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Temporal and Organ-specific Responses in NUE Traits to N Fertilization, Fungicide Intensity and Early Sowing in Winter Wheat Cultivars

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Abstract: Fungicide intensity and sowing time influence the N use efficiency (NUE) of winter wheat but the underlying mechanisms, interactions of plant traits, and the temporal effects are not sufficiently understood. Therefore, organ-specific responses in NUE traits to fungicide intensity and earlier sowing were compared at two nitrogen (N) levels for six winter wheat cultivars in 2017. Plants were sampled at anthesis and at maturity and separated into chaff, grain, culms, and three leaf layers to assess their temporal contribution to aboveground dry matter (DM) and N uptake (Nup). Compared to the control treatment, across cultivars, the treatment without fungicide mostly exerted stronger and inverse effects than early sowing, on grain yield (GY, -12% without fungicide, $+8\%$ n.s. for early sowing), grain Nup (GNup, -9% n.s., $+5\%$ n.s.) as well as on grain N concentration ($+4\%$, -2% n.s.). Grain yield in the treatment without fungicide was associated with similar total DM, as observed in the control treatment but with lower values in harvest index, thousand kernel weight, N use efficiency for GY (NUE) and N utilization efficiency. Lower GNup was associated with similar vegetative N uptake but lower values in N translocation efficiency and N harvest index. In contrast, early sowing tended to increase total DM at anthesis and maturity as well as post-anthesis assimilation, at similar harvest index and increased the number of grains per spike and total N use efficiency. Total N uptake increased after the winter season but was similar at anthesis. Although the relative N response in many traits was lower without fungicide, few fungicide \times interactions were significant, and the sowing date did not interact either with N fertilization for any of the N and DM traits. The results demonstrate the positive effects of fungicides and earlier sowing on various traits related to yield formation and the efficient use of nitrogen and are discussed based on various concepts.

Keywords: sowing date; reduced fungicide; leaf pathogens; nitrogen translocation; dry matter allocation; nitrogen allocation; yield components; post-anthesis assimilation; nitrogen harvest index

1. Introduction

Wheat contributes about 20% to the global calorie and protein production, but substantial advances in grain yield (GY) are required to meet the rising demand of a growing population with its altered consumption patterns [1]. However, efficient nitrogen (N) use, both in N-limited environments for stabilizing yields and in regions where high amounts of N fertilizers lead to detrimental environmental effects, is a major challenge to be addressed [2,3]. On the one hand, breeding progress increases N use efficiency (NUE) [4,5] through exploiting present genetic variation in NUE [6] and contributes to selecting suitable cultivars for specific growing regions [7]. On the other hand, agronomic measures must be optimized and adapted for modern cultivars. This includes optimal sowing densities and seed placement [8], sowing date [9,10], adapted timing and dosing of N fertilizers [11,12] as well as optimum fungicide strategies [13,14].

Early vigor is considered to improve the water use efficiency by increasing the use of available soil water in drought-prone environments and under climate change scenarios [15,16]. Genotypic differences in early vigor are substantial [17], but the timing of sowing and the interaction with N fertilization are equally important for the early development and final yield potential in different environments [9,10,18]. Winter wheat tolerates a large interval for sowing time, but pre-winter growth can increase the yield potential through enhanced tillering, pre-winter N uptake (Nup), better root growth and thus better acquisition of water and nutrients [19]. In addition, shifted phenology [20] may contribute to escape common drought and heat conditions, especially during grain-filling [21]. Higher pre-winter N uptake can reduce N leaching during winter [22] and thus increase NUE. In contrast, pre-winter growth can only be partly controlled so that early sowing can increase the risk of frost damage and pathogens if pre-winter growth is too strong [23,24]. Still, various studies found advantages in Nup and GY also at maturity [25–29]. Ferrise et al. [10] assessed the influence of the sowing date in interaction with N fertilization on dry matter and nitrogen translocation in durum wheat and reported stronger effects on GY than on GNup, altered grain N concentration but similar translocation efficiencies. In contrast, decreased dry matter translocation efficiencies were reported for late sowing in winter barley [30]. Other studies found increased dry matter production and Nup but a lower harvest index so that GY was little altered in early sowing [19,25]. In contrast, delayed sowing was recommended for Chinese conditions for maintaining NUE and GY [31,32].

Under humid growing conditions, adapted fungicide use is essential for maintaining GY [33,34]. Leaf fungicides can prolong the grain-filling phase by retarding senescence, both through curtailing disease-driven carbon loss and a physiological ‘greening effect’, thus enhancing assimilation [35]. Fungicide effects depend on the growing conditions that include temperature, precipitation and leaf moisture, and the optimum timing of the application [36,37]. Since diseases profit from higher N nutrition of the plant, fungicide effects often positively interact with the N fertilization [38–41].

Besides yield effects, positive effects on NUE were found [14,40,42–45]. Extended greenness increased kernel weight, post-anthesis N uptake (PANup), N harvest index and N translocation efficiency (NTEff) of vegetative N into the grain, and therefore GNup in a similar way as GY. Thus, no strong dilution effect on the grain N concentration (GNC) was reported [13]. Rather, the effects were related to longer greenness instead of higher rates of C and N accumulation. Disease-influenced variation in GNup was found to correlate more with PANup than with N translocation (NT) [45]. However, the use of fungicides is costly and increasingly questioned due to pathogen resistance, admission of chemical compounds, consumers’ acceptance, and the uncertainty of the economic benefit [46,47]. Thus, identifying potential detrimental effects of reduced fungicide use on GY and NUE, depending on N fertilization, and comparing cultivars for their response to reduced fungicide is important. Moreover, this study aims at identifying plant traits that influence yield formation and NUE and may help to understand genotypic differences.

Considering the knowledge gap in the traits contributing to NUE, GNup, and GY in response to reduced fungicide treatments and earlier sowing, we compared GY and GNup to a ‘normally’ sown and fungicide-treated control under two contrasting N regimes. Organ-level dry matter and N traits were determined at anthesis and maturity to address the magnitude and mechanisms of how treatments altered the target traits.

2. Materials and Methods

2.1. Study Site and Experiment

The field trial was conducted as a split-split-plot design with control (Cont)/reduced fungicide (RF)/early sowing (sowing date 1: SD1) on the main plot (MP), N level (N) on the sub-plot (SP) and cultivar (Cv) on the sub-sub-plot during the 2016/2017 growing season in southeastern Germany (48.406N, 11.692E). The field consisted mostly of homogeneous Cambisol type of silty clay loam.

The average annual precipitation in this region is approximately 800 mm, with an average annual temperature of 7.5 °C. The previous crop was grass-clover, treated with glyphosate before plowing.

A set of six winter wheat cultivars (Table 1) was mechanically drilled in a depth of 3 cm with a row spacing of 12.5 cm at 350 kernels m⁻². The plots were 1.5 m wide and 6.4 m long. The winter wheat cultivars comprised three high-performance commercial German line cultivars and three hybrid cultivars, registered and released by the national authorities. Additionally, three other cultivars were included in all main plots but neglected due to the previous sampling during early plant development [48], and another 11 cultivars, included only in Cont and not destructively sampled. The cultivars include three quality groups for winter wheat (E: Highest baking, A: High baking quality and B: Bread wheat) and differ in yield components, disease susceptibility and phenology (Table 1). Especially the line cultivars are frequently grown by German farmers.

Table 1. List of cultivars and ratings of the German official registration authority [49]: Yield components, baking quality class (E: Highest baking, A: High baking quality, and B: Bread wheat), heading, senescence and susceptibility (Suscep.) for main diseases, from 1 (low) to 9 (high).

Cultivar	Year of Release	Cultivar Group	Spikes/m ²	GNS	Kernel Weight	Quality Class	Heading	Senesc-ence	Suscep. Septoria	Suscep. Yellow Rust	Suscep. Fusariosis
Hybred	2003	hybrid	4	8	5	B	5	6	6	-	4
Hyland	2009	hybrid	4	9	4	B	4	5	3	2	4
Hystar	2007	hybrid	5	6	5	B	-	4	-	-	-
Impression	2005	line	7	4	5	A	5	6	5	3	3
JB Asano	2008	line	5	5	7	A	4	4	6	5	5
Kerubino	2004	line	7	4	5	E	4	5	4	-	4

Sowing date was 28 September for SD1 and 23 October for Cont and RF. The seeds of the line cultivars were dressed with Landor CT (2.4% fludioxonil, 1.9% difenoconazole and 0.5% tebuconazole), the seeds of the hybrid cultivars with Vibrance Gold (4.63% sedaxane, 2.32% fludioxonil and 2.32% difenoconazole, both Syngenta, Basel, Switzerland), to avoid seed-borne diseases. Each cultivar was cultivated at two N levels (N1, N2) within each of the three main plots, and in four replicates per N level, resulting into four replicates for each factor combination (MP × N × Cv). Nitrogen was fertilized as granulated ammonium nitrate at tillering (27 March, N1: 30 and N2: 60 kg N ha⁻¹), and at stem elongation (18 May, N1: 30 and N2: 60 kg N ha⁻¹). Total nitrogen amounts were kept low due to the pre-crop effect of the grass-clover mixture. An adequate supply of K, P, and S was ensured, and integrated pest management kept the field weed-free. A straw shortener based on chlormequat chloride and trinexapac-ethyl was applied to all plots. Foliar fungicide was applied twice in Cont and SD1 plots, but not in RF plots:

18 May, 2017 at Zadok's growth stage 39 (end of stem elongation): 0.5 L ha⁻¹ Input@Xpro (100 g L⁻¹ prothioconazole, 250 g L⁻¹ spiroxamine, 50 g L⁻¹ bixafen) + 0.7 L ha⁻¹ Skyway@Xpro 75 g l⁻¹ bixafen, 100 g L⁻¹ prothioconazole, 100 g l⁻¹ tebuconazole (both Bayer CropScience, Leverkusen, Germany).

30 May, 2017 at Zadok's growth stage 49 (end of booting): 2.0 L ha⁻¹ Ceriax®(41.6 g L⁻¹ epoxiconazole, 41.6 g L⁻¹ fluxapyroxad, 66.6 g L⁻¹ pyraclostrobin (BASF, Ludwigshafen, Germany).

The study year was characterized by overall favorable weather conditions with above-average temperatures and global radiation during March, May, and June in spite of below-average conditions in April (Figure 1). Conversely, precipitation was above average during April and July but lower than in the previous years during May and June, leading to mild drought stress and accelerated senescence in some cultivars during the grain-filling phase.

