

Nutrient Economy in Annual and Perennial Crops

Comparisons Between and Within Crop Species in a Sustainability Context

Fereshteh Pourazari

*Faculty of Natural Resources and Agricultural Sciences
Department of Crop Production Ecology
Uppsala*

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2016

Acta Universitatis agriculturae Sueciae

2016:70

Cover: Drawing of potato, timothy, red clover, maize and wheat
(Art: F. Pourazari)

ISSN 1652-6880

ISBN (print version) 978-91-576-8642-8

ISBN (electronic version) 978-91-576-8643-5

© 2016 Fereshteh Pourazari, Uppsala

Print: SLU Service/Repro, Uppsala 2016

Nutrient Economy in Annual and Perennial Crops. Comparison Between and Within Crop Species in a Sustainability Context

Abstract

Nitrogen use efficiency (NUE) of agricultural crops is related to crop nitrogen (N) uptake and thereby the amount of N that is removed from agro-ecosystems through crop harvest. As the N removal through harvest is linked to the fertilization requirements and the risk of N leaching, the crop NUE is an important aspect of sustainability in agriculture. Crops with different life strategies, photosynthetic pathways, and selection and breeding histories are expected to have different NUE; and the N content of the harvested crop fractions (*e.g.* total aboveground, grain or tuber) is linked to the N removed from the agro-ecosystem. Therefore, crop traits and desired end use (*e.g.* fodder, energy or industry use) are expected to impact the NUE and sustainability of crop production (*sensu* N removal). The aim of this thesis was to evaluate the variation in NUE between and within several crops commonly grown in Sweden, and to identify the most N efficient crops for specific end uses.

Various NUE components of maize, winter wheat, mixed perennial ley and potato crops were compared in field and pot experiments. In wheat and potato, the NUE was further investigated by comparing different varieties. The yield output per harvested N (*i.e.* N removal from agro-ecosystem) was assessed in relation to different end uses, *i.e.* crude protein and energy output (wheat, maize and ley) or amylose content (two potato varieties). In wheat, the concentration of plant N was further investigated in relation to the concentrations of other elements (P, K, Ca, Mg, S, Mn, Fe, Cu, Na) during two growth periods with different weather and after different preceding crops.

On a growing-season basis, the highest and lowest harvested biomass was found in potato and wheat, respectively. Ley produced moderate yields with moderate N concentrations coupled with a low N uptake, making ley the most sustainable (*sensu* N removal) crop for fodder production. In contrast, moderate biomass production in maize was associated with high N uptake and low yield N concentration, making maize the most sustainable crop for energy production. A potato line genetically modified (GM) for high tuber amylose content had a higher tuber yield and N uptake efficiency than its non-GM parent. Ancient wheat varieties responded weakly to increased N availability and had a higher N uptake efficiency and grain N concentration than modern varieties; suggesting that those varieties can be interesting material for breeding. Element concentration pattern in wheat was strongly affected by developmental stage and weather, but not by preceding crop; N displayed a strong influence on the concentration pattern for all elements. Overall, the assessment of the functional links between crop yield, yield quality and N removal from the agro-ecosystem can contribute to the development of a more sustainable agriculture.

Keywords: nitrogen use efficiency, sustainability, stoichiometry, *Triticum aestivum*, *Zea mays*, *Trifolium pratense*, *Phleum pratense*, *Solanum tuberosum*, genetic modification.

Author's address: Fereshteh Pourazari, SLU, Department of Crop Production Ecology,
P.O. Box 7043, SE-750 07 Uppsala, Sweden
E-mail: Fereshteh.Pourazari@slu.se

Dedication

To Björn; who is a part of me forever

*I shall plant my hands in the garden
And I will grow, I know, I know oh I know
And in my hands' inkstained hollow
The swallow
Shall lay its eggs.*

Forough Farrokhzad

Contents

List of Publications	8
Abbreviations	10
1 Introduction	13
2 Aims and hypotheses	15
3 Background	17
3.1 Nitrogen use efficiency (NUE) in different crops	18
3.1.1 NUE in crops with different life strategies	18
3.1.2 NUE in crops with different photosynthetic pathways (C ₃ and C ₄)	18
3.2 Nitrogen efficient crops - Influence of breeding	19
3.2.1 Crop domestication from ancient to modern varieties	19
3.2.2 Modern crop improvements	20
3.3 Nutrient concentration pattern during life cycle of winter wheat as affected by crop sequences	21
4 Material and methods	23
4.1 Plant material	24
4.2 Sampling and nutrient analysis	26
4.3 Assessment of NUE and N-related sustainability indicators	28
5 Results	30
5.1 Yield and NUE in different crops (Papers I & III)	30
5.2 Variation in grain yield and NUE in winter wheat varieties (Papers II)	31
5.3 NUE and tuber yield in potato lines (Paper III)	32
5.4 Nitrogen-related end use ratios (Papers I & III)	33
5.5 Element concentration patterns (Paper IV)	33
6 Discussion	35
6.1 Nitrogen use efficiency concept	35
6.2 What are the differences between ley, maize, potato and wheat in terms of yield and N economy?	36
6.3 What are the differences between ancient and modern varieties in terms of their N economy?	37
6.4 Is a higher tuber yield in GM potato lines associated with a higher NUE?	38

6.5	What is the influence of crop characteristics and end use on N-related sustainability?	38
6.6	What are the influences of environmental conditions on NUE and its components?	39
6.7	Is the element concentration pattern in wheat mirrored by its N concentration?	40
6.8	What are the impacts of growth condition on element concentration patterns?	40
7	Conclusions	41
8	Implications and future perspectives	43
	References	45
	Acknowledgements	49

List of Publications

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

- I Pourazari, P., Båth, B., Vico, G. & Weih, M. Nitrogen-based crude protein and energy ratios as sustainability indicators in wheat, maize and grassland ley grown for fodder or energy (Manuscript)
- II Pourazari, P., Vico, G., Ehsanzadeh, P. & Weih, M. (2015). Contrasting growth pattern and nitrogen economy in ancient and modern wheat varieties. *Canadian Journal of Plant Science*, 2015, 95(5): 851-860.
- III Pourazari, F., Weih, M. & Andersson, M. Yield and nitrogen economy of genetically modified high amylose and oil potato lines compared to their parents – effect of growing conditions (Manuscript).
- IV Weih, M., Pourazari, F. & Vico, G. Nutrient stoichiometry in winter wheat: Element concentration pattern reflects developmental stage and weather more than preceding crop (Submitted manuscript).

Papers II is reproduced with the permission of the publisher.

The contribution of Fereshteh Pourazari to the papers included in this thesis was as follows:

- I Participated in designing the experiments, carried out the sampling and laboratory work, analysed the data and wrote the majority of the manuscript with the guidance of the co-authors.
- II Participated in designing the experiments, carried out the experiments and sampling with the guidance of the co-authors, analysed the data and wrote some sections of the paper.
- III Carried out the sampling in both experiments, analysed the data and wrote majority of the paper with the guidance of the co-authors.
- IV Carried out the sampling and laboratory work and commented on the text.

Abbreviations

N	Nitrogen
NUE	Nitrogen use efficiency
U_N	Nitrogen uptake efficiency
$E_{N,yield}$	Yield specific N efficiency
$C_{N,yield}$	Yield N concentration at final harvest
N_S	N content of the initial biomass
N'	Mean plant N content during growth period
R_N	Mean relative N accumulation rate during the main growth period
N_{yield}	Yield N content at final harvest
B_{yield}	Yield biomass at final harvest
GM	Genetically modified

1 Introduction

Nitrogen (N) is one of the most vital elements in crop production. Crops need to take up a substantial amount of N in order to maintain their growth, survival and reproduction. For example, wheat crops require approximately 120 kg N ha⁻¹ to achieve a grain yield of 6.5 ton ha⁻¹ under Swedish conditions (Börjesson and Tufvesson 2011). The harvesting of crops contributes substantially to the depletion of N resources in the agroecosystem, as a high proportion of N is removed with the harvested product. This is one of the primary reasons why fertilizer needs to be applied regularly, an agricultural practice associated with a number of negative environmental impacts such as N leaching and enhanced greenhouse gas emissions (Canfield et al. 2010). In this context, the N use efficiency (NUE) of a crop, which is its ability to accumulate biomass and yield with as little N resources as possible, is of great relevance (Fageria et al. 2008). Thus, identification and production of N-efficient crops and crop genotypes can improve the sustainability of the crop production.

Wheat, maize, grass/clover leys and potato are grown worldwide for food, feed or as feedstock for fuel and other industries; and are important sources of carbohydrate, starch and/or protein (FAO 2013). These crops have different life strategies, selection histories and photosynthetic pathways, which may influence their NUE. Thus, assessment of N economy and productivity in those major crops and their varieties can provide important insights into a more sustainable agriculture. Moreover, since these crops are produced for different end uses, *e.g.* energy and fodder production or as feedstock for certain industries, their N economy has to be assessed in relation to their productivity for specific end uses. Furthermore, the assessment of plants' N concentration in relation to the concentrations of other essential elements in the growing plant may provide a better understanding of the influence of this essential element on the concentrations of other elements, and thus on crop productivity.

