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Towards sustainable spring wheat cultivation: enhancing nitrogen use efficiency through strategic breeding

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Abstract

Nitrogen (N) is essential for plant growth; however, the frequent use of N-based fertilizers to maintain high crop yields is associated with several environmental risks. Sustainable agriculture requires crops that maintain high yields with less fertilizer. Spring wheat, a promising staple crop in Nordic regions, was used as a model to evaluate the breeding potential for N use efficiency traits, which are not typically targeted in crop breeding programs. The overall objective of this thesis was to explore the application of N use efficiency traits in spring wheat breeding programs for high latitude environments. The aims were to (i) explore the relationships between breeding target and non-target traits across various varieties grown in Central Sweden over a two year period; (ii) assess the genetic and environmental influences on early-vigor root traits; (iii) develop a methodology to rapidly assess N use efficiency traits; and (iv) identify genomic regions and candidate lines with desirable trait combinations in a population of 183 recombinant inbred lines (RILs). Stable associations were found between certain N use efficiency traits and breeding targets, as wheats bred for high yield were characterized by high N utilization efficiency. High N uptake efficiency was not linked to any breeding target. Early-vigor root traits were strongly influenced by both variety and environmental conditions, but unassociated with breeding target. A simple methodology for phenotyping N use efficiency traits in large populations was developed based on allometry, and promising candidate lines with favorable trait combinations were identified, along with 186 genomic regions associated with N use efficiency. The results demonstrate the potential of incorporating N use efficiency traits as breeding targets for crops, thereby supporting a more sustainable agriculture

Keywords: spring wheat, nitrogen use efficiency, breeding target, allometry, genomic regions.

Mot en hållbar odling av vårvete: Förbättring av kväveeffektivitet genom strategisk växtförädling

Sammanfattning

Växter behöver kväve för att växa, men ökad användning av kvävebaserade gödselmedel i jordbruket för att öka skördarna är förknippad med miljörisker. Ett mer hållbart jordbruk bör baseras på grödor som möjliggör höga skördar med mindre användning av gödsel. Vårvete är en lovande basgröda för de nordiska regionerna och användes här som en modell för att utvärdera växtförädlingspotentialen för olika grödegenskaper kopplade till kväve-effektivitet, vilka idag inte används inom växtförädlingen. Det övergripande målet med avhandlingen var att undersöka användningen av växtegenskaper kopplade till kväve-effektivitet i växtförädlingsprogram för vårvete anpassat till nordiskt klimat. Målen var att (i) utforska samband mellan grödegenskaper som idag används och sådana som inte används inom växtförädlingen, genom odling av olika vetesorter i Mellansverige under två år, (ii) bedöma effekter av genetik och miljö på rotegenskaper hos unga plantor, (iii) utveckla en praktiskt användbar metod för att snabbt kunna bedöma viktiga växtegenskaper kopplade till kväve-effektivitet, och (iv) identifiera genomiska regioner kopplade till kväve-effektivitet och kandidatlinjer med önskvärda kombinationer av växtegenskaper i en population av 183 rekombinant inavlade vårvetelinjer. Stabila samband hittades mellan vissa egenskaper kopplade till kväve-effektivitet och växtförädlingsmål, då vete som förädlats för hög avkastning karakteriserades av hög kväveutnyttjande-effektivitet. Däremot hittades inget samband mellan hög kväveupptagningsförmåga och något växtförädlingsmål. Rotegenskaper hos groddplantor påverkades starkt av genetik och miljö, men var inte kopplade till något växtförädlingsmål. En enkel metod för kartläggning av egenskaper för kväve-effektivitet i stora populationer utvecklades baserat på allometri. Lovande kandidatlinjer med önskvärda kombinationer av växtegenskaper, och 186 genomiska regioner för kväve-effektivitet identifierades. Resultaten indikerar att växtegenskaper kopplade till kväve-effektivitet har potential att användas i växtförädlingsprogram för ett mer hållbart jordbruk.

Nyckelord: allometri, genomiska regioner, kväveutnyttjande-effektivitet, vårvete, , växtförädling.

Rumbo a un cultivo sostenible de trigo de primavera: mejora de la eficiencia del uso del nitrógeno mediante el mejoramiento estratégico

Resumen

El nitrógeno (N) es vital para el crecimiento de las plantas, pero el uso intensivo de fertilizantes nitrogenados genera riesgos ambientales. La agricultura sostenible exige cultivos productivos con menos fertilizantes. El trigo de primavera, alimento básico en regiones nórdicas, fue empleado para estudiar el mejoramiento de rasgos relacionados con la eficiencia en el uso de N, poco priorizados en los programas de mejoramiento. El objetivo general de esta tesis fue explorar la aplicación de los rasgos de eficiencia en el uso de N en programas de mejoramiento de trigo de primavera para latitudes altas. Los objetivos fueron (i) explorar las relaciones entre los rasgos objetivo y no objetivo del mejoramiento en las variedades cultivadas en Suecia central durante dos años; (ii) evaluar las influencias genéticas y ambientales en los rasgos de vigor temprano de las raíces; (iii) desarrollar una metodología para evaluar rápidamente los rasgos de eficiencia en el uso de N; y (iv) identificar regiones genómicas y líneas candidatas con combinaciones de rasgos deseables en una población de 183 líneas endogámicas recombinantes. Se encontraron asociaciones estables entre algunos rasgos de eficiencia en el uso de N y objetivos de mejoramiento, ya que el trigo mejorado para alto rendimiento se caracterizó por una alta eficiencia en la utilización de N. La alta eficiencia de absorción de N no se relacionó con ningún objetivo de mejoramiento. Los rasgos de vigor temprano de la raíz fueron influenciados por la variedad y las condiciones ambientales, pero no se asociaron con el objetivo de mejoramiento. Se desarrolló una metodología simple para fenotipificar los rasgos de eficiencia en el uso de N en grandes poblaciones con base en alometría, y se identificaron líneas candidatas con combinaciones de rasgos favorables, junto con 186 regiones genómicas relevantes. Los resultados resaltan el potencial de incorporar rasgos de eficiencia en el uso de N como objetivos regulares de mejoramiento para cultivos para el respaldo de una agricultura más sostenible.

Palabras clave: trigo de primavera, eficiencia del uso de N, objetivo de mejoramiento, alometria, regiones genómicas.

Dedication

To my beloved ones, especially my Mom.

“Aut viam inveniam aut faciam” (I shall either find a way or make one)

Hannibal

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Guardia-Velarde, L., Hui, L., Cope, J. E., Westerbergh, A., and Weih, M. (2023). Differential breeding targets in wheat influence non-target traits related to grain quality, but not crop nitrogen requirement. Frontiers in Agronomy, 5:1151015.

II. Guardia-Velarde, L., Cope, J. E., Metzler, H., Westerbergh, A. and Weih, M. Same with less: A method to reduce destructive sampling to estimate nitrogen use efficiency components using allometric relationships in spring wheat (submitted)

III. Guardia-Velarde, L., Cope, J. E., Rahimi, Y., Westerbergh, A., Vallenback, P., and Weih, M. Identification of genomic regions for traits related to nitrogen use efficiency in a spring wheat RIL population (manuscript)

IV. Jovanović, I., Guardia-Velarde, L., Frantová, N., and Weih, M. Genotype adaptive patterns in spring wheat reveal drought-induced differentiation in root morphology (submitted)

Paper I is published open access.

The contribution of Lorena de Jesús Guardia Velarde to the papers included in this thesis was as follows:

I. Main author. Participated in the conceptualization, methodology, investigation, data analysis, writing (original draft preparation).

II. Main author. Participated in the conceptualization, methodology, data collection and analysis, investigation, writing (original draft preparation).

III. Main author. Participated in the conceptualization, methodology, data collection and analysis, investigation, writing (original draft preparation).

IV. Co-author. Participated in the methodology, investigation, writing (review and editing).

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Abbreviations

N	Nitrogen
NAE	Nitrogen accumulation efficiency
RILs	Recombinant inbreed lines
SNP	Single nucleotide polymorphism
QTL	Quantitative trait loci
GWAS	Genome-wide association studies

1. Introduction

1.1 The art and purpose of plant breeding

Plant breeding is an applied science that utilizes human effort to direct natural processes, resulting in permanent and heritable changes in plants for our benefit. Like other non-exact scientific fields, plant breeding also incorporates an element of artistry for its success. Early domesticators depended exclusively on their experience and intuition to select and cultivate plants with desirable traits. Today, with expanding knowledge and technological advancements, modern breeders rely increasingly on scientific methods to minimize the guesswork in selection, if not eliminate it entirely (Acquaah, 2009, Luo et al., 2022).

Humans have improved certain aspects of plants for decades, and as a result, more suitable plant varieties are currently available, therefore, why does this still matter to us? To put it simply, humans have depended on plants for food and resources since the earliest days of our existence. Due to a rapidly growing world population, plant breeding has become a valuable tool to address the increasing demand on the food supply. Therefore, plant breeders must continuously improve plants in a changing world, where breeding objectives are constantly evolving (Acquaah, 2009, Luo et al., 2022). During the 1950s and 1960s, the main goal for plant breeders was to achieve a higher yield by adapting crops to their production environment, followed by resistance to biotic stress. Quality traits, such as fiber strength in cotton and milling and baking properties in wheat, were also valuable traits that were considered in early breeding programs. Later, abiotic stress resistance, such as drought, salinity, and extreme temperatures, was also considered important. Throughout the 1990s, crop yield remained the main breeding target. However, as better practices and analytical instrumentation became more accessible, nutritional quality became a key breeding objective (Acquaah, 2009). Advancements in technology are leading to more precise definitions of quality traits in breeding goals. Moreover, to decrease both the global carbon footprint and environmental pollution, alternative fuel sources and the reduction of agrochemicals have been incorporated into the list of the latest breeding objectives (Acquaah, 2009, Luo et al., 2022). Additionally, biofortification breeding has gained significant attention as a strategy to enhance the nutritional value of staple crops (Velu et al., 2017).

Furthermore, breeding for nutrient use efficiency has become increasingly relevant as it addresses various challenges such as nutrient deficiencies in the soil, overuse of fertilizers, and significant nutrient losses through leaching, gaseous emissions, and soil fixation. By tackling these issues, it also has the potential to reduce environmental pollution (Baligar et al., 2001). Today, plant breeders and geneticists face continuous pressure to sustain and enhance food production through innovative breeding and agronomic strategies. This includes introducing minor crops that are well adapted to marginal lands and capable of providing nutritional value by tolerating both abiotic and biotic stresses (Al-Khayri et al., 2019). Although it may appear ambitious, the potential to shape plant development to meet human needs has yet to reach its full extent (Doebley et al., 2006).

1.2 Wheat breeding

As mentioned above, high crop yield has been, and still is, one of the major breeding objectives, mostly because the human population is increasing at an alarming amount. Between the groups of plants for breeding, four staple crops can be considered crucial to feed the world: wheat, rice, corn, and potato. These crops have their own mode of reproduction and breeding approach. While wheat and rice are self-pollinated crops, maize is cross-pollinated, and potatoes possess a vegetative reproduction (clonal) (Bradshaw, 2017).

The cultivation of wheat began during the “Neolithic Revolution”, approximately 10,000 years ago. The first specimens of these plants had either a diploid genome (einkorn – Am Am) or a tetraploid genome (emmer – AABB) (Dubcovsky and Dvorak, 2007). Both emmer (*Triticum dicoccon*) and einkorn (*Triticum monococcum*) originated from wild species in the Fertile Crescent; however, einkorn populations were primarily located further north compared to emmer (Allaby et al., 2017). From there, the cropland extended to West Asia (Near East), and this is where the domestication of hexaploid wheat took place, within the Fertile Crescent (Dubcovsky and Dvorak, 2007, Shewry, 2009). During domestication, desirable traits were selected to suit particular environments or agricultural practices, including the loss of spike shattering and the development of free-threshing naked seeds, which are traits that significantly enhanced harvest yield (Shewry, 2009, Charmet, 2011).

Pure line cultivars were originally selected from diverse landraces and subsequently developed and then improved by crossing and human selection following inbreeding. Improvement continued through repeated cycles of crossing and selection, primarily within emerging elite germplasm, leading to the creation of new and enhanced inbred cultivars that included the wheat cultivars of the 'Green Revolution,' developed between 1945 and 1978 (Pingali, 2012). This work emphasized the critical role of the selection environment, germplasm choice, and breeding methods (Bradshaw, 2017).

Since wheat is a staple food, it plays an imperative role in achieving the United Nations' second Sustainable Development Goal, “Zero Hunger”. This goal seeks to end hunger, ensure food security, enhance nutrition, and promote sustainable agriculture worldwide. However, the growing global population presents a serious challenge to achieving this objective, with it estimated to reach 9 billion by 2050 (Cohen, 2001, Le Blanc, 2015).

1.3 Adaptation to Swedish conditions

In high northern latitudes, crop growth and productivity are primarily limited by low temperatures and short growing seasons (Olesen et al., 2011); however, wheat has adapted to these conditions. Wheat is a widely grown arable crop in many high latitude regions such as Sweden, Norway, Denmark, and Canada (north of 55° N) (Liu et al., 2021). The typical climatic and environmental factors of Sweden have promoted the adaptation of two wheat types, spring and winter wheat. Winter wheat is generally recognized for its higher grain yield potential, while spring wheat is valued for its superior baking quality (Koppel and Ingver, 2008). Due to Sweden’s geographical location, certain breeding targets are more aligned and relevant than others. Significant breeding target traits are related to adaptation to low temperatures, phytosanitary management, allelopathy, and nutrient (namely nitrogen) use efficiency, in addition to the main target traits of high grain yield and high grain protein content for bread wheat. This thesis explores nitrogen (N) use efficiency as a critical trait for spring wheat breeding programs in high latitude environments.

1.4 Nitrogen use efficiency

Nutrients, especially N, play a vital role in the growth and development of all plants. The lack or imbalanced proportion of any nutrient during plant growth can become the primary limiting factor in its development (Reich et al., 2014, López-Arredondo et al., 2017, Weih et al., 2021). In cereal crops, such as wheat, optimizing the balance between maximizing grain yield and grain quality, along with a minimal environmental impact of the crop, requires a fertilization strategy that aligns N supply with crop demands, while also considering both temporal and spatial variability (Foulkes et al., 2009). Nevertheless, N use efficiency is often suboptimal due to mismatches between soil nutrient availability, fertilizer application, and the crop’s physiological needs. Additionally, the lack of real-time adaptation of N management to the crop's developmental stages and changing weather conditions intensify the issue. Improving N use efficiency is expected to enhance crop yields while simultaneously reducing environmental pollution from N losses (Ravier et al., 2017). Therefore, N fertilizer must be applied cautiously to increase profits, diminish the impact of diseases and pests, improve crop quality, and reduce the overall environmental impact (Schröder et al., 2000, Ravier et al., 2017).

Moll et al. (1982) described N use efficiency as the ratio of grain dry matter yield to the total N available from soil and fertilizer. Although this approach is widely recognized, this method only accounts for crop N and grain biomass at harvest, and both are influenced by developmental processes throughout the growth period. Alternatively, Weih et al. (2011, 2018) introduced a different approach to assess N use efficiency in terms of N accumulation efficiency (NAE), which incorporates three components: the amount of N uptake in relation to the initial N content in the sown seed grain, the efficiency of converting plant internal N into grain biomass, and N re-translocation to the harvested grain. This approach connects N use at different growth stages, thus considering the entire plant life cycle.

Enhanced N use efficiency in crops is considered as a key indicator of progress towards ending hunger, ensuring food security, improving nutrition, reducing pollution, and fostering sustainable agriculture (Fageria et al., 2008, Weih et al., 2017).

1.5 A Swedish spring wheat breeding program focused on N use efficiency along with other main breeding targets

A well-designed and strategically effective breeding program should focus on preserving and enhancing established target traits while also addressing N use efficiency. Ensuring an optimal supply of N during plant development can reduce the overuse of N and other nutrients. Consequently, it not only minimizes N leaching into groundwater but also mitigates environmental pollution, including the eutrophication of the Baltic Sea (Larsson and Granstedt, 2010, Chawade et al., 2018, McCrackin et al., 2018).

N use efficiency has emerged as a critical trait in modern agriculture due to its connection with sustainable food production and environmental preservation. Despite its importance, there are currently no dedicated breeding programs for improving N use efficiency in spring wheat under high latitude conditions, principally because it is considered to be a complex polygenic trait, and as many traits selected in breeding programs, it might show low heritability (Fontaine et al., 2009, Chawade et al., 2018, Vukasovic et al., 2024). Furthermore, root traits need to be considered in the pre-breeding research due to their significance in mitigating abiotic stresses such as low temperatures and drought, which represent some of the major challenges associated with climate change (Johansson and Mobjörk, 2009).

1.5.1 High grain yield vs high grain protein content

Wheat contributes to approximately 18% of the global caloric intake and supplies about 20% of the protein consumed by people worldwide (Chen et al., 2022). Therefore, grain yield and grain protein content are the two main breeding targets for wheat production (Bogard et al., 2010, Guardia-Velarde et al., 2023). Nitrogen is a fundamental component of proteins, therefore, soil N availability can have a significant impact on grain protein content. Nitrogen fertilization can substantially increase both grain yield and grain protein content; however, the extensive use of commercial fertilizers increases cropping expenses and enhances the risk of environmental pollution (Chen et al., 2022). Nevertheless, increasing both grain yield and protein content is still considered a difficult task due to the frequently negative correlation between the two traits (Fowler, 2003, Chen et al., 2022). Thus, a decrease in protein content is often reported in association with increased grain yield, which is likely caused by the dilution of N compounds when carbohydrate synthesis is enhanced during photosynthesis (Jenner, 1991, Mirosavljević et al., 2020). This suggests that developing high-yielding cultivars with elevated grain protein content is more complex than merely incorporating yield-enhancing genes into a high grain protein content genetic background, or vice versa (Fowler, 2003).

As higher grain yields often correlate with reduced protein content, wheat breeders must prioritize grain quality or yield to develop cultivars suited to specific food markets (Peña et al., 2002, Amiri et al., 2018). Hence, high grain yield and high protein content are two major targets, for which independent breeding programs have been developed not only in Nordic environments but also worldwide. Nevertheless, innovative efforts are being made to integrate both breeding targets into one single variety. For example, Chen et al. (2022) characterized two new Q alleles (the Q gene is involved in several significant traits, including grain yield and grain protein content) that presented a single missense mutation and revealed that the inverse relation between grain yield and grain protein content can be broken by one of the new alleles using a non-transgenic method. Additionally, the relationship between grain yield and grain N (or protein) content is also influenced by management practices. Strategies that promote arbuscular mycorrhizal fungi (AMF) colonization in wheat, particularly after flowering, could help mitigate the negative correlation between these two traits (Yang et al., 2022). To further explore the relationships between the most common and less common breeding targets related to N use efficiency, part of this thesis focuses on these associations (**Paper I**).

1.5.2 Root traits and their significance under osmotic stress and low-temperature conditions

Continued climate change is projected to significantly reduce water availability for agriculture in many regions globally. In Sweden, for instance, the frequency and intensity of summer droughts are expected to increase (Johansson and Mobjörk, 2009). The timing of a drought plays a critical role in determining its impact on wheat production. Drought-induced osmotic stress early in the growing season can delay the formation of floral structures, ultimately reducing the number of grains produced (Blum, 2010). From a N accumulation efficiency (NAE) perspective, a decline in yield directly affects the plant’s ability to convert N into grain biomass, specifically impacting the yield-specific N efficiency (E_N), as defined by Weih et al.

(2011, 2018). Moreover, the performance of wheat under drought conditions is influenced not only by the timing of water stress but also by the effects of genotype and genotype by environment interactions (Asplund et al., 2014). Low temperature has also been reported as a factor that decreases the efficiency of N uptake by plant roots (Glass, 2003). Thus, low temperatures are likely to favor less efficient, low-affinity N fluxes. Although acclimation to low temperatures may modify the magnitude of this effect, a reduction of plant N demand under low-temperature conditions potentially increases fertilizer N losses (Glass, 2003).

Rapid root growth early in the growing season, often defined as early (seed) vigor, is a crucial crop property for water and nutrient uptake of spring crops, especially under suboptimal conditions such as low temperatures and drought. Spring wheat varieties characterized by early or high seed vigor present more efficient root spread, thereby enhancing resilience in water-stress environments (Liu et al., 2021). Low vigor seeds contain lower levels of essential storage components such as proteins, lipids, and carbohydrates, which leads to poor performance under stress (Andrade et al., 2020). High seed vigor is therefore expected to support drought tolerance, nutrient uptake, and yield stability, particularly in high latitude regions with short growing seasons (Chawade et al., 2018). Selecting varieties with early-vigor root traits can boost drought resilience and nutrient efficiency, which is vital for maintaining stable yields in the face of climate change (Palta and Watt, 2009).

The architecture of the root system is fundamental to nutrient and water uptake, anchorage, nutrient storage, and plant-microbe interactions (Atkinson et al., 2015, Bishopp and Lynch, 2015). However, despite their direct impact on grain yield, root traits are rarely included in breeding programs due to the various challenges of studying roots in soil (Atkinson et al., 2015). Additionally, the presence of reduced height (*Rht*) genes in many modern varieties has been shown to limit root growth, complicating the efforts to optimize root systems (Bai et al., 2013). Recognizing the importance of root traits is essential to address future food demands. Enhancing root biomass and improving nutrient acquisition efficiency can contribute to higher yields (Atkinson et al., 2015, Bishopp and Lynch, 2015). Moreover, optimizing N uptake through improved root traits can reduce the amount of fertilizer, providing significant environmental benefits and contributing to sustainable agriculture (Atkinson et al., 2015). Due to the importance of early root traits for N-use efficiency, part of this thesis focuses on the genetic and environmental (drought and low temperatures) influences on early root traits in spring wheat (**Paper IV**).

1.5.3 Crop phenotyping and the utilization of allometric relationships between traits

A critical step in any breeding program is the collection of field data for target traits across various growth stages. However, this process is labor intensive, time consuming, resource demanding, and often involves destructive sampling methods (Golzarian et al., 2011). Given that large plant populations are typically required for breeding programs (Bernardo, 2004), there is a need for more efficient and less laborious phenotyping techniques, notably for complex traits such as N use efficiency-related traits. The phenotyping of complex traits can be significantly improved by more rapid and non-destructive phenotyping methods, which save time and resources while maintaining high accuracy (Zhao et al., 2016, Lemaire et al., 2019).