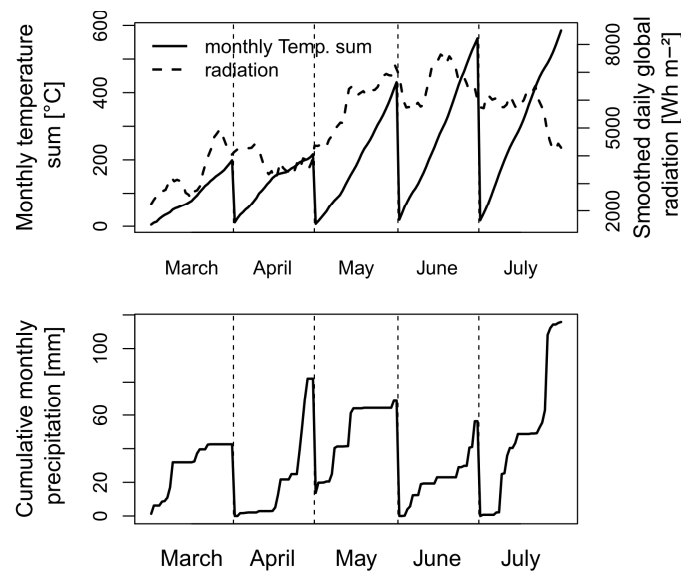


Figure 1. Weather conditions during the main wheat-growing period from March to July 2017 at the experimental site. On 9–10 June, anthesis was on average in the control and reduced fungicide plots and on 6–7 June in the earlier sown plots.

2.2. Plant Sampling and Sample Analysis

Biomass was sampled at mid-flowering (Zadok's growth stage 65 [50], similar to the BBCH-scale [51]) and at physiological maturity (growth stage 95). Sampling dates were determined for each genotype within each subplot by visual scoring. Thirty and 60 randomly selected spiked shoots were cut directly at the stem base at anthesis and maturity, respectively. The plants were manually separated into flag leaves, flag leaves-1 (second leaves from above), 'other leaves' (remaining leaves), culms including leaf sheaths, and spikes.

Plant samples were oven-dried at 50 °C until a stationary weight was reached for subsequent determination of dry weight [52]. All dry matter (DM) traits were standardized to 100% DM content. The vegetative plant samples were homogeneously ground (1 mm) to assess the N concentration (NC) by near-infrared spectroscopy (NIRS) using a FOSS NIRS 6500 (Foss, Silver Spring, MD, USA) and an FT-NIRS (Bruker, Billerica, MA, USA). Grains were analyzed as complete kernels. After plant sampling at maturity, all plots were harvested (1–2 August, 2017) using a combine harvester, and moisture-corrected grain yield (0%) was determined for each plot. Grain number per spike (GNS) and thousand kernel weight (TKW) were determined. By combining the information of yield per spike and yield per plot, the number of spikes m^{-2} (spike density: SD) was calculated. Nitrogen uptake (Nup) was calculated by multiplying nitrogen concentration (N%) with DM. Plant height was determined on the plot level using an ultrasonic sensor [53] on 4 July.

2.3. Calculation of Derived Plant Traits

Derived plant traits were calculated for yield components, DM and N translocation as well as the efficiency of N uptake and utilization as described in [52]: See Table 2 for a list of considered plant traits.

Table 2. List of plant traits considered in this study, grouped by trait groups. See Table 3 for equations of derived, calculated plant traits. Corresponding trait complexes are indicated as described in Equation (1).

Trait Group	Plant Trait	Abbreviation	Description	Trait Complex
Dry matter traits [kg ha ⁻¹]	organ-specific dry matter (vegetative)	DM [organ-name]	at anthesis (Ant) and maturity (Mat) for flag leaf, flag leaf-1, 'other leaves', culms, and summed up for the three leaf layers, at anthesis for spikes and at maturity for chaff	
	total dry matter	DM _{Ant} , DM _{Mat}	sum of organ-specific DM-traits at anthesis and maturity	(d), (i)
	grain yield	GY (DM grain)		(a), (b), (c), (d)
N concentration traits [%]	organ-specific N concentration (vegetative)	NC [organ-name]	at anthesis and maturity for flag leaf, flag leaf-1, 'other leaves' and culms, at anthesis for spikes and at maturity for chaff	
	grain N concentration	GNC		(a)
N uptake traits [kg ha ⁻¹]	organ-specific N uptake (vegetative)	Nup [organ-name]	at anthesis and maturity for flag leaf, flag leaf-1, 'other leaves', culms, and summed up for the three leaf layers at anthesis for spikes and at maturity for chaff	(j), (k)
	total N uptake	Nup _{Ant} , Nup _{Mat}	sum of organ-specific DM-traits at anthesis and maturity	(h), (k)
	N uptake straw	Nup straw	sum of organ-specific vegetative N uptake at maturity	
	grain N uptake	GNup		(a), (g), (h)
Derived DM traits	harvest index	HI	relative dry matter partitioning to the grain	(d)
	organ-specific dry matter harvest index (vegetative)	HI [organ-name]	relative dry matter partitioning to vegetative organs at anthesis and maturity	
	post-anthesis assimilation	PAA		(c)
Derived DM traits	total dry matter	DMTEff		(i)
	translocation efficiency	DMTEff		
	organ-specific dry matter translocation efficiency	[organ-name]		
	contribution of post-anthesis assimilates to grain-filling	CPAA		
	total dry matter translocation	DMT		(c)
	organ-specific dry matter translocation	DMT [organ-name]		
	grain number per spike	GNS		(b)
	thousand kernel weight	TKW		(b)
	spike density	SD		(b)
	N utilization efficiency for total dry matter at maturity	NutEff _{total}		
N utilization efficiency for grain yield	NutEff _{grain}			
N use efficiency for total dry matter at maturity	NUE _{total_Mat}			
N use efficiency for grain yield	NUE _{grain}			
kernels per m ²	-			
yield per spike	-			
Derived N traits	post-anthesis N uptake	PANup		(g)
	contribution of post-anthesis nitrogen to total nitrogen uptake	CPNup		
	total N translocation	NT		(g)
	organ-specific N translocation (vegetative)	NT [organ-name]		
	N translocation efficiency	NTEff		(m)
	organ-specific N translocation efficiency	[organ-name]		
	N harvest index	NHI		(h)
	organ-specific N harvest index (vegetative)	NHI [organ-name]	relative N partitioning to the grain relative N partitioning to vegetative organs at anthesis and maturity	
	apparent N uptake efficiency at anthesis	NupEff _{Ant}		
	apparent N uptake efficiency at maturity	NupEff _{Mat}		
Other traits	plant height	-		
	anthesis date	-		

Table 3. List of derived plant traits, which were calculated from ‘direct’ dry matter (DM) and N-uptake (Nup) traits. Dry matter translocation, N translocation, dry matter translocation efficiency, N translocation efficiency, harvest index, and N harvest index were calculated on the total aboveground plant level and on the organ-level.

Equation	Plant Trait and Description
$DMT = \frac{DM(spikes + stems + leaves)_{anthesis} - DM(chaff + stems + leaves)_{maturity}}{DM(chaff + stems + leaves)_{maturity}}$	Dry matter translocation (DMT): Absolute amount of translocated pre-anthesis accumulated assimilates from vegetative plant organs into grains between anthesis and maturity, in kg ha ⁻¹ [54]
$DMTEff = DMT/DM_{anthesis}$	DMT-efficiency (DMTEff): Relative amount of translocated pre-anthesis accumulated assimilates into grains [54].
$PAA = DM_{maturity} - DM_{anthesis}$	Post-anthesis assimilation (PAA)
$CPAA = PAA/DM_{(grain)_{maturity}}$	Contribution of post-anthesis assimilates to grain-filling (CPAA)
$HI = DM_{grain}/DM_{total}$	Harvest index (HI): Ratio of grain DM to total DM at maturity
$NTEff = NT/Nup_{anthesis}$	Absolute amount of translocated pre-anthesis accumulated nitrogen from vegetative plant organs into grains between anthesis and maturity in kg ha ⁻¹ (NT) [55]
$PANup = Nup_{maturity} - Nup_{anthesis}$	NT-efficiency (NTEff): Relative amount of translocated pre-anthesis accumulated nitrogen into grains [55]
$CPNup = PANup/Nup_{total}$	Post-anthesis nitrogen uptake (PANup)
$NHI = Nup_{grain}/Nup_{total}$	Contribution of post-anthesis nitrogen to total nitrogen uptake (CPNup)
$NupEff = Nup_{total}/N_{fertilized}$	N-Harvest index (NHI): Ratio of grain nitrogen uptake (Nup) to total Nup at maturity
$NutEff_{grain} = DM_{grain}/Nup_{total}$	Apparent nitrogen uptake efficiency, calculated for anthesis and maturity as ratio of total Nup to total N fertilized [56]
$NutEff_{total} = DM_{total}/Nup_{total}$	Nitrogen utilization efficiency (NutEff): Efficiency of the internal conversion of N into grain and total DM [56].
$NT = \frac{Nup(spikes + stems + leaves)_{anthesis} - Nup(chaff + stems + leaves)_{maturity}}{Nup(chaff + stems + leaves)_{maturity}}$	(1)

Dry matter (DM) units of all plant components corresponding to the number of sampled shoots were scaled-up to kg ha⁻¹ using spikes per m² values for each plot. The following parameters were assessed to compare the temporal DM and N accumulation (Table 3).

Besides the calculation for the total above-ground plant, the following traits were calculated at the plant organ level (Supplementary Table S1): DMT, DMTEff, NT, NTEff, HI and NHI for the vegetative organs, both at anthesis and at maturity. Beside the values for the three leaf layers, aggregated values for ‘all leaves’ were included.

2.4. Statistical Analysis

Statistical analysis was conducted in R 3.4.2 (R Stat. Core Team, 2017). All traits were subjected to analysis of variance (ANOVA): The main plot treatment (MP) representing three agronomic scenarios was considered as early sowing (sowing date 1, SD1), control (second sowing date, full fungicide treatment) and the reduced fungicide (second sowing date, no leaf fungicide applied). The N fertilization (N) on the sub-plot was present within each main plot and comprised six cultivars (Cv) on the sub-sub-plot. A random replicate effect was considered nested within the main plots. Type III sums of squares were calculated using the lmer function (lmerTest) in R using Satterthwaite’s approximation for the main and interaction effects. In addition to the analysis on the whole trial, the ANOVA analysis was conducted for two subsets, comprising the control (Cont) together with the reduced fungicide (RF, Cont + RF) main plots and Cont together with the sowing date 1 (SD1,

Cont + SD1) main plot, respectively. The estimated marginal mean values were calculated based on the model for the whole trial, using the emmeans function and compared by Tukey's post-hoc test (i) for the three MP treatments, (ii) the two N treatments across the main plots, (iii) the two N treatments within the main plots and (iv) the cultivars across main plots. The percentage treatment response was calculated between RF and Cont, between SD1 and Cont and between N2 and N1 within the three main plots for comparing the response strength.

Trait combinations were tested within each sub-plot (SPs) within the main plot combinations for linear relationships, and for linear and quadratic relationships across the 'whole trial' (WT) using estimated marginal mean values and are shown for selected traits corresponding to different trait complexes (Equation (1)).