2 Aims and hypotheses

The overall aim of this thesis was to improve the sustainability in agriculture by identifying crops with the best N economy under different circumstances. Specific objectives were to:

a) Compare NUE and growth between crops and crop varieties; and b) identify the links between plants' N concentration and the concentration of 10 other nutrients, and determine temporal patterns in these nutrients in winter wheat as influenced by preceding crop and weather conditions. The following hypotheses were tested:

1. Different crops commonly grown in Swedish agriculture (wheat, maize, grass/clover ley and potato) vary in terms of NUE and its components: N uptake efficiency, yield production per unit of plant N, and N concentration of the yield. Moreover, variation in N economy and productivity is linked to the differences in end use (fodder, energy or amylose) and the ratio between specific yield (crude protein, energy or amylose) and N removal from the system by harvesting (Paper I).
2. Compared to modern wheat varieties, ancient varieties have characteristics suitable for higher biomass production at low N availability and a weaker growth response to higher N availability; whereas the modern varieties produce higher yield under high N availability (Paper II).
3. Potato line genetically modified (GM) for high amylose starch content in tubers is more productive in terms of tuber yield than its parent; the higher yield in the GM potato line is associated with concurrent changes in NUE and its components: N uptake

efficiency, harvested tuber per absorbed N and tuber N concentration (Paper III).

4. In winter wheat, N has the strongest influence on the concentration pattern of other elements (*i.e.* P, K, Ca, Mg, S, Mn, Fe, Cu, Na). Moreover, the element concentration pattern in growing crops greatly varies across the life cycle of the crop, with the largest deviation from seed concentration pattern in early spring. The element concentration pattern is affected by preceding crop type and weather condition (Paper IV).

3 Background

Increasing yield production, while minimizing the N depletion and N fertilizer input, are important aspects in crop production (Spiertz 2010). As crop harvest removes a substantial amount of N resources from the agroecosystem, a better understanding of crop characteristics influencing N removal, *i.e.* crop NUE, has the potential to enhance the sustainability related to N depletion (Karp and Shield 2008, Brodt et al. 2011). Crops differing in life strategies, selection histories and photosynthetic pathways may have different characteristics in terms of N and biomass allocation and thus, NUE (Hawkesford et al. 2014). For example, the growth of many annual crops is strongly dependent on high N inputs, while some perennial crops can produce abundant dry matter yield with minimal N fertilization due to their efficient use of internal N (Karp and Shield 2008). Of biomass produced by crops, different crop fractions (hereafter referred to as the ‘harvested product’) may be desired for the final end use. For example, wheat grain is frequently used for human and animal consumption and energy production, while the aboveground parts of maize and ley are often used for energy and fodder production. Furthermore, the choice of harvested product is defined by its desired quality; *e.g.* for sugar and starch production, a high carbon storage in the harvested product is desirable, while a high N re-translocation to the harvested product is preferable in protein production. As another example, a high N concentration in the harvested product is a negative factor for energy crop production, since it causes NO_x emissions during the biogas production process (Borjesson and Tufvesson 2011). Thus, the choice of crop influences the quantity and quality of the end product, and the NUE of the cultivated crop.

3.1 Nitrogen use efficiency (NUE) in different crops

3.1.1 NUE in crops with different life strategies

Annual and perennial plants have different life histories and strategies, characterized by different biomass and N allocation patterns (Aragón et al. 2009, Jaikumar et al. 2013). Annual crops have been selected for a high resource allocation to the reproductive parts, probably at the expense of allocation to below-ground organs (Van Tassel et al. 2010). At the same time, perennial crops invest a higher proportion of their carbohydrates in the storage organs (*e.g.* rhizomes and stolon) and vegetative reproduction. This investment in below-ground compartments provides perennial crops with better access to water and nutrients, which benefits the crop in buffering variations in growing conditions and results in more stable yield production than in annual crops (Vico et al. 2016). Moreover, having storage organs helps perennial crops maintain their internal N resources. Thus, it has been proposed that perennial crops may utilize resources such as N more efficiently than annual crops (Aragón et al. 2009, Crews et al. 2016). These substantial differences between annual and perennial crops influence their NUE and thus, the N-related sustainability of their production for different end uses (Paper I).

3.1.2 NUE in crops with different photosynthetic pathways (C₃ and C₄)

Nitrogen is an important element in the structure of the enzyme ribulose 1,5 biphosphate carboxylase (Rubisco), which is responsible for carbon fixation in plants and also functions as an oxygenase depending on the concentrations of carbon dioxide (CO₂) and oxygen (O₂) in the mesophyll. The process of oxygenation by Rubisco, which is known as photorespiration, occurs frequently in C₃ plants and reduces the efficiency of carbon fixation (Bräutigam and Gowik 2016). The C₄ photosynthetic pathway inhibits photorespiration by increasing the intercellular concentrations of CO₂. Thus, C₄ plants can utilize the Rubisco enzyme more efficiently than C₃ crops and the efficiency of N use at the leaf level can be expected to be higher in plants with the C₄ photosynthetic pathway than in C₃ plants. These differences between crops with different photosynthetic pathways may therefore influence their NUE and thus the N-related sustainability (related to N depletion) of their production.

In Sweden, the advantage of growing C₄ cereals such as maize for energy or fodder production has begun to be exploited during the past decade (Börjesson and Tufvesson 2011, Eckersten et al. 2012); and maize production increased sharply from 2000 ha in 2002 to 170000 ha in 2009 (Jordbruksverket 2016). The thermal growing season for maize in Sweden runs approximately from

mid-April to mid-September and thus maize currently does not reach maturity in Sweden. However, with predicted climate change, the growing season is expected to be extended for summer growing crops such as maize (Eckersten et al. 2012). Understanding of the N economy of C₄ and C₃ crops may therefore be useful in estimating the future productivity and N-related sustainability of these crops in temperate climates such as Sweden, an issue investigated in Paper I of this thesis.

3.2 Nitrogen efficient crops - Influence of breeding

3.2.1 Crop domestication from ancient to modern varieties

With the development of agriculture, the wild varieties were domesticated and selected for a greater productivity under favourable growing conditions *i.e.* optimal resource availability. However, high resource use efficiency was not a highly prioritised trait in the selection of modern varieties (Chapin 1980, Castagna et al. 1996). Thus, the evolution from wild varieties to domesticated crops, and then to the modern varieties as a result of plant breeding, has increased the harvest index, but with the associated negative effects of reducing quality traits *e.g.* grain protein concentration, and increased resource requirements (Evans and Dunstone 1970). The reliance on resources such as fertilizers and pesticides leads to higher production costs and greater environmental risks (Gioia et al. 2015).

In this context, ancient varieties that are capable of higher yield and higher protein production than modern varieties under unfavourable conditions, *e.g.* low nutrient availability, can be interesting material for breeding high NUE cultivars. For example, hulled wheats were among the earliest domesticated wheat plants, originating from Eurasia more than 10 000 years ago (Nesbitt 2001). Today, ancient hulled wheats are grown mainly in marginal areas, reflecting their tolerance to unfavourable growing conditions, *e.g.* high altitude, cold winters and heavy soil (Nesbitt 2001). In Iran, for example, some native tetraploid hulled wheat varieties (*Triticum turgidum ssp. dicoccum*; Figure 1) are adapted to marginal, mountainous areas (Ehsanzadeh et al. 2009), and have been shown to be more tolerant to salinity stress than modern cultivars (Tabatabaei and Ehsanzadeh 2015). In a field study performed in Iran and presented in Paper II, these ancient varieties were compared with modern varieties in terms of grain productivity and protein concentration along an N fertilizer gradient. In contrast to the modern varieties, hulled varieties did not respond to ample fertilization in terms of their grain yield; this may indicate a higher NUE of those varieties under low nutrient supply. Thus, it was hypothesized that ancient hulled wheat varieties have characteristics suitable

for low N availability and a weak growth response to improved N availability (Paper II).



Figure 1. A) Hexaploid free threshing modern wheat varieties (*T. aestivum* var. Olivin); and B) Tetraploid hulled ancient wheat variety (*T. turgidum* spp. *dicoccum* var. Joneghan 1). Photo by F. Pourazari

3.2.2 Modern crop improvements

Genetic modification (GM) of crops is one of the tools suggested for achieving the increase in food production necessitated by the demands of the growing global population (Areal et al. 2013). GM generally involves genetic engineering by transferring specific genes from one organism into another. In breeding, it is extremely difficult to control exactly which of the millions of genes that parental lines will pass on to their offspring. By selecting and transferring specific genes, the GM approach tends to be far less time consuming and more precise than conventional breeding approaches. So far, the majority of GM work has been used in order to improve crop yield through enhancing crop resistance to pests (Andow and Zwahlen 2006) and enabling symbioses between crops and N fixating bacteria (Van Dillewijn et al. 2001). However, the modification of specific traits by transferring genes may be associated with unexpected effects on non-targeted traits. These unintended effects can have both negative and/or positive consequences for the crop's viability, and thus on the crop production. For example, indirect effects on NUE have been reported in maize, with the genetic modification for pest resistance being associated with higher yield production (Haegele and Below 2013). Other examples can be found in studies by Hofvander et al. (2016) and Menzel et al. (2015), where different potato cultivars were genetically modified to increase amylose and oil content in tubers. Oil accumulation was targeted to improve the nutritional values of tubers, while high amylose content is favourable for certain industries, *e.g.* film formation and bioplastics. The altered carbon allocation in these GM potato lines was found to be associated with a higher fresh tuber yield and lower starch content (Menzel et al. 2015,

Hofvander et al. 2016), and can therefore be expected to influence the economy of resources that often limit plant growth, *e.g.* nitrogen. Given that crop functional traits and N economy can greatly impact the production system, and thus ecosystem services, there is a great need for research evaluating the agronomic and ecological impacts of the novel traits associated with GM (Cellini et al. 2004, Kolseth et al. 2015).

The claim that altered carbon allocation in potato crops is associated with a higher NUE was tested in Paper III, where two potato lines genetically modified for high oil or high amylose content in tubers are compared with their non-GM parents in terms of their N economy. Only the results for the high amylose GM potato line ‘T-2012’ and its parent ‘Dinamo’ are discussed in this summary of the thesis. This is mainly because the high amylose and high oil GM potato lines had similar N economy when grown in the greenhouse, but the high oil potato lines were not grown in the field. For full results and discussion concerning the high oil GM potato line, see Paper III.