Allometry in plant growth analysis refers to a convenient approach in which the relative growth of a particular structure is calculated in proportion to the growth of another structure or the entire organism (Bakhshandeh et al., 2012). Allometric relationships can be practical tools from an agricultural perspective (Qin et al., 2013), and are useful for estimating relevant traits for plant breeding. Thus, various plant traits, such as plant height and shoot biomass, have been previously associated with allometric methodology due to their evident relationships (Sileshi et al., 2023). Another example is the well-documented link between leaf chlorophyll contents, measured with chlorophyll meters (SPAD meters), and the N content in plant leaves (Peltonen et al., 1995, Spaner et al., 2005, Xiong et al., 2015, Asplund et al., 2016). In theory, it should also be possible to explore the allometric relationships between traits such as grain biomass, or even more complex traits such as NAE and its components, and crop characteristics that can be measured using non-destructive methods, to facilitate rapid phenotyping of complex traits. Therefore, practical allometric approaches could influence agronomic studies regarding phenotyping in plant production systems by using non-destructive estimation for yield and biomass estimations (Weiner, 2004). Such an approach is explored as part of this thesis (**Paper II**), aiming at the prediction of the NAE components by Weih et al. (2018) with the non-destructive assessment of traits that show predictable allometric relationships to them.

1.5.4 Breeding for nitrogen use efficiency

Genetic maps are valuable tools for analyzing economically significant traits that are controlled by the combined action of multiple genes. These traits, known as quantitative or polygenic traits, can be localized within the genome using genetic maps. The study of their genetic basis through those maps is called quantitative trait loci (QTL) mapping. QTL analysis is a statistical approach that associates phenotypic data (trait measurements) with genotypic data (DNA markers linked to specific regions of the genome) (Milczarski et al., 2024). Aside from QTL analysis, genome-wide association study (GWAS) offers an alternative method for analyzing complex traits (Cormier et al., 2014). GWAS proposes an alternative of using a biparental population, including wider populations such as wild species, landraces, and elite cultivars, offering a higher allelic diversity compared to a recombinant inbred line (RIL) population (Cormier et al., 2014, Jia et al., 2024).

The hexaploid nature of wheat makes it more challenging to study compared to most other crops with lower ploidy levels. This genetic complexity means the identification of QTLs for N use efficiency is still limited, in part because of the restricted number of mapping populations available (Shi et al., 2022). Identifying QTLs for N use efficiency in wheat will allow us to reveal genomic components of the traits related to N use efficiency, particularly the interactions among loci. This knowledge is essential for developing molecular markers to monitor the introgression of loci associated with the improvement of N use, for their application in future breeding programs for wheat (Habash et al., 2007).

Due to the importance of finding the genomic regions controlling N use efficiency traits for wheat breeding, another focus of this thesis is the identification of genetic markers and QTLs associated with NAE, its components, and related traits (**Paper III**). This analysis uses a RIL population at the F6 generation, derived from 77 lines of two elite spring wheat varieties, ‘Happy’ and ‘Boett.’ These parental lines were selected for their pronounced phenotypic variability in traits linked to N use efficiency (Liu et al., 2022), providing a starting point for the detection of genetic regions that can be used in breeding programs.

2. Aims and Hypotheses

Using spring wheat as the model plant for this research, the overall objective of this thesis was to explore the use of N accumulation efficiency and its components as potential traits in breeding programs targeting spring wheat grown in high-latitude environments. **Four Papers** are included in this thesis and are interconnected (Figure 1). The following four specific aims were addressed:

(1) To explore the relationships between breeding targets and non-target traits across various groups of wheat varieties field-grown in Central Sweden over two years (**Paper I**);
(2) To assess genetic and environmental influences on early-vigor related root traits (**Paper IV**);
(3) To propose a suitable methodology to rapidly assess N accumulation efficiency (NAE) and its components using spring wheat as an example for cereal crops (**Paper II**); and
(4) To identify genomic regions related to N accumulation efficiency and its components to assist the breeding of spring wheat in high latitude conditions (**Paper III**).

The following hypotheses were explored:

(H1) The relationships between target and non-target traits of Swedish spring wheat varieties are stable across different environments (**Paper I**)

(H2) The non-target traits behind nitrogen accumulation efficiency (NAE) and its components are significantly correlated with the main breeding target traits grain yield and/or protein content (**Papers I and IV**)

(H3) Spring wheat varieties bred for different breeding targets show differential early root growth responses to low temperature and osmotic stress, and are related to N use efficiency (**Paper IV**)

(H4) In spring wheat, stem height and the number of tillers at anthesis effectively predict aboveground biomass, while the relative leaf chlorophyll content during anthesis, combined with the corresponding biomass, can be used as a reliable indicator of the plant's N pool (**Paper II**)

(H5) The spring wheat mapping population investigated in this thesis sufficiently segregates in terms of grain biomass and NAE components, so that associations with genomic regions or genetic markers can be found (**Paper III**)

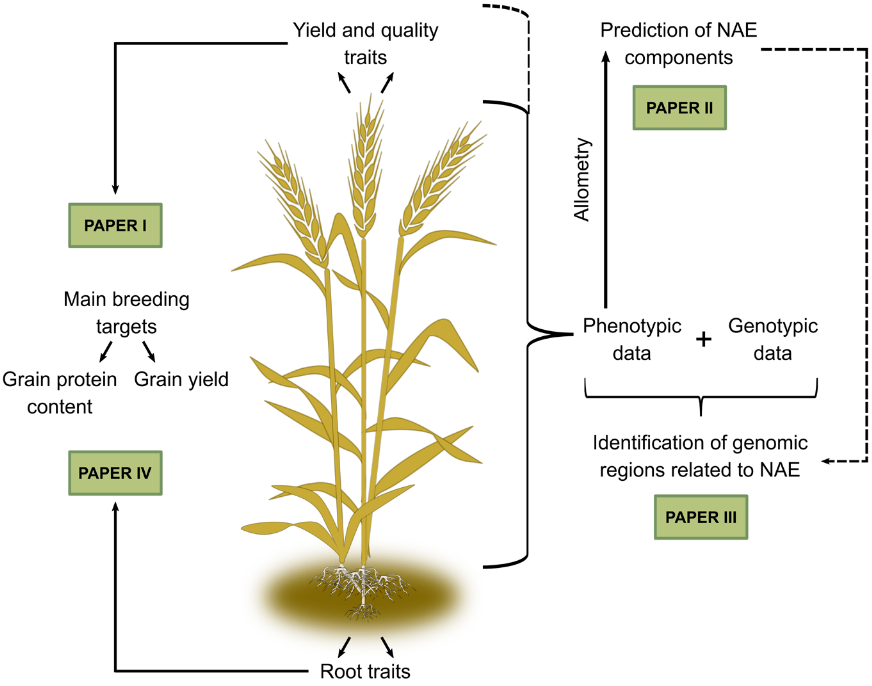


Figure 1. Conceptual diagram of the interconnections between **Papers I, II, III, and IV**, illustrating the various research studies that constitute this thesis. NAE is Nitrogen accumulation efficiency. Illustration: Lorena Guardia Velarde.

3. Materials and Methods

3.1 Plant material

In this thesis, three different sets of plant material were utilized. **Paper I** included nine spring wheat varieties: ’KWS Alderon’ (’Alderon’), ‘Bjarne’, ‘Boett’, ‘Dacke’, ‘Diskett’, ‘Happy’, ‘Quarna’, ‘Rohan’, and a landrace from Dalecarlia ‘Dala’. The varieties were categorized into three different groups in relation to their breeding targets. These groups were (1) high yield, with ‘Alderon’ and ‘Happy’; (2) high protein content, with ‘Bjarne’, ‘Dacke’, ‘Quarna’, and ‘Dala’; and (3) intermediate, with ‘Boett’, ‘Diskett’ and ‘Rohan’. For **Paper IV**, six out of the nine previous varieties were selected and divided into the same breeding target groups: High yield (represented here by ‘Alderon’); high protein content (‘Bjarne’, ‘Dacke’ and ‘Quarna’); and intermediate (‘Diskett’ and ‘Rohan’). **Papers III and IV** were based on a population of 183 spring wheat recombinant inbred lines (RILs) originating from a cross between ’Happy’ and ‘Boett’. F_2 plants were advanced to F_6 generation using the single-seed descent protocol. The parental lines and F_1 hybrid were included in the field trials.

Table 1. List of the spring wheat varieties used in this thesis, their classification according to their main breeding targets, and their contribution to the four Papers of this thesis.

White type	Variety	Paper I	Paper IV	Papers II and III
High yield	Alderon	Alderon	Alderon	
High yield	Happy	Happy		Happy
High protein	Bjarne	Bjarne	Bjarne	↘
High protein	Dacke	Dacke	Dacke	x F_1 → F_6 RIL
High protein	Quarna	Quarna	Quarna	(77 lines)
High protein	Dala	Dala		↗
Intermediate	Boett	Boett		Boett
Intermediate	Diskett	Diskett	Diskett	
Intermediate	Rohan	Rohan	Rohan	

3.2 Study sites and experimental designs

The fieldwork reported in **Papers I, II, and III** was performed in Säby, Uppsala, Central Sweden. For **Paper I**, the field trials were carried out during the 2018 and 2019 growing seasons (April – October). The 2018 growing season was drier and warmer than the corresponding period in 2019 (Weih et al., 2021). Due to a complete lack of rain in the early summer of 2018, artificial irrigation was needed to prevent the loss of plant material (10 mm, 28 and 34 days after planting). The soil was a silty loam (16 % clay, 70 % silt, and 14 % sand) with pH 5.8 and 4 % of organic matter in the top 0.3 m. Nutrient fertilizer (140 kg ha^{-1}N, 24 kg ha^{-1}P, and 46 kg ha^{-1}K) was applied after sowing. The field trials for **Papers II and III** were established in 2022 and 2023 from May to September 2022 and from June to September 2023. The soil was silty clay (44 – 48 % clay, 45 – 51 % silt, and < 4 – 5 % sand) with pH 6.5 and an organic matter content of 2.6 – 3.4 % in the top 0.3 m layer.

The experimental designs in **Papers I to III** were randomized split-plot designs with either four (**Paper I**) or five replicate blocks (**Papers II and III**). In **Paper I**, plots were 12 x 2 m in size and two soil treatments were applied, compaction vs. non-compaction. In **Papers II and III**, micro-plots, 0.62 m x 0.75 m in size, were arranged in six rows, where the central four rows were used for the 183 (2022) or 179 (2023) RILs, bordered by 'Boett' on both sides. Each micro-plot was sown with 45 seeds per row at a density of 550 seeds m-^{2}, with 3-5 repetitions based on seed availability.

Instead of field experimentation, **Paper IV** used the data from a lab experiment conducted at Mendel University in Brno, Czech Republic in collaboration with SLU. The experiment used six commercial varieties ('Bjarne', 'Rohan', 'KWS Alderon', 'Diskett', 'Quarna', and 'Dacke') and included three treatments: optimal temperature (20 °C) without drought ('control'); sub-optimal temperature (10 °C) with osmotic-stress; and closer to the minimum temperature for growing conditions (7 °C, simulating cold stress) with osmotic-stress. Each replication consisted of three Petri dishes, each containing six seeds of the same variety (18 seeds per replication). Each variety was tested with four replications, a total of 72 seeds per variety. In our statistical analysis, each plant was treated as an individual experimental unit, allowing us to consider every plant's value as a single data point. All grains were visually inspected prior to their use in the experiment to ensure a healthy status. Six seeds per variety were selected and sterilized by immersing them in a 3 % sodium hypochlorite solution for 10 minutes, followed by three rinses with distilled water. To evaluate early vigor, the seeds were placed in sterile Petri dishes (90 mm diameter). Osmotic-stress (drought) conditions were simulated by adding 8 ml of polyethylene glycol

(PEG 6000) solution to reach a water potential of -0.5 MPa, as described by (Michel and Kaufmann, 1973). The control group was treated with distilled water. All Petri dishes were sealed to avoid evaporation and incubated in a climate-controlled chamber in complete darkness. The control group followed standard germination procedures according to ISTA guidelines, (20 °C, without osmotic stress) (ISTA, 2007). Germination rates and early vigor were evaluated after 7 and 14 days, with the control group assessed after 7 days.

3.3 Sample analysis

In **Paper I**, for both years (2018 and 2019), five plants were sampled from uniform areas of each plot at tillering (BBCH29), flowering (BBCH65), and spike maturity (BBCH89) stages. The sampled plants were cut about 15 mm above the soil surface, dried at 65 °C for 48 h, and weighed. The 6 m × 2 m central plot area was harvested with a combine harvester to obtain grain yield, with subsamples tested for protein, gluten, starch, thousand kernel weight (TKW), hectoliter weight (HLW), and falling number. The spikes were hand-threshed using a Smooth Chopper 6948A-2, and the dry grains were weighed. Samples were grounded to a 1 mm mesh for nutrient analysis. Measurements included grain yield, TKW, grain protein, gluten, starch, HLW, falling number, and grain concentrations of N, P, Zn, and Fe. The N accumulation efficiency traits (U_N, E_N, C_N, and NAE) were also assessed. The N concentrations in seeds, shoots, and grains from five plants sampled in 0.5 m × 0.5 m plots were analyzed with a LECO CNS72000 analyzer (SS-ISO13878). The P, Fe, and Zn contents were extracted using 32.5 % nitric acid on a heat block, and concentrations were measured via ICP-AES (Spectro Blue FMS 26) following internal protocols (SS028311). The protein, starch, HLW, and gluten contents were determined with a FOSS Infratec NOVA analyzer; TKW was measured using an Opto-Agri machine, and the falling number was determined with a FOSS Alphatec FN° (AACC 56-81B method).

In **Paper II**, the tiller number was counted, and plant height was measured from ground level to the spike tip on the main tiller using a measuring tape, with three plants recorded per replication during anthesis. The chlorophyll content of the second leaf was recorded at the same stage on these plants using a SPAD meter (SPAD-502, Konica Minolta Sensing Inc., Japan), averaging three readings per leaf. Additionally, 72 samples from 14 randomly selected lines and parental lines were collected to analyze shoot biomass and N content. Destructive sampling for biomass determination was conducted at full maturity for all lines cutting five plants at ground level in the middle of each plot. The samples were dried at 70 °C for 48 h, followed by weighing and grain threshing. Nitrogen content was determined for 80 selected RILs (including parental lines and F_1) using a LECO CNS72000 analyzer. Analyses included initial seed samples, shoot samples, and four replicates per line at maturity (for details, see **Paper II**). Furthermore, in **Paper III** the predicted values of Mn and P (SpectraCrop ApS) were recorded at anthesis (Figure 2).

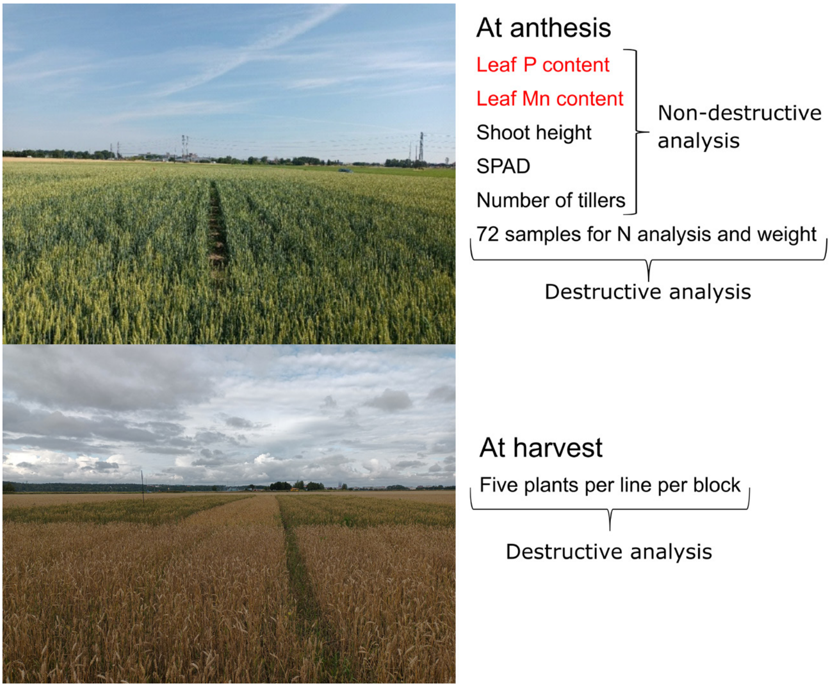


Figure 2. Non-destructive and destructive sampling recorded in field-grown spring wheat at anthesis and harvest stage for **Papers II and III**. The traits in red are related only to **Paper III**. Photos and diagram: Lorena Guardia Velarde.

For **Paper VI**, early vigor of seeds grown in Petri dishes was measured by recording the root lengths (cm) and diameters (mm) after 7 and 14 days. Both traits were assessed by imaging roots with a conventional scanner and subsequent analysis using WinRHIZO (Régent Instruments Inc., Quebec, Canada, version 2020 Arabidopsis).

3.4 Nitrogen accumulation efficiency calculations

In **Papers I to III**, the NAE and its components (U_N, E_N, and C_N) were calculated according to the following equation proposed by Weih et al. (2018):

$$NAE = U_N * E_N * C_N$$

Where U_N (g g^{-1}) represents the N uptake efficiency, calculated as the mean N amount for the whole growing period divided by the N amount in the initial sown seed. The grain-specific N efficiency (E_N, g g^{-1}) is calculated as the grain biomass divided by the mean N amount during the complete growth period. The grain N concentration (C_N, g g^{-1}) is the N content of the grain yield per grain biomass at final harvest.

For **Papers II and III**, an allometry approach was applied and the values of NAE and its components were determined by using the observed data of the initial seed N content and N content in plants at maturity stage, combined with estimated values (using non-destructive measurements) of plant N contents at anthesis. Thus, plant height and tiller number were used to estimate shoot biomass, while SPAD readings in combination with the estimates of shoot biomass were utilized to predict the plant N pools (Figure 3).

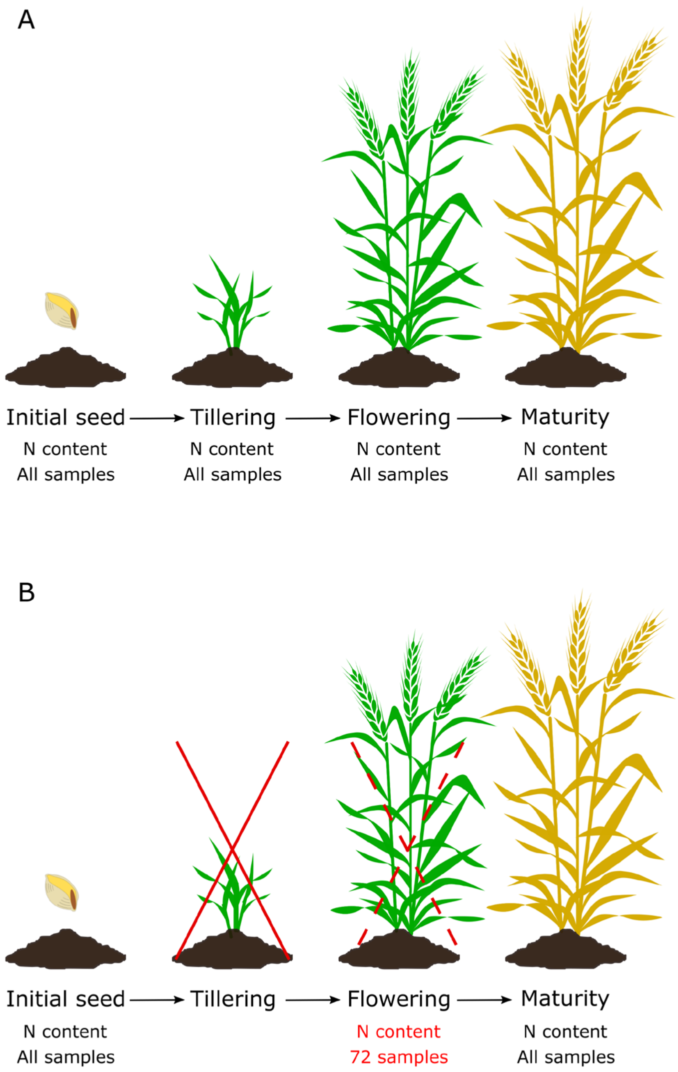


Figure 3. Illustration of the method used to estimate N accumulation efficiency (NAE) and its components developed in **Paper II**. A) The NAE calculation is based on destructive sampling at four developmental stages, as proposed by Weih et al. (2018). B) Shows the approach undertaken in **Paper II**. Destructive sampling was conducted at the initial seed stage and at maturity for 80 lines (with four replicates each). No samples were collected at the tillering stage, while 72 destructive samples were taken at anthesis to calibrate equations for an allometric method using non-destructive sampling. Adapted from **Paper II**. Illustration: Lorena Guardia Velarde.

3.5 Statistical analysis

For processing the data, basic descriptive statistics were conducted, complemented by linear regression (**Papers I, and II**), non-linear regression (**Paper II**), correlation (**Papers I, III, and IV**), ANOVA (**Papers I and II**), and Principal Component Analysis (PCA) (**Papers I and IV**). All statistics were computed using SPSS 26 and 29 (**Papers I, II, III, and IV**) and Python (**Paper IV**). In **Paper I**, a PCA following CATPCA methodology was computed to evaluate the associations between non-target traits and wheat types. Regarding **Paper II**, four regression models (a linear model, two multiple linear models, and a non-linear model) were performed for the estimation of shoot biomass from plant height and tiller number values. For the estimation of shoot N pool from shoot biomass and SPAD measurements, two regression models (multiple linear model and non-linear model) were used. All regression models utilized data from non-destructive assessments (height, tiller number, and SPAD) as well as destructive sampling (plant biomass, N content) for the model equations and calibrations. To verify the suggested equations, a linear regression between the predicted and observed values was calculated to obtain the coefficient of determination and residual sums of squares (RSS). For **Paper III**, to select candidate lines that represent desirable combinations of NAE-related traits with high grain yield, data was normalized and categorized based on a threshold: lines with values ≤ 0.5 for a given trait were categorized as 0, and lines with values >0.5 were categorized as 1. This allowed for a screening procedure, with 1 indicating lines with trait means above the average of all lines, and 0 representing average or below average values for trait means of a given line. For the final screening, five important traits, grain biomass, U_N, E_N, C_N, and NAE, were selected to evaluate individual lines for desirable N use efficiency performance. For some of the assessed traits, genetic markers and genomic regions were identified, and Spearman´s rank correlation analysis was used to assess the relationships between those traits and NAE components.

