic Plasticity of Winter Wheat Heading Date and Grain Yield across the US Great Plains. *Crop Science* 56, 2223–2236. doi:10.2135/cropsci2015.06.0357.

Hochman, Z., Gobbett, D. L., and Horan, H. (2017). Climate trends account for stalled wheat yields in Australia since 1990. *Global Change Biology* 23, 2071–2081. doi:10.1111/gcb.13604.

Højsgaard, S., and Halekoh, U. (2016). doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. *R package version 4.5-15*. Available at: https://cran.r-project.org/package=doBy.

Jaenisch, B. R., de Oliveira Silva, A., DeWolf, E., Ruiz-Diaz, D. A., and Lollato, R. P. (2019).

Plant population and fungicide economically reduced winter wheat yield gap in Kansas. *Agronomy Journal* 111, 650–665. doi:10.2134/agronj2018.03.0223.

Justes, E., Mary, B., Meynard, J.-M., Machet, J.-M., and Thelier-Huche, L. (1994). Determination of a Critical Nitrogen Dilution Curve for Winter Wheat Crops. *Annals of Botany* 74, 397–407. doi:10.1006/anbo.1994.1133.

Kanampiu, F. K., Raun, W. R., and Johnson, G. V. (1997). Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties. *Journal of Plant Nutrition* 20, 389–404. doi:10.1080/01904169709365259.

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models . *Journal of Statistical Software* 82. doi:10.18637/jss.v082.i13.

Legrève, A., and Duveiller, E. (2010). "Preventing potential disease and pest epidemics under changing climate," in *Climate Change and Crop Production*, ed. M. P. Reynolds (CABI), 292.

Leikam, D. F., Lamond, R. E., and Mengel, D. B. (2003). Soil Test Interpretations and Fertilizer Recommendations MF2586. Available at: www.oznet.ksu.edu/agronomy/soiltesting/ [Accessed April 19, 2019].

Lo Valvo, P. J., Miralles, D. J., and Serrago, R. A. (2018). Genetic progress in Argentine bread wheat varieties released between 1918 and 2011: Changes in physiological and numerical yield components. *Field Crops Research* 221, 314–321. doi:10.1016/J.FCR.2017.08.014.

Lollato, R. P., and Edwards, J. T. (2015). Maximum attainable wheat yield and resource-use efficiency in the southern great plains. *Crop Science* 55, 2863–2876. doi:10.2135/cropsci2015.04.0215.

Lollato, R. P., Edwards, J. T., and Ochsner, T. E. (2017). Meteorological limits to winter wheat productivity in the U.S. southern Great Plains. *Field Crops Research* 203, 212–226. doi:10.1016/j.fcr.2016.12.014.

Lollato, R. P., Figueiredo, B. M., Dhillon, J. S., Arnall, D. B., and Raun, W. R. (2019a). Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: A synthesis of long-term experiments. *Field Crops Research* 236, 42–57. doi:10.1016/J.FCR.2019.03.005.

Lollato, R. P., Ruiz Diaz, D. A., DeWolf, E., Knapp, M., Peterson, D. E., and Fritz, A. K. (2019b). Agronomic practices for reducing wheat yield gaps: A quantitative appraisal of progressive producers. *Crop Science* 59, 333–350. doi:10.2135/cropsci2018.04.0249.

López-Bellido, L., López-Bellido, R. J., and Redondo, R. (2005). Nitrogen efficiency in wheat under rainfed Mediterranean conditions as affected by split nitrogen application. *Field Crops Research* 94, 86–97. doi:10.1016/j.fcr.2004.11.004.

Lopez, J. A., Rojas, K., and Swart, J. (2015). The economics of foliar fungicide applications in winter wheat in Northeast Texas. *Crop Protection* 67, 35–42. doi:10.1016/J.CROPRO.2014.09.007.

Maeoka, R. E. (2019). Changes in winter wheat (Triticum aestivum L.) phenotype in response to breeding for yield and in-furrow fertilizer. Available at: https://krex.k-state.edu/dspace/bitstream/handle/2097/39656/RafaelMaeoka2019.pdf?sequence=1&isAllowed=y [Accessed July 11, 2019].

McDonald, G. K. (1989). The contribution of nitrogen fertiliser to the nitrogen nutrition of rainfed wheat crops in australia: A review. *Australian Journal of Experimental Agriculture* 29, 455–481. doi:10.1071/EA9890455.

Moll, R. H., Kamprath, E. J., and Jackson, W. A. (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agronomy Journal* 74, 562–564. doi:10.2134/agronj1982.00021962007400030037x.

Montemurro, F., Convertini, G., and Ferri, D. (2007). Nitrogen application in winter wheat grown in Mediterranean conditions: Effects on nitrogen uptake, utilization efficiency, and soil nitrogen deficit. *Journal of Plant Nutrition* 30, 1681–1703. doi:10.1080/01904160701615541.

NCDC-NOAA (2019). 1981-2010 Climate Normals. National Climatic Data Center - National Oceanic and Atmospheric Administration. Available at: https://www.ncdc.noaa.gov/cdo-web/datatools/normals [Accessed April 20, 2019].

Ortiz-Monasterio, J. I. R., Sayre, K. D., Rajaram, S., and McMahon, M. (1997). Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Science* 37, 898–904. doi:10.2135/cropsci1997.0011183X003700030033x.

Oury, F. X., and Godin, C. (2007). Yield and grain protein concentration in bread wheat: How to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica* 157, 45–57. doi:10.1007/s10681-007-9395-5.

Palta, J., and Fillery, I. (1995). N application increases pre-anthesis contribution of dry matter to grain yield in wheat grown on a duplex soil. *Australian journal of agricultural research* 46, 507–518.

Passioura, J. (2002). Review: Environmental biology and crop improvement. *Functional Plant Biology 29(5)* 29, 537–546.

Raun, W. R., and Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal* 91, 357–363. doi:10.2134/agronj1999.00021962009100030001x.

Reynolds, M. ., and Ortiz, R. (2010). “Adapting crops to climate change: a summary,” in *Climate Change and Crop Production*, ed. M. P. Reynolds (CABI), 292.

Reynolds, M. P. (ed) (2010). *Climate Change and crop production.* , ed. M. P. Reynolds Wallingford, UK,: CABI Available at: https://www-cabdirect-org.ezproxy.lib.purdue.edu/cabdirect/FullTextPDF/2010/20103205516.pdf [Accessed October 16, 2018].

Richards, R. A. (2000). Selectable traits to increase crop photosynthesis and yield of grain crops. *Journal of Experimental Botany* 51, 447–458. doi:10.1093/jexbot/51.suppl_1.447.

Richards, R. A., Rebetzke, G. J., Condon, A. G., and van Herwaarden, A. F. (2002). Breeding Opportunities for Increasing the Efficiency of Water Use and Crop Yield in Temperate Cereals. *Crop Science* 42, 111–121. doi:10.2135/cropsci2002.1110.

Romagosa, I., Borràs-Gelonch, G., Slafer, G., and van Eeuwijk, F. (2013). “Genotype by Environment Interaction and Adaptation,” in *Sustainable Food Production* (New York, NY: Springer New York), 846–870. doi:10.1007/978-1-4614-5797-8_199.

Russell, K., Lee, C., and Van Sanford, D. (2017). Interaction of Genetics, Environment, and Management in Determining Soft Red Winter Wheat Yields. *Agronomy Journal* 109, 2463–

2473. doi:10.2134/agronj2017.02.0126.

Rzewnicki, P. E., Thompson, R., Lesoing, G. W., Elmore, R. W., Francis, C. A., Parkhurst, A. M., et al. (1988). On-farm experiment designs and implications for locating research sites. *American Journal of Alternative Agriculture* 3, 168–173. doi:10.2307/44507020.

Sadras, V. O. (2004). Yield and water-use efficiency of water- and nitrogen-stressed wheat crops increase with degree of co-limitation. *European Journal of Agronomy* 21, 455–464. doi:10.1016/J.EJA.2004.07.007.

Sadras, V. O. (2005). A quantitative top-down view of interactions between stresses: Theory and analysis of nitrogen-water co-limitation in Mediterranean agro-ecosystems. *Crop and Pasture Science* 56, 1151–1157. doi:10.1071/AR05073.

Sadras, V. O., Hayman, P. T., Rodriguez, D., Monjardino, M., Bielich, M., Unkovich, M., et al. (2016). Interactions between water and nitrogen in Australian cropping systems: Physiological, agronomic, economic, breeding and modelling perspectives. *Crop and Pasture Science* 67, 1019–1053. doi:10.1071/CP16027.

Sadras, V. O., and Roget, D. K. (2004). Production and Environmental Aspects of Cropping Intensification in a Semiarid Environment of Southeastern Australia. *Agronomy Journal* 96, 236–246. doi:10.2134/AGRONJ2004.2360.

Sadras, V. O., and Slafer, G. A. (2012). Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. *Field Crops Research* 127, 215–224. doi:10.1016/J.FCR.2011.11.014.

Sanchez-Garcia, M., Royo, C., Aparicio, N., Martin-Sanchez, J. A., and Álvaro, F. (2012).

Genetic improvement of bread wheat yield and associated traits in Spain during the 20th century. *The Journal of Agricultural Science* 151, 105–118. doi:10.1017/S0021859612000330.

Serrago, R. A., Lo Valvo, P. J., and Miralles, D. J. (2019). Is the source-sink ratio at anthesis a driver to avoid yield reductions caused by late foliar disease in wheat? *Field Crops Research* 235, 11–17. doi:10.1016/J.FCR.2019.02.007.

Shewry, P. R., and Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and Energy Security* 4, 178–202. doi:10.1002/FES3.64.

Shroyer, J. P., Kok, H., and Thompson, C. R. (1997). Planting Practices in Wheat Production Handbook, C-529. Available at: http://www.ksu.edu/wheatpage/ [Accessed June 23, 2019].

Simpson, N. L., Brennan, R. F., and Anderson, W. K. (2016). Grain yield increases in wheat and barley to nitrogen applied after transient waterlogging in the high rainfall cropping zone of western Australia. *Journal of Plant Nutrition* 39, 974–992. doi:10.1080/01904167.2014.993475.

Sinclair, T. R., and Park, W. I. (1993). Inadequacy of the Liebig limiting-factor paradigm for explaining varying crop yields. *Agronomy Journal* 85, 742–746.

Slafer, G. A. (2003). Genetic basis of yield as viewed from a crop physiologist's perspective. *Annals of Applied Biology* 142, 117–128. doi:10.1111/j.1744-7348.2003.tb00237.x.

Slafer, G. A., Araus, J. L., Royo, C., and Moral, L. F. G. (2005). Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. *Annals of Applied Biology* 146, 61–70. doi:10.1111/j.1744-7348.2005.04048.x.

Slafer, G. A., Savin, R., and Sadras, V. O. (2014). Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research* 157, 71–83. doi:10.1016/j.fcr.2013.12.004.

Thompson, N. M., Epplin, F. M., Edwards, J. T., and Hunger, R. M. (2014). Economics of foliar fungicides for hard red winter wheat in the USA southern Great Plains. *Crop Protection* 59, 1–6. doi:10.1016/j.cropro.2014.01.009.

USDA-NASS (2018a). 2017 Agricultural chemical use survey wheat. *2018-5*, 1–2. Available at: https://www.nass.usda.gov/Surveys/Guide_to_NASS_Surveys/Chemical_Use/2017_Cotton_Soybeans_Wheat_Highlight/ChemUseHighlights_Wheat_2017.pdf [Accessed April 11, 2019].

USDA-NASS (2018b). State Agriculture Overview for Kansas. Available at: https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=KANSAS [Accessed April 10, 2019].

van Ginkel, M., Calhoun, D. S., Gebeyehu, G., Miranda, A., Tian-you, C., Pargas Lara, R., et al. (1998). Plant traits related to yield of wheat in early, late, or continuous drought conditions. *Euphytica* 100, 109–121. doi:10.1023/A:1018364208370.

van Herwaarden, A. F., Farguhar, G. D., Angus, J. F., Richards, R. A., and Howe, G. N. (1998). "Haying-off", the negative grain yield response of dryland wheat to nitrogen fertiliser. I. Biomass, grain yield, and water use. *Australian Journal of Agricultural Research* 49, 1067–1082.

Wang, Z., Sadras, V. O., Yang, X., Han, X., Huang, F., and Zhang, S. (2017). Synergy between breeding for yield in winter wheat and high-input agriculture in North-West China. *Field*

Crops Research 209, 136–143. doi:10.1016/j.fcr.2017.04.018.

Warton, D. I., Duursma, R. A., Falster, D. S., and Taskinen, S. (2012). smatr 3- an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* 3, 257–259. doi:10.1111/j.2041-210X.2011.00153.x.

Wickham, H. (2009). "ggplot2: Elegant Graphics for Data Analysis," in *Springer-Verlag New York*, 211. doi:10.1007/978-0-387-98141-3.

Zadoks, J., Chang, T., and Konzak, C. (1974). A decimal growth code for the growth stages of cereals. *Weed Research* 14, 415–421.

Chapter 3 - Quantifying the impact of management on winter wheat nutrient accumulation and utilization: I. Macronutrients

Abstract

More information is needed about the impact of management practices on nutrient uptake and utilization efficiency (UtE, yield to nutrient uptake ratio) on winter wheat to develop efficient nutrient management programs. Our main goal was to evaluate the impact of management on yield and uptake of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) in wheat via (i) evaluating trends in nutrient UtE and uptake requirements at different yield levels, (ii) studying the variation in nutrient content, and (iii) examining plant nutritional status via nutritional indices of N, P, and S. The accumulation of N, P, K, and S was measured at five developmental stages using a single bread-wheat genotype grown under a combination of fourteen treatments during two growing seasons in Kansas, US. Treatments were divided into two management systems, standard (SM) and intensive (IM) management. Treatments such as application of fertilizers (N, Cl, and S) and fungicide, plant density, and plant growth regulator were individually added to the SM or removed from the IM treatment controls. Fungicide was the main treatment affecting yield and nutrient uptake, and its removal from IM significantly reduced yield and uptake of nutrients in year with high fungal disease pressure. Nutrients were accumulated at a similar proportion at each growth stage relative to their respective accumulation at the end of the season. The IM seemed to maintain higher shoot concentration of nutrients as compared to the SM control during the growing season. This was emphasized by the significant increase in nutrition indices for N and S from SM to IM control, indicating possible luxury uptake under IM. Hence, crop intensification strategies may alter nutrient uptake at the end of season, but will not affect timing and rate of uptake during the growing season.

