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High-throughput phenotyping of winter wheat under field conditions

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List of abbreviations¹

Abbreviation	Term
ARI	Anthocyan reflectance index
ASN	Ammonium sulfate nitrate
BL	Breeding lines field trial
CH	Commercial heterosis
Cont	Control treatment: Normal fungicide at normal sowing date
CPAA	Contribution of post-anthesis assimilation to grain filling
CPreAA	Contribution of pre-anthesis assimilation to grain filling
CPNup	Contribution of post-anthesis nitrogen to total nitrogen uptake
CPreNup	Contribution of pre-anthesis nitrogen to total nitrogen uptake
Cv	Cultivar
Cv_ag	Field trial for testing cultivars in agronomic treatments
DM	Dry matter
DMT	Dry matter translocation
DMTEff	Dry matter translocation efficiency
E	East
FOV	Field of view
GNC	Grain nitrogen concentration
GNS	Grain number per spike
GNup	Grain nitrogen uptake
GPD	Grain protein deviation
GPS	Global positioning system
GY	Grain yield
H ²	Broad-sense heritability
ha	Hectare
HDP	Hochdurchsatz-Phänotypisierung
HI	Harvest index
HTP	High-throughput phenotyping
HyLi_N	Field trial with hybrid and line cultivars in three nitrogen fertilization levels
IN	Incremental nitrogen fertilization field trial
K	Kelvin
KE	Kornertrag
kg	Kilogram
KNE	Kornstickstoffertrag
K ₂ O	Potash
l	Liter
LAI	Leaf area index
mg	Milligram
N	Nitrogen; nitrogen fertilization; North
n	Number
N0, N1, N2, N3	Nitrogen fertilization level
D1, D2, D4	Names of field sites
NC	Nitrogen concentration [%]
ND	Normalized difference vegetation indices with differing wavebands
NDVI	Normalized difference vegetation index with fixed wavebands

¹ Refer to Table 3, section IV for abbreviations and equations of all spectral vegetation indices

NE	Stickstoffeffizienz
Nfert	Nitrogen dose; temporally split nitrogen fertilization application
NHI	Nitrogen harvest index
NIR	Near-infrared
NNI	Nitrogen nutrition index
NT	Nitrogen translocation
NTEff	Nitrogen translocation efficiency
NUE	Nitrogen use efficiency
NUE _{grain}	Nitrogen use efficiency for grain yield
NUE _{total}	Nitrogen use efficiency for total dry matter
Nup	Nitrogen uptake
NupEff	Nitrogen uptake efficiency
NutEff	Nitrogen utilization efficiency
PAA	Post-anthesis assimilation
PANup	Post-anthesis nitrogen uptake
PLSR	Partial least squares regression
PRI	Photochemical reflectance index
P ₂ O ₅	Phosphate
R ²	Coefficient of determination
RE	Red edge
REIP	Red edge inflection point
RF	Reduced fungicide at normal sowing date
RMSE	Root mean square error
Section I-VII	Thesis sections (numbered in order of usage in this thesis)
SD	Spike density
SD1	Sowing date 1 at normal fungicide treatment
SVI	Spectral vegetation index
t	Metric ton
TKW	Thousand kernel weight
TM	Trockenmasse
UAV	Unmanned aerial vehicle
VIS	Visible light
WBI	Water band index
WMMRS	Weighted mean/maximum rank sums

Summary

Sensor-assisted smart farming holds great potential for increasing the efficiency in using pesticides and fertilizer in precision farming. Facilitated by technical advances and fostered by the need to increase the throughput in the evaluation of field trials, there is an urgent need for optimizing sensor-based methods also for high-throughput phenotyping (HTP) under field conditions. Plant breeding and the evaluation of agronomic factor trials rely on a precise and objective determination of various plant traits, which however is often hampered due to being cost- and labor-intensive, subjective and restricted to narrow timeframes. The adoption of non-destructive sensor methods holds great potential for widening the persisting 'phenotyping bottleneck', but requires a thorough evaluation under field conditions. In wheat (*Triticum aestivum* L.), one of the most important crops both globally and in Germany, increasing the nitrogen (N) use efficiency (NUE) is a major goal both for economic and ecological reasons. Besides maximizing grain yield (GY), targeting grain N uptake (GNup) is becoming more important. Both for increasing NUE per se and for an efficient use of HTP methods, the identification of promising phenotyping strategies is crucial.

Therefore, this thesis pursues the dual purpose of (i) evaluating plant- and plant organ-level variation in dry matter (DM) and N traits of winter wheat in terms of treatment effects and variation during the grain filling phase and of (ii) evaluating HTP methods for the assessment of these traits.

Two triennial field trials were conducted on winter wheat in a high-yielding West European environment in Southern Germany, including a trial with 75 breeding lines (*BL* trial), and a trial with registered hybrid and line cultivars in three N fertilization levels (*HyLi_N* trial). In addition, a selection of six cultivars were evaluated in another trial for the effect of the agronomic treatments N fertilization, fungicide intensity and sowing time (*Cv_ag* trial).

Plants were sampled at anthesis and physiological maturity and analyzed for DM, N concentration (NC) and N uptake (Nup). DM and N translocation were calculated using a difference approach incorporating the results of both sampling dates. Hyperspectral reflectance data was collected at canopy level during multiple growth stages from tillering to dough ripeness using a mobile sensor platform. Spectral data was converted to various spectral vegetation indices, which were compared for the detection of the plant traits.

In addition, a multi-year study was conducted for evaluating the index-based estimation of the pre-anthesis N status.

This thesis comprises seven sections.

Section I compares the performance of the cultivar groups hybrids and lines for various DM and N traits as tested in eight year \times N level combinations in *HyLi_N*. The results suggest a slight relative advantage of hybrids over line cultivars in GY (+5.5%). It was related to a more efficient conversion of

aboveground Nup into GY, but not to higher Nup; to better partitioning to the grain (harvest index +3.5%), but not to higher total DM; predominantly to more grains per spike, as well as to higher post-anthesis assimilation. In contrast, few consistent differences were identified, neither for DM and N traits on the plant and plant-organ level, nor for translocation traits. In contrast, hybrids were afflicted by their relatively lower grain N concentration (GNC; -8.5%), so that no advantage in N acquisition could be identified. In addition, a hypothesized greater advantage of the hybrids under low N conditions was not confirmed. It is concluded that the hybrids' higher seed costs are currently not compensated for under such low-stress high-yielding conditions, but new generations of hybrids may increase their relative advantage.

Section II reports the effects of agronomic treatments on DM and N traits on six cultivars. Earlier sowing by about three weeks tended to increase GY (+8% n.s.) and GNup (+8% n.s.), whereas the omission of leaf fungicide decreased GY (-12%, $p < 0.05$) and by trend GNup (-9% n.s.). The effects of not applying leaf fungicides were predominantly related to less efficient N utilization, DM and N translocation and partitioning as well as post-anthesis assimilation, and lower thousand kernel weight. In contrast, earlier sowing increased the grain number per spike, total DM, particularly from post-anthesis assimilation, and by tendency post-anthesis Nup. In addition, regression analysis emphasized the influence of underlying treatments for the explanation of the target traits. For discriminating genotypic variation, kernel number per m^2 was significant ($p < 0.05$) for GY, and total Nup, but not the N harvest index, for GNup.

Section III reports on the evaluation of DM and N traits similar to those studied in section I and II for dissecting the formation of GY, GNup and GNC in breeding lines. Dry matter translocation was found to contribute substantially to GY from on average 31% under highest-yielding but sink-limited conditions to 44% under conditions of post-anthesis pathogen pressure. However, post-anthesis assimilation was always better correlated with GY. At maturity, the accumulation of total aboveground DM was more descriptive for GY than the harvest index. Among yield components, only the kernel number per m^2 revealed moderate correlations with GY. NUE for GY was better described by N uptake efficiency than by the N utilization efficiency. GNup was closer related to total Nup than to the N harvest index, and better to GY than to GNC. The post-anthesis Nup was strongly year-dependent, substantially lower than the N translocation, but always positively correlated with GNup. It is concluded to further optimize traits of DM and N accumulation, rather than partitioning traits, and to consider post-anthesis characteristics including stay-green traits, both for maximizing GY and GNup. While breeding for GY would also maximize GNup, the selection of lines purely high in GNC is not suggested in order to maintain NUE.

Section IV evaluates 48 literature-based spectral vegetation indices for the in-season early assessment of the traits reported in section III by linear regression analysis. A quantitative ranking

based on the regressions on all measurement dates was introduced for comparing trait-specific indices. The milk ripeness stage revealed to be the most suitable for estimating GY, GNup and various other traits. In contrast, phenological shifts counteracted predictions at heading/anthesis, and the vegetative phase provided generally low relationships. For GY (maximum $R^2 = 0.26^{***}$ – 0.51^{***} in the three years) and for most DM traits, indices related to the water absorption band at 970 nm, followed by indices situated in the near-infrared (NIR)/red edge (RE) domain, provided better relationships than the normalized difference vegetation index (NDVI). For GNup and other Nup traits, NIR/RE indices ranked highest. In addition, indices were validated across years and compared to Partial Least Squares Regression (PLSR). Though the estimation of most organ-level and translocation-related traits was less precise, it may offer new information on the formation of target traits, which would otherwise not be available from destructive methods. While PLSR decreased the prediction error for many traits, the relative discrimination as expressed by the R^2 was not substantially improved for many traits.

Section V appraises the methods established in section IV for the N traits of section II, compared by the influence of the underlying treatment factors. The section confirms the lower suitability of the heading/anthesis phase and the suitability of the milk ripeness, respectively, while the dough ripeness was even better suited—notably under the influence of treatments with senescence effects. Under conditions of N fertilization, also the stem elongation phase provided useful estimations. The index rankings for GNup from section IV were generally confirmed. Several traits were moderately detectable in data subsets influenced by different fungicide applications, but, as in section IV, not for genotypic effects, including most organ-level NC traits, N translocation efficiency and N harvest index—mostly only by specific senescence-sensitive indices.

Section VI assesses the influence of the spectral resolution on the assessment of GY, GNup and GNC in the *HyLi_N* and the *Cv_ag* trials. A contour map analysis was applied for evaluating normalized difference indices from all possible pairwise index combinations of the original hyperspectral reflectance data and simulated multispectral data in correlations on multiple measurement dates. While the seasonal patterns of sections IV and V were confirmed, the trait differentiation through different N fertilization levels was well sensed during stem elongation as well. Correlations of simulated multispectral sensor data, corresponding to those used by actual satellites, differed by traits and dates. The advantage of hyperspectral data and of multispectral sensors with a higher number and more specific bands, notably in the RE and water band domain, increased with decreasing R^2 -levels and was higher during heading, anthesis and late senescence, and higher for GNC and lowest for GNup. The best multispectral sensor, Sentinel-2, minimized information loss compared to the hyperspectral data through covering spectral bands similar to those of a water band index in a NIR/NIR combination for GY and by its specific band in the upper RE region for GNup and GNC. It was followed by the WorldView-2 and RapidEye configuration, whereas the Quickbird and Landsat configurations ranked

lowest. It is concluded that given optimal placement and sufficiently narrow bands, it is promising to transfer findings based on hyperspectral data to simpler, more ready-to-use multispectral sensors.

Section VII evaluates the estimation of wheat N status in the vegetative phase regarding the influence of cultivars, N fertilization level, target trait level and spectral vegetation indices—tested on simple-ratio indices and the Red Edge Inflection Point (REIP). Particularly the assessment of DM and NC was substantially affected by growth stage-specific scattering when relationships were tested across years and growth stages. In contrast, Nup and the N nutrition index (NNI) were better estimated than NC and DM within growth stages. In most cases, the influence of different cultivars distorted the relationships. Based on the NNI, the data range was divided into two intervals of low and high N status, respectively. While at low N status, all indices performed similarly, RE-based indices were clearly better suited for N-related traits than the NIR/VIS (visible light) and water band indices at high N status. It is concluded that the development and use of sensors should consider the level of target traits and N conditions.

The **general discussion** compares the findings between sections and to the key literature. It is concluded that traits related to accumulation, i.e. total DM and Nup, are more indicative for discriminating GY and GNup, respectively, than the partitioning traits, i.e. DM and N harvest index. In addition, post-anthesis dynamics are crucial for both target traits. The NUE variation in registered cultivars and breeding genotypes is substantial and deserves to be exploited in conjunction with optimized agronomic treatments. Spectral HTP methods are promising to be used for both breeding lines in a breeding context for discriminating NUE traits, including GY and GNup, and for the effects of agronomic treatments such as N fertilization, cultivar selection, fungicide and sowing time. The use of indices with RE and water band information rather than of the widespread NDVI appears crucial for detecting the N status and DM, notably GY, respectively. The dense temporal evaluation allowed recommending milk ripeness as the overall most suitable growth stage.

Zusammenfassung

Sensorgestütztes Smart Farming birgt großes Potenzial für die Effizienzsteigerung in der Anwendung von Pestiziden und Düngemitteln. Durch den technischen Fortschritt und gefördert durch die Notwendigkeit, den Durchsatz bei der Auswertung von Feldversuchen zu erhöhen, ergibt sich der Bedarf sensorische Methoden auch für die Hochdurchsatz-Phänotypisierung unter Feldbedingungen zu optimieren. Die Pflanzenzüchtung und die Auswertung agronomischer Faktorversuche basieren auf der präzisen und objektiven Bestimmung verschiedener Pflanzenmerkmale, welche jedoch oft dadurch erschwert wird, dass sie kosten- und arbeitsintensiv, subjektiv und auf enge Zeitfenster beschränkt ist. Die Nutzung nicht-destruktiver Sensormessungen erscheint vielversprechend, um den fortbestehenden Phänotypisierungsengpass aufzuweiten, erfordert aber eine gründliche Bewertung unter Feldbedingungen. Bei Weizen (*Triticum aestivum* L.), einer weltweit wie auch in Deutschland sehr wichtigen Kultur, stellt die Steigerung der Stickstoff (N)-Effizienz aus wirtschaftlichen und ökologischen Gründen ein wichtiges Ziel dar. Neben der Steigerung des Kornertrags (KE) gewinnt die Maximierung des Korn-N-Ertrags (KNE) an Bedeutung. Sowohl für die Erhöhung des KNE als auch für einen effizienteren Einsatz von Phänotypisierungsmethoden ist die Identifizierung phänotypischer Strategien auf Merkmalsebene entscheidend.

Daher verfolgt diese Arbeit einerseits den Zweck, (i) die Variation der Trockenmasse (TM) und der N-Merkmale von Winterweizen in Bezug auf Zielmerkmale und Behandlungseffekte auf Ebene des Bestandes und der Pflanzenorgane während der Kornfüllungsphase zu bewerten, und andererseits (ii) Phänotypisierungsmethoden für die Erfassung dieser Merkmale zu untersuchen. Hierzu wurden zwei dreijährige Feldversuche mit Winterweizen an einem süddeutschen Hohertragsstandort durchgeführt, darunter ein Versuch mit 75 Zuchtlinien (Versuch BL) und ein Versuch mit registrierten Hybrid- und Liniensorten in drei N-Düngestufen (Versuch *HyLi_N*). Darüber hinaus wurde in einem weiteren Versuch (*Cv_ag*) eine Auswahl von sechs Sorten bezüglich der Wirkung der agronomischen Behandlungen N-Düngung, Fungizidintensität und Saatzeitpunkt untersucht.

Die Pflanzen wurden zur Blüte und physiologischen Reife beprobt und bezüglich TM, N-Gehalt und N-Aufnahme analysiert. Die TM- und N-Translokation wurde mittels Differenzmethode berechnet, in welche die Ergebnisse beider Beprobungstermine einfließen. Hyperspektrale Reflexionsdaten wurden während mehrerer Wachstumsstadien von der Bestockung bis zur Teigreife mit einer mobilen Sensorplattform auf Bestandesebene erfasst. Die Spektraldaten wurden zu verschiedenen spektralen Vegetationsindizes verrechnet und für die Schätzung der Pflanzeigenschaften verglichen. Darüber hinaus wurde eine mehrjährige Studie zur Auswertung der indexbasierten Schätzung des N-Status in der vegetativen Phase durchgeführt.

Die vorliegende Arbeit besteht aus sieben Kapiteln.

Kapitel I vergleicht die Leistung von Hybrid- und Liniensorten für verschiedene TM- und N-Merkmale, wie sie in acht Jahr x N-Stufen-Kombinationen im *HyLi_N*-Versuch getestet wurden. Die Ergebnisse deuten auf einen leichten relativen Vorteil der Hybriden gegenüber Liniensorten im KE hin (+5,5%). Dieser stand in Zusammenhang mit einer effizienteren Umwandlung der oberirdischen N-Aufnahme in Korn-TM, aber nicht mit einer effizienteren N-Aufnahme, mit verbesserter TM-Allokation in das Korn (Ernteindex +3,5%), aber nicht mit höherer Gesamt-TM, und war meist zurückzuführen auf mehr Körner pro Ähre sowie auf eine höhere Nach-Blüte-Assimilation. Im Gegensatz dazu wurden nur wenige konsistente Unterschiede für TM- und N-Merkmale auf Ebene der Pflanzenorgane oder für Translokationsmerkmale festgestellt. Hybride zeigten niedrigere Korn-N-Gehalte (relativ: -8,5%), so dass kein Vorteil in der N-Abfuhr festgestellt werden konnte. Darüber hinaus wurde ein postulierter größerer Vorteil der Hybriden unter niedrigerer N-Versorgung nicht bestätigt.

Kapitel II vergleicht die Auswirkungen agronomischer Behandlungen auf TM- und N-Merkmale anhand von sechs Sorten. Eine frühere Aussaat um etwa drei Wochen führte tendenziell zu höherem KE (+8% n.s.) und Korn-N-Ertrag (+8% n.s.), während der Verzicht auf Blattfungizide zu niedrigerem KE (-12%; $p < 0.05$) und KNE (-9% n.s.) führte. Die Fungizideffekte waren auf höhere Werte in der N-Nutzungseffizienz, TM- und N-Translokation, relativen Allokation in das Korn, der Nachblüteassimilation sowie des Korngewichts zurückzuführen. Im Gegensatz dazu erhöhte sich durch frühere Aussaat die Kornzahl pro Ähre, die Gesamt-TM—insbesondere durch die Nachblüteassimilation—und tendenziell die Nachblüte-N-Aufnahme. Darüber hinaus wurde in der Regressionsanalyse der Einfluss der zugrundeliegenden Behandlungen auf die Erklärung der Zielmerkmale deutlich. Zur Unterscheidung der Sorten erwies sich die Kornzahl pro m² bezüglich KE sowie die Gesamt-N-Aufnahme, aber nicht der N-Ernteindex, bezüglich KNE als signifikant.

Kapitel III umfasst die Bewertung von TM- und N-Merkmalen, ähnlich derer in Kapitel I und II, die zur Erklärung der Bildung von KE, KNE und Korn-N-Gehalt in Zuchtlinien im Kontext verschiedener Merkmalskomplexe untersucht wurden. Es zeigte sich, dass die TM-Translokation wesentlich zum KE beiträgt, durchschnittlich von 31% unter ertragreichen, aber ‚sink‘-begrenzten Bedingungen, bis 44% unter Bedingungen stärkeren Krankheitsdrucks nach der Blüte. Allerdings war die Assimilation nach der Blüte stets enger mit dem KE korreliert. Zur Endreife war die Menge der oberirdischen TM deutlich indikativer für den KE als der Ernteindex. Unter den Ertragskomponenten zeigte nur die Kornzahl pro m² moderate Korrelationen mit KE. Die N-Effizienz bezüglich des KE wurde besser durch die N-Aufnahmeeffizienz als durch die N-Nutzungseffizienz erklärt. KNE war besser mit der gesamten N-Aufnahme korreliert als mit dem N-Ernteindex, sowie besser mit KE als mit dem Korn-N-Gehalt. Die Nachblüte-N-Aufnahme war stark jahresabhängig, wesentlich niedriger als die N-Translokation, aber immer positiv mit KNE korreliert. Es erscheint zielführend, eher Merkmale der TM- und N-Akkumulation als solche der Allokation zu optimieren und ‚Stay-Green‘-Merkmale sowohl für die

Maximierung von KE als auch von KNE zu berücksichtigen. Während die Züchtung bezüglich KE auch KNE maximieren würde, erscheint die Auswahl proteinstarker Genotypen kontraproduktiv, um die N-Effizienz zu erhalten.

