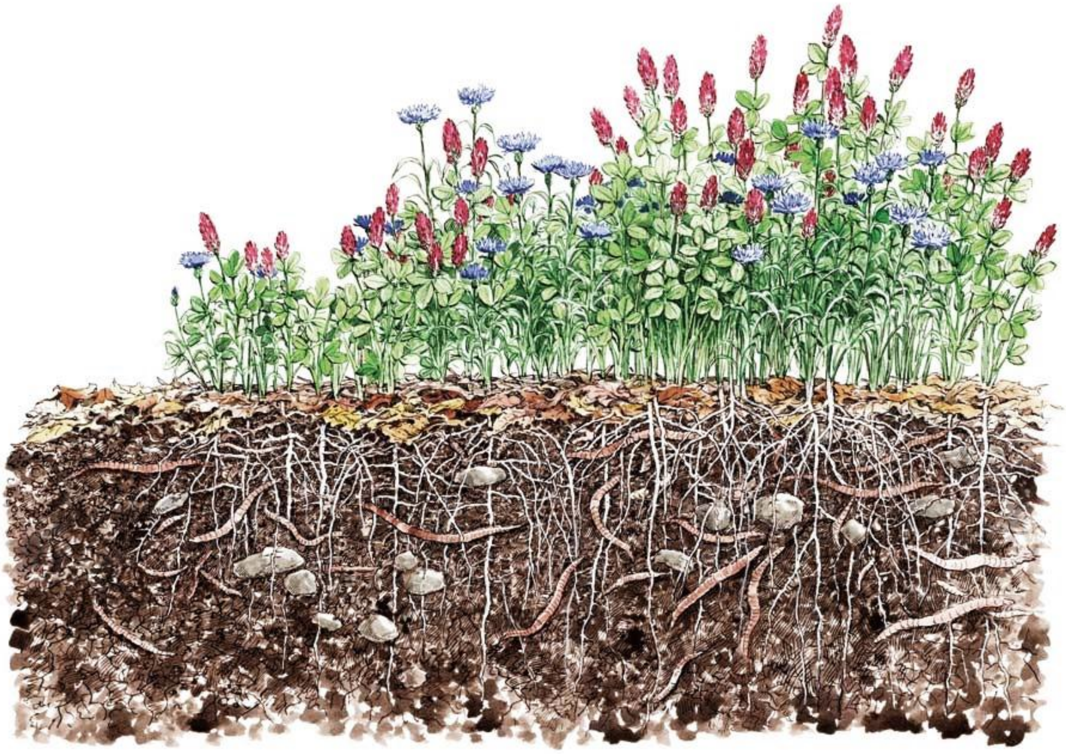
An explorative case study on soil micronutrient availability in the province Groningen, The Netherlands



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36 ECTS

MSc thesis Farming Systems Ecology chair group

August 2015 – June 2016

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Study program: MSc Organic Agriculture

Timeframe: July 20th 2015 – June 2016

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Preface

I am pleased to present you my thesis, written for the MSc program Organic Agriculture at the Wageningen University. This study is evaluating the effects of non-inversion tillage and green manures on micronutrient availability and specifically focusses on the effects of soil biological properties on micronutrient availability in the soil and micronutrient density in crops. The research is conducted in the North of the Netherlands, in the Province Groningen at the organic farm Horaholm, where farmer Erwin and Harm Westers are practicing non-inversion tillage with intensive use of green manures. The thesis was under supervision of dr. ir. Egbert Lantinga and dr. ing. Johannes Scholberg (Wageningen University, Farming System Ecology group).

The idea for the thesis topic germinated slowly and was inspired by the two following events. I attended the Wageningen Academy lecture: Healthy food; a call in the dessert (Nederlands: *"Gezonde voeding, roepen in de woestijn"*) at 26 November 2014. Wageningen Alumni from both plant science and food and nutrition science shared their thoughts on how to increase the nutrient intake of the Dutch society: eating more fruits and vegetables or simply increase the nutrient density of our food, in order to support a society in need of nourishment. Talks about breeding, bio fortification and supplementation followed. The 'soil' was missing in their stories and I wondered, is not every nutrient we eat, once born in the soil?

"As we approach the end of the twentieth century – a century of extraordinary and technological achievements, it is becoming clear that the continued survival of our civilization depends more than ever upon our relationship with the land and soil" (Wood, 1995).

Then, in the spring of 2015 I read the article of organic arable farmer Erwin Westers (Dijkhuis, 2015), reflecting upon their green manuring practices. One of the sentences I found fascinating was: *'het is nog nooit onderzocht, maar het zou mij niets verbazen als onze groenten (..) meer voedingsstoffen bevatten'; 'it has never been researched, but I would not be surprised if our vegetables would contain more nutrients".* Could it be that their produce were of a higher nutrient density than comparable produce from their conventional neighbours? And not healthier because they contain no traces of pesticides or insecticides but healthier because they contain more vitamins and minerals? Could this be due to their soil management and fertilization practices (ed. farmer Harm Westers calls it proudly a *slow food diet,* on which his crops flourish).

I felt the thesis would be a perfect moment for an exploration of the ultimate question: '*does healthier soil produce healthier food?*'. Curious but relatively ignorant I started researching this topic, as I am not schooled in the relationships between soil and human wellbeing. At first the topic seemed non-existent in the current realms of science and knowledge. Soil seems to be studied by soil scientist and human nutrition seems to be studied by nutritionists. However, soon I found confirmation of the importance and urgency of the theme by researchers as Wim van der Putten, Wietse de Boer, Gerard Oomen, Jan Diek van Mansvelt, Anton Nigten and Jaap Bloem. I am certain many more scientists and agricultural practitioners are actively involved in the topic.

During my thesis I was greatly supported by many, who I would like to recall below. First of all I would like to thank my mother, who created the foundation of my knowledge and interest in food and nutrition – "*food should not be filling but nourishing*". Then, I would like to acknowledge with great appreciation my supervisors: Egbert Lantinga and Johannes Scholberg. They have been a great support from beginning to the end of my thesis, showed interested in this relatively new research topic and due to their varied expertise always shed new and refreshing light upon my thoughts and writings. Furthermore, I would like

to acknowledge farmers Dirk Wijk, Kato Gaaikema and Hans Knook. They showed great hospitality and allowed me on their land and have been very open and informative about their farming practices and without their collaboration this research would not have been possible. Then, I would like to thank Dine Volker, Oscar de Vos and Hennie Halm, who have assisted me in the lab with soil and crop analysis and An Vos and Jaap Bloem, who performed the PLFA soil analysis for me, which is greatly appreciated.

Finally I would like to thank family Westers for their hospitality and openness and I would like to thank specifically Harm Westers – for being a great mentor and inspirer. Harm is a man with an, so it seems, endless amount of energy, time and brainpower. He farms with deep respect for all creatures in and around his land and found ways to connect his intuition with a rational mind, which resulted in a wonderful environmentally respectful, responsible and innovative agro-ecosystem.

Bravo for Harm!

With gratitude,
Fogelina
July 2016

"And those who were seen dancing were thought to be insane by those who could not hear the music"
- Friedrich Nietzsche

Executive summary

In arable cropping systems, there is a wide range of tillage and fertilization practices, such as conventional tillage, non-inversion tillage or no-till. Similarly, fertilization can consist out of inorganic fertilization on soil and/or leaf, solid or liquid animal manure, compost and/or the use of green manures. Each of these management practices has its own direct and/or indirect effect on the soils biological, chemical and physical properties. The chosen management practices create a unique soil environment, which has its effects on crop quantity and quality. This study evaluated the effects of tillage (non-inversion tillage) and fertilization practices (green manures) on soil micronutrient availability and specifically focussed on the effects of soil biological properties on micronutrient density in crops. The first relation assessed was the effect of farm management, specifically fertilization and tillage practices, on soil biota and specifically those functional groups which activities show a strong relationship with the ecosystem service nutrient cycling. The second relationship assessed was the effect of soil biota on ecosystem service (micro-) nutrient cycling, availability and uptake.

The study was set up as an explorative case-study design, wherein quantitative research was performed and soil and crop samples were collected on an organic arable farm practicing non-inversion tillage and intensively using green manures. On this farm, no other fertilization was used besides green manure crops. In order to analyse between-case evidence, soil and crop samples were collected on three conventional farms practicing conventional tillage (mouldboard plough) and using inorganic fertilization and several organic amendments, such as compost and crop residues. All four farms were situated on an Entisol with moderate to heavy loam, in the North of the Netherlands, in the province Groningen. Besides the collection of quantitative data also qualitative data on farm management and farmers perception on management practices was collected by use of semi-structured interviews with the farmers.

Soil and crop sampling was performed in August - October 2015 in oat, carrot and potato fields. Four samples were collected, three soil samples and one crop sample. It included an earthworm pit of 30x30x20 cm which was in-situ hand-sorted for earthworm counts, soil probes of 7 cm diameter in the 0-10 cm and 10-20 cm for mycorrhiza root colonization counts and bulk soil samples of the 0-10 cm and 10-20 cm soil layer for biochemical analysis. Crop yield was determined in a 50x50 cm plot. After making a random bulk crop sample, dry matter and further chemical analysis were performed on the crop tissue.

Earthworm density and biomass were assessed. Mycorrhizal root colonization was analysed in the root mass found in the soil probes. The colonization percentage was quantified under a compound microscope using an adapted version of the grid-line intersection method. Concentrations of bacterial and fungal groups were analysed by phospholipid fatty acid (PLFA) fingerprinting. Soil pH was measured in a KCL extraction. Soil organic matter was determined by the loss on ignition method (LOI). Concentrations of nitrate, ammonium, phosphorus, phosphate and potassium were determined spectrophotometrically using a segmented-flow system. Soil sulphur, calcium, magnesium, copper, zinc and iron were analysed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) in a $CaCl_2$ extractant. Plant tissue was analysed for the acid extractable fractions of nitrate, phosphorus, potassium, zinc, iron and copper by use of inductively coupled plasma atomic emission spectroscopy (IC-AES). Statistical analysis were performed using a one-way analysis of variance (ANAVO) and correlations between variables were tested using a Spearman's rank correlation analysis.

When reviewing the first relationship assessed, farm management practices > biodiversity, one sees larges differences between the two farming systems. Earthworm density and biomass was higher in the organic fields. Also the mycorrhizal root colonization was observed to be higher in the organic fields, an effect which was especially visible in the oat crop in the 0-10 cm soil layer. The microbial community abundance was enhanced in the organic oat and potato field however reduced in the organic carrot field. This result was not according to earlier hypothesis, as it was expected that the microbial community would be larger in all organic fields, due to the larger and more diverse food sources from the green manures, both from above ground biomass as from the root exudates.

The second relationship assessed was the effect of biodiversity on ecosystem service (micro-) nutrient cycling, availability and uptake. It is challenging to say concluding words about this relationship, as it was not possible to assess the effects of the two farming systems in time. However, one can see that there is an effect of the management practices, as all fields in the organic farming system had higher mean available and total levels of soil and crop nutrients. Exception to this is soil available sulphur which abundance was slightly reduced in the organic farming systems, as were the concentrations of crop nitrate.

Generally, the results showed promoting effects of the organic farming systems (with non-inversion tillage and green manures) on soil biological life in terms of earthworms, mycorrhiza and microbial biomass. This effect was observed to be largest when comparing the organic and conventional oat field. Furthermore, the organic farming system had an overall enhancing effect on soil and crop macro-, meso- and micronutrient availability, with larger nutrient availability in the organic farming system of 11 out of 13 measured soil and crop nutrients.

The research confirms the results of recent studies, which similarly compare crop mineral densities of conventional and organic farming systems. The current research shows large differences in crop mineral densities between the farming systems and the trend is that the organic crops have an overall higher mineral density per unit of dry weight. It is promising, to know that it might be possible to increase mineral densities of crops by adjusting farm management practices. It is promising, as it is exactly the trace elements which are so often lacking in diets around the world, both in Western countries as in the countries in the Global South. It is promising, as the research shows positive results in terms of soil biological life and carbon stocks. It is promising, as the farm management practices possibly responsible for this positive effects require less fuel (due to less tillage) and no inorganic fertilizers or organic amendments. This again requires less energy as no inorganic fertilizers have to be produced or mined and no fuel has to be spend on transport.

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Glossary

Bio fertilizer [6]	Or *'microbial inoculants'* is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.
Nutrients *(H)[1]	A general term for proteins, carbohydrates, fats, vitamins and minerals, necessary for growth and maintenance of life.
Nutrient content **(P)[2]	The total amount of nutrient per edible part of plant or total plant.
Nutrient concentration (P)[4]	A measurement of the nutrient content of a food relative to the unit of weight (expressed in e.g. $g\ kg^{-1}$ dry weight or %).
Nutrient density (H)[1]	A measurement of the nutrient content of a food or diet relative to the energy yield (expressed in e.g. g 1000 kcal or MJ).
Macronutrients (H)[1]	Those nutrients required in large amounts: protein, carbohydrates and fats.
Micronutrients (H)[1]	Those nutrients required in small amounts: vitamins and minerals
Minerals (H)[3]	The minerals (inorganic nutrients) that are relevant to human nutrition: water, sodium, potassium, chloride, calcium, phosphate, sulphate, magnesium, zinc , iron, copper, manganese, iodine, selenium, molybdenum, boron, cobalt, silicon, tin, vanadium.
Minerals, trace (H)[1]	Those minerals present in the body, and required in the diet, in small amounts (parts per million): copper, manganese, iodine, selenium and molybdenum; although required in larger amounts, zinc and iron are sometimes included with the trace minerals.
Minerals, ultra-trace (H)[1]	Those minerals present in the body, and required in the diet, in extremely small amounts (parts per thousand million or less); boron, cobalt, silicon, tin and vanadium.
Soil microbes [5]	Soil organisms with body width <100μm: bacteria, fungi, archaea

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[1] Definitions from: Bender, D. A. (2014) *A dictionary of Food and Nutrition (3 ed.),* Oxford University press

[2] Definition from: Rengel et al., (1999) *Agronomic approaches for improving the micronutrient density in edible portions of field crops* Field Crop Research, vol 60, issues 1-2; 27-40

[3] Definition from: Jennet, S. (2008) *Dictionary of Sport and Exercise Science and Medicine by Churchill Livingstone*, Elsevier limited

[4] Definition from: Fageria N. K. (2009) *The use of nutrients in crop plants,* CRC press, Taylor and Francis group

[5] Definition from: Wurst et al., (2012) Soil biodiversity and functions. In: Wall, D. H. (eds.) *Soil ecology and ecosystem services,* pp. 28. Oxford University press, Oxford

[6] Definition from: Vessey, J.k. (2003) *Plant growth promoting rhizobacteria as bio-fertilizers.* Plant Soil 255, 571-586

Soil microfauna [5]	Soil animals with body width <100µm: nematodes, protozoa, rotifera

* (H): terminology used in human health and nutritional sciences.

** (P): terminology used in plant sciences.

1. Introduction

The following chapter will present the theoretical context in which this research has been performed. It firstly presents the problem statement in which the necessity and urgency of this research is emphasised, followed by a historical perspectives on the research theme. The chapter will furthermore give a brief overview of the current literature on the theme and theoretical underpinnings for the initial hypothesis. The chapter concludes with an introduction on the research objectives and research questions.

* Words indicated in orange are defined in the glossary

1.1 BACKGROUND AND RATIONALE

1.1.1 Problem statement

Over the last 100 years the pressure on the soils productive capacity has rapidly increased in Europe and several other regions, due to growing population levels and advances in agricultural technology. Europe entered its second agricultural revolution in the first half of the twentieth century, characterized by the use of several new means of agricultural production, naming motorization, mechanization, chemicalization, selection and specialization (Mazoyer & Roudart, 2006). Especially the use of mechanized tillage practices and synthetic fertilizers, which rose exponentially after the second world war, have altered soils physical and chemical properties severely, hereby optimizing the medium for increased crop production levels which paved the way for the elimination of undernourishment in Europe and decreased the dependency on food imports (Zobbe, 2001). Critical questions arise though, whether the increasing yields has altered food quality, in this case perceived as a negative trend and often expressed in '*nutrient concentration*' ; the amount of nutrients present in food relative to its unit of dry weight (Thomas, 2007; Davis, 2009; Fageria, 2009). A combination of outcomes of three historical food composition studies point out evidence of a median decline of 5% to 40% and more in mineral concentrations in vegetables in the UK and USA over the past 50 to 70 years and evaluate vitamin and protein concentrations with similar trends (Davis et al., 2004; Mayer, 1997; White and Broadly, 2005). Although declines in individual crops and nutrients might be partly caused by errors in historical data and/or current optimized nutritional analysis, the consistent evidence is pointing to an overall trend that is difficult to dismiss. Breeding practices and yield increases, caused by several farm management techniques, are stated in the above mentioned papers as the two main reasons of the apparent nutrient declines (Davis, 2009). It is generally referred to as the *'dilution effect' or 'genetic dilution effect'*, the concept in which an increased dry matter accumulation is not met with equal increased proportions of nutrient accumulation (Jarrell and Berverly, 1981).

The apparent consequences of current agricultural practices on food quality, in this case on nutrient concentrations, are not beneficial for overall human health. Heated debates arise when talking about health and a healthy diet and in the previous decade many health food claims have been validated and invalidated once again. However, a diet plentiful of fresh fruit and vegetables seems to be beneficial in battling non-communicable diseases (NCD) and became a general guideline agreed on by many international and national health institutions, including the World Cancer Research Fund, American Cancer Society, American Heart Association, the WHO and Dutch Health Council (WHO, 2013; Gezondheidsraad, 2006). Also public awareness about health and nutrition is rising. Healthy, high quality food is becoming more and more of a trend, both in the Netherlands as in many other countries in the world. Public health institutes, healthcare practitioners and also governments are shifting slowly towards preventive measurements (Berg van den, 2013). Some realize that disease prevention, as opposed to disease treatment, is the way forward in improving ones wellbeing and pleasure in life ensuring the possibility of healthy aging and last but not least reducing the ever growing health costs. With prevention comes, amongst others, a healthy diet.

Yet, we seem to struggle with eating healthy food. From a broad, national food consumption survey conducted in 2007-2010 by the Dutch Ministry of Health, Welfare and Sport some shocking results became known. A very low percentage of the population (5%) met the lower limits of the daily recommended vegetable and fruit consumption whilst it is exactly these food groups that appear to have a disease protecting ability (WHO, 2013; RIVM, 2011). Consequence of this fact, is a majority of society failing to meet the recommended dietary allowance (RDA) of micronutrients (nutrients required by humans in small amounts; vitamins and minerals) leading to micronutrient deficiencies in the daily diet (RIVM, 2011). That micronutrients have a vital role in sufficient human nutrition, is not to be questioned. Take minerals for example, supporting many physical and mental processes in the body. A large number of peer reviewed research papers written between 1941 and 2003 report of significant correlations between various mental illnesses and mineral deficiencies and imbalances. Table 1.1 summarizes the found results from the data extracted from 225 published peer-reviewed papers from various well known scientific journals such as the American Journal of Psychiatry, Canadian Journal of Psychiatry, Journal of the American College of Nutrition, British Journal of Psychiatry etc. (Thomas, 2007). Interestingly, these results and the outcomes of other recent research will be applied from on next year (2016) by the Dutch Ministry of Justice in seven prisons in the Netherlands, where aggressive prisoners will be treated with food supplements. The Ministry suspects the aggression rates show correlations with the often insufficient diet of the prisoners (de Visser, 2015; Zaalberg et al, 2009).

Table 1.1 Collected data from peer reviewed research papers showing correlations between mental illnesses and mineral imbalances and deficiencies
(from: Thomas, 2007)

	Cr	Cu	Fe	I	K	Mg	Mo	P	Se	V	Zn
ADHD		X				X		X			X
Anxiety					X	X		X	X		
Aggression			X		X						X
Bipolar disorder			X	X	X	X	X			X	
Depression	X	X	X	X	X	X			X	X	X
PMS		X	X			X					X
Schizophrenia		X	X	X		X			X		

When summarizing the facts mentioned in the section above, one can identify certain trends. First of all, there are the rapidly increasing care costs in The Netherlands which have in 10 years increased with 71,4%, being 52,5 billion in 2001 up to 90 billion in 2011 (CBS, 2015). It is putting pressure on public health institutes, healthcare practitioners, governments and the society in general and the awareness of the necessity of preventive measurements is rising. Then, the recognition of the merits of a diet plentiful of fruits and vegetables as preventive measurement is growing, however it seems to be a struggle to reach even the lower limit of 400 gram of fruits and vegetables daily (RIVM, 2011). Furthermore there is the apparent nutrient decline of the last 50 to 70 years, which is further endangering general human health (Davis, 2009).

Questions arise what is the best way forward. Is it best to strive for more fruit and vegetables in our diet, complement our diet with supplementation or simply increase the nutrient concentration of our food? Is it possible to increase the nutrient concentration of our food?

These questions brings us back to the soil, where this story has started. Plants can be seen as a mirror of the soil; if the soil is in a vital and balanced condition, the plants will often be vital, healthy and in a balanced condition and a nutritious food source for people. Many have emphasized this important relationship, for example the Greek philosopher Aristotle (384 - 322 BC) as he once said: *"the soil is the stomach of plants"*, *'.. plantarum ventriculus est terra ..'* He believed the soil and its bacterial and fungal communities are the external stomach of a plant, as these organisms break down organic matter into soluble and available nutrients for plant uptake (Agren, 2012). Rudolf Steiner (1861 – 1925), considered to have been one of the key founders of organic agriculture, pointed out how it was the overall health of the soil that determines the health of the plants, animals and humans. Contemporary proponent of this interrelationship is e.g. the International Foundation for Organic Agriculture (IFOAM) which emphasizes the relation as follows: *"the health of individuals and communities cannot be separated from the health of ecosystems - healthy soils produce healthy crops that foster the health of animals and people"* (IFOAM, 2015). If one perceives the above mentioned relationship as true - a healthy soil is necessary to produce healthy crops – then several questions arise; is our soil in Western Europe healthy enough to grow nutritious crops which can support a healthy diet? And if this might not be the case, then how to *'culture'* healthy soils that can produce crops with desired nutritive value? This study aims to explore the potentials of *'culturing'* farm management practices that enhance soil life, which might potentially influence nutrient concentration of our food. Before introducing the research framework in paragraph 1.2 which will guide the study, the following sections 1.1.2 and 1.1.3 shed light on the historical and current perspectives on the theme soil – human health.

1.1.2 Linking soil and human health: a historical perspective

The awareness that human health is connected to the condition of the soil goes far back in time. The Greek physician Hippocrates (460 - 377 BC) included soil properties of local agricultural lands into a list of things that should be reviewed in medical evaluations. Others followed and men slowly started observing the relations between soils and human health (Brevik, 2015). For many centuries these ideas were based upon observations by a few instead of systematic, scientific explorations. That started to change in the 1900's when English physician and nutritionist Robert McCarrison (1878 – 1960) published the book '*Studies in Deficiency Diseases'* (McCarrison, 1921) which was considered notable at the time as he was the first scientist assessing the relationships between disease, diet and (mal)nutrition. Pioneer McCarrison thoughts were further institutionalized by a medical committee in the United Kingdom consisting of 31 doctors who published the *Medical Testament* (Kerr et al., 1939). In this document the committee concluded (amongst others) that poor health in the United Kingdom was partly due to insufficient nutrition and furthermore speculated that this malnutrition was a consequence of undesired agricultural practices, mining the soil of essential nutrients. One of their main conclusions was that reduction of human illnesses would only be possible by restoring inherent soil fertility (Brevik, 2015). McCarrison gave speeches at the earliest presentations of the *Medical Testament,* together with English botanist Sir Albert Howard (1873 – 1947) who is renowned as a principal figure in the early development stages of the organic agricultural movement. In 1940 the latter published the *Agricultural Testament* and in 1947 the book *The soil and health: a study of organic agriculture.* Both works are known for their influence on the organic agricultural movement and contain several chapters on soil fertility and its impact on human health. His quote: *"the health of soil, plant, animal and man is one and indivisible"* has become guiding in one of the four principles (principle of health) of the International Foundation for Organic Agriculture (IFOAM, 2015). Lady Eve Balfour (1898 – 1990) continued this line of thought in her book *The Living Soil* (1943), wherein she discusses the importance of the condition of the soil to the nutrient content of food crops and thus human health. She founded her thoughts on initial findings of the Haughley Experiment in Suffolk (UK), the first long-term scientific comparison trial of an organic and non-organic farming system (IFOAM, 2015).