Introduction

Sustainable increases in grain yield from current cropland are needed (Borlaug and Dowswell, 2003, Fischer et al., 2014) to help support the worldwide growing-population, as an increase of c. 50% in cereal production will be required to attain food security in the next decades (Reynolds et al., 2012). Adequate nutrient management is essential to reduce nutrient losses to the environment (Harris et al., 2016) and to improve nutrient use efficiency (i.e., yield per unit of fertilizer applied) of farming systems. While the effects of nutrient management on crop nutrient uptake pattern are well understood (Papakosta, 1994; Malhi et al., 2006), the impact of other agronomic management practices (e.g., plant density, foliar fungicides, or the integration of different practices) on crop nutrient dynamics has not been widely investigated. Crop response to agronomic practices vary with environmental conditions (Jaenisch et al., 2019), further challenging the implementation of strategies that will improve the target nutrient demand for each potential productivity region (Tilman et al., 2002). Hence, evaluating the impacts of management practices on plant uptake dynamics may help to achieve greater wheat yields.

Although nitrogen (N) is the nutrient required in larger quantities for wheat grain production (Spiertz and Vos, 1983), crops require several other nutrients in large quantities (e.g., macronutrients such as K, P, and S) to complete its life cycle. Multiple factors can affect the development of a well-balanced nutrient management program, such as environmental conditions (Altenbach, 2012), target yield (Setiyono et al., 2010), agronomic traits of each genotype (Russell et al., 2017), crop and disease management practices (Bancal et al., 2008), soil properties (Malhi et al., 2006), nutrient role within the plant (Clarkson and Hanson, 1980), and plant nutrient accumulation patterns (Bender et al., 2013). Thus, quantifying variations in nutrient efficiencies and requirements as function of yield level can help to develop effective nutrient management programs and sustainably improve crop productivity.

The majority of the studies evaluating the effects of agronomic practices on crop nutrient uptake dynamics have focused on nutrient management. For instance, Hamnér et al. (2017) evaluated the effects of N management on macro- and micro-nutrient uptake of wheat. Salvagiotti et al. (2009b) evaluated the effects of different S and N rates on wheat N uptake and UtE. Likewise, Malhi et al. (2012) investigated the effects of P rates on N and P uptake. Lollato et al. (2019) evaluated the interactions of N, P, and K fertilization on wheat yield and grain N removal. A few studies have evaluated the impacts of other agronomic management practices on the uptake dynamics of different nutrients, such as plant population (Ciampitti et al., 2013) and hybrid maturity (Bender et al., 2013) in maize (*Zea mays* L.), sowing date in soybeans (*Glycine max* (L.) Merr.) (Gaspar et al., 2017), and fungicide and sowing date on N uptake in wheat Gooding et al. (2005) and Ehdaie and Waines (2001). However, there is limited information available on the effects of agronomic practices on uptake of N, P, K and S in modern hard red winter wheat production systems.

Likewise, there is evidence that nutrient requirements change based on yield level for soybeans (Gaspar et al., 2017) and maize (Setiyono et al., 2010), and that wheat yield response to inorganic fertilizer rates depend on environmental yield level (Lollato et al., 2019). However, to our knowledge, only two studies conducted in China have attempted to quantify wheat nutrient requirements as affected by yield level in wheat (Liu et al., 2006; Chuan et al., 2013). These studies suggested that wheat has being grown with greater amounts of N than needed (i.e. luxury N uptake), either excessive or deficiency in P, and deficiency in K. However, these two studies are restricted for old and modern cultivars grown in China and therefore, more information about changes in requirements of macronutrients (other than N) due to management practices is needed for other wheat growing regions in the world.

Nutrient utilization efficiency (i.e., yield per unit of nutrient uptake, UtE) is the physiological component of nutrient use efficiency (i.e., yield per unit of nutrient supplied, UE; Ciampitti and Vyn, 2014). NUtE is generally negatively associated with nutrient concentration in the dry matter due to nutrient dilution at greater biomass levels (Plénet and Lemaire, 2000). Plants require a minimum concentration of nutrient to maximize growth, which is known as critical nutrient concentration (Justes et al., 1994). Critical nutrient concentrations are typically derived from dilution curves that account for the allometric relationships between nutrient concentration and crop biomass (Gastal et al., 2015). Evaluation of critical nutrient concentration and accumulation dynamics helps to quantify plants' nutrient demand during the growing season (Lemaire et al., 2007; Cadot et al., 2018). Nutrient nutrition index (NI) (i.e. measured nutrient concentration to critical nutrient concentration ratio) is among the most robust tools to assess in-season nutritional status of plants while considering the dilution of nutrients as plant biomass increases (Sadras and Lemaire, 2014), and can be used to assess the effects of management practices on crop nutrient status (Hoogmoed et al., 2018). Furthermore, understanding the changes in nutrient-specific dilution patterns at different management practices helps to establish a balanced nutrient management program in a wide range of cropping systems.

The overarching objective of this research was to evaluate the impact of management practices and yield levels on the uptake of four macronutrients (i.e. N, P, K, and S) in winter wheat. The specific objectives to accomplish this goal were to: (i) evaluate trends in nutrient utilization efficiency and uptake dynamics at different yield levels, (ii) quantify the variation in nutrient uptake at varying plant growth stages via dissection of nutrient concentration as function of biomass, and (iii) examine plant nutritional status via nutritional indices of N, P, and S as affected by management against published literature critical nutrient concentrations.

Materials and Methods

General Experiment Information

The details of the experiment have been described previously (Jaenisch et al, 2019). Briefly, experiments were conducted under field conditions at Kansas State University Research Stations during the 2015-2016 and 2016-2017 growing seasons, hereafter referred to as 2016 and 2017. Experiments were conducted in Manhattan (39°12' N,133 96°35' W, altitude 350 m) on an Aloha silt loam soil (fine-silty, mixed, superactive, mesic cumulic hapludolls) and in Belleville (39°48' N, 97°48' W, altitude 450 m) on a Crete silt loam soil (fine, smectitic, mesic pachic udertic argiustolls) for a total of four site-years. The experiment was arranged as a randomized complete block design with an incomplete factorial treatment structure consisting of six replications, but only three replications were used for aboveground biomass harvesting and nutrient concentration analysis.

Treatment description

The wheat variety 'Everest' released in 2009 and the most widely variety grown in Kansas for five consecutive years (USDA-NASS, 2017) was used to compare fourteen treatment combinations including two controls for each of the management systems evaluated. The treatment controls were a standard management (SM) in which N rate was calculated for a yield goal of 4.7 Mg ha^{-1}; and intensive management (IM) with N rate calculated for a yield goal of 8.1 Mg ha^{-1} (Leikam et al., 2003). The N rate accounted for soil NO_3-N availability prior sowing in in the profile (0 – 60 cm), N credits from organic matter, previous crop, and tillage system according to the nutrient management guide from Kansas State University (Leikam et al., 2003).

Therefore, the actual amount of N applied to reach this yield goal was different in each site-year (Jaenisch et al, 2019).

The other management practices evaluated consisted of six individual additions to, or removals from, the SM and IM controls, and included different agronomic or fertilization practices. These were: i) application of N at sowing and at tillering (Zadoks growth stage 26; Zadoks et al., 1974) to achieve a yield goal of 4.7 Mg ha^{-1}, versus this practice plus additional N application at beginning of stem elongation (Zadoks 30) for a yield goal of 8.1 Mg ha^{-1}. Addition or omission of 45 kg ha^{-1} of ii) S as gypsum (160 g S kg^{-1}) or iii) Cl as potassium chloride (450 g Cl kg^{-1}) at Zadoks 30; iv) plant population (low vs. high seeding rate with 2.8 vs. 4.0 million seeds ha^{-1}), v) addition or omission of fungicide application at jointing or Zadoks 31 (Picoxystrobin, 63 g a.i. ha^{-1}) and at heading or Zadoks 59 (Picoxystrobin + Cyproconazolez, 119 g a.i. ha^{-1}), and vi) addition or omission of plant growth regulator (Zadoks 31) (Trinexapac-ehtyl, 250 g a.i. ha^{-1}).

The SM control treatment was sown at the recommended seeding rate of 2.8 M seeds ha^{-1} and had the low rate N treatment. Then, each treatment was individually *added* to the SM control for a total of seven treatments within the SM system. The IM control treatment consisted of the high rate of N, higher seeding rate, sulfur, chloride, two fungicide applications, and plant growth regulator. Then, each treatment was individually *removed* from the IM control treatment for a total of seven treatments within the IM system (supplementary Table C1).

Biomass harvesting

Aboveground biomass was determined from plants harvested from one linear meter per experimental unit at beginning of stem elongation (Zadoks 30), at flag leaf emergence (Zadoks 39), at heading (Zadoks 59), at soft dough stage of grain development (Zadoks 85), and at physiological maturity (Zadoks 93). Plants were dried for one week at 60ºC, weighed, ground to pass a 2 mm sieve, and sent to the laboratory for nutrient concentration analysis. Nitrogen and S concentration in plant tissue were determined via combustion using the LECO TruSpec CN and S analyzer (LECO, St. Joseph, MI, USA). Phosphorus and K concentration in plant tissue were determined via the inductively coupled plasma spectrometry method (AOAC, 2000). Plant nutrient uptake was estimated by multiplying nutrient concentration and biomass of each plant component on a dry weight basis. Harvest index (HI) was determined as the ratio of grain yield by aboveground biomass at maturity.

For each treatment, nutrient utilization efficiency (UtE, kg grain kg nutrient^{-1}) was estimated as the ratio of grain yield to aboveground uptake of each nutrient at maturity (Moll et al., 1982). Magnitude of response in nutrient UtE among treatments was estimated as the difference in yield to difference in nutrient uptake (Ciampitti and Vyn, 2011). To compare differences in rate of uptake of N versus the other nutrients (i.e. P, K, and S), we estimated the amount of nutrient uptake at each growth stage relative to the uptake at physiological maturity (i.e. relative rate of uptake = nutrient uptake at each growth stage divided by uptake at maturity), and regressed the relative rate of N uptake at each sampling time (dependent variable) against the relative rate of P, K, and S uptake at each corresponding sampling time (independent variables). The slopes of the regressions were then compared to the 1:1 line to determine whether the different treatments affected the uptake rate of a given nutrient as compared to another. The uptake ratio of each nutrient relative to N during the growing season was estimated by dividing N uptake by P, K, and S uptake, respectively.

We estimated nutrient nutrition index (NI) at anthesis by dividing measured shoot nutrient concentration by critical nutrient values established from previously described equations (Eq. 1).

$$\mathrm{NI} = \frac{\text{measured shoot nutrient concentration at anthesis}}{\text{critical nutrient concentration at anthesis}} \qquad \text{(Eq. 1)}$$

For critical N concentration, where $\mathrm{Nc}=5.35*\mathrm{biomass}^{-0.442}$ using winter wheat (Justes et al., 1994). For critical P concentration, where $\mathrm{Pc}=4.44*\mathrm{biomass}^{-0.41}$ using winter wheat (Cadot et al., 2018). For critical P concentration, $\mathrm{Sc}=0.37*\mathrm{biomass}^{-0.169}$ using spring red wheat (Reussi et al., 2012). We did not find previously described equation to estimate critical K concentration, and thus, we were not able to compare our data with a reference line neither to calculate NI for K.

Statistical Analysis

For the entire data set, a descriptive statistical analysis was conducted via the R packages "doBy" and "dplyr" with estimation of the mean, standard deviation, maximum, minimum, and 0.25 and 0.75 percentiles (n=168) (Højsgaard and Halekoh, 2016; Wickham et al., 2018).

To assess overall trends in nutrient UtE and uptake requirements as a function of yield levels, we first identified two subdatasets of low and high yield levels across all treatments and site-years based on the 25th (yield < 3.75 Mg ha^{-1}) and 75th (yield >5.77 Mg ha^{-1}) percentiles of yield. Then, we calculated nutrient UtE for each data-point, as well as nutrient requirement to produce 1 Mg of grain (i.e., the reciprocal nutrient UtE). Groups were compared for grain yield and nutrient UtE and requirement using two tailed t-tests.

Levene's test was used to assess the homogeneity of variance of treatments across site-years using the R package "car" (Fox and Weisberg, 2011). To estimate treatment effects on the traits measured, we used linear mixed models considering treatment as fixed effect, and site-years, and block nested within site-year as random effect using the R packages "lme4" (Bates et al., 2015) and "lmerTest" (Kuznetsova et al., 2017). Analysis of homogeneity of variance indicated a significant interaction between treatment and year ($p<0.05$), but no interaction between treatment and sites. For each year, we compared mean differences between treatments within each management system using Tukey test (*$p \leq 0.05$*) in the R package “multcomp” (Hothorn et al., 2008). Within each management system, treatments were contrasted against both IM and SM controls; and the SM and IM control treatments were compared against each other (Jaenisch et al., 2019). Within each growth stage, we compared the mean differences between the main treatments affecting yield and nutrient uptake (i.e. SM control vs. SM plus fungicide, SM control vs. IM control, and IM vs. IM minus fungicide) using least square mean differences ($p \leq 0.05$).

We executed pairwise comparisons to test for treatment differences on the slopes of the relationship between the rate of uptake of N versus other nutrients using standardized major axis in the “smatr” package in the R software (Warton et al., 2012). Then we evaluated the relationship among the rates of uptake of each nutrient versus N across all treatments using the “lm” function in the R package “ExpDes” (Ferreira et al., 2018). Shoot nutrient concentration values were plotted against its respective biomass including the entire dataset to evaluate the impacts of treatments on nutrient dilution as biomass increased.

Results

Data description

Our data shows a normal distribution and wide-ranging values for grain yield, and N, P, K and S uptake at maturity, with two distinct populations representing each year (except for P uptake that overlapped across years, Fig 1A-E). In 2016, average yield across all treatments was 4 Mg ha-1 with standard deviation of 0.9 Mg ha-1. The range (i.e., the difference between the lowest and highest values measured) of values were 4 Mg ha-1 for grain yield and 250, 30, 222, 26 kg ha-1 for N, P, K, and S uptake, respectively. In 2017, average yield across all treatments was 5.6 Mg ha-1 with standard deviation of 1.1 Mg ha-1. The range of values were 6 Mg ha-1 for grain yield and 240, 28, 304, 30 kg ha-1 for N, P, K, and S uptake, respectively.