Kapitel IV bewertet 48 aus der Literatur ausgewählte spektrale Vegetationsindizes für die Erfassung der in Kapitel III beschriebenen Merkmale mittels linearer Regressionsanalyse. Für den Vergleich merkmalspezifischer Indizes wurde ein quantitatives Ranking eingeführt, das auf den Regressionen aller Messtermine basiert. Es wurde deutlich, dass das Stadium der Milchreife am besten geeignet ist, um KE, KNE und verschiedene andere Merkmale zu erfassen. Im Gegensatz dazu schien ein phänologischer Versatz den Vorhersagen im Zeitraum Ährenschieben–Blüte entgegenzuwirken, und die vegetative Phase lieferte im Allgemeinen niedrige Beziehungen. Für den KE (maximales $R^2 = 0,26^{***} - 0,51^{***}$ in den drei Jahren) und die meisten TM-Merkmale lieferten Indizes, die die Wasserabsorptionsbande bei 970 nm nutzen, gefolgt von Indizes aus Banden im Nahinfrarot (NIR) in Kombination mit Red Edge (RE)-Banden, bessere Beziehungen als Kombinationen aus NIR-Banden und Banden im sichtbaren Spektrum (VIS), wie etwa dem NDVI. Für KNE und andere N-Aufnahmemerkmale waren NIR/RE-Indizes am besten geeignet. Eine Validierung mittels PLS-Regression im Vergleich zu Indizes zeigte für etliche Merkmale verbesserte Vorhersagefehler, bei aber meist ähnlichen R^2 -Werten. Obwohl die Erfassung der meisten Organ- und Translokations-bezogenen Merkmale weniger genau als die des KE war, kann sie das Verständnis der Bildung von Zielmerkmalen erweitern, was mittels destruktiver Methoden nicht möglich wäre.

Das **Kapitel V** bewertet die in Kapitel IV angewandten Methoden bezüglich der N-Merkmale des Kapitels II unter Berücksichtigung der zugrundeliegenden Behandlungsfaktoren. Es bestätigte sich die geringere Eignung der Phase des Ährenschiebens und der Blüte sowie die Eignung des Milchreifestadiums, während die Teigreife jedoch noch besser geeignet war – nicht zuletzt unter dem Einfluss von Behandlungen mit Auswirkung auf den Seneszenzverlauf. Im Falle differenzierter N-Düngung ergaben sich auch während des Schossens brauchbare Bezüge. Die Index-Rankings für KNE aus Kapitel IV wurden generell bestätigt. Etliche Merkmale waren über beide Fungizidstufen hinweg mäßig gut erfassbar, in Übereinstimmung mit Kapitel IV aber nicht für genotypische Unterschiede. Dies umfasste die meisten N-Gehaltsmerkmale auf Organebene, die N-Translokationseffizienz und den N-Ernteindex—meist für wenige seneszenz-sensitive Indizes.

Kapitel VI bewertet den Einfluss der spektralen Auflösung auf die spektrale Erfassung von KE, KNE und Korn-N-Gehalt in den Versuchen *HyLi_N* und *Cv_ag*. Normalisierte Differenzindizes aus allen möglichen paarweisen Indexkombinationen der hyperspektralen Reflexionsdaten sowie aus simulierten multispektralen Daten wurde zu mehreren Messdaten mit den Zielmerkmalen korreliert. Während die saisonalen Muster der Kapitel IV und V bestätigt wurden, wurde die Differenzierung der Merkmale durch verschiedene N-Düngestufen auch während des Schossens gut erfasst. Die Eignung

simulierter multispektraler Sensordaten, die denen realer Satelliten entsprechen, unterschied sich je nach Merkmal und Termin. Der Vorteil hyperspektraler Daten und multispektraler Sensoren mit einer höheren Anzahl und spezifischeren Bändern, insbesondere im RE- und Wasserbandbereich, nahm mit abnehmenden Korrelationsniveau zu. Er war während des Ährenschiebens und der Anthese und der späten Seneszenz höher, bei Korn-N-Gehalt höher sowie bei KNE niedriger. Der beste Multispektralsensor, Sentinel-2, minimierte den Informationsverlust im Vergleich zu den hyperspektralen Daten durch die Abdeckung der Wasserbande für KE und durch seine optimierte zweite RE-Bande für KNE und Korn-N-Gehalt. Es folgten die Konfigurationen der WorldView-2 und RapidEye-Sensoren, während die Quickbird- und Landsat-Konfigurationen am schlechtesten abschnitten. Es zeigte sich, dass auch multispektrale Sensoren bei optimaler Bandenplatzierung und ausreichend spezifischen Banden geeignet sein können.

Kapitel VII untersucht die Erfassung des Weizen-N-Status in der vegetativen Phase in Bezug auf den Einfluss von Sorte, N-Düngungsniveau, Niveau des Zielmerkmals und der Vegetationsindizes, die an einfachen Verhältnisindizes und dem Red Edge Inflection Point (REIP) getestet wurden. Insbesondere die Erfassung von TM und N-Gehalt wurde durch den Versatz zwischen Messterminen erheblich beeinflusst, wenn Beziehungen über Jahre und Wachstumsphasen hinweg getestet wurden. Im Gegensatz dazu wurden die N-Aufnahme und der N-Nutrition Index (NNI) innerhalb der Wachstumsstadien besser geschätzt als der N-Gehalt und die TM. In den meisten Fällen verzerrte der Einfluss verschiedener Sorten die Beziehungen. Basierend auf dem NNI wurde der Datenbereich in zwei Intervalle mit niedrigem bzw. hohem N-Status unterteilt. Während bei niedrigem N-Status alle Indizes ähnlich abschnitten, waren bei hohem N-Status RE-basierte Indizes eindeutig besser für N-bezogene Merkmale geeignet als die NIR/VIS- und Wasserbandenindizes. Somit sollten Sensoreinsatz und -entwicklung das Niveau der Zielmerkmale und die N-Bedingungen berücksichtigen.

Die **übergeordnete Diskussion** vergleicht die Ergebnisse der Kapitel im Kontext relevanter Literatur. Es wird der Schluss gezogen, dass Merkmale der TM- und N-Akkumulation eher indikativ für die Unterscheidung von KE und KNE sind als die Verteilungsmerkmale, d.h. TM- und N-Ernteindex. Darüber hinaus ist die Nachblüteentwicklung für beide Zielmerkmale entscheidend. Die beträchtliche Variation des KNE bei registrierten Sorten und bei Zuchtmaterial sollte in Verbindung mit optimierten agronomischen Behandlungen genutzt werden. Spektrale Hochdurchsatz-Phänotypisierungsmethoden sind vielversprechend, um sowohl für Zuchtlinien zur Diskriminierung von N-Effizienzmerkmalen, als auch für die Auswirkungen agronomischer Behandlungen wie N-Düngung, Sortenleistung sowie Fungizid- und Saatzeit eingesetzt zu werden. Die Verwendung von RE- und Wasserbandenindizes anstelle des weit verbreiteten NDVI scheint entscheidend für die Erfassung von N-Status und TM-Merkmalen, insbesondere von KE, zu sein. Die dichte zeitliche Auswertung empfiehlt das Milchreifstadium für eine möglichst genaue Bestandese Erfassung.

1 General introduction

1.1 Current and future challenges in cropping systems

Increasing the output from crop production while minimizing the associated environmental impact is one major challenge of the 21st century. By 2050, the world population is projected to increase by another 2 billion to 9.7 billion and may exceed 11 billion by 2100 (Gerland et al., 2014). In addition, changing consumption patterns were projected to more than double the food demand by 2050 compared to 2005 (Tilman et al., 2011). In addition, the limited cropping area and the concomitant detrimental effect of climate change (Asseng et al., 2015; Lobell et al., 2011; Olesen et al., 2011; Ray et al., 2012) demand to considerably increase the productivity on the existing cropland. However, yearly yield increases of the major crops are not sufficient to meet the required yield growth (Grassini et al., 2013; Ray et al., 2013), thus that a second green revolution may become necessary (Zeigler and Mohanty, 2010).

At the same time, agricultural practices need to become more sustainable to reduce the impact on natural ecosystems (Chen et al., 2014; Garnett et al., 2013). Rockström et al. (2009) examined nine key 'earth system processes' in the human-environmental nexus. Besides biodiversity loss and climate change, the nitrogen cycle was identified to critically exceed the planetary boundaries for a sustainable development. Today, about one in two humans globally relies on food produced using industrially fixed reactive nitrogen (N; Erisman et al., 2008), being associated with substantial ecological impacts (Zhang et al., 2015). The unequal distribution of global N use is associated with an overuse particularly in industrial and emerging countries (Zhang et al., 2015), whereas N use is still sub-optimal in many developing countries (Mueller et al., 2012).

Globally, wheat (*Triticum aestivum* L.) is one of the three major crops together with rice and maize. It is the most commonly grown crop and the most important direct food source, contributing about 19% and 20% to the global calorific and protein consumption, respectively (FAOSTAT, 2014). In 2017, the global wheat produce of 771 Mio t originated mainly from Asia (43.5%), Europe (35%) and America (13.8%; FAOSTAT, 2019). In Europe, Germany was the third most important wheat producer behind France and the Ukraine. With a production area of 3.2 Mio ha in 2017, winter wheat occupied more than half of the cereal production area in Germany (Statistisches Bundesamt, 2019).

1.2 The need for increasing the nitrogen use efficiency

Globally, both the average cropping system and wheat cropping remove only about 42% of the applied N through the harvest of produce. In Europe, at a removal efficiency of only 52%, the N surplus from cropping amounts to about 7 Mio t annually, compared to 100 Mio t globally (Zhang et

al., 2015). Wheat accounts for about 17% to the N pollution from crop production activities. The accumulation of surplus N in natural ecosystems causes major damage in terms of biodiversity loss, eutrophication of terrestrial and aquatic ecosystems, as well as human health (Brink et al., 2011; Conley et al., 2009; Galloway and Cowling, 2002; Lassaletta et al., 2014; Schlesinger, 2009; Zhang et al., 2015). In addition, agriculture, notably excessive fertilizer N use, accounts for about 80% of the global emissions of nitrous oxide (IPCC, 2019), which is a potent greenhouse gas (Reay et al., 2012). In Europe and particularly in Germany, recent political initiatives focus on reducing the groundwater nitrate content, which exceeds threshold values in many regions (Velthof et al., 2014). Recently adapted measures by the German fertilization ordinance aim at increasing the N use efficiency (NUE). For wheat, it stipulates reductions in the allowed amount of fertilized N, thus forcing farmers to adopt measures for increasing NUE.

1.3 Plant sensors in precision farming

Sensor-based precision farming is increasingly adopted for considering the site-specific yield potential and the crop nutritional status for the dosing of fertilizers and pesticides (Barker and Sawyer, 2010; Cao et al., 2015; Dellinger et al., 2008; Govaerts, 2010; Mayfield and Trengove, 2009; Mulla, 2013; Schmidhalter et al., 2008). Some authors perceive digitalization in agriculture, 'agriculture 4.0', or 'smart farming' as new green revolution (Schmidt, 2018; Walter et al., 2017; Wolfert et al., 2017). Hitherto, precision crop management has been dominated by ground-based, 'online' sensors, and soil mapping approaches (Ebertseder et al., 2005; Neudecker et al., 2001). However, the ambiguous economic profitability has hindered a more prevalent adoption of such methods in spite of obvious ecological benefits (Colaço and Bramley, 2018).

Unmanned aerial vehicles (UAVs) and satellites provide new opportunities in recent years (Adão et al., 2017; Thessler et al., 2011; Zhang and Kovacs, 2012). For example, the advent of freely available multispectral Sentinel satellite data with improved spectral, spatial and temporal resolution is promoting precision farming applications (Clevers and Gitelson, 2013; Vizzari et al., 2019). Due to the often dominant effect of nitrogen fertilizer on plant growth, its relatively high costs and its environmental impacts, many precision farming applications focus on N management (Diacono et al., 2013; Geesing et al., 2014; Samborski et al., 2009). Here, precision farming contributes to higher NUE and to avoiding both under- and overfertilization. Still, many recent developments are primarily technology-driven, fostered by rapid developments in the realm of satellite sensing, UAV platforms or autonomous field vehicles (Furbank and Tester, 2011). However, usage and implementation of these methods require proper referencing with destructively determined plant data, including extensive 'low-throughput' methods. While sensor data is becoming available faster and at lower costs, data management and analysis must keep pace as well (Shakoor et al., 2019).

1.4 Towards high-throughput field phenotyping

An emerging field of application of plant sensors is the realm of high-throughput phenotyping (HTP). Plant phenotyping as the 'quantitative and qualitative evaluation of functional plant traits' has been the integral method for plant selection and breeding over thousands of years (Kumar et al., 2015). The term 'phenome' describes phenomic traits as a whole; and phenotyping methods are often subsumed in the term of 'phenomics', analogous to 'genomics'. 'Forward phenomic' approaches aim at discriminating superior germplasm, whereas 'backwards phenomics' aim at characterizing traits of the best genotypes, thus improving the mechanistic understanding of the formation of target traits (Kumar et al., 2015). Depending on the target crop, the test environment and the underlying treatments, the requirements for sensing and data analysis can substantially differ between HTP and precision farming (Chawade et al., 2019).

In the context of advanced high-throughput genomic methods being developed and successively adopted in plant breeding (Lorenz et al., 2011), the availability of sufficient and precise phenotypic data is still scarce (Araus and Cairns, 2014; Houle et al., 2010). Being time-consuming and expensive, destructive plant sampling followed by laboratory analysis can often not provide sufficient data with reasonable efforts. The same applies to manual and visual plant scoring, which in addition relies on trained, experienced workforce and suffers from being subjective, error-prone and restricted to visible, manifest trait characteristics (Roitsch et al., 2019). Nonetheless, in plant breeding and in agronomic field trials, a large number of experimental plots have to be evaluated for various traits and need to be harvested. The application of genomic methods suffers from false positive quantitative trait loci, which may be detected in controlled environments, but may fail in target environments (Kumar et al., 2015; Tardieu et al., 2017). Therefore, reliable HTP methods in relevant field environments are crucial both for improving genomic methods and for making field phenotyping more efficient. Furthermore, new plant traits may be determined, providing previously unavailable information (Rebetzke et al., 2019).

Besides applications for plant breeding, phenotyping methods could be utile for the efficient evaluation of agronomic field trials for assessing the effects of agricultural treatments such as fertilization levels or the performance of registered cultivars (Hyman et al., 2017; Shi et al., 2016). Thus, due to frequently strong environmental interactions, such trials must be repeated in different target environments (Tardieu et al., 2017), thereby multiplying the effort to reliably phenotyping particular traits. In this context, HTP methods could be a cost-effective, objective tool also for agronomic trials (Hallett et al., 2014).

Phenotyping takes use of a broad field of techniques from the plant cell to the canopy (Cobb et al., 2013). Currently, substantial efforts are undertaken to improve phenotyping in controlled environments (Basu et al., 2015). Plant screening *ex situ* in growth chambers and greenhouses holds the advantage of optimum control of growth factors and minimization of environmental disturbance

(Cobb et al., 2013). However, it immanently suffers from limited transferability to the relevant target environment. Thus, complex traits such as NUE and yield formation are formed from multiple contributing traits, and therefore often show substantial genotypexenvironment interactions (Cormier et al., 2013). Moreover, plants often cannot be grown until relevant later growth stages are reached in controlled environments.

Therefore, it appears inevitable to screen such traits under field conditions *in situ*, similar to those in the target growth environment. On the downside, this implies that test conditions are less stable, however. Thus, plant growth is impacted by soil heterogeneities and weather conditions (Heil et al., 2018). Field trials demand for adequate experimental design and technical trial management in terms of sowing, pest control, fertilizing and yield determination.

Breeding is to a large extent a culling approach, rejecting genotypes which are not suitable for reasons of pathogen susceptibility, morphology, phenology and insufficient yield potential (Acquaah, 2007). Therefore, one main goal in HTP is to keep a promising percentile of genotypes containing as few as possible false positives while rejecting low-performing candidates. This relative differentiation approach would to some degree tolerate absolute estimation errors (Garriga et al., 2017), which are likely higher under field conditions due to the less controlled growing and measurement conditions.

1.5 Spectral methods in high-throughput phenotyping

Various measurement principles are applied in HTP, but the vast majority is based on imaging and spectral methods. Notably, reflectance-based methods are used in the visible (VIS), near-infrared (NIR) and partly short-wave infrared range. Thermal measurements are useful notably for detecting drought and heat stress. Fluorescence measurements relate to the amount and concentration of chlorophyll and the photosynthetic capacity. While other methods such as RADAR and LIDAR are based on the time of flight principle, spectral methods measure the intensity of the backscattered radiation (Araus et al., 2018; Araus and Cairns, 2014). Either active and passive sensors are used (Erdle et al., 2011). While passive sensors use the ambient light as source for incident radiation, active sensors are equipped with an artificial light source. Therefore, active sensors can be used more independently, whereas measurements of passive sensors are more affected by daytime, sun position, and cloud cover (Fitzgerald, 2010). In turn, due to their weaker light source, active sensors are more sensitive to the measurement distance and are limited in penetrating dense canopies (Kipp et al., 2014a). While multispectral sensors only measure in few wavebands, hyperspectral sensors provide gapless measurements in narrow bands (Backer et al., 2005; Das and Seshasai, 2015).

In field environments, which are less controllable than indoor environments, conflicts of goals can occur in the application of HTP methods. Thus, speed of measurement is crucial for screening a high number of trial plots and may counteract with the achieved accuracy. Optimum measurement

conditions may be restricted to narrow time frames. Moreover, sensors should tolerate changes in illumination. Therefore, sophisticated, precise methods are often not directly transferable to field conditions (Hallett et al., 2014). Consequently, higher precision achieved by some methods such as hyperspectral imaging or LIDAR sensors in controlled environments may counteract with measurement speed and sensor robustness. This includes the choice of imaging versus non-imaging point sensor systems, hyperspectral versus multispectral sensors, and active versus passive systems as well as the choice of scale on the leaf or canopy level. In addition, the sensor choice depends on suitable sensor platforms, including hand-held systems, ground-based mobile vehicles and airborne systems (Deery et al., 2014; Zecha et al., 2013).

Commonly, the reflectance information of only a few spectral bands is used for calculating spectral vegetation indices (SVI). Often basic equations are applied for simple-ratio indices (Pearson and Miller, 1972) or the wide-spread normalized difference vegetation index (NDVI; Rouse et al. 1973). Many SVIs are based on the reflectance contrast between the red and the NIR spectrum. While the reflectance in the red decreases with increased chlorophyll due to stronger absorption, both on the leaf and canopy level, the NIR reflectance increases with increased physiologically active biomass due to light scattering in the palisade tissue (Hatfield and Prueger, 2010; Pinter et al., 2003).

The NDVI is widely used for retrieving structural canopy traits, notably leaf area index (LAI), radiation absorption and biomass (Hatfield and Prueger, 2010). However, the NDVI is affected by saturation at high values in dense canopies beyond LAI values of approximately 3 (Haboudane et al., 2004). Simple-ratio indices were less affected by saturation (Barmeier and Schmidhalter, 2017; Yu et al., 2012). In the VIS spectrum, the green spectrum is most sensitive to chlorophyll content at high levels (Ustin et al., 2009). With increasing chlorophyll content, the red edge (RE) position shifts rightwards (Hatfield et al., 2008). Therefore, RE indices such as the red edge inflection point (REIP) are widely used for estimating chlorophyll content (Vogelmann et al., 1993). Although only a fraction of the N is allocated to leaf chlorophyll, the leaf N status is often a reasonably good proxy for the chlorophyll content (Schlemmer et al., 2013) and holds the advantage of easier reference analysis. Moreover, RE indices are sensitive for estimating N uptake (Nup) and were more sensitive in dense canopies than NIR/red indices—also for the estimation of biomass (Frels et al., 2018; Nguy-Robertson et al., 2012; Pavuluri et al., 2015). Simple-ratio RE indices performed similarly or better than the REIP for biomass and N traits (Erdle et al., 2011). Another group of indices uses VIS information for retrieving pigment-related information, mainly in the green/red region, including the photochemical reflectance index (PRI; Peñuelas et al., 1995) and the anthocyan reflectance index (ARI; Gitelson et al., 2001). Furthermore, the water absorption band at 970 nm is exploited by NIR/NIR combinations such as the water band index (WBI; Peñuelas et al., 1993) for estimating the water status (Sims and Gamon, 2003).

1.6 Yield formation and nitrogen use efficiency: Concepts, genotypic variation and contributing traits

Many plant traits are of interest to be estimated by HTP approaches. This thesis focuses on traits related to the formation of NUE, in particular grain yield (GY) and grain N uptake (GNup). Unlike in genomics, which potentially allows the characterization of the whole genome, phenomics need to be prioritized to relevant traits. Moreover, the interaction of phenotypic traits with the environment and with growth stages demand a more dynamic approach, including the consideration of phenology and growth stages suitable for detecting trait expression and measurements (Houle et al., 2010; Kumar et al., 2015).