Equation (1): Concepts applied to dissect *NUE* into grain yield (*GY*), grain N uptake (*GNup*), grain N concentration (*GNC*) and selected contributing traits: *GNup* as the product of *GY* and *GNC* (a), *GY* as the product of the yield components spike density (*SD*), grain number per spike (*GNS*) and thousand kernel weight (*TKW*, b), *GY* as the sum of dry matter translocation (*DMT*) and post-anthesis assimilation (*PAA*, c), *GY* as the product of total DM and harvest index (*HI*, d), nitrogen use efficiency (*NUE*) as product of N uptake efficiency and N utilization efficiency with respect to *NUE* for total DM production (e) and *NUE* for *GY*, respectively (f), *GNup* as the sum of N translocation (*NT*) and post-anthesis N uptake (*PANup*, g), *GNup* as the product of N uptake (*Nup*) and the N harvest index (*NHI*, h), dry matter translocation (*DMT*) as the product of *DM* at anthesis and *DM* translocation efficiency (*DMTEff*, i), *Nup* at anthesis as the sum of organ-level *Nup* (j), *Nup* at maturity as the sum of organ-level *Nup* (k), N translocation (*NT*) as the sum of partial *NT*s by organs (l), *NT* as the product of *Nup* at anthesis and N translocation efficiency (*NTEff*, m).

$$GNup = GY \times GNC \quad (1a)$$

$$GY = SD \times GNS \times \frac{TKW}{1000} \quad (1b)$$

$$GY = DMT + PAA \quad (1c)$$

$$GY = DM_{Mat} \times HI \quad (1d)$$

$$NUE_{total} = NupEff \times NutEff_{total} \quad (1e)$$

$$NUE_{grain} = NupEff \times NutEff_{grain} \quad (1f)$$

$$GNup = NT + PANup \quad (1g)$$

$$GNup = Nup_{Mat} \times NHI \quad (1h)$$

$$DMT = DM_{Ant} \times DMTEff \quad (1i)$$

$$Nup_{Ant} = Nup_{spikes} + Nup_{culm} + Nup_{flag\ leaf} + Nup_{flag\ leaf-1} + Nup_{other\ leaves} \quad (1j)$$

$$Nup_{Mat} = Nup_{grain} + Nup_{chaff} + Nup_{culm} + Nup_{flag\ leaf} + Nup_{flag\ leaf-1} + Nup_{other\ leaves} \quad (1k)$$

$$NT = NT_{spike} + NT_{culm} + NT_{flag\ leaf} + NT_{flag\ leaf-1} + NT_{other\ leaves} \quad (1l)$$

$$NT = Nup_{Ant} \times NTEff \quad (1m)$$

3. Results

3.1. Treatment Effects

ANOVA was conducted to assess the influence of the main plot (MP) treatments 'sowing date 1' (SD1) and 'fungicide application' (RF) compared to the control (Cont), the 'N level' (N) treatments on the sub-plot and the 'cultivar' (Cv) on the sub-sub-plot on the dry matter (DM) and nitrogen (N) traits, both for the whole trial and two subsets, comprising the control (Cont) together with the reduced

fungicide (RF) main plots and Cont together with the sowing date 1 (SD1) main plot, respectively (Table 4).

For almost all traits, cultivar effects were significant in the whole trial as well as in both trial subsets with the exception of N concentration (NC) of flag leaf-1 at maturity (Mat.), grain N uptake (Nup), N utilization efficiency (NutEff) at anthesis and total N use efficiency (NUE), and only few other traits within both trial subsets. Among direct DM traits, the main plot treatments showed an effect for the whole trial for anthesis DM only for 'other leaves' (leaves excluding both uppermost layers), at maturity most strongly for grain DM (GY) in the whole trial and in Cont + RF, for DM of culms both in the whole trial and in Cont + RF, for 'other leaves' in the whole trial, and in Cont + SD1 and for the total above-ground DM only for the total trial. No two-way MP \times N interactions were found in the three models and MP \times Cv interactions only for the whole trial and for Cont + SD1 mainly for anthesis traits. N \times Cv interactions were present for some traits of total DM at anthesis (Ant) for all three models. For N concentration (NC traits), N effects were found for all traits in the three models while MP effects were mainly limited to the maturity sampling date. Within the trial subsets, MP \times N interactions were significant for 'maturity flag leaf' and for 'anthesis other leaves' in Cont + RF. The MP \times Cv interactions were found for most anthesis NC traits in Cont + SD1.

Influenced both by the MP and N effects on NC traits, the direct N uptake (Nup) traits responded more to MP and especially N treatments than the DM-related traits, especially for the maturity sampling. Within the trial subsets, MP \times N interactions were only found in Cont + RF for 'anthesis other leaves', grain Nup and 'maturity flag leaf', MP \times Cv interactions, especially for anthesis Nup traits in Cont + SD1.

Among the three direct yield components, spike density (SD), grain number per spike (GNS) and thousand kernel weight (TKW), only GNS responded to N in the whole trial and in Cont + RF, whereas the aggregated yield component kernels per m² showed an N effect in all three models. MP was significant for all direct yield components in the whole trial and in Cont + RF, only for GNS in Cont + SD1, never for kernels per m² but always for yield per spike. Within the trial subsets, MP \times Cv was significant in Cont + SD1. With respect to traits related to DM translocation (DMT, DMTEff) and post-anthesis assimilation (PAA, CPAA), N was always significant, but MP and MP \times N never; MP \times Cv was significant in Cont + SD1 but not in Cont + RF.

Considering the N use efficiency for grain (NUE_{grain}) as the product of N uptake efficiency (NupEff) and N utilization efficiency for grain (NutEff_{grain}), only N and Cv-main effects were found for NupEff. For NutEff_{grain}, most main effects were significant, but no interactions were observed within trial subsets for both traits. The harvest index (HI) showed the effects of MP in Cont + RF and in the whole trial. As for DMT and PAA, the MP effect was not significant for N translocation (NT) and post-anthesis N uptake (PANup). However, N was always significant for PANup, its contribution to total Nup (CPNup), but for NT only for the whole trial and in Cont + SD1.

All main effects were significant for the N translocation efficiency (NTEff) with the exception of MP in Cont + SD1. Within the trial subsets, MP \times N was significant for NT in Cont + RF and MP \times Cv for PANup, CPNup, NT, and NTEff in Cont + SD1. For the N harvest index (NHI), no N and interaction effects but an effect of MP in the whole trial and in Cont + RF, and of Cv in all models was found. For anthesis date, MP and N were significant with the exception of MP in Cont + RF, the MP \times N interaction only in Cont + SD1 and in the whole trial. For plant height, effects of N were present in all models and of MP and MP \times N in Cont + RF.

Table 4. ANOVA results for significant (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$) effects of the main plot (MP), N level (N) and cultivar (Cv) as well as two-way interactions, SD1: Sowing date 1, Cont: Control, RF: Reduced fungicide. Models are reported for the whole trial and for the combined main plots Cont + RF and Con t + SD1 for assessing the effect of reduced fungicide and SD1 in comparison to Cont, respectively. The main effects are shaded in gray and interaction effects in blue. For anthesis date, no cultivar effects are reported due to missing data. ‘Other leaves’ denotes the leaves, excluding both uppermost leaf layers, ‘Ant.’ anthesis and ‘Mat.’ maturity. See Supplementary Table S1 for results of further indirect DM and N traits related to organ-specific DM and N allocation, DM and N translocation and translocation efficiencies.

		Whole Trial			Cont + RF			Cont + SD1			Whole Trial			Cont + RF			Cont + SD1			
		MP	N	Cv	MP	N	Cv	MP	N	Cv	MP x N	MP x Cv	N x Cv	MP x N	MP x Cv	N x Cv	MP x N	MP x Cv	N x Cv	
DM traits [kg ha ⁻¹]	Ant. spikes		*	***			***			***		*	*					***	*	
	Ant. culms			***		*	***	*	***	***		*	**			*		***	*	
	Ant. flag leaf		***	***		**	***		***	***		***						***		
	Ant. flag leaf-1			***		***	***	*	***	*	***		*					***		
	Ant. other leaves	***		***		*	***	**	***	***		**	**			**		***	*	
	Ant. leaves			***		***	***		***	*	***		*					***		
	Mat. grain (GY)	***	***	***	*	***	***		***	***								***		
	Mat. chaff		**	**		**	*		**	*										
	Mat. culms	*	*	***	***	**	***		**	***									*	
	Mat. flag leaf		***	***		**	***		***	***									**	
	Mat. flag leaf-1		*	***		***	***	*	***	*	***								*	
	Mat. other leaves	*		***		***	***	*	***	***									*	
	Mat. leaves		*	***		***	***		**	***									*	
	Ant. total			***		***	***		***	***			*			*		***	*	
Mat. total	*	***	*		***	***		***	**									***	*	
NC traits [%]	Ant. spikes	*	***	***		***	***	*	***	**		***	***		***			***	***	
	Ant. culms		***	***		***	***		***	**		***	***		***			*		
	Ant. flag leaf		***	***		***	***		***	***		*	***		*			**	**	
	Ant. flag leaf-1		***	***		***	***		***	***		***	***		***			*	**	
	Ant. other leaves	**	***	***	**	**	***		***	***				*	*			*	**	
	Mat. grain (GNC)	**	***	***	*	***	***		***	***				*	*					
	Mat. chaff	***	***	***	***	***	***		***	***				*	*					
	Mat. culms	***	***	**	***	***	**		***	***		*			*			*		
	Mat. flag leaf	***	***	***	***	***	***		***	***	**	***		**	***	*			*	
	Mat. flag leaf-1	***	***	***	***	***	***		***	*		*								
	Mat. other leaves	***	***	***	***	***	***	***	***	***		**							***	
	Nup traits [kg ha ⁻¹]	Ant. spikes			***		**		**	***		**	*		*				***	
		Ant. culms		***	*		***	***		***	***								**	
		Ant. flag leaf		***	***		***	***		***	***		**						***	
Ant. flag leaf-1		*	***	***		***	***		***	***		*			*			***		
Ant. other leaves		***		***	**	***	***	*	**	***	**	*	***	*	*	***		***	*	
Ant. leaves			***	***		**	***		***	***		*						***		
Mat. grain (GNup)		*	***			***	***		***	***	**			*						
Mat. chaff			***	***	*	***	***		***	***				**						
Mat. culms		***	***	***	***	***	**		***	***										
Mat. flag leaf		***	***	***	***	***	***		***	***	**	***		**	***	*				
Mat. flag leaf-1		***	***	***	***	***	***		***	*										
Mat. other leaves		***	***	***	***	**	***	***	***	***									*	
Nup traits [kg ha ⁻¹]		Mat. leaves	***	***	***	***	***	***		***	***		***		**				***	
		Ant. total		***	***		**	**		***	***		*						***	
	Mat. total		***	**		***	***		***	*	*									
	Mat. straw	***	***	***	***	***	***		***	***										
Derived DM traits	HI	***		***	***	***	***		***	***								*	**	
	PAA		***	***		***	***		***	***	**			*				***		
	DMTEff		***	*		***	**		**									**		
	CPAA		***	***		***	***		***	***	*			*				***		
	DMT		**	***		**	***	*	**		*							***		
	GNS	***	*	***	*	*	***	***		***	***			***				***		
	TKW	***		***	***	***	***		***	***	*									
	spike density	*		***	*	***	***		*	***								*		
	NutEff_total	*	***	**		***	***		***	*				*				*		
	NutEff_grain	***	***	***	***	***	***		***	***	*									
	NUE_total	*	***	***	***	***	***		***	**										
	kernels per m2		***	***		***	***		***	***	**								*	
	yield per spike	***	*	***	***	***	***	**	***	***		***						***		
	NUE_grain	***	***	***	*	***	***		***	***	**			*						

Table 4. Cont.