3.3 Nutrient concentration pattern during life cycle of winter wheat as affected by crop sequences

Nutrient elements are frequently re-translocated from vegetative plant parts to the grain during the grain filling stage in cereal crops, and are essential for the initial growth of the embryo during germination and establishment (Liptay and Arevalo 2000). A crop growing under optimal or near optimal conditions can be expected to have a similar element concentration pattern as the seed (Liptay and Arevalo 2000). However, when a crop is exposed to unfavourable growth conditions, its element concentration is expected to deviate from that in the seed. For example in autumn sown crops such as winter wheat, the element concentration pattern in growing plants may deviate from that in the seed in early spring when winter wheat experiences its most rapid growth. As the crop grows, other factors such as the availability of elements in soil, plant developmental stage and future demand also influence nutrient accumulation and thus the element concentration pattern in the growing crops (Burns et al. 1997, Malhi et al. 2006). The availability of nutrient elements to the wheat in crop sequences can be influenced by the preceding crop; which may in turn influence the crop yield. A number of studies have reported that the wheat yield significantly increased when grown after unrelated species such as legumes (Børresen 1999, Bakht et al. 2009). Thus, the effect of preceding crop on nutrient availability is possibly reflected by the element concentration pattern in the following crop.

Element stoichiometry is an established concept in plant physiology that is frequently used for assessment of the relationships between the concentrations of different elements. Most previous stoichiometric studies on crops have focused on a few major elements such as carbon C, N and P (Ågren 2004, Sadras 2006). This is mainly because of the assumption that these major elements are the most important for growth; and that the minor elements with lower concentrations in the crop are taken up in amounts relative to the major elements. However, there is a lack of studies supporting this assumption, and thus, it is important to assess the stoichiometry of a comprehensive selection of the plant elements from a “seed to seed” perspective. Moreover, since N is one of the most abundant elements in crops and it is relatively easy to assess, it would be beneficial to identify how strong the correlations between the N concentration and the overall element concentration pattern in crops are (Paper IV).

4 Material and methods

To test the hypotheses, experiments were performed in outdoor growth containers, a greenhouse and the field, and sampling was conducted in two of the Swedish long-term field trials (Table 1). In summary:

1. Field data were collected over a two-year period (2014-2015) from fertilized ($150 \text{ kg N ha}^{-1} \text{ year}^{-1}$) and unfertilised grassland ley plots (mixture of *Trifolium pratense* and *Phleum pratense*) in one of the long-term trials (here referred to as trial E1); and over a three-year period (2013-2015) in monocultures of maize (*Zea mays*) and winter wheat (*Triticum aestivum*) in trial E2. Both trials located in Uppsala (Paper I).
2. An outdoor growth container experiment was carried out in summer 2012, in Uppsala, Sweden, to compare two ancient hulled wheat varieties native to Iran with two modern wheat varieties from Sweden. All wheat plants were exposed to four different N treatments (0, 20, 80 and 200 kg N ha^{-1}). The data obtained in the container experiment were compared with data obtained in a field study with the same hulled wheat varieties performed in 2008, in Isfahan, Iran (Paper II).
3. Two genetically modified (GM) potato (*Solanum tuberosum*) lines (modified for high amylose and high oil content) and their parental lines were compared (Paper III) in two experiments:
 - A greenhouse pot experiment was conducted in 2014, where two GM potato lines and their parental varieties were grown (note that only the high amylose potato line and its parent are discussed in the summery part of this thesis).

- A field experiment (E3, Table 1) was carried out in summer 2015 to compare the high amylose potato line and its parent (same amylose potato lines as in the pot experiment).

4. Field data were collected over a two-year period (2013-2014) for wheat grown in monocultures and in crop rotations: monoculture and flax as preceding crops in 2013, and monoculture, ley and flax as preceding crops in 2014. These data were collected from the same field trial in which samplings were performed for Paper I (trial E2, Table 1) (Paper IV).

Table 1. Summary of growth experiments presented in Papers I-IV.

Experiments	paper	Treatments/ factors	Number of varieties/Crops	Replicates /Years
Field trial (E1)	I	Two levels of N treatments	Two components (red clover, timothy)	4/2013-15
Field trial (E2)	I, IV	Crop rotations and monoculture	Two crops (maize, winter wheat)	4/2013-14
Growth container	II	Four levels of N treatments	Four varieties (ancient and modern wheat varieties)	3/2012
Greenhouse	III	-	Four lines (high amylose and oil GM potato lines and their parents)*	5/2014
Field trial (E3)	III	-	Two lines (high amylose GM potato line and its parent)	10/2015

* Only the high amylose potato line and its parent are discussed in this summary

4.1 Plant material

For the growth container experiment (Paper II), the used old wheat varieties (*Triticum turgidum* ssp. *dicoccum* var. ‘Joneghan1’ and ‘Joneghan2’) were collected from remote mountainous areas of central Iran (Isfahan and Chaharmahale Bakhtiyari provinces; Ehsanzadeh et al. (2009)). Modern wheat varieties used in this experiment were *T. aestivum* var. ‘Granary’ and ‘Quarna’; which are commonly grown in Sweden (Figures 1 & 2). In the field studies (Papers I and IV), ‘Olivin’, ‘Active’, ‘Nancy’ and ‘Switch’, were the varieties of wheat, maize, red clover and timothy, respectively. Those varieties are commonly grown in Sweden (Figure 3).



Figure 2. Outdoor container experiment established in summer 2012, in a net yard by the Ecology Centre, SLU, Ultuna, Uppsala. Four wheat genotypes, two modern free-threshing varieties and two ancient hulled wheat varieties, were grown in the containers (Paper II). (Photos: F. Pourazari and G. Vico)



Figure 3. A) Field trial E1 (Säby, Uppsala, Sweden), under-sown barley is followed by three years of a mixed grass/clover ley (Photo: N. Nilsdotter-Linde), B & C) Field trial E2 (Säby), wheat and maize are grown in monocultures and crop rotations (Bergkvist et al. 2011). (Photos: F. Pourazari) (Papers I and IV)



Figure 4. A) Greenhouse pot experiment conducted in September-December 2014, at SLU Alnarp, Sweden. Four potato varieties were grown, two potato lines genetically modified (GM) for a high amylose or high oil content, and their parental varieties. B) Field experiment conducted in May-October 2015, at Borgeby, Sweden. Two potato varieties were grown; the same high amylose GM potato line and parent as in the greenhouse experiment (Paper III). (Photos by F. Pourazari and M. Andersson).

Genetically modified potato line and its parent, used in Paper III (Figure 4), were developed in a study by Hofvander et al. (2004) in which the parental potato cultivar ‘Dinamo’ was genetically modified for higher amylose starch content by introducing a modification inhibiting two starch branching enzymes. This resulted in the GM line ‘T-2012’, which has 23 % higher amylose content of the total starch than its parent.

4.2 Sampling and nutrient analysis

Aboveground biomass (and tubers in potato) in annual crops i.e. wheat, maize and potato were sampled at the following developmental stages (as classified by Witzemberger and Hack 1989): three leaf stage, BBCH 13 (S0, performed only in the field experiment, in wheat and maize); spikelet initiation for wheat and maize and tuber initiation stage for potato, BBCH 30 (S1); flowering, BBCH 55-69 (S2); and maturity, BBCH 88-99 (S3). Sampling in perennial ley took place at: before winter (S1), in early spring (S2) and during summer when ley cuts are commonly performed in the region (S3 and S4). A schematic representation of all the experimental work performed within this thesis is shown in Figure 5.

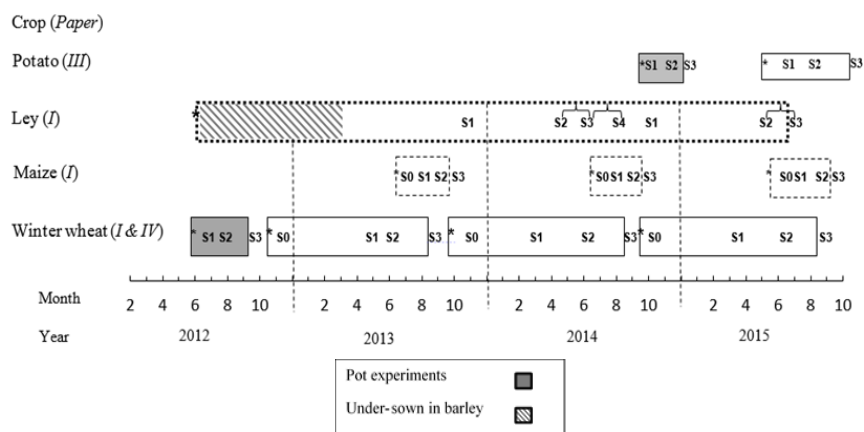


Figure 5. Representation of growth period (bars), sowing dates (stars) and sampling dates (S0-S4) for potato, ley, maize and winter wheat during the four years, 2012-2015. Sampling S0 was only performed in the field, in wheat and maize. The diagonal lines in the illustration for ley represent the period in which it was under-sown in barley, the brackets show the growth periods (two in 2014 and one in 2015). In potato and wheat, the grey bars represent the pot/container experiments, which were performed outdoors for wheat and in the greenhouse for potato.

Collected samples were washed with tap water to remove any soil particles and subsequently oven dried (Heratherm OGS400, Thermo Scientific, USA) at 80 °C for 3-7 days, the length depending on the amount of biomass. Dry weight of aboveground biomass was assessed for all crops at each sampling. For potato, dry tuber weight and aboveground biomass were assessed at each sampling and fresh tuber weight was assessed at the last two samplings. In the greenhouse experiment in Paper III, the root biomass of potatoes were also assessed (Figure 6F). At final harvest in wheat and potato, the harvested aboveground biomass was separated from the harvested yield *i.e.* grain in wheat and tuber in potato crops. The ley samples were divided into their clover and grass components.



