Micronutrients in Cereal Crops

Impact of Nutrient Management and Soil Properties

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Micronutrients in Cereal Crops. Impact of Nutrient Management and Soil Properties

Abstract

Seven elements essential for plants are defined as micronutrients: boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn). Deficiency of these nutrients can cause yield losses in crops and impaired crop quality. The overall aim of this thesis work was to increase the knowledge how micronutrients in Swedish cereal crops are affected by nutrient management and soil properties in order to improve crop status and avoid yield losses. Data from long term and short term Swedish field trials and a Swedish monitoring programme were evaluated to examine impacts of nutrient management and soil properties on crop accumulation. In addition, soil depletion was quantified and methods for prediction of micronutrient availability in soil were assessed.

Although crop production solely with mineral fertilizers may result in depletion of micronutrients in arable soil, results showed that depletion rate is slow and difficult to detect, even over decades. Repeated applications of organic fertilizers caused micronutrient accumulation in soil, but generally did not result in increased micronutrient concentrations in cereal crops. Instead, soil properties affecting micronutrient availability were of greater importance for crop accumulation.

High nitrogen (N) fertilization rates resulted in increased concentrations of most micronutrients in winter wheat, whereas the micronutrient to N ratio generally decreased. Accumulation of micronutrients during crop growth differed from N uptake patterns, possibly due to differing availability in soil. Nitrogen fertilization rate had no or minor effects on the accumulation dynamics or translocation from shoot to grain of micronutrients, except for Fe.

Easily accessible data such as total micronutrient concentration in soil in combination with pH or analysis of grain concentrations can be useful tools for estimation of micronutrient availability in soils. New methods of soil analysis, such as diffusive gradient in thin films (DGT) also showed promising results in predicting Cu uptake in wheat.

The results presented in this thesis can be useful in identification of fields with an elevated risk of micronutrient deficiency in cereal crops.

Keywords: deficiency, DGT, fertilization, field trial, grain, manure, nitrogen, nutrient accumulation, sewage sludge, soil extraction, trace elements, wheat

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Dedication

To the farmers of Sweden!

Some things count in small amounts
Paraphrased from Depeche Mode

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 Kirchmann, H., Schön, M., Börjesson, G., Hamnér, K. & Kätterer, T. (2013). Properties of soils in the Swedish long-term fertility experiments: VII. Changes in topsoil and upper subsoil at Örja and Fors after 50 years of nitrogen fertilization and manure application. *Acta Agriculturae Scandinavica. Section B - Soil and Plant Sciences* 63, 25-36.

II Hamnér, K., Eriksson, J. & Kirchmann, H. (2013). Nickel in Swedish soils and cereal grain in relation to soil properties, fertilization and seed quality. *Acta Agriculturae Scandinavica. Section B - Soil and Plant Sciences* 63, 712-722.

III Hamnér, K. & Kirchmann, H. (2015). Trace element concentrations in cereal grain of long-term field trials with organic fertilizers in Sweden. *Nutrient Cycling in Agroecosystems* 103, 347-358.

IV Hamnér, K., Weih, M., Eriksson, J. & Kirchmann, H. Macro- and micronutrient accumulation during growth of winter wheat as influenced by nitrogen supply. S*ubmitted manuscript.*

V Hamnér, K., Andersson, M., Weih, M., Eriksson, J. & Kirchmann, H. Usefulness of soil and grain analyses for assessment of copper deficiency in winter wheat. *Manuscript.*

Papers I-III are reproduced with the permission of the publishers.

The contribution of Karin Hamnér to the papers included in this thesis was as follows:

I Performed data analysis and interpretation of data concerning trace elements in the soil. Wrote the sections on trace elements.

II Planned the study together with the second author. Performed the study (data preparation, analysis and interpretation) and the writing with some assistance from the co-authors.

III Planned the study together with the co-author. Performed the study (collection of grain samples, data preparation, analysis and interpretation) and the writing, with some assistance from the co-author.

IV Planned the study together with the co-authors. Performed the study (experimental field work, sample preparation, data analysis and interpretation) and the writing, with some assistance from the co-authors.

V Planned the study together with the co-authors. Assisted the second author in sample preparation and experimental laboratory work. Performed data preparation, analysis and interpretation and the writing with some assistance from the co-authors.

Abbreviations

B	Boron
BCF	Bio-concentration factor
Cd	Cadmium
Cu	Copper
DGT	Diffusive gradient in thin films
DM	Dry matter
Fe	Iron
ha	Hectare
HI	Harvest index
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Ni	Nickel
NuHI	Nutrient harvest index
OM	Organic matter
R_G	Relative growth rate
R_{Nu}	Relative nutrient accumulation rate
Se	Selenium
Zn	Zinc

1 Introduction

For all living organisms, a number of elements are essential for maintaining growth and cell functions and for completing the life cycle through reproduction. For plants, there are in total 14 known essential mineral elements, which are mainly accumulated from the soil (Mengel & Kirkby, 1987). Essential elements accumulating in small amounts in crops, usually in the order of grams per hectare, are called micronutrients. For most plants, including cereals, the essential micronutrients are boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn).

Around the world, plant deficiencies due to low micronutrient concentrations are a major concern and large areas of arable land suffer from severe yield losses due to insufficient supply of micronutrients to crops (Alloway, 2008). In addition to reduced yield for farmers, low uptake of micronutrients in plants also results in food and feed products that are poor in minerals. This can be an important cause of malnutrition, especially within populations with a monotonous diet. On the other hand, some areas suffer from excessive uptake of elements, causing toxicity or imbalances in crops or in livestock and humans. Wheat and other cereal crops are major staple foods for billions of people world-wide, and obtaining high yields of high-quality grain is of major importance in feeding the world´s growing population and preventing malnutrition.

In Sweden, severe micronutrient deficiency or toxicity problems in plants occur occasionally, but are not common. However, hidden deficiencies causing small to moderate yield losses are likely to exist and need attention. In order to identify fields where crops are at risk of developing micronutrient deficiency, there is a need for increased knowledge on the extent to which different micronutrients in soil are available to plants and how different nutrient management regimes influence crop accumulation and soil concentrations over time. It is also important to develop analytical methods for accurate and cost-efficient prediction of crop micronutrient status.

Micronutrient demand for obtaining optimal yield of major cereal crops grown in Sweden is the main focus of this thesis. However, this subject is closely related to quality issues concerning use of cereal grain as food and feed, which are also partly addressed. The different parts included in the thesis are illustrated in Figure 1.

The work has been carried out using long-term and short-term field trials in southern and central Sweden, combined with evaluation of data from the Swedish monitoring programme on arable soils. Winter wheat was the major crop evaluated in the studies, but spring barley and oats were also included. All field trials were located in the major arable regions of Sweden on relatively fertile soils. The results and conclusions are thereby restricted to cool temperate conditions and cereal crops adapted to Swedish conditions, although some of the results can be applicable to other environments as well.

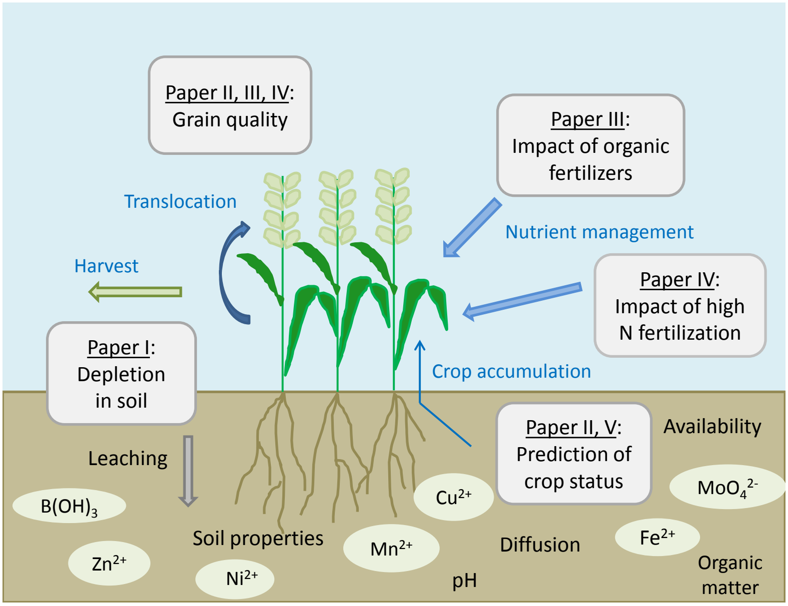

Figure 1. Schematic illustration of the different parts included in the thesis (K. Hamnér)

2 Aim and objectives

The overall aim of this thesis work was to increase the knowledge how micronutrients in Swedish cereal crops are affected by nutrient management and soil properties, in order to improve crop status and avoid yield losses. Achieving a cereal crop with optimal concentrations of micronutrients is important to maximize yield and to produce food and feed products of high quality. Specific objectives of the work were:

- To quantify depletion of micronutrients in Swedish arable soils over time following fertilization solely with macronutrients.

- To assess the influence of different organic fertilizers and soil properties on micronutrient uptake and grain content in major cereal crops grown in Sweden.

- To increase understanding of how nitrogen fertilization rate influences crop accumulation of micronutrients and translocation to grain in winter wheat grown in Sweden.

- To evaluate different methods of soil analysis and grain status for assessment of micronutrient deficiency in cereal crops.

3 Background

3.1 Micronutrients, trace elements and heavy metals

A micronutrient can be defined as an element essential for all higher plants where the requirement and accumulation are small, usually measured in milligrams per kilogram of soil or biomass or in grams per hectare. Trace elements are elements, including micronutrients, that are present in small amounts in soil, water, air or organisms such as microorganisms, plants, animals or humans (Alloway, 2013). Within this thesis, the term trace elements is used when elements not essential to plants, but of importance (essential or toxic) for humans and animals are included.

Heavy metals are often defined as a group of metals with high density (>5 g cm^{-3}) and with toxic effects if they accumulate in organisms (Alloway, 2013). However, even though heavy metals are most often associated with their negative impact on organisms, it is important to note that the majority of the micronutrients also fall within the category of heavy metals.

3.1.1 Macronutrients and micronutrients

Carbon (C), oxygen (O) and hydrogen (H) are the most abundant elements in plants and are recovered mainly as carbon dioxide and water. Beside these elements, plants need a number of mineral nutrients that are accumulated mainly from the soil. For higher plants, such as cereal crops, 14 elements have been proven to be essential to fulfil the life cycle of these plants (Mengel & Kirkby, 1987). The essential mineral nutrients are often divided according to the amounts recovered by plants, where nitrogen (N), potassium (K), sulphur (S), phosphorus (P), calcium (Ca), magnesium (Mg) and chlorine (Cl) are considered macronutrients and iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), boron (B) and nickel (Ni) are considered micronutrients. Chlorine is sometimes included among the micronutrients, but this nutrient is often accumulated in plants in amounts equivalent to macronutrients and is thus not dealt with in this thesis.

The amount of nutrients taken up by plants varies to a large degree. For example, a winter wheat crop can accumulate 150-200 kg of N and K per hectare, whereas the corresponding figure for the micronutrients B and Ni is often only a few grams per hectare. Despite the small amounts present in plants, micronutrients play essential roles in many cell processes and components within the plant (Marschner, 2012). All of them except B are involved in a number of enzymatic reactions, *e.g.* Fe, Cu and Mn are important within several redox systems including photosynthesis and Zn plays an important role in protein synthesis and detoxification of superoxide radicals. Molybdenum and Ni are components within enzymes regulating nitrogen metabolism, whereas B is crucial to the structure of cell walls and membranes.

3.1.2 Trace elements of importance for humans and animals

All the micronutrients essential to plants, with the possible exception of B, are known to be essential also to humans and animals (Hunt, 2012; Mertz & Underwood, 1986). In addition, plants accumulate a number of trace elements not of major importance for the plant itself, but for the humans and animals that consume the harvested product. For elements essential for the consumer, plant accumulation can thereby be an important source to fulfil the nutritional need. Or the other hand, it can also imply a risk when plants accumulate elements considered to be toxic for humans. Two examples of elements within this category are selenium (Se) and cadmium (Cd), both of which have received attention in Sweden and elsewhere due to their impact on human health.

Selenium is a trace element proven to be essential to humans and animals. There are indications that it is also essential to plants, but this has not been proven (Sors *et al.*, 2005). Even though daily dietary intake of Se is small in humans (40-50 µg day^{-1}; NFA, 2014; WHO 1996) it has been shown to be of great importance for the immune system and Se deficiency can cause disease susceptibility and problems in maintaining optimal health (*e.g.* Rayman, 2000).