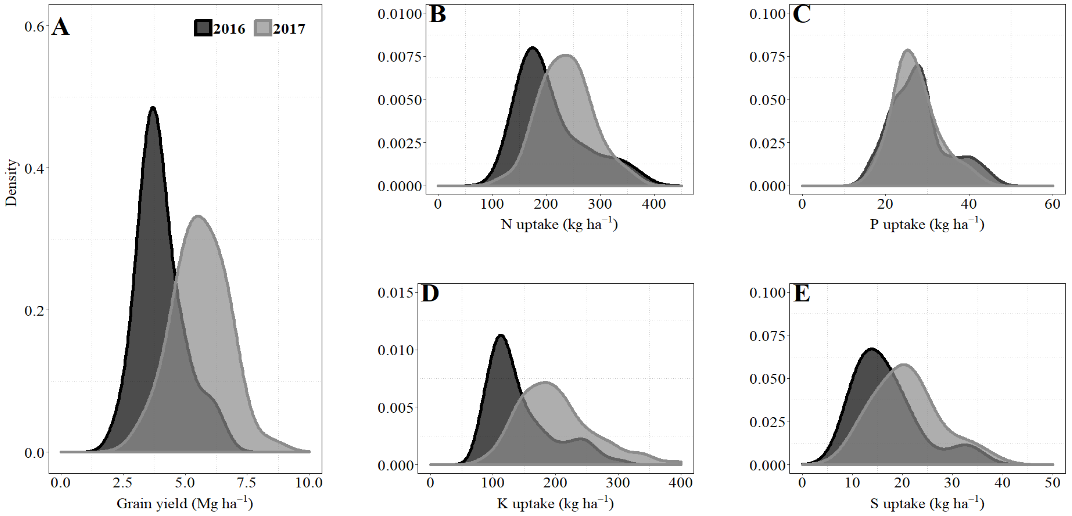


Figure 3.1 Distribution of observations for grain yield (panel A), and uptake of nitrogen (N, panel B), phosphorus (P, panel C), potassium (K, panel D) and sulfur (S, panel E) for each harvest year.

Across all treatments and site-years, the nutrient UtE (calculated for each treatment) ranged from 12 to 31 kg grain kg plant N^{-1}, 106 to 264 kg grain kg plant P^{-1}, 14 to 43 kg grain kg plant K^{-1}, and from 122 to 455 kg grain kg plant S^{-1} (supplementary Table C2). In 2016, the average UtE of N, P, K, and S was 20, 145, 30, 255 kg grain per kg nutrient uptake^{-1}, and the average nutrient uptake required to produce 1 Mg of grain was 52, 7, 36, and 4 of N, P, K, and S. In 2017, the average UtE of N, P, K, and S was 24, 208, 30, 268 kg grain kg nutrient uptake^{-1}, and the nutrient required to produce 1 Mg of grain was 42, 5, 36, and 3.7 for N, P, K, and S.

Across all treatments and site-years, the mean UtE of N and P was smaller at low yielding levels (yield < 3.75 Mg ha^{-1}) as compared to high (yield < 5.77 Mg ha^{-1}) yielding levels and did not change for K and S. Whereas, the average N and P requirements to produce 1 Mg grain was greater at low yield levels relative to high yield levels and did not change for K and S (Table 1). At the low yield levels, the nutrient required to produce 1 Mg of grain was 51, 7, 37, and 4 kg nutrient of N, P, K, and S. At the high yield levels, the nutrient required to produce 1 Mg was 43, 5, 35, and 4 kg nutrient of N, P, K, and S (Table 1).

Table 3-1 Nutrient uptake, utilization efficiency (UtE), and reciprocal utilization efficiency (RUtE) at low (25%Q) and high (75%Q) yield levels, and relative decrease in UtE and increase in RUtE from low to high yield levels for each nutrient across years (2016 and 2017).

Nutrient	25%Q (Yield < 3.75 Mg ha-1)			75%Q (Yield > 5.77 Mg ha-1)			Relative values	
	Uptake	UtE	RUtE	Uptake	UtE	RUtE	UtE	RUtE
	kg ha^{-1}	kg kg^{-1}	Mg kg^{-1}	kg ha^{-1}	kg kg^{-1}	Mg kg^{-1}	%	%
N	166	20	51	277 *	24 *	43 *	17	-16
P	22	152	7	33 *	203 *	5 *	34	-25
K	120	30	37	231 *	29 *ns*	35 *ns*	-1	-3
S	13	264	4	25 *	280 *ns*	4 *ns*	6	-7

Note: Within yield level, nutrient utilization efficiency was calculated as kg grain yield produced per kg nutrient uptake. Nutrient reciprocal utilization efficiency was calculated as kg nutrient uptake per Mg of grain. For each nutrient, *ns* means no significant difference between yield levels and asterisks (*) means significant difference (at $p<0.05$) between yield levels.

Treatment effects on yield and nutrient uptake

In 2016, there were treatment differences for the main variables measured (i.e. yield and uptake of N, P, K, and S) (Table 2 and supplementary Table C3). However in 2017, there were treatment differences for uptake of N, K, and S, without differing for yield and P uptake.

Across management systems in 2016, the IM treatment control increased grain yield by 0.9 Mg ha^{-1} (Table 2) and increased the uptake of K by c. 30% and S by c. 60% relative to SM control (while N and P uptake did not change) (Fig. 2A). The magnitude of response in UtE of N, P, K, and S increased by 15 kg grain kg N^{-1}, 304 kg grain kg P^{-1}, 24 kg grain kg K^{-1}, and 125 kg grain kg S^{-1} from SM to IM practices (difference between IM minus SM treatment controls) (Table 3). These results suggest that plants required larger amounts of nutrients to produce grain under IM as compared to SM system. The IM control increased the amount of nutrients required to produce 1 Mg of grain by c. 70, 3, 41, and 8 kg nutrient Mg grain^{-1} for N, P, K, and S, respectively, as compared to the SM control (Table 3).

The magnitude of response in UtE was larger within the SM system as compared to the IM system, and the fungicide management was the most important treatment affecting yield in our study. Within the SM system, the additional fungicide application to the SM control had a marginal effect on yield (*p=0.08*) and did not affect the uptake of the nutrients evaluated. Within the IM system, however, removal of fungicide application from the IM treatment control reduced yield by 1 Mg ha^{-1}, and consequently, the uptake of all nutrients (Fig. 2A). The removal of fungicide application from the IM control reduced the amount of nutrient uptake required to produce 1 Mg of grain by 46, 6, 32, and 4 kg nutrient uptake Mg grain^{-1} for N, P, K, and S, respectively (Table 3). In 2016, the IM control increased nutrient concentration in the grain of N and S, while reduced grain K concentration and the harvest index of K from 18 to 15% and S from 51 to 43% relative to the SM control. The removal of fungicide from IM control had no effect on grain nutrient concentration. Likewise, the fungicide management (either addition or removal) did not affect nutrient partitioning to the grain (*data not shown*).

Table 3-2. Treatment effects on grain yield for the trials established in Manhattan and Belleville (KS) during the 2016 and 2017 winter wheat growing seasons.

		2016		2017	
Management	Treatments	Yield			
		Mg ha^{-1}			
Standard	1.SM control	3.7	b	5.7	*ns*
	2.Split-N	3.2		5.1	
	3.Sulfur	3.4		5.4	
	4.Cl	3.6		5.5	
	5.Pop	3.8		6.0	
	6.Fung	4.3	a	5.7	
	7.Pgr	3.5		4.9	
Intensive	8.IM control	4.6	A*	6.4	
	9.Split-N	4.2		5.7	
	10.Sulfur	4.3		5.7	
	11.Cl	4.4		5.7	
	12.Pop	4.5		5.1	
	13.Fung	3.6	B	5.3	
	14.Pgr	5.0		6.4	

Note: Lower case letters; standard management treatment is significantly different from standard plus fungicide treatment at $0.05 \geq p \leq 0.10$. *; standard management treatment is significantly different from intensive management treatment at $p<0.05$. Upper case letters; intensive management treatment is significantly different from intensive minus fungicide treatment at $p<0.05$. *Ns*, treatments were not significant different from its respective treatment control.

In 2017, the IM control significantly improved the uptake of N, K, and S relative to SM control (Fig. 2B), however; improvements in the uptake of these nutrients were not translated into yield (average yield across treatments: 5.6 Mg ha^{-1}). Because changes in UtE usually mirror changes in yield, the nutrient requirements did not change within the SM system (values were near zero). For the IM system, the removal of fungicide application reduced the uptake of N, while it did not change the uptake of P, K and S (Fig. 2B). The removal of fungicide from the IM control reduced the amount of nutrient uptake required to produce 1 Mg of grain by c. 46, 6, 30, and 3.3 for N, P, K, and S, respectively. The IM control increased nutrient concentration in the grain for N and S and reduced the HI of S relative to the SM control, whereas the IM system did not affect P and K concentration in the grain neither HI of N, P, and K. For both management systems, fungicide did not affect nutrient concentration in the grain, neither harvest indices.

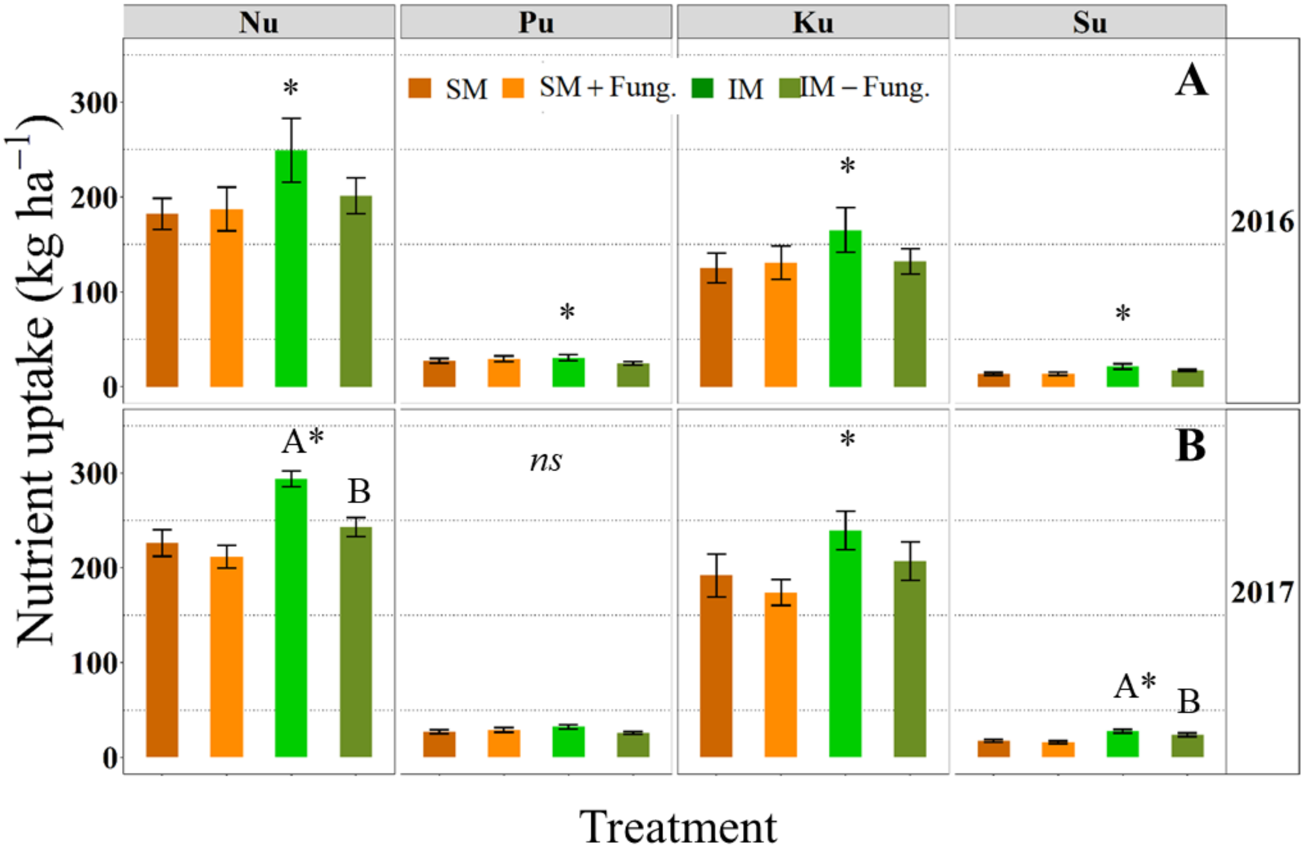


Figure 3.2 Treatment effects on nutrient uptake of N, P, K, and S in 2016 (A) and 2017 (B) harvesting years. *; standard management (SM) is significantly different from intensive management (IM) at *p<0.05*. Upper case letters indicate significant difference between IM and IM minus fungicide at *p<0.05*.

Table 3-3 Magnitude of response in nutrient utilization efficiency (UtE) and requirements among treatments for N, P, K, and S in 2016 and 2017.

		Nutrient UtE				Nutrient requirement			
Year	Treatment	N	P	K	S	N	P	K	S
		kg grain kg nutrient^{-1}				kg nutrient Mg grain^{-1}			
2016	SM+Fung. – SM	121	333	112	3230	8	3	9	0
	IM – IM–Fung.	22	174	32	250	46	6	32	4
	IM - SM	15	304	24	125	69	3	41	8
2017	SM+Fung. – SM	0	1	0	0	0	1024	0	0
	IM – IM–Fung.	22	169	34	305	46	6	29	3
	IM - SM	11	143	16	74	91	7	64	13

Differences in nutrient uptake dynamics during the growing season

For both years, the IM treatment control increased the aboveground biomass and uptake of N, K, and S during the growing season relative to the SM treatment control, and differences among treatments within growth stage became more prominent as the season progressed (Fig. 3 A-E). Meanwhile, there was no treatment effect on the uptake dynamics of P in any of the growth stages evaluated (Fig. 3C).

The IM significantly increased the uptake of S almost during the entire season (from Zadoks 39 to 93) relative to the SM control, and removal of fungicide from IM reduced S uptake at maturity only in 2017 (Fig. 3E). We observed similar treatment impacts on N and K uptake during the growing season: In 2016, there was no significant difference among treatments in uptake of N and K from Zadoks 30 (i.e. early stem elongation) to 59 (i.e. anthesis) (Fig. 3 panels B and D). The IM control increased N and K uptake as compared to the SM control at Zadoks 85 (i.e. soft dough) and 93 (i.e. physiological maturity), and the removal of fungicide from IM control significantly reduced their uptake at Zadoks 93. In 2017, the IM increased N uptake at Zadoks 39 (i.e. flag leaf emergence), 85, and 93. The removal of fungicide from IM control significantly reduced N uptake at Zadoks 85 and 93. The IM increased K uptake at Zadoks 85 and 93, and removal of fungicide from IM reduced K uptake at the Zadoks 85.