Figure 6. A) Sampling of winter wheat plants (S1). B) Cleaning and separating the winter wheat plants from weeds. C) Sampling in ley plots. D) Seed potato tubers prepared for sowing in the field study. E) Sampling of potato plants in the field (S3). F) Separating the roots and tubers of potato plants grown in the pot experiment.

Dried grain samples and the collected aboveground and tuber samples from all experiments were milled using a cutting mill. Nitrogen analysis was carried out on all samples with a LECO CNS/2000 analyzer (LECO 1994) using a standard method (SS-ISO13878). Wheat plant samples used for the assessment of nutrient concentrations (Paper IV) were analysed for their contents of Ca, K, Mg, Na, N, P, S, Cu, Fe, Mn and Zn in vegetative plant parts during the main growth season and in harvested grains. This was done using 32.5 % nitric acid on a heat block and the concentrations of different element were determined using the ICP-AES technique (Spectro Blue FMS 26, Spectro Analytical Instruments, Kleve, Germany).

4.3 Assessment of NUE and N-related sustainability indicators

The method developed by Weih et al. (2011) was used to assess NUE. Accordingly, NUE is defined as the N in the harvested yield (N_{yield}) per unit N in the initial biomass (N_s ; seed for wheat and maize, stolon or seeds for potato plants and pre-wintering biomass for perennial leys). Harvested yield was taken as grain yield in wheat, aboveground biomass in ley and maize, and the tuber biomass in potato. The components of NUE are: U_N , which represents the N uptake efficiency; $E_{N,\text{yield}}$, which is yield-specific N efficiency, representing the efficiency of converting the accumulated N into harvested biomass; and $C_{N,\text{yield}}$, which is the efficiency of N re-translocation to the harvested product. These are calculated as:

$$\text{NUE} = \frac{N_{\text{yield}}}{N_s} = U_N \cdot E_{N,\text{yield}} \cdot C_{N,\text{yield}};$$

$$U_N = N' \cdot N_s^{-1}, \text{ where } N' \text{ is the mean plant N content during growth period}$$

$$E_{N,g} = B_{\text{yield}} \cdot N'^{-1}, \text{ where } B_{\text{yield}} \text{ is the harvested biomass yield, and}$$

$$C_{N,\text{yield}} = N_{\text{yield}} \cdot B_{\text{yield}}^{-1}, \text{ where } N_{\text{yield}} \text{ is the N content in the harvested yield}$$

Mean plant N content (N') in Papers I and III was calculated based on the entire growth period of plants, while in Paper II, N' in wheat varieties was based on the main growth period *i.e.* the period between the stem elongation and the anthesis stages (for details see Papers I & II). In this summary, I

present the calculations based on both main growth period and the entire growth period for wheat varieties.

The N-related sustainability indicators are defined as the amount of final end use (energy, crude protein or amylose) output per unit of harvested (*i.e.* removed) N from the soil. The N-related energy ratio is defined as $\text{Energy ratio}_N = \text{Energy yield} \cdot N_{\text{yield}}^{-1}$. For the calculation of energy (here, ethanol) production, a higher heating value of 18.4 MJ kg⁻¹ for wheat grain (without straw) and 17.6 MJ kg⁻¹ for total aboveground biomass (B_{ag}) of maize and mixed ley were extracted from Börjesson and Tufvesson (2011). The N-related crude protein ratio, indicating the final crude protein production per unit N removed from the soil by biomass harvest ($\text{Crude protein ratio}_N = \text{Crude protein yield} \cdot N_{\text{yield}}^{-1}$), was calculated using a conversion factor of 0.11 for wheat grain, 0.08 for maize and 0.16 g kg⁻¹ dry matter for mixed ley (converting values were extracted from Walsh et al. 2008).

For potato, the amylose output per unit N removed from the system by harvesting, here called Amylose ratio_N, was compared for the parent and GM lines and discussed in this summary. The amylose content was taken to be 19 % and 37 % of tuber dry matter for ‘Dinamo’ and ‘T-2012’, respectively (converting values extracted from Menzel et al. 2015).

5 Results

5.1 Yield and NUE in different crops (Papers I & III)

Crops (potato, ley, maize and wheat) grown in the field in 2015, are compared in terms of harvested product, NUE and its components (Table 2). The harvested product was dry tuber biomass at final harvest (S3; Figure 2) for potato, aboveground biomass for ley and maize and grain for wheat. On average, potato plants had the highest harvested biomass per unit area (B_{yield}), followed by unfertilized ley (Ley_0) and maize. Potato and wheat ranked similar in their initial biomass N content (N_S), mean plant N content over the growing period (N'), N uptake efficiency (U_N), yield specific N efficiency ($E_{N,\text{yield}}$), and overall NUE. However, potato produced more yield, while wheat had a higher yield N concentration ($C_{N,\text{yield}}$). Maize had the highest U_N and $E_{N,\text{yield}}$ and overall NUE, but the lowest N_S . Conversely, N_S and N' and yield production were highest in Ley_0 after the potato, but Ley_0 also had a low U_N and $E_{N,\text{yield}}$.

Table 2. Mean (\pm 95% CI) N content in initial biomass (N_S), mean plant N content during the entire growing period (N'), yield biomass (B_{yield}), N uptake efficiency (U_N), yield specific N efficiency ($E_{N,\text{yield}}$), yield N concentration ($C_{N,\text{yield}}$) and N use efficiency (NUE) in wheat (var. 'Olivin'), maize, grass/clover ley when fertilized (Ley_{150}) and unfertilized (Ley_0), and potato (var. 'Dinamo') grown in 2015, in field experiments (experiments E1-E3, see Table 1). Different superscript letters within the rows indicate significant differences (Tukey HSD test, $\alpha = 0.05$).

	Wheat	Maize	Ley_{150}	Ley_0	Potato
N_S (g m ⁻²)	0.38 ^b \pm 0.12	0.03 ^c \pm 0.05	1.59 ^a \pm 2.00	1.47 ^a \pm 2.17	0.41 ^b \pm 0.11
N' (g m ⁻²)	11.37 ^a \pm 4.24	5.45 ^b \pm 1.70	6.82 ^b \pm 2.54	12.50 ^a \pm 4.66	13.32 ^a \pm 2.95
B_{yield} (g m ⁻²)	656.3 ^{cd} \pm 1.18	827.0 ^{bc} \pm 1.26	502.6 ^d \pm 1.26	904.6 ^b \pm 1.26	1243.6 ^a \pm 1.16
U_N (g g ⁻¹)	27.3 ^b \pm 1.58	134.8 ^a \pm 1.58	3.4 ^c \pm 1.58	5.7 ^c \pm 1.58	31.9 ^b \pm 1.54
$E_{N,\text{yield}}$ (g g ⁻¹)	65.0 ^b \pm 1.38	151.6 ^a \pm 1.38	93.2 ^b \pm 1.38	91.6 ^b \pm 1.38	93.3 ^b \pm 1.23
$C_{N,\text{yield}}$ (g g ⁻¹)	0.018 ^a \pm 1.20	0.012 ^{bc} \pm 1.20	0.016 ^{ab} \pm 1.20	0.015 ^{ab} \pm 1.20	0.010 ^c \pm 1.38
NUE (g g ⁻¹)	36.04 ^b \pm 1.70	286.1 ^a \pm 1.70	5.32 ^d \pm 1.70	9.03 ^c \pm 1.70	30.25 ^b \pm 1.70

5.2 Variation in grain yield and NUE in winter wheat varieties (Papers II)

In the greenhouse experiment, modern wheat varieties had higher B_{yield} but lower grain N concentration ($C_{\text{N,yield}}$) than ancient varieties, regardless of N treatment (Table 3). The ancient varieties had higher total leaf area (data not shown), while the modern varieties had higher mean leaf chlorophyll content (SPAD). Under low fertilizer supply, higher N uptake efficiency (U_{N}) and $C_{\text{N,yield}}$ resulted in higher overall NUE in the ancient than in the modern varieties. However in contrast to the ancient varieties, the modern varieties were more responsive to an increased N supply. In modern varieties grown with a high rate of fertilization (20 g N m^{-2}), the combination of high leaf chlorophyll content and increased leaf area resulted in significantly higher yield production per absorbed N ($E_{\text{N,yield}}$), and higher final grain yield and NUE compared with the ancient varieties (Table 3).

Similar patterns were found in the U_{N} and $E_{\text{N,yield}}$ of the wheat varieties in the growth container experiment, whether the calculations were based on the main growth period (as in Paper II) or on the entire growing period. When N' was based on entire growth period, the modern varieties grown without N fertilization had the lowest U_{N} (mean value of 46.9 g g^{-1}); while the highest value for U_{N} was observed in unfertilized ancient varieties (61.8 g g^{-1}). The $E_{\text{N,yield}}$ was highest in fertilized modern varieties (mean value of 94.8 g g^{-1}) and lowest (41.5 g g^{-1}) in the fertilized ancient varieties. Thus, a higher U_{N} but a lower $E_{\text{N,yield}}$ values were observed when the calculations were based on the entire growth period, compared to the NUE components assessed based on the main growth period.

Table 3. Mean values of grain biomass, NUE and its components in ancient and modern wheats varieties grown under two N treatments (0 and 20 g N m^{-2}) in growth containers, Uppsala, in 2012. Different superscript letters within columns indicate significant differences (Tukey HSD test, at $\alpha = 0.05$).