Nitrogen use efficiency relates the amount of total biomass (NUE_{total}) or harvestable biomass, i.e. grain biomass (NUE_{grain}) to available or fertilized nitrogen. NUE can be further dissected into N uptake efficiency (NupEff), linking Nup to the available or fertilized amount of N, and to the N utilization efficiency (NutEff), which links the amount of overall or harvestable biomass with the Nup (Cassman et al., 2002; Foulkes et al., 2009; Moll et al., 1982). Further NUE concepts exist, notably the physiological NUE, which relates the additional grain biomass by fertilization to the additional Nup (Garnett et al., 2015), and the agronomic efficiency, which relates the additional grain biomass by fertilization to the amount of fertilized N (Delogu et al., 1998). Both concepts require non-fertilized control plots and are not applied in this thesis.

An optimized agronomic management enhances NUE (Garnett et al., 2015). The possibly most influential measure is the optimization of the amount of fertilized N, which requires to match the yield potential (Godard et al., 2008). Regionally adapted sowing dates influence the pre-winter development and are a potential escape strategy against climate change (Semenov et al., 2014). Optimized chemical plant protection, notably fungicides against fungal pathogens, can increase the green leaf area duration, late Nup and the allocation of N to the grain (Gooding et al., 2005). The choice of regionally adapted, efficient cultivars represents another commonly applied measure for increasing NUE. For wheat, new potentials arise from recently advanced hybrid cultivars. While the exploitation of heterosis has achieved considerable yield gains in allogamous crops like rye and maize (Birchler et al., 2010), heterosis is lower in autogamous crops (Longin et al., 2012; Mahajan et al., 1999; Morgan et al., 1989). Still, hybrid wheat is recently fostered by plant breeders due to expected stress tolerance in drought-prone environments (Becker, 2017), but also for commercial reasons. The advantage of hybrid cultivars was related to sturdier root growth (Sinha and Khanna, 1975; Yao et al., 2005), which potentially could also increase the NUE.

Most of these agronomic measures potentially influence NUE through both, NupEff and NutEff, but the quantification of the effects on both components could guide agronomists and breeders for prioritizing available measures.

When genotypes are tested in the same N conditions, the variation in GY represents that in NUE_{grain} , whereas NupEff is a direct function of total Nup. Maximizing the amount of 'harvested' N, i.e. GNup, is a major goal for reducing the N surplus in wheat (Dawson et al., 2008; Hawkesford, 2014), but was often only indirectly targeted through breeding for GY and grain N concentration (GNC). However, GNC and GY are negatively correlated, owing to the high carbon costs of protein assimilation and the dilution of protein in high-yielding genotypes (Yang et al., 2007). In wheat, GNC is associated with the protein concentration, which is often associated with baking quality and therefore a major target trait for breeding (Gabriel et al., 2017; Hawkesford, 2014).

Dissecting GY and NUE formation into the contributing traits could disclose new strategies in plant breeding. For example, both NupEff and NutEff contributed to the NUE breeding progress (Cormier et al., 2013). However, their influence differ between N conditions (Foulkes et al., 2009; Gaju et al., 2011; Hawkesford, 2014). Furthermore, linking phenotyping to the underlying traits can broaden the understanding of observed empirical relationships with main target traits like GY and GNup.

Being highly compensatory, yield formation and its breeding progress could not be ascribed to one of the components spike density (SD), grain number per spike (GNS), or thousand kernel weight (TKW; Cormier et al., 2013). In some studies, increased numbers of spikes and kernels per m² were most relevant for variation in GY (Shearman et al., 2005). Yield components interact with crop management and are important for characterizing cultivars. Furthermore, GY formation can be dissected into the pre-anthesis contribution, i.e. the dry matter (DM) translocation (DMT), and post-anthesis assimilation (PAA). The contribution of PAA to grain filling (CPAA) has often been reported to be well above 50% (Savin and Slafer, 1991). In contrast, the contribution of DMT to GY approached 60% under heat stress (Plaut et al., 2004) and 100% under drought stress (Inoue et al., 2004; Van Herwaarden et al., 1998). In recent decades, yield gains are more attributed to increased total DM than to better relative partitioning of assimilates in the grain as expressed by the harvest index (HI; Cormier et al., 2013; Shearman et al., 2005).

GY can be limited by sink size (Borrás et al., 2004; Fischer, 2007), which is predominantly determined until anthesis (Foulkes et al., 2011; Slafer et al., 2014). Consequently, NutEff could be indirectly predicted based on anthesis canopy traits (Demotes-Mainard and Jeuffroy, 2004; Foulkes et al., 2009). Increasing yield by improving NutEff may involve stay-green characteristics. Unlike for GY, the major component of GNup is not the post-anthesis Nup (PANup; Barraclough et al., 2014; Kong et al., 2016; Masclaux-Daubresse et al., 2010), but N remobilized and translocated during the grain filling phase (N translocation: NT; 60–95%; Hirel et al., 2007). Being associated with senescence, NT following RuBisCo degradation could counteract yield formation and in turn reduce NutEff (Foulkes et al., 2009; Gaju et al., 2011; Masclaux-Daubresse et al., 2010). For quantifying NT, either ¹⁵N labeling or the difference method, which considers the losses in vegetative Nup from anthesis to maturity as

'apparent' NT, was applied. While the difference method does not account for root-shoot exchange and internal turnover, it is relatively simple and, therefore, is often adopted (Kong et al., 2016). Nitrogen translocation is influenced mainly by pre-anthesis Nup and its translocation efficiency (NTEff; Barbottin et al., 2005). In addition, culm and spike N, which are predominantly translocated during grain filling, act as reserve pools and delay leaf NT (Foulkes et al., 2009; Pask et al., 2012). NTEff varied from 43% to 92%, depending on the organ, the environment, and disease pressure (Barbottin et al., 2005; Foulkes et al., 2009; Kong et al., 2016). In contrast to carbon accumulation, NT and PANup are mostly source-limited (Bancal, 2009; Foulkes et al., 2009; Martre et al., 2003; Masclaux-Daubresse et al., 2010). However, the N sink size influences the temporal NT dynamics and senescence. Moreover, PANup seems to be prioritized over NT under conditions of abundant N and excessive N fertilization can affect NTEff (Dreccer et al., 2000; Kong et al., 2016).

Post-anthesis growing conditions influence PANup as well (Bancal et al., 2008; Hawkesford, 2014; Kong et al., 2016). PANup seems to be a key driver for variation in GNup in spite of the lower absolute contribution (Bogard et al., 2011; Cox et al., 1985). Final GNup is furthermore the product of total Nup and the N harvest index (NHI), which is the ratio of GNup to total Nup at maturity (Sinclair, 1998). Since the bulk of GNup originates from NT, NHI was reported to be closely correlated to NTEff (Fageria, 2014; Hirel et al., 2007). In wheat, genetic differences in NHI are relevant and ranged from 0.40–0.70 (Guttieri et al., 2017). In spite of rising grain yields, increases in NHI by 0.13% per year have contributed to stable GNC values over the last 2–3 decades (Cormier et al., 2013), but it remains unclear if higher NHI is due to better NTEff or higher PANup. An earlier analysis found NHI to exceed HI by approximately 0.2 and observed a steady increase in NHI over five decades of breeding (Rooney and Leigh, 1993 in Hay, 1995).

1.7 Plant traits in the phenotyping context

Spectral HTP has been targeted for efficient evaluation of the performance of plant breeding germplasm (Araus and Cairns, 2014; Furbank and Tester, 2011). Target traits in HTP include on the one hand (i) particular morphological and physiological traits and on the other hand (ii) 'integral traits' such as GY, GNup and NUE. The former (i) includes traits related to plant drought and heat tolerance (Rischbeck et al., 2016; Tardieu et al., 2017) or morphological traits such as plant height, which is well estimable from actively measuring distance sensors (Barmeier et al., 2017). These traits are often no direct target traits in breeding but can be useful 'secondary' traits for indirect selection (Fischer and Rebetzke, 2018). This would require sufficiently high heritability values, correlations with the target traits and a robust, reliable assessment. Various studies found SVIs to be highly heritable (Babar et al., 2007; Frels et al., 2018; Prasad et al., 2007a) or to be related to quantitative trait loci associated with GY (Gizaw et al., 2016a).

Several studies optimized the spectral estimation of the leaf area index (LAI) and chlorophyll content, however often from a remote sensing perspective on a landscape scale or on a leaf level scale, respectively (Gitelson et al., 2005, 2003; Verrelst et al., 2015), which both cannot be directly transferred to canopy proximal sensing, however. The same applies to findings based on satellite remote sensing, both owing to differing sensor configurations and atmospheric effects. Moreover, the relationship of vegetation indices are often likely indirect, which limits a mechanistic interpretation (Glenn et al., 2008) and thus demands empirical testing under various conditions. Although the spectral reflectance signal is primarily influenced by LAI and pigment content, the estimation of these traits is not sufficient for predicting GY and GNup. Instead, total DM and Nup, DM and N allocation and their translocation over time, senescence dynamics as well as yield components need to be considered as well (Foulkes et al., 2011; Reynolds et al., 2009).

1.8 Optimizing spectral grain yield prediction

Various authors evaluated in-season spectral measurements for the indirect estimation of the GY potential in durum (Aparicio et al., 2000; Royo et al., 2003), spring barley (Barmeier et al., 2017; Rischbeck et al., 2016) or wheat (Babar et al., 2006b, 2006c; Babar et al., 2007; Freeman et al., 2003; Gizaw et al., 2016b, 2016a; Moges et al., 2004; Pinter, 1981; Prasad et al., 2007a, 2007b; Raun et al., 2001). This would enable plant breeders to focus on a limited set of promising genotypes for further rating, or even make yield determination of dismissed lines dispensable (Garriga et al., 2017). The method would be similarly utile for trials, which evaluate agronomic treatment factors (Nguyen and Kant, 2018).

The use of such HTP methods requires knowledge about suitable growth stages for measurements particularly for 'integral' traits, which are formed in a complex manner from various components over time (see 1.6). In addition, the application of sensors needs to be evaluated in terms of sensor platforms, the configuration of spectral bands and the measurement geometry.

Previous studies were predominantly defined to the estimation of GY, thus limiting causal explanations. Therefore, in order to elucidate 'black box' approaches, contributing plant traits (section 1.6) were included in this thesis. This includes organ- and plant-level N and DM traits at both anthesis and maturity, N and DM translocation traits, accumulative traits, related to N and DM per m², and 'partitioning' traits such as HI and NHI. On the one hand, this approach aims at widening the understanding about the transfer of the information gathered by the sensors to the retrieval of target traits and can contribute to a physiological breeding approach (Pask et al., 2012; Reynolds et al., 2009). On the other hand, the estimation of some of these traits is of direct interest, for example for breeding for total vegetative biomass for bioenergy crops (Busemeyer et al., 2013).

Several previous studies, which were often conducted in warm or drought-prone environments, strengthened the suitability of NIR/NIR water band indices, due to the relation of canopy water mass with biomass and water status with assimilation, respectively, as well as the lower saturation of these bands (Babar et al., 2006b; Becker and Schmidhalter, 2017; Garriga et al., 2017b; Gizaw et al., 2016a; Gutierrez et al., 2010; Rischbeck et al., 2016). However, the suitability of NIR/NIR indices or NIR/VIS indices such as the NDVI depended on the wheat type and environment (Gizaw et al., 2016b).

Besides SVIs, hyperspectral information was exploited using multivariate methods, notably partial least squares regression (PLSR), which mostly improved yield prediction for different crops (Barmeier et al., 2017; Elsayed et al., 2015; Hansen et al., 2002; Overgaard et al., 2013; Rischbeck et al., 2016; Weber et al., 2012). However, it can be affected by overfitting, so that it requires profound validation.

In addition to the spectral optimization of the methods, the optimum choice of measurement dates or growth stages is equally important. Most of previous studies focused on the period from booting until early milk ripeness and few measurement dates. Several studies reported closer relationships until milk ripeness (Babar et al., 2006a; Christopher et al., 2014; Freeman et al., 2003; Gutierrez et al., 2010). While relationships peaked at anthesis for a rain-fed trial, they increased until maturity under irrigated conditions, but were generally lower due to saturation effects in denser canopies (Aparicio et al., 2000).

Given that GY formation depends on the cumulative radiation absorption and the light use over time (Reynolds et al., 2009), some authors reported improved estimations through combining information from multiple measurements dates (Christopher et al., 2014; Crain et al., 2016; Raun et al., 2001).

1.9 Optimizing spectral phenotyping of nitrogen traits

The previous estimation of N traits focused on the vegetative N status for optimizing the agronomic management (Diacono et al., 2013; Muñoz-Huerta et al., 2013). Most studies, which assessed traits related to N status in relation to N fertilization, predominantly agreed on the usefulness of RE bands for vegetative N concentration (NC; Li et al., 2010; Mistele and Schmidhalter, 2008a), Nup (Guo et al., 2017; Li et al., 2013; Mistele and Schmidhalter, 2010, 2008b), N status, DM and LAI (Hansen and Schjoerring, 2003), as well as GNC and Nup. In contrast, band combinations in the VIS were recommended for pigment-related traits such as chlorophyll content (Gitelson et al., 2002; Hansen and Schjoerring, 2003; Peñuelas et al., 1995).

1.10 The estimation of secondary plant traits

Underlying 'secondary' traits (section 1.6) were less frequently addressed with spectral methods, particularly for the discrimination of genotypes. Evaluating the spectral estimation of organ-level DM and Nup traits at anthesis and dough ripeness in spring barley, Barmeier & Schmidhalter (2017) recommended the R780/670 simple-ratio index for DM traits to overcome the saturation of the NDVI. Dry matter and Nup of leaf blades followed by those of culms were mostly better predicted than those of spikes and leaf sheaths. Using RE-based SVIs in winter wheat breeding lines grown in small plots, Frels et al. (2018) observed mostly weaker relationships with DM and Nup at anthesis and maturity, as well as with NHI, NutEff, NupEff and PANup. They recommended the RE Maccioni index and identified the early grain filling stage as most promising. Testing many SVIs for predicting GY, GNup and NUE traits, Pavuluri et al. (2015) confirmed RE-indices such as the R780/R740 (Mistele et al. 2004) and observed closer correlations under reduced N fertilization, what was ascribed to the higher sensitivity in thinner canopies.

Being a major target trait, the estimation of GNC, or of the directly related grain protein content (Mossé, 1990), would be useful as well. Apan et al. (2006) reported good predictions of GPC using PLSR, however across N and sulfur fertilization levels. In contrast, no useful relationships were found for spring barley cultivars (Barmeier et al., 2017).

Contrary to the variation driven by altered N application, the detection of genotypic variation is likely to be more affected by the influence of varying morphology, shifted phenology and differing contributions of underlying DM and N traits. Moreover, even if the methods are also promising in high-yielding environments (Gizaw et al., 2016b), many of the studies on spectral grain yield prediction were conducted on spring wheat or in environments with low yield potential (Gutierrez et al. 2010; Babar et al. 2007; Sultana et al. 2014; Babar et al. 2006). Given that weaker relationships were often reported from irrigated trials or denser canopies compared to drought-stress trials, this limits the transferability to high-yielding winter wheat (Aparicio et al. 2000; Babar et al. 2006; Becker & Schmidhalter 2017; Frels et al. 2018). Moreover, the optimized selection of SVIs depends on the availability of suitable sensors—characterized by band number, narrowness and placement—and measurement stages (Thenkabail et al., 2000).

2 Thesis objectives and outline

This thesis pursues the dual purpose of evaluating traits of yield formation and NUE and the possibility of their estimation by means of spectral HTP methods. The analysis of the plant traits comprises the organ- and plant-level DM and N traits at anthesis and maturity for regarding their response to agronomic treatments, their variation between genotypes and their contribution to NUE target traits, notably GY and GNup. It aims at extending previous knowledge by considering traits, which have rarely been evaluated for hybrid wheat cultivars (section *I* and *II*), for different agronomic treatments under the same environmental conditions (section *II*), and for a comparably large number of breeding lines (section *III*), especially under high-yielding European conditions. Sampling at anthesis and maturity allows assessing the influence of the pre- and post-anthesis phases. The assessment of spectral HTP methods focuses on multi- and hyperspectral SVIs and on the suitability of growth stages for measurement. It aims at appraising the possibility to detect traits, which have rarely been estimated in HTP before. Testing various hyperspectral SVIs intends at identifying both suitable spectral bands and index equations, which could contribute as well to extending the methods to simpler and robust multispectral sensors.

This thesis is structured cumulatively in seven sections, corresponding to round symbols in Figure 1, numbered by Roman numerals. Sections *I–III* comprise the quantification and comparison of DM and N traits, related to the NUE formation, notably to GY and GNup. Sections *IV–VI* report on the spectral detection of these traits. Section *VII* assesses the sensitivity of SVIs for estimating vegetative N status.

Section *I* originates from a three years' study conducted to compare established, registered hybrid wheat cultivars to line cultivars in three N fertilization regimes for organ- and plant-level DM and N traits at anthesis and maturity (hybrid and line cultivar N fertilization trial, *HyLi_N*). Based on the reported stress tolerance of hybrids, it was hypothesized that hybrids are more efficient in N use, particularly under low N conditions.

Section *II* originates from a study (agronomic cultivar trial, *Cv_ag*) conducted in the 2016/17 growing season with six cultivars. The aim of the study was to assess the treatment effects and trait relationships for three agronomic conditions, corresponding to early sowing, reduced fungicide intensity at normal sowing date, and normal fungicide intensity at normal sowing date, with each of the three blocks comprising two N fertilization levels.

Section *III* originates from a three years' study (breeding lines trial, *BL*). It evaluates the variation and contribution of multiple organ- and plant-level DM and N traits at anthesis and maturity for explaining variation of NUE in breeding lines, notably with respect to GY, GNup and GNC.

Section *IV* evaluates the spectral estimation of the traits described in section *III*. It includes the comparison of 48 SVIs and the assessment of suitable growth stages for spectral measurements.

Section V evaluates the same SVIs for the estimation of the N traits of section II.

Section VI reports the spectral detection of GY, GNup and GNC using SVIs based on contour map analysis from hyperspectral and simulated multispectral band configurations. The section comprises data from the second and third year of the *HyLi_N* trial and from the *Cv_ag* trial, including from both studies additional cultivars, which had not been destructively sampled in sections I and II.

Section VII reports the evaluation of selected SVIs for the estimation of the pre-anthesis N status, including seven years' data with eight incrementally increased N levels (trial *IN*: incremental N fertilization trial). It aims at comparing the influence of the level of the target traits, of cultivars and of differing growth stages.

All sections correspond to peer-reviewed published articles. Section summaries are included after the 'Methods' chapter.

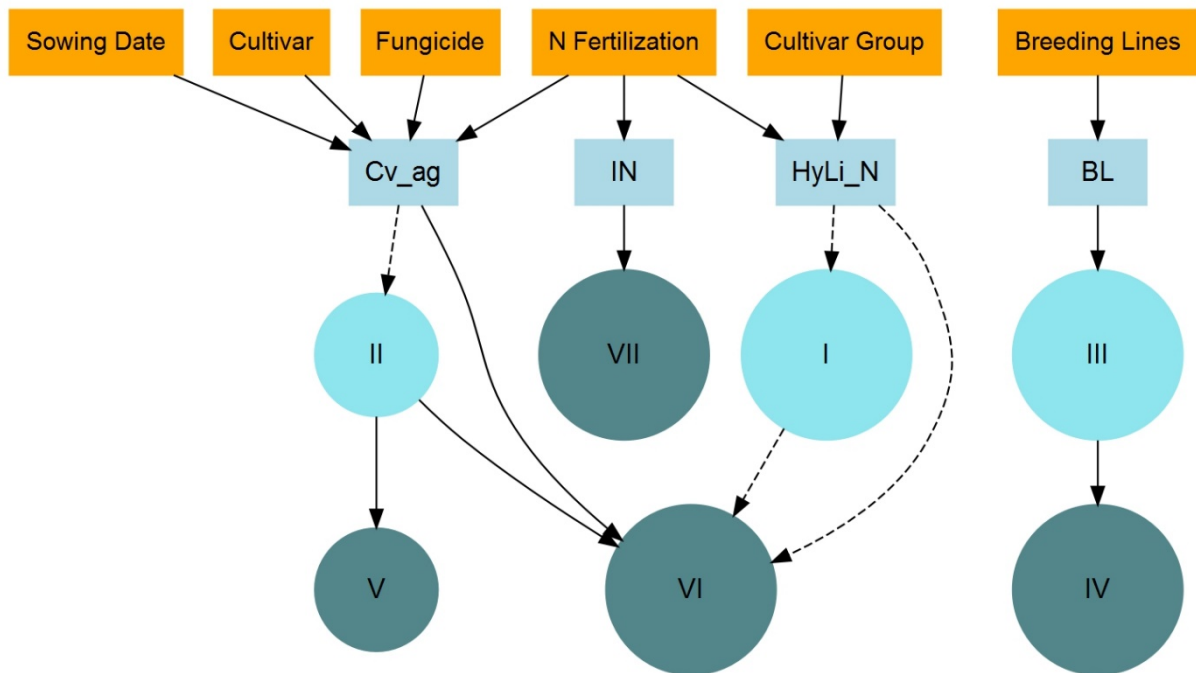


Figure 1: Scheme on field trials (blue rectangles), experimental treatment factors (orange rectangles) and sections in this thesis (circles). Circles colored in light aquamarine indicate the sections on dry matter and nitrogen reference traits and circles colored in dark aquamarine the sections on spectral phenotyping. Small circles indicate one year's data and large circles data of three or more (section VII) years. Arrows link the treatments applied in the field trials to the data used in the different sections. Field trials are abbreviated as *Cv_ag* (agronomic cultivar trial), *IN* (incremental N fertilization trial), *HyLi_N* (hybrid and line cultivar N fertilization trial) and *BL* (breeding lines trial). Dashed lines indicate when the data of not all trial plots was used.