Cadmium is a heavy metal known to be toxic to humans even at very low concentrations and accumulation has been related to e.g. kidney disorders and cancer (Johri *et al*., 2010; Julin *et al*., 2012). Even though Cd can also be toxic to plants, this is not a major concern since plants are much less sensitive to elevated Cd levels than humans. Despite successful agronomic measurements in Sweden to reduce the content of Cd in crop production, the issue of Cd accumulation in soils and crops is still a concern for Swedish agriculture.

3.2 Micronutrients in arable soils

Soil is of major importance for life since it represents a source of both water and nutrients for plants and soil-living microorganisms and animals. Quantitatively, micronutrients are negligible constituents of soils, but the concentrations and availability of these nutrients are still of great importance for plant development and for obtaining high yields in crop production.

Soil micronutrients are derived from minerals in the geological parent material, where the composition of the bedrock, the texture and the degree of weathering of the mineral soil have a large influence on the amount of micronutrients stored and released. In addition to these background concentrations, total micronutrient concentrations in arable soils are influenced by anthropogenic inputs such as mineral and organic fertilizers, liming products, agrochemicals and atmospheric deposition. Unwanted heavy metals such as Cd can also accumulate in soils through these pathways.

3.2.1 Concentrations in Swedish arable soils

Due to the latest glaciation of Scandinavia and northern Europe, Swedish arable soils can be considered relatively young compared with those in many other areas of the world. This means that Swedish arable soils are less leached and depleted in nutrients than *e.g.* many tropical soils. However, the majority of Swedish arable soils originate from granite and gneiss, which are known to be low in several essential micronutrients and slow in weathering (Alloway, 2013). Since the Swedish climate is relatively humid, there can also be substantial leaching of some nutrients through the soil profile, especially in coarse-textured soils (Andersson *et al*., 1988).

Repeated surveys of the concentrations of micronutrients and trace elements in Swedish arable soils have been conducted in recent decades within the Swedish monitoring programme on arable soils (Eriksson *et al*., 2000; 2010). Average topsoil concentrations of micronutrients in arable soils according to these surveys are shown in Table 1. When comparing mean values from the surveys with average soil concentrations in other parts of the world (Kabata-Pendias, 2001), it can be concluded that Swedish micronutrient concentrations in soil are in the low or average range. However, the variation is large, both in Sweden and in other countries.

3.2.2 Impact of specialisation in agricultural production on long-term changes in soil

During the past 50 years, there has been a specialisation within agriculture into farms with and without animal husbandry. Crop production without animals is often characterized by large inputs of mineral fertilizers, no addition

Table 1. *Average topsoil and wheat grain concentrations (mg kg^{-1}), removal with harvested grain (g ha^{-1}) and field balance for mineral fertilization (20 kg P ha^{-1}) and fertilization with cattle manure (g ha^{-1}) for the micronutrients B, Cu, Mn, Mo, Ni and Zn (data from Hamnér et al., 2012; Eriksson et al., 2010)*

Element	Average concentration (mg kg^{-1})		Amount (g ha^{-1})		
	Topsoil	Wheat grain[a]	Removal with grain[a]	Balance for mineral P	Balance for cattle manure
B	MD[b]	0.9	4.5	(+16)	+180
Cu	15	3.9	20	-14	+128
Mn	461	29	148	-72	+541
Mo	1.5	1.0	5.2	-5.0	+10
Ni	13	0.2	0.9	-0.6	+7.7
Zn	59	26	136	-58	+488

a) Corresponds to a grain yield of 6 000 kg DM ha^{-1}
b) Missing data

of organic material and low inputs of micronutrients. This may lead to slow depletion of essential micronutrients in arable soils on farms without manure application.

The risk of micronutrient deficiency and reduced crop yield, as well as decreased quality of food crops for human consumption, could thereby increase. Several studies have indeed reported declining trace element concentrations in crops over recent decades (Ekholm *et al.*, 2007; Kirchmann *et al.*, 2009). Depletion of trace elements in soil, together with high usage of NPK fertilizers to increase yields, has been suggested as one possible explanation for this decline (Thomas, 2003).

On the other hand, farms with livestock often have intensive production with large numbers of animals. On these farms, minerals added to feeds and application of large amounts of manure cause accumulation of micronutrients in soil. On arable farms without animals, application of sewage sludge to fields can be an alternative to manure addition, providing both organic matter and nutrients. The supply of micronutrients with sewage sludge can indeed be positive for both yield and crop quality, but also raises concerns about unwanted heavy metals such as Cd, which may accumulate in soils and crops in the long term (McBride, 1995).

3.2.3 In- and output flows for a Swedish wheat field

On a field scale, major flows of micronutrients to and from fields are governed by inputs through fertilizers and atmospheric deposition and outputs through removal with the harvest product and leaching through the soil profile (Figure 2).

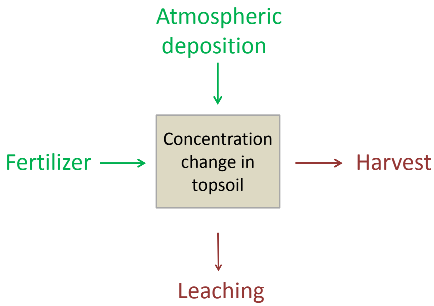

Figure 2. Schematic illustration of sources of inflows and outflows of micronutrients to a field (K Hamnér).

While the amounts of micronutrients added with deposition or lost by leaching can be substantial and can vary between sites, most of the variation in the total balance is determined by differences in fertilization practices and yield levels (crop removal). Flows and balances for different fertilizers (mineral fertilizer and different organic fertilizers) at different yield levels were calculated by Hamnér *et al*. (2012), using data from the Swedish environmental monitoring programme on arable soils and other data from national surveys (Eriksson *et al*., 2010; Steineck *et al*.,1999). The results showed that at a normal yield level for winter wheat in Sweden (6000 kg ha^{-1}), crop micronutrient removal varied between 1 and 150 g ha^{-1} and field balances exhibited different trends depending on type of fertilizer used (Table 1). Crop production with application of only mineral fertilizers resulted in a negative balance for almost all micronutrients studied, whereas fields with application of organic fertilizers such as manure or sewage sludge ended up with a positive balance (Hamnér *et al*., 2012). Similar trends have been shown in other studies although the variation can be large depending on the crops included the calculations (Bengtsson *et al*., 2003).

3.3 Crop accumulation of micronutrients

Plants accumulate nutrients mainly from soil by the roots but also through the leaves. Accumulation of micronutrients through foliar uptake can be substantial for some elements and atmospheric deposition can thereby be important for crop concentrations, as shown for Cu and Ni (Dalenberg & van Driel, 1990; Yläranta, 1996). For Se, large annual variations in crop concentrations could possibly be due to fluctuations in Se deposition (Kirchmann *et al*., 2009). When measuring and comparing crop micronutrient concentrations, the impact of atmospheric deposition should therefore not be neglected. Nevertheless, supply and crop uptake of micronutrients from the soil are essential to fulfil plant requirements and this pathway is the main focus in this thesis.

3.3.1 Mass flow, diffusion and active uptake

Mass flow of nutrients, whereby nutrients in the soil solution are taken up by roots through water flow, is driven by root pressure and plant transpiration (White, 2012). For anions such as nitrate and sulphate and the cations Ca and Mg, the concentration in the soil solution is often high enough to fulfil crop requirements. However, for the micronutrients (as well as for P and K), the crop nutrient requirements can most often not be fulfilled by uptake through mass flow only. Instead, crops are dependent on the diffusion of ions moving through soil across a concentration gradient. The supply to the crop is thereby determined by both the rate of mobilization of nutrients at the surface of soil particles and the diffusion rate of the ion through the soil matrix (Kirkby & Römheld, 2004). The diffusion rate is dependent on the specific ion, but also on the water content of the soil. Since diffusion rates in general are often slow for these elements, the importance of large root volume has been pointed out (Luster *et al*., 2009). The presence of roots is also important for active nutrient uptake, where roots exude chelates and phytosiderophores to increase nutrient uptake. Presence of arbuscular mycorrhiza can also be of importance for plant nutrient uptake, where the external fungal hyphae can absorb non-mobile nutrients and translocate them to the plant (Smith & Read, 2008).

3.3.2 Importance of plant availability in soil for crop uptake

The total concentrations of micronutrients in arable soils are often high in comparison with crop uptake. However, for plants to utilize these nutrients, they need to be in accessible form and thus only a small fraction of the total soil content can be utilized by the plant during the growing season. To optimize crop yields in both the short and long run, it is therefore important to achieve sufficient availability of micronutrients in soil during the cropping season, but also to ensure that the supply of nutrients can continue for a long time, in order to maintain soil fertility.

There are several soil properties that influence the plant availability of micronutrients in soils: (1) Soil pH is one of the most important properties. Since most micronutrients appear as cations in the soil solution, while soil particles have a net negative charge, their solubility and availability for plant uptake increases with decreasing pH. The exception is Mo, where the anionic properties result in increasing solubility at increased pH. Under normal conditions for arable soils, (2) redox potential is important mainly for Mn and partly also for Fe. Plants take up these nutrients in divalent forms which are created under reducing conditions. When the soil enters an oxidizing state, these elements are transformed into higher oxidized stages not accessible to plants; (3) Organic matter (OM) content can release nutrients during mineralization, but the high adsorption capacity of organic compounds can also bind micronutrients, making them less plant available; (4) The interactions with other nutrients can be important, since they can affect binding capacity and crop uptake of the ions due to competition between elements with similar properties; (5) mycorrhiza symbiosis can improve nutrient availability to plants.

Regular application of organic fertilizers such as manure or sewage sludge often results in accumulation of micronutrients in soil (see chapter 3.2.4) and annual inputs of micronutrients through these amendments can be substantial compared with crop requirements. However, since the inputs are often small in relation to total concentration of micronutrients in soil, the effect of the amendment on plant availability is crucial for the influence on crop uptake. In the literature, the reported effects of organic fertilizers on concentrations of micronutrients and other trace elements are inconsistent. Some studies report increased uptake of some trace elements (Codling, 2014; Marcato *et al*., 2009; Pascual *et al*., 2004) whereas others have found no significant differences or even a decline in metal concentrations upon application of organic fertilizers compared with mineral fertilizer or unfertilized controls (Andersson, 1976; Hanc *et al*., 2008; Singh *et al*., 2010).

3.3.3 Relationship to nitrogen

Nitrogen is one of the most abundant elements in crops and often the most limiting nutrient in crop production. High N fertilization rates, in conjunction with advances in plant breeding and pest control, have enabled a major increase in biomass production and grain yields during recent decades.

An understanding of how nutrients accumulate during crop growth is essential for good nutrient management and fertilization timing. Concerning N, this issue has been widely studied, both in theory and in field trials of wheat within Sweden (*e.g.* Ågren, 1985; Engström & Bergkvist, 2009). However, the accumulation dynamics can differ between nutrients, as shown for maize (Bender *et al*., 2013; Ciampitti *et al*., 2013; Ciampitti & Vyn, 2013; Xue *et al*., 2014). For cereal crops, studies usually refer to macronutrients only or have used old varieties that may differ considerably from modern varieties (Hocking, 1994; Lasztity *et al*., 1984; Malhi *et al*., 2006).

Nutrient stoichiometry in plants is an established concept in plant physiology that is frequently used to describe relationships between elements in plants and how these are influenced by crop type and environmental factors

(Sterner & Elser, 2002). Since crop N is of major importance for several essential processes in plants and most often determines plant growth, it is of interest to study how other nutrients interact with N. However, most stoichiometry studies in crops to date have focused on C:N or C:N:P ratios only (Ågren, 2008; Sadras, 2006). Nutrient stoichiometry studies including more than the above-mentioned elements are few, but available for willow (Ågren & Weih, 2012) and maize (Ciampitti *et al.*, 2013; Ciampitti & Vyn, 2013; Riedell, 2010).

There are sometimes indications and concerns about high N fertilization rates causing lower micronutrient concentrations due to dilution in crops at high yields (Jarrell & Beverly, 1981; Thomas, 2003). However, the opposite effect has also been shown in several studies for Zn and Fe (Cakmak *et al.*, 2010) and nitrogen fertilization has thereby been proposed as a way to biofortify cereal grain.

3.4 Micronutrient concentrations in cereal crops grown in Sweden

Within the Swedish monitoring programme on arable soils, numerous samples of winter wheat, spring barley and oat grain have been analysed for micronutrients and other trace elements. Average grain concentrations from this survey are presented in Table 1. The results reveal that, like in the neighbouring countries Finland, Denmark and Norway, average grain concentrations of Cu, Mn, B and Ni are relatively low in Sweden compared with in other parts of Europe (Hamnér *et al.*, 2012; Kabata-Pendias, 2001). The concentrations of Mo and Zn are in general moderate or high compared with those in many other countries, although there are large variations within regions and countries.