Variety	N (g m^{-2}) treatment	B_{yield} (g m^{-2})	NUE (g g^{-1})	U_{N} (g g^{-1})	$E_{\text{N,yield}}$ (g g^{-1})	$C_{\text{N,yield}}$ (g g^{-1})	SPAD
Ancient wheats	0	538.5 ^{AB}	129.6 ^{AB}	42.6 ^A	73.1 ^B	.043 ^{AB}	45.6 ^B
	20	455.7 ^B	112.1 ^{BC}	41.3 ^{AB}	60.6 ^B	.045 ^A	45.8 ^B
Modern wheats	0	684.9 ^{AB}	97.6 ^C	32.3 ^B	78.7 ^{AB}	.038 ^C	52.2 ^A
	20	818.6 ^A	143.6 ^A	33.3 ^{AB}	124.1 ^A	.042 ^B	54.6 ^A

5.3 NUE and tuber yield in potato lines (Paper III)

The observed pattern in N use and tuber production in the potato lines differed when they were grown under different growing conditions, *i.e.* greenhouse or field. In the field study, ‘T-2012’ had a higher fresh tuber yield ($Tuber_{FB}$) and tuber N concentration ($C_{N,yield}$) than ‘Dinamo’. Moreover, in the field, ‘T-2012’ had a lower N content in initial biomass (N_S), but removed 24% more N from the soil (U_N) than its parent (Figure 7). In the greenhouse, ‘T-2012’ had a higher belowground establishment at the tuber initiation stage than ‘Dinamo’ (data not shown). In the greenhouse, unlike the field study, yield production per unit of absorbed N ($E_{N,yield}$) was higher in ‘Dinamo’, while other traits were similar in both lines (Figure 7).

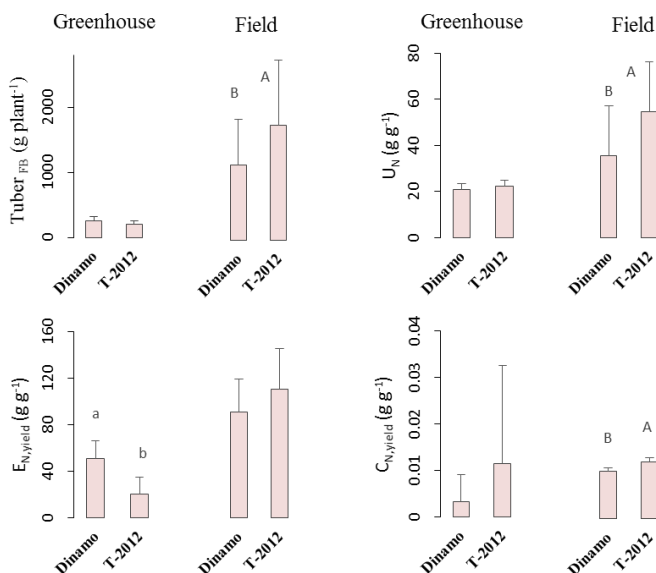


Figure 7. Mean fresh tuber weight at final harvest ($Tuber_{FB}$), N uptake efficiency (U_N), yield specific N efficiency ($E_{N,yield}$) and yield N concentration ($C_{N,yield}$) of the high amylose GM potato lines ‘T-2012’ and its parent ‘Dinamo’ grown in the greenhouse (left; 2014) and in the field (right; 2015). Error bars indicate 95% confidence intervals, letters show results of Tukey HSD test at $\alpha=0.05$.

5.4 Nitrogen-related end use ratios (Papers I & III)

Out of maize, wheat and ley (fertilized or unfertilized), maize had the highest energy output per unit N removed from soil, while unfertilized ley had the highest crude protein production per unit N removed (Figure 8A & 8B). Among potato lines, the GM line ‘T-2012’ had higher amylose production per unit N removed from soil than its parent ‘Dinamo’ (Figure 8C).

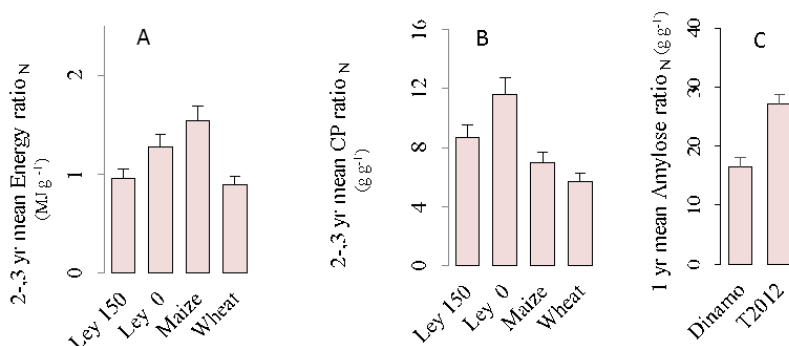


Figure 8. Mean A) energy production per unit N removed from the system (Energy ratio_N) and B) crude protein production per unit N removed from system (CP ratio_N). The mean energy and crude protein were calculated over three years (2013-15) for wheat and maize and two years 2014-15 for fertilized (Ley₁₅₀) and unfertilized ley (Ley₀), grown in Uppsala, Sweden. C) mean amylose production per unit N removed from the system (Amylose ratio_N) in GM potato line ‘T-2012’ and its parent ‘Dinamo’ grown in the field in Borgeby, Sweden. Error bars show 95% confidence interval. Confidence intervals are based on the whole data set, not the yearly mean values.

5.5 Element concentration patterns (Paper IV)

The Principal Component Analysis (PCA) was used to group the samples of winter wheat based on their element concentrations (Figure 9). The N concentration explained most of the variations in the elements concentrations (96 % along PCA dimension 1); while the other elements explained between 30 % (Zn) to a maximum of 90 % (S) of the variations. The developmental stage had a strong influence on the element concentration pattern, with the greatest variation between the concentrations in the grain and in the aboveground plant parts at the beginning of stem elongation stage (aboveground samples taken in spring; Figure 9). At anthesis stage, the aboveground element stoichiometry in growing plants was similar to that in the harvested grain. The yearly variations in weather were reflected in the element concentration pattern in plants at stem elongation and anthesis stage, and in the

grain yield (mean values of 323 and 656 g m⁻² for wheat monocultures in 2013 and 2014; respectively). The preceding crop had only a weak influence on the element concentration pattern (Figure 9).

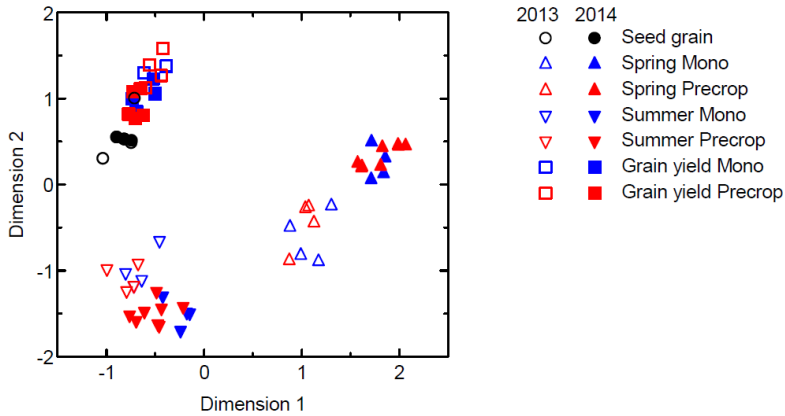


Figure 9. Grouping of samples according to Principal Components Analysis (PCA). Samples are replicates of element concentrations in winter wheat at different developmental stages, *i.e.* seed grain, above ground biomass in spring (BBCH 31) and summer (BBCH 61), and grain yield. Wheat was field-grown in Central Sweden during two growing seasons (2013, open symbols; and 2014, closed symbols) and with different preceding crops. Eigenvalues 7.78 for dimension 1 (*i.e.* explanatory power 71 %), and 1.76 for dimension 2 (*i.e.* explanatory power 16 %). For more details regarding this figure, see Paper IV.

6 Discussion

6.1 Nitrogen use efficiency concept

In this thesis, crop NUE was assessed based on a concept developed by Weih et al. (2011), which is referred to in this discussion as NUE_{Weih} . The advantages and disadvantages of NUE_{Weih} are reviewed, in Weih et al. (2011), Asplund et al. (2014) and Paper II. The concept allows for separation of effects originating from internal plant characteristics, *e.g.* N economy and growth patterns, from effects of external factors, *e.g.* soil N; thus enabling a plant-based assessment of crop N use pattern. For example, if the NUE had been assessed based on the soil available N (*sensu* Moll et al. 1982), the observed patterns in N uptake in relation to the N in initial biomass would have been concealed when crops with different life strategies were compared. Thus, NUE_{Weih} facilitated the work presented in this thesis by allowing comparisons between different crops with different harvested products and end uses.

On a negative note, the NUE_{Weih} concept requires more plant material samples than the other concepts, *e.g.* NUE concept suggested by Moll et al. (1982), and sampling has to be performed at specific phenological development stages. Due to the great variation in the phenology of crop varieties and possible differences in plant phenology depending on environmental factors, it is not always easy to determine phenological development stages. The assessment of NUE_{Weih} is facilitated by a tool developed by Weih (2014), which makes the calculations possible even when the sampling is not performed at the exact developmental stages. Moreover, development work is underway on a model for predicting the growth of cereal crops, *e.g.* wheat, under Nordic conditions, which would facilitate the investigation of crop N dynamics at critical developmental stages. Additionally, there is a knowledge gap in the N economy and biomass accumulation of perennial crops during their long growing season. Given that

the perennial crops are potentially interesting materials for a more sustainable agriculture, further research about their N economy is necessary. Moreover, further development of the NUE_{Weih} concept may be necessary to make even more accurate and easier comparisons among crops. For example, the mean plant N content during the main growth period (*i.e.* between the stem elongation to anthesis) is an important element in the NUE_{Weih} conception, recognizing that plant growth is greatly N limited during that period; and this is how NUE was determined in Paper II dealing with cereals. However, specifically in cereals, the period after the main growth period is also important for yield (grain) growth and grain filling, which is a strong argument for the grain filling period to be considered in the calculation of the mean N content relevant to cereal NUE. Therefore, in this summary of thesis, the NUE_{Weih} conception was modified in the calculations for wheat in Paper I, by considering the mean plant N content during the entire growth period rather than main growth period for the calculation of the mean plant N content (N'). If the modified methodology had been applied in Paper II, the mean plant N content would have been 1.4 times higher than in the original Paper II. As a consequence, the U_N would increase and $E_{N,yield}$ would decrease 1.4-fold compared to the corresponding figures in the original Paper II. This confirms that the N uptake in wheat primarily appears to occur during the main growth period; but some N uptake also occurs after anthesis. As there is a trade-off between the main components of NUE_{Weih} , *i.e.* $E_{N,yield}$ and U_N , the chosen reference period for the calculation of mean plant N influences the $E_{N,yield}$ and U_N but not the overall NUE.