3 Methods

3.1 Field trials

This thesis predominantly comprises data from four study years. Field trials were conducted in the growing seasons 2013/2014 (*HyLi_N*), 2014/2015 (*HyLi_N, BL*), 2015/2016 (*HyLi_N, BL*) and 2016/2017 (*Cv_ag, BL*). Given that all data were sampled in the main growing period during spring–summer, the study years are referred to as 2014, 2015, 2016 and 2017, respectively. The *IN* trial (section VII) comprised data from 2009, 2010, 2011, 2013, 2014, 2016 and 2018. Field trials were conducted at the Dürnast field research station of the Chair of Plant Nutrition of the Technical University of Munich, approximately 2 km west of Freising (see Figure 2 for the *BL* trial in 2015 and 2016). The site represents a high-yielding, temperate environment with an annual precipitation of on average approximately 800 mm.

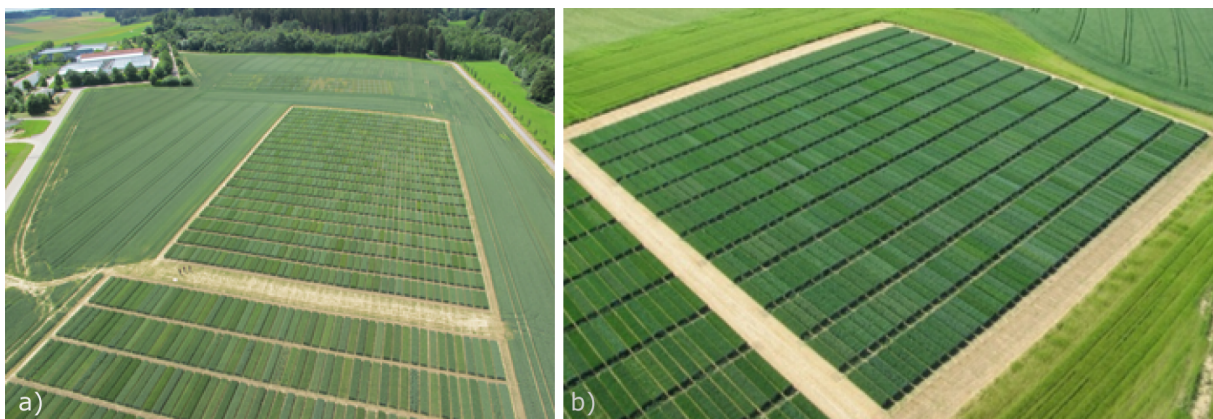


Figure 2: Overview of the breeding lines (*BL*) trials in 2015 (a: background) and 2016 (b). Parts of the *HyLi_N* trial are visible in the foreground (a) and on the left side (b).

The soil type is diluvial Cambisol of loamy clay. The site is located in the Bavarian tertiary Molasse-Hills at approximately 460 m above sea level and characterized by hilly terrain. Details on the trial performance are listed in Table 1. The field trials were conducted on three closely neighbored fields (D1, D2 and D4). The soil value numbers according to the German soil inventory, ranging from zero to one hundred, were 56–66 for D1, 63 for D2 and 56–63 for D4. Analyzed in April 2017, all fields showed optimum supply of phosphate (P_2O_5 : 11, 18 and 20 mg 100 g⁻¹ at D1, D2 and D4, respectively) and potash (K_2O : 12, 11 and 14 mg 100 g⁻¹ at D1, D2 and D4, respectively), as well as optimum pH values (6.4, 6.6 and 6.8, respectively). The soil organic carbon content was on average 1.18%. In all trials, experimental plots were sown continuously within trial rows and mechanically delimited to fixed plot lengths at the beginning of the growing season using a rotary harrow.

Except for the *BL* trial in 2015 (Figure 2 a), which was laid out in two replicates, all trials comprised four replicates. The included treatments differed between years and between sections (Figure 1; Table 1).

The *HyLi_N* trial was designed as modified split-plot trial with 'N level' on the main plot and 'genotype' on the subplot. In all years, the trial *HyLi_N* comprised the N fertilization levels N1 with in total 100 kg N ha⁻¹ applied, N2 with in total 160 kg N ha⁻¹ applied and N3 with in total 220 kg N ha⁻¹ applied. In 2014, a none-fertilized N level (N0) was included, however neglected for the analysis. The trial comprised 20 cultivars in 2014 and 24 cultivars in both 2015 and 2016. While GY, GNC and GNup were determined for all cultivars, only a selection of 10, 13 and 13 cultivars in 2014, 2015 and 2016, respectively, was studied in detail including destructive plant sampling. Thus, only these cultivars were included in section I, but all cultivars in section VI. For section I, N1 was missing in 2015.

The *Cv_ag* trial was designed in a split-split plot design with the agronomic conditions 'sowing date 1 at normal fungicide' (SD1), 'reduced fungicide at normal sowing date' (RF) and 'normal fungicide at normal sowing date' (Cont; control) on the main plot, N fertilization (N) on the sub-plot and cultivar (Cv) on the sub-sub-plot.

The *IN* trial was designed as split-plot experiment with 'cultivar' or 'growth regulator' on the main plot, and 'N fertilization' on the sub-plot. The trial represents a long-term experiment, aiming at evaluating the response to eight incremental N fertilization levels ranging from zero to 420 kg N ha⁻¹, as well as the effects of over-fertilization. It comprised one to three cultivars in the different years and two levels of growth regulator for one cultivar in 2014, respectively.

The *BL* trial was designed as alpha lattice experiment in 2015 and as row-column experiment in 2016 and 2017. Originating from a joint project with the Bavarian State Research Center for Agriculture (Landesanstalt für Landwirtschaft; LfL), the *BL* trial comprised 400 double haploid breeding lines in 2015. The trial was successively reduced to in total 101 and 36 breeding lines in 2016 and 2017, respectively. Among these lines, a random selection of 75, 75 and 32 genotypes was considered for destructive analysis in 2015, 2016 and 2017, respectively. The first two years comprised the same sampled genotypes and the third year a subset of them. Additionally, it included three common registered cultivars, JB Asano, Julius and Elixer, in all years. In 2015 and 2016, the trial was fertilized with 200 kg N ha⁻¹ and in 2017 with 130 kg N ha⁻¹, accounting for high soil N values that year.

The pre-crop for the *HyLi_N* and *BL* trials was winter wheat in 2015 and in 2016, and silage maize for the *HyLi_N* trial in 2014. For the 2017 trials, the pre-crop was mulched grass-clover. The effective soil N supply was derived from the non-N-fertilized plots (N0) in the *IN* trial, which was conducted next to the other trials in 2014–2017. Due to differences in the pre-crop and the mineralization and weather conditions, GY and GNup in N0 plots differed substantially. GY amounted on average to 2.4, 1.5, 3.7 and 7.0 t ha⁻¹ and GNup to 27, 20, 44 and 111 kg N ha⁻¹ from 2014 to 2017 (Table 1).

Table 1: Technical description for the main field experiments Cv_ag, BL and HyLi_N (Figure 1). Dates are indicated as month/day. Additionally, grain yield (GY) and grain N uptake (GNup) in non-fertilized plots in the IN trial (NO, n = 8) conducted next to the other trials are listed. Nfert I, II and III refer to the three N dressings. Coordinates are indicated for the approximate center position of the trials. Data for the IN trial are indicated in section VII. D1, D2 and D4 refer to field names. AN: Ammonium nitrate; ASN: Ammonium sulfate nitrate; CCC: Chloromequat chloride; S: Section; IN: incremental N

Field trial	2014			2015			2016			2017		
	HyLi_N	BL	HyLi_N	BL	HyLi_N	BL	HyLi_N	BL	HyLi_N	BL	Cv_ag	
Location	D2: 48.4049 N/ 11.6970 E	D1: 48.4071 N/ 11.6937 E	D1: 48.4066 N/ 11.6965 E	D4: 48.4026 N/ 11.7022 E	D4: 48.4038 N/ 11.7036 E	D1: 48.4063 N/ 11.6927 E	D1: 48.4063 N/ 11.6927 E	D1: 48.4063 N/ 11.6927 E	D1: 48.4063 N/ 11.6927 E	D1: 48.4063 N/ 11.6927 E	D1: 48.4069 N/ 11.6945 E	
Genotype number included by section (n)	S I: 10	S III, S IV: 78	S I: 13; S VI: 24	S III, S IV: 78	S I: 13; S VI: 24	S III, S IV: 35	S III, S IV: 35	S III, S IV: 35	S I: 13; S VI: 24	S III, S IV: 35	S II: 6; S VI: 20	
Pre-crop	Silage maize	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Winter wheat	Grass-clover	Grass-clover	
Plot length [m]	11.75	6.5	11.25	6.5	9.25	6.5	9.25	6.5	9.25	6.5	6.4	
Replicates (n)	4	2	4	4	4	4	4	4	4	4	4	
Sowing date	10/22	11/04	11/04	10/13	10/13	10/24	10/13	10/24	10/13	10/24	09/28 + 10/24	
Sowing density [kernels m ⁻²]	350	350	350	350	350	350	350	350	350	350	350	
Leaf fungicide [date; l ha ⁻¹]	03/31: Juwel Top ³ 1.0	05/12: Opus Top ¹ 1.0 + Pronto Plus ² 1.0	05/12: Opus Top ¹ 1.0 + Pronto Plus ² 1.0	04/22: Aviator Xpro ² 0.80 + Fandang ^o 0.8	04/22: Aviator Xpro ² 0.80 + Fandang ^o 0.8	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	05/18: Input Xpro ² 0.5 + SkywayXpro ² 0.7	
Growth regulator [date; l ha ⁻¹]	05/06: InputXpro ² 1.5	05/27: Amistar Opti ⁴ 1.5 + Input Classic ² 0.8	05/27: Amistar Opti ⁴ 1.5 + Input Classic ² 0.8	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	06/07: Input Classic ² 1.25	05/30: Ceriax ¹ 2	
N fert I	03/31: CCC ¹ 1.0	04/21: CCC ² 0.5	04/21: CCC ² 0.8	04/04: CCC ² 0.6	04/04: CCC ² 0.6	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	04/11: CCC ² 0.7; 05/18: Moddus ⁴ 0.3	
N fert II	02/27: Carbamide ⁵	03/19: AN	03/19: AN	03/22: ASN	03/22: ASN	03/27: ASN	03/27: ASN	03/27: ASN	03/22: ASN	03/27: ASN	03/27: AN	
N fert III	04/15: AN	05/11: AN	05/11: AN	04/29: AN	04/29: AN	05/18: AN	05/18: AN	05/18: AN	04/29: AN	05/18: AN	05/18: AN	
N fert I	05/20: AN	06/11: AN	06/11: AN	05/23: AN	05/23: AN	06/08: AN	06/08: AN	06/08: AN	05/23: AN	06/08: AN	06/08: AN	
N fert II	30/60/90	80	30/60/90	80	30/60/90	50	30/60/90	50	30/60/90	50	30/60	
N fert III	30/60/90	60	30/60/90	70	30/60/90	30	30/60/90	30	30/60/90	30	30/60	
GY NO (IN) [t ha ⁻¹]	2.4	1.5	1.5	3.7	3.7	7.0	3.7	7.0	3.7	7.0	7.0	
GNup NO (IN) [kg ha ⁻¹]	27	20	20	44	44	111	44	111	44	111	111	
Sampling dates anthesis [days in June]	n.a.	11–16	9–18	2–18	2–18	5–19	6–18	5–19	6–18	5–19	3–14	

¹BASF, Ludwigshafen, D; ²Nufarm, Purr Ridge, IL, USA; ³Syngenta, Basel, CH; ⁴SKW Piesteritz, Piesteritz, D

⁵Three values for HyLi_N and two values for Cv_ag correspond to the different N levels, respectively

According to local best practice, the N fertilizer was supplied in three N doses at the beginning of growth in spring, at early stem elongation and at booting-flowering, respectively, in all trials and years except for *Cv_ag* in 2017, when the last application was skipped because of strong soil N supply (Table 1). N fertilizer was predominantly applied as combined ammonium nitrate granulate (AN; 50% ammonium-N and 50% nitrate-N). In some years, ammonium sulfate nitrate (ASN; Table 1) granulate was used instead for the first dressing for additional sulfur supply. To avoid lodging, predominantly chlormequat-based growth regulator was applied in all years. In all years, seeds were fungicide-dressed to prevent seed-borne diseases. With the exception of RF in the *Cv_ag* trial, leaf fungicide was sprayed two to four times, using different commercial products based on azoles, carbamates and strobilurins as listed in Table 1. Sowing dates were mostly mid of October until beginning of November, corresponding to local practice. The *Cv_ag* trial additionally included an early sowing date at end of September (SD1; Table 1).

3.2 Weather conditions

2014 and 2017 exhibited good growing conditions at the start of the vegetation in March with a higher temperature sum than in the other years (Figure 3).

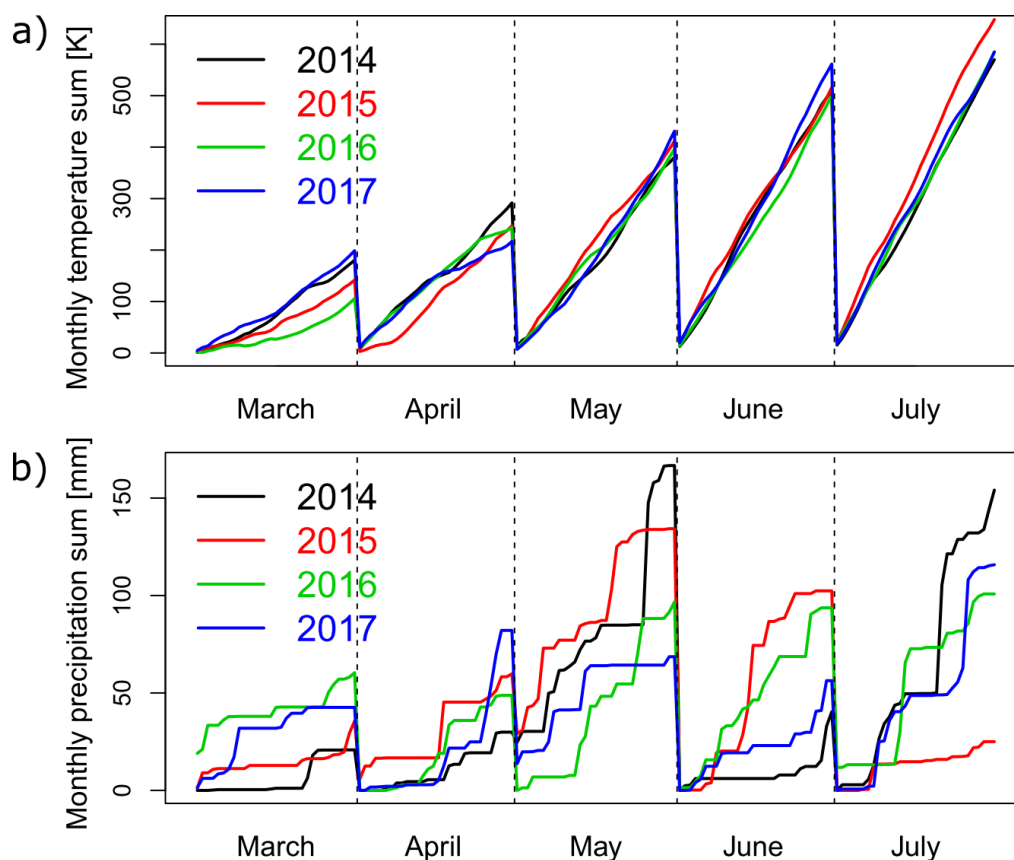


Figure 3: Weather conditions in the four main experimental years: Monthly temperature sum of temperatures > 0°C (a) and cumulative monthly precipitation (b) during the principle months of the four growing seasons from March to July (data source: Bayerische Landesanstalt für Landwirtschaft, 2018).

April was relatively cold in the first half in 2015. Temperature sums in May and June were highest in 2017, whereas July temperatures were clearly highest in 2015 and similar in the other years. In all years, precipitation was sufficient during the vegetative phase until end of May. Low precipitation during June in 2014 was compensated by above-average precipitation in May. Strong precipitation in May together with unfavorable, wet soil conditions during sowing and pre-winter development resulted into visible stagnant moisture effects on some plots in 2015. In contrast, due to the presumably limited root growth, low precipitation and high temperatures, heat and drought stress became apparent during the later grain filling phase in July in 2015. In all years, anthesis took predominantly place in the first half of June, but differed in length depending on the weather conditions (Table 1).

3.3 Plant sampling, sample analysis and calculation of plant traits

In all trials, GY was determined after physiological maturity by harvesting the whole plots by a combine harvester, predominantly in the first week of August. Dry matter content was determined based on oven-dried subsamples in 2014–2016 and by a built-in capacitive moisture sensor in 2017.

Destructive plant sampling was conducted in the trials *HyLi_N*, *BL* and *Cv_ag* in a similar manner at anthesis (Zadok's growth stage 65; Zadoks et al., 1974) and at physiological maturity (Zadok's growth stage 92). Growth stages were regularly recorded for each genotype to meet the accurate sampling date, particularly at anthesis. For each sampling, a fixed number of spiked shoots, which were randomly selected across each experimental plot, were cut directly above the ground. In order to increase the precision in the determination of the spike density, mostly more stems were sampled at maturity than at anthesis. The number of stems was 30, 20 and 30 at anthesis and 30, 30 and 60 at maturity in 2014, 2015 and 2016, respectively, for the *HyLi_N* trial; 20, 30 and 30 at anthesis and 30, 50 and 50 at maturity in 2015, 2016 and 2017, respectively, in the *BL* trial as well as 30 at anthesis and 60 at maturity in the *Cv_ag* trial in 2017. No differentiation between main shoots and primary tillers was made.

Within the following approximately three days, the plant samples were manually dissected into leaf blades, culms¹ including leaf sheaths, as well as spikes. In 2016, only the samples of a random selection of 34 genotypes were separated, whilst the remaining samples were analyzed as complete aboveground samples at anthesis and were separated into kernels opposed to vegetative biomass at maturity. For the *Cv_ag* trial, the leaf samples were additionally separated into flag leaves (uppermost leaf layer), flag leaves-1 (second leaf layer from above) and the remaining, lower leaves (Figure 4). In 2015, additional sampling was conducted at milk ripeness in the *BL* trial by cutting 20 shoots per plot. At maturity, the spikes were additionally threshed to separate kernels from chaff, using a laboratory

¹ used synonymously to 'stems'

thresher. Plant samples were oven-dried at 50°C until a stationary weight was reached for subsequent determination of dry matter (DM) weight using a balance (d = 0.01 g). DM was standardized to 0% moisture content.



Figure 4: Separation of plant samples into plant organs spikes, culms and three leaf layers in the Cv_ag trial in 2017.