Treatment did not change the timing of nutrient accumulated during the growing season. All nutrients were accumulated at a similar rate during the growing season regardless of treatment (as there was no significant difference in the slope among treatments, $p>0.05$) with 20% of the aboveground nutrient uptake at maturity being accumulated at beginning of stem elongation, 50% at flag leaf emergence, and 70% heading (Fig. 3). Thus, management affected the overall accumulation of each nutrient (nutrient demand), but did not alter the relative dynamics (i.e. timing) of nutrient uptake at different growth stages.

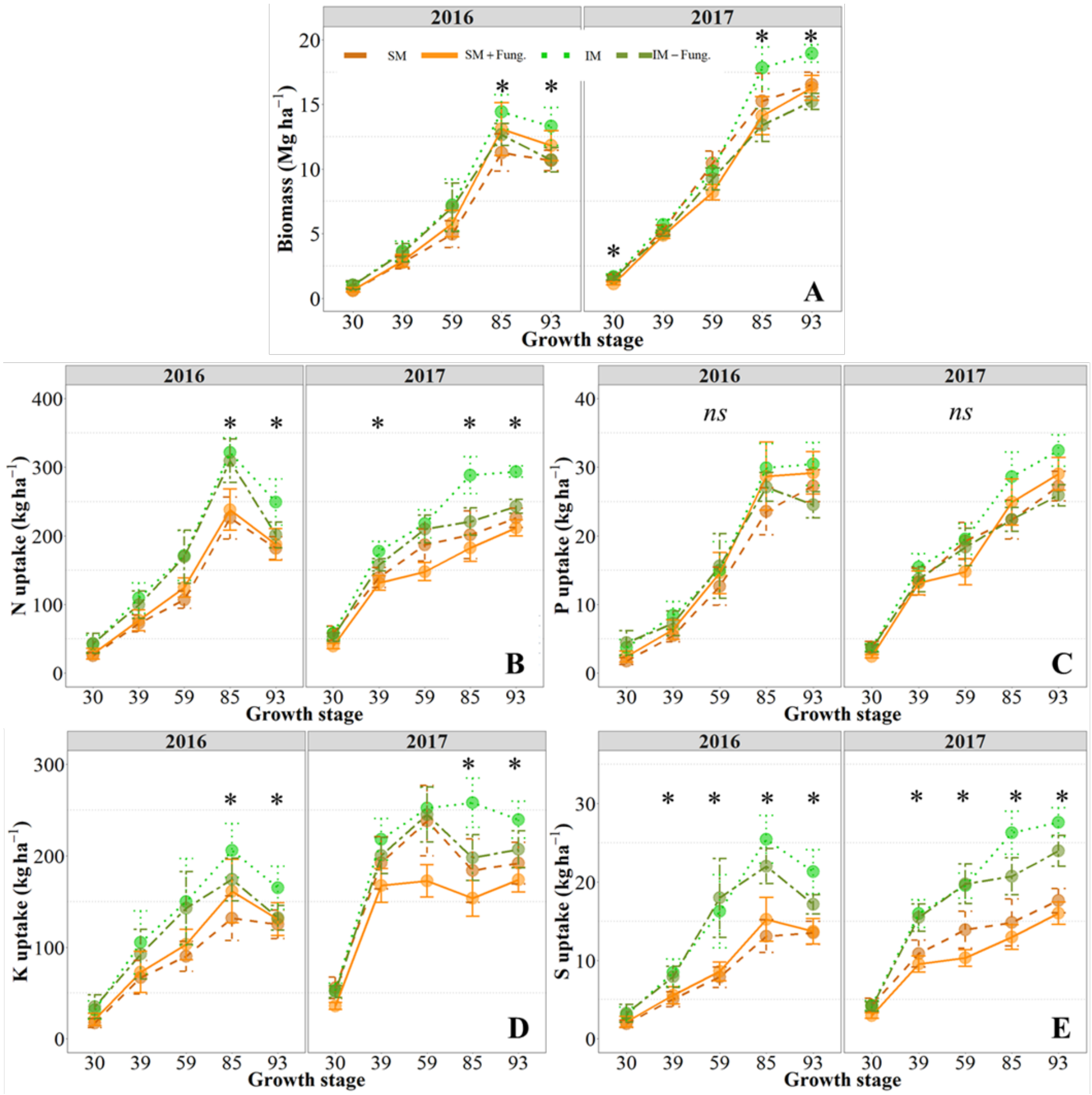


Figure 3.3 Treatment effects on aboveground biomass (A) and nutrient uptake during the growing season for N (B), P (C), K (D), and S (E) in harvest years 2016 and 2017. Within growth stage, asterisk (*) indicates significant treatment difference at $p<0.05$. Growth stage is in Zadoks scale.

Pairwise comparisons from the standardized major axis analysis indicated no significant differences in slopes among treatments for the relationship between rate of N uptake and rate of P, K, or S uptake (supplementary Table C4). Thus, an overall regression equation is reported across all treatments for the relationship between the rates of uptake of N versus the other nutrients (Fig. 4). By comparing the relationship between the rates of uptake at each growth stage relative to maturity for the nutrients evaluated in our study, we observed that nutrients had different rates of uptake during the growing season relative to N, and there were some variation among years (Fig 4). Overall, the N accumulation rate was greater than the P accumulation rate, but slightly lower as compared to K and somewhat similar to S across managements. In 2016, removal of fungicide from the IM showed a trend of reduced rate of N uptake as compared to the other treatments and year. This trend, however, was not significantly different from the other slopes. Subsequently, the ratio of nutrient uptake relative to N during the growing season did not change across years, and plants accumulated approximately 8 times more N relative to P, 0.9 times more N relative to K 10 times more N relative to S (Fig. 5A-C).

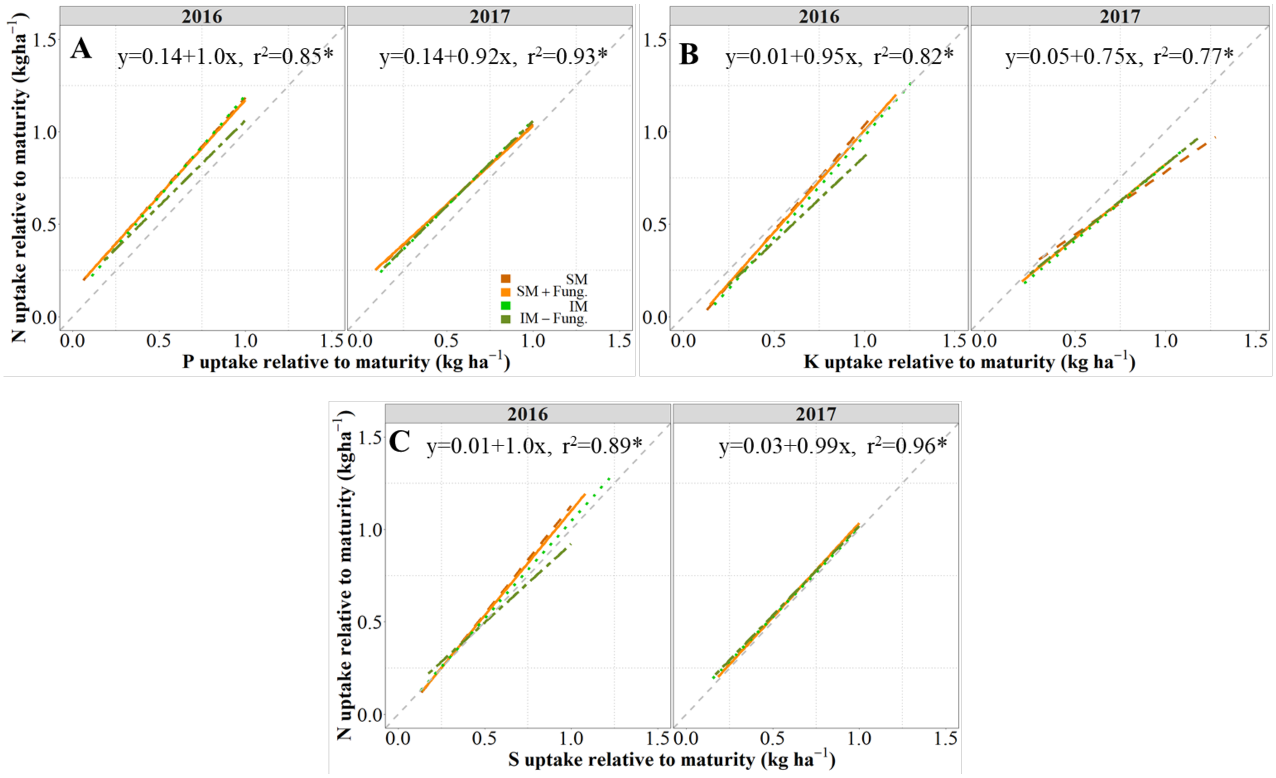


Figure 3.4. Relationship between nitrogen uptake rate for each growth stage relative to the aboveground nutrient uptake at maturity and P rate uptake (A), K rate uptake (B), and S rate of uptake (C) across treatments [standard (SM) and intensive (IM) management systems with (SM+Fung.) or without (IM-Fung.) fungicide] during the growing seasons in 2016 and 2017 harvest years.

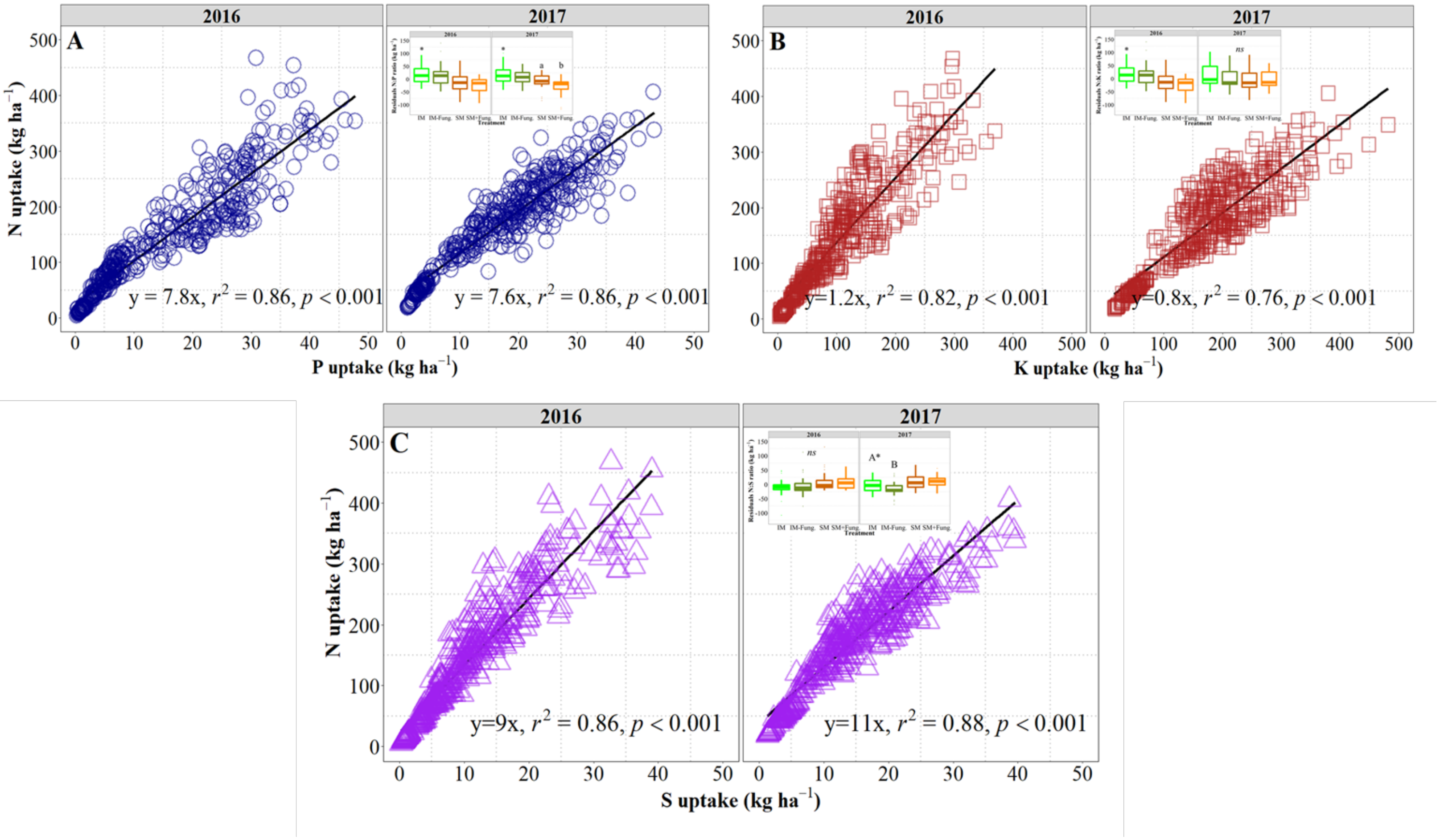


Figure 3.5 Relationship between aboveground nitrogen uptake and uptake of P (A), K (B), and S (C) during the growing season for 2016 and 2017 harvest years. Treatment impacts on the residuals of the relationship between N uptake and P (A, inset), K (B, inset), and S (C, inset) for 2016 and 2017 harvest years.

Treatment impacts on nutrient dilution curve of N, P, K, and S

For both years, as expected, the concentration of all nutrients decreased with increasing shoot biomass during the growing season (Fig. 6). The shoot nutrient concentration was greater for the IM as compared to SM system for all nutrients, except for P with no differences across treatments (Fig. 6 and supplementary Table C5). Differences in N and S concentration for particular biomass levels indicate that changes in N and S uptake due to management resulted from changes in nutrient concentration whereas changes in nutrient uptake of P and K were mainly driven by changes in shoot biomass. For both years, the IM control significantly increased NNI and SNI at anthesis relative to the SM control (from 0.57 to 0.69 for NNI and from 0.47 to 0.66 for SNI) demonstrating the positive effect of the IM on maintaining adequate N and S concentrations required for plants to maximize growth at the anthesis period (Figs. 6A, D insets).. However, there was no significant difference across treatments on average PNI at anthesis indicating that plants did not require additional P to maximize growth at the anthesis (Figs. 6B insets).