6.2 What are the differences between ley, maize, potato and wheat in terms of yield and N economy?

As expected, crops with different photosynthetic pathways (potato, ley and wheat are C_3 plants; maize is C_4) and life strategies (potato, maize and wheat are annual crops; ley is perennial) had different N economy and yield productivity. The results showed that unfertilized ley had high internal N concentration coupled with low N uptake, revealing an ability to maintain internal N throughout its growing period despite a lower soil N concentration than in fertilized ley. It was found that the N fertilizer application resulted in lower N uptake efficiency and final yield production in ley, probably due to the suppressing effect of enhanced N supply on clover growth (and probably on its N-fixation ability or the lesser competitive advantage derived from N-fixation ability in low N conditions; Haynes 1980, Luscher et al. 2014). While perennial ley had a high internal N provided from previous years, annual crops

had a high N uptake from the soil. The combination of a high N uptake and relative growth rate (data not shown) in maize, resulted in it having the highest biomass production per unit of absorbed N of all crops studied. Potato and wheat ranked similarly in their N in initial biomass, mean plant N content and N uptake efficiency. However, in potato, the mean plant N was re-translocated to tubers and diluted in greater harvested biomass than wheat, resulting in a lower yield N concentration in potato compared with wheat. In general, these plant-based differences in N allocation and growth pattern between the crops influenced their NUE and will therefore influence N removal from the agro-ecosystem based on those crops and their final end uses.

6.3 What are the differences between ancient and modern varieties in terms of their N economy?

It was expected that the ancient wheat varieties would produce higher yield than modern varieties under low N supply. However, the modern wheat varieties maintained a yield advantage over the ancient wheat varieties under both high and low N availability conditions. This can be a result of the enhanced crop harvest index and resistance to lodging, traits that have been the primary target in most breeding approaches for cereal crops (Wacker et al. 2002, Ma et al. 2012). There was considerably higher N uptake in the ancient varieties studied here (by 20%) than in the modern varieties, especially under low N supply (similar results were found by Foulkes et al. 1998). This finding is in line with the general expectation that varieties adapted to N-poor environments have traits that enable a higher N uptake from the environment (Chapin 1980, Newton et al. 2010). A well-developed root system and symbiotic relationships with arbuscular mycorrhiza can be considered factors determining high N uptake and both of these traits are reported to be present in old landraces (Newton et al. 2010). However, the root traits were not studied in this thesis and further investigations are required in terms of root traits of the ancient varieties studied here. In agreement with other studies on wheat (Abdelaal et al. 1995, Marconi et al. 1999), the ancient varieties studied in this thesis re-translocated more N to the harvested product, especially under low N availability. In general, these findings confirm that improved grain yield has been the major focus of wheat breeding programmes, indicating a need for a greater focus on the grain quality factors in future breeding programmes.

6.4 Is a higher tuber yield in GM potato lines associated with a higher NUE?

Improved yield has not only been the direct focus of breeding approaches, but can be an indirect consequence of breeding and genetic modifications, for example for quality aspects for a specific end use (e.g. studies by Hofvander et al. 2004, Menzel et al. 2015). In this thesis, the N economy of a high amylose GM potato line ‘T-2012’ and its parent ‘Dinamo’ were compared, and it was found that the altered starch allocation in ‘T-2012’ was associated with higher tuber production. These results were a consequence of higher early below ground establishment and N uptake (U_N) in ‘T-2012’ during the critical developmental stages for tuber production, the period after flowering. Moreover, ‘T-2012’ re-translocated more N to the final tuber, which along with a high N uptake efficiency resulted in a greater overall NUE in ‘T-2012’ than in its parent. Thus, due to its higher U_N , ‘T-2012’ removes more N from agroecosystem and may require more fertilizer than its parent. An interesting question is whether the GM line ‘T-2012’ produced more desired yield fraction (*i.e.* amylose) per unit N removed from soil than its parent; an issue discussed in the next section.

6.5 What is the influence of crop characteristics and end use on N-related sustainability?

The N-related sustainability ratios in Paper I were calculated for ley, maize and wheat, with the assumption that the crops will be used for crude protein (animal feed) or energy production. It was found that ley has characteristics such as high yield production with a high N concentration, making it more sustainable (*sensu* N depletion; Brodt et al. 2011) for (crude protein) fodder production compared with the other crops. Maize proved to be more appropriate for energy production, due to its high biomass production per unit N taken up, and low yield N concentration. This is not in itself surprising, as maize has long been bred and used for energy, and ley for fodder production. However, there is a large difference between knowing that something is good and knowing *why* it is good. By studying the mechanisms responsible for the N economy of those crops in relation to their end use, it is possible to understand why one crop is more suitable for certain end uses. Consequently, we understand which aspects of the crops can be improved to enhance their viability for those end uses and make them more resource conserving thus enabling more sustainable production. For example, in the potato study described in Paper III, by only observing NUE and its components it was concluded that GM potato line ‘T-2012’ may need more fertilizer due to its

higher N uptake. However, 'T-2012' had higher amylose output per unit of absorbed N, and will therefore be more sustainable (*sensu* N removal) in terms of amylose production than its parent 'Dinamo'. Therefore, the calculation of NUE without making the link to the end use may not reveal a complete picture of the N economy of crops grown for certain end uses.

The assessment of NUE is time-consuming and costly, whereas the assessment of ratios developed in this thesis can be performed directly, using data on biomass and N removal by the harvested crop. This makes them useful tools as sustainability indicators for different end uses.

6.6 What are the influences of environmental conditions on NUE and its components?

Great variation was observed in the N economy of winter wheat and potato crops when grown under different growing conditions, *e.g.* wheat grown outdoors in growth containers and in the field (Papers I, II and IV) and potato grown in pots in the greenhouse and in the field (Paper III).

In wheat, grain yield, NUE and its components were higher in plants grown in containers than in the field-grown plants, which can be explained partly by genotypic and seasonal variations, and partly by the superior substrate used, and more controlled environment in the container experiment, compared with the field conditions. It should also be noted here that the calculations of the NUE components in the container experiment (Paper II) were based on the mean plant N content during the main growth period, while in the plants grown in the field the calculations were based on the mean plant N during the entire growth period. However, these differences in calculations did not influence the general patterns observed in the growth container and field experiments.

In the potato experiments, higher fresh tuber yield, N uptake efficiency and yield specific N efficiency were observed in the field than in the greenhouse. This pattern can be a consequence of the limiting effect of pots (7.5 L) on N uptake and tuber development in the greenhouse. In contrast to the tuber yield, the mean aboveground biomass was higher in the greenhouse (in line with Bones et al. 1997). This can be ascribed to the higher temperature coupled with a low light irradiance in the greenhouse than in the field; which negatively influences the tuber development, while stimulate aboveground biomass production. Therefore, similar to many other studies on various crops (Timlin et al. 2006, Nippert et al. 2007), it was found that the growth conditions have considerable impact on the yield and N economy of wheat and potato crops. This is an issue that should be considered when studies are performed under different experimental set-ups.

6.7 Is the element concentration pattern in wheat mirrored by its N concentration?

When the N economy of wheat was studied and compared with those of other crops, the next complementary step was to assess the relationships between the N concentration and that of other nutrient elements in wheat; and whether this relationship changed throughout the growth period. According to the results of Paper IV, the N concentration in plants explained most of the variation in the element concentration pattern, indicating that N was the most limiting factor for wheat growth in this study. These results support the motivation for NUE assessments in crops; since assessment of the use efficiency of an element is reported to be meaningful when that element is the most growth-limiting factor (Hawkesford et al. 2014).

6.8 What are the impacts of growth condition on element concentration patterns?

Paper IV also examined how deviations from optimal growth conditions, *e.g.* during winter, influence the element concentration pattern in wheat. The base assumption was that wheat seeds have a concentration mixture that is optimal for plant growth (Liptay and Arevalo 2000). It was found that after winter, the nutrient element concentrations in the growing wheat crop were higher than those in the grain. This can be explained by nutrient uptake occurring during the winter without significant crop growth. The element stoichiometry in plants at anthesis stage did not differ from that in the seed, suggesting that the wheat plants at anthesis were growing under near optimal growth conditions.

In our study, winter wheat was grown in two years with contrasting weather condition (dry in 2013; humid in 2014); which resulted in a great variation in the grain yield between the two years, and in the concentration of elements at the stem elongation and to some extent at anthesis stage. Moreover, in contrast to the expectations from previous studies, *e.g.* Angus et al. (2015), the results obtained in this thesis did not provide any evidence of a preceding crop effect on soil nutrient availability to the main crop and thus on crop yield. However, a two-year study period is too short for drawing any definite conclusions, considering the fact that the preceding crop effect on wheat yield depends on the weather and local growing conditions (Sieling and Christen 2015). A follow-up study is planned for further assessment of the element stoichiometry in wheat crops as influenced by growing and/or weather conditions.