Subsequently, the samples were mechanically ground using rotary mills. N concentration (NC) was determined by mass spectrometry using an Isotope Radio Mass Spectrometer with an ANCA SL 20-20 preparation unit (Europe Scientific, Crewe, UK) in 2014, and by means of near-infrared spectroscopy (NIRS) using a FOSS NIRS 6500 (NIR System, Silver Spring, Md.) and an FT-NIRS (Bruker, MPA, Germany) in the other years. A subset of the samples was additionally analyzed by Mass Spectrometry to develop and validate PLSR-models, which were optimized for each plant organ by sampling date and incrementally improved over the three years. For NIRS analysis, vegetative plant samples were homogeneously ground using a 1 mm sieve, whilst grains were analyzed as complete kernels. For each plot, spike density per m² (SD) was calculated by dividing GY per m² determined by combine harvester by the average GY per spike. For all organs, DM per unit ground area was calculated by multiplying the DM per sampled shoot by SD. N uptake (Nup) was calculated by multiplying DM with NC. In addition to these traits, traits referred to as ‘direct DM traits’, ‘direct NC traits’, ‘direct Nup traits’, ‘derived DM traits’ and ‘derived N traits’ were calculated as follows (Prey et al., 2019c).

The absolute amount of pre-anthesis accumulated assimilates translocated from vegetative plant organs into grains between anthesis and maturity in kg ha⁻¹ (Dry matter translocation, DMT; Papakosta and Gagianas, 1991):

$$DMT = DM(\text{spikes} + \text{stems} + \text{leaves})_{\text{anthesis}} - DM(\text{chaff} + \text{stems} + \text{leaves})_{\text{maturity}}$$

The relative amount of translocated pre-anthesis accumulated assimilates into grains (Papakosta and Gagianas, 1991): DMT-efficiency (DMTEff):

$$DMTEff = DMT/DM_{\text{anthesis}}$$

Post-anthesis assimilation (PAA): $PAA = DM_{\text{maturity}} - DM_{\text{anthesis}}$

The contribution of pre-anthesis assimilation to grain filling (CPreAA):

$$CPreAA = DMT/DM(\text{grain})_{\text{maturity}}$$

The ratio of grain DM to total DM at maturity (Harvest index, HI):

$$HI = DM_{\text{grain}}/DM_{\text{total}}$$

The absolute amount of pre-anthesis accumulated nitrogen translocated from vegetative plant organs into grains between anthesis and maturity in kg ha^{-1} (N translocation NT; Cox et al., 1985):

$$NT = Nup(spikes + stems + leaves)_{anthesis} - Nup(chaff + stems + leaves)_{maturity}$$

Accordingly, 'partial' NT was calculated for spikes, stems and leaves.

The relative amount of translocated pre-anthesis accumulated nitrogen into grains (Cox et al., 1985) (NT-efficiency, NTEff):

$$NTEff = NT / Nup_{anthesis}$$

Accordingly, 'partial' NTEff was calculated for spikes, stems and leaves.

Post-anthesis nitrogen uptake (PANup):

$$PANup = Nup_{maturity} - Nup_{anthesis}$$

The contribution of pre-anthesis nitrogen to total nitrogen uptake (CPreNup):

$$CPreNup = Nup_{anthesis} / Nup_{maturity}$$

The ratio of grain nitrogen uptake (Nup) to total Nup at maturity (N Harvest index, NHI):

$$NHI = Nup_{grain} / Nup_{total}$$

Apparent nitrogen uptake efficiency, calculated for anthesis and maturity as the ratio of total Nup to total N fertilized (Moll et al., 1982):

$$NupEff = Nup_{total} / N_{fertilized}$$

The efficiency of the internal conversion of N into total DM (Nitrogen utilization efficiency, NutEff_{total}) and grain DM (NutEff_{grain}; Moll et al., 1982), where NutEff_{total} was calculated for anthesis and maturity:

$$NutEff_{grain} = DM_{grain} / Nup_{total}$$

$$NutEff_{total} = DM_{total} / Nup_{total}$$

The efficiency of the conversion of the fertilized N into total DM (Nitrogen use efficiency, NUE_{total}) and grain DM (NUE_{grain}; Moll et al., 1982), where NUE_{total} was calculated for anthesis and maturity:

$$NUE_{grain} = DM_{grain} / N_{fertilized}$$

$$NUE_{total} = DM_{total} / N_{fertilized}$$

The soil N component was neglected for the calculation of NupEff and NUE.

For *Cv_ag* (section II), several traits were additionally calculated, including the partitioning of DM and Nup to the vegetative organs, referred to as partial HIs and partial NHIs, respectively, as well as organ-level DMT and DMTEff.

3.4 Spectral measurements

Spectral canopy measurements were conducted using the mobile sensor platform *Phenotrac 4* (Figure 5), developed and optimized by the Chair of Plant Nutrition since 1998. It is equipped with various plant sensors, including three active multispectral sensors, thermal sensors, an ultrasonic and a laser distance sensor, as well as a hyperspectral spectrometer (tec5, Oberursel, D). Spectral data used in this work were captured using the hyperspectral sensor in the range of 300–1000 nm. It is a passive,

bidirectional MMS1 silicon diode array spectrometer (Zeiss, Oberkochen, D), capturing simultaneously the incident downwelling radiation by a cosine-corrected diffuser and the backscattered upwelling radiation at a nominal resolution of 3.3 nm.



Figure 5: Mobile sensor platform Phenotrac 4 (a) and sensor carrier arm attached to the front of the sensor platform (b). The aperture of the hyperspectral sensor is positioned on the right side and connected with the actual sensor (silvery box in the left image) via optical fibers.

Reflectance was calculated as the upwelling radiation divided by the downwelling radiation, multiplied by the calibration factor, which was determined by calibrating using a gray reflectance standard prior to each measurement campaign. Measurements were registered at a frequency of 5 Hz along with the global positioning system (GPS) coordinates using a TRIMBLE RTK-GPS (real-time kinematic global positioning system; Trimble, Sunnyvale, CA, USA). The sensor was positioned approximately 80 cm above the top of the canopy. The sensor's one-sided circular field of view (FOV) was 12° , resulting in a scanned strip at the top of the canopy of approximately 30–35 cm in width in the middle of the plot. Plot face sides were excluded to minimize edge effects. The measurement speed of about 1.4 m s^{-1} allowed for a continuous sampling of the plots in longitudinal direction with typically 15–25 spectra per plot. Measurements were conducted at solar noon \pm approximately 2.5 hours in order to reduce the impact of the sun position. A similar bidirectional hand-held hyperspectral sensor of comparable spectral resolution (Handyspec, tec5, Oberursel, D) was used instead on some dates in the *IN* trial (see Section VII). Spectral measurements were conducted during several growth stages starting from leaf development to dough ripeness in the *HyLi_N*, *BL* and *Cv_ag* trials with a focus on the grain filling stages, and during vegetative growth stages in *IN*. Measurements included five dates in *HyLi_N* in 2015, four dates in *BL* in 2015, 14 dates in *HyLi_N* in 2016, 12 dates in *BL* in 2016, 12 dates in *Cv_ag* in 2017 and 10 dates in *BL* in 2017. Due to poor results on May 11 (Section VI), this date was not further included in Section V. Less measurement dates per year were included for the *IN* trial (see Section VII).

3.5 Data analysis

Spectral data was pre-processed for correcting for a spatial shift between the GPS and the sensor position and for incorporating the calibration information, using custom-made LabView-Applications (National Instruments, Austin, Tex., USA). Subsequently, the data was extracted on a plot basis using

polygons drawn for the plot target area in ArcView3.3 (ESRI, 2002, Redlands, CA, USA). Thereafter, one average reflectance spectrum was calculated per plot and used for further analysis.

Data was analyzed using the *R* statistical software (R 3.4.2; RCoreTeam, 2017). For DM and N data, analysis of variance was conducted for analyzing the treatment effects (Sections I–III), followed by group comparison by post hoc test, using Tukey's honest significance test in Sections I and II ($p < 0.05$). Normal distribution of the residuals was visually inspected. Broad-sense heritability (H^2) was calculated for expressing the ratio of genotype-related trait variation to the total variation for the breeding lines' DM and N traits (Section III) and SVIs (Section IV).

Three sections (IV, V and VII) contain the comparison of SVIs, which were selected based on previous publications. Prior to index calculation, the spectra were smoothed using a 5-band moving average filter to remove spectral noise (Mistele and Schmidhalter, 2010). Figure 6 depicts the position of the spectral bands included for the SVI calculation in sections IV and V for exemplary reflectance data, recorded during milk ripeness in 2017 in the *BL* trial.

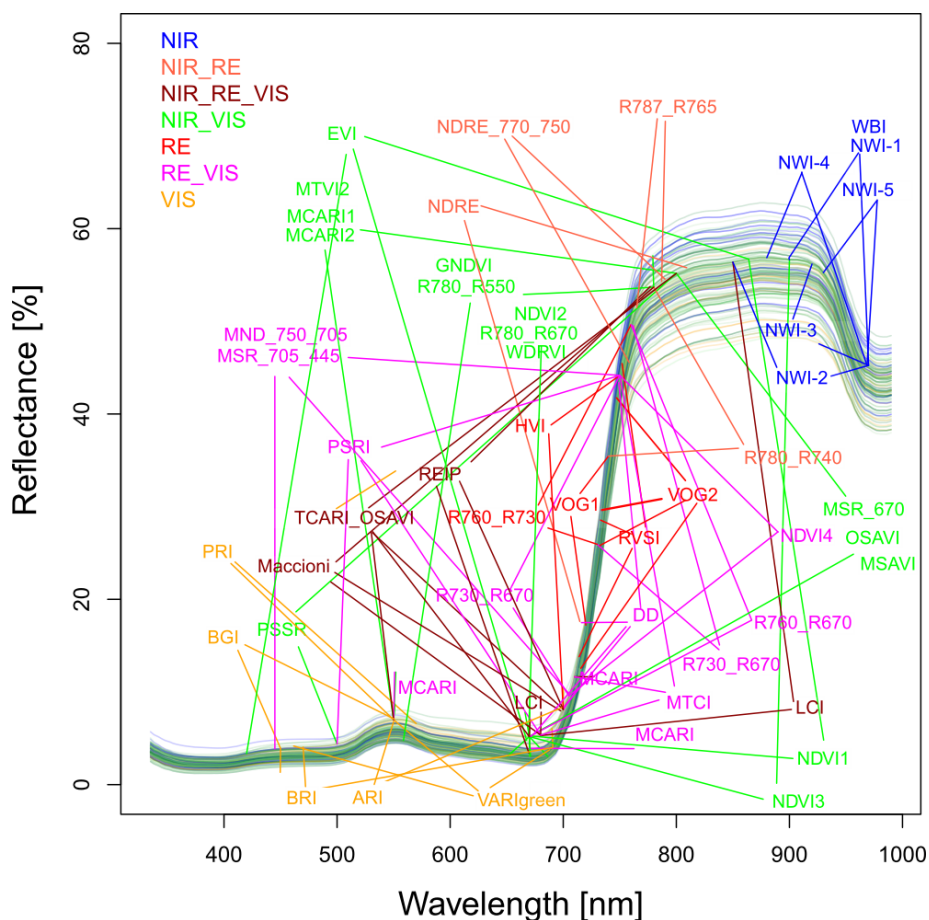


Figure 6: Spectra acquired on 21 June 2017 with band position of vegetation indices, colored by spectral regions: Near-infrared (NIR: > 765 nm), visible (VIS: < 700 nm) and extended red edge (RE: 700–765 nm). The spectra are colored by grain yield from yellow (lowest yield) to green (medium yield) and blue (highest yield). Figure from Prey et al., 2019b: Supplementary Figure 1.

The indices were grouped by the included spectral ranges (visible light *VIS*, the extended red edge *RE* and near-infrared *NIR*), with the *VIS* < 700 nm, *RE*: 700–765 nm and the *NIR* > 765 nm (Figure 6). In section *VII*, only a selection of these indices was considered, including the indices *R780_R550* ('*NIR/green*'), *R780_R670* ('*NIR/red*'), *R760_R730*, *R780_R740*, *WBI* ('*R900_R970*') and the *REIP*.

The indices were predominantly compared for their ability to assess the plant traits via simple linear regression models using the *lm*-function in *R*. The linear fits were compared by coefficients of determination (R^2), and root mean squared error (*RMSE*) between reference values and fitted values in the trait~index relationships. In addition, quadratic relationships were tested in section *VII* in order to account for obvious saturation issues of some indices. In addition, *PLSR* models were calibrated in each year and validated in the other years in section *IV*, using the *pls*-package in *R*. Spectra were pretreated by *Savitzki-Golay* first order derivation.

In section *VI*, a contour map analysis was conducted for testing all possible pairwise band combinations combined for calculating normalized difference vegetation indices (*ND*) in the equation of the *NDVI* as. The *ND*-indices were analyzed by *Pearson's* correlation analysis and compared by their coefficients of determination. In addition to the hyperspectral data, multispectral sensor data were simulated via resampling the hyperspectral data by the sensor response curves of multispectral satellite sensors, including the sensors of the *Landsat-8*, *Quickbird*, *RapidEye*, *Worldview-2* and the *Sentinel-2* satellites, using the *hsdar*-package in *R*.

Refer to the methods chapter in each section for specific methods descriptions.

4 Manuscript overview

This thesis includes seven sections in the format of articles. This paragraph reports the publication information, author contributions and brief summaries.

4.1 Section I: Nitrogen use efficiency and carbon traits of high-yielding European hybrid vs. line winter wheat cultivars: Potentials and limitations

Prey, L., Kipp, S., Hu, Y., Schmidhalter, U., 2019. Nitrogen Use Efficiency and Carbon Traits of High-Yielding European Hybrid vs. Line Winter Wheat Cultivars: Potentials and Limitations. *Frontiers in Plant Science* 9, 1988. DOI:10.3389/fpls.2018.01988.

URL: www.frontiersin.org/article/10.3389/fpls.2018.01988/full

Author contributions

SK, YH, LP and US conceived and designed the experiments. LP and SK performed the experiments. LP analyzed the data. LP and US wrote the paper.

Summary

In contrast to allogamous crops like rye and maize, for which hybrid cultivars are well established, hybrid wheat has only recently been fostered by breeding companies in Europe. Hybrid wheat is considered promising to ease yield depressions and fluctuations caused by weather extremes and associated plant stress, due to its reportedly enhanced stress resistance, e.g. under drought conditions. While reported heterosis for NUE was mainly based on comparisons with the hybrids' parental lines, NUE traits were rarely systematically compared to high-performance line cultivars. In addition, higher heterosis was reported in drought-stressed than in control treatments, but the effect of the N fertilization level is still unclear. However, the hybrids' reportedly sturdier root growth might also contribute to better N acquisition.

In this study, four high-yielding European hybrid and nine line winter wheat cultivars were grown in two to three N regimes and compared over three years at anthesis and maturity for 53 DM and N traits. Commercial heterosis (CH) was calculated as the relative average performance of the group of hybrids compared to those of the line cultivars.

Commercial heterosis was positive for only about one third of the 53 direct and indirect N and DM traits. Higher GY of the hybrids (CH +5.5 %) went along with consistently lower GNC (−8.5 %). Therefore, no advantage in GNup was observed in the eight year x N level combinations. Among contributing yield-related DM traits, the slightly higher yield was ascribed to a higher harvest index (+3.5 %) rather than to differences in total DM. Hybrids were characterized by higher post-anthesis assimilation (+16 %) at lower DM translocation (−10.7%), more grains per spike (+11.5%) as well as higher N utilization efficiency regarding total DM (+4.3%). Most values of further N traits did not differ significantly ($p < 0.05$) between the cultivar groups in most test cases or were lower for hybrids. Notably, N translocation and the temporal Nup of the different plant organs was comparable. In addition, no consistent absolute advantage was observed regarding N acquisition in lower N fertilization levels.

The estimated GY heterosis does not exceed findings from several previous studies. It is concluded that hitherto, attempts to boost hybrid cultivars did not outpace the progress in line breeding. Given the variation and degree in GY heterosis under such high-yielding, relatively low-stress conditions, it currently appears to be too low to compensate for higher seed costs. However, the hybrids' competitive advantage will also depend on the agronomic management regarding sowing density, target grain quality and its payment, available reference line cultivars, and fertilization strategies. Therefore, comparing the cultivar groups in further environments and considering more genotypes will be necessary. Moreover, hybrid breeding benefits from increased investments in recent years and is likely to show higher effects in less favorable growing regions.

4.2 Section II: Temporal and organ-specific responses in NUE traits to N fertilization, fungicide intensity and early sowing in winter wheat cultivars

Prey, L., Germer, M., Schmidhalter, U., 2019. Temporal and Organ-specific Responses in NUE Traits to N Fertilization, Fungicide Intensity and Early Sowing in Winter Wheat Cultivars. *Agronomy* 9, 313. DOI:10.3390/agronomy9060313. URL: www.mdpi.com/2073-4395/9/6/313

Author contributions

LP and US conceived and designed the experiment. LP and MG conducted the experiment. LP analyzed the data. LP and US wrote the manuscript.

Summary

Besides cultivar selection and optimized N fertilization, fungicide intensity and sowing time influence the N use efficiency (NUE) of winter wheat, but often interact mutually. While agronomic trials focus on GY effects, the underlying mechanisms, interactions of plant traits and the temporal effects are not sufficiently understood. Therefore, six winter wheat cultivars, which were included also in the *HyLi_N* trial, were tested in 2017 in their plant-level and organ-specific responses to these agronomic treatment factors.

Compared to the control treatment, across cultivars, the treatment without leaf fungicide mostly exerted stronger and inverse effects than early sowing, on GY (−12% without fungicide; +8% n.s. for early sowing), GNup (−9% n.s.; +5% n.s.), as well as on GNC (+4%, $p < 0.05$; −2% n.s.). The effect of not applying fungicides on GY was associated with lower values in harvest index, thousand kernel weight, NUE and NutEff, but similar total DM. The by trend decreased GNup was related to similar vegetative Nup but lower values in NTEff and NHI. The rather weak fungicide effects were associated with low pathogen pressure due to the favorable weather and the pre-crop grass-clover. Thus, one application would likely have been sufficient for most cultivars. In contrast, early sowing increased GNS and total NUE, and tended to increase total DM at anthesis and maturity, as well as PAA, however at similar HI. Total Nup was increased after the winter season, but similar at anthesis. Few fungicide \times N interactions were significant ($p < 0.05$) and the sowing date neither interacted with N fertilization for any of the N and DM traits, albeit the relative N response in many traits tended to be lower without fungicide.

The results demonstrate the positive effects of fungicides and earlier sowing on various traits but indicate differing causal effects. However, interactions of fungicide intensity and sowing date can be relevant as well and aspects of management and weather conditions must be considered in practice. Still, earlier sowing should be considered for curtailing over-winter N losses and for possible beneficial effects both on GY and GNup. The missing interactions between sowing date and N level suggest that no specific fertilization timing would be necessary.

4.3 Section III: Temporal dynamics and the contribution of plant organs in a phenotypically diverse population of high-yielding winter wheat: Evaluating concepts for disentangling yield formation and nitrogen use efficiency

Prey, L., Hu, Y., Schmidhalter, U., 2019. Temporal dynamics and the contribution of plant organs in a phenotypically diverse population of high-yielding winter wheat: Evaluating concepts for disentangling yield formation and nitrogen use efficiency. *Frontiers in Plant Science* 10, 1295.

DOI: 10.3389/fpls.2019.0129. URL: www.frontiersin.org/articles/10.3389/fpls.2019.01295/full.

Author contributions

YH, LP and US conceived and designed the experiments. LP performed the experiments. LP analyzed the data. LP and US wrote the paper.

Summary

This section evaluates trait-based strategies for exploiting the variation in breeding lines. Notably, the understanding of beneficial strategies concerning the temporal dynamics of NUE and GY formation and the role of plant organs is still scarce. Therefore, forty-six NUE and yield formation traits were assessed in a population of 75 breeding lines over three years from 2015 to 2017 in terms of DM, NC and Nup at anthesis and maturity, both at the aboveground-plant and plant organ levels. Several multiplicative and additive trait complexes were evaluated.

Significant ($p < 0.05$) genotype effects were observed for all traits. DMT contributed substantially to GY by 31–44%, but GY variation was more related to PAA. At maturity, total aboveground DM as opposed to the HI predominantly determined GY. NUE_{grain} was better described by NupEff than by NutEff_{grain}. Similarly, for maximizing total DM, NupEff was more indicative than NutEff_{total}. Due to only moderate correlations from grain number per m², the use of yield components was confirmed to be not promising for the selection for GY. GNup was greatly influenced by variation in total Nup, but not in that of the NHI, and by that in GY rather than by that in GNC. Post-anthesis Nup was low in comparison to NT and strongly differed by year. However, PANup was always correlated with GNup, suggesting considering also associated stay-green characteristics under temperate growing conditions. Among anthesis traits, both total DM and Nup correlated moderately with GY and with GNup but were not consistently exceeded for explaining GY by organ-level DM and Nup traits, neither by anthesis NC traits. The only weak negative correlation between GNC and GY was accompanied by substantial variation in GNup. While the selection for GY would mostly also select genotypes superior in GNup, few stable correlations were found between GNC and directly determinable traits.