Also in the United States awareness on these topics started to rise and in 1940 the US Department of Agriculture (USDA) created the Plant, Soil and Nutrition Research Unit (PSNRU) on the Cornell University with the mission of conducting research on the linkages between human nutrition and agriculture and currently soils and human health is still a major research area at the University. Jeremy Irving Rodale (1898 – 1971) and William Albrecht (1888 – 1974) followed the line of thinking of Howard and Balfour and contributed greatly to further knowledge generation and promotion of the theme in the United States, by several books and studies (e.g. Albrecht, 1945, 1951; Rodale, 1945). In the second half of the 20th century studies on soil-human health relationships continued. The prior investigations on the positive effects of soil fertility and its nutrient content on human wellbeing were widely expanded with studies on the hazardous effects of soils on human health, e.g. the effect of radioactive elements and heavy metals present in the soil and the effect of soil pathogens causing illnesses (Brevik, 2015; Pepper, 2013). Until the mid-1900's the topic was mainly investigated by nutrition and agricultural specialists, which then stretched out to soil science, chemistry, geography, geology and biology.

1.1.3 Current research and knowledge gaps

All above mentioned efforts, from early day observations to modern day research led to the current recognition that soils influence human health by (1) food availability, (2) food quality (human nutrient supply), (3) human contact with heavy metals, (4) human contact with organic chemicals, (5) human contact with soil pathogens, (6) medicines derived from soil and soil organisms, (7) airborne dust and (8) water quality (Brevik, 2014; 2015). This research aims to contribute to knowledge generation on the relationship between soils and food quality and specifically the influence of farm management practices on soil conditions and nutrient concentrations of food – referring to number two in the above mentioned list. Hereafter, recent research efforts and current perceived knowledge gaps on this specific topic will be briefly highlighted.

The nutrient composition of food is determined by the quantity, range and quality of the organic macronutrients (protein, fat, carbohydrates), organic micronutrients (vitamins) and inorganic micronutrients (minerals), wherein this research the emphasis lies on the inorganic micronutrients in the edible parts of crops. Several factors can influence the nutritive value in terms of micronutrient concentration (amount of micronutrients per unit of dry weight) of plant-based foods: (1) genetics (plant crop and cultivar); (2) environment (soil type, soil structure, fertilizer type and application method, climate, soil microbial populations and management practices); (3) post-harvest practices (harvest time, storage, processing methods and conditions) (Bourn & Prescott, 2002). The environmental factors are the focus of this research and in particular the growing conditions influenced by farm management practices.

The influence of growing conditions on food quality is a heavily debated item, a debate which is often concentrated on the differences in food quality of organic and conventional produce. Over the last 20 years, a large body of scientific work has covered this question; whether or not organic production methods lead to significant differences in desired concentrations of beneficial minerals and secondary metabolites (e.g. antioxidants and vitamins) and undesired presence of excessive levels of nitrate, toxic metals and agricultural chemical residues (Termine, et al, 1987; Warman 1997; Herencia, 2005; Bender et al., 2009; Hunter et al., 2011). There is a large variation in the types of studies and study design (Bourn & Prescott, 2010) and the scientific opinion remains divided so far on whether there is a significant difference. Results remain controversial, however most recent meta-analyses come to the overarching conclusion that organic food usually scores higher, which is the case in the presence of undesired substances and is mostly the case in the presence of desired substances (FIBL, 2015). Two out of the in total five meta-analyses performed on this topic in the last five years (since 2011), assessed and compared the mineral content of produce from the two farming systems and are thus of specific interest in this study (Hunter et al., 2011; Baranski et al., 2014).

The study of Hunter et al. (2011) evaluated 769 screened comparisons of minerals (B, Cu, Ca, Fe, Mg, Mo, K, P, Se, Na, Zn) in vegetables, fruits, cereals and legumes from 23 studies for the period 1980-2007. The levels of mineral micronutrients were found to be significantly higher in organic produce and expressed as a mean percentage difference of + 5.5% ($P < 0.001$, range − 1% to + 48% with n=769; Figure 1.1).

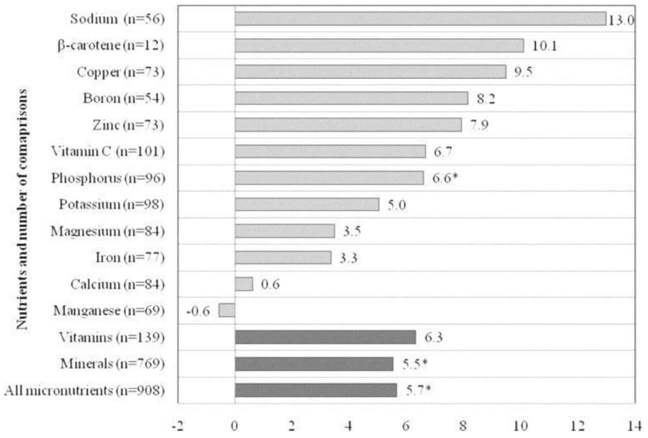


Figure 1.1 Mean percentage difference of micronutrients between organic and conventional produce.
*P < 0.001 *(from: Hunter et al. 2011)*

The second meta-analyses which assessed the mineral composition of organic and conventional crops, is the Baranski study (2014). Whereas the Hunter study solely concentrated on vitamins and minerals, the Baranski study focused on antioxidants, carbohydrates, protein, amino acids, toxic metals, nitrate, nitrite, pesticides and minerals. In terms of minerals, the study found significantly higher levels of Mo and Rb (65%; 82% respectively) and slightly higher concentrations of Mg and Zn (4%; 5%) in organic crops (Baranski et al. 2014).

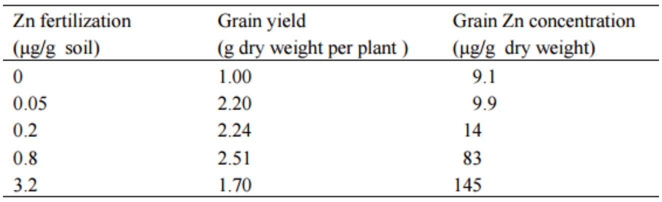
Besides alternative farming systems that significantly alter growing conditions and consequently crop micronutrient concentrations, there are targeted agronomic approaches that optimize growing conditions to meet crop micronutrient supply, which are outlined in Table 1.2. Furthermore, the addition of macronutrients N, P and K can be seen as a vehicle which supplies indirectly micronutrients to crops, as the macro elements promote root and shoot development, which generally increase the uptake of all nutrients. Increased macronutrient supply shows, in general, positive correlations with micronutrient supply, uptake and micronutrient concentration (Zebarth et al. 1992; Verma et al. 1987).

Table 1.2 Current used agricultural techniques and underlying processes that support crop micronutrient supply.

Technique	Process involved	Citation
Micronutrient soil fertilizer application	Increased micronutrient soil concentration	Rengel et al. 1999
Micronutrient foliar fertilizer application	Increased micronutrient uptake	Kannan, 1990
Biofertilizers **(I) – bacteria**	Increased availability (by e.g. solubilisation, mobilization)	Crowley et al. 1994
Biofertilizers **(II) - mycorrhiza**	Increased uptake (by e.g. mobilization, enhanced vigour of plants and extended root system)	Kothari et al. 1991
Soil amendments **(I) - organic matter, composts**	Increased availability (e.g. Fe solubilisation due to fulvic acid formation by OM decomposition)	Lindsay, 1991
Soil amendments **(II) - sewage sludge**	Increased soil micronutrient concentration	Sommers, 1977
Soil amendments **(III) - Gypsum**	Reducing soil pH; increasing micronutrient bioavailability	Singh et al. 1987

There is a relatively large body of research and practical agricultural experience with the above mentioned agricultural practices, especially on the applications of micronutrient soil and foliar fertilizers. Their main aim is sound crop establishment and eventually yield increase, by eliminating crop micronutrient deficiencies. The other techniques in Table 1.2 are mostly used to maintain general soil fertility and increase N and P supply. Their effects on - and utility for supplying micronutrients to increase crop micronutrient concentration is relatively unknown (Rengel et al. 1999). Even though the techniques in Table 1.2 are not deliberately designed to increase micronutrient concentrations for the purpose of human consumption and health, fertilization practices can lead to such effects; an increase in grain nutrient densities as shown in Table 1.3.

Table 1.3 Concentration and content of Zn in wheat (*Triticum aestivum*) grain as influenced by Zn fertilization.
(from: Rengel et al., 1999)



Zn fertilization (μg/g soil)	Grain yield (g dry weight per plant)	Grain Zn concentration (μg/g dry weight)
0	1.00	9.1
0.05	2.20	9.9
0.2	2.24	14
0.8	2.51	83
3.2	1.70	145

An example of an agronomic approach that is designed to intentionally raise crop micronutrient concentrations in human food, is the nationwide addition of sodium selenite to general fertilizers in Finland, which started in 1984 (Alftan et al. 2013). In the 1960's several diseases of both animals and humans were linked to severe selenium (Se) deficiencies, which was soon linked to the exceptionally low dietary Se intake of the Finnish population. In 1969 selenium supplementation, in the form of inorganic Se (selenite) to animals became standard, eliminating most diseases related to Se deficiencies. Studies in the 1970's associated the prevalence of cardiovascular diseases and cancer to the low daily Se intake and since 1984 the addition of 16 mg Se/kg fertilizer (in the form of sodium selenite) became common agronomic practice (Koivistoinen, 1986). Since 1998, 10 milligram of selenium is added per kilogram of fertilizer which has increased to 15 mg Se/kg fertilizer in 2007. The average daily Se intake in 1970 of 0.025mg/day/10 MJ has tripled to a 0.08 mg/day/ 10 MJ in 2013, hereby meeting the EU and US dietary recommendations (Alftan et al. 2015). The practice of deliberately adding micronutrients to the soil with the objective of improving human health, has been unique in the world so far.

In this example and the above mentioned list in Table 1.2 of existing optimization practices to meet crop micronutrient requirements the idea of 'supplying' is central. This can either be the addition of micronutrients in soluble, inorganic form or the addition of amendments containing micronutrients in organic form. Although the main aim is yield increase and crop health and thus not intentionally human nutrition (with the example of Finland as exception), crops do show increases in micronutrient concentrations when fertilized with synthetic micronutrient fertilizers (Rengel et al. 1999). However, this increase is only seen in some crop species and is heavily dependent on the micronutrient and soil properties, such as organic matter content, CEC and pH and cannot be generalized as a linear relationship. Furthermore, micronutrient fertilization is not perceived as sustainable by some, as it adds one sided, synthetic nutrients, meaning the nutrients have been 'mined' from a source not being the farm thus entering the farming system as external input, which is not desired in some farming communities (e.g. organic agriculture, biodynamic agriculture). Also, the addition of plant available micronutrients might not be truly necessary, as there are enormous stocks of mineral micronutrients present in the soil as total element content. However, for crop supply it is not this number which directly counts as only the bioavailable content is of concern as this is available for plant uptake. For example, for Zn and Cu the bioavailable amount is just 0.1 to 1 % of the total amount present in the soil (Bussink, 2012).

An alternative to 'traditional' ways of adding crop micronutrients (inorganic or organic) might be the use of soil microbes (e.g. bacteria and fungi) by adding them deliberately as bio fertilizers or by supporting their abundance and functioning through adjusted farming practices. Soil microbes are one of the factors influencing micronutrient cycling, next to pH, CEC, organic matter and clay content, temperature and the interaction with other macro and micronutrients (Wurst et al. 2012). Some soil microbes and their association with crops are already being used by application through seed or soil (e.g. N_2 fixing bacteria such as *Azotobacter, Rhizobium* and *Anabaena azollae* and P solubilizing and mobilizing bacteria and fungi such as species of *Pseudomonas* and *Aspergillus*) (Mohammadi and Sohrabi, 2012). They are currently used to increase crop health and consequently yield however, not yet explored to boost crop micronutrient densities for human nutrition. There is a considerable body of evidence linking soil microbes to crop nutrient uptake. However, very few studies focussed on the potentials of using soil microbes to enhance human nutrition. Furthermore, even fewer studies focussed on the edible portions of the crops. Another 'knowledge gap' is how to culture future healthy soils; what might be the best management practices that support soil biological functioning which might potentially optimize food quality? (Antunes et al. 2012)

This brings us to the scope of this study; which aims to explore the potentials of farm management practices that enhance soil life and via this pathway possibly influence the mineral micronutrient concentrations of crops. Some theoretical underpinnings followed by the research questions and hypotheses will be highlighted in following paragraph 1.2 - Research framework.

1.2 RESEARCH FRAMEWORK

The scope of this study is to explore the potentials of farm management practices to influence crop micronutrient concentrations, with specific attention to the role of soil biological properties in soil micronutrient availability and mobility. First relationship assessed (A in Figure 1.2) is the effect of farm management, specifically fertilization and tillage practices, on soil biota and specifically those functional groups which activities show a strong relationship with the ecosystem service nutrient cycling. This includes the microfoodweb (microflora and microfauna), the litter transformers (meso- and macrofauna) and the root/rhizosphere biota (N-fixers, free living N-fixers, mycorrhiza) (Kibblewhite et al. 2008). Second relationship assessed is (E) the effect of soil biota on ecosystem service (micro-) nutrient cycling, availability and uptake (Figure 1.2).

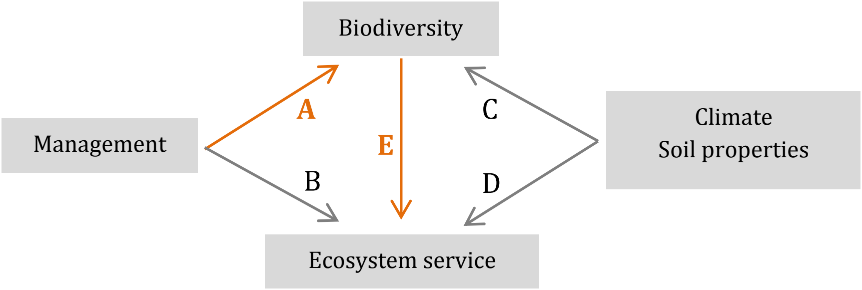


Figure 1.2 Impacts of farm management on biodiversity (A) and provision of ecosystem services (B) are constrained by climate and soil properties (C, D). All impacts, together with the impact of biodiversity on ecosystem services (E) varies considerably among ecosystem type and ecosystem service.
(Adopted from: Cavigelli et al., 2012)

The effect of farm management on soil biota is in this research assessed based on the sets of standard management practices known in West-European arable organic and conventional farming systems. The crops in the organic farming system are cultivated according the EU regulations on organic production (Council Regulation No. 843/2007) (EU, 2007). Additionally, the organic system in this research is characterized by its use of non-inversion tillage (NIT) and the high level input of organic amendments (solely green manure). The conventional system is characterised by the use of conventional tillage (CT) up to 30 cm deep and the use of mineral fertilizers and some additional organic amendments. Further details about the treatments are to be found in chapter 2: *Materials and methods*.

The effects of organic and conventional farm management on soil biota **(relation A)** is specified by selecting two soil biological groups for further evaluation naming the decomposers (fungi and bacteria with special attention to mycorrhiza) and the litter transformers, in this research including solely the earthworms.

Earthworms have numerous beneficial effects on overall soil fertility and have a major impact on the nutrient cycle in general. Secondly, soil microbes are expected to have an influence on the micronutrient cycle through their influence on organic matter turnover and mineral immobilization and dissolution. Microbes near the root hairs (rhizobial microbes) solubilize micro and macronutrients from the mineral and organic micronutrient pools. The amount, the type and the total soil organic matter content are defined as critical soil factors determining microbial diversity and abundance. A farming system which is 'feeding' the soil with raw organic matter is expected to have a different and potentially enhanced microbial community in comparison with a farming system using inorganic nutrients, and this is expected to affect different farming system outputs: in terms of food quality (crop micronutrient densities). A study by Nelson et al. (2011) confirms that hypothesis. The field study compared the effect of an organic and conventional management system on grain micronutrient concentrations and soil microbial profiles (by PLFA analysis). Similar wheat cultivars were grown under organic and conventional management practices and the organic system had higher grain Zn, Fe, Mg and K levels but lower Se and Cu levels. In both systems there was a positive relationship between grain Fe and Zn, leading to the assumption that the nutrients share similar uptake mechanisms. The organic system had elevated levels of fungi wherein the PLFA biomarkers for fungi were correlated with grain Cu. Lastly, the effect of AM fungi on the micronutrient cycle is explored. It is expected that there will be a higher root colonization of mycorrhizal fungi in organic systems, due to the non-use of inorganic P (Mäder, 2002). AM fungi is known for increasing the below ground surface area of plants, wherein the plant can acquire more nutrients, wherein AM fungi increase the absorption of immobile nutrients as Zn and Cu (Lambert et al. 1979). Furthermore, AM fungi can alter rhizosphere chemistry by root exudates which change local soil pH, and by this making ions more mobile.

The second relationship **(E)** is specified by selecting the availability and uptake of iron (Fe), copper (Cu) and zinc (Zn) for further evaluation. The three minerals all show relative similar behaviours in the soil, which inhibits further generalization of the processes of micronutrient mobility, availability and uptake. There is acknowledged significance of the three nutrients in terms of sustaining human health, whereby zinc plays a crucial role in stimulating the activity of more than 300 enzyme reactions in the body and developing and sustaining a well-functioning immune system, copper is essential for blood vessel formation and brain development and iron is an integral part of many enzymes that maintain the regulation of cell growth. Furthermore, the currently observed shift from diets based on animal food sources towards plant based diets in certain segments of West European societies lead to an exploration for plant based sources of micronutrients. Animal based foods are excellent sources of zinc and iron whereby plant based food sources show lower availability of these nutrients. It is urgent to understand the possibilities of optimizing plant based food sources, in terms of micronutrient content. The relations (**A&E**) will be elaborated in the following section.

1.2.1 Theoretical underpinnings

1.2.1.1 Relation A: tillage and fertilization and its effect on earthworms and microbial communities

Management strongly influences and defines the abundance, functioning and diversity of soil biota. In this research, the specific effects of fertilization and tillage practices are evaluated (Cavigelli et al. 2012). Fertilization with organic amendments, compared to mineral fertilizer, is expected to give an increase in earthworm density and biomass. This effect is shown in Mäder et al. (2002) where earthworm biomass and abundance was higher by a factor 1.3 to 3.2 in organic plots amended with composted farm yard manure and slurry compared with conventional plots amended with NPK fertilizers and slurry (FIBL, 2000). The effect might be due to enriched food sources and a more favourable habitat for the earthworm, due to decreased soil temperatures and increased moisture in the top soil thanks to the semi-permanent layer of organic amendments. The total soil microbial community is expected to increase in biomass, diversity and activity in farming systems with high levels of organic matter application (Mäder et al. 2002) as soil microbes need regular application of SOM to survive in the soil (Hoorman and Islam, 2010). Additionally, the non-use of inorganic P fertilizers in organic farming systems is likely to positively affect root AM colonization (Mäder et al. 2002; Clapperton et al. 1997).

Tillage disturbs, inverts and mixes soil layers with each other and in doing so it has both a direct effect on soil biotic communities by killing and/or redistributing them in the soil profile as an indirect effect by changing their habitat (Miller & Jastrow, 1990). The effects of tillage are proportional to the body size of the soil organisms (Wardle, 1995) and significant differences are expected in macrofauna (body width > 2mm) when comparing conventional tillage (CT) and reduced tillage (RT) plots, an effect shown in Kuntz et al. (2013) where in RT soils significant higher earthworm densities and biomass were found compared to counts in CT soils. Besides effects on macrofauna, tillage practices have an impact on the abundance and community structure of microbes (<100 µm e.g. bacteria and fungi), microfauna (<100 µm e.g. protozoa and nematodes) and mesofauna (100 µm – 2 mm e.g. collembola and mites). Tillage practices favour organisms with high metabolic rates (Andrén & Lagerlof, 1983) and hereby select a specific soil biota community structure which thrives in these circumstances. Additionally, tillage and residue management alter the chemical soil properties e.g. SOC stocks and hereby indirectly influencing soil microorganisms food provision and habitat.

1.2.1.2 Relation E: earthworms and microbial communities and their effects on the micronutrient cycle

Second relationship assessed (E in Figure 1.2) is the effect of soil biota on ecosystem service (micro-) nutrient cycling (Zn, Fe, Cu). The following section will briefly assess their behaviour in the soil matrix by looking at the nutrient generic properties, nutrient pools, processes and influential factors engaged in the micronutrient cycle. Zinc, iron and copper are (transition) metals, defined as being an element with a silvery luster and a good conductor of heat and electricity (McLean and Bledsoe, 1992). All three are cationic and fall in the first transition series of Period 4 in the periodic table (Sharma, 2006). Transition metals differ from main group metals (e.g. N, P, K, S, Mg and Ca) where transition metals are more electronegative, forming complexes with excess number of negative ions. Main group metals form salts in which they have enough negative ions to balance the charge on the positive ions. Salts of main group metal ions dissolve relative easily in water to form aqueous solutions, whereas salts of transition metals form further complexes, when in contact with neutral molecules such as water or ammonia.

The total element content and composition of transition metals in the soil is largely related to the geology of the parent material from which the soil has been formed. Parent material weathering adds micronutrients to ecosystems over a long period of time and is in this process influenced by several environmental conditions. Metals appear in one of the four following forms (pools) in the soil: present in the structure of primary and secondary minerals, associated with insoluble soil organic matter, adsorbed (bound to inorganic soil constituents) or dissolved in the soil solution. The two largest pools of metals in soil are the metals bound in minerals and in soil organic matter (SOM) and thus unavailable for uptake by plants. The third largest pool are the metals sorbed to soil surfaces (oxides and clay). A fraction of the total metal content is dissolved in the soil solution and available for root uptake which forms the fourth pool (Jones and Jacobsen, 2009). Generally, the metals must be in free ion form for successful root uptake (Fe^{2+}, Fe^{3+}, Zn^{2+}, Cu^{2+}). However, all three metals can form complexes with soluble organic compounds called 'chelates'. Chelation increased the solubility of the metals and prevents the formation of insoluble complexes (pool 1, 2 and 3). Although chelated metals are not immediately available for root uptake, they are relatively mobile and can convert easily to plant available form. The metal concentration in the soil solution (and chelates) is governed by three processes including precipitation/dissolution, adsorption/desorption reactions and mineralization/immobilization.

Precipitation occurs when metals in dissolved forms separate from the soil solution to form a solid, inorganic mineral. Dissolution is the opposite of precipitation and occurs when inorganic minerals release water soluble metal forms from solid reserves into solution where they become available for plant uptake. Adsorption (or sorption) is defined as the accumulation of metal ions at the interface between a solid and water phase, associated with the surface of soil particles. Metals can be adsorbed on organic matter, clay minerals, oxides and silicates (McLean and Bledsoe, 1992). Mineralization of soil organic matters releases minerals into the soil solution and also micronutrients.

The processes governing the micronutrient cycle are being influenced by a wide range of chemical, physical and biological factors, concerning both plant and soil properties. The specific interest in this study lies in the biotic factors influencing the above mentioned processes and specifically wants to explore the potential role of soil biota in improving micronutrient mobility, availability and uptake. The functional groups known to influence (general) nutrient cycling processes in the soil are the microfoodweb (microflora and microfauna), the litter transformers (meso- and macrofauna) and the root/rhizosphere biota (N-fixers, free living N-fixers, mycorrhiza)(Brussaard, 2012). Currently used soil microorganisms as biofertilizers include Rhizobium, phosphate solubilizing bacteria, mycorrhizal fungi, non-mycorrhizal fungal endophytes and cyanobacteria (e.g. *Anabaena azolla*)(Renger et al. 1999). These are often deliberately applied and not much is known about the influences of resident microbes on crop production and specifically crop micronutrient densities. In this research the focus lies on the resident soil biota.