		Whole Trial			Cont + RF			Cont + SD1			Whole Trial			Cont + RF			Cont + SD1			
		MP	N	Cv	MP	N	Cv	MP	N	Cv	MP × N	MP × Cv	N × Cv	MP × N	MP × Cv	N × Cv	MP × N	MP × Cv	N × Cv	
derived N traits	PANup		***	*		***	***		***	*		**	*			*		***	***	***
	CPNup		***	**		***	***		***	**		**	*			*		***	***	***
	NT		**	**			**		***	***	*	*	*					***	***	***
	NTEff	***	***	***	***	***	***		**	*									*	
	NHI	***	***	***	***	***	***		***	***									***	***
	NupEff Ant. NupEff Mat.		***	***		***	**		***	***									***	***
other traits	plant height anthesis date	***	***	-	**	***	***	***	***	***	*		*				***	-	-	-

3.2. Comparison of Trait Values and Trait Relationships

Treatment means were compared by Tukey’s post-hoc-test and represented for selected traits, which are described by different trait complexes (Equation (1)) as estimated marginal mean values (Table 5). The relationships of selected pairwise combinations of traits included in trait complexes were assessed for the variation by cultivars in the six differing MP × N combinations, and across the whole trial (WT).

3.2.1. Trait Complex a: GNup = GY × GNC

Across the whole trial (WT), GNup was linearly related to grain DM at maturity (GY), explaining 51% of the variation (Figure 2a). For the GNC/GNup relationship, mainly the N fertilization effect was captured in WT (Figure 2b), whereas GNC and GY tended to correlate negatively within the sub-plots (Figure 2c). Within the MP × N level combinations, most relationships were weak, not consistent with trait combinations or treatments, and rarely significant due to the low number of cultivars (n = 6).

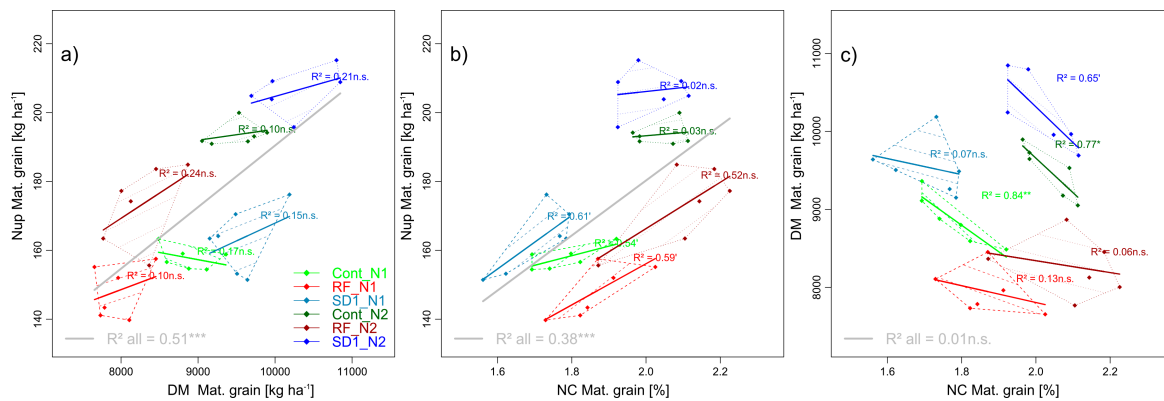


Figure 2. Pairwise trait relationships in trait complex a: The relationships between grain Nup and grain yield (a), grain Nup and grain N concentration (b) and grain yield and grain N concentration (c). Data points correspond to average cultivar values. N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP × N-combinations. Colored regression lines indicate the linear trait relationships within MP × N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). P-values of regression slopes are indicated as $p < 0.01$ (***) , $p < 0.01$ (**) , $p < 0.05$ (*) and $p < 0.1$ (').

Grain yield (GY, grain DM at maturity) increased only moderately in response to a doubling of the applied N fertilizer from 60 to 120 kg N ha⁻¹, from 8.8 t ha⁻¹ on average (rounded up to 1 decimal) to 9.3 t ha⁻¹ (Table 5, Figure 2). Reducing fungicide (RF) decreased GY by 1.1 t ha⁻¹ on average (-12%)

compared to the control treatment (Cont), whereas earlier sowing tended to increase GY by 0.7 t ha^{-1} (+8%, n.s.). Within all main plots, GY increased between both N levels (interaction n.s.), in Cont and SD1 by 7%, but in RF by only 4%. With the stronger reaction of the grain N concentration (GNC) to N fertilization by 13%–14% (N2 compared to N1 in Cont and RF) and 18% (SD1), grain N uptake (GNup) increased by 23% (Cont), 17% (RF) and 26% (SD1). Conversely to GY, GNC was higher in RF than in Cont but slightly lower (n.s.) in SD1 than in Cont. Due to the MP*N-interaction, GNup was significantly lower in RF than in Cont and SD1 only in N2 (not shown), and amounted, on average, to 176, 161 and 185 kg N ha^{-1} in Cont, RF, and SD1, respectively. GY was lowest for the line cultivar Kerubino followed by JB Asano and Impression, and highest for the three hybrid cultivars Hystar, Hybred and Hyland, which reached lower GNC, however. Thus, no cultivar differences were found for GNup across all treatments (Table 4).

Table 5. Estimated marginal mean values and grouping letters of Tukey’s post-hoc-test by treatment factors for selected traits. See Equation (1) for the relationship of traits within trait complexes. Cells colored from red (strong negative reaction) to green (strong positive reaction) indicate the relative response in the trait value between Cont and RF and between Cont and SD1 as the main plot effects and between N1 and N2 within the main plots. PANup was not included for coloring due to extreme relative responses, however on low levels only. For the anthesis date, independent data was lacking for statistics within the main plots.

Treatment	DM Mat. Grain (GY)	DM Ant. total	DM Mat. total	HI	PAA	DMTEff	DMT	GNS	TKW	Spike Density	NutEff Total	NutEff Grain	NUe Mat. Total	NUe Mat. Grain	NC Mat. Grain (GNC)	Nup Ant. Spikes	Nup Ant. Culms	Nup Ant. Flag Leaf	Nup Ant. Flag Leaf-1	Nup Ant. Other Leaves	Nup Mat. Grain (GNup)	Nup Mat. Chaff	Nup Mat. Culms	Nup Mat. Flag Leaf	Nup Mat. Flag Leaf-1	Nup Mat. Other Leaves	Nup Ant. Total	Nup Mat. Total	PANup	NT	NTEff	NHI	NupEff Mat.	Plant Height	Anthesis (Days in June)
Cont	9189b	13091a	17340a	0.53b	4267ab	0.37a	4930a	36.4a	38.6b	663ab	83.94ab	44.49b	214a	114b	1.9a	44a	70a	36a	28ab	18b	176ab	9a	15a	3a	2a	3a	196a	209a	13a	163a	0.83b	0.84b	2.52a	0.65a	9.5b
RF	8107a	13477a	16884a	0.48a	3407a	0.34a	4717a	35.1a	33.6a	697b	82.09a	39.43a	211a	101a	2b	43a	76a	37a	29b	15a	161a	10a	20b	8b	4b	4b	201a	207a	6a	155a	0.77a	0.78a	2.52a	0.69b	9.5b
SD1	9895b	13956a	18706b	0.53b	4712b	0.37a	5154a	40.2b	40b	629a	86.56b	45.87b	231b	122c	1.9a	43a	73a	34a	26a	21c	185b	10a	15a	3a	2a	4b	197a	219a	21a	163a	0.83b	0.85b	2.63a	0.67ab	6.7a
Cont->SD1	8%	7%	8%	0%	10%	-2%	5%	10%	4%	-5%	3%	3%	8%	8%	-2%	-4%	5%	-4%	-6%	16%	5%	2%	1%	-8%	-2%	29%	1%	5%		0%	0%	0%	5%	3%	
N1	8787a	13658a	17178a	0.51a	3527a	0.38b	5264b	36.8a	37.3a	653a	90.33b	46.27b	286b	146b	1.8a	43a	68a	34a	27a	18a	156a	9a	15a	4a	3a	4a	189a	191a	2a	154a	0.82b	0.82a	3.18b	0.65a	8.3a
N2	9340b	13358a	18108b	0.52a	4730b	0.34a	4603a	37.7a	37.6a	673a	78.06a	40.25a	151a	78a	2.1b	44a	78b	38b	29b	18a	191b	10b	18b	5b	4b	4b	207b	232b	25b	166b	0.8a	0.82a	1.94a	0.69b	8.9b
cv. Hyland	9461c	13546b	17982a	0.53cd	4450b	0.37ab	5024abc	42.2c	35.1a	645b	85.74b	45.18b	222a	117c	1.9ab	43ab	74ab	36bc	29bc	19c	176a	9a	17ab	4a	3a	4ab	201ab	212ab	11ab	165b	0.82c	0.83c	2.55ab	0.68bc	8.7c
cv. Hybrid	9217bc	14281b	18125a	0.51ab	3827ab	0.37ab	5379bc	40.7c	35.9ab	639b	83.58ab	42.46a	224a	114bc	1.9bc	48c	72ab	40c	31c	20c	176a	11d	17ab	7b	3b	4c	210b	219b	9ab	167b	0.8ab	0.81a	2.64b	0.66b	10.5f
cv. Hystar	9228bc	11989a	17049a	0.54d	4987b	0.35ab	4182a	37b	37.4bc	678bc	86.25b	46.7b	212a	114bc	1.8a	39a	65a	35b	22a	15ab	165a	9ab	15a	4a	3ab	3a	177a	199a	22b	142a	0.81abc	0.83bc	2.41a	0.6a	4.3a
cv. Kerubino	8634a	14380b	17362a	0.5a	2984a	0.39b	5652c	32.1a	37.9c	715c	82.2a	40.85a	216a	107a	2d	43ab	76b	37bc	30bc	23d	174a	9abc	18b	4a	3a	4bc	209b	212ab	3a	170b	0.81bc	0.82ab	2.59ab	0.69bcd	8.5b
cv. Impression	8939ab	13572b	17994a	0.5a	4457b	0.33a	4517ab	31.8a	38.7cd	728c	84.34ab	41.8a	223a	111ab	1.9cd	43abc	78b	38bc	28b	13a	175a	10bc	18b	6b	3ab	4bc	200ab	216b	16ab	159ab	0.79a	0.81a	2.61ab	0.69cd	10.3e
cv. JB Asano	8902ab	13279ab	17350a	0.51bc	4066ab	0.36ab	4848abc	39.7bc	39.7d	571a	83.06ab	42.58a	215a	110ab	2cd	45bc	72ab	29a	28b	16b	176a	10cd	15a	4a	3a	4abc	191ab	211ab	20ab	156ab	0.82bc	0.83c	2.54ab	0.71d	9d
Cont N1	8872a	13198a	16760a	0.53a	3585a	0.4b	5304b	36.2a	38.5a	645a	89.64b	47.47b	280b	148b	1.8a	43a	64a	33a	27a	17a	158a	9a	13a	3a	2a	3a	185a	188a	3a	155a	0.84b	0.84a	3.13b	0.64a	-
Cont N2	9505b	12984a	17921b	0.53a	4948b	0.35a	4557a	36.7a	38.7a	680a	78.24a	41.5a	149a	79a	2b	45a	75b	38b	30b	18a	194b	10b	16b	4b	3b	3b	206b	230b	23b	170b	0.82a	0.84a	1.91a	0.67b	-
RF N1	7949a	13942b	16683a	0.48a	2739a	0.37b	5219b	34a	34a	696a	87.63b	41.79b	278b	133b	1.9a	44a	73a	36a	28a	16a	148a	10a	18a	7a	4a	4a	198a	191a	-7a	156a	0.78b	0.78a	3.18b	0.68a	-
RF N2	8264b	13011a	17086a	0.48a	4074b	0.31a	4215a	36.1b	33.3a	698a	76.55a	37.07a	143a	69a	2.1b	43a	78a	39a	30a	15a	173b	11b	22b	9b	4b	4a	204a	224b	20b	154a	0.75a	0.78a	1.87a	0.7a	-
SD1 N1	9539a	13833a	18093a	0.53a	4256a	0.38a	5270a	40.1a	39.4a	617a	93.72b	49.57b	301b	159b	1.7a	41a	66a	32a	24a	20a	163a	9a	13a	3a	2a	4a	183a	194a	10a	153a	0.83a	0.84a	3.23b	0.64a	-
SD1 N2	10250b	14079a	19319b	0.53a	5168b	0.35a	5037a	40.3a	40.6b	641a	79.39a	42.18a	162a	86a	2b	44a	80b	37b	29b	22b	206b	10b	17b	4b	3b	4b	210b	244b	32b	173b	0.82a	0.85a	2.04a	0.69b	-
Cont: N1->N2	7%	-2%	7%	0%	38%	-13%	-14%	2%	0%	5%	-13%	-13%	-47%	-47%	14%	5%	16%	15%	12%	7%	23%	13*	22%	24%	29%	22%	12%	22%		10%	-2%	0%	-39%	5%	
RF: N1->N2	4%	-7%	2%	2%	49%	-15%	-19%	6%	-2%	0%	-13%	-11%	-48%	-48%	13%	-4%	7%	8%	4%	-8%	17%	16*	17%	34%	19%	8%	3%	17%		-1%	-4%	0%	-41%	3%	
SD1: N1->N2	7%	2%	7%	1%	21%	-7%	-4%	0%	3%	4%	-15%	-15%	-46%	-46%	18%	6%	20%	17%	17%	10%	26%	12*	25%	41%	28%	18%	15%	26%		13%	-1%	1%	-37%	8%	