3.6 Genotypic data

In **Paper III**, a genotypic analysis of the RILs and the parental lines was carried out using the Illumina 25 XT Infinium array for wheat (TraitGenetics, Gatersleben, Germany; requested by Lantmännen). The genotypic data was prepared by filtering and discarding markers that showed a low call rate (<80

%) and low minor allele frequency (<10 %), which also eliminated the lines that displayed a low rate of marker return (<80 %) or large heterozygosity (false discovery rate <10 %). A linkage map was built based on the selected markers by using the MAP function of QTL IciMapping v4.0 (Meng et al., 2015) with the inclusive composite interval mapping of additive QTL (ICIM-AA) method for the detection of QTL. To perform a genome-wide association studies (GWAS) analysis, the R program (R Core Team, 2021) was used. The GAPIT package version 3 (Wang and Zhang, 2021) was used and the FarmCPU (Liu et al., 2016) method was applied.

4. Results and Discussion

Agricultural practices contribute significantly to environmental contamination worldwide. The overuse of fertilizers to maximize crop yields, combined with the limited nutrient uptake capacity of plants, particularly for N, has led to nutrient leaching into groundwater. This process has tangible environmental impacts, such as the eutrophication of the Baltic Sea observed in Northern Europe (Jansson et al., 2019). Implementing strategies to mitigate N pollution while also ensuring that farmers meet crop yield and quality expectations is vital.

Each Paper in this thesis contributes a partial aspect toward achieving the primary goal of this thesis: establishing N accumulation efficiency (NAE) and its components, N uptake efficiency (U_N), yield-specific N efficiency (E_N), and grain N concentration (C_N), as potential traits to be considered in breeding programs targeting spring wheat grown in high-latitude environments. Combined, these studies provide a basis for a clearer understanding of the genetic and phenotypic mechanisms underlying NAE and its components, while also demonstrating their practical relevance for wheat breeding serving a sustainable agriculture.

As an additional challenge, climate change presents an ongoing and likely growing threat to agricultural productivity and food security, as increasing temperatures and unpredictable weather interfere with the optimal conditions which are necessary for crop growth and development (Steensland and Zeigler, 2021). Moreover, modern agriculture, with its widespread use of commercial fertilizers, pesticides, and herbicides, has detrimental effects on the climate and environment, directly contributing to issues such as soil erosion, nutrient leaching, and increased greenhouse gas emissions (Tudi et al., 2021). Therefore, crop N use efficiency is a key aspect that must be considered in any efforts to decrease the risks of environmental pollution in modern agricultural production.

Crop and genotype performance in different environments is often assessed in terms of trait stability, most prominently yield stability. In this thesis, a static stability approach, aiming at similar performance across different environments was adopted, as opposed to a dynamic stability approach (Knapp et al., 2017). The static stability approach was selected because all plant material used in this thesis was developed for the high latitude environmental conditions in Sweden. Trait stability is accomplished through phenotypic plasticity, which is the spectrum of phenotypes a single genotype can exhibit in response to different environmental conditions (Nicotra et al., 2010). This flexibility in phenotype expression provides a defense against rapid climate shifts and facilitates swift adaptation, making it a key component of plant responses in both the short and long-term (Nicotra et al., 2010). The results in this thesis suggest that the selected varieties may possess an ability to sense environmental changes and partly adapt to them through plastic responses, thereby supporting their primary breeding targets despite changing conditions. Since phenotypic plasticity has a genetic component and can be targeted through breeding (Martre et al., 2015), it is likely that the wheat varieties studied in this thesis differed in their levels of trait stability and/or plasticity.

4.1 Wheat type and environmental impacts on trait stability (**Paper I**)

To explore the correlation pattern between traits and evaluate the associations between different spring wheat types classified according to their main breeding targets (1 - High yield, 2 - Organic high protein, and 3 – Intermediate) and their trait expressions in contrasting environments (here represented by two years with contrasting weather conditions), a Principal Component Analysis (PCA) was conducted (Fig. 4). Similar patterns in the majority of traits were observed in both environments (years), indicating that even under the droughty conditions of one year (2018), grain yield and starch content clustered together with wheat type 1 (high yield) and 3 (intermediate), while grain protein, gluten, hectoliter weight (HLW), Zn, N, P, and falling number were linked to wheat type 2 (Organic high protein) (Figure 4A). In the year with less extreme weather conditions (2019), grain protein, gluten, Zn, N, and P were still linked to wheat type 2 (Organic high protein), while grain yield, starch, thousand kernel weight (TKW), falling number, and HLW were associated with wheat type 1 (High yield) (Figure 4B). Thus, the wheat type 1 (High yield) consistently aligned with high grain yield, high starch content, and high TKW in both years, while wheat type 2 (Organic high protein) correlated with high grain protein, gluten, and macro and micro elements. The wheat type 3 (Intermediate) generally performed similar to wheat type 1 (High yield) in both years. Similar to the correlation analysis results reported in **Paper I**, HLW and falling number showed contrasting patterns in the PCA across the two years, as both were associated with different wheat types in 2018 and 2019 (Figure 4).

This thesis demonstrated that target traits in spring wheat breeding (i.e., high grain yield and high protein content) and most non-target traits (including grain starch and nutrient contents) seem to be linked to each other across different environments (here weather conditions). However, the results also show that certain traits (e.g. HLW, falling number) are more affected by the environmental conditions than the breeding target. These traits seem to be linked to different breeding targets, depending on the environmental conditions.

The results support hypothesis 1 (H1), which stated that the relationship between target and non-target traits is stable across different environments. The robust associations between many target and non-target traits observed here may have been caused by those traits having been co-inherited during the domestication process (Doebley et al., 2006). Regarding HLW and falling number and their inconsistent relationships with specific breeding targets and wheat types, the results of this thesis are in line with the findings of Koppel and Ingver (2008) and Mut et al. (2010), which suggests that these traits do not show static stability and are highly affected by the environmental conditions.

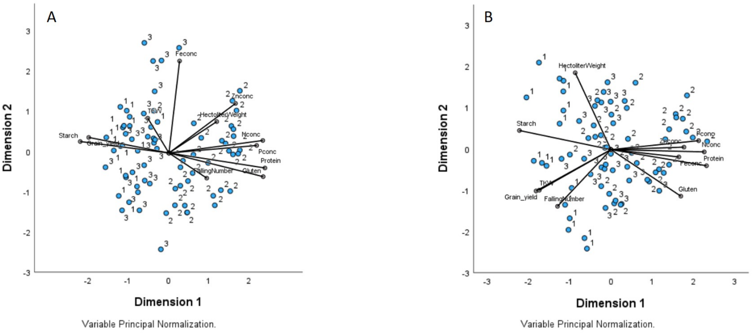


Figure 4. Biplot showing a group of variables by Principal Component Analysis (PCA) for 2018 (A) and 2019 (B) datasets, based on trait values from three wheat type groups: 1 - High yield, 2 - Organic high protein, and 3 - Intermediate, across nine varieties in Sweden. In 2018, dimension 1 had an Eigenvalue of 5.7 (57 % explanatory power) and dimension 2 had 1.5 (15 %). In 2019, dimension 1 had 6.2 (62 %) and dimension 2 had 1.5 (15 %). Traits analyzed: grain yield, protein, starch, gluten, hectoliter weight, TKW thousand-kernel weight, falling number, and concentrations of nitrogen Nconc, phosphorus Pconc, zinc Znconc, and iron Feconc. The figure is reprinted from **Paper I** under the Creative Commons CC BY license (https://creativecommons.org/licenses/by/4.0/).

4.2 Associations between NAE components, root traits, and wheat types (**Papers I, III, and IV**)

4.2.1 Variation in NAE components across wheat types and breeding targets

Mean values of grain biomass, grain protein content, NAE and its components U_N, E_N, and C_N were compared between nine spring wheat varieties field-grown in Uppsala during two years (**Paper I**, Figure 5). Significant differences were found between wheat types regarding grain biomass, grain protein content, E_N, and C_N. As expected, varieties of the High-yield type showed higher grain biomass and E_N, while varieties belonging to the Organic high protein type revealed higher grain protein content and C_N. A tight link between grain protein content and C_N was expected, because grain protein content is usually a function of grain N content, e.g., Weih et al. (2021). The results support the second hypothesis (H2) of this thesis, which stated that the non-target traits related to NAE and its components show significant correlations with the main breeding target traits grain yield and/or protein content. However, U_N and NAE did not show any clear association with any of the wheat types, therefore conflicting with H2. To evaluate the possibility of genotype x environment interaction (G x E) for U_N and NAE, their mean values were also plotted separately for the two years (Figure 6), but still did not reveal any apparent associations between these two traits and the wheat types. The lack of any apparent association between NAE (or U_N) and wheat type suggests that the traits related to N accumulation, grain yield, and grain protein content are inherited independently, and are linked to individual varieties rather than specific wheat types. Significant genotype effects on U_N and NAE were reported in **Paper I**, and in previous studies (Weih et al., 2021, Liu et al., 2022).

The NAE data used for **Paper I** are the same as those reported by Liu et al. (2022). However, while Liu et al. (2022) did not consider grain quality aspects, and based their study on variety differences, **Paper I** also included grain quality aspects and grouped the same varieties according to their breeding targets (i.e., High yield, Organic high protein, Intermediate). The consideration of breeding targets in this thesis enabled an exploration of the links between the traditional breeding targets grain yield and grain protein content on the one hand, and the addition of NAE and its components as regular traits alongside the primary breeding targets. For instance, if the goal is to enhance E_N or U_N, identifying the corresponding wheat type and its associated breeding target provides a clear and efficient way to achieve the desired outcomes. Although N use efficiency traits are considered complex and polygenic (Habash et al., 2007, Fontaine et al., 2009, Vukasovic et al., 2024), the E_N and C_N assessed in this thesis demonstrated associations with specific wheat types. The results of this thesis confirmed that wheat varieties bred for high grain yield are characterized by the ability to efficiently convert N into grain yield (i.e., high E_N), while the N uptake efficiency (U_N) was variable within the group of High yield wheats (Figure 6). From an environmental sustainability perspective, a desirable combination of traits would be a high grain yield along with high E_N but low U_N, because this particular combination of characteristics would represent a high-yielding crop requiring relatively low N fertilization (Pourazari et al., 2018). In this thesis (**Paper III**), I explored a population of 183 spring wheat recombinant inbred lines (RILs), originating from a cross between the commercial varieties ’Happy’ and ‘Boett‘, to find lines that would possibly combine high grain yield with high E_N and low U_N. Interestingly, one line (RIL 145) seemed to represent this exact combination of traits and is therefore proposed as a promising germplasm donor. Thus, RIL 145 could serve as a key candidate line in future breeding programs focusing on enhanced E_N, reduced U_N, and above-average grain yield (**Paper III**).

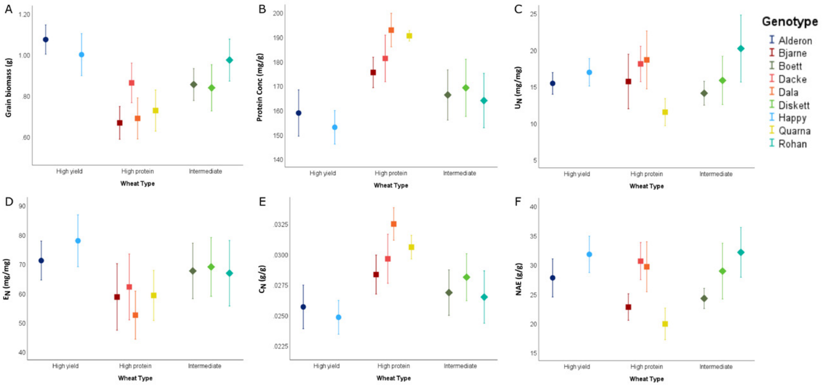


Figure 5. Mean values and 95 % confidence intervals for six traits measured on nine spring wheat varieties field-grown in Uppsala for two years (2018, 2019). The varieties are grouped according to the three wheat types: High yield, Organic high protein, and Intermediate. (A) grain biomass, (B) grain protein, (C) N uptake efficiency (U_N), (D) yield-specific N efficiency (E_N), (E) grain N concentration (C_N), and (F) N accumulation efficiency (NAE). Adapted from **Paper I**.

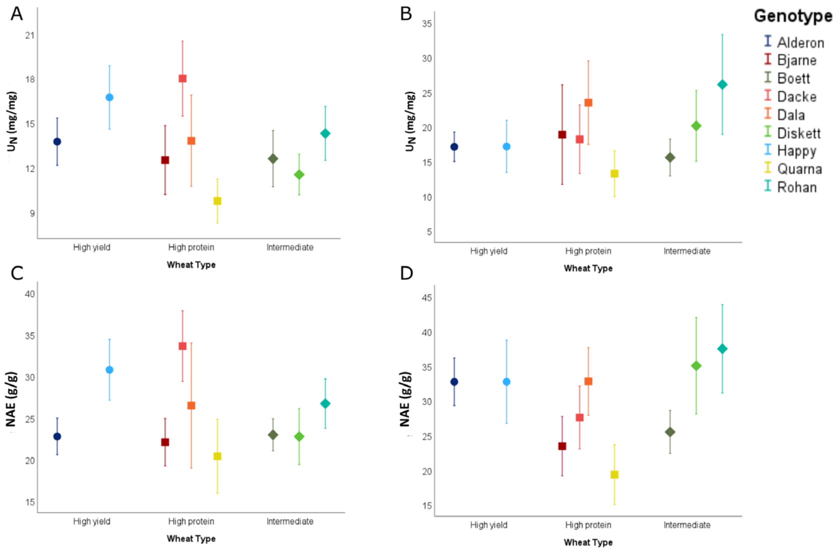


Figure 6. Mean values and 95 % confidence intervals for two traits, U_N (A, B) and NAE (C, D), measured on nine spring wheat varieties field-grown in Uppsala for two years, 2018 (A, C) and 2019 (B, D). The varieties are grouped according to the three wheat types: High yield, Organic high protein, and Intermediate. Adapted from **Paper I**.

4.2.2 Root traits and their associations with wheat types and NAE components

To assess the possible associations between wheat types, NAE components, and (juvenile) root traits, data from **Papers I, and IV** was combined. In **Paper IV**, the root length and diameter of six spring wheat varieties belonging to the same wheat types that were assessed in **Paper I**, were evaluated shortly (7 and 14 days) after germination in a controlled laboratory environment representing drought and cold stress treatments in comparison to a control (optimal temperature without drought stress). Only in the control conditions, the high-yield variety 'Alderon' exhibited shorter roots than the high protein wheat type and intermediate (except for ‘Rohan’), and the high-protein type varieties (‘Bjarne’, ‘Dacke’, ‘Quarna’) displayed the longest roots, while this pattern was not observed in the drought and cold stress treatments (Figure 7). Additionally, the intermediate variety ‘Rohan’ consistently showed low root lengths across all the scenarios (Figure 7). Root diameters were greatest in ‘Alderon’ for most treatments, while two out of three high-protein varieties ('Bjarne' and 'Quarna') exhibited the lowest values in the control and drought treatments (Figure 8).

These findings suggest that, while some similarities exist between wheat types (especially under optimal conditions), the presence of only one variety within certain types (e.g., the high-yield variety 'Alderon') makes it challenging to draw general conclusions about patterns in root length and diameter at early growth stages under optimal and stress conditions. Consequently, the results indicate that changes between varieties are likely variety-specific, highlighting the importance of studying these traits and treatments at the variety level rather than grouping them by wheat type, which does not provide any clear support to hypothesis 3 (H3) of this thesis. For example, 'Bjarne' developed long and thin roots particularly under the control conditions in **Paper IV**, which indicates an ability to rapidly reach water and nutrients from subsoil layers (Palta and Watt, 2009). Developing thinner roots and more metaxylem vessels can be considered as conferring increased resilience to cope with restricted water access, such as different drought scenarios (Liu et al., 2022).

The variety differences in early root growth observed (**Paper IV**) in the control treatment could be more pronounced under suboptimal conditions, such as the osmotic stress (simulating drought) and low temperature treatments applied in this thesis. The varieties in this thesis have shown different performances across treatments (e.g. 'Dacke' in root diameter), exemplifying the interaction between genotype and environment (G x E). However, a deeper understanding of the physiological and genetic factors driving G x E, particularly in the context of climate change, is still missing (Aspinwall et al., 2015).

In contrast to 'Bjarne', 'Alderon' developed relatively short and thick roots in most treatments (Figures 7, 8; **Paper IV**), which is consistent with the findings from a field study in which a similar set of varieties was investigated for linking root traits to NAE and its components (Liu et al. (2022). Liu et al. (2022) found that U_N increased with smaller root diameters, while E_N increased with larger root diameters. In this thesis, 'Alderon' was associated with higher E_N (Figure 5), supporting the conclusions by Liu et al. (2022). However, no significant associations were detected between root diameter and U_N for any variety in this study (Figures 5 and 6).

In addition, **Paper IV** also investigated the genetic factors influencing early root development, focusing on specific genes and their allelic differences that affect different aspects of plant growth, including root traits. The key genes studied include PPD-D1, which is related to photoperiod sensitivity and flowering (Foulkes et al., 2004, Gomez et al., 2014), and VRN-1, which is involved in the vernalization pathway (Deng et al., 2015). In this thesis, ‘Bjarne’ was shown to carry the photoperiod-insensitive allele (Ppd-D1a), which is linked to early flowering and potentially early root production under control conditions, as shown in Figure 7A. Contrastingly, the other varieties were shown to carry the photoperiod-sensitive allele (ppd-D1b) (**Paper IV**). While mutations in VRN-D1 indicate that, all genotypes might display a facultative growth habit, even though some carry winter alleles at other loci (**Paper IV**).

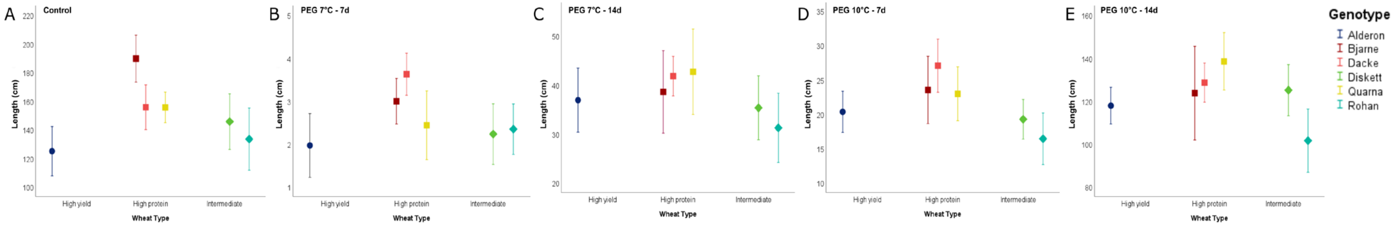


Figure 7. Mean values and 95 % confidence intervals of root length after 7 and 14 days for 6 spring wheat varieties grown in a short-term lab experiment and exposed to three treatments, (A) Control, (B) drought 7 °C – 7 days, (C) drought 7 °C – 14 days, (D) drought 10 °C – 7 days, and (E) drought 10 °C – 14 days. The varieties were grouped into three wheat types (High yield, Organic high protein, and Intermediate).

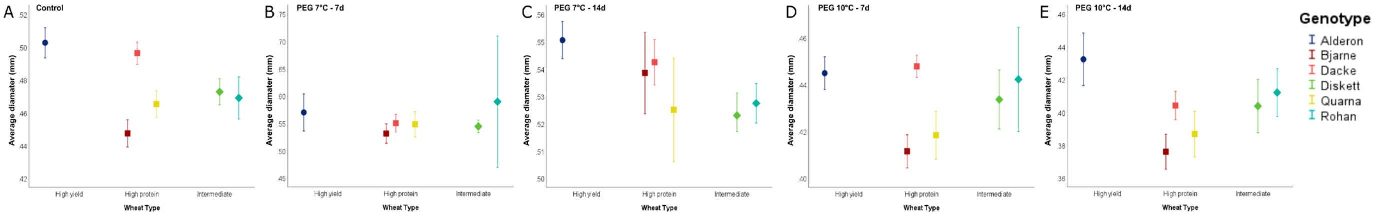


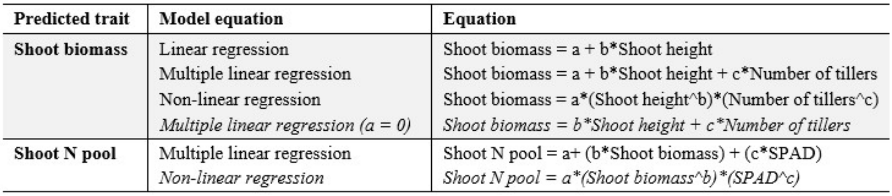
Figure 8. Mean values and 95 % confidence intervals of root diameter after 7 and 14 days for 6 spring wheat varieties grown in a short-term lab experiment and exposed to three treatments, (A) Control, (B) drought 7 °C – 7 days, (C) drought 7 °C – 14 days, (D) drought 10 °C – 7 days, and (E) drought 10 °C – 14 days. The varieties were grouped into three wheat types (High yield, Organic high protein, and Intermediate). PEG, drought treatment.

4.3 Prediction of NAE components using non-destructive assessments and allometry: a method development to support rapid breeding towards higher N use efficiency (**Paper II**)

Using spring wheat as a model crop, **Paper II** aimed at developing a simple methodology for utilizing allometric relationships to reduce the amount of destructive sampling when assessing important N use efficiency characteristics of crops. The two steps involved were (1) the estimation of crop shoot biomass from measurements that can be performed non-destructively (shoot height and number of tillers); and (2) the estimation of shoot N pools from non-destructive chlorophyll meter measurements coupled with the shoot biomass estimates. The methodology was developed to assess crucial aspects of N use efficiency using the concept of N accumulation efficiency (NAE) (Weih et al., 2018), for which the assessment of shoot N pool at flowering is needed. Four regression models were explored to accomplish the first step: a linear regression model, two multiple linear regression models, and a non-linear regression model. A multiple linear regression model with a fixed constant at zero (Table 2) was considered as the most suitable for the data at hand because this model is robust with also relatively small data, avoids subtraction and does not generate negative constants. The model parameters were determined separately for each year (Table 3), to accommodate the large environmental (weather) differences between the two investigated years. Although requiring specific calibration for every given environment, this step is essential to accurately capture environmental variations and provide reliable model equations. Using the above model, the estimated shoot biomass, here based on the non-destructively assessed shoot height and tiller number, adequately predicted the destructively assessed shoot biomass (Figure 9), indicating a good model fit.