1.2.2 Research objective

The overall objective of this thesis is to explore the outcomes of alternative farm management practices (which consider soil biodiversity in management decisions) on the functioning of soil biota as governors of ecosystems functioning: (micro)nutrient cycling. The premise of the research is that alternative farm management practices (non-inversion tillage and application of organic amendments) have impacts on the abundance and community composition of soil biota. It is expected that these impacts contribute to the functioning of several ecosystem services, wherein this research expected outcomes of one ecosystem service in particular will be investigated, naming (micro)nutrient status of soil and crop. The study aims to explore the relationships 'management – soil biodiversity – ecosystem service', ultimately exploring ecosystem service outputs in terms of food nutritional value; in this case the micronutrient output (Fe, Cu, Zn) of alternative farming systems compared to conventional farming systems.

1.2.3 Research questions

How do farm management practices (tillage and fertilization) influence soil biological properties and what is the effect of this on crop quality in terms of nutritional value (micronutrient concentration)?

a. What is the effect of tillage and fertilization practices on ecosystem structure in terms of:
a1) earthworm community abundance, activity and diversity?
a2) microbial community abundance, activity and diversity?
a3) arbuscular mycorrhizal (AM) root colonization?

b. How does ecosystem structure (abundance, diversity and composition of the three soil biological parameters mentioned above) influence ecosystem functioning in terms of micronutrient cycling (mobility, availability and uptake) and ecosystem services (crop quality)?

1.2.4 Hypotheses

1) Earthworm density and biomass will be higher in farming systems using reduced tillage and high levels of organic matter application, compared to farming systems using conventional tillage and inorganic fertilizers (Kuntz et al., 2013).

2) Microbial community abundance, activity and diversity will be increased in farming systems using reduced tillage and high levels of organic matter application, compared to farming systems using conventional tillage and inorganic fertilizers (Mader et al. 2002).

3) Arbuscular mycorrhizal fungi (AM) colonization will be greater in farming systems using reduced tillage and high levels of organic matter application, compared to farming systems using conventional tillage and inorganic fertilizers due to non-use of P fertilizers (Clapperton et al. 1997, Mader et al. 2002).

4) Farm management practices that increase **earthworm** density and biomass will lead to increased OM breakdown and mineralization rates of macronutrients and potentially micronutrients, and might lead to higher bioavailable amounts of crop micronutrients Zn, Cu, Fe.

5a) Farm management practices that stimulate **microbial growth** (e.g. high addition levels of raw organic matter) will lead to soils with increased biological activity (soil microbes) leading to higher mineralization rates of OM, which leads to increased release of micronutrients and higher bioavailable amounts of crop micronutrients Zn, Cu, Fe.

5b) Farm management practices that stimulate **microbial growth** will lead to higher microbial activity in the rhizosphere, promoting micronutrient availability by solubilisation of micronutrients Zn, Cu, Fe (Altomare et al., 1999).

6) Farm management practices that support **arbuscular mycorrhizal fungi** (AM) root colonization will lead to increased percentages of AM root colonization, leading to an increased access and absorption of less mobile micronutrients (Lambert et al., 1979).

1.3 LIMITATIONS OF THE RESEARCH

Several limitations were faced when conducting this research. The first limitation was the selection of one organic and three conventional farms. Unfortunately it was not possible to select a conventional farm with a same crop selection as the organic farm which led to the selection of three conventional farms, resulting in more variance in the data set, as inherent soil properties differed between farms. Second limitation was the small financial budget of the research, which led to a one-time measuring of soil and crop parameters which means the development of several soil and crop parameters over time could not be assessed. It furthermore led to the selection of 'only' three micronutrients, whilst other micronutrients might strongly interact and are also of importance to assess, to get a complete picture. The third limitation was that the main soil and crop sampling was performed in mid-summer. Due to the hot and dry weather this period is not ideal to assess microbial soil communities and might have underestimated the soil microbial community.

1.4 AUDIENCE AND USE OF FINDINGS

The intended audience for this report are scientists and current and future students. The research provides current data on the effects of an innovative arable farming system on soil biological quality and its effects on food quality. The research has the potential to assist current and future students interested in this topic with their (field)work and studies. Second intended audience are soil associations and other advocates for responsible soil management. The research and its data could potentially support their campaigning activities and information flows to the broader public. Thirdly, the research hopes to inform current and future policy makers about sustainable soil management. In this report the effects of alternative ways of soil management are assessed, which can inform policy makers about the effects of farm practices on certain soil parameters important for society, such as the organic matter status or biodiversity in the soil. Fourthly, this report hopes to inform farmers about the effects of a set of farm management practices on soil and food quality.

1.5 REPORT STRUCTURE

The report is organized as follows. Chapter two: *Materials and methods* provides information on how the case study was selected, research site conditions and gives an overview of farm management practices differentiation between the 4 farms. It furthermore provides information on sampling procedures and analytical techniques used during the research. In chapter three: *Results* the findings are presented whilst being divided in results on soil properties and results on crop properties. In chapter four: *Discussion* the results are reviewed and placed in the context of existing literature. Chapter five: *Conclusion* links the initial hypothesis with the found results and presents the general conclusions on the outcomes of the research. Finally, the recommendations for further research are shared. The appendices contain first of all a Dutch summary of the research. They present all the results in detailed tables and give an overview of the research locations.

2. Materials and Methods

The following chapter contains information about the case study selection procedure and information about the case study location. Information about farm management practices is given in paragraph 2.4. The chapter concludes with a paragraph containing information on the sampling and analysis procedure.

* Words indicated in orange are defined in the glossary

2.1 CASE STUDY SELECTION

The research approach used was selected since it employs an exploratory case study design, wherein quantitative data will be collected on both organic and conventional arable farms with the aim to analyse between-case evidence (Yin, 1981). The selected organic farm will serve as 'lead' case study which is called 'Horaholm', an arable farm located in Hornhuizen (53° 23' N, 6° 22' E, one meter above sea level) in the province Groningen in the North of the Netherlands (see Figure 2.1).

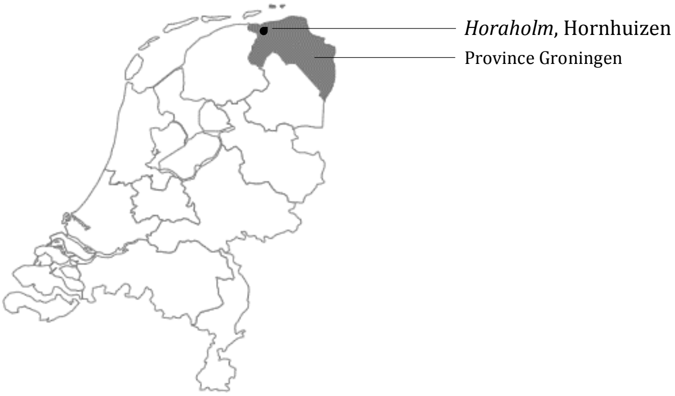


Figure 2.1 Location of the research area and specific location of first case study site: Farm Horaholm in Hornhuizen

Horaholm is operated by the family Westers with Harm Westers and son Erwin Westers being the active farmers. The farm is organically certified since 2002 and currently includes gluten-free oats, potatoes (seed), carrots, tulips, fodder radish (seed), yellow mustard (seed) and several green manure crops. Total farm size is 85 hectares of cropped fields with an additional 32 hectares of natural, tidal marshlands (Westers, 2015). Since 2004 the farmer initiated on-farm testing and evaluation trials including non-inversion tillage and cover crops. Since 2010 the use of ploughing at the farm level has been dis-continued with the selling of the mouldboard plough. These developments resulted in an innovative non-inversion till based farming system where currently also no animal manure or other organic amendments are being imported while the nutrient balance is sustained by on farm in situ produced green manure crops. This system is still evolving and technical innovations are investigated, but since 2010 no animal manure has been applied. The farm has been selected as case study first of all since it is one of the most innovative farming systems in the Netherlands. Secondly, one of the intrinsic objectives of the farmer, next to productivity and efficiency, is investing in soil quality with specifical reference to soil life. The farmers, as part of being agricultural pioneers, have encountered failures and difficulties in while optimizing their farming system. This research might contribute to an improved understanding of the processes and performance of their farming system, specifically in terms of soil life functioning relative to conventional farming systems. The third reason for selection is that the farming system appears to be well-established and fully functional which is an inherent requirement for the specific research theme. This since soil's biological parameters governing nutrient cycles, may take years to adapt to changing farming practices.

Since this farm has been experimenting with soil life improving measures for over 10 years, it is expected that may have affected soil life abundance, activity and diversity. Another ground for the choice of this farm is related to the strong interest that the farmers have in the relationship between farm management, soil functioning and crop quality. It is expected that this may greatly enable the communication and dialogue between researchers and farmers. Three crops grown on this farm have been selected for between-case comparisons including oat (*Avena sativa* var. Max), potato (*Solanum tuberosum* var. Connect) and carrot (*Daucus carota* subsp. *Sativus* var. Nerac). They have been selected due to sampling practicalities (early fall) and are relevant for further analysis as they include three plant families naming the grass family *Poaceae*; the carrot family *Umbelliferae* and nightshade family *Solanaceae.* These crops thus have family-specific root system, nutrient acquisition and nutrient storing capabilities, in grain, root and tuber. It appeared not possible to select one single conventional farm in the region with similar crops in rotation. Therefore, three conventional arable farms in the region have been selected for comparison, which had a match with the three crops and varieties cultivated on farm Horaholm resulting in four case study locations (see Table 2.1).

Table 2.1 Overview of case study details

Farm	Contact person	Farming system	ha[a]	Crops in rotation	Crop selected
Mts[b] Westers - *Horaholm*	Harm Westers	ORG	85 (32)	Potato, carrot, oat, tulip, yellow mustard (seed), fodder radish (seed), green manure	Oat, carrot, potato
Mts Wijk	Dirk Wijk	CON	81 (1)	Potato, winter wheat, winter barley, oat, sugar beet, grass (seed)	Oat
Bv[c] Koop landbouw	Hans Knook	CON	409 (31)	Potato, winter wheat, sugar beet, carrot, onion	Carrot
Mts Gaaikema-Olsder	Cato Gaaikema	CON	79 (6)	Potato, winter & summer wheat, sugar beet, grass (seed)	Potato

[a] Arable land in ha, with ha extensively managed land/nature/marches in (..)
[b] Mts (maatschap) is a partnership form to be translated as ...
[c] Bv (besloten vennootschap) is a partnership form to be translated as Ltd (Limited).

2.2 SITE CONDITIONS

The four case study locations are located within a 25 km distance range from each other, in the province Groningen in the North of the Netherlands (see Figure 2.2). The mean annual temperature and mean annual precipitation were 11.5 °C and 621 mm respectively in the year 2014, however show relatively large fluctuations in mean annual precipitation, ranging from 1020 (2001) to 621 mm in 2014, measured at the weather station Lauwersoog which is located ± 20 km from the research area (Weerstatistieken, n.d.).

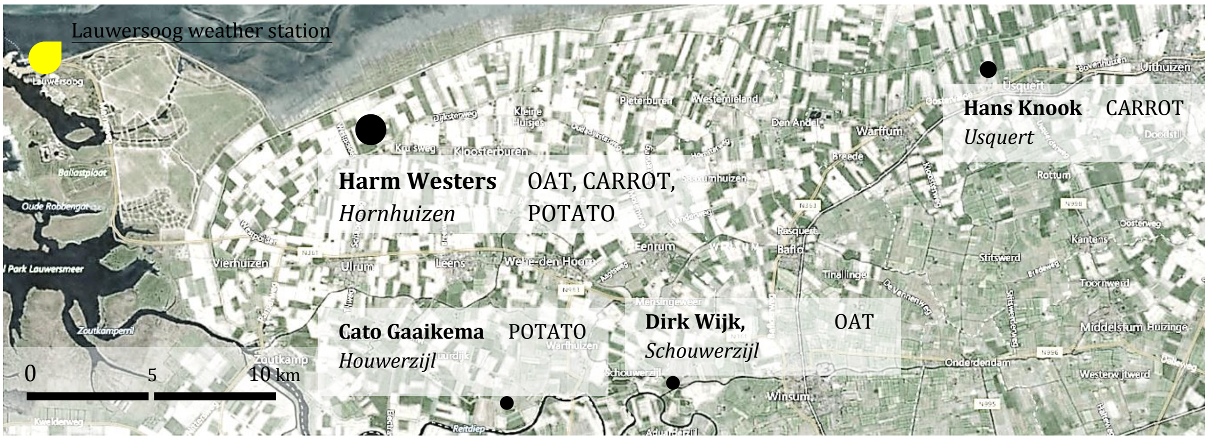


Figure 2.2 Locations of case study sites. *Source: google.nl*

The region where the farms are located is known as *het Hogeland* (English: *the High Land*) due to the slightly raised ground level (1 m. a. s. l.) compared to the surrounding regions. The regions underlying soil strata were formed during the Pleistocene era, via depositions of gravely clay (Nederlands: *keileem*) packages which are currently located at a soil depth of around two meters (Knottnerus, n.d.). This deposit was overgrown by peat marches around 2000 BC, which in 1000 BC became permanently buried under marine deposits (Schepers et al. 2013). The drowned but present boulder clay platform ensured further sedimentation by the Wadden Sea, a natural process which was followed by an anthropogenic, systematic reclamation of the marsh areas by use of dykes, a process which started in 1000 AD (Bazelmans et al. 2009). All case study locations are situated within one morphogenetic unit; naming the raised marches and plains (Nederlands: *kwelderwallen en bijbehorende vlakten*), one soil order; the vague soils (Nederlands: *zeekleigronden*) and one suborder; the hydrovague soils which is according to the Dutch system of soil classification (Atlas van Nederland, 1985). In the soil classification system of the USDA the approximate equivalent is the Entisol and in the World Reference Base of the FAO they are named Fluvisols (Harteminkt & Bakker, n.d; FAO, 1998). Main diagnostic property of the Fluvisols are their weak horizons and parent material derived from marine sediments (ISRIC, 2015). Despite the strong geomorphological similarities, minor differences between soil type and soil textures can be identified. The main differences in inherent soil properties as texture and clay content and other soil properties as $CaCO_3$ (%) and humus content can be found in Table 2.2. In subgroup level all soils can be classified as either calcium rich or calcium poor "Polder" vague soils (Nederlands: *kalkrijke en kalkarme Poldervaaggronden*), wherein polder is referring to the type of reclamation and is vague referring to the profile development (Siderius & Bakker, 2003).

Soil maps of the four farms can be found in Appendix II.

Table 2.2 Overview of several soil geophysical and chemical properties
Source: *private soil analysis of the farms, conducted by BLGG Agroexpertus* (different timing – see last column)

Crop	**Farming system**	**Texture class**[ab]	**Clay:silt:sand**[bc] (%)	**$CaCO_3$**[b] (%)	**pH** KCl	**OM**[b] (%)	**CEC**[b] (mmol+/kg)	**Year**[d] (mm-yyyy)
Oat	ORG	Heavy loam	**20**	6.8	7.5	1.8	..	08-2000
	CON	Heavy loam	**21** : 40 : 36	0.8	7.3	..	165	02-2015
Carrot	ORG	Heavy loam	**18**	5.0	7.6	2.2	..	09-1999
	CON	Moderate loam	**17** : 36 : 42	3.7	7.5	1.8	151	11-2015
Potato	ORG	Heavy loam	**24**	6.7	7.5	1.9	..	09-2001
	CON	Moderate loam	**14**	7.7	7.5	1.9	124	08-2010

[a] Moderate loam: 12.5 – 17.5 %; heavy loam: 17.5 – 25 % - categorisation adopted from STIBOKA (1968)
[b] Determined in soil layer 0-25 cm
[c] Clay (Nederlands: *Lutum*): fraction < 2µm; silt: fraction 2-50 µm; sand: fraction 50-2000 µm
[d] Year of soil sampling and analysis

The region is characterised by a flat and open, agricultural landscape dominated by arable farming (LTO, 2012). Being in close proximity to the sea, the region is ideal for the production of high quality seed potatoes for an international market due to its fertile soil and low disease pressure. Moreover, seed potatoes are the corner stone of agriculture in this region as it generates the largest revenue stream for most farms in this area. From the total cultivated arable land in this agricultural region, 33% is being allocated to potato production in 2014 of which 91% was seed potatoes (CBS StatLine, 2015). Complementary crops in the overall rotation include cereals (primarily winter wheat and summer barley) and sugar beets, accounting for 45% and 13% of the arable area in 2014, respectively. Together, these crops (potatoes, wheat/barley, sugar beet) make up for 90% of the arable area in this region. The remaining 10% consists of carrots, onions and grass for seed production and a small percentage is considered fallow. It results in a prevalent crop rotation of 1:3 or 1:4 with potato; cereal; sugar beet; potato or potato; cereal; sugar beet; cereal; potato. Specific crops rotations and sequences of the case study farms will be discussed in the following paragraph.

2.3 FARM MANAGEMENT

The general description in previous paragraph of the regional specific arable farming applies largely for the four case study farms. Seed potato production is the corner stone of all four farms and is grown in a 1:4 frequency. Sugar beets appear also in a 1:4 frequency in all conventional crop rotation schemes but are absent in the organic crop rotation. Winter wheat, summer wheat, winter barley, oat or grass for seed production is used as grain crop in between the tuber and root crops; potato, carrot, sugar beets and onion (see Table 2.3). On the organic farm yellow mustard and fodder radish are cultivated, both for seed production which furthermore serves as 'resting' crop in between the tuber and root crops.

Table 2.3 Crop rotation schemes of the four case study farms.

Crop	Farming system	Crops in rotation	#	Rotation
Oat, carrot, potato	ORG	Potato, carrot, oat, tulip, yellow mustard (seed), fodder radish (seed), green manure	7	Potato – oat (or carrot/tulip) – seed production - GM
Oat	CON	Potato, winter wheat, winter barley, oat, sugar beet, grass (seed)	6	Potato – winter wheat – sugar beet - winter wheat (or oat/barley/grass)
Carrot	CON	Potato, winter wheat, sugar beet, carrot (1:6), onion (1:8)	5	Potato – winter wheat – sugar beet – carrot (or onion)
Potato	CON	Potato, winter & summer wheat, sugar beet, grass (seed)	5	Potato – grass (seed) – sugar beet – winter wheat (or summer wheat)

The farmers make use of green manure crops, which appear in the rotation either as intercrop with the primary crop (e.g. clover/common vetch mix in the organic oat and *Festuca rubra* in the conventional oat), as secondary crop sown directly after the primary crop has completed its growth cycle (e.g. GM crop after pumpkin) or serving as primary crop itself, hereby taking up a full cropping season (see Table 2.4). The conventional farmers practice the first two uses of GM crops, depending on the primary crop, harvest date and soil condition in the autumn. The organic farmer distinguishes himself by using GM crops in a more intensive matter and uses GM as primary crop in the rotation.

Table 2.4 Crop sequence over the past 4 years.

Crop	Farming system	2012	2013	2014	2015
Oat	ORG	Potato - GM[a]	Leguminous GM[b]	Yellow mustard	Oat + GM
	CON	Potato	Sugar beet	Winter wheat	Oat + GM
Carrot	ORG	Potato - GM	Oat - GM	Pumpkin - GM	Carrot
	CON	Potato	Winter wheat	Winter wheat	Carrot
Potato	ORG	Oat - GM	Carrot - GM	Leguminous GM	Potato
	CON	Winter wheat	Sugar beet	Winter wheat	Potato

[a] + GM: green manure intercropped with primary crop; - GM: green manure sown after primary crop

[b] Consists of mixture up to 20 species, dominated by leguminous species as common vetch (*Vicia sativa*), broad bean (*Vicia faba*), winter pea (*Pisum Sativum*) and several clover species.

Figure 2.3 Green manure mixture undersown in organic oat crop (a); slashing the green manure crop (b)
Source: Fogelina Cuperus (a) and Harm Westers (b)

2.3.1 Tillage practices

At the conventional farms tillage treatments were performed by using a mouldboard plough which inverted the upper 25 – 30 cm, with small differences in soil depth and timing of tillage depending on the succeeding crop, clay content and general condition of the soil (Table 2.5). At the organic farm no inverting tillage practices are performed and in general, main soil cultivation practices are performed in autumn after the harvest. It consists firstly of using a cultivator (Nederlands: *Smaragd Vleugelschaar cultivator*) at a soil depth of 8 cm where after secondly the Paragrubber (also called sub-soiler or flat-lifter) is used, which intends to break up the soil by lifting the soil at 25 cm depth. The Paragrubber forms a combination with the cultivator (Nederlands: *kopeg*) and sowing machine, hereby preparing the seedbed and seeding in the green manure crop for winter. This is the general procedure but can differ between fields depending on inherent soil properties (clay content), field conditions and crops as can be seen in Table 2.5. The oat and potato crop in the organic system did not receive a primary soil cultivation treatment as a green manure and yellow mustard crop were the preceding crop. These fields were slashed (see Figure 2.3) and treated with a cultivator, either at 4 or 8 cm depth, instead of the *Vleugelschaar* cultivator and Paragrubber.

Table 2.5 Soil cultivation practices preceding the researched crop cycle.

Crop	Farming system	Timing	Cultivation treatment	Depth
Oat	ORG	April 2015	Cultivator ‘*kopeg’*	4
	CON	October 2014	Mouldboard plough	25
Carrot	ORG	September 2014	Paragrubber + cultivator ‘*kopeg’*	25 & 4
		May 2015	Cultivator ‘*kopeg + frees’*	4 & 8
	CON	September 2014	Mouldboard plough	23
Potato	ORG	April 2015	Cultivator ‘*frees*’	8
	CON	April 2015	Mouldboard plough	27

2.3.2 Fertilization practices

Fertilization treatments on the organic farm consisted solely of in situ produced green manures, either a green manure with a produce (yellow mustard), which preceded the oat crop or a green manure without a produce, cultivated prior the carrot and potato crop. All green manure crops were slashed (Nederlands: *klepel*) in April – early May and hereafter the stubble was treated by either a cultivator at a soil depth of 2-4 cm (*kopeg*) to 8 cm (*frees*). The green manure quantities are shown in Table 2.6 on the following page.

Table 2.6 Fertilization treatments in the growing season 2015 applied in the oat, potato crop in the organic (ORG) and conventional (CON) farming system.
source: kennisakker.nl (N content green manure) & koch.eurolab.nl (N content additional organic amendments)

Crop	Farming system	Timing	Type	Application rate Kg ha^{-1}	N_{tot} Kg N ha^{-1}	P Kg P_2O_5 ha^{-1}	K Kg K_20 ha^{-1}	
Oat	ORG	April 2015 (slashed)	Green manure (remains of previous yellow mustard crop; ±2% N)	2.000 (DM)	40	-	-	
					40	**0**	**0**	**=TOTAL**
Oat	CON	October 2014	Chicken manure (solid)(± 20 kg N/ton)	10.000 (Fresh)	200	242	133	
		May 2015	Amonium nitrate	30	30	-	-	
		June 2015	Mantrac Manganese (500 gr Mn/L)	1	-	-	-	
					230	**242**	**133**	**=TOTAL**
Carrot	ORG	April 2015 (slashed)	Green manure (mixture of *Brassica rapa rapa* and rye; ±2.5 % N)	5.000 (DM)	125	-	-	
					125	**0**	**0**	**=TOTAL**
Carrot	CON	January 2015	Kali 60 (60% K)	400	-	-	240	
		April 2015	Top mix wortel (5-9-22) + Ca, Mg, S	227	11.3	20.4	50	
		June 2015	NK mix (16-0-32)	200	32	-	64	
			Top trace Borium (150 gr B/L)	0.2	-	-	-	
		July 2015	EPSO micro (16 MgO; 31 SO_3 + B, Mn)	5	-	-	-	
			NK mix (16-0-32)	250	40	-	80	
			Top trace Magnesium nitrate (135 gr MgO/L)	0.5	0.035	-	-	
		August 2015	Top trace Magnesium nitrate (135 gr MgO/L)	2	0.14	-	-	
			NK mix (16-0-32)	125	20	-	40	
			Top trace Magnesium nitrate (135 gr MgO/L)	1	0.07	-	-	
			EPSO micro (16 MgO; 31 SO_3 + B, Mn)	2	-	-	-	
		September 2015	Top trace Magnesium nitrate (135 gr MgO/L)	0.5	0.035	-	-	
			EPSO micro (16 MgO; 31 SO_3 + B, Mn)	5	-	-	-	
					103.7	**20.4**	**416**	**=TOTAL**
Potato	ORG	April 2015 (slashed)	Autumn 2013 – summer 2014: mix A*	7.500 (DM)	225	-	-	
			Summer 2014 – spring 2015: mix B*	**(solely mix B)**	**225**	**0**	**0**	**=TOTAL**
Potato	CON	October 2014	Champost (±6.0 kg N/ton fresh product)	20.000 (Fresh)	120	72	174	
		May 2015	APP - Ammonium polyphosphate (10-34-0)	180	18	61	-	
			Patentkali (0-0-30) + 42% SO_3 + 10 % MgO	600	-	-	180	
			UREAN (60-0-0)	180	108	-	-	
					246	**133**	**354**	**=TOTAL**

* A: rye, winter pea and winter Vicia (10 t DM ha^{-1}; ± 3.5% N)

* B: oat, pea, broad bean, mungo bean, sun flower, phacelia, lupine, sorghum, Persian-, red-, white-, honey-, Alexandrine-clover, alfalfa, caraway, mallow, flax (7.5 t DM ha^{-1}; ± 3% N)

As can be seen in Table 2.6, the N_{tot} application rate of the green manure crops was around 40 kg N/ha for the oat and carrot crop and around 450 kg N/ha in the potato crop. Note hereby is that the 15 ton DM/ha is the accumulated weight of an 18 month period and thus the 450 kg N/ha is released in this period. Fertilization treatments on the conventional farms consisted of a combination of organic amendments and synthetic fertilizer. The conventional oat crop was fertilized with autumn applied solid chicken manure (10 t/DM/ha), synthetic N fertilizer and a manganese micronutrient foliar fertilizer. The conventional carrot crop was fertilized with synthetic N, K, S, Ca, Mg, B and Mn appearing both in solid form as dissolved foliar spray. No additional organic amendments were used in the carrot crop. The conventional potato crop was fertilized in autumn 2014 using champost (20 t/ha) and in May 2015 using synthetic fertilizer with an N, P, K mixture supplemented with Mg and S. The exact timing and rates can be found in Table 2.6.