7 Conclusions

1. The life strategies and photosynthetic pathways of different crops greatly affect their NUE and thus the sustainability (related to N depletion) of cropping systems that include these crops. For example, maize as a C₄ crop has characteristics that are more appropriate for energy production, while the characteristics of perennial ley are more desirable for fodder production.
2. The significantly higher N uptake efficiency and yield N concentration in ancient than modern wheat varieties, suggests that ancient varieties can be potentially interesting materials for breeding crops with high NUE.
3. Genetic modification for increased amylose starch content in potato tubers influences non-target traits such as fresh tuber weight, through increased N uptake efficiency. Thus, more N will be required for growing the high amylose GM potato line than its non-GM parent, but less N will be required per gram of amylose produced.
4. In winter wheat, the variation in ten different nutrient concentrations, across various developmental stages and two years with contrasting climate, is closely mirrored by the N concentration pattern.
5. The developmental stage strongly influences the element concentration pattern in winter wheat, with greatest variation occurring between the concentrations in the grain and in the vegetative plant in spring.

8 Implications and future perspectives

In this thesis, the assessments of NUE and the ratio between a desired yield (*e.g.* energy, crude protein or amylose) and the N removal from the agroecosystem, were employed to show how N use and growth strategies of different crops and crop varieties can influence crop sustainability when grown for specific end uses. The sustainability indicator employed here is an easily assessable measure, which links the crop N use characteristics to the production system and end use; a link that is missing in many agricultural and breeding programs. Moreover in this thesis, the crop NUE was assessed at the plant-level, where the individual plant was regarded as an input-output system. However, NUE assessment at different scales of plant, field and agroecosystem is necessary when aiming for agricultural sustainability improvements.

Most previous studies investigating sustainability in cropping systems claim that the sustainability in crop production can be improved by a high plant N uptake from the soil, in order to avoid N leaching (*e.g.* see the review by Hirel et al. 2011). However, this reflects only one aspect of sustainability, because increased N uptake from the soil by plants results in a higher N removal by the harvest, which in turn necessitates higher N fertilizer input to the system. The production of inorganic N fertilizers is not only energy consuming, but the continuous application of N fertilizers can lead to more N leaching and environmental problems (Canfield et al. 2010). Consequently, it is a considerable challenge for the agricultural sector to increase the crop yield without significantly increasing the need for N inputs and thus exacerbating the environmental impacts. The efficient growth strategies of perennial crops, which are based on internal partitioning and recycling of N and C throughout their life cycle, can potentially be utilized to make cropping systems more resource conserving. Therefore, additional studies are needed on the N economy and functional traits of perennial species that are agronomically interesting. Moreover, additional research is required in order to uncover the

physiological basis of NUE in crops, and the functional traits associated with those physiological processes. Identification of genetic variability in the traits associated with NUE within crop species, can make a valuable contribution to the genetic improvements of crops. In conclusion, more integrative studies, including breeding, agronomic and ecological approaches, are required in order to improve a highly complex and multigenic traits such as those determining crop NUE.

References

- Abdelaal, E. S. M., P. Hucl, and F. W. Sosulski. 1995. Compositional and nutritional characteristics of spring einkorn and spelt wheats. *Cereal Chemistry* **72**:621-624.
- Andow, D. A., and C. Zwahlen. 2006. Assessing environmental risks of transgenic plants. *Ecology Letters* **9**:196-214.
- Angus, J. F., J. A. Kirkegaard, J. R. Hunt, M. H. Ryan, L. Ohlander, and M. B. Peoples. 2015. Break crops and rotations for wheat. *Crop & Pasture Science* **66**:523-552.
- Aragón, C. F., M. Méndez, and A. Escudero. 2009. Survival costs of reproduction in a short-lived perennial plant: live hard, die young. *American Journal of Botany* **96**:904-911.
- Areal, F., L. Riesgo, and E. Rodriguez-Cerezo. 2013. Economic and agronomic impact of commercialized GM crops: a meta-analysis. *The Journal of Agricultural Science* **151**:7-33.
- Asplund, L., G. Bergkvist, and M. Weih. 2014. Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. *Plant and Soil* **374**:829-842.
- Bakht, J., M. Shafi, M. T. Jan, and Z. Shah. 2009. Influence of crop residue management, cropping system and N fertilizer on soil N and C dynamics and sustainable wheat (*Triticum aestivum* L.) production. *Soil and Tillage Research* **104**:233-240.
- Bones, A. M., B. r. I. Honne, K. M. Nielsen, S. Visvalingam, S. Ponnampalam, P. Winge, and O. P. Thangstad. 1997. Performance of transgenic plants of potato (*Solanum tuberosum* cv. Laila) grown in vitro in greenhouse and in a field trial. *Acta Agriculturae Scandinavica B—Plant Soil Sciences* **47**:156-167.
- Brodt, S., J. Six, G. Feenstra, C. Ingels, and D. Campbell. 2011. Sustainable agriculture. *Nature Education Knowledge* **3**:1.
- Bräutigam, A., and U. Gowik. 2016. Photorespiration connects C3 and C4 photosynthesis. *Journal of Experimental Botany*:erw056.
- Burns, I. G., R. L. Walker, and J. Moorby. 1997. How do nutrients drive growth? *Plant and Soil* **196**:321-325.
- Börjesson, P., and L. M. Tufvesson. 2011. Agricultural crop-based biofuels - resource efficiency and environmental performance including direct land use changes. *Journal of Cleaner Production* **19**:108-120.
- Børresen, T. 1999. The effect of straw management and reduced tillage on soil properties and crop yields of spring-sown cereals on two loam soils in Norway. *Soil and Tillage Research* **51**:91-102.
- Canfield, D. E., A. N. Glazer, and P. G. Falkowski. 2010. The evolution and future of earth's nitrogen cycle. *Science* **330**:192-196.
- Castagna, R., C. Minoia, O. Porfiri, and G. Rocchetti. 1996. Nitrogen level and seeding rate effects on the performance of hulled wheats (*Triticum monococcum* L, *T-dicoccum* Schubler and *T-spelta* L) evaluated in contrasting agronomic environments. *Journal of Agronomy and Crop Science-Zeitschrift Fur Acker Und Pflanzenbau* **176**:173-181.

- Cellini, F., A. Chesson, I. Colquhoun, A. Constable, H. V. Davies, K. H. Engel, A. M. R. Gatehouse, S. Karenlampi, E. J. Kok, J. J. Leguay, S. Lehesranta, H. P. J. M. Noteborn, J. Pedersen, and M. Smith. 2004. Unintended effects and their detection in genetically modified crops. *Food and Chemical Toxicology* **42**:1089-1125.
- Chapin, F. S. 1980. The mineral-nutrition of wild plants. *Annual Review of Ecology and Systematics* **11**:233-260.
- Crews, T. E., J. Blesh, S. W. Culman, R. C. Hayes, E. S. Jensen, M. C. Mack, M. B. Peoples, and M. E. Schipanski. 2016. Going where no grains have gone before: From early to mid-succession. *Agriculture, Ecosystems & Environment* **223**:223-238.
- Eckersten, H., A. Herrmann, A. Kornher, M. Halling, E. Sindhøj, and E. Lewan. 2012. Predicting silage maize yield and quality in Sweden as influenced by climate change and variability. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* **62**:151-165.
- Ehsanzadeh, P., M. S. Nekoonam, J. N. Azhar, H. Pourhadian, and S. Shaydaee. 2009. Growth, chlorophyll, and cation concentration of tetraploid wheat on a solution high in sodium chloride salt: Hulled versus free-threshing genotypes. *Journal of Plant Nutrition* **32**:58-70.
- Evans, L. T., and R. L. Dunstone. 1970. Some physiological aspects of evolution in wheat. *Australian Journal of Biological Sciences* **23**:725-&.
- Fageria, N. K., V. C. Baligar, and Y. C. Li. 2008. The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition* **31**:1121-1157.
- FAO. 2013. State of diversity of major and minor crops.
<http://www.fao.org/docrep/013/i1500e/i1500e14.pdf>.
- Foulkes, M., R. Sylvester-Bradley, and R. Scott. 1998. Evidence for differences between winter wheat cultivars in acquisition of soil mineral nitrogen and uptake and utilization of applied fertilizer nitrogen. *The Journal of Agricultural Science* **130**:29-44.
- Gioia, T., K. A. Nagel, R. Beleggia, M. Fragasso, D. B. M. Ficco, R. Pieruschka, P. De Vita, F. Fiorani, and R. Papa. 2015. Impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilization. *Journal of Experimental Botany* **66**:5519-5530.
- Haegerle, J. W., and F. E. Below. 2013. Transgenic corn rootworm protection increases grain yield and nitrogen use of maize. *Crop Science* **53**:585-594.
- Hawkesford, M. J., S. Kopriva, and L. J. De Kok. 2014. *Nutrient use efficiency in plants: concepts and approaches* preface. Springer International Publishing.
- Haynes, R. 1980. Competitive aspects of the grass-legume association. Pages 227-261 *Advances in Agronomy*.
- Hirel, B., T. Tétu, P. J. Lea, and F. Dubois. 2011. Improving nitrogen use efficiency in crops for sustainable agriculture. *Sustainability* **3**:1452-1485.
- Hofvander, P., M. Andersson, C. T. Larsson, and H. Larsson. 2004. Field performance and starch characteristics of high-amylose potatoes obtained by antisense gene targeting of two branching enzymes. *Plant Biotechnology Journal* **2**:311-320.
- Hofvander, P., T. Ischebeck, H. Turesson, S. K. Kushwaha, I. Feussner, A. S. Carlsson, and M. Andersson. 2016. Potato tuber expression of Arabidopsis WRINKLED1 increase triacylglycerol and membrane lipids while affecting central carbohydrate metabolism. *Plant Biotechnology Journal*.
- Jaikumar, N. S., S. S. Snapp, and T. D. Sharkey. 2013. Life history and resource acquisition: photosynthetic traits in selected accessions of three perennial cereal species compared with annual wheat and rye. *American Journal of Botany* **100**:2468-2477.
- Jordbruksverket. 2016. Swedish Board of Agriculture.
<http://www.jordbruksverket.se/amnesomraden/odling/andrajordbruksgrador/majs/skordestatistik.html>. (in Swedish).
- Karp, A., and I. Shield. 2008. Bioenergy from plants and the sustainable yield challenge. *New Phytologist* **179**:15-32.