While severe yield losses due to lack of micronutrients are considered rare in Sweden, a number of field trials and observations report low concentrations and deficiencies caused by micronutrients. Concerning Cu, low concentrations in cereal crops grown on organic and coarse-textured soils was reported already in the 1940´s (Stenberg *et al.*, 1949) and this issue have received attention since then. Critical threshold concentrations for grain have not been determined, but the literature suggests that Cu concentrations below 2-3 mg kg^{-1} could cause yield limitation (Fageria *et al.*, 2002; Kabata-Pendias, 2001; Kirchmann & Eskilsson, 2010; Sinclair & Edwards, 2008). Using these figures to evaluate the Cu status of Swedish cereal crops, up to 25% of the arable land in Sweden has too low Cu concentrations for obtaining optimal crop yield. Older Swedish field trials have indeed reported yield increases upon Cu fertilization on several sites (Carlgren, 2003; Lundblad & Johansson, 1956). Furthermore, in nearby countries such as Denmark, Finland and Germany, 20-25% of the arable land is considered to cause Cu deficiency in susceptible crops such as cereals (Sinclair & Edwards, 2008).

Yield losses due to low Mn concentrations in cereal crops are known to occur in different parts of Sweden, mainly on alkaline and coarse textured soils (Swedish Board of Agriculture, 2015). Visible deficiency symptoms are not uncommon and foliar fertilization with Mn is regularly applied to cereals and other sensitive crops. Grain samples from the monitoring programme also indicate Mn concentrations within the critical range of deficiency, 10-15 mg Mn kg^{-1} DM, according to Alloway (2008) and Bergmann (1992).

Zinc concentrations in Swedish cereals are often above the critical range and deficiency problems are not a major concern. However, studies from recent field trials have observed low Zn concentrations at some sites (unpublished data).

3.5 Methods of analysis for assessment of micronutrient deficiency

The ability to predict and assess the micronutrient status in crops is essential in order to avoid and counteract deficiencies and thereby yield losses. Analysing plant tissue during the growing season gives a good indication of the current status and concentrations can be related to critical thresholds for deficiency (Bussink & Temminghoff, 2004). However, these analyses are time-consuming and expensive and only give a picture of the momentary status of the crop. In addition, if low tissue concentrations are detected, yield losses may already be inevitable. It would therefore be useful to be able to predict the availability of micronutrients in soil. A number of different methods for soil analysis have thus been developed for this purpose.

3.5.1 Soil analysis based on extraction methods

There are a number of different soil extraction methods for determination of micronutrient concentration and availability. Extraction with strong acids such as HNO_3, HCl or a combination of these (*aqua regia*) extracts a major part of the soil micronutrient content, often referred to as the pseudo-total. Although the pseudo-total content of micronutrients can give some information about the potential supply from soil, it does not determine the proportion available to plants. In Sweden, pseudo-total analysis (extraction with 2M HCl) is nevertheless performed to estimate Cu supply and a soil concentration below 7 mg Cu kg^{-1} is considered to imply a risk of deficiency in susceptible crops, such as cereals (Swedish Board of Agriculture, 2015). Results from the Swedish monitoring programme show that while there seems to be an increased risk of deficiency at low Cu soil concentrations, there is large variation in crop concentrations at these low soil levels (unpublished data).

To get a better estimate of the plant-available content of micronutrients in soil, extractants using weaker acids with chelating agents (EDTA; DTPA), dilute salt solutions ($CaCl_2$, KCl) or water (hot or cold) have been developed and are used in different parts of the world. Sometimes two different extractions are combined, one measuring the directly available micronutrient concentrations in soil solution (intensity) and one the quantity that can readily be mobilized and enter the soil solution to replace the ions removed by crop uptake (capacity) (Bussink & Temminghoff, 2004). However, a general problem with all soil extraction methods is their inability to represent plant conditions, as they involve destruction of soil structure through shaking of the sample, use a high solution to soil ratio and do not take into account the extent of plant roots within the soil.

3.5.2 Soil analysis using diffusive gradient in thin films (DGT)

The diffusive gradient in thin film (DGT) technique is based on the diffusion of ions through the soil matrix. The DGT device contains a diffusive gel that allows free movement of ions, while a resin gel accumulating the ions acts as an infinite sink and thereby mimics nutrient root uptake (Figure 3). The contact between the device and soil locally lowers element concentrations in the soil solution and ions of the element are then resupplied from the labile pool in the solid phase (Zhang *et al*, 2001). Thus, this technique measures both element concentrations in the soil solution and the mobilization capacity of ions from the solid phase (Degryse *et al.*, 2009). Another advantage compared with extraction methods is that DGT more closely resembles plant conditions, since the soil structure is kept more or less intact. Therefore, the DGT technique has been proposed as a way to predict the plant availability in arable soils of micronutrients, as well as phosphorus and heavy metals such as Cd (*e.g.* Mason *et al.*, 2010; Nolan *et al.*, 2005; Tandy *et al.*, 2011).

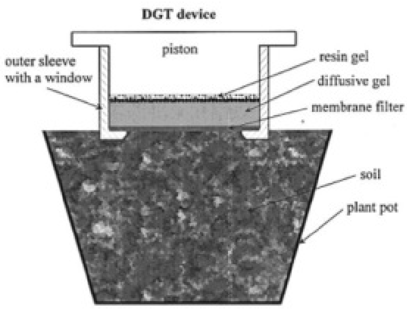

Figure 3. Schematic cross-sectional diagram of the device used in the diffusive gradient in thin film (DGT) technique deployed in a pot of soil (Zhang, 2001).

4 Material and Methods

The studies presented in this thesis were based on long-term and short-term field trials and on data from the Swedish monitoring programme on arable soils. Winter wheat (*Triticum aestivum* L) was the major crop evaluated in the studies, but spring barley (*Hordeum vulgare* L) and oats (*Avena sativa* L) were also included. The following paragraphs provide an overview of the methods used within each study. For full details, see the paper(s) referred to in each headline.

4.1 Evaluation of monitoring data (Paper II)

The monitoring programme was started in 1988 to evaluate the status of Swedish arable soils at ten-year intervals and to detect possible changes over time by analysing soils and crops. Sampling points were fixed and selected using a stratified method to represent all arable land throughout Sweden. The data used within this project included approximately 1 900 soil and grain samples (approximately 900, 600 and 400 samples of winter wheat, spring barley and oats, respectively) from two sampling periods (1988-1995 and 2001-2007). Samples were analysed within the programme for all major trace elements as well as a number of soil parameters, such as pH, organic matter and clay content. Compilations of the data-sets have been published in national reports (Eriksson *et al*., 2010; 2000).

4.2 Field trials, description and sampling

Several long-term and short-term field trials in southern and central Sweden were selected to evaluate the different objectives set for this thesis work (Figure 4). All trials selected were situated on mineral soils with a clay content of 11-56%, low to moderate organic matter (OM) content (2.1-10.5%) and slightly acid to neutral pH (Table 2). All trials had a randomized block design.

Table 2. *Soil properties (soil type, clay content in topsoil (%), organic matter content, OM (%) and pH at start (H_2O) for the field experiment sites*

Site	Soil type	Clay (%)	OM (%)	pH (H_2O)
Long-term field trials (Papers I, II, III)				
Bjertorp	Silty clay	30	4.4	6.4
Ekebo	Sandy loam	14	6.2	6.8
Fjärdingslöv	Sandy loam	17	2.8	7.5
Fors	Silt loam	18	4.4	7.7
Högåsa	Silty sand	10	4.8	5.9
Kungsängen	Gyttja clay	56	4.2	7.1
Lanna	Silty clay	42	4.0	6.7
Mellby	Loamy sand	6	5.2	6.0
Orup	Sandy loam	13	4.8	6.2
Petersborg	Sandy loam	14	2.4	6.8
S:a Ugglarp	Silty loam	8	3.0	6.6
Vreta Kloster	Silty clay	50	4.2	6.7
Örja	Sandy clay loam	15	2.2	7.2
N fertilization trials (Papers IV, V)				
Falkenberg	-	14	3.2	7.3
Glyttinge	-	31	2.8	6.8
Grillby	-	38	5.8	6.4
Grästorp	-	33	4.3	6.5
Höjagården	-	29	3.5	7.1
Karlsfält	-	23	10.5	6.1
Klagstorp	-	19	1.5	7.5
Kårby	-	22	4.8	6.8
Nybble	-	32	3.1	6.6
Skofteby	-	11	2.7	6.3
Skultuna	-	20	2.1	6.7
Strömsholm	-	49	5.0	6.0
Teckomatorp	-	16	3.0	7.6
Tjustorp	-	15	3.6	6.3

4.2.1 Swedish long-term soil fertility experiments (Papers I-III)

The Swedish long-term soil fertility experiments were started between 1956 and 1966. For the studies described in this thesis, 10 different sites in southern and central Sweden were used (Figure 4). A detailed description of the experimental design is given by Carlgren & Mattsson (2001). The sites differ

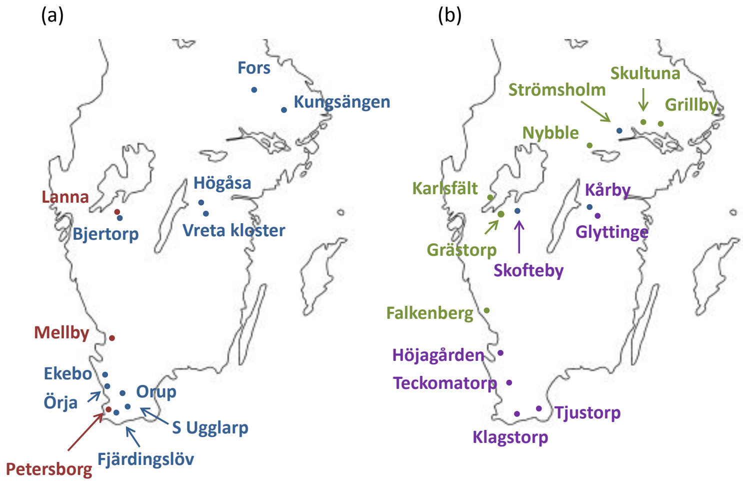

Figure 4. Experimental sites in southern and central Sweden used in (a) Papers I, II and III (blue=soil fertility experiments, red=other sites) and (b) Papers IV (green) and V (green and purple) (K. Hamnér).

in terms of climate and soil properties (Table 2). For grain analysis, archived samples were used.

For Paper I, the trial at Örja in the very south of Sweden, on a sandy loam soil, was used. Two treatments were included in this study: (A) No fertilization and (B) NPK fertilization (150 kg N, 30 kg P, and 60 kg K ha^{-1}). Soil sampling was carried out in May 2010 in the two replicate plots of each treatment, with five mixed subsamples taken from each experimental plot. Topsoil (0-20 cm) and subsoil (20-25, 25-30, 30-35 and 35-40 cm) samples were taken with an auger.

For Paper II, 10 sites were included: Bjertorp, Ekebo, Fjärdingslöv, Fors, Högåsa, Kungsängen, Orup, S. Ugglarp, Vreta kloster and Örja. Treatments included were the same as in Paper I (see above). Since only one pooled grain sample from each year was available, stored samples of winter wheat grain collected on 5-6 occasions between 1970 and 1995 were used as replicates. Topsoil samples (0-20 cm), taken in the same or in an adjacent year to the grain samples, were used for soil analysis (five mixed subsamples from each experimental plot).

In Paper III, nine of the sites listed for Paper II were included (Fors site excluded) and the following treatments were included: (A) No fertilization; (B) mineral fertilization with 150 kg N as calcium nitrate, 30 kg P as single superphosphate and 60 kg K as KCl ha^{-1} $year^{-1}$; (C) cattle manure at 25 Mg fresh weight ha^{-1} every four years; and (D) mineral fertilizer plus cattle manure (B+C). Since only one pooled grain sample from each year was available, grain samples of winter wheat from three different years between 1995 and 2003 were used. Topsoil samples (0-20 cm), taken in the same or an adjacent year to the grain samples were used for soil property analysis (five mixed subsamples from each experimental plot).