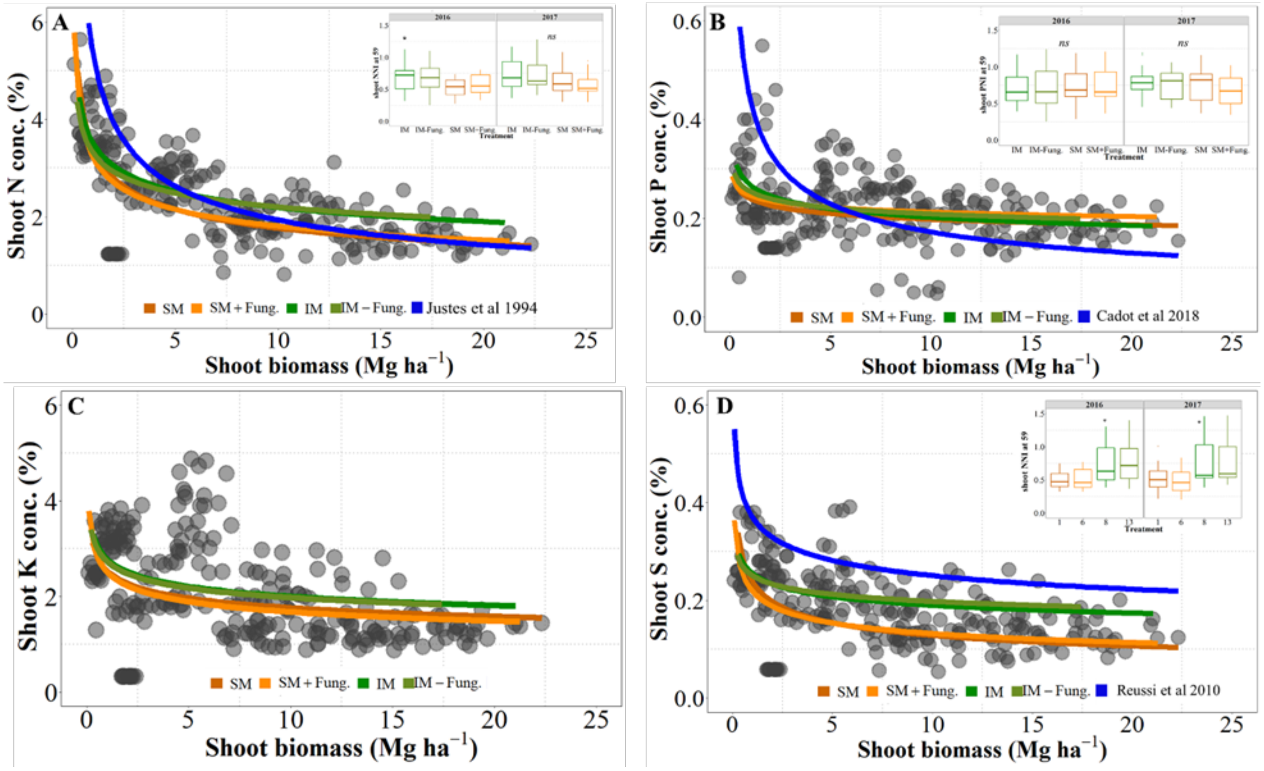


Figure 3.6. Shoot nutrient concentration of N (A), P (B), K (C), and S (D) as affected by biomass during the growing season for standard (SM) and intensive (IM) management systems with (SM+Fung.) or without (IM-Fung.) fungicide applications across years (2016 and 2017). Treatment impacts on nutrition index at anthesis of N (A, inset), P (B, inset), and S (D, inset) across years (2016 and 2017).

Discussion

Nutrient utilization efficiency and uptake requirements

The management practices evaluated in this study provided a representative range of values to estimate maximum and minimum UtE of four macronutrients for hard red winter wheat grown in the U.S. southern Great Plains at yield levels ranging from 2.2 to 8.6 Mg ha^{-1}. On average of site-years and treatments, our results suggested UtE of 22 kg grain kg N^{-1}, 177 kg grain kg P^{-1}, 30 kg grain kg K^{-1}, and 270 kg grain kg S^{-1}. Two studies analyzing N, P, and K uptake requirements in winter wheat in China evaluating published and unpublished experiments suggested greater UtE for N (c. 11.3 – 91.8 kg grain kg N^{-1}), P (c. 48.4 -614.9 kg grain kg P^{-1}) and K (c. 12.8 – 185.8 kg grain kg K^{-1}), likely due to the greater yield levels attained in their experiments (Liu et al., 2006; Chuan et al., 2013). Similarly, greater NUtE was reported in higher yielding European environments as compared to lower yielding Mediterranean conditions (Savin et al., 2019). Expectedly, the average nutrient requirement to produce 1 Mg of grain in our research (c. 46 kg N Mg grain^{-1}, 6 kg P Mg grain $^{-1}$, and 36 kg K Mg grain $^{-1}$) were greater than previous findings. Giambalvo et al. (2010) reported an average NUtE of 41 kg grain kg N^{-1} for durum wheat genotypes released in 1990's. Chuan et al. (2013) reported average UtE of 40, 190, 56 kg nutrient kg grain^{-1} for N, P, and K, which was equivalent to a requirement of 26 and 22 kg nutrient Mg grain^{-1} for N and K, and similar results were found for P with 6.5 kg P Mg grain^{-1}. The greater requirement for N, P (Liu et al., 2006) and K (Chuan et al., 2013) in our study could be due to several factors, including greater nutrient availability in the soil in our experiments as compared to the others, differences in genotype, yield potential and yield limiting factors.

Impacts of crop management practices on grain yield and uptake of N, P, K, and S

The larger nutrient demand of higher-yielding crops call for reevaluation of management of macronutrients other than N, including P, K, and S. The general belief of Liebig's 'law of the minimum' where yield can be limited by a single factor (e.g., typically water limitation in dryland production systems) leads producers to adopt conservative approaches and reduce the use of inputs; preventing them from maximizing yield when possible (Sadras et al., 2016). Proving this theory is flawed, evidences suggest improvements in agronomic management can attend plant nutrient requirements and increase yield in dryland environments (Raza and Saleem, 2013). Inadequate fertilization of S and micronutrients contribute to lack of yield gain in multiple crop production systems (Kihara et al., 2017). Previous studies have investigated the impacts of crop production (e.g. plant density), fertilization (e.g. N and S fertilization), and disease management (e.g. fungicide) practices (individually or coupled in two or three practices) on wheat yield and nutrient uptake (Ehdaie and Waines, 2001; Gooding et al., 2005; Hamnér et al., 2017; Salvagiotti et al., 2009b). However, to our knowledge, there have been no attempt to comprehensively evaluate the combined effects of these practices on wheat macronutrient uptake dynamics. In general, our results suggest that implementation of an intensive management program in dryland areas need to account for the different nutrient requirements according to the potential yield of a system. This is in line with previous findings where increases in yield and N uptake were followed by increase in uptake of several nutrients (Hamnér et al., 2017).

Foliar fungicide impacts on wheat yield and nutrient accumulation

Foliar fungicide was the main treatment influencing yield and nutrient uptake in our study. Yield protection resulting from fungicide application is expected when a susceptible genotype is grown in the presence of foliar fungal diseases (Cruppe et al., 2017; Prey et al., 2019; Lollato et al., 2019b). However, in our study, the foliar fungicide seemed to be more important for the crop under IM (average yield of 4.4 Mg ha^{-1}) than under SM as yield significantly decreased with removal of fungicide from the IM control. Similarly, Cruppe et al. (2017) reported that fungicide application resulted in an yield gain of 0.6 Mg ha^{-1} in high yielding environments (yield >3.2 Mg ha^{-1}), while it did not increase yield when yield levels were below 3.2 Mg ha^{-1}. The authors attributed the lack of effect on grain yield to the low disease pressure due to the extremely dry conditions.

Moreover, in our study, the fungicide treatment increased the UtE of N and S without reducing their concentration in the grain (*data not shown*). Likewise, Gooding et al. (2005) found that fungicide increased UtE of N without a reduction in grain N concentration, and attributed that the increased remobilization of nutrients from vegetative parts to the grain and extended accumulation of nutrients during grain filling period, and increased root growth (Dimmock and Gooding, 2002; Ford et al., 2006). This is demonstrated by a decline in the relative uptake of N in reference to the relative uptake of P, K, and S, with the removal of fungicide treatment under intensive management systems in our study (even including situations where fungicide marginally affected yield). On the other hand, adequate levels of nutrient in the plants are needed to sustain photosynthesis of the green canopy resultant from fungicide application (Gregory et al., 1981), which may explain the decrease in nutrient uptake in our study in the 2017 year due to additional fungicide application to the SM treatment.

Nutrient fertilization practices

Greater N rates can increase N uptake and concentration in the plants when nutrient requirements have been reached and there is marginal or none yield gain from N (Subedi et al., 2007; Cruppe et al., 2017; Lollato et al., 2019a) (i.e. luxury uptake). In our study, the additional N application did not increase yield or N uptake under standard management but increased N concentration, in both grain and vegetative components. Likewise, studies have reported that most of the N is accumulated in the grain when surpassing plant N requirements to produce yield (Uhart and Andrade, 1995; Gooding, 2007), while others have shown that N concentration increases in vegetative parts (Cassman et al., 1992; Ehdaie and Waines, 2001). Our data suggests that N application may have benefited grain formation and helped to maintain photosynthetic capacity of plants later in the season (Sinclair and Jamieson, 2006).

Previous studies have indicated increasing concerns on S deficiency in wheat-producing regions (Salvagiotti et al., 2009), but wheat yield response to S fertilization is inconsistent (Rasmussen et al., 1975; Dhillon et al., 2019). Relative to the other macronutrients, S is required in smallest amounts by plants, and consequently, its deficiency is easily corrected. In our study, S application neither affected yield nor S uptake under standard management, however; the removal of S decreased S uptake under IM system, suggesting that soil S was enough to attend crop requirements under SM and that crop S requirements increased for IM system (Jaenisch et al., 2019). Consequently, application of S reduced SUtE, while its removal from intensive management control improved SUtE. Sulfur deficiency negatively affects plant N uptake by reducing nitrate reductase activity (Anderson et al. 1990), and consequently grain protein content (due to lack of N) and composition (due to lack of S) (Zhao et al., 1999). In agreement with our findings, Salvagiotti et al. (2009) observed a synergism between N and S and no impact of S fertilization on UtE of N. The lack of effect of S fertilization practices could be explained because our experiments were not conducted under extreme nutrient deficiency (i.e. mean NNI=0.62, PNI =0.49, SNI= 0.56), even under standard management systems, which is also shown by the lack of significance among treatments (except for the SM vs IM control) in NNI, PNI and SNI at anthesis in our study.

Conclusion

Our research showed that crop intensification will require a reassessment of plant nutrient requirements as plants may change its demand and UtE according to management practices and potential yield of an environment. Fungicide application was the key yield-driving factor in our study and would allow for a more conservative intensification of management for genotypes that are susceptible to most fungal diseases. In general, all nutrients accumulated at a similar proportion at each growth stage relative to their respective accumulation at the end of the season. The IM increased yield and uptake of nutrients at maturity relative to SM, and reduced the decline in concentration of nutrients as biomass increased as compared to the SM during the growing season. Our data suggested that intensification of crop management may alter total nutrient accumulation as related to yield levels, but it might not affect timing of nutrient uptake during the growing season or the relative ratio to maturity uptake among nutrients, with exceptions when the absence of foliar fungicide decreased the relative N uptake due to reduction in biomass. Nutrient recommendations should be adjusted according to the potential yield level with the adoption of an integrated pest management program to attend the demand of a higher-yielding crop while improving nutrient use efficiency.

References

Altenbach, S.B. 2012. New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. Journal of Cereal Science 56: 39–50Available at https://ac.els-cdn.com/S0733521012000124/1-s2.0-S0733521012000124-main.pdf?_tid=45ec6be2-e5bb-4afd-8488-f35273141cba&acdnat=1526508212_6a12a93734f945274602d505c59ab3bc (verified 16 May 2018).

Bancal, M.O., R. Roche, and P. Bancal. 2008. Late foliar diseases in wheat crops decrease nitrogen yield through N uptake rather than through variations in N remobilization. Annals of Botany 102(4): 579–590.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using **lme4**. Journal of Statistical Software 67(1): 1–48Available at http://www.jstatsoft.org/v67/i01/.

Bender, R.R., J.W. Haegele, M.L. Ruffo, and F.E. Below. 2013. Nutrient uptake, partitioning, and remobilization in modern, transgenic insect-protected maize hybrids. Agronomy Journal.

Cadot, S., G. Bélanger, N. Ziadi, C. Morel, and S. Sinaj. 2018. Critical plant and soil phosphorus for wheat, maize, and rapeseed after 44 years of P fertilization. Nutrient Cycling in Agroecosystems 112(3): 417–433Available at http://link.springer.com/10.1007/s10705-018-9956-0 (verified 2 July 2019).

Cassman, K.G., D.C. Bryant, A.E. Fulton, and L.F. Jackson. 1992. Nitrogen Supply Effects on Partitioning of Dry Matter and Nitrogen to Grain of Irrigated Wheat. Crop Science 32(5): 1251–1258Available at https://www.crops.org/publications/cs/abstracts/32/5/CS0320051251.

Chuan, L., P. He, J. Jin, S. Li, C. Grant, X. Xu, S. Qiu, S. Zhao, and W. Zhou. 2013. Estimating nutrient uptake requirements for wheat in China. Field Crops Research 146: 96–104Available at http://dx.doi.org/10.1016/j.fcr.2013.02.015 (verified 6 June 2019).

Ciampitti, I.A., J.J. Camberato, S.T. Murrell, and T.J. Vyn. 2013. Maize nutrient accumulation and partitioning in response to plant density and nitrogen rate: I. macronutrients. Agronomy Journal 105(3): 727–734.

Ciampitti, I.A., and T.J. Vyn. 2011. A comprehensive study of plant density consequences on nitrogen uptake dynamics of maize plants from vegetative to reproductive stages. Field Crops Research 121(1): 2–18Available at http://www.sciencedirect.com/science/article/pii/S0378429010002649 (verified 25 April 2015).