2.3.3 Weed, pest and disease management

The weed management on the organic farm consisted of mechanical weed control by harrowing all three crops. In the carrot crop this was supplemented by burning the weeds. In the conventional system (carrot and potato) the mechanical weed control was further supplemented by the use of several non-selective herbicides, ranging from two different herbicides in the potato crop up to seven in the carrot crop. In the conventional oat crop no herbicides were used. Pest and disease management on the conventional farms consisted of using several synthetic pesticides and fungicides against louse and the cereal leaf beetle (Ned: *Graanhaantje)* in the oat crop; louse, silver scurf (Ned: *zilverschurft*), late blight and rhizoctonia in the potato crop and nematodes in the carrot crop. Mineral oils against louse damage was used in the conventional potato crop.

2.4 SAMPLING PROCEDURES AND ANALYSIS

2.4.1 Sampling procedures

At the end of the 2014/15 cropping season (August 2015 – October 2015) crop and soil samples were collected. Soil sampling was done prior to harvesting of the root crops and directly after harvesting the oat crop. Crop sampling was done in the week prior to final harvest (Table 2.7).

Table 2.7 Overview of harvest (specific and DAS) and sampling timing for the oat, carrot and potato crop during 2015

Crop	Operation	Date	DASa # days
Oat	Soil sampling	17-08/23-08	
	Crop sampling[b]	10-08/16-08	
	Harvest ORG	18-08	121
	Harvest CON	08-08	146
Potato	Soil sampling	31-08/06-09	
	Crop sampling	31-08/06-09	
	Harvest ORG	03-09	114
	Harvest CON	29-09	159
Carrot	Soil sampling	05-10/11-10	
	Crop sampling	05-10/11-10	
	Harvest ORG	13-10	136
	Harvest CON	14-10	154

[a] DAS: days after sowing

[b] Crop sampling only performed at ORG as harvest in CON was already executed.

Sampling was executed to distinguish the status of the indicators outlined in Table 2.8.

Table 2.8 Indicators for soil and crop sampling.

Soil	**Crop**
1. N, P, K, S, Ca, Mg, Cu, Fe, Zn	7. Crop biomass and DM
2. SOM	8. Cu, Fe, Zn
3. pH	
4. Soil microbial abundance	
5. Earthworm abundance	
6. Mycorrhizal root colonization	

The indicators identified in Table 2.8 were grouped in four categories, based on a field sampling protocol with similar sampling procedures (Table 2.9 – following page). Thus, in each field four different types of samples were collected. For soil biochemical analysis (indicator 1 – 4) the following protocol was used. Before entering the field, the possibility of a gradient (e.g. clay content, organic matter content, height, and compaction) was discussed with the farmer. Once having identified this gradient, the field was separated in four similar sized blocks, wherein 30 soil cores were taken per block, following a W shape resulting in 120 sampling probes per field (see Figure 2.4a). The samples were collected using a soil core sampler (2.5 cm diameter) and separate soil samples were collected for three soil depth intervals corresponding to the 0-10, 10-20 and 20-30 soil layer. A composite sample of approximately 30 soil cores for each sampling depth interval was made by bulking the soil cores and thoroughly mixing them. Hereafter the sample was stored in an airtight bag at 4°C until further processing and analysis.

A second type of sample was collected for earthworm measurements (indicator 5). Earthworm populations were assessed for eight sample sites in the field, whereby earthworm pits (oat field: 30 x 30 x 20 cm (WxLxH), $\boldsymbol{V}$=0,018 m^3, $\boldsymbol{A}$=0,09 m^2; potato field, in the ridge: 55 x 20 x 20 (WxLxH), $\boldsymbol{V}$=0,011 m^3, $\boldsymbol{A}$=0,11 m^2; carrot field, in the ridge: 60 x 20 x 20 (WxLxH) $\boldsymbol{V}$= 0,012m^3, $\boldsymbol{A}$= 0,12 m^2) were manually excavated using a quick levering action with a spade. Two samples for each field sampling unit were obtained using the same blocks according to existing field gradients discussed earlier (see Figure 2.4). The location of the earthworm pits within each block were set 25 meters from the border of the field, to reduce increased variability's due to edge-effects. The excavated soil was placed on a large, light coloured plastic sheet and the entire soil volume was hand sorted for earthworms in the field. To standardize the sorting procedure, time for hand sorting the soil was set on 45 minutes per soil monolith in the oat field and 30 minutes in the carrot and potato field. Less time was needed in the fields of the root crops as the excavated soil was loos and crumbly, contributing to an easy hand sorting process. Prior to earthworm sampling, a mustard extraction was prepared using a modified recipe from Valckx et al. (2009), based on actual sampling surface of this experiment (A= 0,09, 0,11 and 0,12 m^2; ± 0,1 m^2). 2 L (3 gr mustard powder/L) was poured on the bottom of the earthworm pit. After 10 minutes a second 2 L was poured in the earthworm pit. All earthworms reaching the surface within earthworm square within 20 minutes, were collected. Then, 2 L (6 gr mustard powder/L) was poured where after 10 minutes a second 2 L of this solution (6 gr/L) was poured. The earthworms were preserved alive in an air tight container with a small amount of soil at 4°C until further analysis, for a maximum of two days.

For mycorrhiza root colonization measurements intact soil cores were taken at four sample sites in the field, at two depth intervals (0-10, 10-20 cm) using a hand probe (7 cm diameter). The locations of the sample sites was determined according to Figure 5b, with one sample site within each block 1, 2, 3 and 4. The hand probe was placed directly next to the crop row with one side of the probe next to one side of the plant. The intact soil cores were stored at 4°C until further processing.

Table 2.9 Sampling procedures.

	Indicators			
	Biochemical	**Earthworm**	**Mycorrhiza**	**Yield, DM**
Sampling dimension (cm)	2.5 (diameter)	30x30	7 (diameter)	50x50
Sampling depth (cm/interval)	10	20	10	-
Intervals (#)	3	1	2	-
Max. sampling depth (cm)	30	20	20	-
Probes/samples (#/field)	120	8	4	8 (oat), 4 (carrot/potato)
Total samples per field (# interval x # sample/field)	3 (bulk samples)	8	8	8

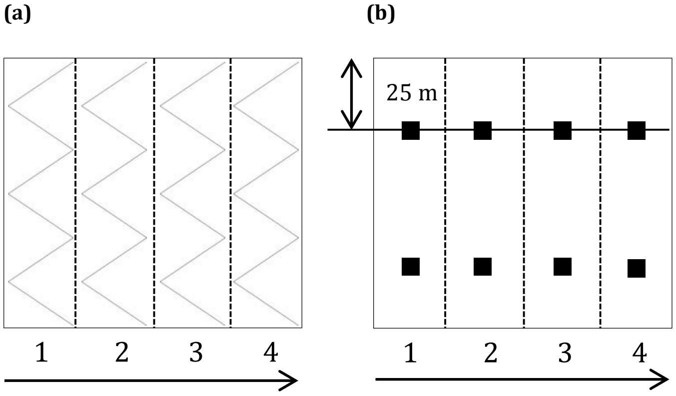


Figure 2.4 Sampling scheme for bulk soil samples (a) and sampling scheme for earthworm, mycorrhiza and crop samples (b). Arrow indicates a potential gradient in the plot.

The fourth and final sample taken from the field is the crop sample for yield, DM and nutrient analysis on the N, P, K, Cu, Zn and Fe content of the edible parts. The location of the sampling sites for this sample was identified according the procedures described for indicator 5 - earthworms. Once identified, a metal frame (50 x 50 cm) was placed on top of the crop whereafter all biomass within the metal frame was cut with sharp harvesting scissors at a height of 1 cm above soil level (oat). The same procedure was followed in the potato and carrot crop however with a harvesting fork whereby all biomass of the root crops within the frame, below and above ground, was collected. The biomass was stored in airtight bags at 4°C until weighing and processing for further analysis.

2.4.2 Soil chemical, soil biological and crop analysis

Biochemical analysis (soil)

The collected bulk soil samples were used to determine total and available macronutrient fractions (N, P, K) and available micronutrient concentrations (S, Ca, Mg, Cu, Zn, Fe), SOM, pH and microbial abundance. A well-mixed, 100 gram subsample of the bulk soil of the 0-10 soil layer was used to quantify soil microbial abundance by Phospholipid fatty acid (PLFA) fingerprinting. The soil was passed through a 5 mm mesh and afterwards frozen at -40°C until further analysis. For the PLFA analysis, a second homogenous subsample of 100 gram was taken from the 0-10 cm soil layer and dried overnight in a furnace at 105°C to determine the DM content of the soil. The PLFA was conducted at the laboratory of Animal Ecology, one of the laboratories of the Environmental Science chair group at Wageningen University. The remaining part of the bulk soil samples was dried for 48 hours at 40°C and grinded. From this sample, chemical analysis were executed on macro-, meso- and micronutrient concentrations, SOM and ph. Soil available N (N_{min}, kg N ha) was determined using a 0.01 M $CaCl_2$ extractant followed by an spectrophotometrically analysis using a segmented flow system. Soil available P (P-PAE)(kg P_2O_5 ha^{-1}) and soil available K (K-PAE)(kg K_20 ha^{-1}) were determined using a 0.01 M $CaCl_2$ extractant, where after P was analysed spectrophotometrically using a segmented-flow system and K was analysed by flame emission spectrophotometer. Total SOM (g SOC kg) was analysed according the loss on ignition methods (LOI) by drying the soil in a furnace at 550 °C, wherein the weight loss of the sample represents the amount of organic carbon. Soil pH was determined by a pH KCL extraction. Above mentioned procedures on macronutrients, SOM and pH were conducted by the laboratory of the Farming Systems Ecology chair group at the Wageningen University. Bioavailable magnesium (Mg), sulphur (S), copper (Cu), zinc (Zn) and Iron (Fe) were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by use of extractant 0.01 M $CaCl_2$. Bioavailable Ca was determined by use of a water extractant. Analyses on meso- and microonutrients were processed at the CBLB (Nederlands: *Chemisch Biologisch Laboratorium Bodem*), one of the laboratories of the Environmental Science chair group, situated at the Wageningen University.

Earthworm analysis

Earthworms were rinsed in water and carefully blotted using tissue paper to remove additional moisture, after which they were grouped into adults and juveniles, based on the presence of the clitellum. Sub-adults, having an emerging but not fully grown clitellum were grouped as juveniles. Both adults and juveniles were identified to ecological group level and categorized as epigeic (litter and surface dwelling species), endogeic (top soil dwelling species) and anecic (deep burrowing species). However, due to the limited occurrence of anecic earthworms (specifically the *Lumbricus terrestris*) this ecological group was left out during statistical analysis. Although the adults of *Lumbricus rubellus* and *Aporrectodea calliginosa* are known for their ability to perform anecic burrowing behavior (Felten & Emmerling, 2009), in the context of the current research they were grouped as epigeic (*L. rubellus*) and endogeic (*A. calliginosa*) earthworms, respectively. Overall grouping was based on the generally accepted ecological classification of Bouché (1972). The groups were counted for density (No. of individuals m^{-2}) and weighed for biomass (gram m^{-2}) measurements. Biomass measurements includes gut content.

Mycorrhiza analysis

Roots contained in the intact soil cores were carefully rinsed to remove soil particles with tap water using wet sieving above a 1 mm mesh-sized sieve to prevent loss of fine roots. The cleaned roots were stored in 70% ethanol until staining (Vierheilig et al. 2005). The staining of the roots was done according the procedure advised by Walker (2005). In short, roots were cut into pieces of approximately 2 cm where after placed in a preheated (90°C) 10 % KOH solution during 30 minutes. Air bubbles between the roots were removed by use of a vacuum press, where after the sample was placed in a 0.1 N HCl solution for 5 minutes. Hereafter, the roots were stained by placing them in a preheated (90°C) solution of glycerol, H_20, HCl and ink (Parker Quink Fountain Pen Ink, permanent blue™).

After staining, the arbuscular mycorrhizal fungi root infection was quantified using the magnified intersection method, which estimates AMF colonization by inspecting intersections between roots and the microscope crosshair at a magnification x 200 using a compound microscope (McGonigle et al. 1990). This method allows the identification and quantification of mycorrhizal structures in terms of arbuscles, hyphae, spores and vesicles. This method is also suitable to asses AMF in root textures which are suboptimal (e.g. old roots) which was relevant as the roots were collected at the end of the growth cycle.

From each root sample, 40 root segments of 2 cm each were selected randomly. This amount slightly differs from what is advised in Sun and Tang (2012) where the minimum quantity of roots segments for this method is 150 (1 cm each; 150 cm in length). Root segments were placed on a microscope slide (10 segments per slide) and aligned perpendicular to the long axis of the slides resulting in 4 slides per root sample. The slides were examined by a compound microscope x 200 magnification (Zeiss, Germany, Axioskop 95FX.0506) according to the procedures of McGonigle et al. (1990). Three intersections per root segment were examined (segments x intersections=120 intersections per sample) on the presence of AMF at the intersection of root x microscopic field of view (Figure 2.5). The location of the microscopic field of view was placed using a systematic interspacing. The presence or non-presence of mycorrhiza in the microscopic field of view was classified as follows: 'negative' (no fungal material in root), 'arbuscles' (including fungal clumps), 'vesicles', 'spores' and 'hyphae'.

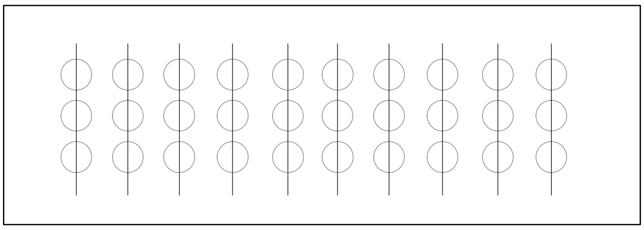


Figure 2.5 Microscope slide, placement of the roots and microscope field of view

Crop analysis

The biomass samples (oat *n=8*, potato; carrot *n=4*) were weighted for fresh weight of kernel, stems and leaf of crop and stems and leaf of green manure in the oat crop and the below-ground crop and above-ground biomass of the potato and carrot crop. The above-ground biomass of the potato crop at all sampling locations was removed before sampling and thus not taken into considerations for further analysis. After fresh weight measurements, a homogeneus subsample was obtained and dried at 65°C for 48 hours and then once again weighted for dry weight determination. Hereafter the dried sample was ground in a stainless steel mill to pass a 1 mm sieve. Samples were used for subsequent plant tissue analysis based on acid extractable fractions of N, P, K, Zn, Fe and Cu (mg kg DM^{-1}) in the edible parts of the crop. Samples for P, K, Zn, Fe and Cu analysis were digested in a concentrated HNO_3, HCl and H_2O_2 solution in microwave containers wherefter the mineral concentrations was determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES). Plant N was determined by (... will ask Hennie). Analysis on the N, P and K fractions were conducted at the laboratory of the Farming Systems Ecology chair group at the Wageningen University and analysis on Cu, Zn and Fe concentrations was conducted at the CBLB (Nederlands: *Chemisch Biologisch Laboratorium Bodem*).

2.4.3 Statistical analysis

Results (where having enough measurements) were analysed using the one-way analysis of variance (ANOVA) in order to determine statistical significance between farming systems at p=0.05. Furthermore, a Spearman's rank correlation matrix was created for the following soil chemical and biological parameters: soil organic matter (SOM), available nitrogen (N), total nitrogen (N_{tot}). available phosphorus (P), total phosphate (P_{tot}), available potassium (K), available calcium (Ca), available magnesium (Mg), pH, earthworm density ($EW_{density}$) total bacterial ($Bact_{tot}$), saprotrophic fungi (fungi), arbuscular mycorrhizal fungi hyphae (AMF_{hyphea}), arbuscular mycorrhizal fungi spores (AMF_{spore}) and actinomycetes (Act). A second Spearman's rank correlation matrix was created for the following soil chemical and biological parameters and crop nutrient density: SOIL: soil organic matter (SOM), available nitrogen (N), total nitrogen (N_{tot}). available phosphorus (P), total phosphate (P_{tot}), available potassium (K), available calcium (Ca), available magnesium (Mg), available copper (Cu), pH, earthworm density ($EW_{density}$) total bacterial ($Bact_{tot}$), saprotrophic fungi (fungi), arbuscular mycorrhizal fungi hyphae (AMF_{hyphea}), arbuscular mycorrhizal fungi spores (AMF_{spore}), actinomycetes (Act) and CROP: nitrogen (N), phosphate (P), potassium (K), copper (Cu), iron (Fe) and zinc (Zn).

3. Results

3.1 SOIL PROPERTIES

3.1 Earthworms

The first biological soil parameter investigated was the abundance of earthworms. The results of the collected data is being presented as total earthworm density (individual's m^2) and biomass (gr m^2) in the topsoil (0-20cm). The aggregated data (total density and biomass values) is divided by ecological group level (epigeic and endogeic). A further subdivision is created by separating the ecological groups per development stages (adults and juveniles) (Table 3.1). The final two columns shows the percentage of adults and epigeic earthworm compared to the total earthworm count.

Table 3.1 Mean densities and standard deviations (individual's m^2) of the total earthworm community, divided at ecological group level (epigeic and endogeic) and age categories (adult and juvenile) in an oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system. **Sampling period:** mid-August – early October.

		Earthworm community			**Epigeic**			**Endogeic**				
		Individuals m^2			**Individuals m^2**			**Individuals m^2**			**%**	
		Total	**Adult**	**Juveniles**	**Total**	**Adult**	**Juveniles**	**Total**	**Adult**	**Juveniles**	**Adult**	**Epigeic**
Crop	**System**											
Oat	CON	351 ± 109	62 ± 30	289 ± 109	72 ± 31	28 ± 25	44 ± 19	279 ± 107	35 ± 22	244 ± 113	*18*	*21*
	ORG	575 ± 176	156 ± 76	419 ± 214	64 ± 61	7 ± 12	57 ± 61	512 ± 152	149 ± 75	363 ± 170	*27*	*11*
Significance[a]		**	**	n.s.	n.s.	*	n.s.	**	***	n.s.		
Carrot	CON	127 ± 28	31 ± 11	96 ± 17	26 ± 17	9 ± 6	17 ± 12	101 ± 27	22 ± 10	79 ± 19	25	21
	ORG	229 ± 55	43 ± 22	186 ± 42	44 ± 19	17 ± 12	27 ± 13	185 ± 41	26 ± 13	160 ± 32	*19*	*19*
Significance		**	n.s.	***	n.s.	n.s.	n.s.	***	n.s.	***		
Potato	CON	89 ± 24	28 ± 14	62 ± 14	27 ± 11	4 ± 4	23 ± 12	62 ± 17	24 ± 12	38 ± 11	*31*	*30*
	ORG	123 ± 47	23 ± 11	100 ± 44	44 ± 23	6 ± 4	38 ± 22	79 ± 29	17 ± 10	62 ± 27	*19*	*36*
Significance		n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s	n.s.	*		

[a] Differences between treatments were n.s. not significant, *, **, *** significant at $p \leq 0.05$, 0.01 and 0.001, respectively

3.1.1.1 Earthworm density

The mean total density of earthworms was significantly higher in the oat and carrot crop in the organic system compared to the similar crop grown in the conventional system (Table 3.1). In all three ORG-crops the total earthworm density was higher than in the CON-crops, by 63% (oat), 80% (carrot) and 38% (potato). When looking at a lower aggregation level (ecological group) we saw in both farming systems and all crops a dominant presence of endogeic species (Figure 3.1). When looking proportionally (epigeic species of total earthworm density), largest epigeic species abundance was observed in the ORG-potato (36%), CON-potato (30%), CON-carrot (21%), CON-oat (21%), ORG-carrot (19%) and ORG-oat (11%) fields. When reflecting the results non-proportionally we saw that epigeic species density is 68% and 64% higher in the ORG-carrot and ORG-potato field. It is 9% lower in the ORG-oat field, when compared to the CON-cropping system. Endogeic species density was higher in all ORG-fields with 84%, 83% and 27%, in carrot, oat and potato field respectively.

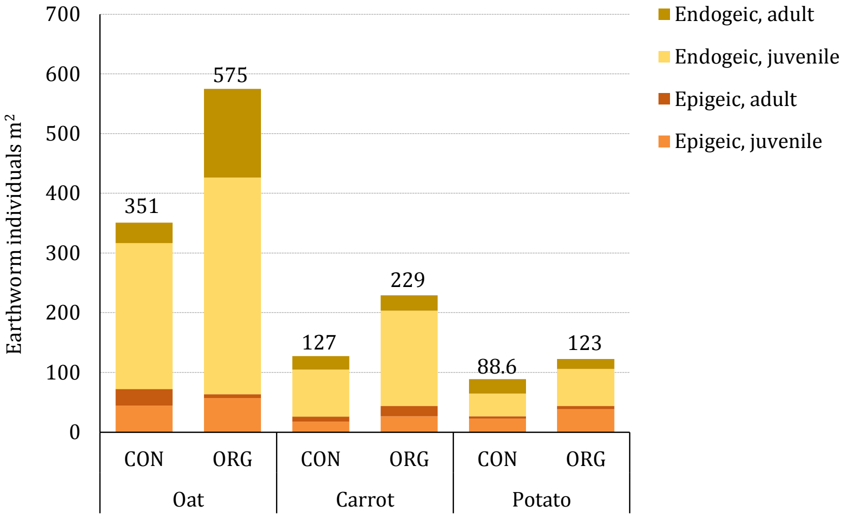


Figure 3.1 Means (individuals m^2) of the total earthworm community, divided in functional group (endogeic; epigeic) and age category (adult; juvenile). *n*(sample sites/field)=8

The results of the development stages showed significant differences between adult densities in ORG-oat and CON-oat fields, juvenile densities of ORG-carrot and CON-carrot and juvenile densities of ORG-potato and ORG-potato (Table 3.1). Overall, we observed a relatively young population with mean adult percentages ranging from 31% to 18% in CON-potato and CON-oat respectively. CON-oat had the highest number of juveniles per adult (4.6) closely followed by ORG-potato (4.4) and ORG-carrot (4.4)(Figure 3.2). In both root crops we saw a similar pronounced shift towards higher adult:juvenile ratios.