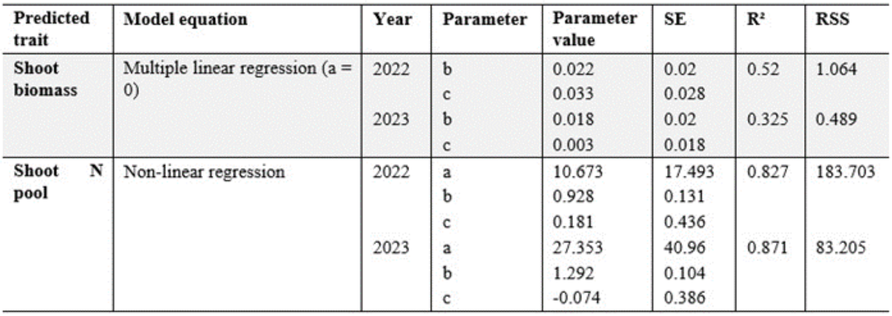
To accomplish step 2 of the methodology, namely the estimation of shoot N pool without using destructive sampling, the estimated shoot biomass (from step 1) was combined with chlorophyll meter (SPAD) values assessed non-destructively at anthesis, exploring multiple linear and non-linear regression models (Table 2). Due to a higher coefficient of determination (R^2) and a lower residual sum of squares (RSS), the non-linear regression model was selected. Parameters were also calculated separately for each year to accommodate inter-annual variation (Table 3). A regression of the estimated values (based on estimated shoot biomass and measured SPAD) against observed shoot N pools showed a good model fit (Figure 10). Thus, by linking together two different regression models (step 1 and 2 above), it was possible to use only non-destructive measurements to accurately estimate shoot N pool at flowering, which is an essential input variable for the calculation of the NAE components N uptake efficiency (U_N) and grain specific N efficiency (E_N).

Table 2. Summary of the equations used for the prediction of shoot biomass and shoot N pool in spring wheat lines field-grown in Uppsala, Sweden, for two years (2022 and 2023). The selected equation for each trait is shown in italics. Adapted from **Paper II.**



Predicted trait	Model equation	Equation
Shoot biomass	Linear regression	Shoot biomass = a + b*Shoot height
	Multiple linear regression	Shoot biomass = a + b*Shoot height + c*Number of tillers
	Non-linear regression	Shoot biomass = a*(Shoot height^b)*(Number of tillers^c)
	Multiple linear regression (a = 0)	_Shoot biomass = b*Shoot height + c*Number of tillers_
Shoot N pool	Multiple linear regression	Shoot N pool = a+ (b*Shoot biomass) + (c*SPAD)
	Non-linear regression	_Shoot N pool = a*(Shoot biomass^b)*(SPAD^c)_

Table 3. Summary of statistics from the equation's parameters of shoot biomass and shoot N pool at anthesis in wheat lines grown in Uppsala, Sweden in 2022 and 2023. Parameter estimation, standard error (SE), coefficient of determination (R^2), and residual sum of the squares (RSS) for the different regression models per year. Adapted from **Paper II**.



Predicted trait	Model equation	Year	Parameter	Parameter value	SE	R^2	RSS
Shoot biomass	Multiple linear regression (a = 0)	2022	b	0.022	0.02	0.52	1.064
			c	0.033	0.028		
		2023	b	0.018	0.02	0.325	0.489
			c	0.003	0.018		
Shoot N pool	Non-linear regression	2022	a	10.673	17.493	0.827	183.703
			b	0.928	0.131		
			c	0.181	0.436		
		2023	a	27.353	40.96	0.871	83.205
			b	1.292	0.104		
			c	-0.074	0.386		

As a final proof, the estimated (with non-destructive methods) N pools at anthesis were combined with destructively assessed data on initial (sown seed) and final (at crop maturity) plant N pools, and the two NAE components U_N and E_N were calculated according to Weih et al. (2011, 2018). The data based on partly (at anthesis) non-destructively assessed N pools (here called predicted U_N or E_N) were plotted against the corresponding data that were entirely based on solely destructively assessed data (here called observed U_N or E_N) in a linear regression, showing a good fit of the predicted U_N and E_N (Figure 11).

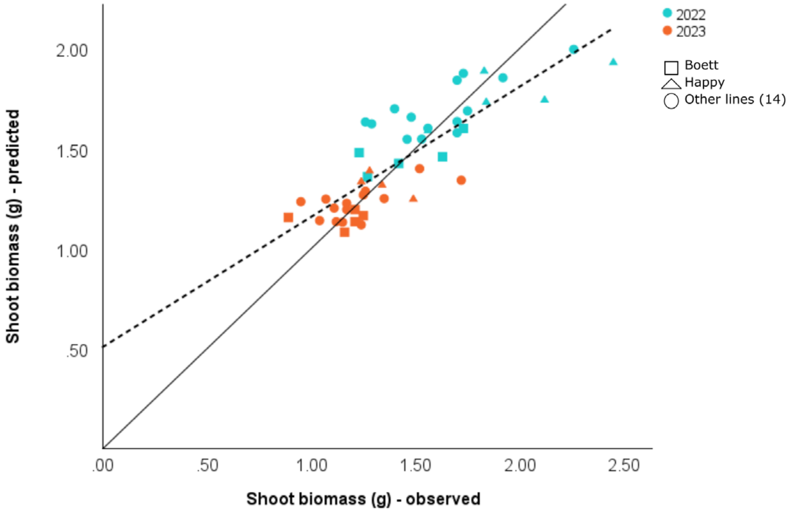


Figure 9. Linear regression of predicted values for shoot biomass from mean stem height and mean tiller number against observed (destructively assessed) values of shoot biomass at anthesis. The predicted values were calculated using a multiple linear regression model (Table 2, in italics). The solid one-to-one line at the origin represents the ideal prediction and the dashed line shows the regression line from our model prediction. Different colors represent data from different years (sky blue: 2022, orange: 2023). Adapted from **Paper II**.

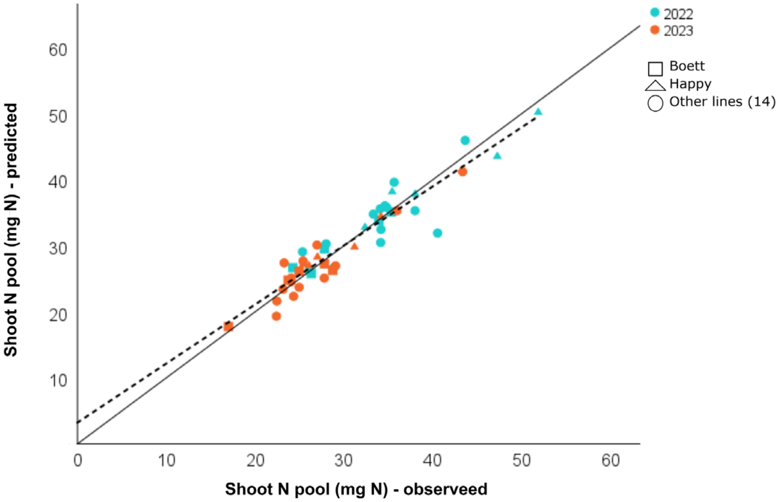


Figure 10. Linear regression of predicted (using non-destructive assessments) and observed values of shoot N pool at anthesis. The predicted values were calculated using a non-linear regression model (Table 2, in italics). The solid one-to-one line at the origin represents the ideal prediction and the dashed line shows the regression line from our model prediction. Different colors represent data from different years (sky blue: 2022, orange: 2023). Adapted from **Paper II**.

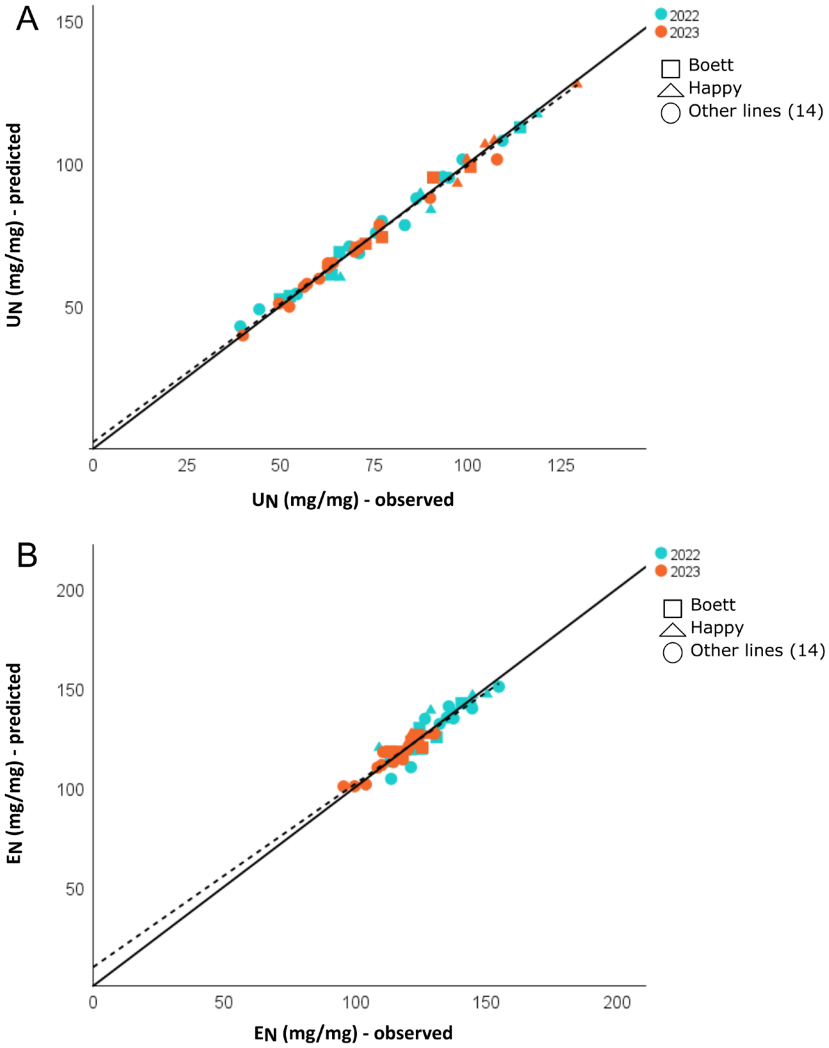


Figure 11. Linear regressions of predicted against observed data of (A) N uptake efficiency (U_N) and (B) grain-specific N efficiency (E_N). The solid one-to-one line at the origin represents the ideal prediction and the dashed line shows the regression line from our model prediction. Different colors represent data from different years (sky blue: 2022, orange: 2023). Adapted from **Paper II**.

Allometric relationships between traits that can be assessed non-destructively, such as plant height for estimating shoot biomass, have been previously described (Bakhshandeh et al., 2012, Sileshi et al., 2023). In **Paper II**, the allometric associations between some non-destructively assessable traits (i.e., shoot height, tiller number, and leaf chlorophyll content) and the normally destructively assessed traits shoot biomass and N pool were examined, to facilitate the calculation of shoot N pool at anthesis, which is an important input variable for the calculation of NAE and its components. The results support the fourth hypothesis (H4) which stated that the stem height at the flowering stage and the number of tillers are good predictors of aboveground biomass; and the relative leaf chlorophyll amount during flowering, combined with the corresponding biomass, is a good predictor of plant N pool. Additionally, it was proven that the important NAE components U_N and E_N can be reliably estimated by partly using non-destructive methods to estimate shoot N pool at the flowering stage, thus considerably reducing the need of destructive sampling for the assessment of NAE components according to the methodology by Weih et al. (2011, 2018). This methodology traditionally requires major destructive samplings at three developmental stages, which is both highly time-consuming and labor-intensive. The approach proposed in this thesis successfully reduces the amount of destructive sampling for the assessment of NAE, thereby reducing labor costs, time, and the demand for chemical N analyses. The findings have important implications for the phenotyping of breeding populations for NAE and its components, particularly the use of these traits in breeding programs targeting spring wheat grown in high latitude environments.

Phenotyping is a key element in plant breeding and genetics research. To improve phenotypes by optimizing desirable traits with genetic tools, it is essential to document repeated evaluations of phenotypes and genotypes in a cost and time-efficient manner (van Eeuwijk et al., 2019, Crossa et al., 2021). Moreover, suitable models that enable the prediction of phenotypic variation from different environments are required. This thesis demonstrates that the allometric approach is an effective tool to accelerate phenotyping while reducing the amount of destructive sampling.

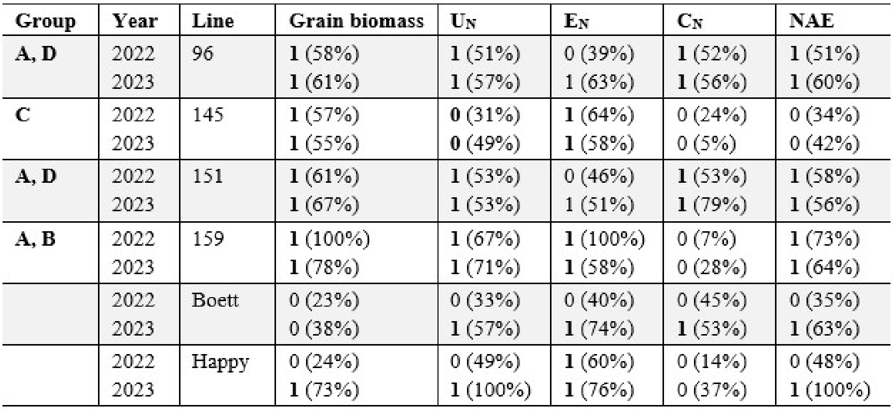
In breeding and pre-breeding programs, large mapping populations are needed to decrease false discovery rate (FDR) and increase the breeding power (Bernardo, 2004). Evaluating NAE and its components in a mapping population typically requires many destructive samplings at the tillering, anthesis, and maturity stages for each individual line and for all repetitions, making the phenotyping for NAE a formidable challenge especially in breeding populations. Additionally, this process makes it impossible to re-use the same plant across different crop stages. To accelerate the acquisition of phenotypic data in large breeding populations, the allometric approach proposed here could be complemented with remote sensing technologies. Such an integration with modern phenotyping technologies would transform the traditional use of destructive sampling into a more efficient method with a reduced dependency on destructive sampling (Furbank and Tester, 2011). Many traits that are important for breeding can now be assessed using digital imaging, which provides significant advantages for the monitoring of plants throughout their entire life cycles and reduces the need for manual scoring, saving both considerable time and effort (Furbank and Tester, 2011). Many of the traits relevant in plant breeding are complex, including plant size, color, biomass, along with other characteristics related to biotic and abiotic stress (Poorter et al., 2010, Furbank and Tester, 2011, Golzarian et al., 2011, York, 2019). To accelerate and simplify crop phenotyping, regression models are frequently used to link breeding-relevant crop characteristics that are difficult to assess to other characteristics that are easier to monitor using simple methods (Martre et al., 2015). In this thesis, two reliable model equations were developed based on allometry to facilitate a possible routine assessment of important N use efficiency characteristics. While regression models based on allometric relationships have been previously used (Spitters, 1989), the combination of trait assessments developed in **Paper II** for the more rapid estimation of important crop N use efficiency characteristics (here NAE and its components) is an important achievement of this thesis. This becomes particularly evident in the context of **Paper III**, where genomic regions for NAE and its components were identified, highlighting the importance of efficient phenotyping methodologies to routinely use these traits in breeding.

4.4 Identification of candidate lines and genomic regions for strategic breeding towards high-yielding spring wheat varieties supporting sustainability in agriculture (**Paper III**)

A population of 77 recombinant inbred lines (RILs) and their parental lines ‘Happy’ (a high-yield variety) and ‘Boett’ (an intermediate variety) were field-grown for two years with contrasting weather and phenotyped for various traits related to shoot biomass, leaf chlorophyll status (SPAD), grain yield, and NAE and its components E_N, U_N and C_N. To explore the population for potentially desirable candidate lines to be used in a future breeding program, all RILs were scored and classified according to five evaluation groups. The grouping was based on specific combinations of the desirable traits grain biomass, NAE, E_N, U_N and C_N, scoring lines with 1 for above-average performance and 0 for average or below-average performance of the corresponding trait (Table 4). In the first group (A), a high grain biomass along with high NAE was desired, and three RILs (lines 96, 151, and 159) fulfilled the criteria for both years (Table 4). The second group (B) consisted of RILs combining high grain biomass with high U_N and E_N, and one line (line 159) was found to fulfil this criteria for both years. Evaluation group C was special because it combined high grain yield with a potentially low N requirement. Thus, a high grain biomass along with high E_N but low U_N was desired, and one RIL (line 145) qualified for this group. For the evaluation group D, desiring a combination of high grain biomass along with high U_N and C_N, two lines (lines 96 and 151) were identified to fulfil the criteria for both years. Evaluation group E considered high grain biomass along with low U_N and high C_N across both years as desirable, but none of the lines investigated qualified for this group. Therefore, only four, instead of five, evaluation groups are considered from here.

The most interesting combination considered was the evaluation group combining high grain biomass with high E_N and low U_N, because this particular combination of traits could support the breeding towards high grain yield through high N conversion efficiency, but without a large N requirement. A reduced fertilizer requirement could support an important sustainability goal in agriculture, which is reduced resource depletion (Brodt et al., 2011). Interestingly, one individual (line 145) out of the 77 RILs investigated in this thesis showed this specific trait combination, suggesting that this line possesses an interesting germplasm that may be promising for breeding programs focusing on high-yield varieties through high N conversion efficiency but relatively low N uptake. In a participatory approach, local farmers could be encouraged to participate in the evaluation of RIL populations in the field, and their input could be taken into account for the selection of new materials (vom Brocke et al., 2010). It is worth noting that line 159 presented an interesting combination of traits as well, including high grain biomass, high E_N, and high U_N. However, its high heterozygosity in the selected markers reduces its reliability as a germplasm source (**Paper III**, Table 3). In contrast, line 145 seems to align more closely with the parental lines, with lower U_N values derived from ‘Happy’ and higher E_N values from ‘Boett’ (**Paper III**, Table 3). These findings provide valuable insights into the markers associated with NAE components and their potential application in future breeding programs.

Table 4. Overview of four evaluation groups (A to D) combining various desirable yield and nitrogen accumulation efficiency (NAE) properties for spring wheat lines field-grown in Central Sweden for two years (2022, 2023). Normalization of 5 traits, with a score of 1 indicating above-average performance and a score of 0 indicating average or below-average performance followed by the percentage in brackets in each year (2022 or 2023). The prioritized combinations for each evaluation group (A to E) are presented separately in bold for both 2022 and 2023. Group A: Grain biomass (1) – NAE (1), group B: Grain biomass (1) – U_N (1) – E_N (1), group C: Grain biomass (1) – U_N (0) – E_N (1), and group D: Grain biomass (1) – U_N (1) – C_N (1). U_N N uptake efficiency, E_N grain-specific N efficiency; C_N grain N concentration. Adapted from **Paper III**.



Group	**Year**	**Line**	**Grain biomass**	**U_N**	**E_N**	**C_N**	**NAE**
A, D	2022	96	**1** (58%)	**1** (51%)	0 (39%)	**1** (52%)	**1** (51%)
	2023		**1** (61%)	**1** (57%)	1 (63%)	**1** (56%)	**1** (60%)
C	2022	145	**1** (57%)	**0** (31%)	**1** (64%)	0 (24%)	0 (34%)
	2023		**1** (55%)	**0** (49%)	**1** (58%)	0 (5%)	0 (42%)
A, D	2022	151	**1** (61%)	**1** (53%)	0 (46%)	**1** (53%)	**1** (58%)
	2023		**1** (67%)	**1** (53%)	1 (51%)	**1** (79%)	**1** (56%)
A, B	2022	159	**1** (100%)	**1** (67%)	**1** (100%)	0 (7%)	**1** (73%)
	2023		**1** (78%)	**1** (71%)	**1** (58%)	0 (28%)	**1** (64%)
	2022	Boett	0 (23%)	0 (33%)	0 (40%)	0 (45%)	0 (35%)
	2023		0 (38%)	**1** (57%)	**1** (74%)	**1** (53%)	**1** (63%)
	2022	Happy	0 (24%)	0 (49%)	**1** (60%)	0 (14%)	0 (48%)
	2023		**1** (73%)	**1** (100%)	**1** (76%)	0 (37%)	**1** (100%)

The same RIL population that was explored for candidate lines was also used to identify genomic regions and Quantitative Trait Loci (QTL) markers associated with NAE and its components. Thus, the phenotypic data on NAE and its components observed in the field in two different environments (years with contrasting weather) were associated to genotypic data to create a linkage map and identify genomic regions related to NAE and its components. Generally, a prerequisite for the identification of genetic markers and genomic regions for traits is that the investigated population segregates sufficiently in terms of the traits under consideration. In this thesis, a total of 186 genomic regions associated with NAE-related traits were identified, including 10 QTLs and 176 marker-trait associations, supporting the fifth hypothesis of this thesis (H5) which stated that the spring wheat mapping population investigated here segregates sufficiently in terms of grain biomass and NAE components.

The QTL analysis revealed the following results: with LOD values > 3.0, 10 QTLs were identified for 16 traits for two years (2022 and 2023). QTLs for grain N concentration (C_N) and SPAD were detected for both years. SPAD was associated with one QTL in 2022 (chromosome 5B) and two QTLs in 2023 (chromosomes 2D and 7D), while C_N was associated with two QTLs in 2022 (chromosomes 2B and 3A) and one QTL in 2023 (chromosome 5B). Additionally, in 2023, four QTLs were detected for straw biomass (chromosome 3B), straw N concentration (chromosome 7B), and P predict (chromosomes 2A and 5B) (Figure 12). Furthermore, out of the 21693 SNP markers evaluated, 1072 were excluded due to low call rates (< 80 %) and 15843 for having a low minor allele frequency (< 10 %). A GWAS was performed using the FarmCPU model (Liu et al., 2016), and significant markers were identified for all traits, however, they were not consistently observed in both years. Markers associated with NAE and its components were also detected. In 2022, markers for U_N, E_N, and NAE were identified, while markers for C_N were mostly found in 2023. Most associated regions were located on chromosome 5A, followed by 1B, 2B, 3A, 3D, 5B, and 6A (Table 5).