3.2.2. Trait Complex b: $GY = SD \times GNS \times TKW$

Lower GY in RF than in Cont was mainly related to lower thousand kernel weight (TKW) in RF (33.6 g, -13%) whereas SD1 reached a higher grain number per spike (GNS, $+10\%$) and by tendency higher TKW ($+4\%$) but fewer spikes per m^2 (Table 4). Across the main plots, none of the three direct yield components differed between N levels, whereas kernel number per m^2 increased (Figure 3). Interestingly, within the main plots, N fertilization increased mainly GNS in RF ($+6\%$) in contrast to TKW ($+3\%$) and spike density ($+4\%$, n.s.) in SD1, and spike density ($+5\%$, n.s.) in Cont, as well as kernel number per m^2 (Figure 3), but never yield per spike. Influenced by the strong scattering within MP*N level-combinations and the weak influence of N on the spike density, all relationships between spike density and GY were weak (not shown). For the whole trial, GNS was related to GY ($R^2 = 0.37^{***}$, Figure 3). TKW was related especially to the difference in GY between RF and Cont ($R^2 = 0.35$), whereas within MP \times N level combinations, the relationships were negative. The kernel number per m^2 explained little in GY across the main plots but 65%–85% of the GY variation between cultivars within the MP \times N level-combinations.

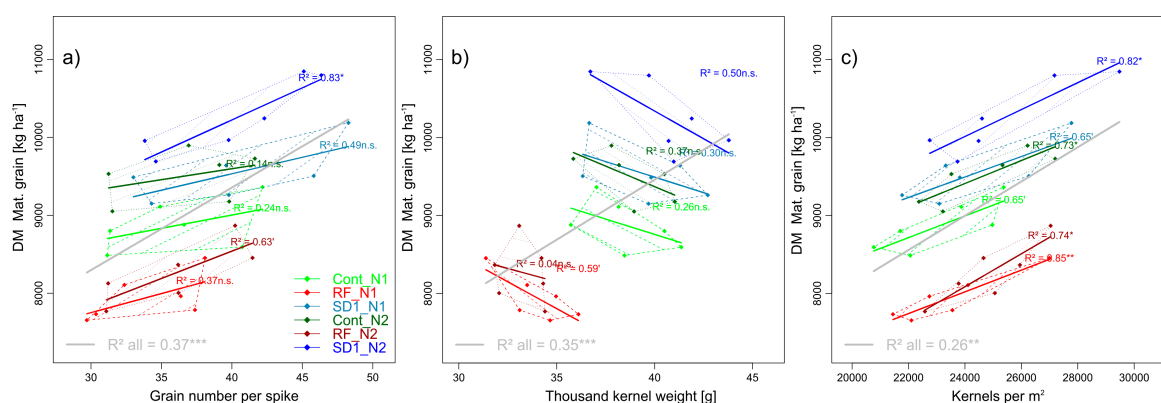


Figure 3. Selected pairwise trait relationships in trait complex *b*: DM of grain at maturity (GY) in relation to the direct yield components grain number per spike (a) and thousand kernel weight (b) and the combined yield component kernels per m^2 (c). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP \times N-combinations. Colored regression lines indicate the linear trait relationships within MP \times N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). *P*-values of regression slopes are indicated as $p < 0.01$ (***) , $p < 0.01$ (**) , $p < 0.05$ (*) and $p < 0.1$ (·).

3.2.3. Trait Complex c: $GY = DMT + PAA$

The pre-anthesis component of GY, i.e., DMT, contributed strongly to GY by 53% (SD1), 54% (Cont) and 58% (RF, not shown). DMT ($4.9 t ha^{-1}$ in Cont) was only slightly decreased in RF (-4% , n.s.) and increased in SD1 ($+5\%$, n.s.) compared to Cont, but PAA reacted stronger (-20% in RF and $+10\%$ in SD1, Table 4, Figure 4). However, substantial differences between cultivars were observed in both traits. While N fertilization increased PAA strongly within all main plots, DMT tended to decrease contrariwise, especially within Cont and RF. Due to the weak effects of MP and N treatments on DMT compared to the differing cultivar effects, DMT was not significantly related to GY (not shown), in contrast to PAA ($R^2 = 0.43$, Figure 4).

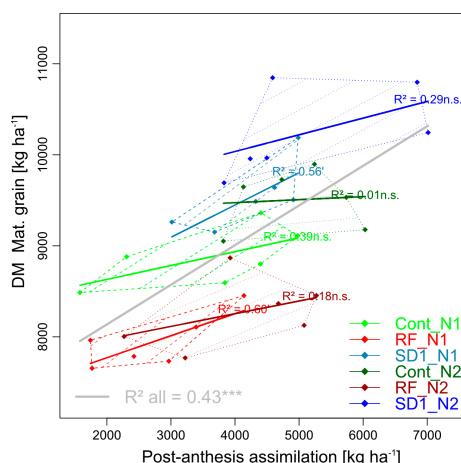


Figure 4. Selected pairwise trait relationship in trait complex c: DM of grain at maturity (GY) in relation to its additive component post-anthesis assimilation (PAA). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP × N-combinations. Colored regression lines indicate the linear trait relationships within MP × N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). P-values of regression slopes are indicated as $p < 0.01$ (***), $p < 0.01$ (**), $p < 0.05$ (*) and $p < 0.1$ (·).

3.2.4. Trait Complex d: GY = DM × HI

Lower GY (−12%) in RF than in Cont was related to a lower harvest index (−9%) whereas total above-ground DM decreased only moderately (−3%, Table 4, Figure 5). Instead, GY in SD1 increased by tendency (+8%, n.s.) rather than from higher total DM (+8%, n.s.) at a similar harvest index (0.53). With GY reacting to N fertilization weakest in RF, the increase in total DM (+2%, n.s.) was moderate as well compared to Cont and SD1 (both +7%), while HI remained stable within the main plots between N levels. Within MP × N level combinations, harvest index was more descriptive for the variation in GY between cultivars than total DM (Figure 5).

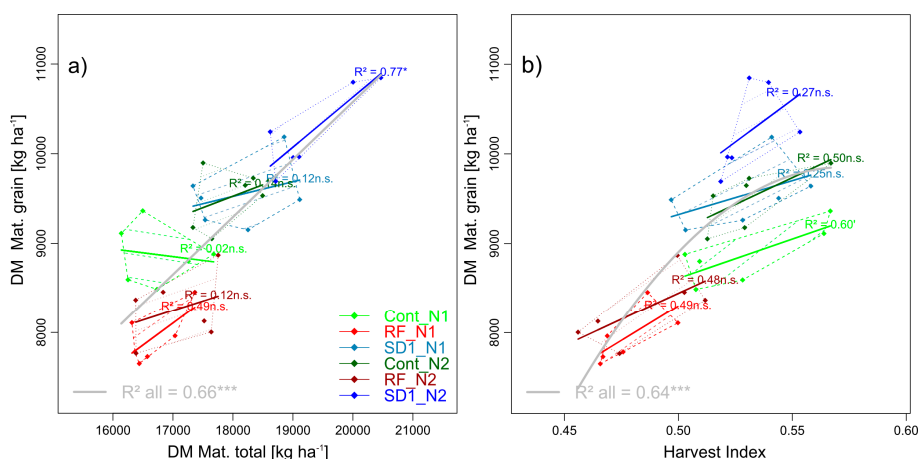


Figure 5. Selected pairwise trait relationships in trait complex d: DM of grain at maturity (GY) in relation to its two multiplicative components total dry matter at maturity (DM Mat. total, (a)), and harvest index (unitless, (b)). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data

range covered by cultivars within MP \times N-combinations. Colored regression lines indicate the linear trait relationships within MP \times N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). P -values of regression slopes are indicated as $p < 0.01$ (***) , $p < 0.01$ (**) , $p < 0.05$ (*) and $p < 0.1$ (·).

3.2.5. Trait Complex e: $NUE_{total} = NupEff \times NutEff_{total}$ and Trait Complex f: $NUE_{grain} = NupEff \times NutEff_{grain}$

As total DM at maturity, the apparent efficiency of the conversion of applied nitrogen into total DM (NUE_{total}) was higher in SD1 (231 kg DM/kg N) than in both other main plots (Table 4, Supplementary Figure S1). Due to the weak effect of N on total DM, NUE_{total} almost halved with the doubling of N fertilization. Due to the low fertilization level but the strong soil mineralization, which was not included in the calculation, the apparent N uptake efficiency ($NupEff$) was high (2.52 kg Nup/kg N_{fert} both in Cont and 2.63 kg Nup/kg N_{fert} in SD1). The internal conversion of Nup into total DM ($NutEff_{total}$) was only slightly lower in RF (82 kg DM/kg N, -2%) and higher in SD1 (+3%) than in Cont, but decreased in a similar way (13%–15%) from N1 to N2 in all main plots.