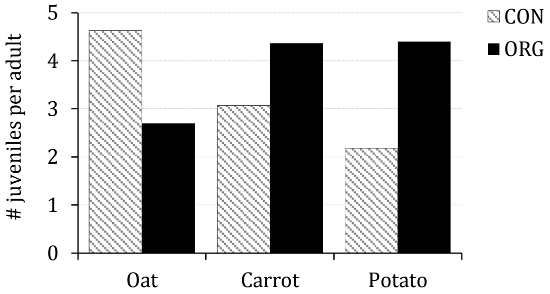


Figure 3.2 Juvenile : adult ratio

3.1.1.2 Earthworm biomass

The mean total biomass was significantly higher in the ORG-oat and ORG-carrot crop compared to the similar conventional crops. Earthworm biomass was higher by 96%, 67% and 15% in ORG-oat, ORG-carrot and ORG-potato, respectively (Table 3.2). This can mainly be attributed to a difference in endogeic earthworm biomass of 83% (+147% ORG-oat; +88% ORG-carrot; +15% ORG-potato) compared to a difference in epigeic earthworm biomass of 12% (-18% ORG-oat; +37% ORG-carrot; +18% ORG-potato). From the total earthworm weight, epigeic biomass percentages ranged from 42% in CON-carrot to 13% in ORG-oat (Table 3.2).

Table 3.2 Mean biomass (gr m^{-2}) and standard deviations of the total earthworm community, divided at ecological group level (epigeic and endogeic) and age categories (adult and juvenile) in an oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system. **Sampling period:** mid-August – early October.

		Earthworm community $g\ m^{-2}$			**Epigeic** $g\ m^{-2}$			**Endogeic** $g\ m^{-2}$			**%**	
Crop	**System**	**Total**	**Adult**	**Juveniles**	**Total**	**Adult**	**Juveniles**	**Total**	**Adult**	**Juveniles**	**Adult**	**Epigeic**
Oat	CON	49.6 ± 14	21.1 ± 10	28.5 ± 13	15.2 ± 10	11.7 ± 9	3.5 ± 3	34.4 ± 12	9.4 ± 7	25 ± 12	*43*	*31*
	ORG	97.4 ± 23	56.4 ± 35	41.0 ± 24	12.5 ± 12	3.1 ± 5	9.5 ± 10	84.9 ± 26	53.3 ± 35	31.6 ± 15	*58*	*13*
significance		***	*	n.s.	n.s.	*	n.s.	***	**	n.s.		
Carrot	CON	17.3	9.2	8.1 ± 4	7.3 ± 3	5.4 ± 2	2 ± 1	10 ± 4	3.8 ± 2	6.1 ± 3	*53*	*42*
	ORG	28.8 ± 12	13.9 ± 8	14.9 ± 5	10 ± 6	7.2 ± 5	2.8 ± 2	18.8 ± 7	6.7 ± 4	12.1 ± 4	*48*	*35*
significance		*	n.s.	**	n.s.	n.s.	n.s.	**	n.s.	**		
Potato	CON	15.7 ± 5	10.3 ± 5	5.4 ± 1	4.0 ± 1	2.1 ± 2	1.9 ± 1	11.7 ± 4	8.2 ± 4	3.6 ± 1	*66*	*25*
	ORG	18.0 ± 5	9.6 ± 4	8.4 ± 2	4.7 ± 2	3.4 ± 2	1.2 ± 0.6	13.4 ± 3	6.2 ± 4	7.2 ± 2	*53*	*26*
Significance		n.s.	n.s.	**	n.s.	n.s.	n.s.	n.s.	n.s.	***		

[a] Differences between treatments were n.s. not significant, *, **, *** significant at $p \le 0.05$, 0.01 and 0.001, respectively

3.1.1.2 Earthworm biomass (continuation)

Figure 3.3 shows a similar distribution in earthworm biomass as the earthworm density distribution shown in Figure 3.1, with highest earthworm biomass in ORG-oat followed by CON-oat > ORG-carrot > ORG-potato > CON-carrot > CON-potato. When looking at the development stage of the earthworm community we observed the highest adult percentages of total earthworm biomass in the CON-potato crop (66%), interestingly the crop with the lowest total earthworm biomass.

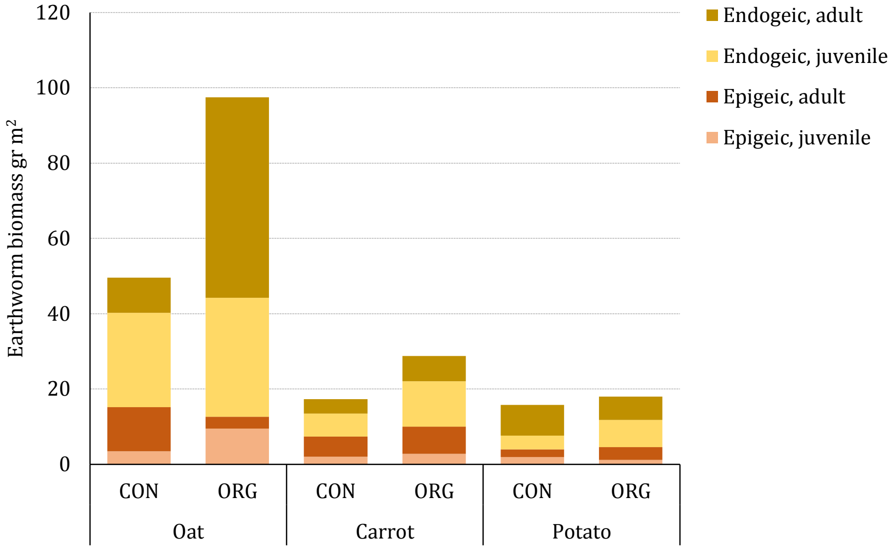


Figure 3.3 Means (biomass gr m^2) of the total earthworm community, divided in functional group (endogeic; epigeic) and age category (adult; juvenile).
n(sample sites/field)=8

Table 3.3 shows that the average weight of adult earthworms is higher in the ORG-fields compared with the CON-fields. Juvenile earthworm weight is similar in the ORG- and CON-oat and carrot field however lower in the ORG-potato field, by 11%.

Table 3.3 Average weight of adult and juvenile earthworms mean differences in an oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system.

		Average weight g	
		Adult	**Juvenile**
Crop	**System**		
Oat	CON	0.34	0.10
	ORG	0.36	0.10
▪ **Difference**		**+6%**	**-**
Carrot	CON	0.29	0.08
	ORG	0.33	0.08
▪ **Difference**		**+14%**	**-**
Potato	CON	0.37	0.09
	ORG	0.42	0.08
▪ **Difference**		**+14%**	**-11%**

3.1.2 Mycorrhiza

The second biological soil parameter investigated was the presence of arbuscular mycorrhizal fungi (AMF). The results of the collected data are presented as the percentage of root colonized by arbuscles, vesicles, spores and hyphae. As the samples were taken late in the season, it is likely that arbuscles start to degenerate and therefore, fungal clumps have been included in the arbuscles category (AC, see note [a]). Note that the sum of the percentages of categories AC, VC, SC and HC might be more than the total percentage root colonized (column: positive) as in a given microscopic field of view a score can be given to more than one category.

Table 3.4 Mean percentages of arbuscular colonization (AC), vesicular colonization (VC), spore colonization (SC) and hyphal colonization (HC) (AMF hyphae) and non-colonization (positive) in an oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system and standard deviations. **Sampling period:** mid-August – early October.

		Root colonization 0-10 cm %					**Root colonization 10-20 cm %**				
		AC[a]	**VC[b]**	**SC[c]**	**HC[d]**	**Positive[e]**	**AC**	**VC**	**SC**	**HC**	**Positive**
Crop	**System**										
Oat	CON	7.8 ± 4.4	5.2 ± 1.2	0.6 ± 0.7	22.1 ± 9.4	**20.1** ± 8.4	3.8 ± 1.9	1.1 ± 1.13	0.1 ± 0.7	15.3 ± 5.7	**13.9** ± 6.1
	ORG	35.7 ± 7.5	10.0 ± 7.9	1.1± 0.5	63.2 ± 21.7	**35.2** ± 10.2	16.3 ± 6.5	5.3 ± 5.3	0.4 ± 0.5	55.7 ± 10.7	**54.2** ± 15.6
Carrot	CON	3.8 ± 0.6	2.3 ± 1.2	0.0 ± 0.0	13.8 ± 3.3	**16.3** ± 3.5	7.3 ± 3.5	2.3 ± 0.9	0.0 ± 0.0	9.6 ± 3.1	**11.4** ± 1.3
	ORG	6.7 ± 4.1	3.8 ± 2.9	0.0 ± 0.0	14.3 ± 9.5	**15.9** ± 9.5	6.5 ± 3.3	2.7 ± 1.8	0.0 ± 0.0	13.1 ± 0.7	**15.4** ± 1.3
Potato	CON	2.2 ± 0.9	0.0 ± 0.0	0.2 ± 0.5	11.8 ± 4.5	**15.1** ± 5.3	1.8 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	7.3 ± 1.9	**11.8** ± 1.4
	ORG	2.7 ± 1.8	0.2 ± 0.5	0.5 ± 0.7	14.2 ± 4.4	**18.1** ± 4.8	2.1 ± 1.5	0.0 ± 0.0	0.1 ± 0.5	9.2 ± 4.8	**14.2** ± 3.9

[a] Arbuscles: scored when in microscopic field of view there was identified at least one arbuscle or fungal clump (degenerated arbuscle)

[b] Vesicles: scored when in microscopic field of view there was identified at least one vesicle

[c] Spore: scored when in microscopic field of view there was identified at least one spore

[d] Hyphae: scored when in microscopic field of view there was identified at least one hyphal structure (VAM only – hyphae belonging to other fungi were not scored)

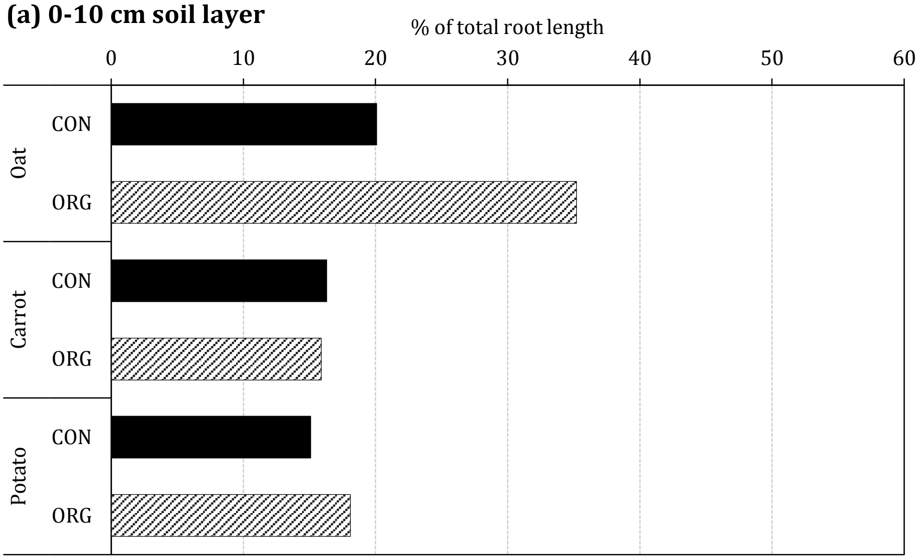
[e] Positive: opposite of negative root colonization: when in microscopic field of view there was no arbuscles nor vesicles nor spores nor VAM hyphae

3.1.2 Mycorrhiza (continuation)

Highest mycorrhizal root colonization counts were observed in the ORG-oat field, with 35% and 54% root colonization in the 0-10 and 10-20 cm soil layer, respectively. The ORG-oat roots had substantially more AMF colonization then all other crops (Figure 3.4). Mycorrhizal root colonization in the ORG- and CON-carrot crop were similar in the 0-10 cm soil layer (Figure 3.4 (a)), however, differ in the 10-20 cm soil layer (+4% more AMF root colonization in the ORG-crop). Mycorrhizal root colonization in the ORG- and CON-potato crop was quite similar in the 0-10 cm soil layer (+3% more AMF root colonization in the ORG-crop) and in the 10-20 cm soil layer (+2.4% more AMF root colonization in the ORG-crop).

Colonization percentage of all crops, except the ORG-oat, were in a similar range. All roots in the 0-10 cm soil layer were within the 15-20% colonization range whereas the roots in the 10-20 cm soil layer were within 9-15% root colonization. Thus, overall there was slightly less colonization in the deeper soil layer, except for the ORG-oat crop, which showed the contrasting trend of having a higher AMF root colonization percentage in the 10-20 cm soil layer.

(a) 0-10 cm soil layer



(b) 10-20 cm soil layer

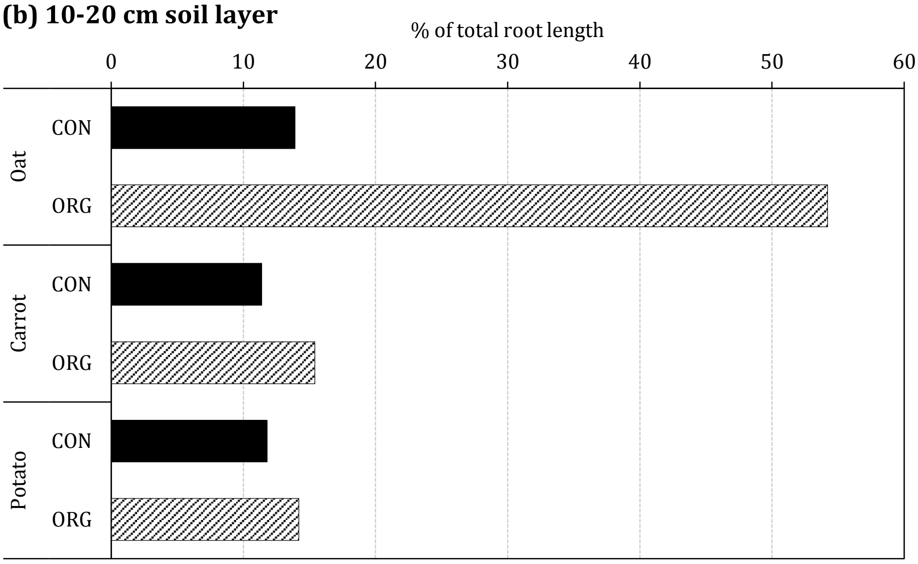
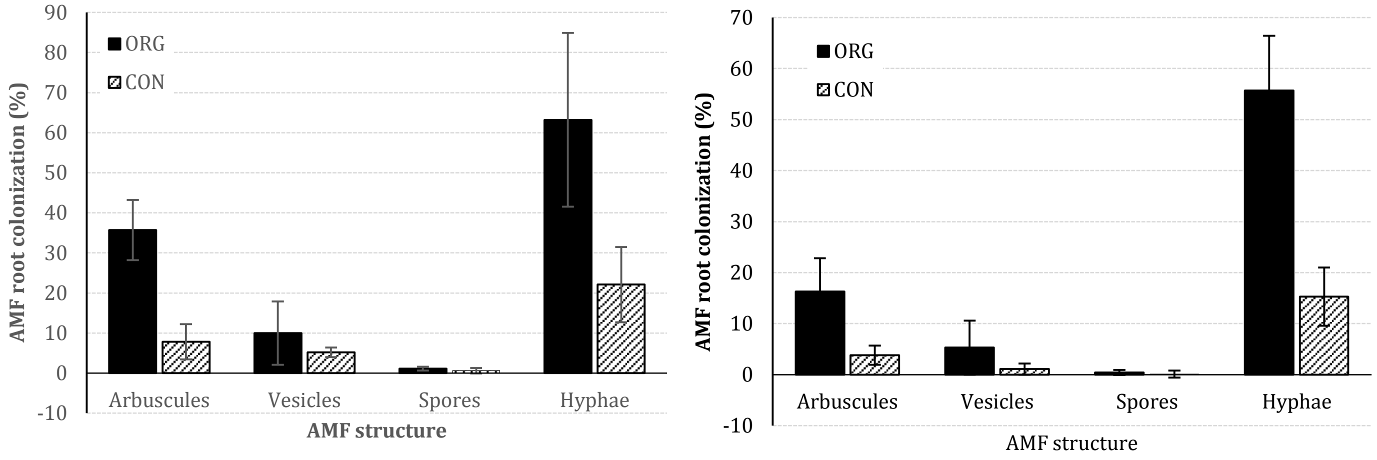


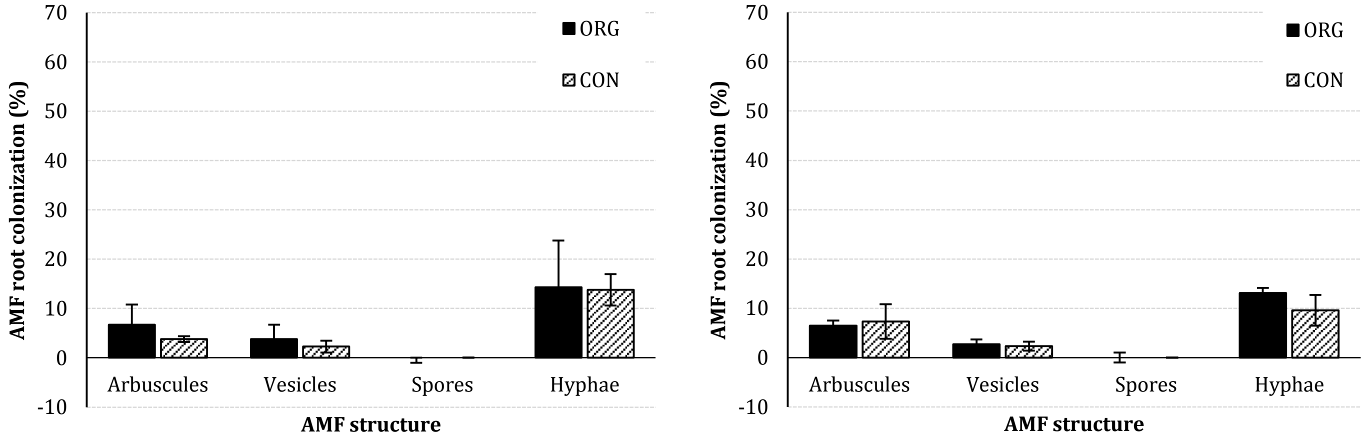
Figure 3.4 Mean percentages of arbuscular mycorrhizal fungi colonization of organic and conventional oat, carrot and potato roots in the 0-10 cm (a) and 10-20 cm (b) soil layer. *n*(sample sites/field)=8

The following figures show the contribution of four different AMF colonization structures to the overall AMF root colonization percentage. A subdivision is made for the 0-10 cm and 10-20 cm soil layer. Figure a1 and a2 (oat, 0-10 and 10-20 cm soil layer) show the high hyphal and arbuscles root colonization in the ORG-crop and reduced root colonization in the CON-oat crop. In both the ORG- and CON-oat crop higher numbers of vesicles are found in the top soil. In both the potato and the carrot crops, low AMF root colonization is found. Very little to no AMF spores were detected in all crops, which may be because crop roots, rather than soil, was investigated in this method. AMF spores are detectable mostly in soil.

(a1) oat: 0-10 cm soil layer **(a2) oat:** 10-20 cm soil layer



(b1) carrot: 0-10 cm soil layer **(b2) carrot**: 10-20 cm soil layer



(c1) potato: 0-10 cm soil layer **(c2) potato:** 10-20 cm soil layer

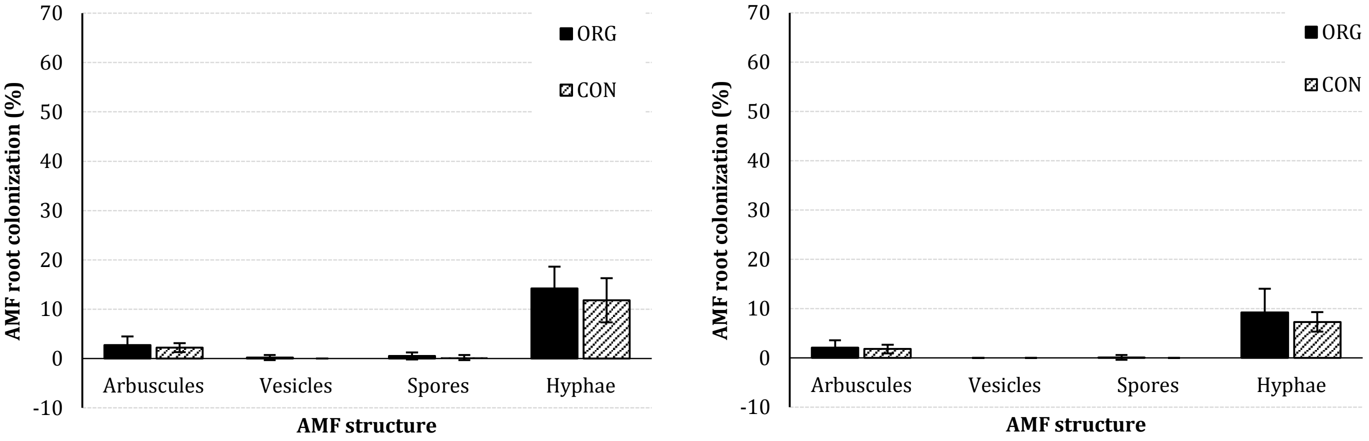


Figure 3.5 Mean percentages of arbuscular mycorrhizal root colonization (arbuscles, vesicles, spores and hyphae) of organic and conventional oat (a), carrot (b) and potato (c) roots in the 0-10 cm and 10-20 cm soil layer. *n*(sample sites/field)=8

3.1.3 Microorganisms

The third biological soil parameter investigated was the total microbial biomass and community composition (0-20 soil layer; in nmol g^{-1} DM soil) by using phospholipid fatty acid analysis (PLFA).

Total microbial biomass and bacterial biomass

Total microbial biomass was highest in the ORG-oat field, with values 281% higher than in the CON-oat field (Table 3.5). The ORG-carrot and potato field had a lower total microbial biomass then in the CON-fields, with values of 31% and 8% respectively. The same trends are shown in the bacterial biomass data.

Total saprotrophic fungal biomass and AMF

Fungal density was numerically highest in the ORG-oat field (Figure 3.6) and was 413% higher than the counts in the CON-oat field. No calculation error could be found that may explain this outlier – which implies the results stem from inherent differences in the samples. PLFA samples originate from the same bulk soil sample wherefrom the chemical analysis have been performed. In the chemical analysis no abnormal differences were found in the ORG-oat sample which further strengthens the belief the differences must be related and specific for this soil sample. The high saprotrophic fungal counts in the ORG-oat field were followed by the ORG-potato > CON-carrot > CON-oat > CON-potato > ORG-carrot. AMF hyphae and AMF spore density was higher in the ORG-oat and carrot fields when compared to the CON-oat and carrot field, however, lower in the ORG-potato field compared to the CON-potato field.

Table 3.5 PLFA[a] and NLFA[b] concentrations (nmol g^{-1} DM soil) of microorganisms (0-20 cm) in the oat, carrot and potato crop in organic (ORG) and conventional (CONV) farming systems.