Ciampitti, I.A., and T.J. Vyn. 2014. Understanding global and historical nutrient use efficiencies for closing maize yield gaps. Agronomy Journal 106(6): 1–11.

Clarkson, D.T., and J.B. Hanson. 1980. The mineral nutrition of higher plants. Ann. Rev. Plant

Physiol 31: 239–298Available at www.annualreviews.org (verified 19 July 2018).

Cruppe, G., J.T. Edwards, and R.P. Lollato. 2017. In-season canopy reflectance can aid fungicide and late-season nitrogen decisions on winter wheat. Agronomy Journal 109(5): 2072–2086.

Dhillon, J., S. Dhital, T. Lynch, B. Figueiredo, P. Omara, and W.R. Raun. 2019. In-Season Application of Nitrogen and Sulfur in Winter Wheat. age 2(1): 0Available at https://dl.sciencesocieties.org/publications/age/abstracts/2/1/180047 (verified 15 August 2019).

Dimmock, J.P.R.E., and M.J. Gooding. 2002. The effects of fungicides on rate and duration of grain filling in winter wheat in relation to maintenance of flag leaf green area. The Journal of Agricultural Science 138(1): 1–16Available at https://www.cambridge.org/core/product/identifier/S0021859601001666/type/journal_article (verified 19 June 2019).

Ehdaie, B., and J.G. Waines. 2001. Sowing date and nitrogen rate effects on dry matter and nitrogen partitioning in bread and durum wheat. Field Crops Research 73(1): 47–61.

Ferreira, E.B., P.P. Cavalcanti, and D.A. Nogueira. 2018. ExpDes: Experimental Designs. R package version 1.2.0. Available at https://cran.r-project.org/package=ExpDes.

Ford, K.E., P.J. Gregory, M.J. Gooding, and S. Pepler. 2006. Genotype and fungicide effects on late-season root growth of winter wheat. Plant and Soil 284(1–2): 33–44Available at http://link.springer.com/10.1007/s11104-006-0028-x (verified 1 August 2018).

Fox, J., and S. Weisberg. 2011. An R companion to applied regression. Second. Sage, Thousand Oaks, CA.

Gastal, F., G. Lemaire, J. Durand, and G. Louarn. 2015. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. p. 161–205. *In* Sadras, V.O., Calderini, D.F. (eds.), Crop Physiology: Applications for genetic improvement and agronomy. 2nd ed. Elsevier.

Giambalvo, D., P. Ruisi, G. di Miceli, A.S. Frenda, and G. Amato. 2010. Nitrogen use efficiency and nitrogen fertilizer recovery of durum wheat genotypes as affected by interspecific competition. Agronomy Journal 102(2): 707–715.

Gooding, M.J. 2007. Influence of foliar diseases and their control by fungicides on grain yield and quality in wheat. Wheat Production in Stressed Environments (2002): 567–581Available at http://centaur.reading.ac.uk/8591/.

Gooding, M.J., P.J. Gregory, K.E. Ford, and S. Pepler. 2005. Fungicide and cultivar affect post-anthesis patterns of nitrogen uptake, remobilization and utilization efficiency in wheat. The Journal of Agricultural Science 143(06): 503Available at http://www.journals.cambridge.org/abstract_S002185960500568X.

Gregory, P.J., B. Marshall, and P. V. Biscoe. 1981. Nutrient relations of winter wheat:3. Nitrogen uptake, photosynthesis of flag leaves and translocation of nitrogen to grain. The Journal of Agricultural Science 96(3): 539–547Available at https://www.cambridge.org/core/product/identifier/S0021859600034493/type/journal_article (verified 19 June 2019).

Hamnér, K., M. Weih, J. Eriksson, and H. Kirchmann. 2017. Influence of nitrogen supply on macro- and micronutrient accumulation during growth of winter wheat. Field Crops Research 213(July): 118–129Available at http://dx.doi.org/10.1016/j.fcr.2017.08.002.

Harris, R.H., R.D. Armstrong, A.J. Wallace, and O.N. Belyaeva. 2016. Effect of nitrogen fertiliser management on soil mineral nitrogen, nitrous oxide losses, yield and nitrogen uptake of wheat growing in waterlogging-prone soils of south-eastern Australia. Soil Research 54(5): 619–633Available at https://www.scopus.com/inward/record.uri?eid=2-s2.0-84981543701&partnerID=40&md5=bbd93f756bd44caa76aeddab6214955d.

Højsgaard, S., and U. Halekoh. 2016. doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. R package version 4.5-15Available at https://cran.r-project.org/package=doBy.

Hoogmoed, M., A. Neuhaus, S. Noack, and V.O. Sadras. 2018. Benchmarking wheat yield against crop nitrogen status. Field Crops Research 222: 153–163Available at http://linkinghub.elsevier.com/retrieve/pii/S0378429017314508 (verified 11 April 2018).

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50(3): 346–363.

Justes, E., B. Mary, J.-M. Meynard, J.-M. Machet, and L. Thelier-Huche. 1994. Determination of a Critical Nitrogen Dilution Curve for Winter Wheat Crops. Annals of Botany 74(4): 397–407Available at https://academic.oup.com/aob/article-lookup/doi/10.1006/anbo.1994.1133 (verified 6 April 2018).

Kihara, J., G.W. Sileshi, G. Nziguheba, M. Kinyua, S. Zingore, and R. Sommer. 2017. Application of secondary nutrients and micronutrients increases crop yields in sub-Saharan Africa. Agronomy Sustainable development 37:25: 1–14Available at http://www.fao.org/faostat/en/#data (verified 20 June 2019).

Kuznetsova, A., P.B. Brockhoff, and R.H.B. Christensen. 2017. **lmerTest** Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software 82(13): 1–26Available at http://www.jstatsoft.org/v82/i13/ (verified 21 April 2019).

Leikam, D.F., R.E. Lamond, and D.B. Mengel. 2003. Soil Test Interpretations and Fertilizer Recommendations MF2586.

Lemaire, G., E. van Oosterom, J. Sheehy, M.H. Jeuffroy, A. Massignam, and L. Rossato. 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? Field Crops Research 100(1): 91–106.

Liu, M., Z. Yu, Y. Liu, and N.T. Konijn. 2006. Fertilizer requirements for wheat and maize in China: The QUEFTS approach. Nutrient Cycling in Agroecosystems 74(3): 245–258.

Lollato, R.P., B.M. Figueiredo, J.S. Dhillon, D.B. Arnall, and W.R. Raun. 2019a. Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: A synthesis of long-term experiments. Field Crops Research 236: 42–57Available at https://www.sciencedirect.com/science/article/pii/S0378429018318495 (verified 19 April 2019).

Lollato, R.P., D.A. Ruiz Diaz, E. DeWolf, M. Knapp, D.E. Peterson, and A.K. Fritz. 2019b. Agronomic practices for reducing wheat yield gaps: A quantitative appraisal of progressive producers. Crop Science 59(1): 333–350Available at https://dl.sciencesocieties.org/publications/cs/abstracts/59/1/333 (verified 19 April 2019).

Malhi, S.S., R. Lemke, Z.H. Wang, and B.S. Chhabra. 2006. Tillage, nitrogen and crop residue effects on crop yield, nutrient uptake, soil quality, and greenhouse gas emissions. Soil and Tillage Research 90(1–2): 171–183.

Malhi, S.S., C.L. Vera, and S.A. Brandt. 2012. Seed Yield Potential of Five Wheat

Species/Cultivars without and with Phosphorus Fertilizer Application on a P-Deficient Soil in Northeastern Saskatchewan. Agricultural Sciences 6(February): 224–231Available at http://www.scirp.org/journal/as%5Cnhttp://dx.doi.org/10.4236/as.2015.62022%5Cnhttp://creativecommons.org/licenses/by/4.0/.

Moll, R.H., E.J. Kamprath, and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal 74(3): 562–564.

Papakosta, D.K. 1994. Phosphorus Accumulation and Translocation in Wheat as Affected by Cultivar and Nitrogen Fertilization. Journal of Agronomy and Crop Science 173(3–4): 260–270.

Plénet, D., and G. Lemaire. 2000. Relationships between dynamics of nitrogen uptake and dry matter accumulation in maize crops. Determination of critical N concentration. Plant and Soil 216(ii): 65–82.

Prey, L., M. Germer, U. Schmidhalter, L. Prey, M. Germer, and U. Schmidhalter. 2019. Temporal and Organ-specific Responses in NUE Traits to N Fertilization, Fungicide Intensity and Early Sowing in Winter Wheat Cultivars. Agronomy 9(6): 313Available at https://www.mdpi.com/2073-4395/9/6/313 (verified 18 June 2019).

Rasmussen, P.E., R.E. Ramig, R.R. Allmaras, and C.M. Smith. 1975. Nitrogen-Sulfur Relations in Soft White Winter Wheat. II. Initial and residual effects of sulfur application on nutrient concentration, uptake and N/S ratio1. Agronomy Journal 67: 224–228Available at https://www.agronomy.org/publications/aj/abstracts/67/2/AJ0670020219?access=0&view=pdf.

Raza, S., and M.F. Saleem. 2013. Potassium applied under drought improves physiological and nutrient uptake performances of wheat (Triticum Aestivun L.). Journal of soil science and plant nutritionnd Plant Nutrition 13(1): 175–185Available at http://www.scielo.cl/scielo.php?pid=S0718-95162013005000016&script=sci_arttext.

Reussi, N., H.E. Echeverría, & Hernán, and S. Rozas. 2012. Stability of foliar nitrogen:sulfur ratio in spring red wheat and sulfur dilution curve. Journal of Plant Nutrition 35(7): 990–1003Available at https://www.tandfonline.com/action/journalInformation?journalCode=lpla20 (verified 4 July 2019).

Reynolds, M.P., J. Foulkes, R. Furbank, S. Griffiths, J. King, E. Murchie, M. Parry, and G.A. Slafer. 2012. Achieving yield gains in wheat. Plant, Cell and Environment 35: 1799–1823Available at http://doi.wiley.com/10.1111/j.1365-3040.2012.02588.x (verified 3 April 2018).

Russell, K., C. Lee, and D. Van Sanford. 2017. Interaction of Genetics, Environment, and Management in Determining Soft Red Winter Wheat Yields. Agronomy Journal 109(6): 2463–2473Available at https://dl.sciencesocieties.org/publications/aj/abstracts/0/0/agronj2017.02.0126.

Sadras, V.O., P.T. Hayman, D. Rodriguez, M. Monjardino, M. Bielich, M. Unkovich, B. Mudge, and E. Wang. 2016. Interactions between water and nitrogen in Australian cropping systems: Physiological, agronomic, economic, breeding and modelling perspectives. Crop and Pasture Science 67(10): 1019–1053.

Sadras, V.O., and G. Lemaire. 2014. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. Field Crops Research 164(1): 54–64Available at http://dx.doi.org/10.1016/j.fcr.2014.05.006.

Salvagiotti, F., J.M. Castellarín, D.J. Miralles, and H.M. Pedrol. 2009. Sulfur fertilization improves nitrogen use efficiency in wheat by increasing nitrogen uptake. Field Crops Research 113(2): 170–177Available at https://www.sciencedirect.com/science/article/pii/S0378429009001105 (verified 19 June 2019).

Savin, R., V.O. Sadras, and G.A. Slafer. 2019. Benchmarking nitrogen utilisation efficiency in wheat for Mediterranean and non-Mediterranean European regions. Field Crops Research, in press.

Setiyono, T.D., D.T. Walters, K.G. Cassman, C. Witt, and A. Dobermann. 2010. Estimating maize nutrient uptake requirements. Field Crops Research 118(2): 158–168Available at https://www-sciencedirect-com.ezproxy.lib.purdue.edu/science/article/pii/S0378429010001309#fig3 (verified 20 June 2018).

Sinclair, T.R., and P.D. Jamieson. 2006. Grain number, wheat yield, and bottling beer: An analysis. Field Crops Research 98(1): 60–67.

Spiertz, J.H.J., and N.M.D. Vos. 1983. Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. Plant and Soil 75: 379–391.

Subedi, K.D., B.L. Ma, and A.G. Xue. 2007. Planting date and nitrogen effects on grain yield and protein content of spring wheat. Crop Science 47(1): 36–44.

Tilman, D., K.G. Cassman, P.A. Matson, R. Naylor, and S. Polasky. 2002. Agriculture sustainability and intensive production practices. Nature 418: 671–677.

Uhart, S.A., and F.H. Andrade. 1995. Nitrogen and carbon accumulation and remobilization during grain filling in maize under different source/sink ratios. Crop Science 35(1): 183–190.

USDA-NASS. 2017. Kansas Wheat Varieties.

Warton, D.I., R.A. Duursma, D.S. Falster, and S. Taskinen. 2012. smatr 3- an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution 3(2): 257–259.

Wickham, H., R. François, L. Henry, and K. Müller. 2018. dplyr: A Grammar of Data Manipulation. Available at https://cran.r-project.org/package=dplyr.

Wuest, S.B., and K.G. Cassman. 1992. Fertilizer-Nitrogen Use Efficiency of Irrigated Wheat: II. Partitioning Efficiency of Preplant versus Late-Season Application. Agronomy Journal 84(4): 689.

Zadoks, J.C., T.T. Chang, and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14(6): 415–421Available at http://doi.wiley.com/10.1111/j.1365-3180.1974.tb01084.x (verified 20 April 2019).

Zhao, F., M. Hawkesford, and S. McGrath. 1999. Sulphur Assimilation and Effects on Yield and Quality of Wheat. Journal of Cereal Science 30(1): 1–17Available at https://www.sciencedirect.com/science/article/pii/S0733521098902417 (verified 10 October 2018).