In general, mapping genomic regions linked to quantitative traits is a highly useful method for unravelling the genetic basis of complex traits in wheat breeding programs (Dogan et al., 2023). In this thesis, two techniques were applied, namely QTL analysis and GWAS, for detecting genomic regions related to NAE and its components. From the QTL analysis, 10 QTLs were found, and only two QTLs, one for SPAD and one for C_N, were repeated in both years. Nevertheless, these QTLs were not found in the exact same positions. QTLs that are presented in different environments are particularly important due to their stability and consistency, and should be the top priority in breeding programs (Liu et al., 2008). The other eight QTLs found can be considered environmentally dependent QTLs (Fontaine et al., 2009), and may be triggered by specific environmental conditions, e.g. those inducing stress conditions for the plant.

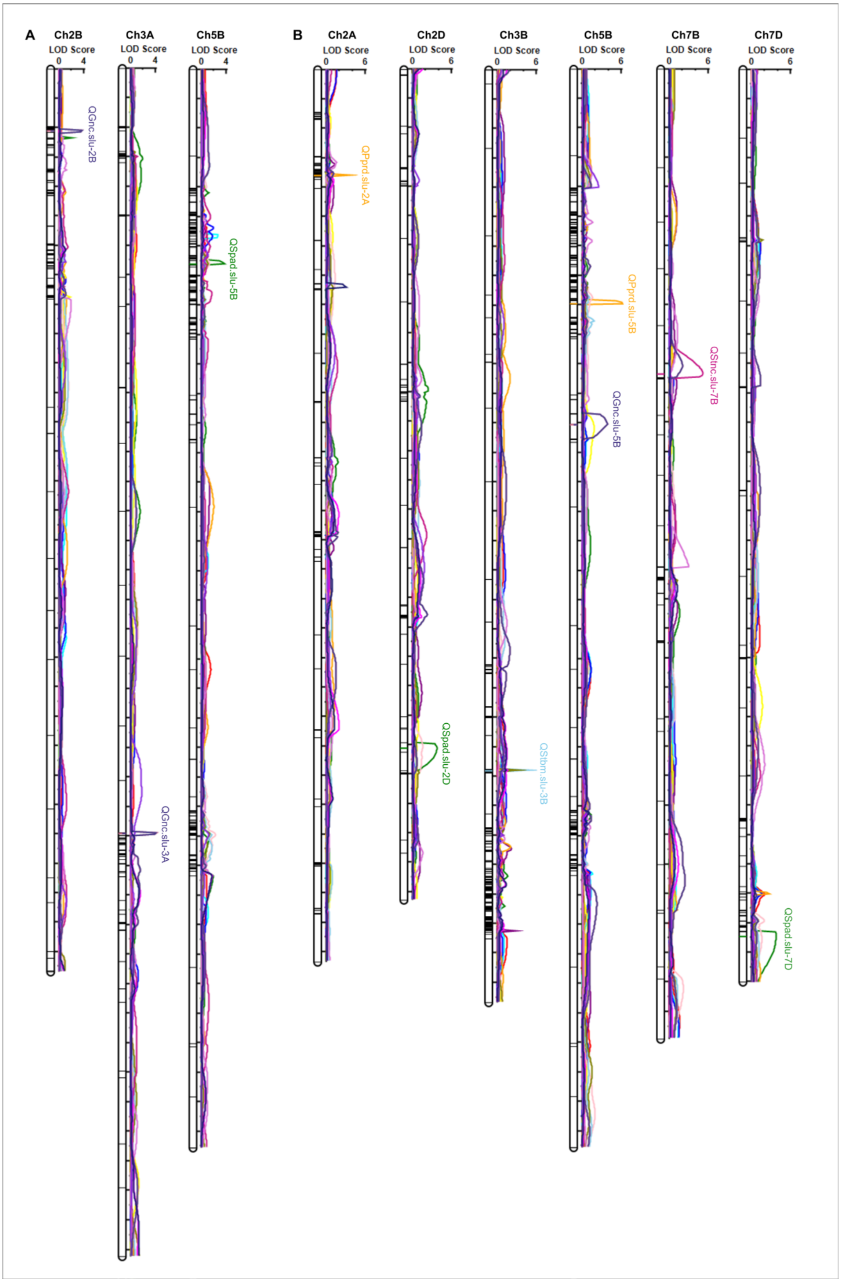
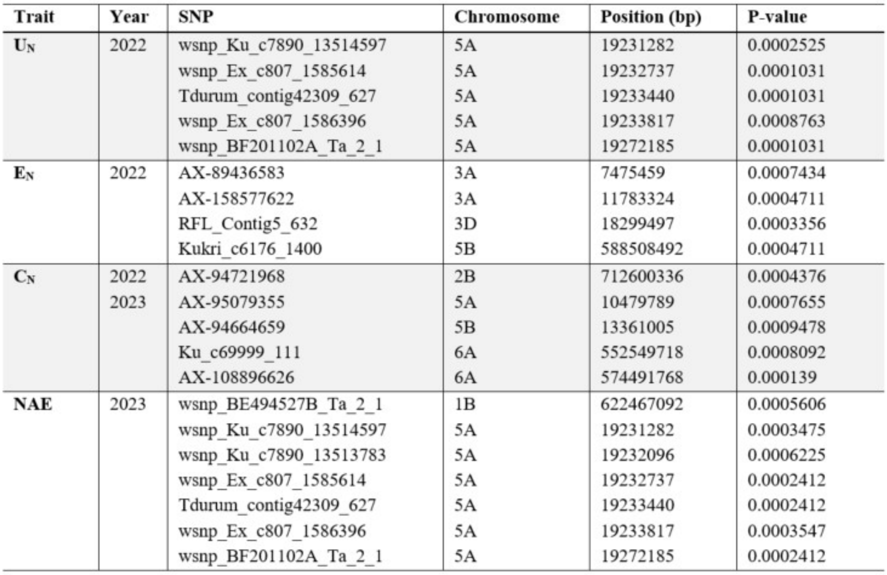


Figure 12. Genetic map of chromosomes 2B, 3A, and 5B in 2022 (A) and 2A, 2D, 3B, 5B, 7B, and 7D in 2023 (B) of a spring wheat mapping population ('Happy' x 'Boett' RIL population) field-grown in Central Sweden for two years. The positions of the QTLs associated with different traits are presented. Adapted from **Paper III**.

The main objective of **Paper III** was the identification of genomic segments that are relevant for controlling NAE and its components. Markers were detected for each component: five for U_N, four for E_N, three for C_N, and seven for NAE (Table 5). I consider these findings to be the central aspect of my thesis, and they provide me with a satisfactory sense of achievement and closure. To compare these results with previous studies, I used the report of Shi et al. (2022) because of the vast amount of wheat accessions (389), wheat markers (660 K SNPs array), and because its focus was on N use efficiency related traits. Additionally, this study also incorporated previous research (Laperche et al., 2007, Fontaine et al., 2009, Guo et al., 2012, Cormier et al., 2014, Deng et al., 2017). Moreover, the reports by An et al. (2006) and Huang et al. (2003) have been included. Detailed results are provided in Supplementary Table 1 and **Paper III**.

Table 5. The name of markers identified in the GWAS analysis in relation to U_N, E_N, C_N, and NAE with the chromosome, physical position and P. value for 2022 and 2023 for a spring wheat mapping population ('Happy' x 'Boett' RIL population) field-grown in Central Sweden for two years. Adapted from **Paper III**.



Trait	Year	SNP	Chromosome	Position (bp)	P-value
U_N	2022	wsnp_Ku_c7890_13514597	5A	19231282	0.0002525
		wsnp_Ex_c807_1585614	5A	19232737	0.0001031
		Tdurum_contig42309_627	5A	19233440	0.0001031
		wsnp_Ex_c807_1586396	5A	19233817	0.0008763
		wsnp_BF201102A_Ta_2_1	5A	19272185	0.0001031
E_N	2022	AX-89436583	3A	7475459	0.0007434
		AX-158577622	3A	11783324	0.0004711
		RFL_Contig5_632	3D	18299497	0.0003356
		Kukri_c6176_1400	5B	588508492	0.0004711
C_N	2022	AX-94721968	2B	712600336	0.0004376
	2023	AX-95079355	5A	10479789	0.0007655
		AX-94664659	5B	13361005	0.0009478
		Ku_c69999_111	6A	552549718	0.0008092
		AX-108896626	6A	574491768	0.000139
NAE	2023	wsnp_BE494527B_Ta_2_1	1B	622467092	0.0005606
		wsnp_Ku_c7890_13514597	5A	19231282	0.0003475
		wsnp_Ku_c7890_13513783	5A	19232096	0.0006225
		wsnp_Ex_c807_1585614	5A	19232737	0.0002412
		Tdurum_contig42309_627	5A	19233440	0.0002412
		wsnp_Ex_c807_1586396	5A	19233817	0.0003547
		wsnp_BF201102A_Ta_2_1	5A	19272185	0.0002412

As mentioned above, several candidate genes for grain N concentration (C_N) were found. Of the 16 genes detected in this specific region, only three genes out of the 16 seem to be related to grain N concentration, and one of them is the transcription factor of the basic leucine zipper (bZIP). The bZIP transcription factor is involved in regulating various stress responses, including N deficiency (Ueda et al., 2020, Yu et al., 2020), suggesting a role in N metabolism and the activation of N stress-related genes. The second gene is the putative ripening-related protein 7, which is linked to fruit and seed development (Pei et al., 2019). The possible activity of this gene in our material suggests that proteins involved in ripening may also play a role in N accumulation during grain filling. Further, the late embryogenesis abundant protein D-34 (LEA), also relevant in our material, is known to participate in stress tolerance related to nutrients such as potassium (K) and calcium (Ca) (Zhao et al., 2011), suggesting a potential influence on N metabolism under stress conditions. Additionally, in **Paper III** is shown a QTL located in chromosome 7B for TKW that can be considered non-specific (Liu et al., 2008), because it is present in our RIL population and in the Chinese wheat panel including 389 accessions (Shi et al., 2022).

In my view, the combination of two methodologies, namely searching for candidate lines based on phenotypic data and performance in the field, and the identification of SNPs to detect genomic regions of interest, is a powerful approach for advancing and improving NAE-related traits in spring wheat for cultivation in high-latitude environments. Phenotypic analysis plays a crucial role in the development of new lines with desirable traits. Regular evaluation and interpretation of field data from mapping populations can provide valuable insights for identifying and selecting lines with optimal performance for traits such as those related to NAE. The approach used in this thesis led to the identification of line 145 as a particularly promising candidate line for future breeding. Indeed, its distinctive combination of favorable characteristics makes it a valuable germplasm resource for future breeding efforts. Moreover, integrating phenotypic data with genotypic information through statistical analysis of the mapping populations enables QTL and GWAS analyses (Tuberosa et al., 2002) to uncover genomic regions associated with desirable traits, here NAE and its components. Once these markers and QTLs are identified, breeding programs can be refined to facilitate crosses and incorporate these regions into elite materials for developing superior lines.

4.5 Limitations and future research opportunities to improve spring wheat in Nordic regions

A limitation of this thesis has been the limited set of environments, repetitions, and samples that could be included in the research. Although larger populations are generally recommended, the inclusion of them is not always possible for practical feasibility reasons, leading to a reduction of breeding improvements due to breeder's selection method (Bernardo, 2004). It is desirable to establish experimental trials in not just one location (here Uppsala), but also in other regions where spring wheat is considered a promising crop. Including data from several locations would provide a more comprehensive understanding of the crop's performance across different environments and should be considered as part of a long-term project. Despite the challenges I faced in this thesis, I chose to view the shortcomings in my work as opportunities. For example, the small number of available samples for the trials was initially a significant setback, but it became the driving force behind the development of an allometric approach for a more efficient phenotyping of NAE components in **Paper II**. This challenge pushed me to innovate my thinking and led to a positive outcome that not only solved the issue but also provided the foundation for further research. By transforming a limitation into an opportunity, I gained valuable insights for the continuation of my work. This experience highlights the importance of adaptability and the potential to turn obstacles into opportunities for progress in both research and practical applications.

Polyploid genomes, such as wheat (hexaploid), present distinctive challenges due to their inherent complexity, complicating their analysis compared to diploid crops. Traits such as N use efficiency, which are influenced by multiple interacting genes, add an extra layer of difficulty to genetic studies (Shi et al., 2022). Due to its complexity, N use efficiency has not yet been the focus of a dedicated breeding program. Instead, improvements have been made indirectly through the selection for grain yield in specific environments as a reference point for alternative approaches (Sadras and Richards, 2014, Cormier et al., 2016). Identifying candidate genes for such complex traits often demands additional techniques such as fine mapping or advanced sequencing approaches.

The variability in QTL detection across studies is another obstacle. Differences in populations, environmental conditions, or experimental designs can lead to inconsistencies in QTL identification, limiting their practical application in breeding programs (Xu et al., 2017). Furthermore, the genetic background of the mapping population plays a significant role in QTL discovery, implying that QTLs identified in one population may not be transferable or relevant to others (Liu et al., 2008; Xu et al., 2017). While extensive research has focused on Chinese (Shi et al., 2022, Jia et al., 2024) and American wheat (Cerit et al., 2023, Dogan et al., 2023), studies on Swedish or Nordic spring wheat varieties are rare.

In **Papers II and III**, two elite Nordic spring wheat varieties with well-characterized differences in N use efficiency and distinct breeding targets were selected to be the parental lines for the 77 RIL population. The parental line ’Happy’ represented high grain yield as the main breeding target, therefore, **Paper III** emphasized the relationships between grain yield and NAE. Increasing the number of mapping populations for Nordic spring wheat should prioritize parental lines with high protein content, which would serve as a valuable basis for future QTL studies. For future research, at least one parental with the desirable target trait should be high protein content, and must be selected for the design of the crossing strategies to be passed to the potential new lines of the RIL population (Peña et al., 2002).

For GWAS, populations with greater genetic diversity, such as Nordic spring wheat landraces, modern varieties, and other regional cultivars could enhance polymorphism detection, thereby offering a broader pool of traits for breeding programs (Jia et al., 2024). Ortiz et al. (1998) examined 75 cultivars from Denmark, Finland, Norway, and Sweden, released between 1901 and 1993, to rediscover phenotypic diversity, with most traits linked to grain yield. Diederichsen et al. (2013) expanded on this research by analyzing 57 Nordic spring wheat cultivars (including some from Ortiz’s study) and 22 landraces preserved by NordGen, focusing on many traits, even biotic stress resistance. However, none of the studies included traits related to N use efficiency. These populations could be highly valuable for GWAS, after data collection on N use efficiency-related traits and genotyping with a large set of molecular markers.

5. Conclusions

In this thesis, I explored the characteristics of spring wheat varieties selected for two differential main breeding targets and examined their traits related to nitrogen (N) use efficiency. The findings indicate that many relationships between target and non-target traits are specific to wheat types and remain consistent across different environmental conditions. Specifically, N conversion efficiency and grain N concentration were linked to high yield and high protein breeding targets, respectively, whereas N uptake and accumulation efficiency showed no clear association with any particular breeding goal.

Seedling root length after 1 to 2 weeks of growth did not show a clear association with the breeding targets of the different varieties under optimal or stress conditions. The results of this thesis revealed no specific pattern but instead highlighted a G × E interaction, as results changed depending on the treatment applied. This suggests that a genotype-specific approach would be the most accurate way to examine these varieties. For instance, 'Alderon' exhibited shorter and thicker roots and was associated with higher E_N, while 'Bjarne' showed the longest roots under optimal conditions.

An important part of this thesis was the development of a methodology for utilizing allometric relationships to reduce the amount of destructive sampling when assessing important N use efficiency characteristics of crops. Thus, by applying an allometric approach, three traits that can be assessed non-invasively (height, tiller number, and leaf chlorophyll content) were used to estimate shoot N pool at anthesis, which is a major input variable for the calculation of important N use efficiency traits. The methodology developed in this thesis facilitates the phenotyping of N use efficiency-related traits in breeding populations while simultaneously minimizing destructive sampling.

A mapping population was explored to find potentially desirable candidate lines for use in a future breeding program, and a notably interesting promising line was identified, combining high grain biomass with low N uptake (potentially reducing fertilizer requirement) and high N conversion efficiency. In the same mapping population, genomic regions associated with N accumulation efficiency and its components were also detected. The successful identification of genomic regions associated with N use efficiency traits highlights the importance of efficient phenotyping methodologies for these traits to routinely use them in breeding. In this thesis, the identification of genomic regions for N use efficiency traits, together with the development of a methodology to accelerate the phenotyping of these traits, is an important step to facilitate the strategic breeding towards productive but less N requiring spring wheat varieties for high-latitude conditions.

The findings from this thesis emphasize the potential of integrating important traits related to N use efficiency as regular traits in breeding programs targeting high-yield varieties of spring wheat to be grown in high latitude environments. In the case of high-protein varieties, more studies are needed. However, selecting a parental to fulfil this breeding target is recommended for the development of a RIL population.

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Popular science summary

While the sufficient supply of nitrogen ensures healthy growth in all plants, the excess of nitrogen fertilizers in agriculture to support high yields often causes environmental contamination. Improving crop nitrogen use efficiency, especially in staple crops such as wheat, will effectively contribute to the maintenance of high yields, a more sustainable food production, and a decrease of environmental contamination caused by current agricultural practices.

When we were kids, we were often told to eat all our food to grow strong and healthy; notably fruits and vegetables, which are rich in nutrients and minerals that are essential for our development. The same principle applies to plants: nutrients are vital for their growth and survival. Among these, one nutrient stands out as the most important and often most growth-limiting nutrient in a plant's life: nitrogen (N).

Nitrogen is crucial for plant growth, playing a critical role in their entire life cycle. For crops that are a staple in the human diet, such as wheat, N is particularly important. However, in a Nordic context, the use of N fertilizers to ensure high yields has increased eutrophication in the Baltic Sea. Therefore, increasing crop N use efficiency has become a high priority in modern agriculture.

The present research focused on N use efficiency in spring wheat, a promising crop for Nordic climates, by using a methodology to separate crop N accumulation efficiency into three components: N uptake efficiency, N utilization efficiency, and grain N content. The thesis examined key traits and breeding targets in spring wheat to gain a better understanding and improve the N use efficiency of the crop when grown under different environmental conditions. A central aspect of the thesis was the development of a simplified, less-destructive method to assess the key traits for improved N use efficiency in breeding populations. The final part of the thesis focused on the analysis of genetic data and the identification of specific regions of the wheat genome that are linked to the crop’s N use.

The results highlight the strong associations between, firstly, the two key breeding goals for spring wheat, i.e., high grain yield and protein content; and, secondly, the three different wheat types in the Nordic region, i.e., high yield, intermediate, and high protein content wheats. While high N utilization efficiency and high grain N content were associated with high-yield and high-protein wheat types, respectively, the N uptake efficiency was not associated with any wheat type or breeding target. Root traits are important for N uptake but were predominantly influenced by variety and environment (including their interaction) rather than wheat type in the investigated plant material. Together with the identification of various genomic regions associated with key traits for improved N use efficiency, the successful development of a more efficient methodology to phenotype these traits will subsequently pave the way for more efficient breeding of spring wheat with improved N use efficiency.

This research suggests the utilization of practical tools and proposes N use efficiency to be used as a regular target for the breeding of spring wheat varieties that are both productive and support environmental sustainability. These advancements can help to reduce the agricultural sector's environmental footprint while simultaneously supporting food sustainability in Nordic regions.

Populärvetenskaplig sammanfattning

Medan en tillräcklig tillgång på växtnäring, särskilt kväve, är en förutsättning för god tillväxt och fröproduktion hos alla växter medför en ökad användning av kvävegödselmedel i jordbruket stora risker för miljöproblem. En förbättrad kväve-effektivitet, särskilt i våra baslivsmedelsgrödor såsom vete, kommer att bidra till att säkerställa höga skördar i en mer hållbar livsmedelsproduktion och en minskad risk för miljöföroreningar inom jordbruket.

När vi var barn fick vi ofta höra att vi skulle äta upp all mat för att bli starka och friska. Detta gällde särskilt frukt och grönsaker som är fulla av näringsämnen och mineraler som är nödvändiga för en god hälsa hos oss människor. Samma princip gäller även för växter- näringsämnen är avgörande för växters tillväxt och överlevnad. Bland dessa utmärker sig särskilt ett växtnäringsämne som ofta blir starkt tillväxtbegränsande för många växter, nämligen kväve.

Kväve är oumbärligt för växter och spelar en avgörande roll under hela livscykeln. För grödor såsom vete, som är en basföda för många människors kost, är kväve särskilt viktigt. Därför används exempelvis i de nordiska länderna mycket kvävegödselmedel för att säkerställa höga skördar, vilket har medfört ökat utsläpp av växtnäring i Östersjön, med miljöproblem som följd. Det har därför blivit en hög prioritet inom det moderna jordbruket att öka grödors kväve-effektivitet.

Forskningen fokuserade på kväve-effektivitet hos vårvete, som är en lovande gröda för nordiskt klimat. För att studera kväve-effektiviteten undersöktes olika aspekter såsom kväveupptagseffektivitet, kväveutnyttjande-effektivitet och kväveinnehåll i kärnorna. Avhandlingen undersökte viktiga växtegenskaper och växtförädlingsmål hos vårvete för att bättre förstå och förbättra grödans kväve-effektivitet vid odling under olika miljöförhållanden. En viktig del i avhandlingen var utvecklingen av en praktiskt användbar metod för att snabbt kunna bedöma viktiga växtegenskaper kopplade till kväve-effektivitet i stora växtförädlingspopulationer. En annan del i avhandlingen fokuserade på analysen av genetiska data och identifieringen av specifika regioner i vetegenomet som är kopplade till grödans kväve-effektivitet.

Resultaten visade stabila samband mellan grödegenskaper som idag används inom växtförädlingen, t.ex. kärnavkastning och proteinhalt, och egenskaper kopplade till kväve-effektivitet som idag inte används inom växtförädlingen. Medan hög kväveutnyttjande-effektivitet kunde kopplas till sorter med hög avkastning, och högt kväveinnehåll i kärnorna kunde associeras med sorter förädlade för höga proteinhalter, var kväveupptagseffektiviteten inte kopplad till något växtförädlingsmål. Rotegenskaper är allmänt viktiga för grödors kväveupptag men påverkades här mest av genetik (sort) och miljö (temperatur och torka) snarare än förädlingsmålet hos det undersökta växtmaterialet.

Identifieringen av olika genomiska regioner kopplade till egenskaper för förbättrad kväve-effektiviteten och den framgångsrika utvecklingen av en effektivare metod för att kartlägga dessa egenskaper kommer att bana väg för en effektivare växtförädling av vårvete med förbättrad kväve-effektivitet. Denna forskning har alltså tagit fram ett praktiskt användbart verktyg för att mer effektivt kunna kartlägga viktiga växtegenskaper kopplade till kväve-effektivitet. Den har också lagt grunden för att växtegenskaper kopplade till kväve-effektivitet ska kunna användas rutinmässigt i växtförädlingen för att ta fram vårvetesorter som är både produktiva och kan bidra till ökad miljömässig hållbarhet. Således kan denna forskning bidra till att minska jordbrukets miljöpåverkan och öka livsmedelssäkerheten i de nordiska länderna.