3.2.6. Trait COMPLEX g: $GNup = NT + PANup$

In spite of the lower absolute contribution, post-anthesis Nup ($PANup$) was more descriptive for differences in $GNup$ that nitrogen translocation (NT) in the whole trial ($R^2 = 0.39^{***}$), but not within MP \times N level combinations (Figure 6).

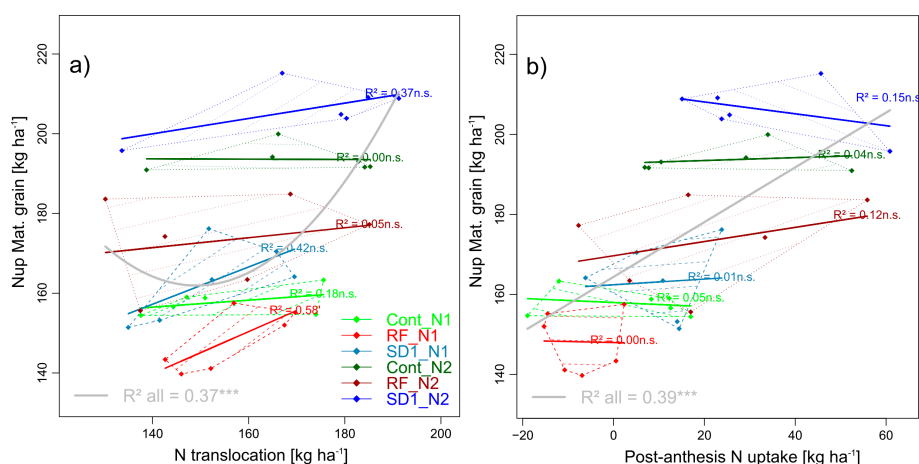


Figure 6. Selected pairwise trait relationships in trait complex g: Nup of grain at maturity ($GNup$) by its two additive components N translocation (NT, (a)) and post-anthesis N uptake ($PANup$, (b)). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP \times N-combinations. Colored regression lines indicate the linear trait relationships within MP \times N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). p -values of regression slopes are indicated as $p < 0.01$ (***) , $p < 0.01$ (**) , $p < 0.05$ (*) and $p < 0.1$ (·).

In contrast to the about equal contribution of pre- and post-anthesis assimilation to GY, $PANup$ was low (3 and 10 kg N ha^{-1} in Cont and SD1 and partly negative in RF). Thus, it contributed only 5.6%, 2.6% and 9.4% to the overall Nup ($CPANup$), and 3.8%, 7.6% and 11.5% to the grain Nup ($GNup$) in Cont, RF and SD1, respectively (not shown). The N translocation (NT) was slightly (−5%, n.s.) lower in RF and similar in SD1 compared to Cont (163 kg N ha^{-1}), respectively. NT increased

between N1 and N2 in Cont and SD1 but not in RF, whereas PANup responded to N in all main plot (+20 – 27 kg N ha⁻¹).

3.2.7. Trait Complex h: GNup = Nup × NHI

The overall relationship of GNup with total Nup ($R^2 = 0.88^{***}$, Figure 7a) was mainly driven by differences in total Nup between Cont and SD1 and between the N levels, but also between cultivars within MP × N level combinations, whereas the overall weak relationship of GNup with N harvest index (NHI) was due to the fungicide effect only (Figure 7). While NHI and total Nup were unrelated in RF, a negative relationship is visible within MP × N level combinations in Cont and SD1 (Figure 7c).

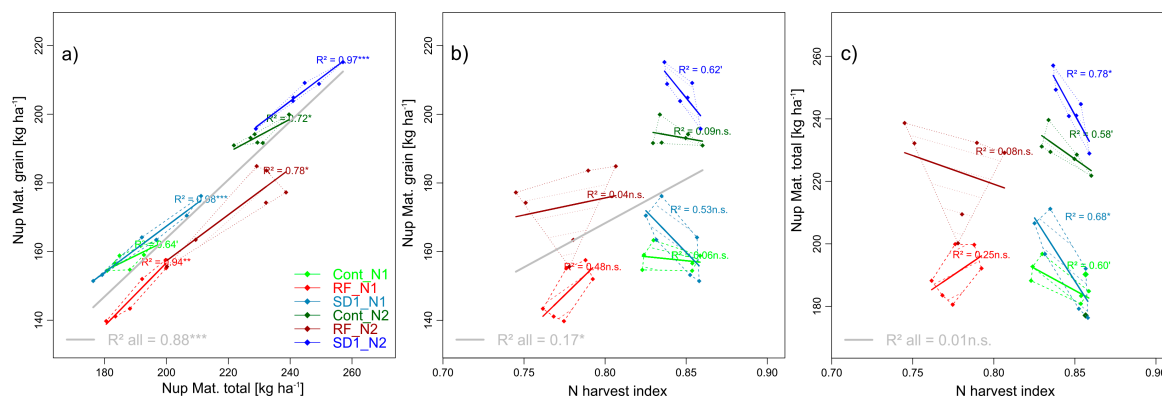


Figure 7. Pairwise trait relationships in trait complex *h*: Nup of grain at maturity (GNup) in relation to its two multiplicative components total Nup at maturity (Nup Mat. total, (a)) and the N harvest index (NHI, unitless, (b)) as well as the relationship of total Nup at maturity with NHI (c). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP × N-combinations. Colored regression lines indicate the linear trait relationships within MP × N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). P -values of regression slopes are indicated as $p < 0.01$ (**), $p < 0.01$ (**), $p < 0.05$ (*) and $p < 0.1$ (').

NHI was markedly higher than the (DM) harvest index (HI), but as the HI, it decreased without fungicide treatment (0.78, Table 5), but not with early sowing compared to Cont (0.84). As for HI also, no N effect was found for NHI. Total Nup in RF was almost equal but tended to be higher in SD (+5%, n.s.) than in Cont. Both traits interacted to the differing GNup in RF (−15 kg N ha⁻¹) and SD1 (+9 kg N ha⁻¹), respectively. Cultivar differences were found for NHI, ranging from 0.81 (Hybrid and Impression) to 0.83 (Hyland and JB Asano) and for total Nup, but not for GNup.

3.2.8. Trait Complex i: DMT = DM_{Ant} × DMTEff

The reaction of total DMT (trait complex c) can be explained by its multiplicative components, the amount and allocation of total above-ground DM at anthesis, and DMTEff. Similar DMT for all main plots was associated with similar total DM at anthesis and, by tendency, lower (−8%) DMTEff in RF, especially due to the decreasing DMTEff of culms and ‘other leaves’ (Figure 8a, Supplementary Table S1). Lower DMT with higher N fertilization, especially within RF and Cont, was mainly due to lower DMTEff (overall 0.38 in N1 and 0.34 in N2). At the organ level, the decrease in DMTEff with higher N fertilization was significant for culms (all main plots) and spikes (only RF). DMTEff differed more between organs than between treatments: Average DMTEff was lowest for spikes (0.23), followed by culms (0.36), flag leaves (0.45), ‘other leaves’ (0.48) and flag leaves-1 (0.51, Figure 8a). However, culms constituted by far the largest DM pool at anthesis with a partial harvest index (HI) of 0.60, followed by spikes (0.20) and similar values for each of the three leaf layers (Figure 8c). Reduced

fungicide decreased and increased the partial HIs at anthesis significantly for spikes and culms, respectively. Earlier sowing decreased partial HIs for spikes and both upper leaf layers in favor of culms and ‘other leaves’. N fertilization shifted the DM allocation (higher partial HI) from culms towards spikes and both upper leaf layers.

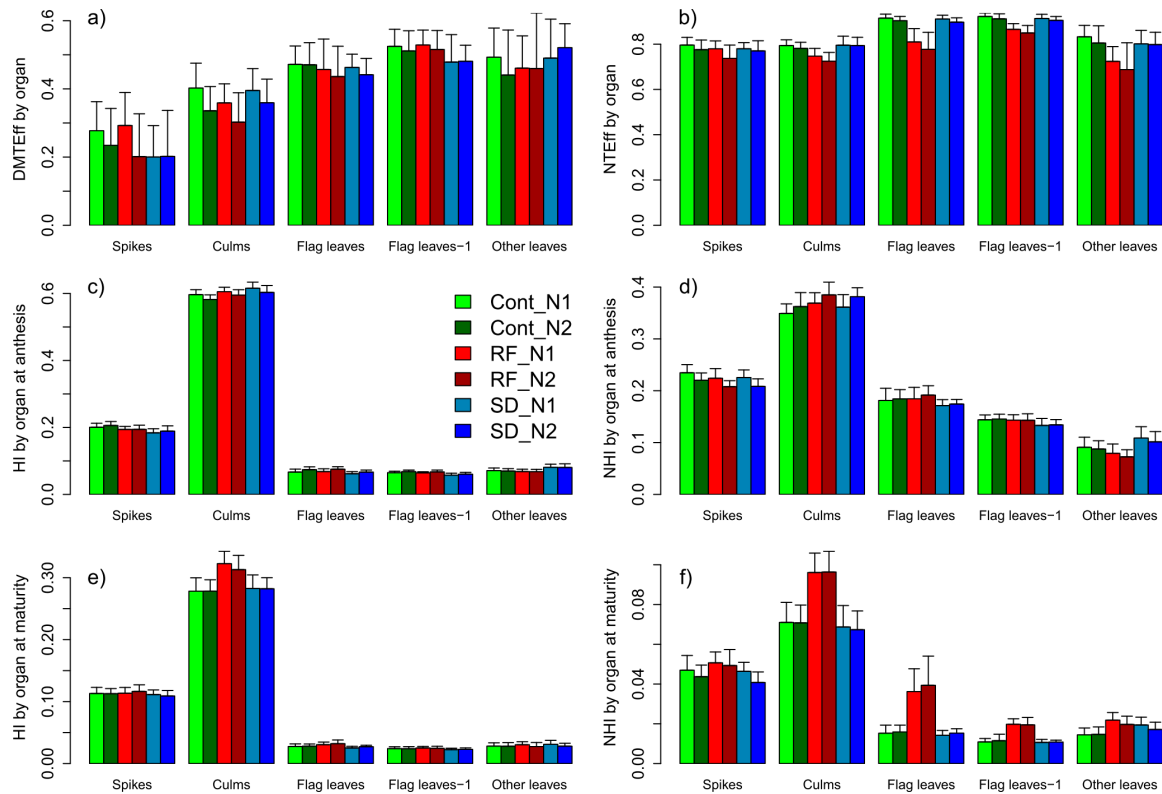


Figure 8. Reaction in the apparent translocation efficiency of dry matter (DMTEff, (a)) and nitrogen (NTEff, (b)) as well as in the proportional organ-level allocation of DM (partial harvest index HI, (c,e)) and nitrogen (partial harvest index NHI, (d,f)), both at anthesis and maturity, by vegetative plant organs and main plot (colors) and N (color intensity) treatments. Error bars are one-sided standard deviations. The ANOVA treatment effects are reported in Supplementary Table S1.

The lower grain HI at maturity in RF compared to Cont was mainly reflected in increased HI values for culms (0.32 instead of 0.28) and flag leaves (Figure 8e). As for DM and DMT-related traits, genotypic differences were present in all partial harvest indices for both sampling dates (Supplementary Table S1).