4.2.2 Lanna site (Papers II, III)

The on-going trial at Lanna research station in south-west Sweden was started in 1996 to study the effect of different organic amendments and N fertilizers on soil organic matter content, soil biological properties and crop yield. The soil at the site is a silty clay (42% clay) and the experiment has four replicate plots per treatment, with each plot measuring 8 m x 14 m. The treatments included in Paper II and III were: (A) No fertilization, (B) $Ca(NO_3)_2$ at 80 kg N ha^{-1} $year^{-1}$ plus single superphosphate at a rate of 40 kg P and 30 kg K as KCl every two years; (C) $(NH_4)_2SO_4$ at 80 kg N ha^{-1} $year^{-1}$ and PK as in B; solid cattle manure at 8 Mg DM ha^{-1} every two years; (E) sewage sludge at 8 Mg DM ha^{-1} every two years and (F) as E + addition of metal salts at a rate of 0.1 kg Cd ha^{-1}, 3.0 kg Cu ha^{-1}, 6.3 kg Ni ha^{-1} and 0.2 kg Zn ha^{-1} together with sewage sludge application. The crop rotation consisted of oats, spring barley and winter wheat. Grain samples of winter wheat and topsoil samples (0-20 cm) were collected from each replicate plot in 2010.

4.2.3 Petersborg site (Papers II, III)

At Petersborg, in the very south of Sweden, a field trial was started in 1981 with the aim of testing the impact of sewage sludge addition on soil and crop properties. The soil is a sandy loam (14% clay). The following treatments were used within the studies: (A) No fertilization; (B) sewage sludge at 4 Mg DM ha^{-1} every four years; (C) sewage sludge at 12 Mg DM ha^{-1} every four years; (D) mineral fertilizer at 140 kg N as $Ca(NO_3)_2$, 18 kg P and 63 kg K as PK fertilizer; and (E) sewage sludge (as C) plus mineral fertilizer (as D). The experiment has four replicate plots in each treatment and a plot size of 6 m x 20 m. Grain samples of winter wheat and topsoil samples (0-20 cm) from each replicate plot were collected in 2010.

4.2.4 Mellby site (Paper III)

The Mellby experimental site is situated in south-west Sweden on a sandy loam. The experiment started in 1989 with the aim of investigating the effects of catch crops and manure addition on nitrogen leaching (see Torstensson & Aronsson, 2000 for details). Plot size is 30 m x 30 m and there are three replicates per treatment (unfertilized control has one replicate). Treatments used in Paper III were: (A) No fertilization; (B) mineral nitrogen at 90 kg N ha^{-1} $year^{-1}$ as NH_4NO_3 and (C) pig slurry at 113 kg tot-N ha^{-1} $year^{-1}$ + mineral nitrogen at 45 kg ha^{-1} $year^{-1}$. Grain samples of spring barley were collected in 2010 and 2013 and analysed. Samples for soil properties (topsoil 0-20 cm) taken in 2005 were used in the study.

4.2.5 Nitrogen fertilization trials (Papers IV-V)

In Papers IV and V, field trials at seven different sites in southern and central Sweden were sampled across one growth cycle of a winter wheat crop, while for Paper V an additional seven sites were included. All field sites consisted of mineral soils with a clay content of 14-49% and an organic matter content of 1.5-10.5%. The trials had four replicates in each treatment and each plot measured 3 m x 12 m. Treatments used in the study were: (1) No N fertilization (N0); (2) 80 kg N ha^{-1} (N80); (3) 160 kg N ha^{-1} (N160); and (4) 240 kg N ha^{-1} (N240). The N fertilizer consisted of NH_4NO_3 with a sulphur (S) supplement corresponding to addition of 11, 22 and 33 kg S ha^{-1} in the N80, N160 and N240 treatments, respectively.

Crop sampling was performed on four occasions during the cropping season of 2014 at four different developmental stages according to the Zadok´s scale (Zadok *et al*., 1974): 1) At tillering, in the beginning of crop growth in spring (ZS23); 2) at stem elongation (ZS37); 3) at flowering (ZS65); and 4) at grain maturity (ZS93). To avoid contamination with soil, all plants were cut approximately 2 cm above the ground and the lowest, wilted leaf level was removed from the samples. For the seven additional sites included in Paper V, sampling was carried out only at ZS37 and in one N treatment (N160).

Topsoil samples (0-20 cm) were taken from each replicate plot on the same occasion as plant sampling at stem elongation (ZS37). Approximately 15 subsamples were merged into one sample from each plot and used for analysis of Cu soil concentrations (paper V). For soil property analysis, soil samples from the different treatments were merged into one pooled sample per site (paper IV and V).

4.3 Plant and grain sample preparation and analysis (Papers II-V)

Papers II and III: Grain samples were digested in concentrated nitric acid (HNO_3) and trace element concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS; Elan 6100 ACP-MS; Perkin Elmer SCIEX instruments). Whole grains were used to avoid contamination at milling. The elements analysed were Ni, Cd, Cu, Mn, Mo, Se and Zn. Dry matter content was determined on every fifth sample by drying at 105 ºC for 24 h.

Papers IV and V: Plant samples were dried (45º C for approximately 48 hours) and milled (Retsch Grindomix GM200) prior to analysis. A titanium blade was used to avoid contamination from the grinder (Dahlin et al., 2012). Shoot and grain samples were digested in concentrated nitric acid (HNO_3) and analysed using an ICP-OES (PerkinElmer Optima 7300DV) for K, P, S, Ca, Mg, Fe, Zn, Mn, Cu and B. To avoid contamination, whole grains were used for the analysis. An LECO analyser (TruMac CN) was used for N analysis. Dry matter content was determined on every fifth sample by drying at 105 ºC for 24 h.

4.4 Soil sample preparation and analysis (Papers I-V)

The samples were dried (oven-dried at 40 ºC) and sieved (< 2 mm). All soil samples were analysed for clay content, organic matter (OM) content and pH (H_2O; 1:5 soil to water ratio).

Paper I: Soil samples from Örja were digested in 7M nitric acid (HNO_3) and analysed for trace elements (ICP-MS).

Paper II: Soil samples from the experimental sites and within the monitoring programme were digested in 7M nitric acid (HNO_3) and analysed for Ni (ICP-MS).

Paper III: Soil samples from Lanna and Petersborg were digested in 7M HNO_3 and analysed for trace elements (ICP-MS).

Papers IV and V: Soil samples were analysed for plant-available concentrations of P, K and Mg, using extraction with ammonium lactate-acetate (AL) (Egnér *et al.*, 1960). For Paper V, extraction with 7M HNO_3 and 0.01 M $CaCl_2$ were made and analysed for Cu concentration. Also additional soil analyses were performed (see section 4.5).

4.4.1 Extraction with HNO_3 and $CaCl_2$

For pseudo-total analysis of trace elements in soil, 10 mL of 7M HNO_3 were added to 2 g soil and left for 24 hours. Samples were then briefly shaken and boiled in three steps: 60 ºC for two hours, 100 ºC for one hour and 130 ºC for two hours. After cooling, the samples were diluted with 50 mL Milli-Q water and filtered. Analysis for Cu content was then performed by ICP-OES.

For extraction with $CaCl_2$ (paper V), 100 mL of 0.01M $CaCl_2$ were added to 10 g soil and samples were shaken for two hours. Samples were then centrifuged, filtered and analysed for Cu content on an ICP-MS (ALS Scandinavia, Luleå, Sweden).

4.5 Diffusive gradients in thin film (DGT) technique (paper V)

4.5.1 Deployment of DGT devices

For measurements with the DGT technique, a device for soils with a diffusive layer thickness of 0.91 mm (0.8 mm diffusive gel plus filter) was used (DGT Research Ltd, Lancaster, UK). A mixed binding layer (MBL) was used since phosphorus analysis was performed using the same devices (Mason *et al.*, 2005). For this, 100 g dry and sieved soil from each replicate plot was placed in a plastic container and Milli-Q water was carefully added until the soil reached the saturation point. The samples were left to equilibrate for 24 hours before deployment of the DGT device. Prior to deployment, the DGT device was smeared with moist soil to ensure good contact with the soil and it was then pushed into the soil surface. Two blank samples without soil were left together with the soil samples during deployment. After a deployment time of 24 hours, the device was taken out, rinsed with Milli-Q water to remove adhering soil and gently cleaned with clean tissues if necessary. The binding gel was removed from the device and eluted in 1 ml of 1M HNO_3. After 24 h of elution, the binding gel was removed and the eluate was sent for analysis by ICP-MS (ALS Scandinavia, Luleå, Sweden)

4.5.2 Calculation of C_{DGT}

The Cu concentration in the eluate is dependent on the volume of the eluate, the proportion of the total amount of the binding gel that is eluted by the acid, the surface area of the device, the deployment time and the thickness of the diffusive gel layer. The average Cu concentration for the two blanks was deducted from the soil sample concentrations before further calculations.

The mass of Cu accumulated in the resin gel (M) was calculated from the volume of the eluate and the gel as follows:

$M = C(V_{acid} + V_{gel})/f_e$

where C is the concentration of Cu measured in the eluate (µg L^{-1}), V_{acid} is the volume of acid used for the eluate (L), V_{gel} is the volume of the resin gel (L) and f_e is the elution factor (0.8).

Measurements made using this technique are often reported as DGT-measured concentration (C_{DGT}) which is the time-averaged concentration (µg L^{-1}) of the element at the interface between the DGT device and the soil solution. C_{DGT} is calculated as follows:

$C_{DGT} = M * \Delta g/(D * A * t)$

where M is the mass of the element accumulated in the binding gel, Δg the thickness of the diffusion layer + filter (cm), D is the diffusion coefficient of the ion (cm^2 s^{-1}), A is the area of the exposure window of the device (cm^2) and t is the deployment time (s) (Zhang *et al.*, 2004).

4.6 Other calculations

4.6.1 Bio-concentration factor (Paper III)

Bio-concentration factors (BCF) for Cd, Cu and Zn were calculated using data from the Lanna and Petersborg trials to evaluate the relative phytoavailability of trace elements in sewage sludge. The BCF value was obtained for each element by dividing crop metal concentration (mg kg^{-1}) by total soil metal concentration (mg kg^{-1}) (Heemsbergen *et al.*, 2010).

4.6.2 Relative nutrient accumulation rate (Paper IV)

The relative accumulation rate is based on the concept of relative growth rate (R_G). The R_G is the (exponential) increase in size relative to the size of the plant present at the start of a given time interval (e.g. growth period). Expressed in this way, growth rates can be compared among species and individuals that differ widely in size, and this concept is considered to be the most widely used way of estimating plant growth (cf. Hunt, 1982). The relative nutrient accumulation rate (R_{Nu}) was calculated for nutrient pools the same way as R_G calculates accumulation rate of biomass, using the formula:

$R_{Nu} = (ln\ Nu_2 - ln\ Nu_1)/(t_2 - t_1)$

where Nu is the amount of nutrient accumulated at t (g) and t is the time (days)

Calculations of R_G and R_{Nu} were performed for three different periods for biomass and all included nutrients: ZS 23-ZS37 (Period I), ZS37-ZS65 (Period II) and ZS 65-93 (Period III).

4.6.3 Harvest index and nutrient harvest index (Paper IV)

The harvest index (HI) and nutrient harvest index (NuHI) are defined as the percentage of total plant content of biomass and nutrient, respectively, that is translocated to the grain at full maturity of the plant.

5 Results and Discussion

5.1 Depletion of micronutrients in arable soils over time (Paper I)

On arable farms without regular application of organic fertilizers, the inputs of micronutrients are often low and field balances negative (Hamnér *et al.*, 2012). However, results from the experimental site at Örja showed no significant differences in micronutrient soil concentrations between unfertilized treatments and treatments with high N fertilization rates at any depth of the profile (0-40 cm). It could be expected that after 50 years of cropping, there would be some depletion of soil micronutrients at high N fertilization rates owing to greater crop removal than in unfertilized or manured treatments. Since the soil at Örja has a relatively low micronutrient concentration compared with many other Swedish soils (Eriksson *et al.*, 2010), these changes could be assumed to be more pronounced there than at some other sites.

To examine the rate of depletion, crop removal of Mn and Zn was used as an example. These nutrients are accumulated in crops at higher rates than other micronutrients and represent, respectively, relatively high (260-410 mg Mn kg^{-1} soil) and low (42-49 mg Zn kg^{-1} soil) concentrations in soil. The total difference in yield between the high N treatment (150 kg N ha^{-1} $year^{-1}$) and unfertilized treatment amounted to roughly 480 Mg biomass per hectare over a 52-year period, corresponding to a difference in removal of a few kg Mn and Zn in total (Table 3). Calculated crop removal for the two nutrients over the years was 0.14-0.35% and 1.1-2.7% of the total amount in soil for Mn and Zn, respectively. This is theoretically equivalent to a change in soil concentration of 0.48 and 1.26 mg Mn kg^{-1} and 0.53 and 1.36 mg Zn kg^{-1} soil for the unfertilized and high N treatment, respectively. Comparisons of these concentrations with those in soil showed that variations within each treatment were higher than the decline caused by crop removal (Table 3).