Artículo de divulgación científica

Si bien el suficiente suministro de nitrógeno garantiza un crecimiento saludable en todas las plantas, el exceso de fertilizantes nitrogenados en la agricultura para mantener altos rendimientos a menudo causa contaminación ambiental. Mejorar la eficiencia del uso del nitrógeno en los cultivos, especialmente en nuestros cultivos básicos como el trigo, contribuirá a mantener altos rendimientos, una producción de alimentos más sostenible y una disminución de la contaminación ambiental causada por las prácticas agrícolas.

Cuando éramos niños, a menudo nos decían que comiéramos todos nuestros alimentos para crecer sanos y fuertes; especialmente frutas y verduras, que están llenas de nutrientes y minerales esenciales para nuestro desarrollo. El mismo principio se aplica a las plantas: los nutrientes son vitales para su crecimiento y supervivencia. Entre ellos, un nutriente se destaca como el más importante y, a menudo, el que más limita el crecimiento en la vida de una planta: el nitrógeno (N).

El nitrógeno es esencial para las plantas y desempeña un papel fundamental en todo su ciclo de vida. Para cultivos como el trigo, que es un alimento básico en la dieta humana, el N es particularmente importante. Sin embargo, en un contexto nórdico, el uso de fertilizantes nitrogenados para garantizar altos rendimientos ha aumentado la eutrofización en el mar Báltico. Por lo tanto, aumentar la eficiencia del uso de nitrógeno en los cultivos se ha convertido en una alta prioridad en la agricultura moderna.

Esta investigación se centró en la eficiencia del uso de nitrógeno en el trigo de primavera, un cultivo prometedor para los climas nórdicos, utilizando esencialmente una metodología que separa la eficiencia de acumulación de nitrógeno en tres componentes: eficiencia de absorción de nitrógeno, eficiencia de utilización de nitrógeno y contenido de nitrógeno en el grano. Esta tesis examinó los rasgos clave y los objetivos de mejoramiento en el trigo de primavera para comprender y mejorar la eficiencia del uso de nitrógeno del cultivo cuando se cultiva en diferentes condiciones ambientales. Una parte central de la tesis fue el desarrollo de un método simplificado y menos destructivo para evaluar los rasgos clave para mejorar la eficiencia del uso de nitrógeno en las poblaciones de mejoramiento. La parte final de la tesis se centró en el análisis de datos genéticos y la identificación de regiones específicas del genoma del trigo que están vinculadas al uso de nitrógeno del cultivo.

Los resultados destacan las fuertes asociaciones entre, por un lado, los dos objetivos clave de mejoramiento para el trigo de primavera, es decir, alto rendimiento de grano y contenido de proteína; y, por otro lado, los tres tipos diferentes de trigo en la región nórdica, es decir, trigos de alto rendimiento, intermedios y de alto contenido de proteína. Si bien la alta eficiencia de utilización de N y el alto contenido de N en el grano se asociaron con tipos de trigo de alto rendimiento y alto contenido de proteínas, respectivamente, la eficiencia de absorción de N no se asoció con ningún tipo de trigo ni objetivo de mejoramiento. Los rasgos de la raíz son importantes para la absorción de N, pero se vieron influenciados principalmente por la variedad y el medio ambiente (y su interacción) en lugar del tipo de trigo del material vegetal investigado aquí. Junto con la identificación de varias regiones genómicas asociadas con rasgos clave para una mejor eficiencia en el uso de N, el desarrollo exitoso de una metodología más eficiente para fenotipificar estos rasgos allanará el camino para un mejoramiento más eficiente del trigo de primavera con una mejor eficiencia en el uso de N.

Esta investigación sugiere herramientas prácticas y propone que la eficiencia en el uso de N se utilice como un objetivo regular para el mejoramiento de variedades de trigo de primavera que sean productivas y respalden la sostenibilidad ambiental. Estos avances pueden ayudar a reducir la huella ambiental de la agricultura y, al mismo tiempo, respaldar la sostenibilidad alimentaria en las regiones nórdicas.

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Differential breeding targets in wheat influence non-target traits related to grain quality, but not crop nitrogen requirement

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Wheat is considered an established crop with a long breeding history. Its varieties are being developed with differential breeding targets, e.g. high grain yield or high grain protein content. The different breeding targets strongly influence performance in the targeted traits, but may also influence non-target traits related to grain quality, biofortification potential, and nutrient accumulation. This influence of non-targeted traits may, in turn, affect the environmental performance and ecological sustainability of the crop. The aim of this study was to evaluate the relationships between breeding target traits such as grain yield and grain quality, and non-target traits for three groups of spring wheat varieties differing in breeding targets, i.e. high yield (I), organic high protein (II), and intermediate (III) wheat types. Data from a field trial with nine spring wheat varieties grown under two soil compaction treatments over two years with contrasting weather were used. We found that wheat type affected most target and non-target traits with partly large effect sizes ($0.874 \leq \eta_p^2 \leq 0.173$), but not nitrogen (N) uptake efficiency ($\eta_p^2=0.006$), which reflects the potential N resource requirement of the crop. Associations shown between target and non-target traits will be advantageous for wheat breeding programs. Wheat type and environment had similarly sized effects on grain yield and quality traits. Grain concentrations of various macro- and micro-nutrients were frequently, but not always, correlated, indicating that the biofortification potential varied between wheat types and was affected by environmental conditions. The grain and starch yields per accumulated plant N were higher in the wheat varieties bred for high grain yields than those bred for high grain protein content; whilst the protein yield per accumulated whole-plant N was similar across all wheat types despite much higher grain N concentrations in the high-protein varieties. Additionally, most of the evaluated traits tended to preserve their static stability across environments. The results link grain yield and grain quality traits to crop nutrient accumulation aspects relevant for the environmental performance and ecological sustainability of the crop. Future breeding strategies should consider the non-target effects in traits that influence the environmental performance of the crop.

KEYWORDS

grain yield, grain quality, target traits, non-target traits, nitrogen uptake efficiency, breeding targets, spring wheat

1 Introduction

One of the main challenges of contemporary agriculture is to fulfil the nutrition requirements of growing populations by producing safe, nutritional, and high-quality food without harming biodiversity or depleting limited resources (Dwivedi et al., 2017). Wheat is recognized as one of the big three cereal crops globally (Baye et al., 2020) and plays a key role as a human food source. Compared to maize and rice, wheat has a greater amount of protein, turning it into one of the most important staple foods worldwide (Mecha et al., 2017).

During the domestication process, selection of desirable traits such as the loss of seed shattering, and the free-threshing naked seed form, were selected because these characteristics increased the chance of achieving higher grain yield (Shewry, 2009). Since then, wheat breeding targets have been focused mostly on the improvement of grain yield and thousand-kernel weight (i.e., two traits that here are considered as "yield traits"), disease resistance, and seed maturity traits. However, the attention has shifted to include grain quality traits, with those enhancing end-use quality becoming vital to wheat breeders (Table 1). Nevertheless, the genetic bottlenecks due to strong directed selection in the breeding of target traits have negatively affected some non-target traits that have potential in tackling challenges such as climate change and food security. Biotic and abiotic stress related traits have been considered main target traits due to economic impact on the yield, while morphological traits have not been extensively identified and exploited yet (Yong et al., 2004; Charmet, 2011; Dempewolf et al., 2017; Amiri et al., 2018; Pourazari et al., 2018).

The definition of wheat quality is a dynamic and complex concept to describe, mostly because it changes depending on the intended end product. Common quality traits of wheat are often based on morphological properties, such as grain size, hectoliter weight, and the absence of grain damage. Wheat intended for human consumption target organoleptic properties, gluten quality and quantity, and grain hardness; with food manufactures including targets such as grain uniformity. In the case of millers, wheat quality is mainly focused on grain morphology traits, grain density and grain hardness (Guzman et al., 2016). The quantity of starch and protein stored in the wheat grain are here considered as quality traits, although they are closely linked to grain yield (Xurun et al., 2015). Moreover, consumers often link quality to end-use traits such as baking properties or nutritional values. Both traits are essential breeding targets in wheat (Koppel and Ingver, 2008; Mecha et al., 2017). However, maximizing quality and yield traits in the same plant is challenging because of a genetic and inverse correlation between them (Fowler, 2003; Amiri et al., 2018). Grain yield establishment is a complex process in which breeders are interested to explore the primary and secondary connections between related traits. Additionally, a high genetic variability should be present in the material for achieve an improvement (Iqbal et al., 2022). Other important grain quality traits are related to the

TABLE 1 List of the nine-spring wheat varieties used in this study, their origin, and the classification to their breeding target.

Genotype	Accession number	Origin	Wheat type
'Alderon'	KWS W185	Germany	High-yield (I)
'Happy'	SW 91003	Sweden	High-yield (I)
'Dacke'	W 26267	Sweden	Organic high-protein (II)
'Dala'	Landrace	Sweden	Organic high-protein (II)
'Quarna'	CH 21112283	Switzerland	Organic high-protein (II)
'Bjarne'	NK 97520	Sweden	Organic high-protein (II)
'Boett'	SW 71034	Sweden	Intermediate (III)
'Diskett'	SW 45456	Sweden	Intermediate (III)
'Rohan'	SW 01198	Sweden	Intermediate (III)

possibility of grain biofortification, for which micronutrients such as zinc (Zn) and iron (Fe) are of major concern (Cakmak, 2008; Gupta et al., 2021). Breeding for biofortification has become increasingly popular and could aid in providing essential nutrients to vulnerable populations that depend on staple crops as their main dietary intake, ensuring food and nutritional security (Guzman et al., 2016; Velu et al., 2017). It has been shown that high-yielding wheat cultivars usually produce grains with low concentrations of Zn and Fe, due to a "dilution effect" (Ortiz-Monasterio et al., 2007; Gupta et al., 2021), which is another example for links between traits with a high relevance for breeding. Due to these links between traits, breeders face a difficult task to deliver varieties that satisfy the demands of different stakeholders and end-users (Guzman et al., 2016). Thus, many grain yield and quality traits might be correlated, either through physiological or genetic links between traits (Weih, 2003); and targeting one of the traits could imply a high likelihood to also modify other trait(s) in the same breeding process. However, little is known about trait correlations among yield and different grain quality traits, and this poor knowledge reduces the possibility to target suites of traits rather than single traits in the breeding.

Previous studies have pointed out that quality traits such as grain protein content in wheat is strongly influenced by the genotype (Simons et al., 2012; Makawi et al., 2013; Taheri et al., 2021), but also by environmental factors (Amiri et al., 2018). The grain starch content is usually correlated to grain yield, and seems to be mainly influenced by the environmental conditions (Labuschagne et al., 2007). Taken together, the previous results suggest that grain quality traits can be expected to be mainly affected by the genotype, while grain yield should be more affected by environmental factors.

Frequent genotype by environment interaction (G x E) is observed for both grain yield and quality traits (Nagarajan et al., 2007), although a desirable prerequisite for viable wheat production is the availability of genotypes with high and stable performance among diverse environmental conditions (Nagarajan et al., 2007; Tayyar, 2010). Trait stability can be either static or dynamic. Static stability is when performance remains similar in different environments (low G x E), while dynamic stability is when performance follows the potential of an environment (high G x E) (Knapp et al., 2017). Static stability of quality traits, reflected by low G x E, favors stable end products and thus ensures that a quality standard can be performed by a given genotype, allowing efficient processing and decreased waste (Mut et al., 2010; Knapp et al., 2017). However, little is known about (static) stability of grain yield and quality traits in wheat varieties that where bred for different breeding targets.

Many grain yield and quality traits are connected to crop nutrition issues, because nutrient elements are important for the generation of crop growth and yield on the one hand, and grain quality traits (e.g., protein content and micronutrient biofortification) on the other hand. Crop nutrition is, in turn, linked to crop requirements for nutrient resources. Higher crop nutrient requirements imply a greater depletion of nutrient resources and thus potentially a negative impact on the ecological sustainability of the crop (Weih et al., 2014). Because of these links, it is possible that wheat varieties bred for high protein contents (i.e., high grain N concentrations) have greater N requirement and a stronger impact on ecological sustainability than varieties bred for high grain yields. In this study, we use the N accumulation efficiency concept (Weih et al., 2011; Weih et al., 2018), in which the N uptake efficiency (U) reflects the mean N amount during the entire growth period (N') divided by the N amount in initial seed; where all variations regarding U were caused by N' changes in this study. In the same conceptual approach (Weih et al., 2011; Weih et al., 2018), grain-specific N efficiency (E_N) quantifies the efficiency of utilizing N to produce grain. As proposed in a previous paper (Pourazari et al., 2018), this concept is here supplemented by the efficiency of utilizing N to produce specific end products; i.e., the protein-specific N efficiency ($E_{N,yp}$) and starch-specific N efficiency ($E_{N,ys}$), quantifying the efficiencies of utilizing N to generate protein and starch yield, respectively.

This paper complements a series of previous papers in which data from the same field trial have been used. Thus, the effects of soil compaction on growth and grain yield as modulated by the weather conditions were investigated by (Liu et al., 2022a); whilst other works addressed the N economy and co-limitation by nutrients other than N especially in relation to root traits (Weih et al., 2021; Liu et al., 2022b). In contrast to our previous papers using the same field trial, the main objective of this study is to evaluate the relationships between target and non-target traits related to grain yield, various grain quality traits, biofortification potential, and N accumulation aspects (indicating N requirement and use) for three groups of spring wheat varieties. The three wheat groups where classified based on their breeding targets: high-yield (type I), organic high-protein (type II), and intermediate (in terms of both grain yield and protein content) (type III) wheats. The nine varieties were grown in a field trial with different soil compaction treatments over two years with contrasting weather. They were assessed for grain yield and quality traits, including the grain macro and micronutrient concentrations, and N accumulation aspects. We explored the following hypotheses: (H1) The relationship between target and not-target traits is specific for a given wheat type and stable across different environments. (H2) Grain quality traits are expected to be mainly affected by the genotype and more stable across contrasting environmental conditions, while grain yield should be more affected by environmental factors. (H3) Wheat varieties bred for high grain protein concentration (type II) have greater N demand and thus greater N uptake efficiency than varieties bred for high grain (starch) yields (type I).

2 Materials and methods

2.1 Study location

Field studies were conducted in Uppsala, Central Sweden (59° 45' N, 17° 42' E) during the 2018 and 2019 growing seasons. Uppsala has a boreal-temperate climate and the growing season lasts from April to October. During the summer months of 2018 the weather was drier and warmer, compared to the cooler and wetter weather of 2019 (Weih et al., 2021). Because of the lack of precipitation in 2018, artificial irrigation of ca. 10 mm of water was required 28 and 34 days after sowing in order to ensure survival of the plants during an extreme drought period. The soil has a silt loam texture (16% clay, 70% silt, 14% sand) and an organic matter content of 4% in the top 0.3 m. A commercial nutrient fertilizer with 140 kg ha^{-1} N, 24 kg ha^{-1} P, and 46 kg ha^{-1} K was added to the soil after sowing in both years. The pH was 5.8, and the particle density was 2.61 Mg m^{-3}.

2.2 Experimental design

The field trial was established in spring 2018 in a randomized split plot design with four replications; the size of each plot was 12 x 2 m (soil treatment, compaction vs. non-compaction). The soil compaction treatment started in April 2018 by double track-by-track passing using a front loader with four wheels and an average wheel load of 42 kN. To ensure crop establishment, the surface of the compacted soil was loosened to a depth of approximately 50 mm with a surface cultivator before sowing. During the field trial in 2019, the same soil compaction areas were used as in 2018 using the same protocol, while the positions of the genotype plots were re-randomized. Further details of the experimental treatments and soil conditions during the two experimental years are presented by Liu et al. (2022a). Nine spring-wheat varieties were selected and grown for the two years: 'KWS Alderon', 'Bjarne', 'Boett', 'Dacke', 'Diskett', 'Happy', 'Quarna', 'Rohan', and the landrace 'Dala'. The varieties were classified at three different sets based on their breeding targets. The groups were high–yield type (type I): 'Alderon' and 'Happy', organic high–protein type (type II): 'Quarna', 'Dacke', 'Dala', and 'Bjarne', and intermediate type (type III): 'Diskett', 'Rohan', and 'Boett' (Table 1).

2.3 Plant sampling and assessments

Five plants were selected in homogeneous areas of each plot at the crop developmental stages tillering (BBCH29), flowering (BBCH65), and spike maturity (BBCH89) (Lancashire et al., 1991) in both 2018 and 2019. The designated plants were cut with scissors at approximately 15 mm above ground, oven-dried at 65°C for 48 h and weighed. The central plot area (6 m * 2 m) was harvested with a combine harvester to assess grain yields, and a sub-sample was taken to assess grain protein, gluten, starch, TKW, HLW and falling number. The spikes of the selected plants were then manually threshed using a Smooth Chopper 6948A-2 (Tupperware, USA), and the dried grains were weighed. These samples (straw and grains separated at maturity) were ground in a stainless steel grinder to pass a 1 – mm mesh before nutrient element analysis. Eleven grain parameters were measured: grain yield and thousand-kernel weight (TKW) (these two traits are here considered as "yield traits"), protein content, gluten, starch, hectoliter weight (HLW), falling number; and the grain concentrations of N, P, Zn and Fe (Table 2), related to biofortification potential. In addition, the N accumulation traits U, E_N, $E_{N,yp}$, and $E_{N,ys}$ were also assessed as indicators of the characteristics affecting environmental performance and sustainability (Table 2). The N concentrations of planted seeds, shots and grains of five representative plants were sampled in the 0.5 m x 0.5 m plots and were analyzed on a LECO CNS72000 analyzer using standard method (SS-ISO13878) in order to determine the N

TABLE 2 Description and units of 15 traits measured in this study.

Quality trait	Description	Units
Grain yield	The grain yield per plot weighted.	Mg/ha
Thousand kernel weight (TKW)	The weight of 1000 kernels from randomly sample seeds per plot.	g
Falling number	Measures indirectly the alpha-amylase activity and specific starch value.	s
Protein	Protein content of the grain.	mg/g
Hectoliter weight (HLW)	Related to fleshiness of the grain.	g/L
Gluten	Most common protein in wheat for bread making. It has two components, gliadin and glutenin.	mg/g
Starch	Determines dough rheological characteristics.	mg/g
Nitrogen (N)	Most limiting nutrient. Linked to quality, yield processes and environmental concern.	mg/g
Phosphorus (P)	Second limiting nutrient. Part of phospholipids and metabolic processes. It storages in the grain as phytic acid.	mg/g
Zinc (Zn)	Related to resistance to diseases, abiotic stress tolerance, improving human nutrition.	ug/g
Iron (Fe)	Related to photosynthesis, hormone biosynthesis. Improving human nutrition.	ug/g
Nitrogen uptake efficiency (U)	Mean N amount during the entire growth period/N amount in initial seed.	mg/mg
Grain-specific N efficiency (E_N)	Grain yield/Mean N amount during the entire growth period	mg/mg
Protein-specific N efficiency ($E_{N,yp}$)	Grain protein yield/mean N amount during the entire growth period.	mg/mg
Starch-specific N efficiency ($E_{N,ys}$)	Grain starch yield/mean N amount during the entire growth period.	mg/mg

use efficiency and related traits. The contents of P, Fe, Zn were extracted using 32.5% Nitric acid on a heat block and concentrations were determined using ICP-AES technique (Spectro Blue FMS 26, Spectro Analytical Instruments, Kleve, Germany) by applying internal standardization protocols (Protocol number SS028311).

Protein, starch, hectoliter weight and gluten content were determined using FOSS Infratec NOVA grain analyser (Foss Analytical A/S. Infratec^{TM}nova), thousand kernel weight was determined on an Opto-Agri Machine (Opto Machines,TSW & Seed Biometry), and falling number was determined on a FOSS Alphatec FN° (Method AACC 56-81B).

2.4 Calculations of nitrogen accumulation efficiency and related traits

The calculations were based on the N pool of five sampled plants and the method presented by Weih et al. (2018). The N uptake efficiency (U; Eq. 1), the mean N amount during the entire growth period (N') and the grain-specific N efficiency (E_N; Eq. 2) were calculated separately for each replicate. For the calculations of protein-specific N efficiency ($E_{N,yp}$; Eq. 3) and starch-specific N efficiency ($E_{N,ys}$; Eq. 4) the E_N equation was adapted.

$$U = N'/N \ \ amount \ \ in \ \ initial \ \ seed \qquad \text{(Eq. 1)}$$

$$E_N = grain \ \ yield/N' \qquad \text{(Eq. 2)}$$

$$E_{N,yp} = grain \ \ protein \ \ pool/N' \qquad \text{(Eq. 3)}$$

$$E_{N,ys} = grain \ \ starch \ \ pool/N' \qquad \text{(Eq. 4)}$$

2.5 Statistical analysis

All the statistical analyses were performed using SPSS version 26. Analyses of Variance (ANOVA) were used for the calculation of the probabilities of significant differences in each of the fifteen plant traits and element concentrations, using fixed effects of year, treatment, wheat type, genotype nested within wheat type and block. This was also used to assess the interactions between year, treatment, and wheat type, as well as their effect sizes (partial eta squared; η_p^2). Correlations between the eleven traits were analyzed using Spearman's rank coefficient. Linear regression analysis was performed to evaluate the relationships between the concentrations of various macro- and microelements. In addition, a Principal Component Analysis (PCA) following CATPCA procedure was performed in order to relate and group traits and wheat types for each year using SPSS version 26. Coefficient of variation (CV) was used to test the environmental static stability of the traits for the three wheat types.

3 Results

3.1 Effects of wheat type, variety and environment on grain yield and quality traits

Significant differences between years, wheat types, genotype nested within wheat type, and the year*wheat type interactions were observed for the grain yield, starch, and TKW (Table 3). In this study, environmental variation is represented by the experimental compaction treatments and the two years with contrasting weather. No significant effect of compaction treatment on grain yield was found, whilst year significantly affected grain yield ($P<0.001$). The effect sizes of year were large for TKW, starch, and yield ($0.427 \leq \eta_p^2 \leq 0.895$). Additionally, the effect sizes of wheat type for the same traits were large as well ($0.616 \leq \eta_p^2 \leq 0.772$). The year*wheat type interaction displayed large effect sizes for starch and TKW ($\eta_p^2 = 0.418$ and 0.205, respectively) and medium effects for yield ($\eta_p^2 = 0.105$).