It is concluded that under the given favorable post-anthesis conditions, the plant anthesis status is not sufficient for predicting final GY and GNup, whereas stay-green traits should be considered as well due to the dominant role of the grain filling phase.

4.4 Section IV: High-throughput field phenotyping traits of grain yield formation and nitrogen use efficiency: Optimizing the selection of vegetation indices and growth stages

Prey, L., Hu, Y., Schmidhalter, U., 2020. High-Throughput Field Phenotyping Traits of Grain Yield Formation and Nitrogen Use Efficiency: Optimizing the Selection of Vegetation Indices and Growth Stages. *Frontiers in Plant Science* 10, 1672. DOI: 10.3389/fpls.2019.01672. URL: <https://www.frontiersin.org/articles/10.3389/fpls.2019.01672/full>.

Author contributions

YH, LP and US conceived and designed the experiments. LP performed the experiments. LP analyzed the data. LP and US wrote the paper.

Summary

This section evaluates the application of SVIs for the assessment of the DM and N traits reported in section III. From 2015 to 2017, hyperspectral canopy measurements were acquired on overall 26 measurement dates during vegetative and reproductive growth and 48 vegetation indices from the VIS, RE and NIR spectrum were tested in linear regression analysis for assessing the influence of measurement stage and index selection on the detectability of the traits. The indices were quantitatively ranked for each trait by 'weighted mean/maximum rank sums' (WMMRS), considering the seasonal R^2 -values achieved.

For most plant traits including GY and GNup, the milk ripeness stage was most reliable under conditions of moderate terminal heat/drought or pathogen stress, whereas the relationships were more stable during dough ripeness in the year with favorable maturation conditions (2017). In contrast, phenological shifts appeared to impact the relationships at heading/anthesis. For GY (maximum $R^2 = 0.26$ – 0.51 , $p < 0.001$, in the three years) and most DM traits, indices exploiting the water absorption band at 970 nm, such as the WBI and the NWI-2, provided better relationships than VIS indices and NIR/VIS indices such as the NDVI. In addition, most indices with RE bands, notably NIR/RE combinations, ranked above the NIR/VIS group. Due to index saturation, the index differentiation was most apparent in the highest-yielding year, 2017. For GNup and total Nup, the MSR_705_445 and the simple-ratio R780_R740 ranked highest, followed by various other indices with RE bands. Among the vegetative organs, R^2 -values were mostly highest and lowest for leaf and spike traits, respectively. Moderate, yet year-dependent, relationships were obtained from RE indices for the N harvest index, N translocation and its efficiency.

The estimation of GY and GNup was explained by the detection of total DM and Nup, respectively, rather than by that of the relative allocation (harvest index). With GNup being closely related to GY but not to GNC, GNC was not reliably estimated. DM and N traits related to maturity canopy status were overall better detected than anthesis traits.

Trait-specific best indices, the NDVI and the REIP were calibrated in comparison to PLSR models from each year's milk ripeness data and validated on the data of the other years. Though PLSR improved the prediction errors of various traits, it showed limited advantage in the R^2 -values over the best indices, yet over the NDVI.

The results suggest the use of sensor-based phenotyping as an effective support-tool for screening of yield potential and NUE and for identifying contributing plant traits—which, due to their expensive and cumbersome destructive determination are otherwise not readily available—both from an organ-level and temporal perspective.

Depending on the sensor, water bands and RE bands should be preferred over NIR/VIS indices for DM traits and N-related traits, respectively, and milk ripeness is suggested as the most reliable stage.

4.5 Section V: Temporal and spectral optimization of vegetation indices for estimating grain N uptake and late-seasonal nitrogen traits in wheat

Prey, L., Schmidhalter, U., 2019. Temporal and Spectral Optimization of Vegetation Indices for Estimating Grain Nitrogen Uptake and Late-Seasonal Nitrogen Traits in Wheat. *Sensors* 19, 4640. DOI: 10.3390/s19214640. URL: www.mdpi.com/1424-8220/19/21/4640.

Author Contributions

LP and US conceived and designed the experiment, LP conducted the experiment, LP analyzed the data, LP and US wrote the manuscript.

Summary

This section extends the methods, which were applied in section *IV* to breeding lines, in an analogous way to the N traits of the Cv_ag trial as reported in section *II*.

The 48 previously described (section *IV*) SVIs were evaluated on 10 measurement days for the estimation of 34 N traits in four data subsets, which represented the variation generated by six high-yielding cultivars, two N fertilization levels, two sowing dates and two fungicide intensities.

Close linear relationships were found for GNup both in response to the cultivar-related variation ($R^2 = 0.52$, $p < 0.001$) and to the other agronomic treatments ($R^2 = 0.53$ – 0.67 , $p < 0.001$), particularly during milk ripeness. Especially NIR/RE indices, such as the NDRE_770_750, outperformed NIR/VIS indices. While index rankings and seasonal R^2 -values were similar for total Nup as for GNup, the NHI was moderately detected ($R^2 = 0.46$, $p < 0.001$) only during dough ripeness, primarily from indices sensitive to the contrasting senescence between different fungicide intensities. These indices, including the R787_R765 and the TCARI_OSAVI, also performed best for NTEff and some organ-level N traits at maturity. The blue/green index (BGI) was more suitable for leaf-level N traits at anthesis, even though GNC was best detected by the REIP. When SVIs were quantitatively ranked by data subsets, a better agreement between rankings was found for GNup, total Nup and GNC than for several contributing N traits. NT could be estimated similarly as the closely related Nup at anthesis. Although leaf information was better detected, the N status of other organs could be moderately estimated as well. However, the mechanisms of the organ-specific detection may be indirect and require further evaluation. Although the milk ripeness stage was generally most useful, moderate relationships already at stem elongation hold the advantage of seasonally earlier estimations, whereas the heading/anthesis phase should be avoided, probably owing to phenological shifts among cultivars.

4.6 Section VI: Simulation of satellite reflectance data using high-frequency ground based hyperspectral canopy measurements for in-season estimation of grain yield and grain nitrogen status in winter wheat

Prey, L. and Schmidhalter, U., 2019. Simulation of satellite reflectance data using high-frequency ground based hyperspectral canopy measurements for in-season estimation of grain yield and grain nitrogen status in winter wheat. *ISPRS Journal of Photogrammetry and Remote Sensing* 149, 176–187. DOI: 10.1016/j.isprsjprs.2019.01.023.

URL: <http://www.sciencedirect.com/science/article/pii/S0924271619300310>

Author contributions

LP and US conceived and designed the experiments, LP performed the experiments, LP analyzed the data, LP and US wrote the paper.

Summary

While proximal hyperspectral sensing offers the potential to detect specific plant traits, the potential of satellite remote sensing is still limited seen the degree of spatial and temporal resolution. Therefore, hyperspectral satellite data are still not readily available for most agricultural applications and ground truth validation remains scarce. In this section, multispectral satellite data were simulated through resampling ground-based hyperspectral reflection data (400 nm–1000 nm) sampled at 5-14 growth stages over the second and third growing seasons in the *HyLi_N* trial and in the *Cv_ag* trial. The study comprised 24 and 20 genotypes from the *HyLi_N* and *Cv_ag* trials, respectively, which varied in morphology and phenology and were grown at varying levels of N application and additionally of sowing time and fungicide intensity in the *Cv_ag* trial. Ground-based reflectance data were resampled to fit the spectral resolution of the multispectral sensors of the satellites Landsat-8, Quickbird, RapidEye, WorldView-2 and Sentinel-2. The resulting spectral bands were used for calculating all possible normalized difference vegetation indices, which were correlated to GY, GNup and GNC.

The index performance depended substantially on the trait, growth stage and year. For GY, maximum coefficients of determination (R^2) obtained from hyperspectral sensing over the season ranged from 0.65 in 2017 to 0.88 in 2015 compared to 0.40–0.79 for the best simulated multispectral sensor, Sentinel-2. In most cases, indices performed better for GNup and less well for GNC. Typically, the relationships decreased around heading before peaking at early to mid-grain filling. The index performance from multispectral compared to hyperspectral data decreased over time during grain filling. The sensor ranking remained consistent with Sentinel-2 followed by Worldview-2 and RapidEye outperforming the other simulated sensors.

Advantages are attributed to the RE band for N-related traits and the better coverage of the NIR range between 800–1000 nm by the Sentinel-2. The results can possibly be extrapolated to the

application of multispectral ground-based, UAV and satellite sensing by elucidating optimized measurement stages and optimizing the spectral sensor properties. An optimal multispectral sensor for the studied traits would dispose of a narrow band in the upper red edge and a NIR band similar to the Sentinel-2-NIR1 close to 780 nm. Two further NIR bands at about 930 and 980 nm, respectively, seem to be promising for assessing GY, whereas VIS bands were largely dispensable for the studied traits. Mostly no trade-off was found between the temporal stability and the R^2 -level. Limitations of multispectral sensors were identified, especially for the later grain filling phase, when band narrowness became more critical. Frequent measurements suggested (i) to avoid the heading/anthesis phase due to phenological shifts (ii) and in contrast to gain the most reliable relationships during the milk ripeness stage.

4.7 Section VIII: Sensitivity of vegetation indices for estimating vegetative N status in winter wheat

Prey, L. and Schmidhalter, U., 2019. Sensitivity of Vegetation Indices for Estimating Vegetative N Status in Winter Wheat. *Sensors* 19, 3712. DOI: 10.3390/s19173712.

URL: www.mdpi.com/1424-8220/19/17/3712

Author contributions

US conceived and designed the experiment, LP analyzed the data, LP and US wrote the manuscript.

Summary

This section evaluates the application of SVIs for assessing the pre-anthesis N status and aimed at a critical assessment of the estimation ability depending on the level of the target traits. It included data from seven years (*IN* trial), comprising overall nine measurement dates from early stem elongation until flowering in eight N regimes (0–420 kg N ha⁻¹) for selected SVIs.

Tested by regressions across dates and years, a pronounced date-specific clustering was found particularly for DM and NC. While only the R900_970 yielded moderate but saturated relationships ($R^2 = 0.47$, $p < 0.001$) for DM, and no index was useful for NC across dates, the nitrogen nutrition index (NNI) and Nup could be better differentiated (REIP: $R^2 = 0.59$, $p < 0.001$, for both traits). Tested across N levels within growth stages, the order of the detection of the traits was mostly Nup \approx NNI $>$ NC \approx DM. Depending on the characteristic and the number ($n = 1–3$) of cultivars included, the relationships improved when testing within instead of across cultivars, with relatively the least cultivar effect on the estimation of DM and the strongest on that of NC.

For assessing the trait estimation under conditions of high–excessive N fertilization, the range of the target traits was divided into two intervals with NNI values < 0.8 (interval 1: low N status) and with NNI values > 0.8 (interval 2: high N status). Although better estimations were found in interval 1, useful relationships were obtained in interval 2, as well as, from the best indices as averaged from the results of the nine dates (DM: R780_R740, average $R^2 = 0.35$, RMSE = 567 kg ha⁻¹; NC: REIP, average $R^2 = 0.40$, RMSE = 0.25%; NNI: REIP: average $R^2 = 0.46$, RMSE = 0.10; Nup: REIP, average $R^2 = 0.48$, RMSE = 21 kg N ha⁻¹). While in interval 1, all indices performed rather similarly, the three RE-based indices were relatively better suited for the three N-related traits in interval 2.

The results are promising for applying SVIs also under conditions of high N status, aiming at detecting and avoiding excessive N use. While in canopies of lower N status the use of simple NIR/VIS indices may be sufficient, RE information appears crucial for conditions of higher N status and in dense canopies. These findings could be transferred to the configuration and use of simpler multispectral sensors under conditions of contrasting N status in precision farming.

5 General discussion

The first part (chapters 5.1–5.3) of this discussion recapitulates and discusses the results observed for the DM and N traits in sections I–III. The second part (chapters 5.4–5.10) compares congruent and opposing findings in the domain of spectral phenotyping.

5.1 The use of hybrid cultivars

Given recent incentives of breeders to foster hybrid cultivars, section I (Prey et al., 2019c) compared the groups of contemporary hybrid and line cultivars in eight year \times N level conditions. Previous recommendations were predominantly based on the hybrids' advantage in GY. The present results showed a relative commercial GY heterosis of the hybrids over the lines by only 5.5%, which in addition was statistically significant ($p < 0.05$) only at the highest N level in 2016. The expected relative advantage of hybrids under low N-conditions due to their reportedly stronger root system (Becker, 2017) was only observable by tendency in 2014 and 2015, while the reverse observation in 2016 suggested instead an advantage under conditions of stronger pathogen pressure, possibly supported by the earlier flowering of some hybrids (section II, Prey et al., 2019a). In contrast, no significant cultivar-group \times fungicide interaction was observed in the *Cv_ag* trial under conditions of weak pathogen pressure (not shown). The by trend positive heterosis in GY was more ascribable to an enhanced 'internal' efficiency rather than to an advantage in DM and N accumulation. Thus, all significant differences between lines and hybrids in direct Nup traits and all but one in direct DM traits revealed lower values of the hybrids. However, hybrids converted the accumulated N more efficiently to total DM and GY, as evidenced by their higher ($p < 0.05$) NutEff in most year \times N level combinations. In addition, DM was allocated more efficiently to grains as expressed by the HI (+3.5%). Similarly, in *Cv_ag* (Section II, Prey et al., 2019a), two hybrid and line cultivars showed highest and lowest values in HI and NutEff_{grain}, respectively. These findings are in line with the hybrids' higher grain number per spike, which might contribute to achieving higher GY at similar amounts of total DM. Advantages in these traits are likely to be more pronounced under less favorable growing conditions (Becker, 2017). A relative yield advantage of the hybrids was observable within all main plot \times N level combinations in *Cv_ag*, with the three hybrids always ranking among the four highest-yielding cultivars (not shown). However, as in the *HyLi_N* trial, it could not be concluded that hybrids respond differently to agronomic treatments, since none of the interactions of cultivar-group with sowing time, N level, and fungicide were significant for GY, GNup or GNC (not shown). In both trials, the hybrids' slight advantage in GY was counteracted by their lower GNC so that no advantage in GNup was found. While the findings on GNC are in line with more recent research (Thorwarth et al., 2018), these authors reported positive grain protein deviations (GPD) and protein yields in experimental hybrids. Possibly, these advantages

are influenced by more recent breeding efforts since the release of the present hybrid cultivars (2003–2009), and by testing in less favorable environments. Based on the present results, considering the costs for seeds and a decreased product price due to lower GNC, hybrids would be economically compatible only if sowing densities were reduced by about 50% to approximately 150–200 kernels m^{-2} without compromising GY, however. In contrast, a sowing density trial conducted in 2016 with each three hybrid and line cultivars indicated decreased GY by 7% when the sowing density was reduced from 350 to 150 kernels m^{-2} (not shown), in addition to the side-effect of increased weed growth.

5.2 Organ-level partitioning and temporal DM and N dynamics during grain filling

For most traits, the values observed for the breeding lines (*BL* trial) were on a comparable level as for the cultivars (*HyLi_N* and *Cv_ag* trials), but were more widely distributed. At anthesis, DM was predominantly allocated to culms at a percentage of 61–63%. The remaining DM was approximately equally allocated to leaves and spikes as compared from the average results of 2015 and 2016 in the *BL* trial (Figure 7 a).

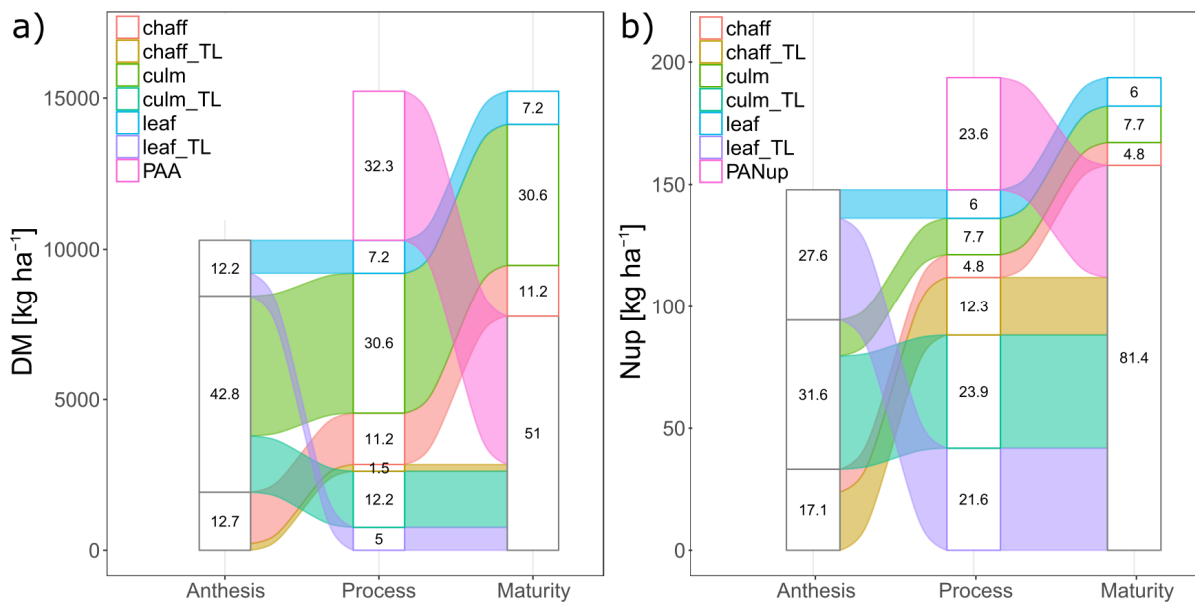


Figure 7: Origin and fate of DM (a) and Nup (b) from anthesis to physiological maturity. Values are averaged from 75 breeding lines and three reference cultivars from the *BL* trial in 2015 and 2016: Y-axis values are absolute values; values within bars indicate relative values [%]. TL: Translocation; PAA: Post-anthesis assimilation; PANup: Post-anthesis Nup. ‘Process’ relates to the post-anthesis dynamics with newly formed DM and Nup (PAA, PANup) and partly translocated DM and N (TL). Relative values refer to the total DM or Nup at maturity and sum up to 100 % for ‘Process’ and ‘Maturity’.

The difference method was applied to dissect GY formation into its pre- and post-anthesis components, i.e. DM translocation (DMT) and post-anthesis assimilation (PAA), and accordingly GNup into N translocation (NT) and post-anthesis N uptake (PANup). In particular, DMT and the associated (apparent) DMT-efficiency (DMTEff) differed substantially between genotypes, years and agronomic treatments. Increased N fertilization tended to decrease DMTEff. Nonetheless, DMT stagnated or increased in *HyLi_N* in response to N because of the increase in vegetative DM at anthesis, but

decreased in the control block and without leaf fungicide in *Cv_ag* due to a weak N-effect on anthesis DM. DMTEff was inversely related to the year-specific yield potential in *BL* and *HyLi_N*. In addition, DMTEff and DMT varied the most between genotypes in 2016, likely associated with the incidence of pathogens (Ayres et al., 1996) in combination with low irradiance and high plant densities (Austin et al., 1977). A detrimental effect of pathogens was supported by the by tendency decreased DMTEff (–8%) in *Cv_ag*, when no leaf fungicide was applied.

The determination of DMT using the difference method is based on the simplified assumption that both PAA and the aboveground post-anthesis DM lost from vegetative organs are completely allocated to grains. Thus, volatile respiratory losses and the root/shoot exchange are neglected and could not be determined in the present trials. Hence, the difference approach is likely to overestimate DMT and underestimate PAA because of neglecting losses by respiration and inevitable leaf shedding, which were quantified to approximately one quarter of the loss of anthesis DM (Austin et al., 1977; Gebbing and Schnyder, 1999; Savin and Slafer, 1991). Thus, notably the apparent DMTEff of the three leaf layers (on average 46–50%) was determined to be clearly higher than that of culms (36%) and chaff (24%) in *Cv_ag* (section II, Prey et al., 2019a). In addition, the measurement of DMT relies on the exact determination of the flowering time, a phase of rapid DM accumulation (section I, Prey et al., 2019c: Figure 3). Slight temporal delays in the anthesis sampling would overestimate notably spike DM and thereby DMT of spikes. The uncertainties for determining DM at anthesis, DMT, DMTEff and reversely PAA resulted in comparably low heritability values of these traits (section III, Prey et al., 2019b).