Appendix A. Supplementary Tables and Figures from Chapter 1

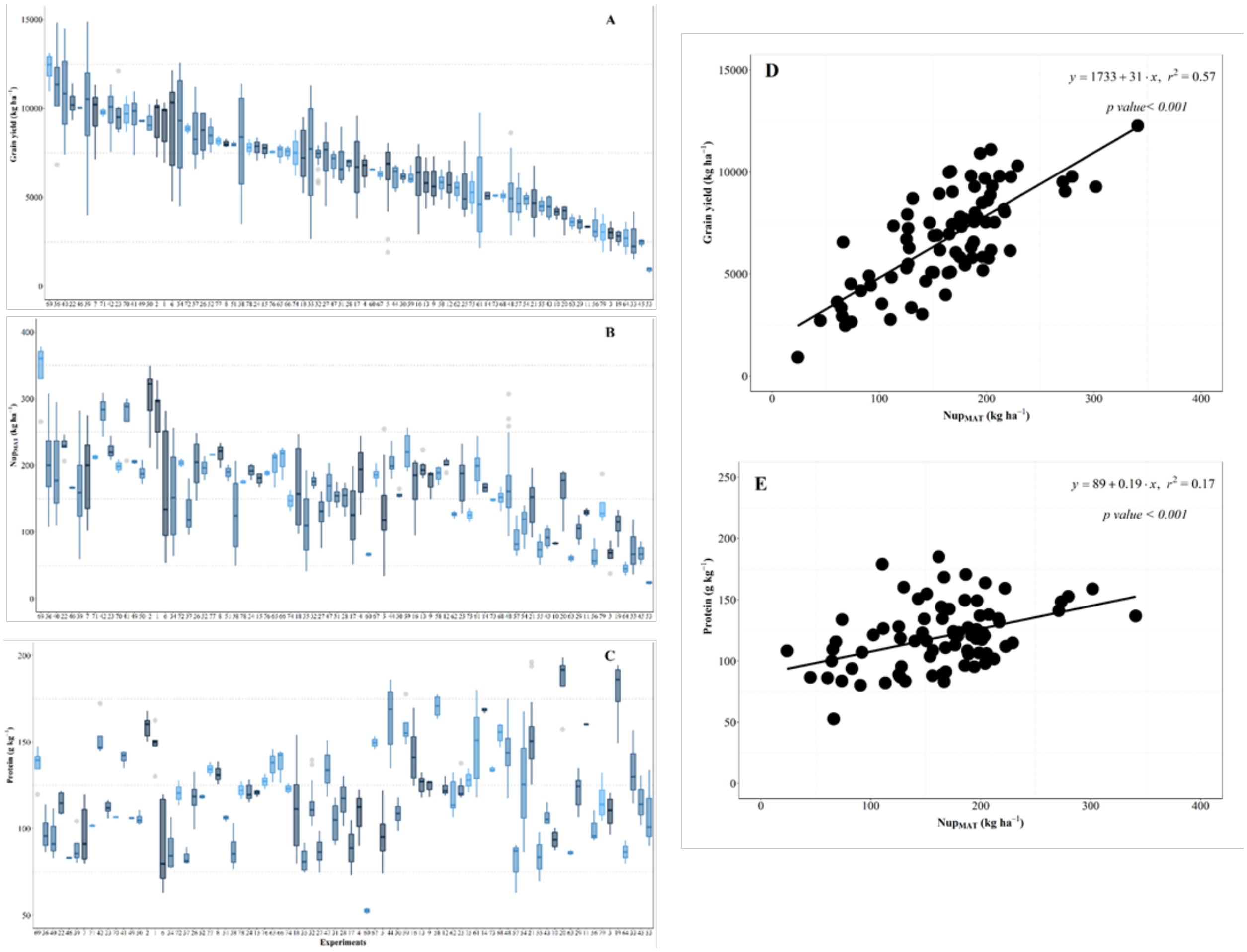
Appendix A. Tables and Figures

Supplementary Table A1. Summary descriptive statistics for raw-data values of the entire database (*n=524*). Mode, mean, standard error of the mean (se), minimum and maximum, and 0.25 (25Q) and 0.75 (75Q) quantiles were determined for each variable collected from published manuscripts.

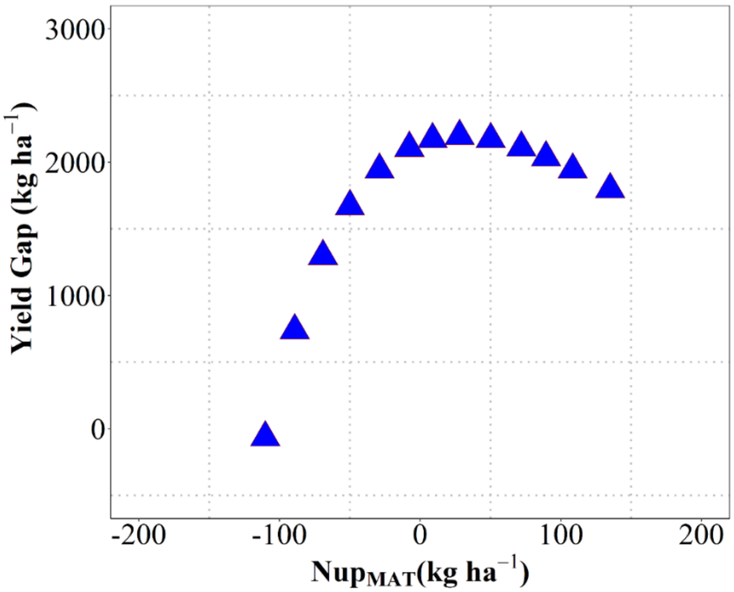
Variables	mode	mean	se	min	25Q	75Q	max
GNY (kg ha^{-1})	104	123	2.0	14	90	150	293
SNC (g kg^{-1})*	4	6	0.2	1	4	8	28
SNY (kg ha^{-1})	36	43	1.0	5	27	54	164
NHI (%)	80	74	0.4	30	70	80	92
HI (%)*	48	43	0.4	15	39	49	59
NUtE (kg kg^{-1})	38	40	0.5	17	33	46	112
STV BM (kg ha^{-1})*	7966	7209	135	1386	5494	8713	18546
BM (kg ha^{-1})*	14300	12722	217	2040	10002	15283	25340

**Variables with n=337.*

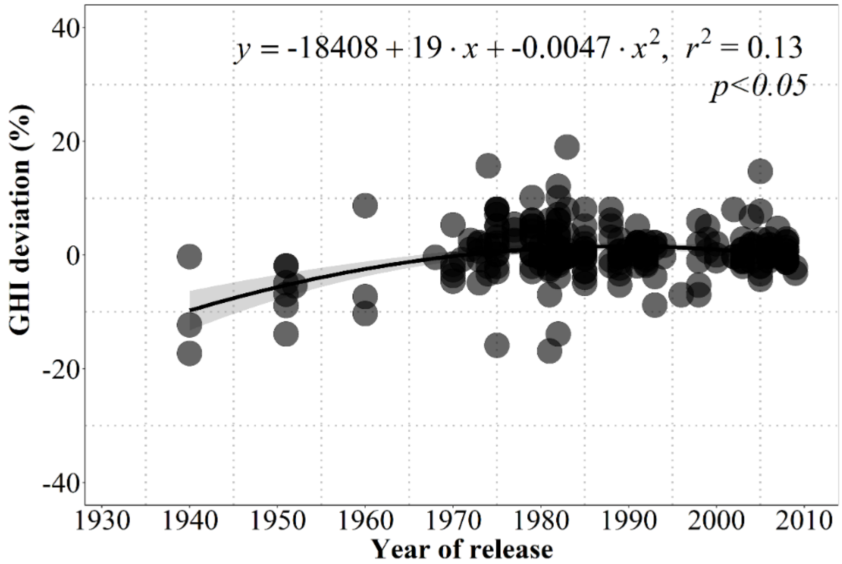
GNY, grain N uptake per unit area at maturity; SNC, stover N concentration at maturity; SNY, stover N uptake per unit area at maturity; NHI, nitrogen harvest index (grain N uptake to aboveground N uptake at maturity ratio); HI, harvest index (grain yield to aboveground biomass at maturity ratio on a dry weight basis); NUtE, N utilization efficiency (grain yield to aboveground N uptake per unit area at maturity ratio); STV BM, stover biomass at maturity on a dry weight basis; BM, aboveground biomass at maturity on a dry weight basis.



Supplementary Figure A1. Left panels show the box-plots for raw-data of grain yield per unit area with 135 g kg^{-1} moisture content (A), aboveground N uptake per unit area at maturity (Nup$_{\text{MAT}}$) (B), and grain protein concentration on a dry weight basis (C) from each experiment considered in the analysis, ordered from highest to lowest average values (*n*=524). Variations within experiments shown in each box-plot within each panel are due to the effects of treatments in each experiment. Right panels illustrate the relationship of either yield (D) or grain protein concentration (E) vs. Nup$_{\text{MAT}}$ across experiments (each data-point is the average across treatments within a single experiment (*n*=79).



Supplementary Figure A2. Gap between Nup$_{MAT}$-limited and actual yield averaged for each interval of aboveground N uptake per unit area at maturity (Nup$_{MAT}$) deviation. The dependent variable is the average Nup$_{MAT}$ for each interval.



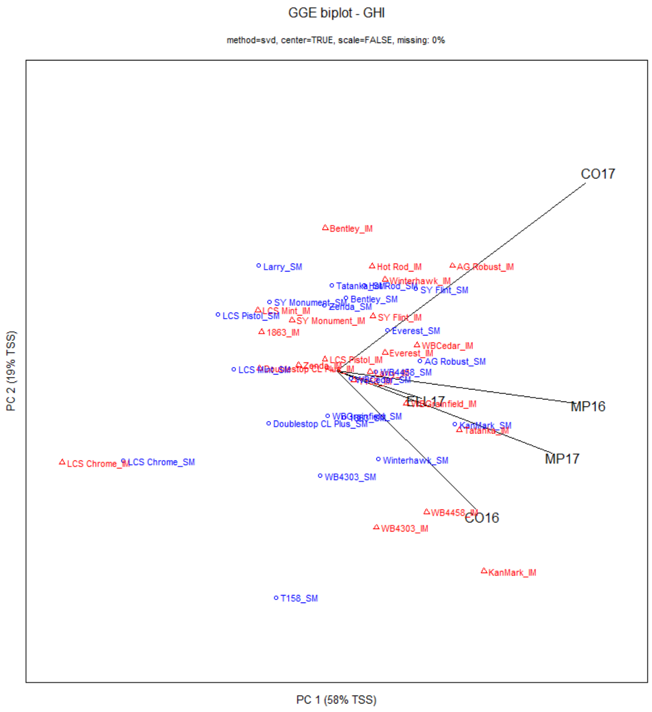
Supplementary Figure A3. Grain harvest index *(grain yield to aboveground biomass at maturity ratio on a dry weight basis)* and year of genotype release from 1930 to 2010 (*n*=330).

Appendix B. Supplementary Tables and Figures from Chapter 2

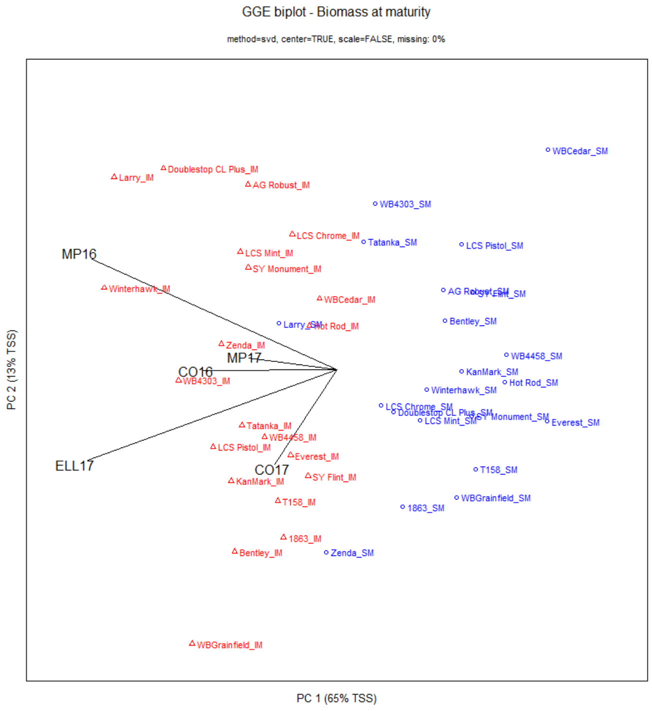
Appendix B. Tables and Figures

Supplementary Table B1. Summary of standardize major axis (SMA) regression analysis for the relationship between grain nitrogen concentration and aboveground biomass at maturity for intensive (IM) and standard (SM) maangement. Differences in slopes and intercepts among management were individually analyzed. [a] Slope differences were estimated by likelihood ratio statistical test (Ho: slopes are not equal) forcing intercept to the origin at $p<0.05$ level. [b] Differences in intercept among management were analyzed by the Wald statistical test (assuming same slopes) at $p<0.05$ level.

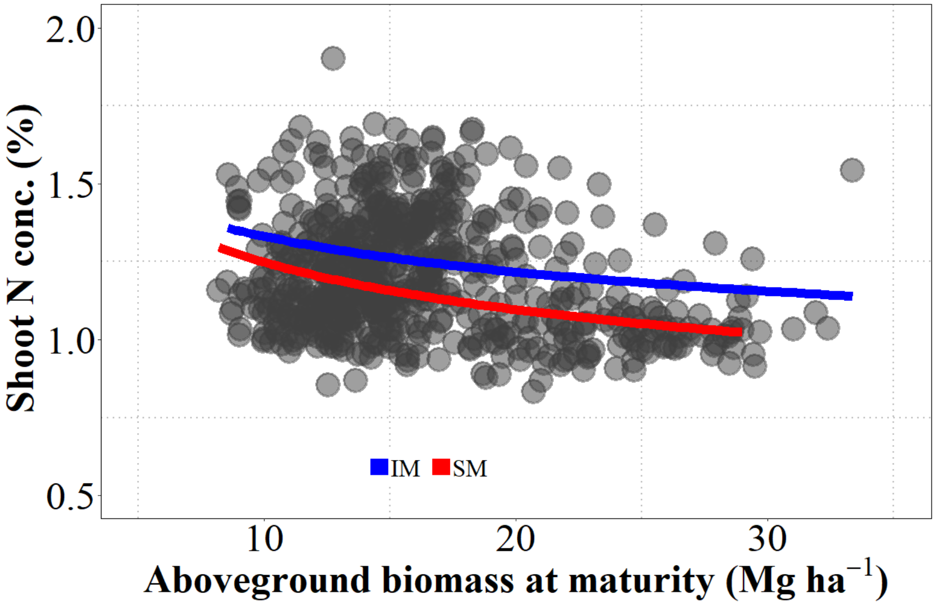
Manag.	**R^2**	**slope (95%CI)**	**[a]statistic for slope (95%CI)**	**Intercept (95%CI)**	**[b]statistic for intercept (95%CI)**
IM	0.0004	0.003 (-0.03; 0.04)	0.38 (0.37; 0.40)	2.17 (1.98; 2.36)	2.26 (2.10; 2.42)
SM	0.03	-0.04 (-0.09; 0.002)	0.43 (0.41; 0.44)	2.24 (2.01; 2.46)	2.08 (1.96; 2.22)



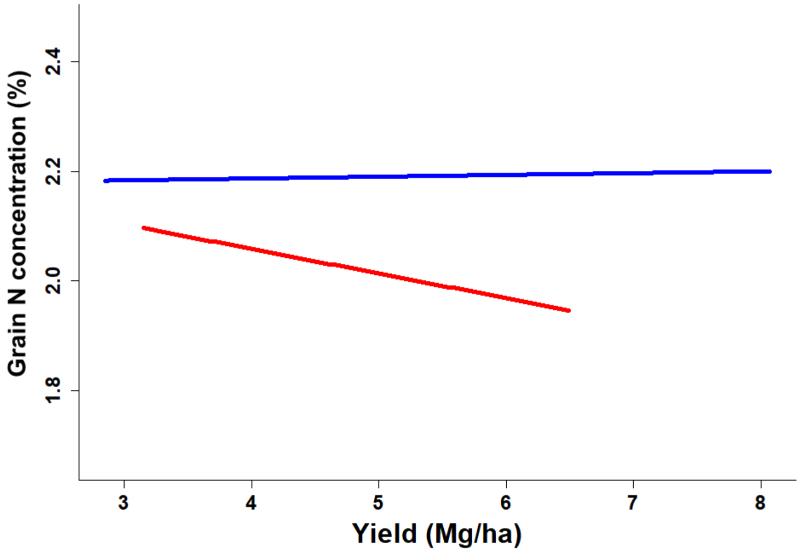
Supplementary Figure B1. Genotype and genotype x environment (GGE) biplot analysis for harvest index (i.e. yield to aboveground biomass at maturity ratio) of 21 genotypes grown in five site-years at intensive management (IM) and standard management (SM) systems.