Table 3. *Crop removal of Mn and Zn from the Örja soil over 52 years compared with concentrations in soil the No N and 150 kg N ha^{-1} treatments*

Element	Treatment (kg N ha^{-1})	Total amount in soil (kg ha^{-1})	Crop removal Σ52 yrs (kg ha^{-1})	Crop removal Σ52 yrs (% of tot)	Depletion Σ52 yrs (mg kg^{-1})	Concentration in soil (mg kg^{-1}) Mean	Min	Max
Mn	0	2387	3.3	0.14	0.48	351	284	407
	150	2475	8.5	0.35	1.26	364	294	472
Zn	0	326	3.6	1.1	0.53	47.9	40.3	54.1
	150	342	9.2	2.7	1.36	50.3	45.2	54.4

Calculations based on concentration to 40 cm depth, bulk density (Kirchmann & Eriksson, 1993), yield differences and mean concentrations in crops (Eriksson et al., 2010, 2000; Andersson, 1992)

It is sometimes claimed that the use of mineral fertilizers and intensive crop production cause depletion of trace elements in soil (*e.g.* Granstedt *et al*., 2009). However, the example from Örja showed that depletion of soil micronutrients is often very slow and difficult to detect due to the fact that too small amounts are removed through crop harvesting in relation to natural variations in these elements in soil. Earlier calculations also showed that the total amount of micronutrients in soil is equivalent to the amounts removed by thousands of harvested crops (Graham, 1978; Sippola, 1978). While this is just an example from one single site, there is reason to believe that the situation is similar in many other arable soils in Sweden with a relatively high total content of micronutrients.

Thus, over a reasonable time of crop production, mining and depletion of micronutrients should not be a major concern, except in cases of extremely low total soil micronutrient concentrations (White & Zasoski, 1999). However, it should be noted that the ability of soils to supply micronutrients to crops in plant-available form is more important and requires special attention.

5.2 Crop micronutrient and Cd accumulation as influenced by nutrient management

5.2.1 Long-term impact of organic fertilizers on concentrations in cereal crops (Papers II, III)

Addition of organic fertilizers such as manure or sewage sludge to arable soils implies accumulation of micronutrients in soil. Even though this accumulation is often slow in comparison with total soil content, it could have an impact on crop uptake if the nutrients are in phytoavailable form. However, results from several Swedish long-term field trials showed that even after 15-30 years of regular application of different organic fertilizers, there was no general increase in grain micronutrient concentration compared with treatments with mineral fertilizers or in unfertilized controls. For Mn and Se, there was no impact of treatment at any of the sites studied while some differences for Cu, Mo and Ni could be assigned to changes in soil properties such as soil pH rather than effects of the organic fertilizers as such (see section 5.3).

Earlier studies on crop responses to addition of organic fertilizers have produced inconsistent results, which can be due to: (1) Variable metal concentrations in the organic amendment, (2) different application rates, (3) single or repeated application, (4) different impacts on soil properties and (5) high native soil metal concentrations overshadowing the impact of metals added with organic fertilizers. Previous studies showing increased crop uptake of micronutrients have often used amendments with very high trace element concentrations or applied them in extremely large quantities (Evanylo *et al*., 2006; Granato *et al*., 2004; Hooda *et al*., 1997). However, studies performed with application rates more relevant to practical agriculture also show some inconsistent results. Results from Papers II and III showed that under Swedish conditions, there is no major impact concerning the above-mentioned elements.

Zinc was the only one of the nutrients studied for which application of organic fertilizers resulted in a positive response in grain concentrations at several sites. Treatments with manure or sewage sludge resulted in higher Zn grain concentrations compared with treatments with mineral fertilizers at both Lanna, Petersborg and Mellby. However, at four sites of the soil fertility experiments application of manure alone or in combination with mineral fertilizers resulted in lower Zn concentrations than in treatments with mineral fertilizers only. Inconsistent results can also be found in the literature, where soluble Zn concentrations in soil and Zn uptake in crops are reported to increase after manure or sludge application (Codling, 2014; Gartler *et al*., 2013) or remain unaffected (*e.g.* Li *et al*., 2007). The reasons for these contradictory results could be differing plant availability of Zn in the amendments or soil Zn availability being altered by the organic amendments. Also differences in yields could be a contributing factor to the results. Calculations of BCF indicated that the phytoavailability of Zn in sewage sludge was not lower than that of native soil Zn. The results indicate that Zn added with organic materials is plant available to some extent and more mobile than other trace elements.

Cadmium grain content after long-term sewage sludge application (Paper III)

Cadmium concentrations in wheat grain from the experimental sites varied between 0.019 and 0.081 mg kg^{-1}, which is within the range reported previously for winter wheat (Eriksson *et al*., 2010). Even in the treatment spiked with Cd salts, grain Cd concentrations were below the maximum limit of 0.1 mg Cd kg^{-1} (EC 1881/2006).

Despite the net input of Cd with manure and sewage sludge amounting to 10-100 g ha^{-1} over the years of the trials, the concentration of Cd in crops did not increase in these treatments compared with the mineral-fertilized plots or unfertilized controls. Considering that the Cd concentration in Swedish sewage sludge is low compared with those in many other countries and that addition of Cd is strictly limited by Swedish regulations to a maximum of 0.75 g Cd ha^{-1} $year^{-1}$ (SNFS 1994:2), the risk of increased crop Cd concentrations through the use of sewage sludge can be considered low. Compared with other studies showing increased crop Cd concentrations after addition of sewage sludge (Evanylo *et al.*, 2006; Granato *et al.*, 2004; Hooda *et al.*, 1997), both Cd concentrations and quantities of the sludge added were much lower in the study in Paper III.

However, calculations of the BCF for Cd showed indications of similar availability in sewage sludge as when the application was in the form of water soluble Cd salts. In addition, there were indications that higher soil Cd concentrations at Petersborg resulted in higher Cd grain concentrations than at Lanna. Together with earlier studies showing increased crop Cd concentrations after application of sewage sludge with a high Cd content, these results show the importance of keeping Cd concentrations low and of maintaining strict regulations for sewage treatment plants and for sludge application to arable fields in order to ensure crop quality.

5.2.2 High N fertilization rates influence crop accumulation (Paper IV)

For all micronutrients (Fe, Zn, Mn, Cu and B) included in the study with different N fertilization rates, the total amounts taken up increased with increasing N supply to the crop. This result was expected, since a crop with higher biomass requires a larger amount of each nutrient and therefore crop uptake through mass flow and increased active uptake by plant roots can be expected to increase. In addition, the concentrations of Fe, Zn and Cu were generally positively correlated to increased N fertilization rate and N concentration throughout crop development (Figure 5). Positive correlations between N concentration and those of other nutrients in the wheat crop showed that nutrient uptake increased more than biomass production in high-yielding crops, *i.e.* there was a synergistic effect (Jerrell & Beverly, 1981). Positive nutrient to N uptake correlations have been reported previously for wheat (Aciksoz *et al.*, 2011; Kutman *et al.*, 2010; Schwartz & Kafkafi, 1978) and maize (Xue *et al.*, 2014). For Mn and B, no positive response to N concentration was detected except at single sites.

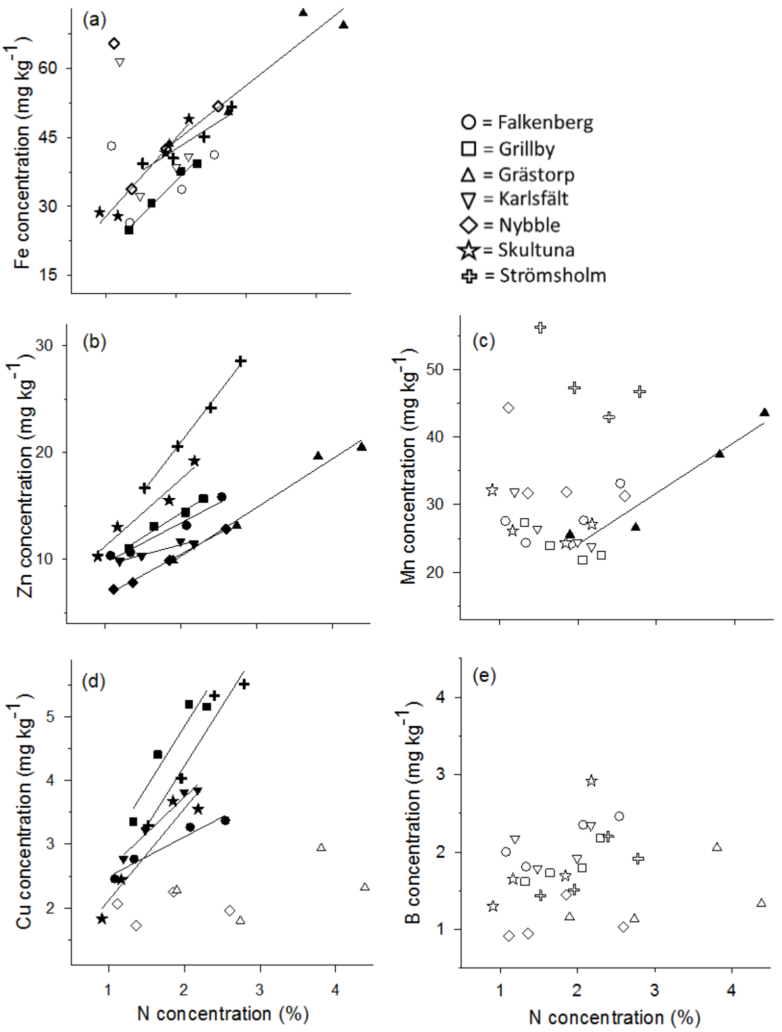

Figure 5. Correlations between micronutrients (Fe, Zn, Mn, Cu and B; a-e) and nitrogen concentrations in whole shoots of winter wheat at stem elongation (ZS37). Closed symbols indicate significant correlations ($p<0.05$) and open symbols non-significant correlations. Diagrams for other developmental stages and macronutrients are included in paper IV.

Despite increased nutrient concentrations, the nutrient to N ratio decreased at high N fertilization rates for all micronutrients studied across all sites. The growth rate hypothesis postulates that the ratio between P and N increases at high yield levels due to increased activity within an organism, and support for this hypothesis has been found for microorganisms and also for higher plants (Ågren, 2004). The same assumption could be made for micronutrients, but this could not be confirmed by the study. Instead, the micronutrient to N ratio in general decreased at high N concentrations and this was also noticed for P.

Increased N supply did not have a major impact on the accumulation dynamics of micronutrients during crop growth. Except for Fe, the accumulation of micronutrients in the wheat crop more or less followed biomass production, irrespective of N fertilization rate (Figure 6). Iron accumulation, unlike that of other micronutrients, more resembled the N accumulation pattern, where accumulation in shoots up to stem elongation was proportionally higher than the production of biomass. This was most pronounced at high N rates. However, unlike N, a high proportion of the total Fe uptake occurred during early crop growth, before the first sampling occasion in spring.

For all nutrients, the impact of N fertilization on relative nutrient accumulation rate (R_N) was most pronounced during early crop growth (period I) (Figure 7). In contrast, accumulation during later stages of crop development (periods II and III) was not significantly influenced by N fertilization for most nutrients. This was revealed in a comparison of the different N treatments within each period (one-way ANOVA; data not shown).

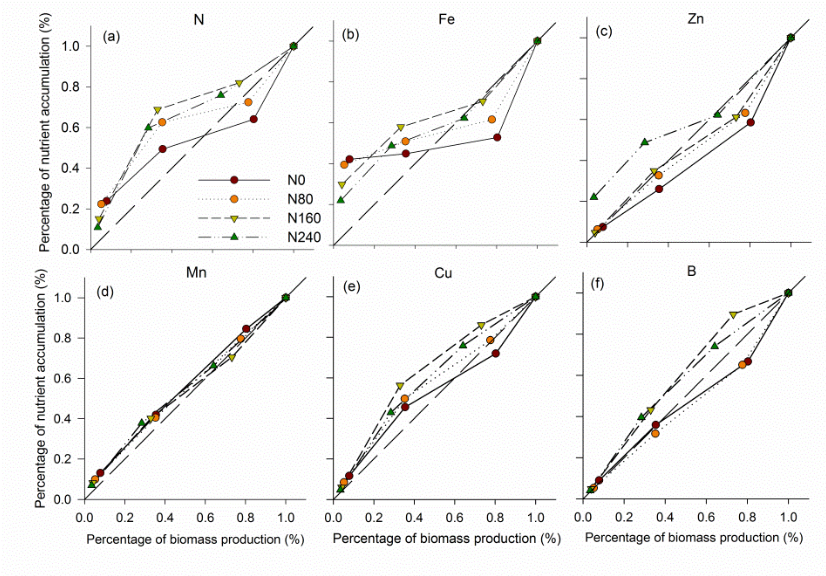

Figure 6. Temporal nutrient accumulation dynamics of N, Fe, Zn, Mn, Cu and B (a-f) versus the dynamics of biomass production in winter wheat during the cropping season. Percentage of total nutrient and biomass accumulation on different sampling occasions is shown. Dashed line indicates a 1:1 relationship. Diagrams for macronutrients are included in paper IV.