		Total[a]	**Bacteria**[a]	**Fungi**			**Fungal bacterial-ratio**[c]	**Actinomycetes**[a]	**Gram+ PLFA**	**Gram- PLFA**	**Gram+/ Gram- ratio**
				Saprotrophic[a]	AMF hyphae[a]	AMF spores[b]					
		nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	nmol g^{-1}		nmol g^{-1}	nmol g^{-1}	nmol g^{-1}	
Crop	**System**										
Oat	CON	55.8	39.1	1.23	4.6	4.0	0.032	3.3	1.3	4.6	0.28
	ORG	212.9	119.7	6.33	17.6	20.6	0.053	9.4	6.0	11.3	0.53
Carrot	CON	53.9	29.9	1.31	3.2	4.6	0.044	2.6	3.5	3.1	1.13
	ORG	37.2	20.8	0.92	2.8	3.5	0.044	1.7	2.1	1.6	1.26
Potato	CON	50.3	29.2	1.04	3.3	1.4	0.036	2.0	1.8	2.4	0.75
	ORG	46.2	24.8	1.39	3.7	2.2	0.056	2.0	1.9	2.0	0.94

[a] Phospholid fatty acids (PLFA): the primary lipids composing cell membranes of living cells
[b] Neutral lipid fatty acids (NLFA): storage lipids (spores, vesicles)
[c] Ratio: saprotrophic fungal biomass/bacterial biomass

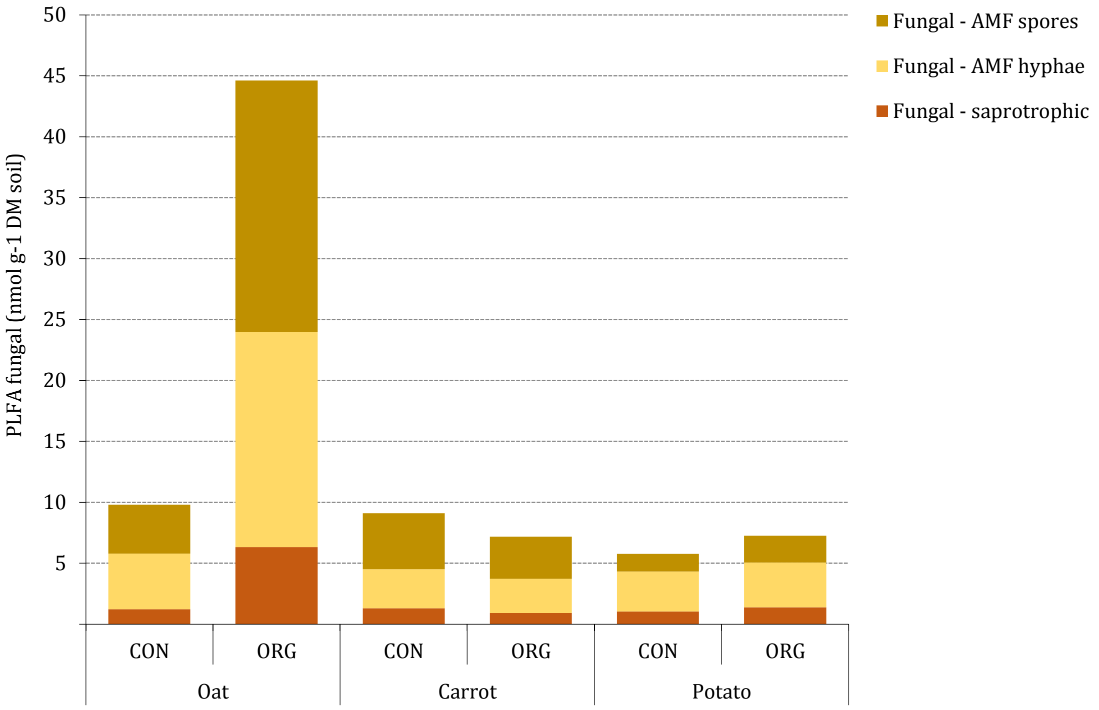


Figure 3.6 Concentration of PLFA biomarkers (saprotrophic fungi: PLFA 18:2ω6,9 and symbiotic fungi AMF hyphae: 16:1ω5) and NLFA biomarker (symbiotic fungi AMF spores: 16:1ω5) for the 0-20 cm soil depth in the organic (ORG) and conventional (CON) oat, carrot and potato field.

Actinomycetes

Actinomycetes density was numerically highest in the ORG-oat field and was 183% higher than the counts in the CON-oat field. In the carrot field highest densities were measured in the CON-field and the ORG-field had 34% less actinomycetes. Actinomycetes counts in the potato fields were very similar (ORG>CON) with 2.0 and 1.98 nmol g^{-1} respectively (Table 3.5).

Fungi : bacteria ratio and Gram+ : Gram– ratio

The fungi : bacteria ratio was higher in the ORG-oat and potato fields compared to the CON-fields, which shows relatively more fungi were present in the ORG-fields (Figure 3.7). The FB ratio was similar in the ORG- and CON-carrot fields. In the carrot fields highest Gram+ : Gram– ratios were measured and this ratio was higher for all three crops in the ORG-fields, meaning more Gram+ bacteria were present relative to Gram– bacteria (Figure 3.7).

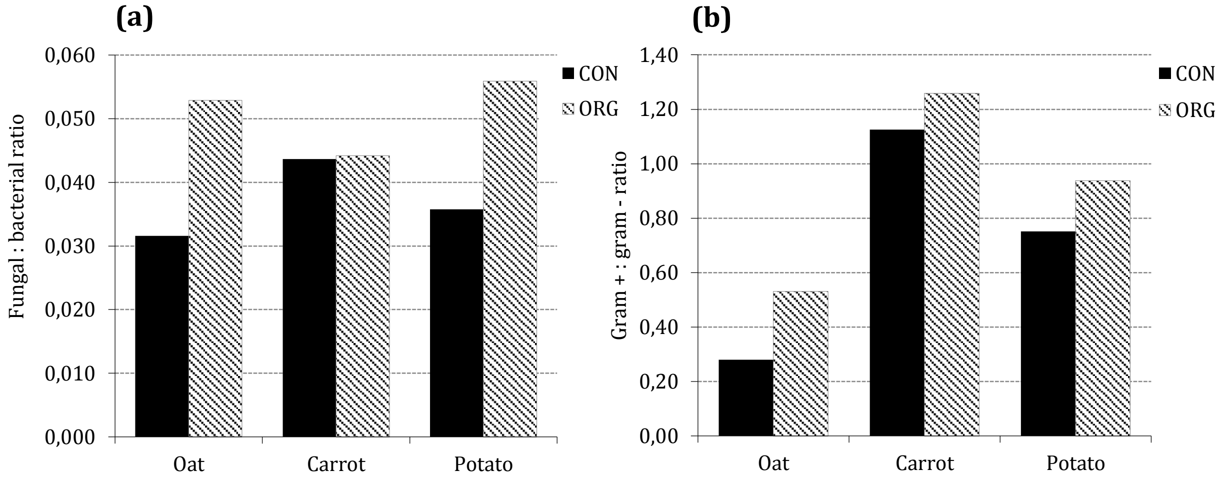


Figure 3.7 Fungi : bacteria ratio (a) and the Gram+ : Gram– ratio (b) for the 0-20 cm soil depth in the organic (ORG) and conventional (CON) oat, carrot and potato field.

3.1.4 pH | SOM | macronutrients | mesonutrients | micronutrients

The pH in all plots (0-20 cm) was close to neutral and did not significantly differ between farming systems. The pH was found to be lowest in the CON-oat and highest in CON-potato field. The SOM measurements showed significant differences between ORG-fields and CON-fields in the 0-10 and 10-20 cm soil layer (Table 3.6).

Table 3.6 SOM (%) and pH-KCl values in different soil layers, prior to the harvest of the oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system. **Sampling period:** mid-August – early October (see also Table 2.7).

		pH		**SOM**		
		KCl		**%**		
		0-20 cm	**0-10 cm**	**10-20 cm**	**20-30 cm**	*Significance ORG vs CON*
Crop	**System**					
Oat	CON	7.3	2.9	3.0	3.1	
	ORG	7.4	5.2	4.5	4.2	**
Carrot	CON	7.4	3.5	3.2	3.4	
	ORG	7.4	4.3	3.9	3.5	*
Potato	CON	7.8	2.8	2.8	2.8	
	ORG	7.5	4.1	3.9	3.3	**

[a] Differences between treatments were n.s. not significant, *, **, *** significant at p ≤ 0.05, 0.01 and 0.001, respectively

Highest SOM stocks in the 0-10 cm soil layer were observed in the ORG-oat field, followed by ORG-carrot > ORG-potato > CON-potato > CON-oat > CON-potato. In the organic plots there was an overall trend line of increasing SOM in the top soil layers which could be detected in oat, carrot and potato with increases of 0.96%, 0.77% and 0.79% respectively, from the 20-30 cm soil layer towards the 0-10 cm soil layer. This trend line was not observed in the conventional plots, where SOM stocks were relatively similar amongst all three soil layers (Figure 3.9).

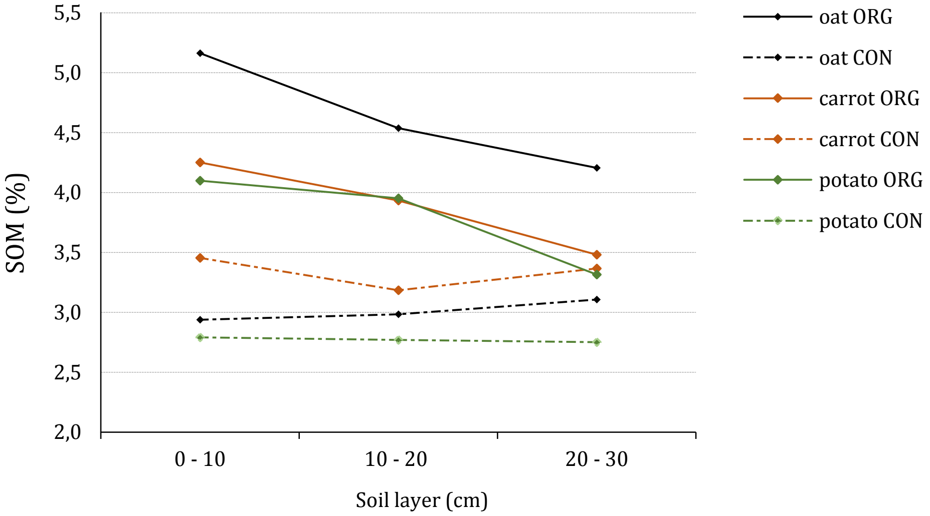


Figure 3.9 SOM (%) in different soil layers, measured in the oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system.

3.1.4 pH | SOM | macronutrients | mesonutrients | micronutrients (continuation)

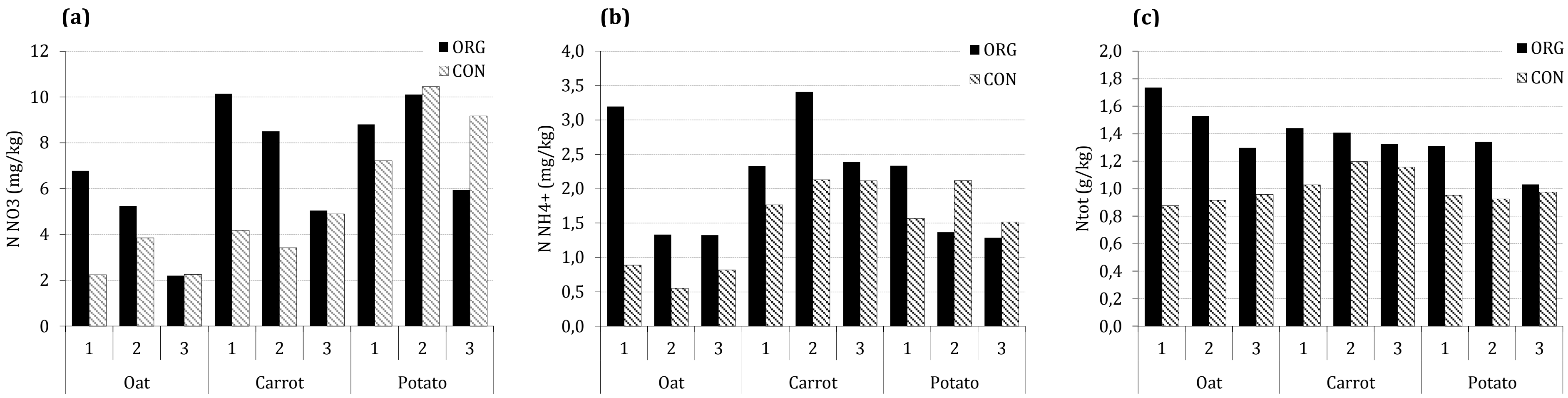


Figure 3.10. Nitrate (NO_3) (a), ammonium (NH_4^+) (b) and total nitrogen (N_{tot}) (c) in soil layers 1 (0-10 cm), 2 (10-20 cm), 3 (20-30 cm) in the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

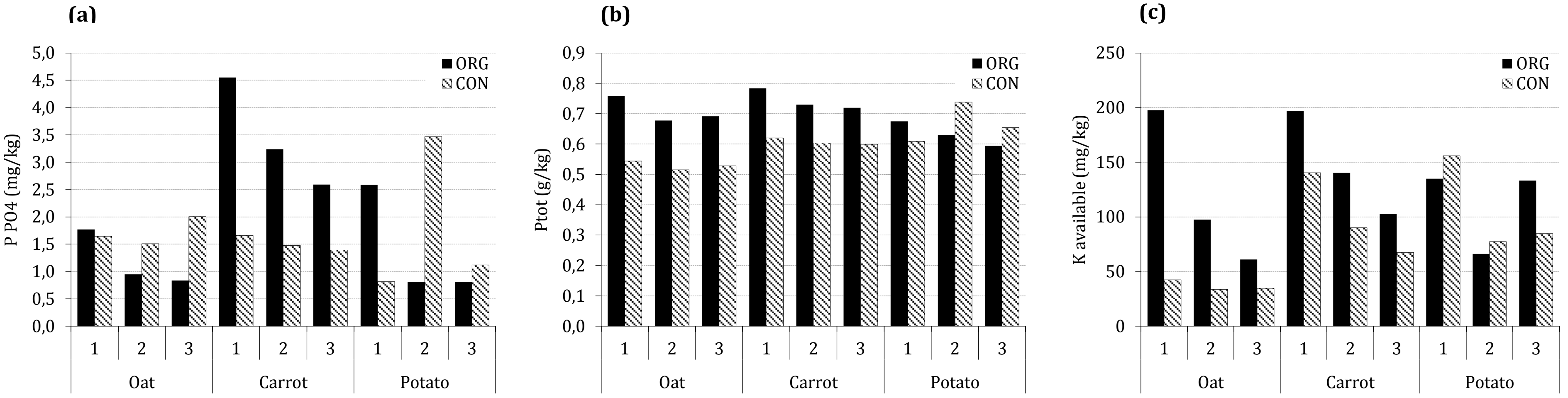


Figure 3.11 Phosphate (PO_4) (a), total phosphorus (P_{tot}) (b) and available potassium ($K_{available}$) (c) in soil layers 1 (0-10 cm), 2 (10-20 cm), 3 (20-30 cm) in the oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system.

3.1.4 pH | SOM | **macronutrients |** mesonutrients | micronutrients (continuation)

Overall, the highest soil nitrate ($N\text{-}NO_3^-$) was found in the ORG-fields, with the exception of soil layer 3 in the oat field and soil layer 2 and 3 in the potato field, where the CON-fields showed the highest soil nitrate content. The differences between the two farming systems, when shown in percentages, ranged from +203% (ORG>CON) in soil layer 1 of the oat field to +3% in soil layer 3 in the oat field (CON<ORG). The overall mean percentage difference for soil nitrate contents across soil layers and crops was +57% (ORG>CON) (Table 3.7). Soil ammonium ($N\text{-}NH_4^+$) test results showed a similar trend, with overall higher ammonium contents in the ORG-fields. However, in soil layer 2 and 3 in the potato field highest ammonium content was found in the CON-field. The largest difference in $N\text{-}NH_4^+$ between the farming systems as a percentage was again found in soil layer 1 in the oat field (+260%, ORG>CON). The lowest percentage difference was found in soil layer 3 in the carrot field (+13%, ORG>CON). The overall mean percentage difference across soil layers and crops was +63% (ORG>CON) for ammonium. Total soil nitrogen (N_{tot} - all forms of inorganic and organic soil N combined) was 40% higher in the ORG-farming system, when averaging all crops and all soil layers. The highest percentage difference was found in the oat crop, with 67% higher levels of N_{tot} in the ORG-oat field. This was followed by 30% and 24% higher N_{tot} levels in the ORG-potato and ORG-carrot field, respectively. A final note on the N_{tot} is the difference in the trend line of the two farming systems. In the ORG-fields we observed decreasing N_{tot} in deeper soil layers, whereas in the CON-fields this trend was not detected and even slightly reversed: with slightly increasing N_{tot} in the deeper soil levels (Figure 3.10). No large differences were present between the farming systems and their N_{min} fraction compared to N_{tot}, with percentages ranging from 0.27% - 1.36%. Overall, the N_{min} fractions were slightly higher in the conventionally managed fields with the exception of the ORG-oat field (soil layer 1) and the ORG-carrot field (soil layer 1 and 2), where the N_{min} fraction was higher.

The highest soil phosphate ($P\text{-}PO_4^{3-}$) contents were found in the ORG-carrot field (all soil layers) and in the CONV-potato field (soil layer 2) and ORG-potato field (soil layer 1). The differences between soil phosphate contents in the two farming systems, when shown in percentages, ranged from +330% (CON>ORG) in soil layer 2 of the potato field to +8% in soil layer 1 in the oat field (ORG>CON). Mean percentage difference for soil phosphate contents across soil layers was -29% (ORG<CON), +127% in carrot (ORG>CON) and +38% in potato (ORG>CON) leading to an overall mean difference across crops and soil layers of +45% (ORG>CON)(Table 3.7).

Table 3.7 Mean differences (cumulated soil layers 1, 2 and 3) in percentages between soil nutrient content in organic fields, when compared with soil nutrient contents in conventional fields.

	$N\text{-}NO_3^-$	**$N\text{-}NH_4^+$**	**N_{tot}**	**$P\text{-}PO_4^{3-}$**	**P_{tot}**	**K**
	%	%	%	%	%	%
Crop						
Oat	+78	+155	+67	-29	+34	+211
Carrot	+98	+35	+24	+127	+23	+49
Potato	-6	-1	+29	+38	-4	+10
Mean +/-	**+57**	**+63**	**+40**	**+45**	**+17**	**+90**

[a] Plus and minus signs refer to conventional crops as the baseline for comparison. E.g. $P\text{-}PO_4^{3-}$ is 38% more abundant in the organic potato crop (conventional 100%, organic 138%).

Total soil phosphorus content was observed to be 17% higher in the ORG-fields when compared to the CON-fields. The highest P_{tot} was found in the ORG-carrot field (soil layer 1) with 0.78 gr P kg. Lowest P_{tot} was found in the CON-oat field (soil layer 2) with 0.52 gr P kg.

In seven of the nine comparisons (soil layer x crop) soil available potassium (K) was to be found highest in the ORG-fields. Higher soil available K in the CON-fields was found in soil layer 1 and 2 in the potato field (Figure 3.11c). Highest soil available K was found in soil layer 1 in the ORG-oat field (197.6 mg K kg) and in soil layer 1 in the ORG-carrot field (196.8 mg K kg). Large differences between soil available K were present in the oat fields, with 211% higher K levels found in the ORG-field. Across crops and soil layers, the mean difference between the organic and conventional fields is +90% (ORG>CON).

3.1.4 pH | SOM | macronutrients | **mesonutrients | micronutrients** (continuation)

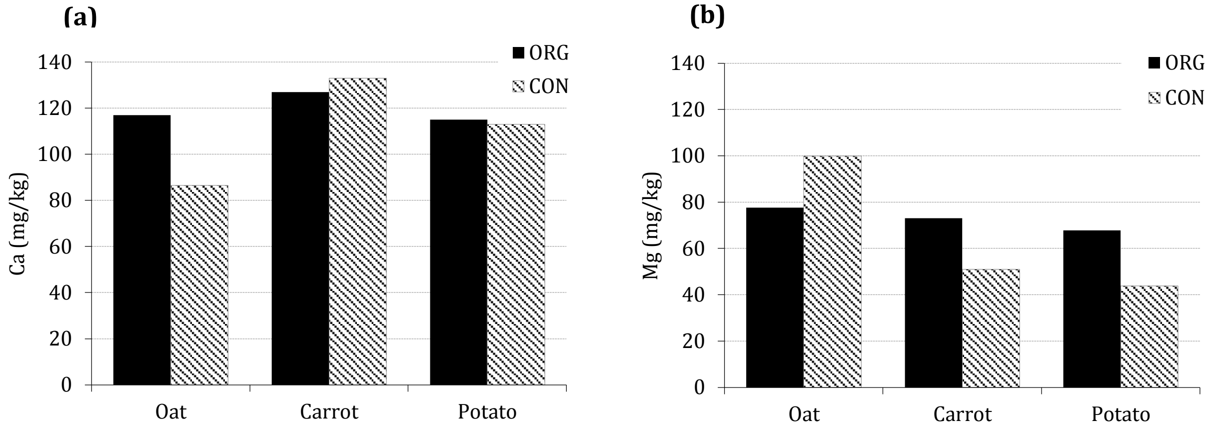


Figure 3.12 Calcium – H_2O extracted (a) and magnesium – $CaCl_2$ extracted (b) in soil layer 0-20 cm in the oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system.

The highest soil calcium (Ca) contents were found in the CON-carrot field with Ca levels of 133 mg kg^{-1} dry soil (Figure 3.12a, see appendix for full data sheet). The ORG-carrot field had 5% less Ca compared to the CON-field. The ORG- and CON-potato fields had similar soil Ca contents of 115 and 113 mg kg^{-1}, respectively. In the oat fields, the CON-field had 35% less soil Ca then the ORG-oat field.

The highest soil magnesium (Mg) contents were found in the CON-oat field (100 mg Mg kg^{-1}), which is 22% more than present in the ORG-oat field (Figure 3.12b). Both the ORG-carrot field as ORG-potato field showed higher soil Mg contents, with differences of 43% and 55% compared to the CON-fields, respectively.

The highest soil sulphur (S) contents were found in the CON-potato field with 11.2 mg S kg^{-1}, which is 70% more S then found in the ORG-potato field. On the contrary, the ORG-carrot and ORG-oat had more soil S compared to the CON-fields (Figure 3.13a – following page).

Soil copper (Cu) was higher in the oat, carrot and potato crop with 100%, 67% and 20%, respectively, compared to the CON-fields, resulting in a mean percentage difference of 62% (Table 3.8). Highest soil Cu levels were found in the ORG-oat and ORG-potato field (Figure 3.13b).

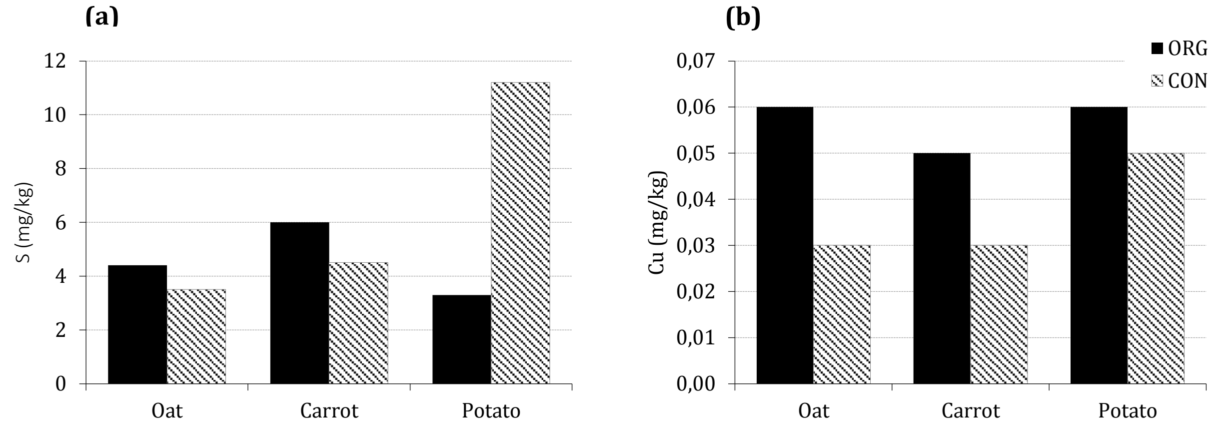


Figure 3.13 Sulphur – $CaCl_2$ extracted (a) and copper – $CaCl_2$ extracted (b) in soil layer 0-20 cm in the oat, carrot and potato crop in an organic (ORG) and conventional (CON) farming system.

No readable soil iron and zinc contents were measured in this analysis. The lowest measurable fraction of plant available iron and zinc is 3 and 0.3 mg kg^{-1}, respectively. Fractions as high as this were not found in the soil samples analyzed in this experiment.

Table 3.8 Mean differences in percentages between soil nutrient content in organic fields, when compared with soil nutrient contents in conventional fields.

	Ca	**Mg**	**S**	**Cu**	**Fe**	**Zn**
	%	%	%	%	%	%
Crop						
Oat	+35	-22	+26	+100	n.d.[b]	n.d.
Carrot	-5	+43	+33	+67	n.d.	n.d.
Potato	+2	+55	-71	+20	n.d.	n.d.
Mean +/-	**+11**	**+25**	**-4**	**+62**	n.d.	n.d.

[a] Plus and minus signs refer to conventional crops as the baseline for comparison. E.g. copper is 67% more abundant in the organic carrot crop (conventional 100%, organic 167%).
[b] No data available for soil iron and zinc as the soil concentrations were below the detection levels.