As summarized for the average results in the *BL* trial (Figure 7 a), notably the DMT of culms, which corresponded to on average 12.2% of the total DM, contributed substantially to final GY, which represented in turn 51% of the DM at maturity, as expressed by the HI. In contrast, on average 32.3% of the total DM was formed from PAA, which thus contributed on average 63% to GY, yet with substantial differences between years and genotypes.

The percentage of PANup with respect to total Nup (CPNup; 23.6%; Figure 7 b) was lower than that of PAA on total DM (Figure 7 a), but more fluctuant between years. Thus, PANup was higher in 2015 in both trials than in 2016, when a post-anthesis N loss was observed in particular for many breeding lines. At about 70–85%, the translocation of N was approximately 2–3 times as efficient as that of DM. However, its determination can be similarly error-prone as that of DMT. As for probable DM losses, N appeared to be released from the straw but to be partly lost during the transport due to diseases in 2016. Thus, the loss was not captured in the (apparent) N translocation efficiency (NTEff), but accounted for as apparent net ‘negative’ PANup (Simpson and Dalling, 1981), similarly as previously reported (Delogu et al., 1998; Ehdai and Waines, 2001; Guttieri et al., 2017; Papakosta and Gagianas, 1991).

Despite its substantial absolute contribution and in contrast to PAA, DMT was not explanatory for the GY variation, neither in the *BL* nor in the *Cv_ag* trial. This is in line with the only moderate correlations of anthesis DM traits with GY (section III, Prey et al., 2019b: Table 3). PANup correlated with GNup in all years (section III, Prey et al., 2019b), confirming results on its importance for GNup and grain protein deviation (Bancal et al., 2008; Bogard et al., 2010; Guttieri et al., 2015; Monaghan et al., 2001). In contrast, neither NT nor PANup well described the genotypic GNup variation in the *Cv_ag* trial (section II, Prey et al., 2019a).

5.3 Accumulation and partitioning of DM and N at maturity

Trait concepts that include maturity traits appeared more useful for explaining the genotypic variation in GY and GNup than translocation-related concepts in both the *BL* and the *Cv_ag* trials. From a net perspective, HI and NHI express the effective efficiency in DM and N partitioning to the grain, respectively, resulting from an efficient translocation and strong post-anthesis accumulation.

5.3.1 Yield-related concepts

Historically, increases in HI were a principle driver for gains in GY (Cormier et al., 2013; Shearman et al., 2005), whereas recent breeding effects were more effective for increasing total DM. A previously hypothesized upper HI limitation of 0.62 (Foulkes et al., 2011; Unkovich et al., 2010) is still clearly above the breeding lines' average values of about 0.51 (Figure 7 a) and the slightly higher values of the pre-selected cultivars. Hybrids were found to have higher HI values (section I, Prey et al., 2019c). However, the values were markedly lower in 2016 than in 2015, indicating inefficient allocation under pathogen-influenced conditions in 2016, thus representing a waste of assimilates. Interestingly, the effect of fungicide use on DMTEff and on the resulting organ-level DM allocation at maturity was more apparent for culms than for leaves, whereas with respect to NTEff, both organs profited similarly (section II, Prey et al., 2019a). The results achieved for the breeding lines confirm the variation in HI to be secondary behind that in total DM for explaining GY (Foulkes et al., 2011; Unkovich et al., 2010). In contrast, though being only partly significant ($p < 0.05$) for the low number of cultivars, HI explained 25–50% of the GY variation within all main plot \times N combinations in the *Cv_ag* trial, whereas total DM was rarely correlated with GY. Correlation analysis in the *HyLi_N* trial within year \times N level combinations supported that unlike to breeding lines, GY was correlated with both HI and total DM for the cultivars (data not shown). HI was always more heritable ($H^2 = 0.74\text{--}0.91$) than GY, but total DM always less heritable, similarly as for the breeding lines.

The exceptionally high GY in *BL* in 2017 was associated with the clearly highest PAA that year, which also exceeded that of *Cv_ag*, possibly due to the higher N fertilization level and better soil quality in *BL*. Unlike to the other years, a trade-off between HI and total DM was observed that year in addition

to low DMTEff values, what may indicate sink-limitation under the conditions of high PAA. In contrast, higher DMTEff in the other years may be an indicator that GY was more source-limited and lower PAA partly compensated by DMT. Thus, breeding for both increased sink and source size appears to be necessary (Foulkes et al., 2011). Increasing the grain number per m² was suggested for increasing the sink size (Shearman et al., 2005). While no 'direct' yield component was explanatory for GY in the *BL* trial, the grain number per m² explained 11–31% of the GY variation (section III, Prey et al., 2019b: Supplementary Figure 1), which was confirmed in *Cv_ag*. Similarly, the hybrids' higher GY was associated with higher grain number per spike at similar spike density.

The efficiency of using fertilized N for grain GY (NUE_{grain}) corresponds to the product of the multiplicative components NupEff, NutEff_{total} and HI, with NutEff_{total} × HI expressing the internal utilization efficiency for GY (NutEff_{grain}; Moll et al., 1982). The effect size of the genotypic variation in NupEff and NutEff_{grain} has been the subject of various investigations. In both the *Cv_ag* and *BL* trials, NupEff, respectively total Nup, was more indicative for NUE_{total} , respectively total DM, than NutEff_{total}. In *BL*, NupEff was as well better related to GY than NutEff_{grain}. However, in *Cv_ag*, NutEff_{grain} but not NupEff was moderately related to NUE_{grain} and GY within N fertilization levels, probably due to the positive effect of the hybrids' higher HI on NutEff_{grain}. The dominant effect of NupEff in the breeding lines is consistent with findings of Latshaw et al. (2016) and Guttieri et al. (2017), whereas Le Gouis et al. (2000) and Gaju et al. (2011) reported NutEff to be more explanatory, particularly under high N supply. Thus, differing conclusions in the literature are likely related to the germplasms used, and if high DM accumulation as a result of high NupEff and NutEff_{total} may have resulted into sink limitation—associated with a decreased HI as observed in 2017 for breeding lines with high total DM.

5.3.2 Concepts related to grain N uptake

More clearly than total DM with GY, total Nup was well correlated with GNup in all trials. In contrast, NHI was less or not (*BL*) correlated with GNup, as found in 2017 in both the *Cv_ag* and *BL* trials due to a negative association with total Nup, indicating sink limitation also for GNup under the condition of high total Nup (Mi et al., 2000). NHI was positively correlated with NTEff, as demonstrated previously (Fageria, 2014; Hirel et al., 2007). In most trial × year combinations, NHI correlated well with NTEff, HI, NutEff_{grain}, PAA and GY, suggesting generally positive effects of the post-anthesis metabolism both on the DM and N balance (Desai and Bhatia, 1978; Ehdaie and Waines, 2001; Guttieri et al., 2017).

The comparably weak inverse correlation between GY and GNC in *BL* compared with previous studies (Monaghan et al. 2001; Bogard et al. 2010; Latshaw et al. 2016; Oury & Godin 2007) was ascribed to a possibly weaker trade-off between GY and GNC in breeding lines than in cultivars, which are either optimized towards GY or GNC. Positive deviations from the negative relationship between GNC, or grain protein content, and GY are commonly expressed in the grain protein deviation, or

alternatively in GNup. Since the selection for GPD may favor genotypes high in GNC, but not superior in GNup (Rapp et al. 2018), the direct use of GNup appears to be preferable (section III, Prey et al., 2019b). Aiming at maximizing GNup, close correlations were found with GY but not with GNC in all years in BL, whereas for the lower fertilization level in *Cv_ag*, only GNC was indicative. Correlations with GNup within N levels in *HyLi_N* revealed distinct differences by years with significant correlations only with GNC in 2014 but only with GY in the other years (not shown).

5.4 Optimum growth stages for spectral measurements

The discussion on spectral phenotyping comprises the aspects of (i) the suitability of growth stages, (ii) the suitability of spectral vegetation indices (SVIs), considering (iii) the spectral resolution, and (iv) the comparison of the assessment of the DM and N traits.

More measurement dates than in most previous studies were tested in order to identify reliable growth stages. The spectral detection of all traits depended substantially on the seasonal measurement time. The variation in the final target traits GY, GNup and GNC is importantly determined during the grain filling phase and the influence of senescence characteristics became apparent in all datasets. Nevertheless, these traits can only be indirectly estimated by spectral measurements—with respect to both the temporal shift and the detected information at the organ level. Although SVIs are primarily influenced by leaf area, chlorophyll content and leaf area distribution (Hatfield et al., 2008), seasonal influences are important and dependent on the time when the variation in the target trait is determined. However, genotypic differences in the phenological development, i.e. time of flowering and accordingly shifts in ripening, influence the spectral signal differently.

For both GY and GNup, best relationships with SVIs were found during milk ripeness when the dominant source of variation was genotypic differences of breeding lines (section IV) or of cultivars in interaction with N fertilization levels (section VI, Prey and Schmidhalter, 2019c). Until this stage, substantial amounts of DM are formed (Supplementary Figure 1) while the influence of senescence is not dominant yet. Previous results support the suitability of the milk ripeness stage (Freeman et al. 2003; Babar et al. 2006; Gutierrez et al. 2010; Christopher et al. 2014; Zhang et al. 2019).

In 2017, the R^2 -peak in *Cv_ag* shifted towards dough ripeness for many traits (section V)—especially for data subsets, which included the agronomic treatment factors N fertilization and fungicide intensity, which resulted in shifted senescence. Both higher N fertilization and fungicide application result in higher GY and GNup and prolonged green leaf area duration (Gooding et al., 2000), which is captured by SVIs (Viña et al., 2004).

In contrast, PAA is reduced under conditions of drought/heat-induced rapid senescence, when early flowering and the translocation of vegetative DM may be an escape-strategy (Inoue et al., 2004; Kipp et al., 2014b; Van Herwaarden et al., 1998). In contrast to 2017, the canopy status at anthesis may

then be more indicative, possibly explaining the relatively better relationships of earlier growth stages in drier environments (Babar et al. 2006; Prasad et al. 2007b; Becker & Schmidhalter 2017).

The in-season—and therefore indirect—estimation of GY and GNup relies on the detection of traits that influence the formation of GY and GNup, respectively. Anthesis DM showed moderate correlations with GY (section III, Prey et al., 2019b), which are likely to be even higher for simultaneous sampling as conducted for the spectral readings and as suggested by the seasonal DM progression curve (Prey et al., 2018a; Supplementary Figure 1). In addition, correlations between total DM or Nup with GY and GNup, respectively, increased progressively during grain filling (Prey et al., 2018a).

Consistently for all analyses that included different genotypes, the phase of heading/early anthesis provided weak relationships, what was attributed to shifted phenological development. The emergence of ears and of colored anthers results in morphological and optical changes, which affect the spectral signal (Pimstein et al., 2009), whereas the time of flowering was rarely correlated with the target traits (sections III and IV). This phenological distortion is likely to be more pronounced with stronger differences in heading and flowering dates, what may explain that also these stages were reported to be suited, depending on the growing conditions, time of measurements and the germplasm used (Aparicio et al., 2000; Becker and Schmidhalter, 2017). In addition, temporal shifts by few days are likely to substantially influence the use of spectral measurements due to the rapid canopy development in this phase. Although plant sampling and spectral measurements were temporally closer at anthesis than for predictions of maturity traits, weaker relationships were found for most anthesis-related traits. On the one hand, these traits are not independent due to the same shoot density values used for up-scaling DM and Nup traits. On the other hand, plants at anthesis were sampled at the same growth stage instead of the same dates, thus the late-flowering genotypes gained on the earlier genotypes (Prey et al., 2018a; Supplementary Figure 1). This approach resulted in lower trait variation than would be detected for sampling simultaneous to spectral sensing, but was necessary for separating the vegetative and generative phases. Occasionally, measurements during grain filling were better suited than at anthesis, notably in 2017 and 2015, resulting in ‘post-dictions’. Anthesis DM was never well detected in *BL* in 2016 (section IV) in spite of good estimations of DM and the vegetative N in the adjacent *IN* trial (section VII, Prey and Schmidhalter, 2019b). In contrast, higher coefficients of determinations were observed also at anthesis in the *Cv_ag* trial, probably due to the comparably strong effect of the agronomic treatments and less apparent phenological shifts of the only six included cultivars.

5.5 Vegetation indices for estimating grain yield and contributing DM traits

Many remote-sensing approaches previously optimized the detection of LAI and chlorophyll concentration (Gitelson et al., 2003; Nguy-Robertson et al., 2012; Xie et al., 2016). However, vegetative

NC traits at anthesis were never significant for predicting GY and rarely for GNup, so that the commonly closely related chlorophyll content likely would neither have been particularly indicative. In addition, the determination of LAI was not feasible for the large number of included genotypes. Among the evaluated SVIs, those previously optimized for LAI, namely MCARI1, MCARI2, MTVI2, EVI and the MSAVI (Haboudane et al., 2004; Hatfield and Prueger, 2010), did not perform substantially better for GY than the NDVI. While the EVI exhibited indeed a specific advantage for DM and Nup of leaves in *BL* as previously reported (Hatfield and Prueger, 2010), this was not confirmed in *Cv_ag*, and not sufficient for the estimation of the more complex GY in both trials. The frequently discussed saturation issue of NIR/VIS indices, such as the NDVI (Frels et al., 2018; Pavuluri et al., 2015; Prasad et al., 2007b), became apparent in all trials (sections *IV–VII*) and was evidenced by the decreasing R^2 -levels with increasing DM from 2015–2017 (sections *IV* and *VI*) in spite of the similar data range.

Replacing the red band by a green band (Mistele and Schmidhalter, 2008b; Viña and Gitelson, 2005) was not generally expedient for increasing the sensitivity for DM. This is in line with the comparison of a UAV-based multispectral sensor in the *IN* trial in 2017 (Prey et al., 2018c), whereas under conditions of high N status, the NIR/green index was more sensitive than the NIR/red index, particularly for N traits (section *VII*, Prey and Schmidhalter, 2019b). Three NDVI equations, which shared the same red band and differed in the NIR band, were tested, but little differences were observed in the index rankings, what is in line with the contour map results (section *VI*, Prey and Schmidhalter, 2019a). The soil-adjusted indices MSAVI and OSAVI did not rank higher than the NDVIs for the majority of traits, probably due to the mostly low soil fraction in the dense canopies, little differences in soil brightness within measurement dates and the low R^2 -level on early days. Simple-ratio instead of normalized difference index equations were suggested for overcoming saturation issues for the estimation of leaf chlorophyll (Yu et al., 2012), DM, Nup (Barmeier and Schmidhalter, 2017) and biomass (Mutanga and Skidmore, 2004). However, only marginal effects were observed for the corresponding index pairs sharing the same wavebands (sections *VI* and *V*).

Instead, improvements were observed from the incorporation of a RE band. Most RE, NIR/RE and NIR/RE/VIS indices ranked higher than NIR/VIS indices for GY and vegetative DM in the *BL* trial. In contrast, the simulated Rapid Eye sensor profited little from its RE band (section *VI*, Prey and Schmidhalter, 2019a), and no clear advantage was identified from RE indices in the *Cv_ag* trial for the genotypic variation within main plot \times N combinations. A temporal trade-off was observed in the *Cv_ag* trial for DM traits between the vegetative phase with a relative advantage of the RE indices, and the senescence-influenced phase, when NIR/VIS indices ranked higher (unpublished results), indicating a certain sensitivity for the senescence status (Germer and Prey, 2018, unpublished). In contrast, the advantage of using RE bands was attributed to their increased sensitivity in dense canopies (Nguy-Robertson et al., 2012). The RE-indices Maccioni index (Frels et al., 2018), R^{-1}_{700} (Pavuluri et al., 2015)

and REIP as well as a NIR/RE simple-ratio index for barley (Barmeier et al., 2017) were also suggested for GY. Higher sensitivity of RE indices compared to the NIR/VIS indices was confirmed by the lower noise equivalent and higher coefficients of determination, especially under conditions of high N status, whereas smaller differences were found under low N conditions (section VII, Prey and Schmidhalter, 2019b). While shifting the NIR band leftwards and keeping the red band provided no improvements in the case of the R760_R670, or even lower rankings for the R730_R670 as compared to the R780_R670 (section IV), the replacement of the red band by a RE band increased the index sensitivity of NIR/RE indices, such as of the R760_R730 (Jasper et al., 2009) and the R780_R740 (Mistele et al., 2004).

The stability in the genotypic ranking in terms of total DM and Nup during the grain filling phase, especially from milk ripeness to (Supplementary Figure 1), appeared to be an important pre-requisite for GY and GNup prediction over time, respectively—given that total DM but not the HI and total Nup but not the NHI were the main determinants, respectively. Therefore, the seasonal R²-pattern for total DM resembled that for GY (sections IV, V), whereas HI was not well estimated. In *Cv_ag*, a moderate detection of HI from the senescence-sensitive R787_R765 was mainly driven by the fungicide effects. However, the comparably low R²-values found for GY in 2016 compared to total DM and GY in 2015 are likely linked to the late-seasonal pathogen pressure and low radiation conditions during grain filling that year—both factors that probably contributed to overall low HI and its significant correlation with GY only that year.

In both the *BL* and *Cv_ag* trials, water band indices ranked clearly highest for GY in the weighted mean/maximum rank sums (WMMRS), notably due to their higher R²-values at milk ripeness and their advantage during the later grain filling phase, when the R²-values of most other indices declined more rapidly. In contrast, no advantage or even weaker relationships were observed during the vegetative phase, being in line with the findings in section VII (Prey and Schmidhalter, 2019b) with respect to the estimation of vegetative DM.

In spite of above-average rankings of the NIR-based water band indices for total DM and DM of culms and spikes in *BL* but not in *Cv_ag*, these indices performed less well than most RE-based and even the NIR/VIS indices for leaf DM at both anthesis and maturity. It might be possible that the reflection in the water absorption band is influenced by water, which is mainly located in culms and—with ongoing grain filling—in kernels, thus confounding the detection of leaf DM. In contrast, given that water-band indices ranked relatively high during the late grain filling, it is also conceivable that they are better in detecting leaf senescence, which—given the strong influence of PAA on GY—should influence GY stronger than total DM. This may explain the relatively stronger advantage of the water band indices for GY than for total DM. Indeed, contour map analysis between canopy hyperspectral measurements and senescence, as referenced via RGB images, in the *Cv_ag* trial revealed close correlations from water band combinations (Germer and Prey, 2019, unpublished).

In addition, water band indices were reported to be less prone to saturation than the NDVI (Sims and Gamon, 2003), as evidenced by their relatively stronger advantage in the higher-yielding years 2016 and 2017 (section IV). Either way, the detection of ‘stay-moist’ characteristics by water band indices rather than of the ‘stay-green’ traits was promising for detecting variation in GY, both for breeding lines (section IV) and across N levels for cultivars (section VI, Prey and Schmidhalter, 2019a). This confirms previous findings from drought-stressed wheat (Babar et al., 2006; Babar et al., 2006b; Becker and Schmidhalter, 2017; Gutierrez et al., 2010) and barley (Rischbeck et al., 2016) also for high-yielding, relatively low-stressed conditions. While Garriga et al. (2017) found the NWI-3 to perform best at anthesis and the WBI at grain filling, the five established water indices performed rather similarly, whereas the newly adapted NWI-5, through shifting the reference band of the NWI-3 (920 nm) to 930 nm, performed slightly better in 2015 and 2016 in *BL* and in *Cv_ag*.

Among vegetative traits, DM of leaves followed by that of culms was better assessed than that of the chaff, as previously found in spring barley (Barmeier and Schmidhalter, 2017). This was ascribed to the nadir position of the sensor, since leaves dominate the signal of most structural indices.

Estimations of further, ‘derived’ DM traits revealed to be more challenging. While no consistently useful indices were identified for the three yield components in the *BL* trial, spike density was moderately assessed by the PSSR index in the *Cv_ag* trial both for the agronomic treatments and the main plot x N approach, and grain number per spike by the BGI (not shown). However, the causality of these relationships is questionable for the genotypic variation within main plot x N blocks and was confirmed neither in *BL* nor in the literature. Instead, recent advances in image analysis based on machine learning are promising for the detection of spike density (Qiongyan et al., 2017). With the other direct yield components being likely ‘hidden’ traits to non-imaging sensors, rather indirect relationships via DM are conceivable.

The spectral estimation of DMT and DMTEff was not consistent, both in the *BL* and *Cv_ag* trials. Similarly, PAA and its contribution to GY (CPAA) were not well assessed in the *Cv_ag* trial (unpublished results), but moderate relationships from water band indices were found for PAA in two years in the *BL* trial. This is in line with the correlations of PAA with GY (chapter 5.2). Yet, PAA is importantly determined during senescence, what limits the estimation from single-day measurements. Similarly, NutEff was not consistently detected by any index.