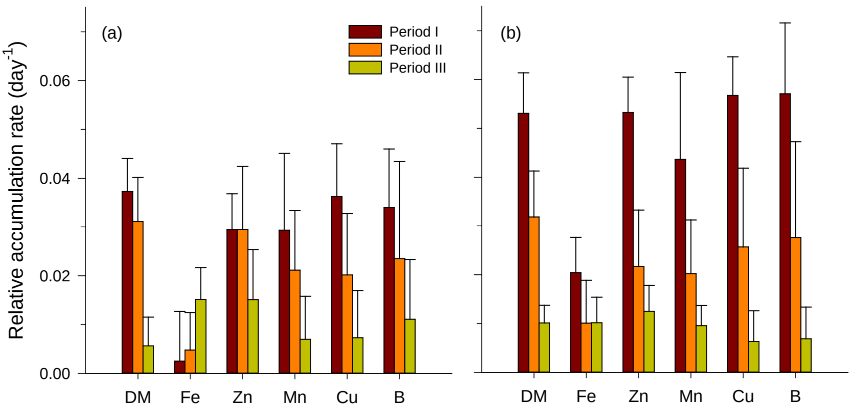

Figure 7. Relative accumulation rate of biomass (DM) and micronutrients at a) No N fertilization (N0) and (b) a high N fertilization rate (N240). Error bars indicate standard deviation. Diagrams for macronutrients are included in Paper IV.

However, the unfertilized treatment exhibited higher accumulation rates of N and Fe after flowering (period III) compared with treatments with high N application.

5.2.3 Do high yields of wheat imply impaired grain quality? (Papers II-IV)

Analysis of grain from the N fertilization trials showed a similar pattern as for shoots of wheat (Paper IV). When all sites were considered, grain Fe, Zn and Cu concentrations were positively correlated to grain N (Figure 8). However, the correlations were weak for Zn and Cu and only significant at single sites when evaluated separately. For Mn and B, there was no general correlation with N, although positive correlations to N were observed at single locations. The effect of increased N fertilization rate on nutrient concentrations in grain was smaller than in vegetative plant parts. The concentration of Fe increased by approximately 19% (from 27 to 32 mg kg^{-1}) from the lowest to the highest N fertilization rate, on average across all sites. As for shoots, the nutrient to N ratio in grain generally decreased with increasing N concentration.

When comparing treatments with high N fertilization rates with unfertilized controls of the long-term field trials, there was a negative impact of N on Ni concentrations at several sites, while there was tendency for increased Cu and Zn concentrations at some sites (Paper III). Use of N fertilization as a way of increasing the mineral concentration in grain has been proposed for Fe and Zn and has been shown to be successful in several studies (Cakmak *et al.*, 2010).

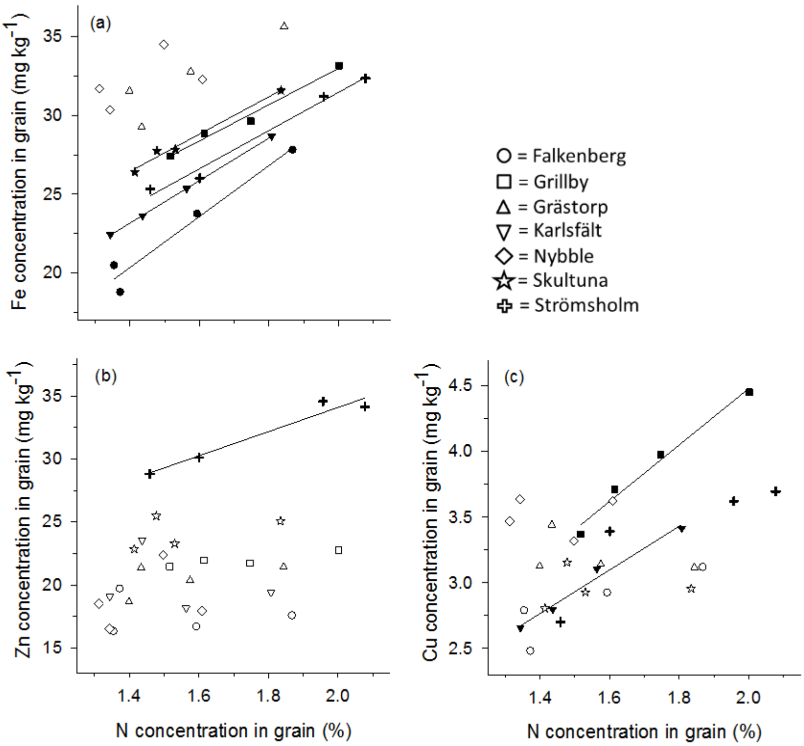

Figure 8. Correlation between micronutrients (Fe, Zn and Cu; a-c) and nitrogen concentrations in grains of winter wheat. Closed symbols indicate significant correlations ($p<0.05$) and open symbols non-significant correlations. Figures on the other included macro-and micronutrients are available in paper IV.

A synergistic effect could arise from two factors: increased uptake at high N supply and/or more efficient translocation of nutrients to grains at high N concentrations in crops. Results from the N fertilization trials (Paper IV) showed that high N fertilization rates increased the nutrient harvest index (NuHI) only for Fe. Therefore, increased Fe grain concentration was a combined effect of both increased uptake and increased translocation to grain. For the other nutrients studied, increased uptake was the major reason for increased grain concentrations.

For Zn, there was a positive correlation to N concentration in grain only at one single site. This site had the highest Zn concentrations in both the vegetative plant parts and in the grain, indicating that a high Zn concentration in shoots is needed to have a positive impact on Zn grain content. This is in agreement with other studies showing that at limited Zn supply from soil, no positive interaction with N in grain is obtained (Kutman *et al.*, 2010; Verma & Bhagat, 1990).

In summary, high N fertilization rates did not result in lower concentrations of minerals in cereal grain. Instead, there were indications of increased concentrations of Fe, Zn and Cu which is consistent with earlier studies (*e.g.* Cakmak *et al.*, 2010; McGrath, 1985). However, unwanted heavy metals such as Cd have also been shown to increase at high N fertilization rate (*e.g.* Eurola *et al.*, 2003), indicating that it is not only the concentration of essential trace elements that may be affected.

5.3 Importance of soil properties for crop micronutrient accumulation

Although there were some differences in micronutrient concentrations in crops between treatments in the long-term field trials, in general these could not be attributed to a direct effect of micronutrient input with organic fertilizers. Instead it was concluded that soil properties, such as pH and organic matter content, are often of greater importance.

5.3.1 Influence of pH on crop concentrations (Papers II, III)

Soil pH is an important factor influencing the availability of micronutrients to crops (*e.g.* Young, 2013). This was confirmed in the long-term field trials for Cd, Mn and Mo (Paper III). For Mo, there were large differences in crop concentrations between sites with differing pH. At some sites, it was obvious that an indirect effect of fertilizer treatments on soil pH determined Mo availability, and not Mo addition with manure and sludge as such. Large effects of pH on Mo crop concentrations have also been reported for forage crops grown in Sweden (Lindström, 2013).

Nickel concentrations were also found to be affected by soil pH (Paper II). In the long-term field trials there was a tendency for treatment with NH_4SO_4 to result in higher Ni concentrations than treatment with $Ca(NO_3)_2$ (representing low and high pH), although this effect was not statistically significant. Evaluation of soil and grain data from the monitoring programme showed a negative correlation between pH and crop Ni concentration (linear regression R^2=0.14; p<0.001). A one-unit increase in soil pH on average resulted in a 33% decrease in grain Ni concentration, although there was large variation in the correlation. These results are in agreement with previous findings on the importance of pH for crop Ni concentrations (Andersson & Christensen, 1988; Echevarria *et al.*, 2006; L-Baeckström *et al.*, 2006; Yli-Halla & Palko, 1987). A similar trend was found for Mn and Zn, where the correlations were particularly strong for concentrations in oats (linear regression R^2=0.54 and 0.26, respectively; unpublished data; Eriksson *et al*., 2010; 2000). For Mo, there was instead a significant positive correlation with soil pH (linear regression R^2=0.11 in oats).

5.3.2 Organic matter – source or sink for Cu and Se? (Paper III)

Addition of organic fertilizers resulted in a net input of Cu during the experimental period (15-30 years) ranging from about 50 g for the low input of cattle manure up to 35 kg Cu for the highest sewage sludge treatment. For several of these treatments, addition of Cu was substantial compared with crop demand. Despite this, there was no significant increase in crop Cu concentrations after long-term addition of manure or sewage sludge compared with mineral fertilized treatments or unfertilized controls. Due to increased soil concentrations and unchanged grain Cu concentrations, the BCF decreased after application of the organic fertilizers. Low phytoavailability of Cu after addition of organic amendments has also been found in other studies, indicating strong Cu adsorption to organic material (*e.g.* Alva *et al*., 2005; Bolan *et al*., 2003).

Regarding Se, overall grain concentrations were low (0.014-0.106 mg kg^{-1}) and were not significantly affected by long-term application of organic fertilizers, despite the fact that manures were enriched with Se through supplementation of cattle feed. Several studies have reported low plant availability of Se in organic fertilizers (Ajwa *et al*., 1998; Gaskin *et al*., 2003) and strong retention of Se by soil organic matter (Johnsson, 1991). Selenium is incorporated into organic matter in soil in a similar way as S and a positive correlation between soil organic matter and soil Se has been reported for Swedish soils (Shand *et al*., 2012). Application of animal manure has been proposed as a method to increase crop Se content in deficient soils (*e.g.* Kabata-Pendias, 2001) and positive correlations between organic matter and Se uptake in crops have been shown for mineral soils (Eich-Greatorex *et al*., 2007). However, this could not be confirmed in Paper III, where no significant correlation between soil organic matter and crop Se concentration could be found (p=0.77). Decomposition of organic matter in soil probably controls plant availability of Se and thus a possible explanation for the lack of positive response could be the insufficient amount of Se released from mineralized organic matter. In addition, the application of organic matter also involves addition of sulphate, which can compete with Se during root uptake and thereby lower the impact of the amendment on crop Se uptake.

5.4 Incidence of low micronutrient and Se concentrations in Swedish cereal crops

The results from the monitoring programme and the numerous field trials included in Papers II-V indicate that concentrations of trace elements in Swedish cereals are within a low to moderate range compared with those reported for other countries. Thus, there is a risk that critical thresholds for optimal yield, proper seed germination or sufficient food quality are not always reached.

5.4.1 Reduced crop yield due to micronutrient deficiency? (Papers III, IV)

The results from the different field trials included in this thesis revealed that crop concentrations of Cu, Zn and Mn were within the critical range for causing crop deficiency at some sites (Reuter & Robinson, 1997). Evaluation of the Swedish monitoring programme showed a similar trend (Eriksson *et al*., 2000, 2010; Hamnér *et al*., 2012). Low concentrations and deficiency of these three micronutrients have been reported in previous field trials and in practical agriculture. Since most of the experimental sites included in this thesis are quite fertile in relation to other arable soils in Sweden, this indicates that hidden deficiencies of these nutrients could be frequent, an issue which requires further attention. For Mo and Fe, crop concentrations were in general adequate for crop growth.

Insufficient supply from soil can cause increased risk of deficiency in high yielding crops

The study on nutrient accumulation during crop growth (Paper IV) revealed differences in the uptake dynamic between nutrients. Supply from the soil could be important, as nutrients with high availability in soil, such as K and Ca, displayed faster accumulation during early crop growth, whereas the accumulation of micronutrients was slower, following biomass production. This was most apparent at the high N fertilization rates where at stem elongation (ZS37) 69% of the N had accumulated (N160) compared with 35, 40, 56 and 43% for Zn, Mn, Cu and B, respectively across sites. At high N fertilization rates, the relative nutrient accumulation rate (R_N) increased during early growth, whereas in unfertilized crops significant uptake occurred after anthesis. This indicates a need for a continuous supply from soil throughout the whole growing period to ensure that crop requirements are fulfilled.