3.2.9. Trait Complex j: $Nup_{Ant} = Nup_{spikes} + Nup_{culm} + Nup_{flag\ leaf} + Nup_{flag\ leaf-1} + Nup_{other\ leaves}$

The allocation of Nup into aboveground plant organs was assessed at anthesis and maturity and related to DM and N concentration (Figure 9). At anthesis, cultivars allocated most N to leaves (partial N harvest index NHI: On average 0.41), followed by culms (0.37) and spikes (0.22, Figure 8d, Figure 9m). Most of the N in leaves was found in the flag leaf layer (0.18) and the flag leaf-1 layer (0.14). Total above-ground Nup at anthesis was similar between the main plots (Table 5), but the allocation to organs differed. Earlier sowing shifted the relative leaf N allocation downwards to lower leaves whereas reduced fungicide resulted into relatively more N in culms at the expense of lower leaves, and spikes (Figure 8d, Figure 9m). Interestingly, N fertilization increased total Nup at anthesis significantly only in Cont (+11%) and SD1 (+15%), but not in RF (Table 5). Moreover, none of the N responses in organ-level Nup was significant in RF, but for culms and for most leaf layers both in Cont

and SD1 (not shown). Conversely, the partial NHI decreased in all main plots between N1 and N2 for spikes in favor of culms (Figure 8d).

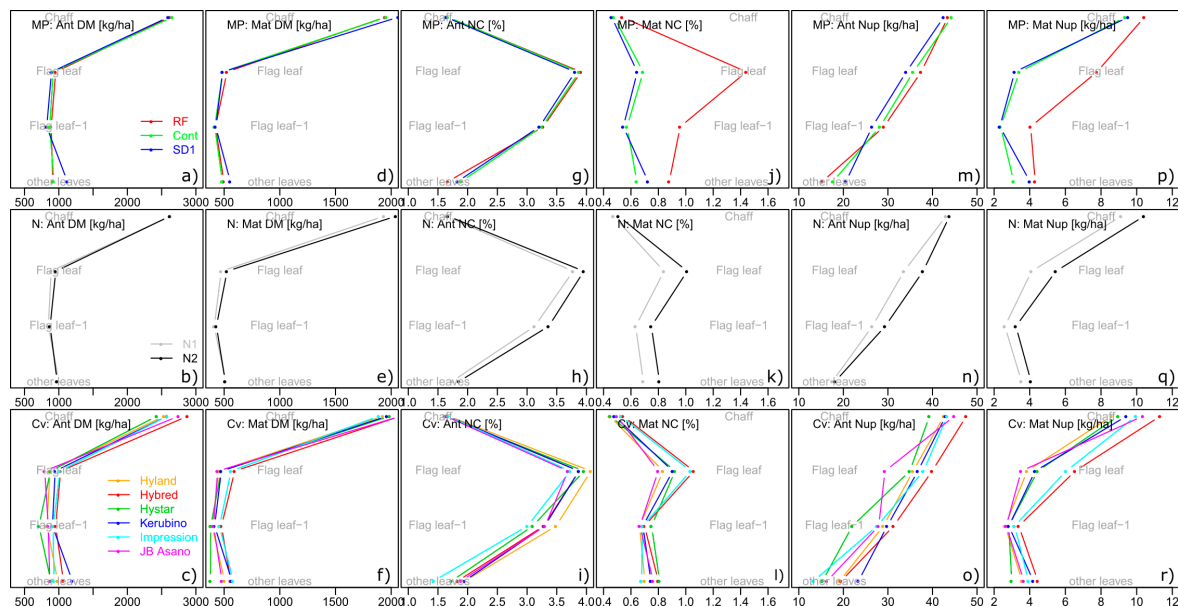


Figure 9. Vertical distribution of dry matter (DM, a–f), N concentration (NC, g–l) and N uptake (Nup, m–r) by sampling dates (Ant: Anthesis and Mat: Maturity): Average values for three MP treatment blocks (above, RF: Reduced fungicide, Cont: Control, SD1: Sowing date 1), the two N levels (middle) and six cultivars (bottom).

3.2.10. Trait Complex k: $Nup_{total} = Nup_{grain} + Nup_{spike} + Nup_{culm} + Nup_{flag\ leaf} + Nup_{flag\ leaf-1} + Nup_{other\ leaves}$

Total above-ground Nup at maturity was not significantly different between the main plots (Table 5). On the plant-organ level, reduced fungicide decreased the NHI for grain (Table 5) so that both relatively (NHI, Figure 8) and absolutely (Nup, Figure 9), more N was allocated to chaff (n.s.), culms and especially to the flag leaf and flag leaf-1 layers. Early sowing increased Nup and NHI only for the ‘other leaves’. In spite of these effects, given the efficient N translocation (NT) from straw to grains between anthesis and maturity by, on average, 77% in RF and 83% in Cont and SD1, the NHI was low for the vegetative organs at maturity (maximum 0.078 for culms, Figure 8). Still, as for the grain NHI, cultivars differed in NHI in all vegetative organs. As for grain NHI, N fertilization had little effect on the vegetative NHI values but increased and decreased the NHI for flag leaves and chaff, respectively. Within all main plots, the N response in Nup was stronger at maturity than at anthesis, strongest for flag leaves (on average +33% at maturity) and lowest for chaff (+14%), at totally increased Nup from 191 to 232 kg N ha⁻¹ (+22%, Table 5, Figure 9). However, as at anthesis, the N responses differed by the main plot treatments: The relative increase in Nup in RF (+17%) was lower overall than in Cont (+22%) and SD1 (+26%).

3.2.11. Trait Complex l: $NT = NT_{spike} + NT_{culm} + NT_{flag\ leaf} + NT_{flag\ leaf-1} + NT_{other\ leaves}$ and trait complex m: $NT = Nup_{Ant} \times NTEff$

N translocation (NT) tended to be lower in RF than in Cont (−5%, n.s., Table 5, Figure 10). With similar total Nup at anthesis in all main plots and increased Nup between N levels (+8 kg N ha⁻¹), the apparent N translocation efficiency (NTEff) decreased significantly from 0.83 (Cont) to 0.77 (RF, unchanged for SD1). NT increased only moderately between N1 and N2 and only in Cont (+10%) and SD1 (+13%), due to the low NTEff in RF in N2. The organs differed in NTEff from on average 0.89 (flag leaf-1), 0.87 (flag leaf) to only about 0.77 of all other vegetative organs (Figure 8b). Relative

NTEff decreases without leaf fungicides were stronger for leaves (overall -10%) than for culms (-7%) and chaff (-4% , Figure 8b). N fertilization significantly reduced total NTEff and NTEff of flag leaves-1 in Cont, NTEff of flag leaves in SD1 and NTEff of all organs in RF (Figure 8b).

While NT had a close linear relationship with the total Nup at anthesis ($R^2 > 0.85$) both for the whole trials and the MP*N level combinations, NTEff was less descriptive for NT (Figure 10).

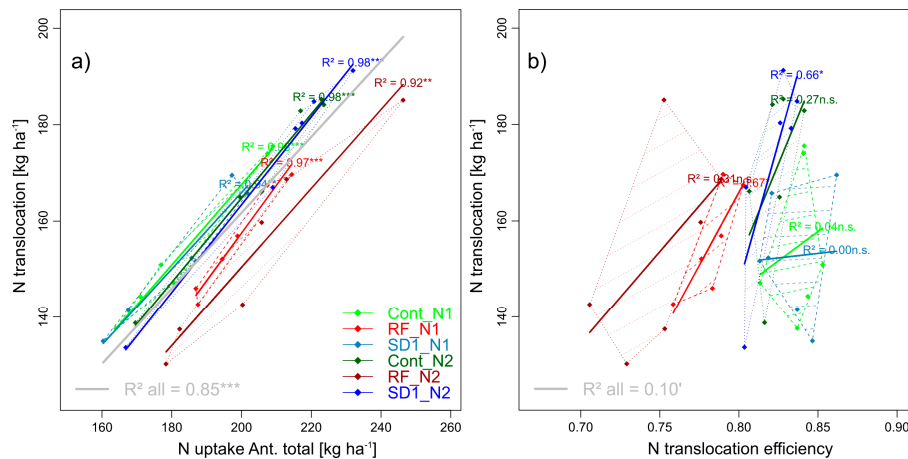


Figure 10. N translocation (NT) and its relation to total Nup at anthesis (left, kg N ha^{-1} , (a)) and the apparent N translocation efficiency (NTEff, (b)). N levels are distinguished by line types and color intensities (N1: Dashed lines and light colors, N2: Dotted lines and dark colors). Dashed and dotted lines delimit the bivariate data range covered by cultivars within MP \times N-combinations. Colored regression lines indicate the linear trait relationships within MP \times N-combinations and gray lines the regression line for the whole trial, calculated across the included data of reduced fungicide (RF), control (Cont) and sowing date 1 (SD1). Gray lines are only drawn for significant relationships ($p < 0.05$). P -values of regression slopes are indicated as $p < 0.01$ (***) , $p < 0.01$ (**) , $p < 0.05$ (*) and $p < 0.1$ (').

4. Discussion

Both the use of fungicides, together with early sowing, were assessed in two N levels as potential measures for increasing N efficiency. Early sowing was expected to increase N-uptake, as well as grain yield through shifted phenological phases. Favorable fungicide effects were expected mainly during grain-filling.

4.1. Trait Level of Dry Matter and N Traits

The treatment factors influenced yield formation during different growth stages, which could be resolved from the response of the contributing traits.

Probably, the strong N-mineralization from the pre-crop grass-clover decreased the use of additional N fertilization for GY. Non-N-fertilized plots in an experiment conducted directly next to the study side yielded approximately 7 t ha^{-1} and took up 111 kg N ha^{-1} of GNup, which is not uncommon for this pre-crop [57], considering that soil mineralization was enhanced by warm conditions with sufficient precipitation. In a similar experiment conducted in the three previous years on the same experimental site with 13 genotypes including the six cultivars of this study, the N response in GY between 160 and 220 kg N ha^{-1} differed between years from a 21% increase under favorable growing conditions and four fungicide treatments to 10% under drought, and only 4% in the year with higher pathogen pressure [52]. The yield level in the present study of 9.5 t ha^{-1} in N2 in Cont was clearly higher than the 8.5 t ha^{-1} reached, on average, from 220 kg N ha^{-1} fertilized N in the previous years [52]. The same was observed for total DM at maturity (17.9 t ha^{-1} in N2 in Cont compared to 15.6 t ha^{-1} in previous years).

Total Nup at anthesis and maturity, grain N concentration and GNup (+33 kg N ha⁻¹) were substantially higher in Cont/N2, than at the highest N level in previous years, in spite of a slightly lower NHI [52]. The study year profited from high radiation during May and June at below-average precipitation, conditions which fostered vegetative growth, enabled Cont and RF plots to compensate for later sowing, and curtailed disease growth. Considering the above-average precipitation in April, sowing date effects are likely to be stronger in drier years.