Although the estimation of some traits is likely indirect via correlations with morphological and phenological traits, causal effects are not required if robust relationships are confirmed. Few of the ‘derived’ DM traits were previously evaluated in spectral phenotyping. Erdle et al. (2013a) reported good index relationships for HI at milk ripeness, however for few cultivars only. For NutEff_{grain}, Frels et al. (2018) found good relationships (max. $R^2 = 0.41$) already at heading in one year, but weaker relationships than in the present study in another year.

5.6 Vegetation indices for estimating grain N uptake and contributing N traits

Besides GY, GNup was addressed as main phenotyping target trait, though being less established in breeding. The similar seasonal R^2 patterns for GNup and GY are in line with the close relationships between both traits (chapter 5.3.2). Just as found for GY with total DM, the estimation of GNup was dominated by that of total Nup as evidenced by the seasonally similar R^2 -values, being in line with Frels et al. (2018), whereas NHI was not reliably assessed for the breeding lines.

Under the present conditions of high N status and dense canopies, RE bands exhibited a clear advantage over the NIR/VIS indices for most N-related traits, including GNup. The benefit of RE bands is well established and was related to the rightward-shift of the RE position with increasing N status (Boochs et al., 1990; Guo et al., 2017; Guyot et al., 1988). The higher ranking of most RE, NIR/RE and the NIR/RE/VIS indices relates to the placement of the lower band at 700–750 nm (Datt, 1999). Notably indices with RE bands in the range 730–750 nm instead of VIS bands performed well (sections IV, V and VII). This is in line with the results of the Sentinel-2 indices among which mostly the combinations of NIR bands with the second RE band were better suited than those with the first RE band (section VI, Prey and Schmidhalter, 2019a). The related Maccioni index was previously suggested for GNup and total Nup efficiency (Frels et al., 2018), the R760_730 for spike Nup (Erdle et al., 2013), the R780_740 for total Nup (Pavuluri et al., 2015), as well as the REIP for GNup (Prey et al., 2018 a)—all indices that ranked best for GNup. The NDRE_770_750 was newly developed based on the results in section VII (Prey and Schmidhalter, 2019b) and was confirmed to perform slightly better than the former indices for many Nup traits in section V (Prey and Schmidhalter, 2019c). Likely, its RE band positioned further right than from most other indices with RE bands improved the estimation in dense canopies, as confirmed by the ‘cross-over’ rankings of the R760_730 and the R780_740 between N status interval in section VII (Prey and Schmidhalter, 2019b).

While GNC was well assessed across N levels from similar indices as for GNup (section VII, Prey and Schmidhalter, 2019b) and, yet on a lower level, in the ‘agronomic’ approach in the *Cv_ag* trial, no consistent relationships were found for the genotypic variation in the main plot \times N approach and for the breeding lines. This is in accordance with results on barley (Barmeier et al., 2017; Rodrigues et al., 2018), whereas Erdle et al. (2013a) found moderate correlations, however only on some dates and with fewer selected wheat cultivars. Apparently, the information gathered by the indices was dominated by DM and Nup, whereas GNC was determined during the late grain filling in interaction with DM and senescence characteristics.

Among vegetative organs, as for DM, Nup of leaves at anthesis was mostly better estimated than that of culms (i.e. stems) and spikes, but not consistently at maturity. Generally, similar R^2 -values were observed for total Nup as for leaf Nup, indicating that the detection of total Nup and that of the other organs may be indirect via correlations with leaf characteristics.

While the estimation of NC at anthesis failed for the breeding lines, NC of the lower leaf layers was moderately detected by VIS indices as the blue/green pigment index (BGI) in the *Cv_ag* trial. At maturity, the NC of vegetative organs is inversely related to the variation in NTEff. Thus, the fungicide-related variation in both NC and NTEff in the full data was captured by indices sensitive to senescence such as the R787_R765 (section V, Prey and Schmidhalter, 2019d). In the *BL* trial, instead water band indices ranked higher for leaf NC at maturity, suggesting indirect detection via DM or leaf senescence.

As for 'derived' DM traits, the estimation of 'derived' N traits was less consistent. The moderate relationships found for NTEff in *BL* differed strongly in the regression slopes and were not confirmed for the genotypic variation in *Cv_ag*. With NT being well correlated to total Nup at anthesis (section II, Prey et al., 2019a), the index rankings of both traits were comparable. In contrast, the post-anthesis Nup was never reliably detected.

5.7 Partial least squares regression

SVIs are the most common way of using spectral canopy data. Being potentially better transferable between sensors, easier to exchange and more robust in terms of overfitting and data pre-treatment, this thesis focused on SVIs rather than multivariate models.

Multivariate PLSR models were evaluated across years for the milk ripeness data in the *BL* trial. In comparison to SVIs, this approach holds the advantage of retaining spectral information of more than a few bands, yet can be prone to overfitting. While PLSR clearly outperformed the NDVI for almost all traits, the advantage in the R^2 -values in the validation over the REIP and especially over the trait-specific WMMRS-based best SVIs was relevant only for some traits. While the R^2 -value in the validation increased on average from 0.36 to 0.43 for total DM at maturity, it slightly decreased for GY and GNup in comparison to the WMMRS-indices, while the prediction error improved substantially for GY, GNup and various other traits. These findings corroborate the use of optimized index selection and are in line with previous results on spring barley (Barmeier and Schmidhalter, 2017) and from the *HyLi_N* trial (Prey et al., 2018a). While relative improvements in the cross-validation by PLSR were higher for GY than for other traits in the *HyLi_N* trial, possibly indicating its use for the 'composite' GY trait, which is related to multiple DM, N and senescence characteristics, this was not confirmed in the test set validation for the breeding lines, indicating a risk of overfitting. The evaluation of influential bands confirmed the RE and both the RE and water bands to be important for GNup and GY, respectively.

5.8 The influence of the underlying treatment factors

The *Cv_ag* dataset was evaluated as a whole and in several subsets for assessing the influence of the treatment combinations (section V, Prey and Schmidhalter, 2019d). Notably for HI, TKW, NutEff, NHI and NTEff, moderate relationships were found across the agronomic treatments, particularly

across both fungicide intensities, which were not transferable to the variation dominated by genotypic differences within main plot \times N combinations. Therefore, substantially different index rankings were identified. The otherwise inconspicuous indices R787_R765 and TCARI_OSAVI excelled by high rankings for these traits in the agronomic approach, indicating a reliable senescence assessment. For most other traits, however, suitable indices identified in the agronomic approach could be transferred to the discrimination of cultivars. In addition, many indices recommended for the breeding lines were confirmed for cultivars, being promising for a more robust application of the tested spectral methods.

5.9 The value of multispectral resolution

While this thesis focused on hyperspectral sensing, the simulation of multispectral data indicated that optimized configuration of multispectral sensors minimizes the information loss. Sensors similar to the Sentinel-2 multispectral sensor were found to cover the relevant spectral regions, notably the higher RE and a water band for reliable N and GY estimations, confirming previous results (Herrmann et al., 2011). While the application of actual satellite data needs to consider further aspects including atmospheric transmissivity, spatial resolution and signal to noise ratio (Hadjimitsis et al., 2010; Herrmann et al., 2011; Julien and Sobrino, 2010; Rembold et al., 2013), the findings can be applied for optimizing ground-based and UAV-based proximal sensing and the configuration of multispectral sensors. Comparing hyperspectral passive data to multispectral active sensing in the *HyLi_N* data (Prey et al., 2018a) indicated that—given a sufficiently strong active light source—a modified ALS N sensor (YARA International, ASA) can almost compete with hyperspectral sensors. Likewise, the NDVI of a two-band GreenSeeker® Handheld Crop Sensor (Trimble, Sunnyvale, CA, USA) performed similarly as the best hyperspectral indices for plant traits in sparse canopies (Prey et al., 2018b). If the information loss is minimized, multispectral sensors may be favored in practice with respect to simpler technical implementation, lower sensor costs and for overcoming the curse of dimensionality of hyperspectral data (Backer et al., 2005; Furbank and Tester, 2011; White et al., 2012). However, further evaluations with actual sensor data are required, notably for detecting genotypic variation.

5.10 Further aspects for applying spectral methods

The ‘structured’ approach of using pre-established SVIs and the ‘unstructured’ contour map approach were mostly coherent in the identification of best wavebands, indicating a limited potential for improving established SVIs. This is in line with mostly little advantage of more sophisticated equations over simple two-band indices. Besides associations with the target trait, SVIs should be more heritable than the trait to be considered as indirect selection criteria (Babar et al., 2006b). High broad sense heritability ($H^2 > 0.80$) was observed for most indices in particular during grain filling (section IV). While lower values were observed for NIR/VIS indices on some dates, notably RE-based, but also water

band indices, reached high values, indicating a good potential to gather repeatable information. Besides the use of optimized SVIs, the time of measurement holds optimization potential. For reducing the risk of missing suitable stages (Magney et al., 2016), which may differ by year and growing condition, multi-day data can be used as averaged or combined to spectral curve parameters (Christopher et al., 2014; Prasad et al., 2007a; Raun et al., 2001).

The generally higher coefficients of determination in section VI (Prey and Schmidhalter, 2019a) than those observed in section IV were mainly due to the ‘stretching’ of the data by two to three N fertilization levels. Still, often lower R^2 -values were found for the breeding lines in *BL* than for the selected cultivars within main plot \times N combinations in 2017 (section V, Prey and Schmidhalter, 2019d) and in the *HyLi_N* trial (Prey et al., 2018a). Compared to the cultivars, the breeding lines were more diverse in phenology and in morphology, including some awned lines and substantial variation in plant height (section III, Prey et al., 2019b). While these characteristics influence the spectral signal, they were rarely correlated with the target traits, what therefore distorted the trait~index relationships. Similarly, cultivar effects influenced the index relationships for the sensing of the vegetative N status (section VII, Prey and Schmidhalter, 2019b).

The index comparison focused on the R^2 -values as a dimensionless measure of the ability to discriminate the target trait variation. Though being sensitive to the data range, a scale-independent discrimination is often sufficient for a relative grouping in phenotyping (Garriga et al., 2017). While no precise GY prediction was achieved particularly for the breeding lines in 2016 and 2017, the method allowed to ‘halve’ the population without losing the best-yielding genotypes—a ‘culling tool’ strategy that would be relevant for plant breeders (Frels et al., 2018; Garriga et al., 2017), aiming at a visual evaluation of only relevant genotypes or even non-harvesting the others.

Conclusions

The evaluation of DM and N traits for hybrid and line cultivars in **section I** (Prey et al., 2019c) suggested to possibly consider hybrids in terms of GY if lower GNC can be tolerated, but currently not for higher GNup.

As evaluated in **section II** (Prey et al., 2019a), besides genotypic variation, agronomic treatments importantly impact various NUE traits. However, treatment effects and interactions depend on growing conditions and goal conflicts may arise, e.g. between NUE and fungicide use and sowing date and plant health.

As evaluated in **section III** (Prey et al., 2019b), the variation in the accumulative traits total aboveground DM and Nup was more descriptive for the genotypic variation than that in the partitioning traits, thus holding more potential for the selection of breeding lines. Nup was more descriptive for NUE than NutEff and in turn GY more for GNup than GNC. Post-anthesis dynamics were important and are likely linked to stay-green traits as also suggested by SVI-relationships in the spectral analyses.

Section IV (Prey et al., 2020) indicated useful trait~SVI relationships, but the selection of SVIs and of measurement dates is crucial. Water band information was superior to RE- and particularly NIR/VIS-SVIs during grain filling, whereas RE information was crucial for assessing the N status. The milk ripeness stage was the most robust, whereas heading and anthesis was not suited.

Section V (Prey and Schmidhalter, 2019c) confirmed most results from section IV for cultivars in addition to spectral applications for agronomic factor trials. Indices detected traits related to partitioning and translocation efficiency as effect of fungicide-induced shifts in senescence.

Section VI (Prey and Schmidhalter, 2019a) suggested that multispectral sensors are promising for transferring results based on hyperspectral data if they are equipped with a water band and a band in the higher RE region. Limitations were identified during later grain filling.

Section VII (Prey and Schmidhalter, 2019b) confirmed the use of RE information for sensing vegetative N traits under conditions of high N status but not that of water bands. Under low N conditions, simpler NIR/VIS sensors may be sufficient.

Further analytic methods, notably multivariate statistics, date and sensor fusion, provide possible options for improvements. Imaging methods provide more information but must be pondered with respect to sensor costs, measurement time and data handling. Thriving UAV-based methods are promising for leveraging the reported spectral methods. However, their evaluation should be contextualized with the growing conditions, treatment factors and the interaction of the contributing traits for further advancing field-based phenotyping.

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Liste der Veröffentlichungen und Tagungsbeiträge

Die Angaben sind chronologisch sortiert. Wenn zutreffend ist die Einbindung in die Dissertation angegeben.

Veröffentlichungen in Zeitschriften mit Peer-Review Verfahren

- Prey, L.,** von Bloh, M., Schmidhalter, U., 2018b. Evaluating RGB Imaging and Multispectral Active and Hyperspectral Passive Sensing for Assessing Early Plant Vigor in Winter Wheat. *Sensors* 18, 2931. doi: 10.3390/s18092931 (*nicht in Dissertation eingebunden*¹)
- Prey, L.,** Kipp, S., Hu, Y., Schmidhalter, U., 2019c. Nitrogen Use Efficiency and Carbon Traits of High-Yielding European Hybrid vs. Line Winter Wheat Cultivars: Potentials and Limitations. *Frontiers in Plant Science*. 9, 1988. doi: 10.3389/fpls.2018.01988 (*Kapitel I*)
- Prey, L.,** Schmidhalter, U., 2019a. Simulation of satellite reflectance data using high-frequency ground based hyperspectral canopy measurements for in-season estimation of grain yield and grain nitrogen status in winter wheat *ISPRS Journal of Photogrammetry and Remote Sensing*. 149, 176–187. doi: 10.1016/j.isprsjprs.2019.01.023 (*Kapitel VI*)
- Prey, L.,** Germer, M., Schmidhalter, U., 2019a. Temporal and Organ-specific Responses in NUE Traits to N Fertilization, Fungicide Intensity and Early Sowing in Winter Wheat Cultivars. *Agronomy* 9, 313. doi: 10.3390/agronomy9060313 (*Kapitel II*)
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- Prey, L.,** Hu, Y., Schmidhalter, U., 2018a. Spectral sensing traits of nitrogen use efficiency in hybrid and line wheat cultivars., in: Brandstetter, A.; Geppner, M. (Ed.), Tagungsband der 68. Jahrestagung der

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Vereinigung der Pflanzenzüchter und Saatgutkaufleute Österreichs, 20.-22. November 2017. Universität für Bodenkultur Wien, Vienna, 59–62.

Prey, L., Weigel, M. & Schmidhalter, U., 2018c. Validierung einer UAV-basierten Multispektralkamera zur Erfassung von Bestandesmerkmalen in Weizen. 38. Wissenschaftlich-Technische Jahrestagung der DGPF und PFGK18 Tagung, München, 258–273.

Tagungsbeiträge

Poster: **Prey, L.:** High-throughput phenotyping of winter wheat under field conditions. 2nd HEZagrar PhD Symposium. Freising, Germany, 26.04.2016.

Poster: **Prey, L.,** Hu, Y., Schmidhalter, U.: Spectral high-throughput phenotyping the nitrogen use efficiency of german hybrid and line wheat cultivars. 7. Agrarwissenschaftliches Symposium, Hans Eisenmann Zentrum. Freising, Deutschland, 22.09.2016.

Poster: **Prey, L.,** Hu, Y., Schmidhalter, U.: Spectral high-throughput phenotyping the nitrogen use efficiency of german hybrid and line wheat cultivars. Deutsche Gesellschaft für Pflanzenernährung, Stuttgart-Hohenheim, Deutschland, 28–30.09.2016.

Poster: **Prey, L.,** Hu, Y., Schmidhalter, U.: Spectral phenotyping for grain yield prediction in winter wheat. 3rd HEZagrar PhD Symposium, Freising, Germany, 25.04.2017.

Vortrag: **Prey, L.,** Hu, Y., Schmidhalter, U.: Spektrale Erfassung von Merkmalen der Stickstoffeffizienz bei Winterweizen. 129. VDLUFA-Kongress, Freising, Deutschland, 13.09.2017.

Vortrag: **Prey, L.,** Schmidhalter, U.: Möglichkeiten der spektralen Erfassung der Stickstoffeffizienz bei Weizen. Seminar zu aktuellen Themen–Nutzpflanzenwissenschaften, Universität Kiel, Deutschland, 14.11.2017.

Vortrag: **Prey, L.,** Hu, Y., Schmidhalter, U.: Spectral sensing traits of nitrogen use efficiency in hybrid and line wheat cultivars. 68th Plant Breeder's Meeting in Gumpenstein. Raumberg-Gumpenstein, Austria, 22.11.2017.

Vortrag: **Prey, L.,** Schmidhalter, U.: Spektrale Sensorik zur Beurteilung der Stickstoffeffizienz. Jahrestreffen der Bayerischen Weizenzüchter an der LfL Freising, Deutschland, 14.12.2017.

Vortrag: **Prey, L.,** Weigel, M., Schmidhalter, U.: Validierung einer UAV-basierten Multispektralkamera zur Erfassung von Bestandesmerkmalen in Weizen. PFGK-2018, München, Deutschland, 09.03.2018.

Vortrag: **Prey, L.,** Schmidhalter, U.: Simulation of satellite reflection data from hyperspectral canopy measurements for in-season estimation of grain yield and grain nitrogen status in winter wheat. 38th annual EARSeL Symposium, Chania, Greece, 10.07.2018.

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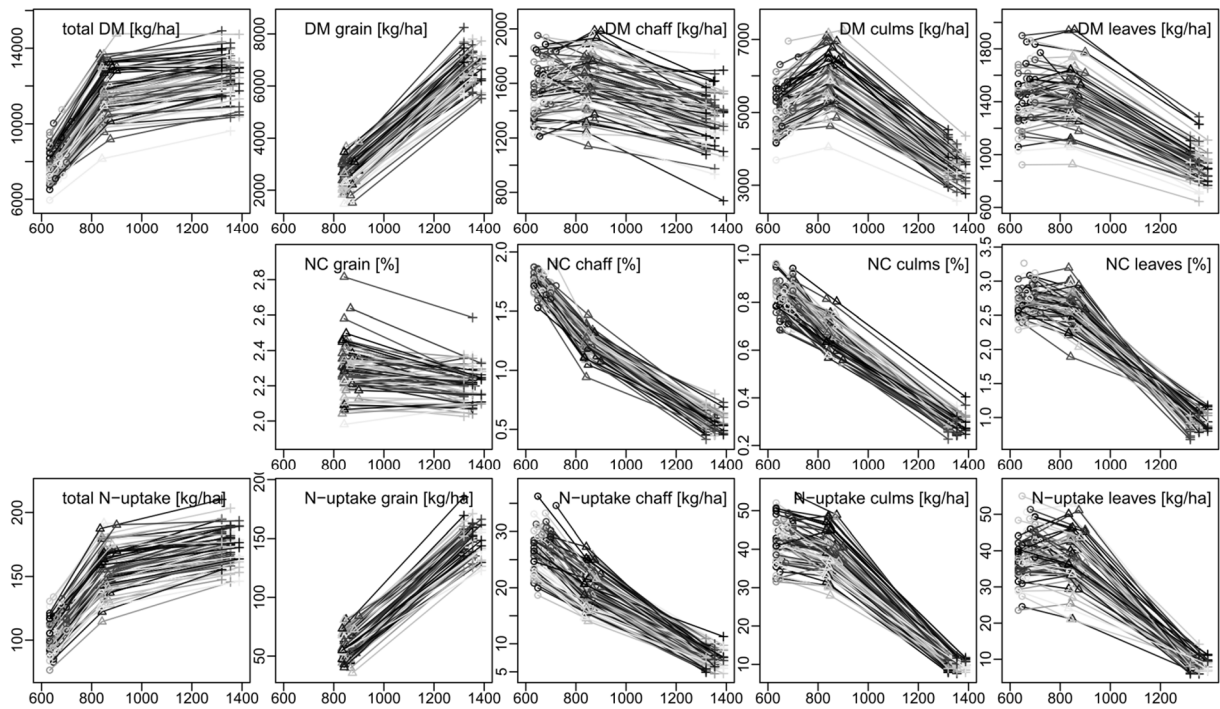
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Supplementary Material



Supplementary Figure 1: Temporal development of dry matter (DM; top), N concentration (NC; middle) and N uptake (Nup; bottom) from anthesis (circles) to milk ripeness (triangles) and maturity (crosses) in the BL trial in 2015 by organs. Lines indicate values by genotypes.