While no decline in nutrient concentrations at increasing N rates could be detected, crop response to high N supply differed between sites. At two sites, Cu concentrations in crops did not increase with N supply. These sites also had the lowest soil Cu concentrations, both when measured as pseudo-total Cu (7M

HNO_3) and when extracted with weak salt solution (0.01 M $CaCl_2$) (Paper V). When analysing Cu accumulation during crop growth, the proportion of the total amount of Cu accumulated at stem elongation was only 15-30%, compared with 45-100% for the other sites (data not shown). A similar trend was detected for B. Furthermore, in the long-term field trials (Paper III), high N fertilization resulted in a decline in Cu concentration compared with the unfertilized controls or manure-treated crops (Figure 9). These three sites (Ekebo, Orup and S Ugglarp) are known to have dense subsoils (Kirchmann *et al.*, 1996, 1999; Kirchmann & Eriksson, 1993), resulting in poor root proliferation and low or no access to nutrients in the subsoil.

It can be concluded that in fertile soils enabling rooting in subsoil, high N fertilization rates may not cause problems with micronutrients, whereas on soils with low availability or supply of micronutrients, there is a risk of deficiency and yield loss at high N input.

5.4.2 Too low Ni concentrations for optimal germination? (Paper II)

The results from the Swedish monitoring programme showed that the mean Ni concentration in Swedish cereal grain were 0.18, 0.07 and 1.20 mg Ni kg^{-1} DM for winter wheat, spring barley and oats, respectively. Almost all samples had concentrations below 5 mg Ni kg^{-1}.

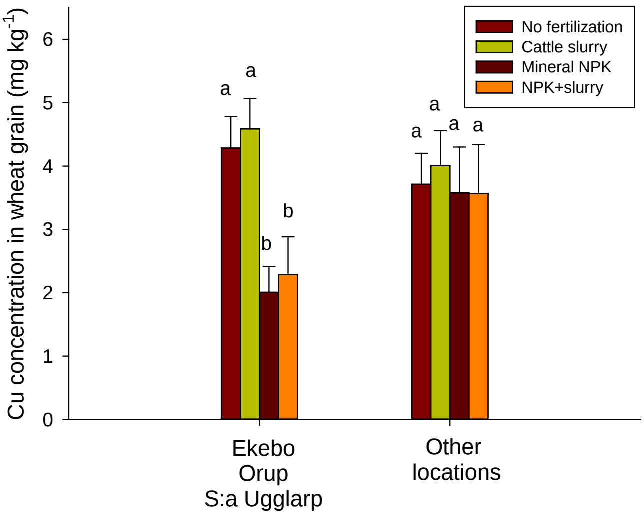

Figure 9. Copper (Cu) concentrations in wheat grain in response to different fertilization treatments at Ekebo, Orup and S. Ugglarp compared with the other six long-term soil fertility experiments. Error bars represent standard deviation.

Even though there were large differences between the different crops, concentrations were generally low, particularly in spring barley. Previously, Brown (2006) reported that Ni concentrations lower than 0.2 mg kg^{-1} have not been found in field-grown crops. However, results from a large Finnish survey reported similarly low Ni concentrations in Finnish as in Swedish cereal grain (Varo *et al.*, 1980). Furthermore, Baeckström *et al.* (2006) reported Ni concentrations as low as 0.14 mg Ni kg^{-1} in wheat grains from a Swedish field trial.

Brown *et al.* (1987) studied the impact of Ni on germination of barley grain and proposed a limit for optimal germination of 0.09 mg Ni kg^{-1} DM. Data from the monitoring programme revealed that as many as 77% of the grain samples of barley analysed were below this limit. For winter wheat, the corresponding figure was 29%, while hardly any (0.2%) of the oat samples analysed had concentrations below the proposed critical limit. Moreover, NPK fertilization resulted in Ni concentrations below the proposed critical concentration in the Petersborg trial and at six sites of the soil fertility experiments. Since only the study by Brown *et al.* (1987) has identified critical Ni concentrations for germination and only for barley, more experimental work is needed to determine whether low Ni concentrations in cereals grown in Sweden are a serious problem. However, several studies have confirmed that addition of small amounts of Ni to growth medium increase germination or seedling growth of sunflower (Ahmad *et al*., 2009), alfalfa (Peralta *et al*., 2001) and mung bean (Srivastava, 2007).

5.4.3 Too low grain Se concentrations to meet daily intake recommendations for humans? (Paper III)

Grain Se concentrations in the long-term field trials were found to be low overall (0.014-0.106 mg Se kg^{-1}) and at similar levels as for grain samples taken within the monitoring programme (0.02 mg Se kg^{-1}; unpublished data). The threshold concentration to meet the daily recommended Se intake for animals and humans is often set to 0.05-0.3 mg Se kg^{-1} fresh weight (Fordyce, 2007). The Swedish population has an average daily intake of Se of about 40-50 µg Se, which is within the critical range for fulfilling the recommended dose (NFA, 2014; WHO, 1996). Among certain groups within the population, such as vegetarians, the average intake is much lower. In Finland, which has similarly low soil Se concentrations as found in Sweden, a Se fertilization programme was implemented in the 1980s and has succeeded in increasing Se intake from 25 µg Se day^{-1} in the 1970s to 80 µg Se day^{-1} in 2013 (Alfthan *et al.*, 2015). In Sweden, Se supplements are given to livestock to avoid Se deficiency, which enriches animal products as well as manure. However, results from Paper III together with earlier studies (Ajwa *et al.*, 1998; Johnsson, 1991) show that this is not sufficient to increase Se concentrations in food and feed and that the issue needs further attention.

5.5 Indicators of micronutrient availability in soil

5.5.1 Methods of soil analysis for prediction of crop status (Papers II, V)

Pseudo-total soil concentrations (measured with *e.g.* HNO_3-extractions) usually do not show a strong correlation to micronutrient uptake by crops. This was confirmed on analysing data from the monitoring programme (Paper II), where total Ni in soil was significantly correlated with Ni concentrations in wheat grain, but with a low predictive power (linear regression R^2=0.13; p<0.001) (Figure 10a). A similar trend was obtained for spring barley (R^2=0.10; p<0.001), while the correlation for oats was somewhat stronger (R^2=0.32; p<0.001). Similar results were found for Cu, but no or very weak correlations between total soil Zn and Mn and grain concentrations (unpublished data). Since several micronutrients are affected by soil pH, a combination of total concentrations and pH can be used in assessment of micronutrient availability in soil.

For Ni, grain concentrations were negatively correlated to soil pH, and multiple regression analysis including both soil Ni concentrations and pH improved the predictive power of the relationship to Ni in grain (R^2=0.34) (Figure 10b), compared with when only soil Ni concentrations were used.

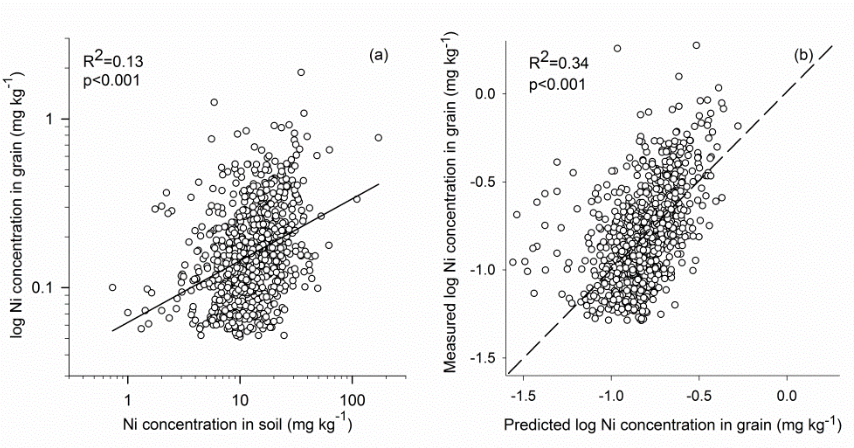

Figure 10. (a) Nickel (Ni) concentrations in grain of winter wheat versus Ni concentration in soil (HNO_3 extraction) (log-transformed data and scale) and (b) measured Ni concentrations in grain of winter wheat versus predicted grain Ni concentrations using Ni concentrations in soil and soil pH as factors in multiple regression (log-transformed data) (n=902).

For Zn and Mn, inclusion of pH also improved the correlation, whereas pH was not an important factor regarding Cu. Even though this approach only explained part of the variation in crop concentrations, the results indicate that prediction of crop micronutrient uptake can be improved by including pH data, which are often easily accessible. Similar findings have been presented earlier, for example in the extensive study by Sillanpää (1982).

In addition, the results from the field trials (Paper V) showed a significant, correlation between HNO_3-extracted Cu in soil and Cu concentration in shoots of winter wheat, also here with a relatively poor predictive power (linear regression R^2=0.28; p<0.05) (Figure 11a). Again, inclusion of pH did not improve the predictive power of the relationship.

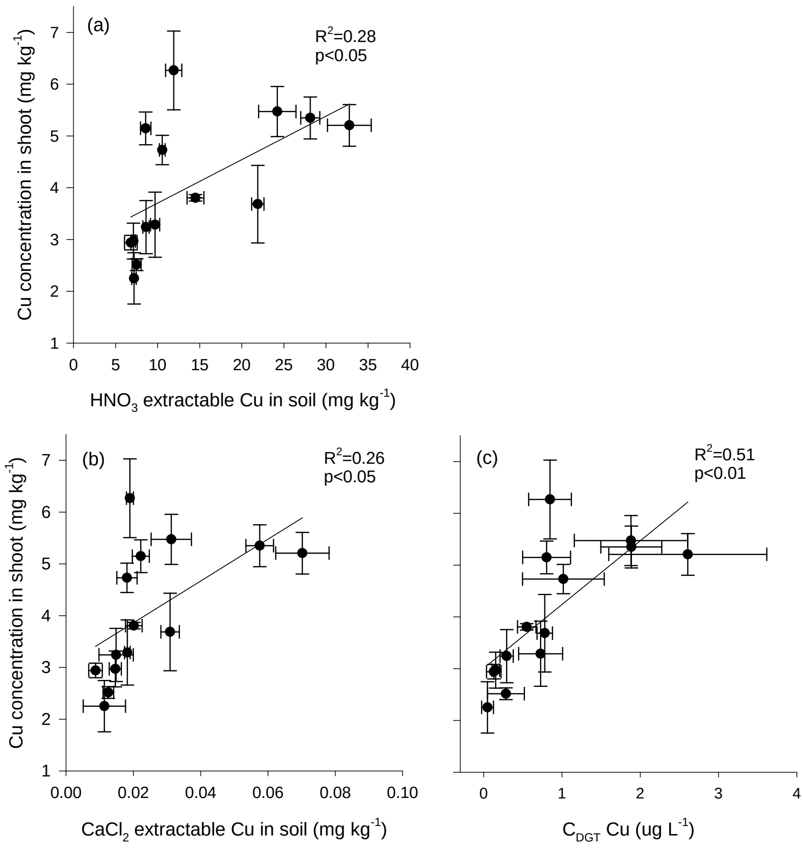

Figure 11.Copper (Cu) concentration in soil measured in (a) extraction with 7M HNO_3, (b) 0.01 M $CaCl_2$ and (c) diffusive gradient in thin film (DGT) and the correlation with Cu concentration in whole shoot of winter wheat at stem elongation (ZS37)(n=14).

Furthermore, extraction with a weak salt solution, $CaCl_2$, did not improve the predictive power of the relationship between soil and crop Cu (linear regression R^2=0.26; $p<0.05$) (Figure 11b) compared with HNO_3 extraction. Earlier studies have reported inconsistent results, with some showing soil analyses based on $CaCl_2$-extraction to be a better predictor of plant availability than total soil concentration (e.g. Menzies et al., 2007), and others showing no or a poor correlation between soil analyses based on $CaCl_2$ extraction and Cu uptake in roots and shoots of barley (e.g. Feng et al., 2005).

Use of the DGT technique improved the predictive power of the relationship between soil and crop Cu concentrations (linear regression R^2=0.51; $p<0.01$) (Figure 11c), as has also been found in earlier studies using pot trials (Tandy *et al*., 2011; Zhang *et al*., 2001). Even though the degree of unexplained variation was still quite high, the data indicate that this technique might have the potential to predict Cu availability for crops more precisely than other methods of soil analysis. Still, there is a need to further evaluate the method including more field sites and also to test it for other micronutrients.

As can be seen in Figure 11, there was a larger variation within sites for the DGT measurements, and to some extent also for the $CaCl_2$ extractions, than for the HNO_3 extractions. This might be explained by easily available Cu in soil varying within short distances, whereas total Cu in soil is more uniformly distributed. It is also worth noting that some sites deviate more than others from the regression line. The Kårby site, for example, had high plant Cu concentrations despite low concentrations measured by all methods of soil analysis.