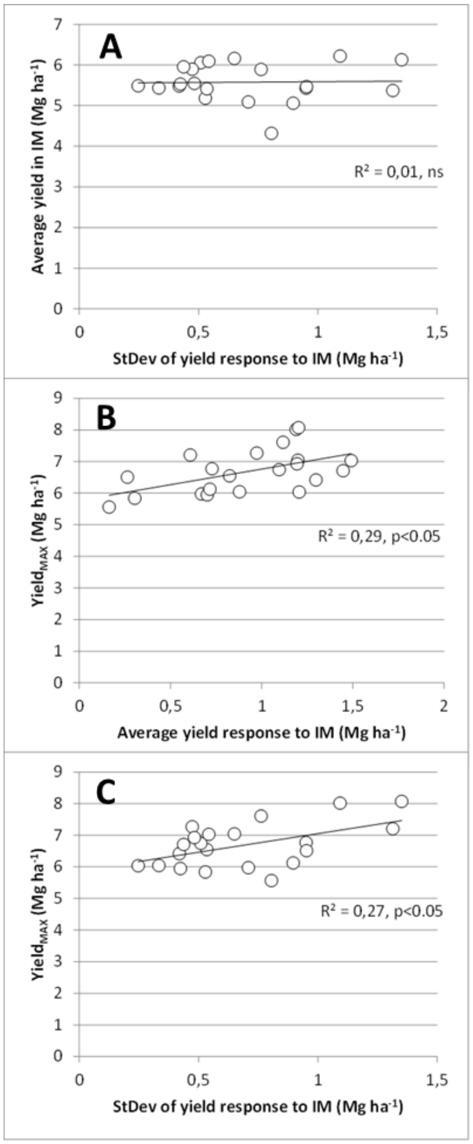
Supplementary Figure B2. Genotype and genotype x environment (GGE) biplot analysis for biomass at maturity of 21 genotypes grown in five site-years at intensive management (IM) and standard management (SM) systems.



Supplementary Figure B3. Shoot nitrogen concentration versus aboveground biomass at maturity across environments, genotypes and management systems intensive management (IM) and standard management (SM) (*n*=210). Power fit equation for IM Shoot N conc. = 1.79*biomass$^{\wedge -0.13}$. Power fit equation for SM Shoot N conc. = 1.92*biomass$^{\wedge -0.18}$.



Supplementary Figure B4. Grain nitrogen (N) concentration versus aboveground biomass at maturity across environments and genotypes for intensive (IM, blue) and standard (SM, red) management systems.



Supplementary Figure B5. Relationship between mean yield in intensive management (IM) and standard deviation of yield response to IM for each of the 21 genotypes tested across site-years (A). Relationship of maximum yield values of each genotype and mean yield response to IM from all site-years (B). Relationship of maximum yield values of each genotype and standard deviation of yield response to IM from all site-years (C).

Appendix C. Supplementary Tables and Figures from Chapter 3

Appendix C. Tables

Supplementary Table C1. Treatment description for standard and intensive management systems including the addition (+) or removal (-) of inputs nitrogen (N) [rates vary with soil profile], sulfur (S) [45 kg ha^{-1} as gypsum (160 g S kg^{-1}) at Zadoks 30, chloride (Cl) [45 kg ha^{-1} as potassium chloride (450 g Cl kg^{-1}) at Zadoks 30], plant population [2.8 and 4.0 million seeds ha^{-1}), fungicide [Picoxystrobin, 63 g a.i. ha^{-1} at jointing or Zadoks 31 and Picoxystrobin + Cyproconazolez, 119 g a.i. ha^{-1} at heading or Zadoks 59], and plant growth regulator (GR) [Trinexapac-ehtyl, 250 g a.i. ha^{-1} at Zadoks 31) to their respective treatment control for 2016 and 2017 harvesting seasons in Kansas.

Management	**Treatment**	**Exception**	**Nitrogen**	**Sulfur (kg ha^{-1})**	**Chloride (kg ha^{-1})**	**Plant Population (million seeds ha^{-1})**	**Fungicide**	**PGR**
Standard	**1.SM control**	None	base + top dress	-	-	2.8	-	-
	2.Split-N	+N	base + top dress I + topdress II	-	-	2.8	-	-
	3.Sulfur	+S	base + top dress	+45	-	2.8	-	-
	4.Cl	+Cl	base + top dress	-	+45	2.8	-	-
	5.Pop	+Population	base + top dress	-	-	4.0	-	-
	6.Fung	+Fungicide	base + top dress	-	-	2.8	+Fung.	-
	7.GR	+GR	base + top dress	-	-	2.8	-	+GR
Intensive	**8.IM control**	None	base + top dress I + topdress II	+45	+45	4.0	+Fung.	+GR
	9.Split-N	-N	base + top dress I	+45	+45	4.0	+Fung.	+GR
	10.Sulfur	-S	base + top dress I + topdress II	-	+45	4.0	+Fung.	+GR
	11.Cl	-Cl	base + top dress I + topdress II	+45	-	4.0	+Fung.	+GR
	12.Pop	-Population	base + top dress I + topdress II	+45	+45	2.8	+Fung.	+GR
	13.Fung	-Fungicide	base + top dress I + topdress II	+45	+45	4.0	-	+GR
	14.GR	-GR	base + top dress I + topdress II	+45	+45	4.0	+Fung.	-

Supplementary Table C2. Treatment effects on nutrient utilization efficiency (UtE) of N, P, K, and S relative to standard (SM) and intensive management (IM) treatment controls for each harvest year 2016 and 2017.

		2016								2017						
Management	**Treatments**	**NUtE**		**PUtE**		**KUtE**		**SUtE**		**NUtE**		**PUtE**		**KUtE**		**SUtE**
					kg kg^{-1}								kg kg^{-1}			
Standard	**1.SM control**	21	b	138	*ns*	31	*ns*	283		25	a	212	*ns*	32	A	331
	2.Split-N	19		155		30		288		21	b	200		25	b	280
	3.Sulfur	20		147		30		233	b	26		217		32		260
	4.Cl	21		142		30		302		28		216		33		364
	5.Pop	19		132		28		267		26		213		30		336
	6.Fung	23	a	149		34		320	a	27		198		33		360
	7.Pgr	20		142		30		293		24		201		33		325
Intensive	**8.IM control**	19	B	153		29		224	B*	22	*	200		28		236
	9.Split-N	22	A	139		32		272	A	25		201		32		296
	10.Sulfur	19		145		30		271	A	23		213		30		335
	11.Cl	19		152		29		190		22		210		28		220
	12.Pop	18		145		27		204		22		214		27		218
	13.Fung	18		147		28		213		22		208		27		229
	14.Pgr	19		149		27		211		22		212		25		225

Note: Within the standard management, lower case letters indicate that treatments were significant different from the SM control (*$p<0.05$)*. Within the intensive management, upper case letters indicate that treatments were significant different from the IM control (*$p<0.05$*). *; indicates that treatment SM control was significantly different from treatment IM control. Letters in italic indicate marginal significant difference among treatments with 0.05>*p*≤0.1.

Supplementary Table C3. Treatment effects on yield and nutrient uptake per unit area of nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) relative to standard (SM) and intensive management (IM) treatment controls for each harvest year 2016 and 2017.

2016

Management	**Treatments**	**Yield**		**N**		**P**		**K**		**S**	
		Mg ha^{-1}				kg ha^{-1}					
	1.SM control	3.7	b	182		27	a	125		14	
	2. +topdress N	3.2		172		21	b	105		11	
	3. +Sulfur	3.4		170		23		118		15	
Standard	**4. +Cl**	3.6		171		26		123		12	
	5. +Pop	3.8		203		29		151		15	
	6. +Fung	*4.3*	*a*	187		29		131		14	
	7. +Greg.	3.5		176		25		125		13	
	8.IM control	4.6	A*	249	A	30	A	165	A*	21	A*
	9. –topdress N	4.2		194	B	30		135	*B*	15	B
	10. -Sulfur	4.3		236		30		154		16	B
Intensive	**11. -Cl**	4.4		241		29		162		24	
	12. -Pop	4.5		262		31		186		23	
	13. -Fung	3.6	B	201	B	25	B	132	*B*	17	B
	14. -Greg	5.0		269		34		197		25	

2017

Management	**Treatments**	**Yield**		**N**		**P**		**K**		**S**	
		Mg ha^{-1}				kg ha^{-1}					
	1.SM control	5.7	*ns*	226		27	*ns*	192		18	
	2.Split-N	5.1		235		25		207		19	
	3.Sulfur	5.4		206		25		177		21	
Standard	**4.Cl**	5.5		200		26		169		15	
	5.Pop	6.0		230		28		202		18	
	6.Fung	5.7		212		29		174		16	
	7.Pgr	4.9		204		24		165		16	
Intensive	**8.IM control**	6.4		294	A*	32		239	A*	28	A*
	9.Split-N	5.7		232	B	29		187	B	20	B
	10.Sulfur	5.7		251		27		204		18	
	11.Cl	5.7		262		27		213		26	
	12.Pop	5.1		230	B	24		197		24	
	13.Fung	5.3		243	B	26		207		24	
	14.Pgr	6.4		294		31		274		29	

Note: Within the standard management, lower case letters indicate that treatments were significant different from the SM control (*$p<0.05$*). Within the intensive management, upper case letters indicate that treatments were significant different from the IM control ($p<0.05$). *; indicates that treatment SM control was significantly different from treatment IM control. Letters in italic indicate marginal significant difference among treatments with $0.05>p\leq 0.1$.

Supplementary Table C4. Results from the standardized major axis analysis on the relationship between relative rate of N uptake versus relative rate of P, K, and S uptake including the estimate, lower and upper limits of the slopes of each treatment, and pairwise comparisons among treatments in 2016 and 2017. Slope differences among treatments were estimated by likelihood ratio statistical test (Ho: slopes are not equal) forcing intercept to the origin at *p<0.05* level.

	N vs P			**N vs K**			**N vs S**		
2016	estimate	lower	upper	estimate	lower	upper	estimate	lower	upper
SM	1.24	1.12	1.37	1.01	0.93	1.11	1.12	1.05	1.20
SM+Fung	1.21	1.12	1.32	1.00	0.93	1.06	1.10	1.04	1.16
IM	1.23	1.14	1.33	0.96	0.89	1.04	1.04	0.98	1.10
IM-Fung	1.22	1.13	1.32	0.98	0.88	1.08	1.07	0.98	1.16
	N vs P			**N vs K**			**N vs S**		
2017	estimate	lower	upper	estimate	lower	upper	estimate	lower	upper
SM	1.11	1.05	1.16	0.80	0.74	0.87	1.04	1.01	1.06
SM+Fung	1.10	1.03	1.17	0.84	0.78	0.91	1.05	1.02	1.08
IM	1.10	1.06	1.16	0.83	0.78	0.89	1.01	0.98	1.05
IM-Fung	1.11	1.05	1.16	0.83	0.77	0.89	1.03	0.99	1.07
Pairwise comparisons									
2016	N vs P	N vs K	N vs S		**2017**	N vs P	N vs K	N vs S	
SM vs. SM+Fung	*ns*	*ns*	*ns*		SM vs. SM+Fung	*ns*	*ns*	*ns*	
SM vs. IM	*ns*	*ns*	*ns*		SM vs. IM	*ns*	*ns*	*ns*	
IM-Fung.	*ns*	*ns*	*ns*		IM-Fung.	*ns*	*ns*	*ns*	

Note: ns; slopes were not significantly different among treatments at *p<0.05* for each year.

Supplementary Table C5. Coefficients of critical shoot nutrient concentration for each treatment across harvest years (2016 and 2017).

Treatment	**N**					**P**					**S**				
	a	**std. error**	**b**	**std. error**	**residual SS**	**a**	**std. error**	**b**	**std. error**	**residual SS**	**a**	**std. error**	**b**	**std. error**	**residual SS**
SM	3.41	0.13	0.29	0.03	22.90	0.24	0.02	0.08	0.04	0.29	0.24	0.01	0.27	0.03	0.19
SM+Fung.	3.21	0.11	0.25	0.02	19.94	0.25	0.25	0.06	0.06	0.22	0.22	0.01	0.22	0.03	0.19
IM	3.52	0.16	0.21	0.03	25.10	0.27	0.02	0.12	0.03	0.24	0.25	0.02	0.13	0.03	0.22
IM-Fung	3.31	0.15	0.18	0.03	24.41	0.25	0.02	0.09	0.04	0.31	0.25	0.02	0.10	0.04	0.27
average	3.36	0.14	0.23	0.02	23.09	0.25	0.07	0.09	0.04	0.27	0.24	0.01	0.18	0.03	0.22