Protein, gluten, HLW, and falling number were significantly affected by year ($P<0.001$), treatment ($P<0.001$, $P=0.004$, $P<0.001$, and $P=0.003$, respectively), wheat type (except HLW) ($P<0.001$), and the interaction between year and wheat type ($P<0.001$) (Table 3). Among these traits, the effect sizes of year and wheat type were largest for protein ($\eta_p^2 = 0.907$ and 0.874, respectively). Similarly, large effect sizes of year and wheat type were found for gluten ($\eta_p^2 = 0.773$ and 0.749, respectively). Falling number and HLW showed large effect sizes for year ($\eta_p^2 = 0.543$ and 0.466, respectively) and large and small effect sizes for wheat type ($\eta_p^2 =$ 0.173 and 0.043, respectively).

Grain concentrations of N, P, Zn, and Fe varied significantly between years, with large effect sizes ($0.577 \leq \eta_p^2 \leq 0.839$). For wheat type, the data showed large effect sizes in grain N, P, and Zn ($0.216 \leq \eta_p^2 \leq 0.744$) (Table 3).

Significant differences between years were reported for U, E_N, $E_{N,yp}$ and $E_{N,ys}$ ($P<0.001$, $P<0.001$, $P=0.001$, $P<0.001$, respectively), wheat type (except U and $E_{N,yp}$), and year*wheat type interaction ($P=0.013$, $P=0.014$, $P=0.001$ and $P=0.007$, respectively). Medium effect sizes of year *wheat interaction were shown by these traits ($0.067 \leq \eta_p^2 \leq 0.102$). Whereas, large effect sizes of year and wheat type were registered for E_N ($\eta_p^2 = 0.393$ and 0.236, respectively) and $E_{Ny,s}$ ($\eta_p^2 = 0.452$ and 0.331, respectively).

3.2 Trait correlations and the influence of genotype and environment on trait stability

Due to the year effect, that had a strong influenced on all traits the correlation analysis was performed separately for each year. In this way, the identification of links between traits across varieties and the wheat types was more effective (Table 4). Most traits showed consistent correlation patterns between years, with the notable exception of the correlations involving falling number

TABLE 3 Analysis of variance (ANOVA) p-value (P) result for grain yield and quality traits and their equivalent effect sizes ($\eta_p{}^2$) for fixed effects.

Trait unit		Year (Y)	Treatment (T)	Wheat type (Wt)	Genotype (within Wt))	Y*T	Y*Wt	T*Wt	Y*T*Wt
Grain yield (Mg/ha)	P	<0.001	0.103	<0.001	<0.001	0.003	0.001	0.363	0.468
	$\eta_p{}^2$	0.4271	0.0214	0.6159	0.3869	0.0685	0.1054	0.0163	0.0122
Protein (mg/g)	P	<0.001	<0.001	<0.001	<0.001	0.814	<0.001	0.220	0.584
	$\eta_p{}^2$	0.9067	0.1387	0.8738	0.5587	0.0004	0.4428	0.0244	0.0087
Gluten (mg/g)	P	<0.001	0.004	<0.001	<0.001	0.451	<0.001	0.033	0.494
	$\eta_p{}^2$	0.7733	0.0671	0.7490	0.3246	0.0047	0.5376	0.0549	0.0115
Starch (mg/g)	P	<0.001	<0.001	<0.001	<0.001	0.223	<0.001	0.804	0.051
	$\eta_p{}^2$	0.7148	0.1437	0.7721	0.6104	0.0122	0.4188	0.0036	0.0479
HLW (g/L)	P	<0.001	<0.001	0.060	<0.001	0.110	<0.001	0.366	0.550
	$\eta_p{}^2$	0.4665	0.1177	0.0435	0.2760	0.0200	0.2540	0.0158	0.0094
TKW (g)	P	<0.001	0.009	<0.001	<0.001	0.942	<0.001	0.921	0.255
	$\eta_p{}^2$	0.8955	0.0557	0.6328	0.8122	0.0000	0.2050	0.0013	0.0223
Falling number (s)	P	<0.001	0.003	<0.001	<0.001	0.151	<0.001	0.443	0.011
	$\eta_p{}^2$	0.5427	0.0722	0.1726	0.5492	0.0169	0.3822	0.0131	0.0707
N conc (mg/g)	P	<0.001	0.078	<0.001	<0.001	0.271	<0.001	0.394	0.367
	$\eta_p{}^2$	0.8388	0.0483	0.7442	0.4601	0.0098	0.1508	0.0150	0.0161
P conc (mg/g)	P	<0.001	0.187	<0.001	<0.001	<0.001	0.005	0.184	0.244
	$\eta_p{}^2$	0.5770	0.0140	0.5974	0.4235	0.1002	0.0812	0.0271	0.0226
Zn conc (ug/g)	P	<0.001	0.739	<0.001	<0.001	0.852	0.618	0.877	0.664
	$\eta_p{}^2$	0.6229	0.0083	0.2160	0.3505	0.0002	0.0063	0.0017	0.0054
Fe conc (ug/g)	P	<0.001	0.281	0.963	0.073	0.392	0.668	0.376	0.323
	$\eta_p{}^2$	0.6399	0.0094	0.0006	0.0882	0.0059	0.0065	0.0157	0.0182
U (mg/mg)	P	<0.001	0.043	0.677	<0.001	0.047	0.013	0.252	0.680
	$\eta_p{}^2$	0.2552	0.0328	0.0063	0.2680	0.0318	0.0682	0.0222	0.0063
E_N (mg/mg)	P	<0.001	0.078	<0.001	0.310	<0.001	0.014	0.555	0.158
	$\eta_p{}^2$	0.3926	0.0250	0.2361	0.0553	0.1384	0.0674	0.0095	0.0295
$E_{N,yp}$ (mg/mg)	P	0.001	0.469	0.114	0.557	<0.001	0.001	0.874	0.086
	$\eta_p{}^2$	0.0886	0.0044	0.0353	0.0390	0.1310	0.1022	0.0022	0.0398
$E_{N,ys}$ (mg/mg)	P	<0.001	0.026	<0.001	0.165	<0.001	0.007	0.593	0.093
	$\eta_p{}^2$	0.4520	0.0404	0.3305	0.0717	0.1415	0.0786	0.0086	0.0385

Year (df=1), treatment (df=1), wheat type (df=2), genotype (wt) (df=6), Y*T (df=1), Y*Wt (df=2), T*Wt (df=2) and Y*T*Wt (df=2). Significant differences are denote by P. Trait abbreviations HLW, Hectoliter weight; TKW, Thousand kernel weight; N conc, Nitrogen concentration; P conc, Phosphorus concentration; Zn conc, Zinc concentration; Fe conc, Iron concentration; U, Nitrogen uptake efficiency; E_N, Grain-specific Nitrogen efficiency; $E_{N,yp}$, Protein-specific Nitrogen efficiency, $E_{N,ys}$, Starch-specific Nitrogen efficiency.

and HLW, for which trait correlations were not consistent and changed from negative to positive from one year to the other (Table 4).

To visualize correlation patterns and specifically evaluate the associations between wheat type and traits for each year, a Principal component analysis (PCA) was performed. The total Eigenvalue was 5.7 for dimension 1 (explanatory power 57%) and 1.5 for dimension 2 (explanatory power 15%) for 2018. For 2019 the total Eigenvalue was 6.2 for dimension1 (explanatory power 62%) and 1.5 for dimension 2 (explanatory power 15%). In 2018 grain yield and starch were part of the same cluster, and the wheat types related to both traits were High yield (I) and intermediate (III) type (Figure 1A). In a different cluster, protein, gluten, HLW, Zn, N, P, and falling number were associated with the high protein (II)

TABLE 4 Spearman's rank correlation coefficients between grain yield and quality parameters per year.

Year	Trait	Protein	Gluten	Starch	HLW	TKW	Falling Number	N conc	P conc	Zn conc	Fe conc
2018	Grain yield (Mg/ha)	-.750**	-.751**	.597**	-.422**	.257*	-.157	-.656**	-.758**	-.409**	-.077
2019		-.603**	-.324**	.554**	.057	.653**	.550**	-.636**	-.623**	-.580**	-.400**
2018	Protein (mg/g)		.943**	-.870**	.420**	-.191	.324**	.861**	.706**	.592**	-.040
2019			.728**	-.891**	-.382**	-.596**	-.369**	.942**	.820**	.599**	.527**
2018	Gluten (mg/g)			-.849**	.363**	-.271*	.254*	.804**	.708**	.506**	-.071
2019				-.583**	-.503**	-.384**	-.140	.566**	.453**	.408**	.419**
2018	Starch (mg/g)				-.329**	.051	-.355**	-.817**	-.687**	-.552**	.004
2019					.442**	.641**	.240*	-.915**	-.715**	-.578**	-.496**
2018	HLW (g/L)					.000	.120	.452**	.348**	.290*	.263*
2019						.121	-.086	-.323**	-.182	-.234*	-.155
2018	TKW (g)						.141	-.202	-.160	-.098	.124
2019							.487**	-.668**	-.497**	-.386**	-.224
2018	Falling Number (s)							.234	.152	.054	-.014
2019								-.435**	-.437**	-.321**	-.277*
2018	N conc (mg/g)								.781**	.771**	.118
2019									.801**	.562**	.502**
2018	P conc (mg/g)									.690**	.144
2019										.774**	.569**
2018	Zn conc (ug/g)										.355**
2019											.610**

** and * denote significant differences at $P<0.01$ and $P<0.005$; respectively. Trait abbreviations HLW, Hectoliter weight; TKW, Thousand kernel weight; N conc, Nitrogen concentration; P conc, Phosphorus concentration; Zn conc, Zinc concentration; Fe conc, Iron concentration.

type. In 2019, protein, gluten, Zn, N, and P remained together as part of the same cluster, and the wheat type most related to these traits was the high protein (II) type, while grain yield, starch, TKW, falling number, and HLW were related to the high yield (I) type (Figure 1B).

High yielders (type I) were associated to high grain yield, starch content, and TKW in both years, while high protein types (II) were related to high concentrations of protein, gluten, and nutrients. The Intermediate wheat types (III) were more related to High yield type (I) during both years. Similar to the results from the correlation analysis, HLW and falling number also showed opposite patterns between the two years in the PCA where HLW and falling number were associated to different wheat types in 2018 and 2019 (Figure 1).

Coefficient of variation (CV), based on five individual plants for each variety, indicated that most of the traits were stable for the three wheat groups for both years (Figure 2). Hectoliter weight (CV 2.1% for type (I), 0.91% for type (II), and 1.3% for type (III)) and starch (CV 2.1%, 1.9%, and 3.7%, respectively) were the most stable traits across the three wheat types, while Fe was the least stable trait with a CV above 60% (CV 69%, 64%, and 78%, respectively) follow by Zn (CV 23%, 19% and 24%, respectively). Macro nutrients N and P showed similar values for type (I) 11%, 12%, for type (II) 9%, 11%, and for type (III) 13%, 15% respectively. Results of high yielders (I), high protein type (II), and intermediate ones (type III) were similar, except for gluten and falling number in high protein wheat (type II). For these two traits, the percentage value in the high-protein wheat was doubled in comparison to the other two wheat types (Figure 2).

3.3 Biofortification potential

Significant positive correlations between grain N and Zn were found for the high yielders (I) and high protein types (II) (Figures 3A, B), while the Intermediate type (III) showed a positive correlation between N and Zn only in 2018. The high yield wheat varieties showed a poor correlation between Zn and Fe in 2018 ($R^2 = 0.004$), but a higher correlation in 2019 ($R^2 = 0.385$) (Figures 3C, D). The correlation between Zn and P during 2018 and 2019 (Figures 3E, F) was positive across all wheat types; however, the strongest correlation was observed during 2019 in the high protein wheats (II). A positive correlation was found between N and Fe in the high protein (II) and intermediate (III) wheat type during 2018, while in 2019 the positive correlation was found in the high yield (I) and high protein (II) wheat groups (Figures 3G, H). Finally, a low correlation between P and Fe in 2018 was registered among all wheat types. During 2019, the correlation between P and Fe was positive, however, the highest correlation was noticed in high protein wheat type ($R^2 = 0.304$) (Figures 3I, J).

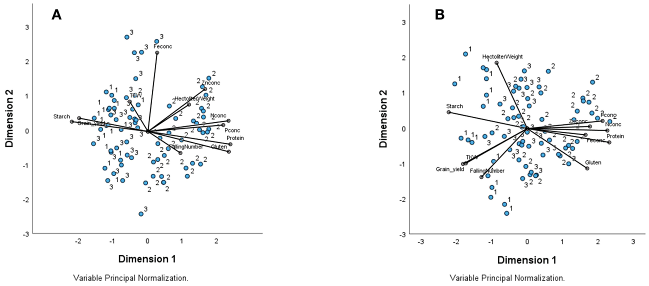

FIGURE 1
Biplot containing grouping of variables according to Principal component analysis (PCA) for 2018 **(A)** and 2019 **(B)** datasets. The variables are the trait values measured on three different wheat type groups: 1 - High yield, 2 - Organic high protein, and 3 – Intermediate for nine wheat varieties field-trial in Sweden. In 2018 the total Eigenvalue was 5.7 for dimension 1 (explanatory power 57%) and 1.5 for dimension 2 (explanatory power 15%). For 2019 the total Eigenvalue was 6.2 for dimension 1 (explanatory power 62%) and 1.5 for dimension 2 (explanatory power 15%). Traits Grain yield, protein, starch, gluten, hectoliter weight, TKW thousand-kernel weight, falling number, Nconc Nitrogen concentration, Pconc Phosphorus concentration, Znconc Zinc concentration, and Feconc Iron concentration.

Greater mean values for N, P and Zn concentrations in the grain were recorded for the high protein wheat type (II) for both years combined (Figure 4).

Due to the positive correlations between N and Zn in the high protein group, the higher mean values and the high environmental effect in N, Zn and Fe; an ANOVA analysis was performed for each variety of the high protein wheat type (II) regarding environmental factors. The four varieties: Bjarne, Dacke, Dala and Quarna, did not present significant differences for N regarding environmental effects, as year ($P=0.139$, $P=0.153$, $P=0.198$ and $P=0.302$ respectively), treatments ($P=0.981$, $P=0.810$, $P=0.852$ and $P=0.664$ respectively) and the interaction of Y*T ($P=0.985$, $P=0.847$, $P=0.894$ and $P=0.972$ respectively). Additionally, all four showed significant differences regarding year for Fe ($P=0.002$, $P=0.012$, $P=0.007$ and $P=0.007$ respectively). Finally, varieties Dala and Quarna did not present significant differences for year ($P=0.199$), treatment ($P=0.966$ and $P=0.367$) and Y*T interaction ($P=0.724$ and $P=0.365$) regarding Zn (Table 5).

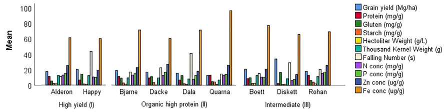

FIGURE 2
Coefficient of variation analysis to determine the static stability of quality traits of nine-spring wheat varieties, grouped in three wheat types due to their main breeding target, grown over two years (2018 and 2019) in a field experiment in Uppsala, Sweden. Genotypes Alderon and Happy are categorized as High yield wheat type (I); Bjarne, Dacke, Dala, and Quarna are categorized as Organic high protein wheat type (II), and Boett, Diskett, and Rohan are categorized as Intermediate wheat type (III).

3.4 Nitrogen accumulation efficiency and its components

For this study, the values of U and N' during the growing season were similar, and therefore, in this study, the variation in U was caused mostly by N' and not by the initial amount of N in the seed. Among all the N accumulation and N efficiency traits assessed, only E_N and $E_{N,ys}$ were significantly different between wheat types (Table 3). Year and the year*wheat type interaction significantly affected the N uptake efficiency (U), E_N, $E_{N,yp}$, and $E_{N,ys}$. Thus, under the drier conditions in 2018, the high yield wheat type (I) accumulated more N, shown by higher U than the other wheat types, while in 2019, the intermediate (III) and high protein (II) types showed the highest U values (Table 6). For E_N and $E_{N,ys}$, high-yield varieties achieved the highest values in 2019 and intermediate varieties had the highest values in 2018. During 2018, no significant differences were found between the three wheat types regarding $E_{N,yp}$. However, in 2019, the high yield type (I) showed a significantly higher $E_{N,yp}$ ($P=0.001$) (Tables 3, 6).

The effect sizes for year regarding U, E_N, $E_{N,yp}$, and $E_{N,ys}$ were between medium and large ($0.089 \leq \eta_p^2 \leq 0.452$). For the year*wheat interaction, the effect sizes were medium for the four traits ($0.067 \leq \eta_p^2 \leq 0.102$) (Table 3).

Mean values of U, E_N, $E_{N,ys}$, and $E_{N,yp}$ were plotted separately for 2018, 2019, and both years combined (Figures 4L–O and 5) (Supplementary Table S1). The N uptake efficiency (U) varied significantly between the wheat types in both years, and was either lower (2018) or similar (2019) in the high-protein varieties compared to the high-yield varieties (Table 6). The grain-specific and yield-specific N efficiencies (E_N and $E_{N,ys}$, respectively) were always much higher in the high-yielding varieties than the high-protein varieties. In contrast, the protein-specific N efficiency ($E_{N,yp}$) was similar between the wheat types in the dry year (2018), but lower in the high-protein varieties than the high-yielding varieties in 2019.

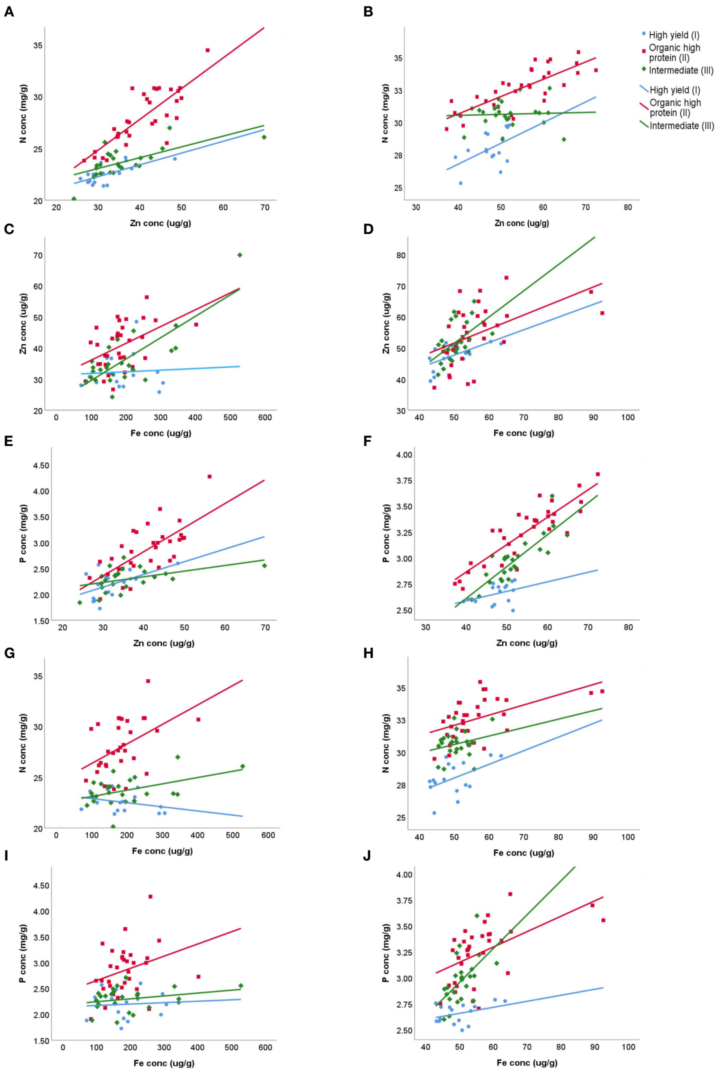

FIGURE 3

Relationships between grain concentrations of various macro- and micro-elements relevant for biofortification potential, plotted for 2018 and 2019 data sets and grouped into three wheat types (of nine spring wheats) grown in Uppsala, Sweden. Shown are the relationships between N and Zn concentrations: **(A)** 2018 and **(B)** 2019, Zn and Fe concentrations **(C)** 2018 and **(D)** 2019, Zn and P concentrations **(E)** 2018 and **(F)** 2019, N and Fe concentrations: **(G)** 2018 and **(H)** 2019, and P and Fe concentrations: **(I)** 2018 and **(J)** 2019Linear regressions: **(A)** High yield R^2 = 0.467, Organic high protein R^2 = 0.663, Intermediate R^2 = 0.451; **(B)** High yield R^2 = 0.238, Organic high protein R^2 = 0.614, Intermediate R^2 = 0.002; **(C)** High yield R^2 = 0.004, Organic high protein R^2 = 0.220, Intermediate R^2 = 0.619; **(D)** High yield R^2 = 0.385, Organic high protein R^2 = 0.252, Intermediate R^2 = 0.269; **(E)** High yield R^2 = 0.276, Organic high protein R^2 = 0.495, Intermediate R^2 = 0.205; **(F)** High yield R^2 = 0.148, Organic high protein R^2 = 0.771, Intermediate R^2 = 0.715; **(G)** High yield R^2 = 0.089, Organic high protein R^2 = 0.209, Intermediate R^2 = 0.211; **(H)** High yield R^2 = 0.241, Organic high protein R^2 = 0.267, Intermediate R^2 = 0.060; **(I)** High yield R^2 = 0.005, Organic high protein R^2 = 0.097, Intermediate R^2 = 0.071; **(J)** High yield R^2 = 0.138, Organic high protein R^2 = 0.304, Intermediate R^2 = 0.306 Traits N conc Nitrogen concentration, Zn conc Zinc concentration, Fe conc Iron concentration and P conc Phosphorus concentration. Wheat varieties High yield wheat type (I) (blue), Organic high protein wheat type (II) (red) and Intermediate wheat type (III) (green).