There are several limitations with the methods of soil analysis used in this study and these constraints are often valid for most soil analyses: (1) Sampling is performed on one occasion only and does not detect changes over time; (2) normally only topsoil is analysed, even though subsoil can be important for crop uptake; (3) variations in soil concentrations within short distances in the field can be difficult to capture; and (4) differing root proliferation due to varying soil structural conditions is not considered.

5.5.2 The usefulness of grain analysis (Paper V)

Analysing grain micronutrient concentrations over several years could potentially be a useful approach to assess the micronutrient status of crops. However, this requires the Cu concentration in grain to actually represent the Cu status of the growing crop, in order to provide a reliable measure of the plant availability in soil. In Paper V, the correlation between Cu concentrations in grain and vegetative shoots of wheat was weak and dependent on the developmental stage of the wheat crop (Figure 12a-c). At stem elongation and

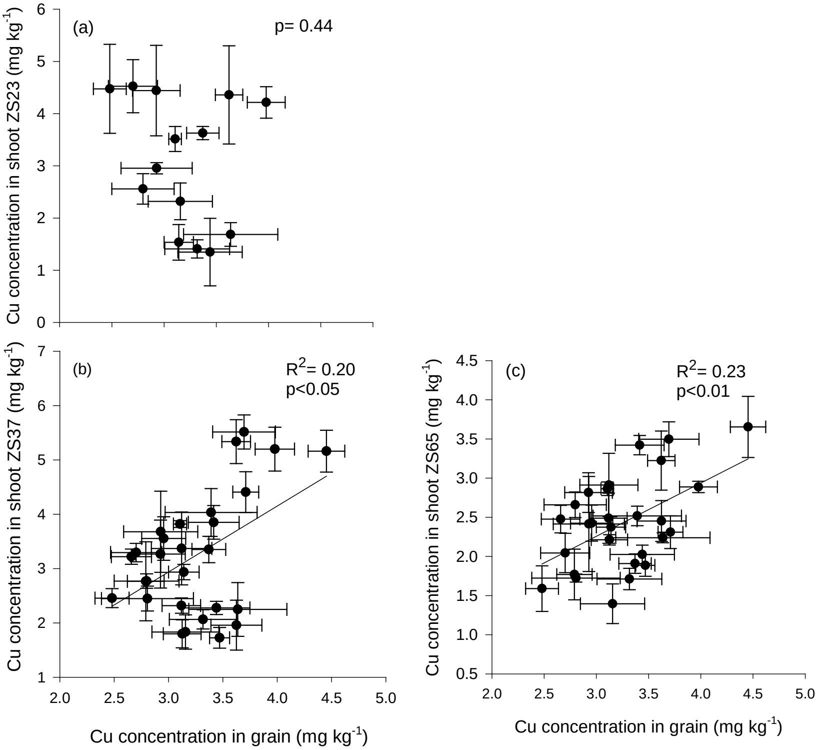

Figure 12. Copper (Cu) concentrations in grain correlated to Cu concentrations in whole shoot of winter wheat at (a) tillering stage (ZS23), (b) stem elongation (ZS37) and (c) at flowering (ZS65).

flowering, the correlation was significant but with a relatively poor predictive power (linear regression R^2=0.20 and 0.23, respectively; $p<0.05$), whereas the correlation at an early growth stage (ZS23) was non-significant. These results indicate that grain analysis can only give an approximate estimate of the Cu status of vegetative tissue during crop growth. Even though the results are not directly comparable to those for soil analysis (due to differences in the data included), they indicate that grain analysis is a method with larger unexplained variations, especially compared with DGT measurements in soil (R^2=0.51).

A limitation of analysing grain is that translocation of nutrients to grain can be influenced by a number of factors not related to soil characteristics. For example: (1) Differences in total concentrations may affect translocation, since fraction of a nutrient can be bound to structural components not accessible for translocation and at low concentrations a relatively small proportion can be translocated to the grain; (2) crop N concentrations may have a profound impact. The more N available for translocation, the more micronutrients will also be translocated, as shown for Fe within this thesis; and (3) crop demand for proper germination and viability in seedlings can be regulating translocation which can therefore be limited if sufficient micronutrient requirements for the seed are already reached.

It can be concluded that while grain analysis could be a simple way of evaluating the micronutrient status of cereals, it does not seem to have high accuracy for Cu (Figure 12). However, for Zn and Mn, regression analysis showed a much stronger predictive power (R^2=0.77 and R^2=0.85 at flowering, respectively; unpublished data) and for these nutrients grain analysis could be of great value.

6 Conclusions

This thesis contributed to new insights regarding micronutrients in soil and cereal crops and can thus enable better assessment of micronutrient deficiency. Major conclusions are:

- Depletion of micronutrients in arable soils is slow and difficult to measure due to natural variation in soil content and low removal rates with the crop in comparison with total soil content (Paper I).
- With the possible exception of zinc, repeated application of organic fertilizers does not increase crop concentrations of micronutrients under Swedish conditions (Paper II and III).
- Supplementation of selenium to livestock feed is not an efficient way to fortify cereal grain with selenium when manure is recycled (Paper III).
- Applying the Swedish regulations for use of sewage sludge on arable land, there is no short-term risk of elevated cadmium concentrations in cereal grain (Paper III).
- High nitrogen fertilization rates increase both total uptake and crop concentrations of several micronutrients in wheat (Paper IV). No dilution of mineral concentrations in grains of high yielding crops could be detected.
- In less fertile soils, high nitrogen fertilization rates may cause micronutrient deficiency in crops. Dense subsoils and low availability in topsoils may contribute to this effect (Paper IV).
- Useful methods to predict micronutrient availability in soil may include total micronutrient concentrations corrected for the impact of pH or measurements with diffusive gradients in thin films (DGT) (Paper V).

7 Implications and future perspectives

The pool of micronutrients in soils can be divided into total concentration and the fraction easily available to plants during the cropping season. While the specialisation in agricultural production systems between arable and animal farms results in changes in soil micronutrient concentrations, this thesis showed that the decrease due to inputs of only mineral fertilizers is slow, with a low risk of micronutrient depletion within a reasonable time scale.

Determining the available pool of micronutrients in soil is of the utmost importance for predicting crop micronutrient accumulation and possible deficiencies. This thesis showed that while repeated applications of organic fertilizers such as manures cause micronutrients to accumulate in soil, the plant availability of these nutrients is limited and crop accumulation therefore does not generally increase, even in the long run. Instead, other soil properties seem to be of great importance and soil pH in particular needs to be taken into account for some nutrients. Another issue concerning nutrient availability in soil is the functioning of roots. Since micronutrients move slowly in soil, vigorous root growth is important for the ability of plants to accumulate micronutrients. In this thesis, dense subsoils were identified as one possible explanation for low micronutrient concentrations in crops. Quantification of the impact of root proliferation on micronutrient uptake would be an interesting future research topic. Soils compacted by heavy machinery or natively dense soils can affect micronutrient status in crops. In addition, micronutrient accumulation from subsoils has not been thoroughly investigated, but is of interest for a fuller understanding of micronutrient accumulation in crops.

This thesis showed that crop concentrations of several micronutrients increase in intensive farming systems using high application rates of nitrogen fertilizers. This indicates that the micronutrient requirements of crops increase disproportionally to the increase in yield. Thus, micronutrient supply from soil needs to be sufficiently high to avoid yield losses. Critical thresholds for micronutrient deficiency in plant tissue might need to be redefined and adjusted to the prevailing nitrogen concentration in the crop. However, even though other studies have shown increased threshold concentrations in high-yielding crops, this issue needs to be further evaluated. If a critical nutrient to nitrogen ratio in crops can be identified, this might be a more accurate tool when interpreting plant analysis data than using micronutrient concentrations alone.

Analytical methods are useful tools for predicting micronutrient concentrations in crops. Regarding soil analysis, the results in this thesis showed that for some nutrients, analysis of total soil content can be of value if combined with data on soil pH. However, the ability to use the same method of analysis for multiple elements is important to keep costs low. The DGT method showed promising results for Cu in this thesis and in other studies, but still needs further evaluation and the inclusion of other elements. Here, prediction of cadmium availability in soil is also of interest. In addition, data from grain analysis are often easily accessible and can be useful for some nutrients.

In order to identify ‘risk fields’ that may cause micronutrient deficiency in crops, there is a need for a holistic approach using both soil and grain analysis as well as other relevant information about the particular site. Development of a ‘toolkit’ combining easily accessible data such as soil properties, yield levels and preconditions for root growth with analytical methods can be one way forward. The results in this thesis can, together with results from other studies, be used as a starting point for this approach.

8 Sammanfattning (Swedish summary)

Växtnäringsämnen som tas upp i grödan i små mängder brukar benämnas mikronäringsämnen. Sju mikronäringsämnen anses vara essentiella för alla växter, inklusive spannmålsgrödor: bor (B), koppar (Cu), järn (Fe), mangan (Mn), molybden (Mo), nickel (Ni) och zink (Zn). Låga halter av dessa ämnen i grödan kan orsaka skördeförluster och medföra försämrad kvalitet när slutprodukten används som livsmedel eller foder.

Målet med denna avhandling var att förbättra kunskapen om mikronäringsämnen i spannmålsgrödor och åkermark i Sverige och därigenom öka möjligheterna att värdera och förutse grödans status och minska risken för brister. Ett antal fältförsök i södra och mellersta Sverige samt data från miljöövervakningsprogrammet ”Mark- och grödoinventeringen” utvärderades i syfte att undersöka hur olika gödslingsstrategier och markegenskaper påverkar upptaget av mikronäringsämnen i spannmålsgrödor samt om och i vilken omfattning mikronäringsämnen utarmas i marken. Även olika analysmetoder för att förutse och bedöma grödans mikronäringsstatus utvärderades.

Även om den totala mängden av mikronäringsämnen i marken ofta är stor i förhållande till grödans behov så är endast en mycket liten del tillgängligt för växterna under en odlingssäsong. Att kunna bedöma tillgängligheten är därför mycket viktigt för att kunna förutse risken för att brist i grödan ska uppstå. Tillförsel av organiska gödselmedel, som stallgödsel eller avloppsslam, medför en ackumulering av mikronäringsämnen i marken. Trots detta visade resultat från långliggande fältförsök att upptaget i grödan generellt inte ökade genom tillförsel av dessa gödselmedel, med undantag för zink på ett antal platser. Låg växttillgänglighet för mikronäringsämnen i organiska gödselmedel är en trolig förklaring till detta. Istället visade det sig att olika markegenskaper påverkar mikronäringsämnenas tillgänglighet i stor utsträckning där markens pH-värde är en viktig faktor.

Intensiva odlingssystem där stora givor mineralkväve tillförs grödan, anses ibland orsaka en ”utspädning” av mikronärings- eller spårämnen i grödan. Detta skulle därmed kunna resultera i en ökad risk för brist i grödorna och försämrad livsmedelskvalitet. Resultaten från denna avhandling visade emellertid att koncentrationen istället ökade för flera mikronäringsämnen, både i den växande grödan och i kärnan. Inga tecken på lägre koncentration av viktiga spårelement i spannmålskärnor kunde påvisas. Däremot visade resultaten att på jordar med begränsad leveransförmåga av vissa mikronäringsämnen uteblev haltökningen i grödan vid höga skördar. Detta kunde hänvisas till jordar med lågt innehåll av mikronäring samt till jordar med kompakterad alv. Resultaten visar på att man bör vara särskilt uppmärksam på denna typ av jordar för att se till att det ökade behovet av mikronäringsämnen i högavkastande grödor kan tillgodoses.

Metoder för analys av mikronäringsinnhåll i jord eller i växten kan vara värdefulla verktyg för att kunna förutse och bedöma växtens status. Resultaten visade att analys av det totala innehållet av mikronäringsämnen i marken i kombination med markens pH-värde kan användas för vissa ämnen, t.ex. koppar och nickel, men att denna metod bara ger en grov uppskattning av grödans status. För mangan och zink visade sig kärnanalyser vara en metod för att långsiktigt bedöma grödans status, medan det för t.ex. järn, koppar och bor endast fanns en svag korrelation mellan kärnhalter och halter i den växande grödan. Den nya jordanalysmetoden DGT visade på lovande resultat för att bedöma tillgänglighet av koppar i marken, men metoden behöver utvärderas vidare.

För att kunna identifiera ”riskfält” som kan orsaka brister i spannmålsgrödor behövs en helhetssyn där analysmetoder kombineras med annan relevant information om den specifika platsen, såsom markens pH, skördenivåer och förutsättningar för rottillväxt. Resultat från denna avhandling kan, tillsammans med resultat från andra studier, utgöra en grund för detta tillvägagångssätt.

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