4.2. Dissecting the Grain Yield Response to Fungicide

Given that the fungicide intensity differed substantially between Cont and RF, the yield response (−12%) was moderate due to the low disease incidence influenced by the rather dry and warm conditions but was within the previously reported range [13,37,39]. While fungicides effectively prevented diseases in the treated plots, moderate disease incidence was detected in RF during grain-filling, caused by mildew (*Erysiphe graminis*), *Septoria tritici* and in some plots by *Puccinia striiformis*. However, image-based color analysis evidenced accelerated senescence also in non-visibly diseased leaves, indicating a combined effect of disease control and a secondary physiological ‘greening effect’ of the fungicides [35].

Fungicide-related yield effects were mainly related to increased kernel weight, confirming previous findings [37–39,42,58], whereas grain number per spike (GNS) was stable since pathogens were relevant only after anthesis [45]. In this phase, both spike density and grain number per spike were mostly determined, but kernel size reacts to the availability of assimilates. Therefore, increasing the source size through delaying the onset of senescence may be the major fungicide effect [42].

Thus, DM until anthesis was not affected by RF, but post-anthesis assimilation (PAA) decreased clearly (−20%). Still, the decrease in total above-ground DM at maturity (−3%, n.s.) was less pronounced compared to the decrease in the harvest index (HI, −9%). The lower HI is partly explained by a decreased dry matter translocation (DMT), due to a significantly lower translocation efficiency (DMTEff) of the culms. Analyzed from 13 trials, a high variability in the HI response was found previously, reaching from no effect to a relative decrease by 37% without fungicide [44], being in line with [38] and [40], but contrasting with [59], who reported stronger reduction in total DM than in HI. Bancal et al. (2007) found clearly lower DMT and its contribution to GY (15 to 36%) than in the present trial, but also no consistent influence from the fungicide treatment. Since the transport of assimilates to the roots and respiratory losses are not considered in the applied difference method, the apparent DMT and DMTEff captured the loss in DM, but it is likely that the effective translocation was overestimated by about one quarter [60], especially in RF, since assimilate losses are likely to be higher as an effect of fungi [61].

Since the total Nup and Nup efficiency were only marginally affected by RF, the response in NUE_{total} reflected mainly the response in NutEff_{total}, which decreased through lower PAA. NUE_{grain} declined due to lower NutEff_{grain}, which decreased through the combined effect of decreasing PAA and HI. This is in contrast to [13], where Nup (−7%) and NutEff_{grain} (−6%) decreased similarly between treated and non-treated plots. Decreased PAA was found to be closely related to lower post-anthesis PAR absorption [44].

Only a few MP*N interactions were significant in the Cont + RF trial subset. Still, the slightly decreased N response for GY in RF may be explained through the fostering of certain pathogens with additional N supply and higher fungicide effects with increased N supply [38–40,62–66].

4.3. Dissecting the Grain Yield Response to Early Sowing

In contrast to RF, yield effects of earlier sowing were mainly attributed to increased grain number per spike (GNS, +10%). Although pre-winter tillering was visibly increased in SD1, the Cont treatment appears to have quickly compensated for the lower tiller number, supported by the high soil N supply, so that spike densities were comparable. In the autumn, the total mineral soil N content in 0–60 cm was about 83 kg N ha⁻¹ in SD1 and only 69 kg N ha⁻¹ in Cont (not shown). The level remained high with 92

and 87 kg N ha⁻¹ in SD1 and Cont during spring, respectively. The dominant effect of early sowing on GNS compared to spike density is in line with findings on durum wheat [10]. In contrast, depending on the growing conditions, rather spike density and TKW [20,67] or spike density and GNS [26] explained the sowing date effects on GY.

Early sowing resulted in earlier anthesis by only 2.8 days compared to seven to nine days found by [10], from a larger time span between sowing dates, however. High temperatures and radiation around anthesis in early June may have led to decreasing phenological shifts.

As for RF, the yield effect across N levels of early sowing was larger for post-anthesis assimilation (PAA) than for dry matter translocation (DMT). A higher GNS was related to only slightly increased spike weight (+4%, n.s.) at anthesis. Thus, plants sown earlier possibly increased GNS rather by maintaining more GNS through higher PAA, during grain-filling than through initially generating more grains. Higher PAA may be associated with better water and nutrient acquisition, if root growth is fostered through the longer vegetative phase, and with an escape strategy through shifting phenological critical phases like flowering towards more favorable periods [68]. While additional DM at anthesis was allocated to culms (+10%) and 'other leaves' (+21%), the higher leaf layers did not profit from earlier sowing, possibly indicating that the leaf area approached saturation. The sowing date's main effects on DM traits were weaker than reported by [10], possibly due to the shorter time interval between sowing dates and the high baseline values in Cont due to the strong soil N mineralization and favorable growing conditions. More DM of culms and 'other leaves' could, however, not be efficiently translocated to the grains. Thus, these organs showed increased absolute and relative (partial HI) DM still at maturity. The overall DMTEff was substantially higher than in durum wheat [10], and also showed no sowing date effect.

In contrast to RF, the (positive) response in NUE_{total} and NUE_{grain} was a combined effect of increased NupEff (n.s.) and increased NutEff_{total} (n.s.) with a similar increase in NUE_{grain} and NutEff_{grain} due to the non-altered HI. Notably, no significant interaction of sowing date and N level was found for any one of the carbon and nitrogen traits, which is in line with [10]. The relative beneficial effect of earlier sowing was similar to the one found under Danish conditions (+0.9–1.1 t ha⁻¹), where it was related to higher pre-winter Nup [29]. If early sowing leads to excessive pre-winter development, it may increase disease susceptibility [23]. However, unlike in RF, the N response was equal as in Cont, indicating that the two fungicide applications effectively compensated for the higher disease risk in SD1, together with the low disease pressure.

4.4. Dissecting the Response in Grain N Uptake to Reduced Fungicide

Considering that more than 90% of total Nup occurred until anthesis in all main plots, it is plausible that the fungicide treatment affected neither Nup at anthesis nor at maturity since disease effects were mostly detected after anthesis. Thus, the decrease in post-anthesis N uptake (PANup) by 6 kg N ha⁻¹ was not significant either and weaker than the one found by [13]. In the present study, GNup tended to decrease by 9% in RF, mainly due to the lower NHI (−8%). This is in line with [40], whereas many trials, found a combined effect on total Nup, N mobilization, and NHI [38,43].

In all main plots, PANup contributed little (only 3%–11%) to GNup, in contrast to about one third found by [13] and [45]. The similar negative response in NT and PANup without fungicide contrasts with Bancal et al. (2008), who found a dominant effect on PANup and a wide response in NT. Concerning genotypic differences, the close relationship of GNup with total Nup but not with NHI, irrespective of the fungicide treatment, is in line with [45].

Bancal et al. (2008) reported a good relationship between leaf NT and GNup across environments without fungicide. Among organ-level NT traits, NT of flag leaves-1 explained genotypic GNup variation by 31–83% in the present data, but only in N1 (not shown). For all data, PANup explained GNup better (Figure 6) than total NT, but in contrast to [45], no relation was found for the variation between cultivars, possibly due to the considerably lower PANup. These authors reported only a few effects of late foliar diseases on NT of culms and chaff compared to leaf blades. Our data

confirm the strongest reactions in leaf NTEff but also shows significantly decreased NTEff of spikes and culms. The level of NTEff (0.83 in Cont and 0.77 in RF) was higher than under English conditions [13] (0.72 and 0.68, respectively), clearly higher than in [14] (0.61–0.67 and 0.57–0.58, respectively) but similar in Cont as in [69], where NTEff decreased to 0.56 without fungicide, however. NTEff in Cont was similar as in previous years when fungal pathogens were well controlled, and on the same level as in another, fungi-affected year [52].

Although fungicide may delay the release of N from leaf blades [14,42,43,45], leaf residual Nup at maturity was increased by about 40% [45] without fungicide, and the disease effect was strongest for the flag leaves and flag leaves-1 [13]. These findings confirm the strong increase in Nup and the partitioning to flag leaves and flag leaves-1 as an effect of decreased NTEff in the present data. With GNup decreasing less than GY, GNC slightly increased without fungicide. In contrast, GNC did not respond to fungicide [13], increased [38,45] or reacted inconsistently [14] and may depend on the dominant fungi. The relative N response for anthesis and maturity Nup and GNup tended to be lower in RF. Other authors reported no N × fungicide interaction for total Nup, NT, and PANup [45], but for GNup [40].

The overall significant decrease in NTEff of all vegetative organs by 1% to 3% with higher fertilization is in line with [29]. In contrast, in a similar experiment conducted at the same site, a negative N effect was found on NTEff of culms but not of leaves [52]. Since both relative N-reactions in GY and GNup tended to be lower in RF, the N response of GNC was comparable in Cont and RF, which is in line with [45] and [70], who reported fungicide × N level interactions for GNC only in few reviewed studies.

4.5. Dissecting the Response in Grain N Uptake to Early Sowing

Early sowing increased total Nup (+5%) less clearly than total DM due to a concomitant decrease in N concentration. Thus, the Nup of most organs changed only marginally. Still, GNup reacted in the same way as total Nup due to the stable N harvest index (NHI) in contrast to RF. Negative relationships between total Nup at maturity and NHI (Figure 7) may indicate some degree of sink limitation for GNup too under high Nup conditions [71].

However, due to the advanced anthesis by two to three days, the slight advantage in total Nup at maturity (+10 kg N ha⁻¹) is likely to originate partly from increased pre-winter Nup, being a major reason to sow earlier to decrease N losses over winter. In the present trial, three other genotypes were evaluated for early vigor [48]. In early spring, Nup was 19.5 kg ha⁻¹ or 5 times higher than in late sown control plots (Supplementary Table S2: 15 March), and the difference increased to 36, 51 and 26 kg N ha⁻¹ on 28 March, 4 April and 3 May, respectively. In contrast to post-anthesis Nup, N translocation and N translocation efficiency were almost unaltered by early sowing in spite of differing contributions of the vegetative organs, being in line with [10]. Since GNup increased less than GY with early sowing, GNC tended to decrease slightly, in contrast to substantially lower GNC in early sowing in [10].

In contrast to RF, the relative N response in SD1 was similar as in Cont for most N-related traits and no significant MP × N interactions were found, suggesting that no adapted fertilization was required for early sowing.

5. Conclusions

Traits contributing to GY and GNup were compared in response to reduced fungicide and earlier sowing at two N levels on six cultivars differing in phenology and pathogen susceptibility. The comparable low effect of the fungicide suggests that one application would have been sufficient for most cultivars. Though interactions of fungicide intensity and sowing date can be relevant [23,24], earlier sowing should be considered for decreasing over-winter N losses and for possible beneficial effects both on GY and GNup under the present conditions with optimum pre-crop and low pathogen incidence. However, the effects of the sowing date differ strongly between environments [72], and aspects of management and weather conditions must be considered in practice as well. The low

interactions between sowing date and N level suggest that no specific fertilization timing would be necessary whereas the N effect was lower for several traits when no leaf fungicide was applied, confirming that levels of both treatments should be adapted together. Assessing the studied treatment factors in differing environments is recommended.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/9/6/313/s1>, Supplementary Figure S1: Pairwise trait relationships of traits of trait complexes e and f, Supplementary Table S1: ANOVA results for further, Supplementary Table S2: Early development from leaf development to early stem elongation.

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