3.2 CROP PROPERTIES

3.2.1 Biomass and dry matter

The oat yield (t ha^{-1}) was higher in the ORG-farming system compared to the CON-farming system, based on field measurements and farmers estimates of the organic crop and farmers estimates of the conventional crop (Table 3.9). When based on farmer's estimations solely, the CON-farming system performed better with a total yield of 7.5 t ha^{-1} versus 6 t ha^{-1} in the ORG-farming system. The yield of the ORG-oat was 7.9 t ha^{-1} based on field measurements. The relatively large variation between the farmers estimates and field measurements might result from the selection and sampling from optimal sites in the centre of the plot which do not represent the actual oat yield in the plot edges, where the crops suffers from a high density of field sow thistles (*Sonchus arvensis,* Nederlands: *Akkermelkdistel*), a weed that creates suboptimal conditions for the oat crop. Additional cause of the variation could be the differences in moisture content at time of weighing. This is a result of different drying periods of the oat crop, as the farmer's estimates are based on oat kernel biomass after a substantial period of drying on the field whereas the field measurements were performed directly after sampling.

The carrot yield and carrot leaf biomass was significantly higher in the CON-farming system, with 138% and 91% respectively (Table 3.9). The DM percentages of both carrot and carrot leaf did not significantly differ. The carrot number m^{-1} was 30 (ORG) and 62 (CON) resulting in an average carrot weight of 166 gr in the ORG system and 187 gr in the CON-system.

The potato yield did not differ significantly however; DM percentages showed significant differences. The fresh potato yield was 20% higher in the CON-system compared to the ORG-system whereas the dry potato yield was only 3% higher in the CON-system compared to the ORG-system. The DM percentage of potatoes in the ORG-system was 4% higher compared to the CON-potatoes. The potato number m^{-1} was 45 (ORG) and 49 (CON) resulting in an average potato weight of 66 gr in the ORG-system and 74 gr in the CON-system.

Table 3.9 Mean yield and above ground biomass (gr m^{-2}) of the oat, carrot and potato crop and their dry matter percentage in an organic (ORG) and conventional (CON) farming system.

		Yield gr m^{-2}		**DM %**	**Above ground biomass gr m^{-2}**		**DM %**	**Yield t ha^{-1}**
		Fresh	**Dry**		**Fresh**	**Dry**		
Crop	**System**							
Oat (*n*=8)	CON	n.d.	n.d.	-	n.d.	n.d.	-	7.5[b]
	ORG	834[cd]	645	77	1517[e]	447	30	**7.9** (6.0[b])
Significance[a]		-	-	-	-	-	-	**-**
Carrot (*n*=4)	CON	11620	1289	11	1806	292	16	**116.2**
	ORG	4886	532	11	946	152	16	**48.9**
Significance		***	***	n.s.	***	***	n.s.	**-**
Potato (*n*=4)	CON	3581	692	19	n.d.	n.d.	-	**51.2**
	ORG	2973	674	23	n.d.	n.d.	-	**42.5**
Significance		n.s.	n.s.	**	-	-	-	-

[a] Treatments were n.s. not significant, *, **, *** significant at $p \leq 0.05$, 0.01 and 0.001, respectively.

[b] As estimated by farmer (de-husked weight).

[c] Yield (kernel weight) is measured after de-husking.

[d] Oat yield is in gr m^{-2}.

[e] Above ground biomass is oat shoot + husk + green manure (excluding kernel weight).

3.2.2 Crop nutritional status – macro and micronutrients

Nitrogen (N) was found to be highest in the ORG-oat crop, with 15.5 g kg^{-1} oats followed by the CON-oat with 14.5 g kg^{-1} (Figure 3.14a). In the carrot crop higher N contents were found in the ORG-crop whereas in the potato crop higher N contents were found in the CON-crop. Phosphorus (P) was observed to be higher in the ORG-oat crop, closely followed by the CON-oat crop (Figure 3.14b). ORG- carrots and potatoes had higher amounts of P then the CON-crops, with 41% and 35%, respectively (Table 3.10 – following page). The highest level of potassium (K) was found in the ORG-carrot crop (Figure 3.14c). The ORG-carrots contained 59% more K than the CON-carrots. The ORG-oat contained 357% more K than the CON-oat, containing 21.7 and 4.7 mg K kg^{-1} respectively. The ORG-potatoes however, contained 10% less K then the CON-potatoes.

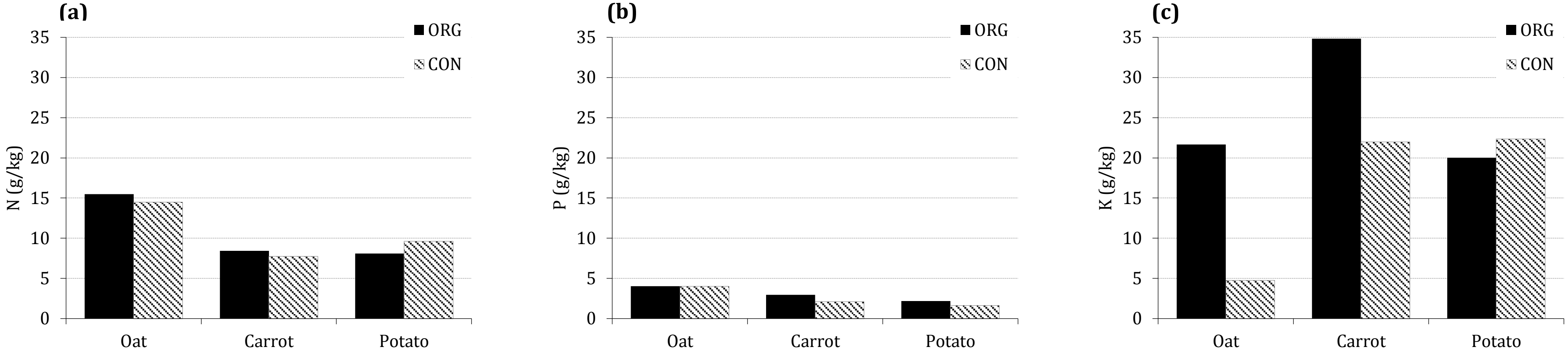


Figure 3.14 Nitrogen (a), phosphorus (b) and potassium (c) (g kg^{-1} dry matter) in the edible plant tissues of the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

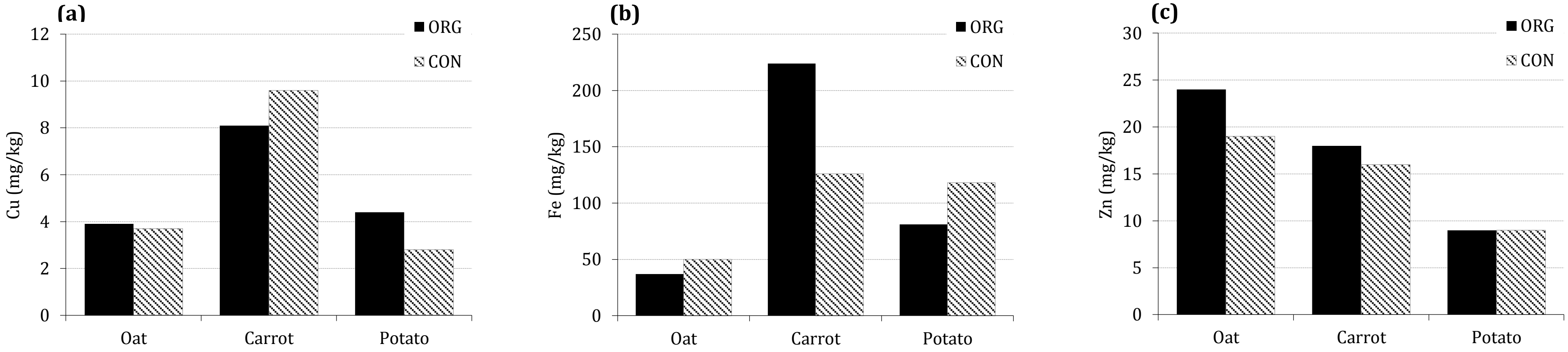


Figure 3.15 Copper (a), iron (b) and zinc (c) (mg kg^{-1} dry matter) in the edible plant tissues of the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

Copper contents were highest in the CON-carrot crop (9.6 mg Cu kg^{-1} dry matter), followed by ORG-carrot (8.1 mg Cu kg^{-1} dry matte)(Figure 3.15a). ORG-carrots contained 16% less Cu than the CON-carrots. Differences in Cu content were relative small in the oat crop (ORG>CON; +5%) and large in the potato crop (ORG>CON; +57%). The crop Cu content was on average 16% higher in the ORG-fields compared with the CON-fields.

Iron contents were highest in the ORG-carrot crop (224 mg Fe kg^{-1} dry matter), followed by CON-carrot (126 mg Fe kg^{-1} dry matter). Fe content was 78% higher in the ORG-carrots (Table 3.10). CON-oat and potato contained more Fe than the ORG-oat and potato, -26% and -31%, respectively. The mean percentage difference in crop Fe content between the farming systems is 7%.

Zinc contents were highest in the ORG-oat crop (24 mg Zn kg^{-1} dry matter), followed by CON-oat (19 mg Zn kg^{-1} dry matter) resulting in a 26% higher Zn content of ORG-oat (Table 3.10). ORG-carrots contained 13% more Zn than CON-carrots whilst no differences in Zn amounts could be detected in the potatoes.

Table 3.10 Mean differences[a] in nutrient content of organic versus conventional crops

	N %	**P** %	**K** %	**Cu** %	**Fe** %	**Zn** %
Crop						
Oat	+7	0	+357	+5	-26	+26
Carrot	+9	+41	+59	-16	+78	+13
Potato	-16	+35	-11	+57	-31	0
Mean +/-	**-1**	**+25**	**+135**	**+16**	**+7**	**+13**

[a] Plus and minus signs refer to conventional crops as the baseline for comparison. E.g., phosphorus is 35% more abundant in the organic potato crop (conventional 100%, organic 135%).

3.3 CORRELATION ANALYSIS

3.3.1 Spearman rank correlation analysis

The correlation analysis in Table 3.11 shows that SOM had a strong positive relation with N$_{tot}$ (r=0.99) and a moderate positive correlation with P$_{tot}$ (r=0.60) and EW$_{density}$ (r=0.60) however no significant relation with other parameters. Available P showed strong negative correlations with all biological parameters, significant in the case of act (r=-0.94), AMF$_{sp}$ (r=-0.83), fungi (r=-0.83), bact$_{tot}$ (r=-0.89) and PLFA$_{tot}$ (r=-0.89) and moderate negative but non-significant in the case of EW$_{density}$ (r=-0.54) and AMF$_{hyp}$ (r=-0.66). Available Mg showed a strong positive correlation with EW$_{density}$ (r=0.89) and negative with pH (r=-0.83). PH had a moderate to strong negative correlation with all soil biological parameters which was significant in the case of EW$_{density}$ (r=-0.89) and AMF$_{sp}$ (r=-0.83).

Table 3.11 Spearman's rank correlation matrix of soil chemical and biological parameters, soil organic matter (SOM), available nitrogen (N), total nitrogen (N$_{tot}$). available phosphorus (P), total phosphate (P$_{tot}$), available potassium (K), available calcium (Ca), available magnesium (Mg), pH, earthworm density (EW$_{density}$) total bacterial (Bact$_{tot}$), saprotrophic fungi (fungi), arbuscular mycorrhizal fungi hyphae (AMF$_{hyphea}$), arbuscular mycorrhizal fungi spores (AMF$_{spore}$), actinomycetes (Act). Values in **orange** are significantly different at $p \leq 0.05$, ** $p < 0.01$, *** $p < 0.001$ (6 comparisons).

N	0.371														
N$_{tot}$	0.986***	0.464													
P	-0.257	0.714	-0.203												
P$_{tot}$	0.600	0.771	0.696	0.371											
K	0.543	0.600	0.638	0.257	0.943**										
Ca	0.543	0.257	0.580	-0.143	0.371	0.543									
Mg	0.429	-0.314	0.290	-0.314	-0.086	-0.143	-0.257								
pH	-0.257	0.714	-0.116	0.657	0.371	0.257	0.029	-0.829*							
EW$_{density}$	0.600	-0.371	0.493	-0.543	0.086	0.143	0.086	0.886*	-0.886*						
PLFA$_{tot}$	0.029	-0.829*	-0.029	-0.886*	-0.371	-0.257	-0.200	0.429	-0.714	0.600					
Bact$_{tot}$	0.057	-0.829*	-0.029	-0.886*	-0.371	-0.257	-0.200	0.429	-0.714	0.600	1.000***				
Fungi	0.429	-0.314	0.406	-0.829*	-0.200	-0.257	0.086	0.200	-0.314	0.314	0.600	0.600			
AMF$_{hyp}$	0.143	-0.486	0.087	-0.657	-0.257	-0.371	-0.543	0.543	-0.486	0.486	0.771	0.746	0.714		
AMF$_{sp}$	0.543	-0.543	0.464	-0.829*	-0.086	0.086	0.429	0.543	-0.829*	0.829*	0.714	0.714	0.543	0.371	
Act	0.200	-0.771	0.116	-0.943**	-0.429	-0.371	-0.143	0.543	-0.771	0.657	0.943**	0.943**	0.771	0.829*	0.771
	SOM	**N**	**N$_{tot}$**	**P**	**P$_{tot}$**	**K**	**Ca**	**Mg**	**pH**	**EW$_{dens}$**	**PLFA$_{tot}$**	**Bact$_{tot}$**	**Fungi**	**AMF$_{hyp}$**	**AMF$_{sp}$**

🟩	Chemical x chemical
🟩	Chemical x biological
🟧	Biological x biological

Besides the correlation between soil chemical x soil chemical and soil chemical x soil biological parameters, soil biological parameters seem to be correlated with one another. All were positively correlated, mostly moderate to strongly. EW$_{density}$ was positively correlated with AMF$_{sp}$ (r=0.83). PLFA$_{tot}$ was significantly correlated with Bact$_{tot}$ (r=1.0) and act (r=0.94). Bact$_{tot}$ was significantly correlated with actinomycetes and moderately correlated with but AMF forms (r=0.746 and 0.714). AMF$_{hyp}$ was was not strongly correlated with AMF$_{sp}$ (r=0.37) however was significantly correlated with act (r=0.829).

Table 3.12 Spearman's rank correlation matrix of soil chemical and biological parameters and crop nutrient density. Parameters consist of SOIL: soil organic matter (SOM), available nitrogen (N), total nitrogen (N$_{tot}$). available phosphorus (P), total phosphate (P$_{tot}$), available potassium (K), available calcium (Ca), available magnesium (Mg), available copper (Cu), pH, earthworm density (EW$_{density}$) total bacterial (Bact$_{tot}$), saprotrophic fungi (fungi), arbuscular mycorrhizal fungi hyphae (AMF$_{hyphea}$), arbuscular mycorrhizal fungi spores (AMF$_{spore}$), actinomycetes (Act) and CROP: nitrogen (N), phosphate (P), potassium (K), copper (Cu), iron (Fe) and zinc (Zn). Values in **orange** are significantly different at p ≤ 0.05, ** p < 0.01, *** p < 0.001 (6 comparisons).

SOM	0.143	0.657	0.086	0.486	-0.143	0.522
N	-0.257	-0.200	0.600	0.200	0.486	-0.406
N$_{tot}$	0.116	0.551	0.203	0.464	-0.087	0.426
P	-0.257	-0.429	0.543	-0.086	0.657	-0.522
P$_{tot}$	0.200	0.143	0.714	0.086	0.257	0.116
K	0.143	0.086	0.829*	0.200	0.371	0.203
Ca	-0.543	-0.086	0.543	0.886*	0.543	0.058
Mg	0.600	0.943**	-0.543	-0.086	-0.543	0.841*
Cu	0.239	0.239	0.000	-0.120	-0.359	-0.061
pH	-0.429	0.771	0.543	-0.086	0.486	-0.870*
EW$_{density}$	0.600	0.943**	-0.314	0.086	-0.486	0.986***
PLFA$_{tot}$	0.600	0.486	-0.543	-0.314	-0.771	0.638
Bact$_{tot}$	0.600	0.486	-0.543	-0.314	-0.771	0.638
Fungi	0.143	0.371	-0.600	0.086	0.714	0.232
AMF$_{hyp}$	0.714	0.600	-0.771	-0.543	-1.000***	0.464
AMF$_{sp}$	0.257	0.657	-0.257	0.371	-0.371	0.812*
Act	0.486	0.600	-0.714	-0.143	-0.829*	0.638
	N	**P**	**K**	**Cu**	**Fe**	**Zn**

A second Spearman's rank correlation matrix was formed, to review the correlations between soil chemical and biological parameters and nutrient density of the different crops (Table 3.12). Soil chemical parameter Mg had a significant correlation with crop P (r=0.94), soil available K had a significant correlation with crop K (r=0.829), soil Ca had a significant correlation with crop Cu (r=0.89) and crop Zn had a significant positive correlation with soil Mg (r=0.84) however, a significant negative correlation with pH (r=-0.87).

Soil biological parameter EW$_{density}$ showed strong significant correlations with crop P (r=0.94) and crop Zn (r=0.99), AMF$_{sp}$ showed significant correlations with crop Zn (r=0.81) and act was negatively correlated with crop Fe (r=-0.83). Crop Fe was also strongly significantly negatively correlated with AMF$_{hyp}$ (r=-1.00).

4. Discussion

The following chapter contains the interpretation of the results presented in the previous chapter. The chapter encompasses three paragraphs, describing the effects of farming system practices (fertilization and tillage) on soil biological and soil chemical properties and thirdly, describing the effect of farming system practices and soil biochemical properties on crop nutrient density.

4.1 EFFECT OF FARMING SYSTEM PRACTICES ON SOIL BIOLOGICAL PROPERTIES

4.1.1 Earthworms

4.1.1.1 Earthworm density

Total earthworm densities (no. m^{-2}) of an average of 189 individuals m^{-2} in the conventional fields (CON) and 309 individuals m^{-2} in the organic fields (ORG) were found, slightly higher than density counts in Kuntz et al. (2013) reporting 262 m^{-2} in reduced tillage (RT) fields on a clay soil in Switzerland and Crittenden et al. (2015) reporting 225 m^{-2} in non-inversion tillage (NIT) fields on a sandy loam in The Netherlands. Earthworm counts in the current study were however lower than found in Marinissen (1992), who found population densities up to 400 individuals m^{-2} in Dutch region the 'Noord Oost polder' on a silty loam soil under RT. Highest earthworm densities were found in the ORG-oat crop (575 m^{-2}) and CON-oat crop (351 m^{-2}). The high earthworm densities in the oat fields can be explained by a combination of factors such as minimum soil disturbance during the crop cycle and a (semi-closed) soil cover. Both investigated farmers had sown the oat crop in early spring, whereafter no soil disturbance took place, with the exception of shallow mechanical weeding procedures. The absence of soil disturbance during the crop cycle seems to greatly benefit the earthworm population, which becomes noticeable when comparing the earthworm counts in the oat fields with those in the potato and carrot crops. The ORG-oat field has been undersown with a leguminous mixture whilst the CON-oat crop was undersown with *Festuca rubra* (Ned: *Roodzwenkgras*). This resulted in an increased above- and belowground biomass in both fields, enhancing the food quantities and diversity and widening the time frame of available food resources for the earthworm population. Furthermore, the used cover crop (mixture) ensured a semi-closed soil cover, which might have decreased soil evaporation and thus increased soil moisture levels. This effect could have contributed to a more favourable environment for the earthworms to mate and hatch during the hot and dry summer months. Earthworms can tolerate cold and wet periods better than hot and dry periods (Gerard, 1967) and in critical times during summer, chances increase that earthworms move to deeper soil layers and become inactive. This state of inactivity is called *quiescence* or *diapause* (Edwards and Bohlen, 1996) in which the earthworm ties itself in a knot, to reduce water loss to a minimum, ultimately resulting in reduced mating and hatching behaviour. More favourable soil moisture levels reduce the chance earthworms go into this state of inactivity.

Higher earthworm counts in all the ORG-fields compared to the CON-fields result in mean higher earthworm densities of 61% in the ORG-fields. Recent research efforts investigating the effect of fertilization and tillage practices on earthworm density and population structure have frequently reported similar results (Capelle et al., 2012; Kuntz et al., 2013; Crittenden et al., 2015). Kuntz et al. (2013) reported a total earthworm density increase of 67% in minimum tillage (MT) plots (chisel plough – loosening the soil to a depth of 15 cm) compared to conventional tillage (CT) plots (mouldboard plough – inverting the upper 15 cm) in Frick, Switzerland. Crittenden et al. (2015) reported a 22% increase in NIT plots compared to CT plots in the Hoeksche Waard, The Netherlands. NIT practices in the latter study are characterised by use of the

Kongskilde Paragrubber to 30-35 cm, a practice comparable to soil cultivation practices on the organic farm in this study however here depth is shallower (18-20 cm). Our results showed that crop and corresponding management practices have, to a certain extent, a larger effect on earthworm densities then the farming system, as in the CON-oat field larger number of earthworms were found then in the ORG-carrot and potato fields. In the CON-carrot field similar earthworm counts were made as in the ORG-potato field. Tillage operations (e.g. seed bed preparation, weeding operations) used in the production of root and tuber crops as potatoes and carrots involve severe soil manipulation, operations which also apply to NIT farming systems.

Effect on ecological groups

Total epigeic and endogeic earthworm densities were found to be higher in the ORG-fields by 40% and 65%, respectively. The earthworm population consisted largely of endogeic species and they represented 76% of the population in the CON-fields and 78% in the ORG-fields, thus showing no significant difference in earthworm community composition between the two farming systems. An increase in epigeic earthworm representation of the earthworm population in the ORG- fields was expected due to higher and more diverse surface organic matter which serves as food source and also habitat for the epigeic species, classed as *detritivores. Detritivores*, consisting of epigeic and anecic earthworms, are feeding at or near the soil surface on animal and plant litter whilst endogeic species - classed as *geophages*, feed deeper in the soil on soil organic matter and dead roots whilst ingesting large amounts of soil (Lee, 1985). This distinct classification of earthworms based on food preference is supported by Doube et al. (1996), distinguishing the food preference of several lumbricids by using a choice chamber method, in which the earthworms can choose between soil, litter, litter + soil, dung, dung + soil, sludge and sludge + soil. It was shown that *A. caliginosa* (endogeic) was the most selective feeder, consuming large quantities of pure soil but little other sources. *L. rubellus* (epigeic) also fed on soil but also fed on soil + organic matter and pure organic matter (Figure 4.1). *L. rubellus* had the most active and vigorous feeding behaviour, having emptied almost all feed tubes at day 7 and being least discriminating regarding their food sources. In the ORG-fields studied large quantities of organic matter were applied to the soil and it was thus expected that in the earthworm communities in the ORG-fields, epigeic earthworm representation would rise.

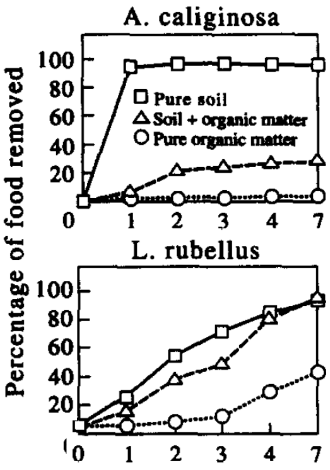


Figure 4.1 Attractiveness of pure soil, a mixture of soil and organic matter and pure organic matter to two earthworm species.
Source: Doube et al. (1996)

Furthermore, increased epigeic earthworm representation was expected due to the enhanced habitat conditions in the ORG-fields caused by the permanent soil cover in the winter by the used green manures. The green manures in winter time ensure a more stable soil temperature with less fluctuations with cold (and hot) weather, compared to bare fields. While *A. caliginosa* (endogeic) hibernates in winter in deeper soil layers, the *L. rubellus* (epigeic) is confined to the topsoil layer (top 10cm), as reported in Marinissen (1992) and thus more prone to cold temperatures.