- Kolseth, A. K., T. D'Hertefeldt, M. Emmerich, F. Forabosco, S. Marklund, T. E. Cheeke, S. Hallin, and M. Weih. 2015. Influence of genetically modified organisms on agro-ecosystem processes. *Agriculture Ecosystems & Environment* **214**:96-106.
- Liptay, A., and A. E. Arevalo. 2000. Plant mineral accumulation, use and transport during the life cycle of plants: A review. *Canadian Journal of Plant Science* **80**:29-38.
- Luscher, A., I. Mueller-Harvey, J. F. Soussana, R. M. Rees, and J. L. Peyraud. 2014. Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* **69**:206-228.
- Ma, B. L., D. K. Biswas, Q. P. Zhou, and C. Z. Ren. 2012. Comparisons among cultivars of wheat, hulled and hullless oats: Effects of N fertilization on growth and yield. *Canadian Journal of Plant Science* **92**:1213-1222.
- Malhi, S., A. Johnston, J. Schoenau, Z. Wang, and C. Vera. 2006. Seasonal biomass accumulation and nutrient uptake of wheat, barley and oat on a Black Chernozem soil in Saskatchewan. *Canadian Journal of Plant Science* **86**:1005-1014.
- Marconi, E., M. Carcea, M. Graziano, and R. Cubadda. 1999. Kernel properties and pasta-making quality of five European spelt wheat (*Triticum spelta* L.) cultivars. *Cereal Chemistry* **76**:25-29.
- Menzel, C., M. Andersson, R. Andersson, J. L. Vazquez-Gutierrez, G. Daniel, M. Langton, M. Gallstedt, and K. Koch. 2015. Improved material properties of solution-cast starch films: Effect of varying amylopectin structure and amylose content of starch from genetically modified potatoes. *Carbohydrate Polymers* **130**:388-397.
- Moll, R. H., E. J. Kamprath, and W. A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. *Agronomy Journal* **74**:562-564.
- Nesbitt, M. 2001. Wheat evolution: integrating archaeological and biological evidence. *The Linnean* **3**:37-59.
- Newton, A., T. Akar, J. Baresel, P. Bebeli, E. Bettencourt, K. Bladenopoulos, J. Czembor, D. Fasoula, A. Katsiotis, and K. Koutis. 2010. Cereal landraces for sustainable agriculture. A review. *Agronomy for Sustainable Development* **30**:237-269.
- Nippert, J. B., P. A. Fay, and A. K. Knapp. 2007. Photosynthetic traits in C-3 and C-4 grassland species in mesocosm and field environments. *Environmental and Experimental Botany* **60**:412-420.
- Sadras, V. O. 2006. The N: P stoichiometry of cereal, grain legume and oilseed crops. *Field Crops Research* **95**:13-29.
- Sieling, K., and O. Christen. 2015. Crop rotation effects on yield of oilseed rape, wheat and barley and residual effects on the subsequent wheat. *Archives of Agronomy and Soil Science* **61**:1531-1549.
- Spiertz, J. 2010. Nitrogen, sustainable agriculture and food security. A review. *Agronomy for Sustainable Development* **30**:43-55.
- Tabatabaei, S., and P. Ehsanzadeh. 2015. Photosynthetic pigments, ionic and antioxidative behaviour of hulled tetraploid wheat in response to NaCl. *Photosynthetica*:1-12.
- Timlin, D., S. Lutfur Rahman, J. Baker, V. Reddy, D. Fleisher, and B. Quebedeaux. 2006. Whole plant photosynthesis, development, and carbon partitioning in potato as a function of temperature. *Agronomy Journal* **98**:1195-1203.
- Wacker, L., S. Jacomet, and C. Korner. 2002. Trends in biomass fractionation in wheat and barley from wild ancestors to modern cultivars. *Plant Biology* **4**:258-265.
- Walsh, K., P. O'Kiely, A. P. Moloney, and T. M. Boland. 2008. Intake, digestibility, rumen fermentation and performance of beef cattle fed diets based on whole-crop wheat or barley harvested at two cutting heights relative to maize silage or ad libitum concentrates. *Animal Feed Science and Technology* **144**:257-278.
- Van Dillewijn, P., M. a. J. Soto, P. J. Villadas, and N. Toro. 2001. Construction and environmental release of a *Sinorhizobium meliloti* strain genetically modified to be more competitive for alfalfa nodulation. *Applied and Environmental Microbiology* **67**:3860-3865.
- Van Tassel, D. L., L. R. DeHaan, and T. S. Cox. 2010. Missing domesticated plant forms: can artificial selection fill the gap? *Evolutionary Applications* **3**:434-452.

- Weih, M. 2014. A calculation tool for analyzing nitrogen use efficiency in annual and perennial crops. *Agronomy* **4**:470-477.
- Weih, M., L. Asplund, and G. Bergkvist. 2011. Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nitrogen use efficiency. *Plant and Soil* **339**:513-520.
- Vico, G., S. Manzoni, L. Nkurunziza, K. Murphy, and M. Weih. 2016. Trade-offs between seed output and life span – a quantitative comparison of traits between annual and perennial congeneric species. *New Phytologist* **209**:104-114.
- Witzenberger, A., and H. Hack. 1989. Erlaeuterungen zum BBCH-Dezimal-Code fuer die Entwicklungsstadien des Getreides-mit Abbildungen. *Gesunde Pflanzen*.
- Ågren, G. I. 2004. The C: N: P stoichiometry of autotrophs–theory and observations. *Ecology Letters* **7**:185-191.

Acknowledgements

First of all, I would like to thank my supervisors, for their support and encouragement during these four years.

Martin: I cannot thank you enough for what I have learned from you. Thank you for welcoming me, both to Sweden and to the academic world and for helping me grow as a researcher these past four years. I have always felt welcome to your place as well as to your office, and I am very thankful for that.

Giulia: Your very constructive and perceptive comments have contributed greatly to my thesis. Thank you for being always there whenever I needed to get some ideas.

Birgitta: I am glad you joined the supervisors group. You brought new ideas and perspectives to my PhD work. Thank you for being such a supportive and welcoming supervisor and thank you for our discussions.

Mariette Andersson: I appreciate all your support in Alnarp, thanks for being so nice and friendly.

Parviz Ehsanzadeh: My master supervisor; thank you for your advice and support during my bachelor, master and PhD education.

Maria Kedmark and Ann-Sofie: I greatly appreciate that you helped me with sampling and lab works. You made the lab work much more enjoyable. Thank you for that.

Johannes and Libere: You are the researchers outside my supervisor group that I visited the most. I felt comfortable asking questions and discussing my work with you; because you are so nice. I cannot thank you enough with your help on the statistics and commenting on my manuscripts.

Lars, Velemir, Theo, Göran, Paola, Anneli and Christine Watson: You are very nice and friendly researchers. I hope to meet more people like you in academia. Thanks a lot for all your support.

Per Nyman: Thank you not only for all the help with IT stuff, but also for being so friendly and always supportive.

Special thanks to the members of ‘**AgResource**’ project for organizing interesting workshops and for financing my PhD project.

To my friends at the ecology centre

I met so many wonderful people at the ecology centre during my time as a PHD. I would like to thank all of you for these wonderful years. There are some that I would like to direct a special thank you to:

Raj: you have been a great officemate and friend to me. Thanks. You were the first who invited me over and introduced me to your friends. I was very lucky to have you and your family. I enjoyed cooking together with you and spending time with you and your family, and I definitely plan for us to spend more time together.

Monika: I always felt good after talking to you. That smile on your face is so nice and welcoming. I hope that smile never fades.

Stefanie: You are such a giving person, I love you for that. Thanks for being such a good friend.

Ida, Kristin and Frauke: Thanks for great times we spent together, cooking, watching movies or playing games. It has been so nice to have you around. Thanks for helping me with organizing my wedding.

Preeti: Thanks for the great times we spent together, watching movies, cooking, going for dance, Katushka and many more.

Sepideh and Sara: I feel like you have become my best friends. I am so glad that I met you and have you.

Daniella, Bodil, Linnea, Iris, Jacob, Victor, Alva, Sophie, Sofia and others; thanks for cheerful chats during the Fika and lunch time.

Pernilla and Davide: I enjoyed hanging around in Japan with you. I was so lucky to have such great company like you with me there.

Romain: we started working at SLU approximately at the same time. Thank you for being a good friend and company in travelling to Iran, going to parties and so forth.

Nicole, Tina, Thomas, Friderike and Martin H: Even though you left Sweden I have great memories with you. You made me think of Germans as welcoming, open and hospitable people. So be proud of yourselves, because you are all very lovely. Hope to keep in contact with you.

To my family

Thanks to **my parents and siblings**, who always wanted the best for me, supported me, and encouraged me to continue my education.

Thanks to my spouse's family; **Britt-Marie, Lars, Ylva, Jimmy, Arne and beautiful Ebba**, for being always supportive. I love you all and I am very glad to have you by my side.

Björn you are the love of my life, my forever best friend and the best thing that I have in the world. Thank you for always being there for me, supporting me, encouraging me and believing in me. Every minutes that I spend with you is a blessing and I can't find words to thank you for that.