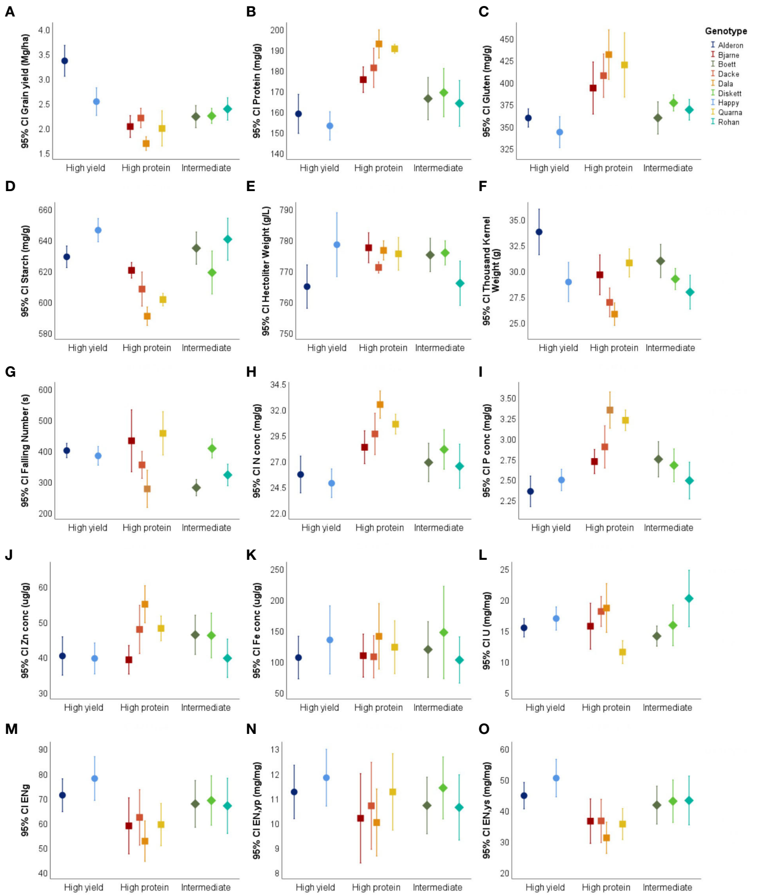

FIGURE 4
Mean values and confidence interval at 95% of fifteen traits **(A)** Grain yield, **(B)** Protein, **(C)** Gluten, **(D)** Starch, **(E)** Hectoliter Weight, **(F)** Thousand Kernel Weight, **(G)** Falling Number, **(H)** N conc, **(I)** P conc, **(J)** Zn conc, **(K)** Fe conc, **(L)** U, **(M)** EN, **(N)** EN,yp, and **(O)** EN,ys of nine spring heat varieties divided by their breeding target in Uppsala, Sweden for combined years (2018 & 2019). Wheat groups High yield wheat type (I), Organic high protein wheat type (II), and Intermediate wheat type (III). Trait abbreviations N conc Nitrogen concentration, P conc Phosphorus concentration, Zn conc Zinc concentration, Fe conc Iron concentration, U Nitrogen uptake efficiency, EN Grain-specific Nitrogen efficiency, EN,yp Protein-specific Nitrogen efficiency, EN,ys Starch-specific Nitrogen efficiency.

TABLE 5 Analysis of variance (ANOVA) p-value for N, Zn and Fe for fixed effects related to environmental variation of the four varieties in high protein wheat type (II).

Wheat type II Varieties	N conc			Zn conc			Fe conc		
	Year (Y)	Treatment (T)	Y*T	Year (Y)	Treatment (T)	Y*T	Year (Y)	Treatment (T)	Y*T
Bjarne	0.139	0.981	0.985	0.050	0.850	0.888	0.002	0.096	0.472
Dacke	0.153	0.810	0.847	0.054	0.720	0.535	0.012	0.306	0.663
Dala	0.198	0.852	0.894	0.199	0.966	0.724	0.007	0.560	0.890
Quarna	0.302	0.664	0.972	0.199	0.367	0.365	0.007	0.716	0.667

TABLE 6 Interaction between Year (2018 & 2019) and Wheat type (Wt; High yield (I), Organic high protein (II), and Intermediate (III)) of nitrogen (N) accumulation efficiency components: U N use efficiency, E_N Grain-specific N efficiency, $E_{N,yp}$ Protein-specific N efficiency and $E_{N,ys}$ Starch-specific N efficiency.

Year * Wheat type interaction								
	Year	P (Wt)	High yield (I)		Organic high protein (II)		Intermediate (III)	
U (mg/mg)	2018	0.008	15.232	± 1.075	13.512	± 0.760	12.808	± 0.878
	2019	0.011	17.129	± 1.075	18.435	± 0.760	20.585	± 0.878
E_N (mg/mg)	2018	0.005	79.422	± 3.215	69.623	± 2.273	81.220	± 2.625
	2019	<0.001	69.596	± 3.215	46.639	± 2.273	54.355	± 2.625
$E_{N,yp}$ (mg/mg)	2018	0.512	11.203	± 0.551	11.979	± 0.396	11.833	± 0.460
	2019	<0.001	11.861	± 0.551	9.116	± 0.389	10.090	± 0.450
$E_{N,ys}$ (mg/mg)	2018	<0.001	51.548	± 2.017	42.113	± 1.451	52.843	± 1.686
	2019	<0.001	43.638	± 2.017	27.896	± 1.426	33.119	± 1.647

Mean, significance (P) and ± SE were produced with an Analysis of variance (ANOVA).

4 Discussion

Based on the analysis of very different spring wheat materials over two years under contrasting weather conditions, this paper evaluated the relationships between grain yield and quality traits, as well as crop nutrient accumulation aspects relevant for the evaluation of environmental performance and ecological sustainability of the crop. Additionally, it incorporated a large number of grain quality traits including four elements relevant to the potential for biofortification. Some of the results are not surprising and have been known for long time, such as the negative relationship between grain yield and grain N or protein concentration (Bogard et al., 2010). Our study goes beyond the established knowledge and investigated the relationships between the two target traits grain yield and grain protein concentration on the one hand, and various non-target traits including grain quality traits, grain nutrient (cf. biofortification) and crop nitrogen use efficiency traits (cf. environmental performance of the crop) on the other hand. Our results have implications for crop breeding, especially in wheat. The limitations of this study are the small number of wheat varieties we analysed and the lack of additional locations, the latter of which could have contributed to a better analysis of the G x E interactions.

Due to the large differences between sowing years, all correlation, regression and PCA analyses were applied separately for the two years. Not surprisingly, this study illustrated that grain yield and quality traits are partly strongly affected by the underlying breeding targets, represented here as wheat types, but also by the contrasting environmental conditions in the two study years. Supporting our first hypothesis (H1), the results indicate that most of the grain quality traits were positively associated with the high protein wheat type (II), and the grain yield was positively correlated with the high yield wheat type (I); confirming the differential breeding targets (Figure 1). The effect size analysis revealed only small differences in stability between grain quality and yield traits. Contrary to our second hypothesis (H2), both trait groups were affected by genetic differences and environmental conditions (Table 3), which partly contradicts the literature (Makawi et al., 2013; Labuschagne et al., 2016). We also found no evidence for high-protein varieties (type II) to have greater N uptake efficiency than varieties bred for high grain (starch) yields (type I), not supporting our third hypothesis (H3).

4.1 Associations among target and non-target traits favor breeding selection

The relationship between target and non-target traits was specific for the three wheat types investigated here, and stable for both years (H1). Whilst starch and TKW were in the same cluster with grain yield, and associated to the high-yield wheat type during both years, macro- and microelements, gluten, and protein content were associated to the high-protein varieties (Figures 1A, B). Our findings suggest that some non-target and target traits may have been inherited together during the breeding process, although the large quantity of significant genotype (within wheat type) effects in our study indicates that most of the traits also have a strong individual genotype-specific component which is discussed elsewhere (Weih et al., 2021; Liu et al., 2022b). Similar to other studies (Koppel and Ingver, 2008; Mut et al., 2010), our study showed that HLW and falling number were strongly affected by the environmental conditions, and the low coefficient of variation (CV) for these two traits (Figure 2) is probably a result of the high values of these traits (high means result in low CV) and can here not be interpreted as indicators of static stability. In 2018, both traits also showed a positive correlation with protein and gluten instead of the positive correlation with yield and starch that was expected and seen in 2019 (Figures 1A, B) (Table 4).

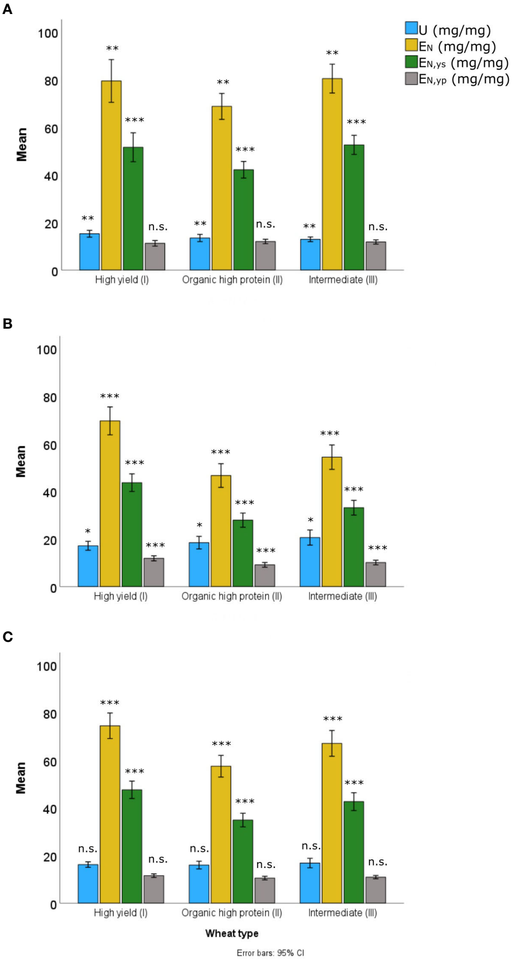

FIGURE 5
Comparison between U Nitrogen uptake efficiency, EN Grain-specific Nitrogen efficiency, EN,yp Protein-specific Nitrogen efficiency, and EN,ys Starch-specific Nitrogen efficiency for three wheat types corresponding to nine spring wheat varieties grown in central Sweden in 2018 **(A)**, 2019 **(B)** and the combined years **(C)**. Bars represent the mean values of each parameter. Wheat varieties High yield wheat type **(I)**, Organic high protein wheat type (II), and Intermediate wheat type (III). ***, ** and * denote significant differences at P<0.001, P<0.01 and P<0.05, respectively, n.s. denotes non-significant effects.

4.2 Positive correlation between Zn and P could jeopardize wheat biofortification

A positive correlation between grain N content (consider as a target trait for the high-protein varieties) and Zn and Fe grain content (non-target traits) was found in our results. This result partially supports our first hypothesis (H1), because it implies that target and non-target traits can be positively correlated due to pleiotropic effects or genetic linkage, facilitating the breeding process (Tabbita et al., 2017). Therefore, breeding for high grain N (e.g. high protein wheat) results most likely in high grain concentrations of Zn and Fe, and a high potential for biofortification with these nutrients (Cakmak, 2008). However, we also found a positive correlation between Zn, Fe and N in cultivars bred for higher yields as was suggested by others (Welch and Graham, 2004; Cakmak et al., 2010; Kutman et al., 2011). Additionally, we found that Zn and Fe was positively correlated with P (which is mainly accumulated as phytic acid in the grain) which could lead into problems of Zn and Fe bioavailability in the grain. Phytic acid acts as an antinutrient hindering Zn bioavailability, which can impact public health by reducing Zn and Fe absorption in the human body (Svečnjak et al., 2007; Shi et al., 2008; Gupta et al., 2015; Xue et al., 2015). Thus, breeding programs need to consider how to prevent high grain concentrations of phytic acid when breeding for high Zn and Fe concentrations in an attempt to achieve biofortification, as 49-80% of P in the grain is accumulated as phytic acid (Svečnjak et al., 2007).

4.3 Environmental and genetic differences influence grain yield and quality traits nearly equally

The relationship between wheat types, quality traits, and their interaction with different weather conditions has been studied before (Koppel and Ingver, 2008). Although wheat varieties with a persistent performance across diverse environments are desirable (Knapp et al., 2017), our results showed significant and large variation in yield and quality traits not only between wheat types, but also between years. Contrary to our second hypothesis (H2) and to other investigations (Labuschagne et al., 2007; Simons et al., 2012; Makawi et al., 2013; Taheri et al., 2021), grain yield was more affected by wheat type than by environment in our study (Table 3), whilst protein and grain N content were more affected by the environment (here weather) than the breeding target (Table 3). This suggests that both genetic and environmental variation influenced grain quality and yield similarly. The effect sizes for differences between years and between wheat types were greatest for grain yield, protein, gluten, starch, N, and P, implying that environment and genotype explained more of the variation than for the other studied traits, a finding that was also reported by others (Amiri et al., 2018).

4.4 High yield wheat type is the most efficient regarding E_N, $E_{N,ys}$ in a sustainability perspective

Pronounced effects of wheat genotype and year on the various N use efficiency traits have been reported and discussed previously for the same material (Liu et al., 2022b). Here we evaluate differences in N uptake and use in relation to the breeding targets of the different wheat varieties (i.e., the wheat groups) and various grain quality traits. The efficiency of converting plant-internal N to grain biomass (E_N) and starch yields ($E_{N,ys}$) was clearly highest in the group of high-yielding varieties, irrespective of the year. This implies that the high-yielding varieties generate higher amounts of the targeted end product per whole-plant N than the other wheat types, which is positive in an environmental performance perspective, because lower amounts of resources (e.g., N fertilizer) are needed to produce the desired end product (Pourazari et al., 2018). If high protein contents are desired, the protein-specific N efficiency ($E_{N,yp}$) is a more relevant measure. Our results indicate that the high-protein types were not more efficient in converting whole-plant N into protein yield than the other wheat types, because $E_{N,yp}$ was similar between the wheat types in the dry year (2018), and lower in the high-protein types in the year with a more normal weather (2019). An interesting measure for evaluating environmental performance is the total amount of N accumulated in the plant representing plant N requirement, here reflected by the N uptake efficiency (U). We hypothesized (H3) that the high-protein varieties should have greater N demand by means of higher U than the high-yield varieties, but surprisingly our results showed that U was either lower (2018) or similar (2019) in the high-protein varieties compared to the high-yield varieties. Breeding for high grain protein concentrations therefore is not necessarily associated with a high N uptake and N fertilizer requirement, although breeding target significantly influenced the U in both years (Table 6).

4.5 Near future breeding perspective

This study adopted an integrated perspective on grain yield and various quality traits in spring wheat. As wheat is one of the most important staple foods globally, the breeding programs have been focusing mostly on two major breeding targets, high yield and high protein content. In addition, the strong selection during wheat domestication and breeding have reduced the allelic diversity of both target and non-target traits so that a commercial cultivar consists of a single genotype. However, neglecting quality traits in breeding could prove crucial in the development of better elite varieties.

Although plant domestication and breeding have reduced the genetic diversity in target and non-target traits, the target traits seem to be linked to some non-target traits either by pleiotropy or linkage disequilibrium, where traits are inherited together as part of the same QTL contributing positively to the phenotypic expression of the target trait. Therefore, it is beneficial to plant breeding if a linkage block is discovered that has for breeders favorable effects on target and non-target traits (Charmet et al., 2005; Wang et al., 2012).

However, conventional and non-conventional plant breeding techniques might have a negative impact on some non-target traits, affecting some crop quality traits. Additionally, both target and non-target traits can be significantly affected by environmental factors.

Based on our results, we suggest the inclusion of biofortification as a regular quality trait in wheat breeding if the varieties show a high content of N, Zn, Fe, do not show a high influence of environmental effects and if do not show a strong correlation with P. Reaching bioavailability of Zn through genetic breeding has been the focus of many studies (Cakmak, 2008; Kutman et al., 2011; Guzmán et al., 2014; Tabbita et al., 2017), as it seems to be the most sustainable and cost-effective approach. Our results confirm a positive correlation between Zn and P across the spring wheat varieties in this study (Shi et al., 2008). If this result is a general pattern, it will cause problems in developing varieties with high Zn and low phytic acid concentrations. However, the development of a variety with this characteristic will be a beneficial long-term breeding goal. There are different strategies to implement biofortification. An approach proposed to cope with phytic acid accumulation in the seed was by increasing the level of the enzyme phytase (Nielsen et al., 2013). It has been reported that phytases are able to increase iron absorption in human intestine. Therefore, breeding for biofortified crops will require low phytic acid and high phytase content (Nielsen et al., 2013). Another method is to interfere in the Phytic acid biosynthesis pathway. Wheat inositol penta*ki*phosphate kinase

(*TaIPK1*) takes part in Phytic acid synthesis. After silencing IPK1 gene, a higher quantity of free phosphate in mature kernels was obtained. This finding was complemented by the rise of Zn and Fe in the grain content. Once more, the reduction of the Phytic acid content in cereal grains is a key step to achieve a greater accumulation and biovailability of Zn and Fe (Aggarwal et al., 2018). Furthermore, breeding efforts can be accompanied by agronomic biofortification. Plants required adequate amount of micronutrients from the soil and many of agricultural lands present complications that decrease availability of them to roots. Therefore, applications of Zn-fertilizers, Zn-NPK fertilizers and foliar sprays of $ZnSO_4$ and $FeSO_4$ suggest a complementary strategy to on-going breeding programs by enriching agricultural soil and by enhancing the re-translocation of Zn from flag leaves to the grain (Cakmak, 2008; Xue et al., 2015). Finally, the positive correlation between N and Zn showed in our results suggest that both traits could share genetic factors influencing the accumulation of Zn and N in the grain, even indicating that genes controlling protein and Zn accumulation in the grain are possibly co-segregated.

Our integrated perspective also considered environmental performance aspects related to crop resource (N) requirement and resource depletion of the production system. Genetic variation in traits related to nutrient use efficiency have been found in different cultivars, and include total N uptake, translocation, and assimilation (Xu et al., 2012). Additionally, total N uptake from soil is affected by the developmental growing stage of the plant, thus with a suitable N fertilization, will not increase N depletion. Moreover, based on the reviewed literature, N uptake and remobilization seem to be independently inherited traits, thus a combination of both alleles will be a positive addition to a cultivar for N uptake efficiency, and it will also allow the combination between low N uptake with high grain protein concentration (Xu et al., 2012).

5 Conclusions

High yield and high protein content are two of the most significant traits in modern wheat breeding. However, non-target traits are also required to breed new varieties, especially because some are related to quality concerns. Our results indicate that grain yield and quality parameters are affected by environmental conditions and wheat type in similar proportions. In addition, our results suggest that correlations between target and non-target traits tend to remain stable within the wheat types depending on their breeding target across different weather conditions, and the majority of the traits generally conserve their static stability (except for Fe). Furthermore, the inclusion of macro- and micro-elements in wheat varieties pose a new perception for quality, and should be further investigated. Moreover, in the case of varieties with a high protein content, biofortification may be considered in future breeding programs as a viable trait especially for varieties in which these traits are not strongly affected by the environmental conditions. Finally, the high-yielding varieties generate higher amounts of the targeted end product per whole-plant N than the other wheat types, and breeding for high grain protein concentrations is not necessarily associated with a high N fertilizer requirement, which has implications for the environmental performance of the crop.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

The field experiment was mainly planned and designed by MW and AW, and all authors were involved in the designing of the study; LG-V.: conceptualization, methodology, investigation, data analysis, writing (original draft preparation); HL: collecting all the data, methodology, writing (review and editing); JC: conceptualization, methodology, supervision, writing (review and editing); AW: conceptualization, methodology, supervision, writing (review and editing); MW: conceptualization, methodology, investigation, analysis of the data, project administration, supervision, writing (review and editing). All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2023.1151015/full#supplementary-material

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Supplementary Material

Differential breeding targets in wheat influence non-target traits related to grain quality, but not crop nitrogen requirement

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1 Supplementary Table

Supplementary Table 1. Mean and ±SE (produced with an Analysis of variance (ANOVA)) for both years (2018 & 2019) by Wheat type (Wt; High yield (I), Organic high protein (II), and Intermediate (III)) of fifteen traits. Trait abbreviations **HLW** Hectoliter weight, **TKW** Thousand kernel weight, **N conc** Nitrogen concentration, **P conc** Phosphorus concentration, **Zn conc** Zinc concentration, **Fe conc** Iron concentration, **U** Nitrogen uptake efficiency, **E_N** Grain-specific Nitrogen efficiency, **$E_{N,yp}$** Protein-specific Nitrogen efficiency, **$E_{N,ys}$** Starch-specific Nitrogen efficiency.

Trait unit		Wheat type (Wt)		
		High yield (I)	Organic high protein (ll)	Intermediate (III)
Yield (TN/ha)	Mean	2,944	1.972	2.283
	±SE	±0.057	±0.040	±0.046
Protein (mg/g)	Mean	155.900	184.839	165.963
	±SE	±0.871	±0.622	±0.720
Gluten (mg/g)	Mean	351.206	413,719	368.336
	±SE	±2.962	±2.114	±2.448
Starch (mg/g)	Mean	637.659	605.068	631.834
	±SE	±1.521	±1.085	±1.257
HLW (g/L)	Mean	771.646	775.054	772.195
	±SE	±1.386	±0.989	±1.145
TKW (g)	Mean	31.338	28.225	29.317
	±SE	±0.176	±0.125	±0.145
Falling number (s)	Mean	391.495	381.775	336.527
	±SE	±9.692	±6.915	±8.009
N conc (mg/g)	Mean	25.248	30.247	27.143
	±SE	±0.229	±0.162	±0.187
P conc (mg/g)	Mean	2.423	3.043	2.633
	±SE	±0.040	±0.029	±0.033
Zn conc (ug/g)	Mean	39.911	47.493	43.983
	±SE	±1.077	±0.761	±0.879
Fe conc (ug/g)	Mean	120.304	119.944	122.641
	±SE	±9.457	±6.687	±7.722
U (mg/mg)	Mean	16.180	15.973	16.696
	±SE	±0.760	±0.537	±0.621
EN (mg/mg)	Mean	74.509	58.131	67.788
	±SE	±2.273	±1.608	±1.856
EN,yp (mg/mg)	Mean	11.532	10.547	10.962
	±SE	±0.389	±0.278	±0.322
EN,ys (mg/mg)	Mean	47.593	35.005	42.981
	±SE	±1.426	±1.017	±1.178

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Nitrogen (N) use efficiency is a vital trait for achieving sustainable food production with reduced environmental impact. This thesis evaluated spring wheat varieties and a breeding population, focusing on key N use efficiency traits, improved phenotyping methods, and the genomic regions controlling them. The findings suggest the incorporation of N use efficiency as a regular target in spring wheat breeding.

Lorena de Jesús Guardia Velarde received her PhD education from the Department of Crop Production Ecology at SLU, Uppsala. She obtained her MSc degree in Plant Biotechnology from Wageningen University & Research in The Netherlands, and a BSc in Biology from Universidad Nacional Agraria La Molina in Peru.

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