Anecic earthworms (e.g. *Lumbricus terrestris, Aporrectodea giardi)* were left out of our analysis as none were identified in both CON- as ORG-fields. The low- to non-existence of *L. terrestris* in arable land has been reported by several authors (e.g. Roarty & Schmidt, 2013; Crittenden et al. 2015). Results comparable with the current study were found in the Hoeksche Waard by Crittenden et al. (2015) with *L. terrestris* counts of 0-3 m^{-2} in NIT plots and up to 12 m^{-2} in adjacent field margins, in a field which had been under NIT operations for two years before earthworm sampling. Roarty & Schmidt (2013) report *L. terrestris* counts of 0-1 m^{-2} in minimum tillage (MT) plots and up to 1.3 m^{-2} in adjacent field margins, in an experiment which had been under MT operations for three years before earthworm sampling. In these cases the field margins seem to be supporting *L. terrestris* as disturbance is non-existent and food sources are, in most cases, of higher quality, quantity, diversity and stability then in the arable field. The earthworms do not seem to disperse out in adjacent NIT and MT plots which might be due to the short time frame of the NIT and MT practices, installed just two-three years before sampling. Nuutinen et al. (2011) reported the spread of inoculated *L. terrestris* in no-till (NT) plots, with a clear spatial gradient across the field developed after 13 years practicing NT. In this study, densities declined from 14 to 1 ind. m^{-2} with distances of 5-9 m to 56-60 m from the field margin, while 7 years after instalment of NT practices no increases outside the field margins were found (Nuutinen et al. 2006). Based on dispersal distances in m^{-1} and earthworm densities in the latter study, the authors estimated the rate of spread of *L. terrestris* at 4.6 m^{-1} $year^{-1}$ which is similar to the mean yearly dispersal distance of 4.5 m^{-1} $year^{-1}$ estimated for *L. terrestris* in Dutch polder pastures (Hoogerkamp et al. 1983). Ligthart and Peek (1997) estimated the dispersal rate of *L. terrestris* to be 6.3 m^{-1} $year^{-1}$. However, others measured active over-surface dispersal distances of *L. terrestris* of up to 19 m^{-1} $night^{-1}$ (Mather & Christensen, 1988).

In the current study the presence of anecic earthworms in the ORG-fields was expected since the fields have not been ploughed (inverted) in the last 5 years and on-farm experiments with NIT have been present for over 10 years. Furthermore, the farmer had observed earthworm burrows large in diameter which further strengthened the expectations. In this study earthworm pits were dug 25 meter from the field margin and according to the reported dispersal rates of 4.6 m^{-1} $year^{-1}$ of Nuutinen et al. (2011) chances were relatively low but present to find the anecic *L. terrestris*. An important difference between the study of Nuutinen et al. (2006, 2011) and the current study are the cultivation practices investigated, NT and NIT tillage practices respectively. Although NIT consist of severely less soil disruption then CT, it is still much more intensive than strict NT systems and NIT practices might be too disruptive to the burrows of anecic species. Their low-branching (Jégou et al. 2000), vertically oriented burrow system is extending to a depth of 2.5 m (Edwards and Bohlen, 1996). Felten and Emmerling (2009) studied the burrowing behaviour of several earthworm species in 2D terraria and reported little burrowing activity by *L. terrestris* after day 6, whilst endogeic and epigeic earthworm species showed an explorative burrowing behaviour, up to day 14. The anecic species seldom created more than one burrow per individual and the energy usage in the creation of a burrow is high as the burrow is used permanently, with the earthworm inhabiting the burrow over several seasons (Shipitalo and Butt, 1999). The disruption of burrows is especially severe in crop rotations including tuber and root crops (e.g. potato, carrot) due to the linked crop cultivation practices. This involves - even in NIT farming systems - sub- and top soil cultivation (loosening), ridge building and harvesting machines which all increases the chance of damaging earthworms in general and proportionally large-sized anecic species the most. Furthermore, the cultivation practices harm the energy-intensive, permanent burrows (Chan, 2001).

Besides land use and related mechanical disturbance, a third predictor variable exists for the earthworm population composition; namely food stocks and the quality of those. As mentioned before, anecic and epigeic earthworms are classed as *detritivores*, feeding at or near the soil surface on animal and plant litter whilst endogeic species are classed as *geophages*, feeding deeper in the soil on soil organic matter and dead roots whilst ingesting large amounts of soil (Lee, 1985). Detritivorous species differ in feeding behaviour (Doube et al. 1996) and show different responses to the food present. In research of Shipitalo et al. (1988) alfalfa leaves and red clover leaves were preferred by both *L. terrestris* and *L. rubellus* (compared to corn- and bromegrass leaves) however induced different weight gains, with alfalfa leaves and clover leaves inducing slight weight gains in *L. terrestris* (25%) and large weight gains in *L. rubellus* (75%-100%). Food consumption rates were three or more times greater for *L. rubellus* then for *L. terrestris*; consuming 52 and 13 mg dry alfalfa leaves gr^{-1} live worm weight day^{-1}, respectively. The estimated litter consumption values of the *L. terrestris* mentioned here are similar to the consumption rates in laboratory settings found in Curry and Bolger (1984) of 15 mg dry Salix leaves gr^{-1} live worm weight day^{-1}. Van Rhee (1983) reported consumption rates of 10 mg dry alder leaves gr^{-1} live worm weight day^{-1}, with values up to 66 mg dry alder leaves gr^{-1} live worm weight day^{-1} in cases where the worms were fed on poor quality litter. When comparing the laboratory results with field conditions, a decrease in litter consumption of 30% is expected under field conditions (Curry and Bolger, 1984) due to suboptimal (e.g. temperature, soil) conditions in the field slowing down metabolic processes in the earthworm. Assuming an average litter consumption of 10 mg gr^{-1} day^{-1} by a population of 10 gr *L. terrestris* m^{-2} a litter requirement of 36.5 gr m^{-2} yr^{-1} accounts and thus 0.36 t DM ha^{-1}. However, Curry and Bolger (1984) came to the conclusion that only 69% of litter is available for earthworms, due to microbial functioning, thus total litter requirement becomes 0.52 t DM ha^{-1} yr^{-1} to sustain an *L. terrestris* population of 10 gr m^{-2}. This estimate is in the same range as the hypothesized annual feeding rates by *L. terrestris* in an alfalfa-orchard grass plot of 0.49 t ha^{-1} yr^{-1} and the 0.22 t ha^{-1} yr^{-1} in a continuous corn crop in a no-till plot (Shipitalo et al. 1988). For an earthworm population (all ecological groups combined) of 60 g m^{-2}, Curry et al. (1995) calculated a food requirement of 3.4 – 10.5 t DM ha^{-1} yr^{-1} (thus: 0.56 – 1.75 t DM ha^{-1} yr^{-1} in an earthworm population of 10 gr m^{-2}). The food requirement for a population of *L. terrestris* of 10 gr m^{-2} (± 2 adult *L. terrestris* species) thus seems to range from 0.22 – 1.75 t DM ha^{-1} yr^{-1}.

These numbers present a range rather than absolute numbers and the litter consumption rates are very variable, taking into account that not only quantity in t ha^{-1} determines earthworm population densities. Hendriksen (1990) found that litter preference by *Lumbricus* species is strongly correlated with C:N ratios and phenolic contents of the litter, both factors related to the palatability of the litter whilst *geophage* numbers were not correlated with litter palatability. Other criteria known to influence earthworm growth rates and diversity is the litter particle size (Boström and Lofs-holmin, 1986; Lowe and Butt, 2003), litter N, litter carbohydrate contents and the microbiota present on the litter.

Periodically insufficient organic matter on the topsoil is highly unfavourable for *detritivores* (Butt et al. 2003). In a laboratory study, Shipitalo et al. (1988) showed a decline in initial biomass of 11% and 35% for *L. terrestris* and *L. rubellus* respectively, after the earthworms inhabited a pot mineral soil where all organic debris bigger than 2 mm had been removed for 32 days. *L. rubellus* seemed most vulnerable to a period with no food, a result which is similar to Hartenstein (1984), who reported *L. rubellus* having the lowest survival rate (75%) after a starvation period of 4 weeks at 15°C, when comparing it with eight other species. Sufficient feed on the soil surface benefits especially *L. terrestris*, as this specie explores a circular area of only 0.28-0.63 m^{-2}, whilst remaining within the burrow with the lower part of its body (Nuutinen and Butt, 2005). A lack of food resources triggers over-surface movement by *L. terrestris*, leaving its burrow for short distance travel to feed and mate (Butt et al. 2003). The fact that in this study in some periods of the year, especially in the potato and carrot crop, no fresh litter was present as food for the *detritivores* can be one of the causes no *anecic* population is currently present. However, an *epigeic* population exists, in both conventional and organic fields who are having similar food preferences and feeding behaviour then *anecic* earthworms (Bouché, 1977; Lee, 1985) and are most vulnerable to periods without food (Hartenstein 1984; Shipitalo et al. 1988). This fact could lead to the hypothesis that mechanical disturbance has the biggest impact on *anecic* earthworms, as *epigeic* species seem to be able to survive the mechanical disturbance. This hypothesis is supported by Pelosi et al. (2009), reporting different earthworm communities in conventional, conventional direct seeding (‘*living mulch system*’) and organic arable farming systems with *anecic* densities being 3.3 and 3.6 times higher in the conventional living mulch system than the organic and conventional systems, respectively.

In addition to the effects of land use, related mechanical disturbance and food stocks there is a fourth predictor variable for the existence of *L. terrestris* in a farming system, namely soil texture. A classification tree predicting the abundance of *L. terrestris* presented in Lindahl et al. (2009) showed the effect of soil texture on *L. terrestris* numbers, wherein fine (silty clay, clay, clay loam, silty clay loam) and coarse textured soils (loamy sand, sand) are found least favourable to support high numbers of *L. terrestris* and medium (sandy clay, sandy clay loam, sandy loam, loam, silt loam, silt) textured soils found to be most favourable, comparing several earthworm articles/reports (n=86). Clay fractions in the soils in this study are not exceeding 25% and all investigated soils fall in the medium texture class ‘loam’ (classified as either moderate or heavy loam) and therefore soil texture is not seen as predictor variable for the large differences in earthworm counts in this study.

Lastly, there is an indication that the use of the combined sampling method (hand sorting followed by a mustard solution as an expellant) might have underestimated the earthworm results, with emphasis on the (non-existent) *anecic* earthworm counts. The excavation procedure might lead to earthworms escaping to deeper parts of the soil profile which especially accounts for the deep vertically burrowing *anecic* species and without strong expellant (e.g. allyl isothiocyanate (AITC) or formalin) it is difficult to ensure the *anecic* species to resurface.

Effect on age classes

The density of juvenile earthworms was significantly higher in the ORG-carrot and ORG-potato crop compared to the CON-plots, with 95% and +65% respectively and differed not significantly in the ORG-oat crop, where juvenile earthworm density was 45% higher in the ORG-field, resulting in a difference of 68%. This is slightly lower than the juvenile density differences of +82% reported by Kuntz et al. (2013) in a RT field compared to a CT field, in Frick, Switzerland. In the current study, highest absolute juvenile density was found in the ORG-oat field with 419 m^{-2} whilst lowest juvenile counts were found in the CON-potato field with 61 m^{-2}. The high juvenile abundances in the ORG-fields in this study might be a result of the enhanced habitat conditions for juvenile earthworms. In the ORG-fields a clear SOM accumulation is to be found in the topsoil, known to increase water retention and soil moisture levels in the topsoil. Juvenile earthworms are more sensitive to drought then adults, as they are too weak and small to efficiently reach subsoil layers in dry periods (Sims and Gerard, 1985) and increased SOM in the top soil thus reduces juvenile mortality. Secondly, increased juvenile densities might be related to the energy allocation of the sexually mature adults. It is known that burrowing entails a high energy investments and as in ploughed field's disturbance is maximal, this destroys the semi-permanent burrowing system, resulting in a high energy and time allocation of the earthworms to the reconstruction of their burrow (Lavelle, 1981). It is assumed this is at the expense of mating and hatching rates (Lee, 1985). Densities of adult earthworms were observed to be significantly higher in the ORG-oat crop by 149%. No significant difference was found in the carrot fields and a slight lower adult earthworm abundance was observed in the ORG-potato field compared to the CON-potato field. On average, earthworm densities were found to be 56% higher in the ORG-fields. We assume that the significant difference in adult earthworm densities in the ORG-oat field compared to the CON-oat field, results from the preceding crops in the rotation, naming a leguminous green manure (2013) and yellow mustard (2014), both crops not entailing severe soil disturbance, compared to root and tuber crops. This increases the chances for adult earthworms to survive autumn and winter and thus sustain a more mature, thus adult, population in the following year.

4.1.1.2 Effect on earthworm biomass

Earthworm biomass measurements showed similar trends as earthworm densities and the biomass was on average 59% higher in the ORG-fields. Total epigeic earthworm biomass was 12% higher whilst endogeic earthworm biomass was 83% higher in the ORG-fields. This result is similar to the findings reported by Ernst and Emmerling (2009), where no significant difference was found in epigeic biomass between different tillage treatments. It seems that epigeic earthworms are found in higher numbers in the ORG-fields however biomass does not differ much. As previously discussed, it can be assumed that NIT practices are still too disturbing for epigeic earthworms, as they habit the litter and top of the soil. With NIT practices the top soil is being loosened and for a fragile organism as the earthworm this might be still too harmful.

4.1.2 Arbuscular mycorrhizal fungi

In the following paragraph the effects of farming system practices on mycorrhizal colonization of the roots of the crops will be discussed. Arbuscular mycorrhizal fungal (AMF) root colonization has been examined and quantified by use of two methods, including a modification of the grid-line intersect (GLI) method and by use of a phospholipid fatty acid (PLFA) analysis. The first method assesses the AMF root colonization at a soil depth of 0-10 cm and 10-20 cm separately whereas soil layers are aggregated for the PLFA analysis thus assessing the 0-20 cm soil layers as a whole. The modified GLI method reports the AMF structures (arbuscles, vesicles, spores, hyphae) separately whilst the PLFA analysis only divides the AMF structures in hyphae and spores. In the following paragraph the results of both the PLFA analysis as the GLI method will be discussed. The PLFA *analysis is expected to give the most precise and reliable results and is therefore the directive of this paragraph. The results of the modified GLI method are then added to the discussion. The results of the latter method are expected to be slightly less reliable, however a valuable addition as the soil layers 0-10 cm and 10-20 cm are subdivided as are the different AMF structures.*

One of the most striking findings of both PLFA and GLI analysis is the extremely high total fungal (saprotrophic (SF) and arbuscular mycorrhizal fungi (AMF)) biomass in the ORG-oat field. Several aspects related to farming system practices might explain this. Firstly, no severe soil cultivation has been performed since autumn 2012 in the ORG-oat field as the cultivated crops consisted of a sequence of green manures (GM) – yellow mustard – oat, all not involving severe disturbance such as root and tuber crops require (ridging and harvest). The CON-oat field was ploughed with a mouldboard (25 cm) in autumn 2014. It is known that within soil microbial community's fungi are especially vulnerable for tillage practices as it disrupts their hyphal networks (Miller and Jastrow, 1990; Lowell and Klein, 2001). Groenigen et al. (2010) reports significant increases in total fungal biomass (SF and AMF) after 8 years of reduced tillage (stubble cultivator; 7-10 cm) in a wheat crop in Ireland. Fungal biomass increased significantly by 30% in the 0-5 cm soil layer in reduced tillage plots (RT) compared to mouldboard ploughed plots whereas fungal biomass increased by 77% in the 5-20 cm soil layer in the RT plots. Other authors report similar results on the effects of reduced tillage on fungal biomass (Doran, 1980; Norstadt and McCalla, 1969). In the current study SF biomass was 413% higher and AMF hyphae and spores were 286% and 415% higher, in the ORG-oat field in the 0-20 cm soil layer which is quite outside of the 'normal' range of fungal biomass increases in RT fields. An explanation of this significant difference beyond the known ranges might be the carbon supply by the roots to the AM fungi. At the time of soil sampling a flowering cover crop was present in the oat field, including mallow and several clover species. This mixed cover crop ensured a high biodiversity, above and below ground, resulting in a diversified and potentially enriched amount of root exudates for the AMF. The enhanced above ground species diversity in the ORG-oat field might lead to a higher species diversity of AMF, potentially leading to an overall higher density as the habitat for the AMF species is enhanced (Kirchmann and Bergstrom, 2008). In a field experiment in Minnesota, USA, plant diversity increased from one to 16 plant species per plot and in parallel sporulation and AMF species diversity increased, especially the larger-spored AMF species (Burrows and Pfleger, 2002). Furthermore, AMF spore germination, one of the stages prior to root colonization, can be sped up by increased host root exudates (Nagahashi and Douds, 2000). Most reports evaluate agricultural management factors, either the effect of tillage or the effect of quality and quantity of fertilizers on fungi however do not take into account a cover crop and a diversified below ground biomass. That might be (one of) the reasons for the high and previously unreported abundance in fungal biomass in the ORG-oat field in the current study.

Another reason for the high fungal biomass in the ORG-oat field might be the P status of the ORG-soil. In the Spearman's rank correlation matrix it was found that SF and AMF spores were significantly negative correlated with soil available P whilst AMF hyphae was not-significantly negative correlated with available P . Total P had a weak negative correlation with SF and AMF biomarkers. Available P was slightly lower in the ORG-oat field compared to the CON-oat field at time of soil sampling and it is expected that available P in the beginning of the growing season was severely lower in the ORG-oat field, caused by the non-application of synthetic fertilizers or manure.

The decrease in soil disturbance, hypothesised increase and diversification of root exudates, reduced soil available P and furthermore the non-use of synthetic pesticides and fungicides might have all benefitted the habitat for AMF and supported the steep increase in the ORG-oat field, compared to the CON-crop. Other, more inherent soil physical and chemical properties, as soil moisture, pH and temperature are known to be influencing the development of AMF (Bellgard, 1993) however little information about soil moisture and soil temperature is known for the researched fields. It may be assumed that soil moisture levels were higher in the ORG-fields due to the cover crops usage and linked reduced soil moisture evaporation (Haramoto and Brainard, 2012) however this remains speculation. No significant differences in soil pH were found between the ORG- and CON-fields, however, in the Spearman's rank correlation matrix a significant negative correlation was found between AMF spores and pH. Due to the strikingly high AMF densities in the ORG-oat field one would almost forget the preceding crop was a *Brassica*, namely yellow mustard. *Brassica's* are non-hosts for AMF resulting in similar effects as one would see during a bare fallow, where AMF is (inactively) present in the soil in the form of spores and hyphae (Bellgard, 1993) and successful AMF root colonization is declined in the following crop (Thompson, 1987). No effect of the *Brassica* crop could be noticed; however strong conclusions cannot be drawn from this observation as many interfering factors were present in the ORG-oat field and no soil samples with AMF measurements were taken during previous crops.

AMF root colonization in the ORG- and CON-potato fields was relatively similar, with slightly raised AMF counts in the ORG-field. The PLFA analysis shows that the AMF spore density in the ORG-field is the main contributor to the overall higher AMF counts in the ORG-field. The AMF hyphae density is only showing a minor positive trend line in the ORG-field. In the oat crop similar results appear and it therefore seems that sporulation benefits more from the ORG-farming system practices than AMF hyphae. The soil disturbance in the ORG-farming system, although practicing non-inversion tillage, might be still too disruptive for the sensitive hyphae.

In the carrot fields, AMF counts were relatively similar, with slightly raised AMF counts in the CON-carrot field, in both the hyphae and spore counts, supported by both the PLFA analysis and results of the GLI method. This result is against prior expectations, as it was expected AMF counts would be higher in the ORG-field compared to the CON-carrot field, due to the non-use of inorganic P fertilizers in the ORG-carrot field. Available P is significantly negatively correlated with AMF spore density and moderately negative correlated with AMF hyphae density. Furthermore, synthetic fungicide was used in the CON-carrot field, including azoxystrobine, boscalid, difenoconazole pyraclostrobin and prothioconazole. Boscalid and difenoconazole generally do not directly reduce AMF root colonization (Meenakshi et al. 2007). The effect of prothioconazole and pyraclostrobin on AMF root colonization has however not been studied or studied very limited (Clapperton, n.d.). One of the few papers on the effects of azoxystrobine on AMF describes no effect on the AMF *Rhizophagus irregularis* in terms of spore germination and root colonization however describes a tenfold reduction in the development of extra-radical mycelium and spore production (Buysens et al. 2015). The reduced soil available P and non-use of synthetic fungicides in the ORG-carrot field supported the earlier hypothesis of finding elevated AMF root colonization counts in the ORG-carrot field. However, no elevated AMF root colonization was found in the ORG-carrot field. A result which remains a mystery for now.

4.1.3 Microbial biomass and community composition

In the third paragraph the effects of farming system practices, specifically fertilization and tillage, on microbial biomass and community composition (PLFA analysis) will be discussed. The results will be addressed separately for total microbial biomass and community composition. In the performed PLFA analysis, arbuscular mycorrhizal fungi (AMF) biomarkers have been assessed, however, these will be discussed briefly as AMF colonization and its relation to farming system practices is discussed extensively in the previous paragraph.

4.1.3.1 Total microbial biomass

Total microbial biomass (nmol g^{-1} DM soil) was 281% higher in the ORG-oat field when compared to the CON-oat field, however, was reduced in the ORG-carrot and potato field with 31% and 8%, respectively, compared to the CON-fields. This result is in contrast with our hypothesis, as it was expected to find a higher microbial biomass in all ORG-fields compared to the CON-field – an expected outcome of the high biomass application rates and the interlinked elevated soil organic matter (SOM) status of the soil in the ORG-farming system. Several long term field experiments reported the positive effect of organic amendments on the SOM status of the soil and soil biological properties, comparing the effects of organic amendments such as farm yard manure (FYM) (Mäder et al. 2002; Edmeades, 2003) and plant-derived organic fertilizers (*vegetal fertilizers*) (Muller and von Fragstein und Niemsdorff, 2006; Heinze et al. 2011) with effects of synthetic fertilizers on soil organic matter and microbial biomass. The application of organic amendments stimulates microbial biomass and activity by increasing the carbon (C) and nitrogen (N) inputs (Fierer et al.

2009). Others report on the beneficial effects of cover crops and green manure (fertilization practices intensively used on the organic farm in the current study) on soil microbial biomass. The use of cover crops and green manure practices generate and maintain SOM and diversify the C inputs in time and space by an extended and diversified rhizosphere environment of the cover crops, both benefitting microbial activity (Schutter et al. 2001; Buyer et al. 2010). Daniel et al. (2014) conducted a meta-analysis of 122 studies on the effects of crop rotation on soil C and N concentration and soil microbial communities and found that when including a cover crop in the rotation, soil microbial biomass C and N pools substantially increased. Thus, the used cover crops and green manures in rotation of the ORG-farming system were expected to support elevated levels of total microbial biomass, due to their high C inputs, compared to the CON-farming system, however this effect is only noted in the ORG-oat field. No C inputs were applied to the carrot and potato fields (both ORG as CON) during the growing season. It might be that this caused the relative low total microbial biomass in the carrot and potato fields as food sources for the soil microbiota might have been limited during the growing season.

Besides the effects of cover crops and green manures it was expected to see a positive effect on microbial biomass from the non-inversion tillage practiced in the ORG-farming system, a hypothesis which is based on earlier findings of increased total microbial biomass in reduced tillage systems (Doran, 1980; Norstadt and McCalla, 1969; Groenigen et al. 2010; Kuntz et al. 2013). This hypothesis was strengthened by the measured SOM accumulation and assumed reduced N mineralization in the top soil, resulting partly from the non-inversion tillage practices (Sapkota et al. 2012). Kandeler et al. (1999) found significant increases of microbial biomass in the top 10 cm of the soil profile after a 4-year period of reduced and minimum tillage treatments, when compared to conventional tillage. In the current study ploughing has been dis-continued since 2010 and in this 5-year period it was expected to see effects in terms of soil microbial biomass.

4.1.3.2 Community composition

Besides total microbial biomass other PLFA biomarkers were assessed including bacteria, saprotrophic fungi, arbuscular mycorrhizal fungi (AMF) – hyphae and spores, actinomycetes, Gram+ and Gram– bacteria. Bacterial biomass reflected similar outcomes as the total microbial biomass and was 206% higher in the ORG-oat crop compared to the CON-oat crop. It was reduced in the ORG-carrot and potato fields by 31% and 15%, respectively, compared with the CON-fields. Saprotrophic fungal (SF) biomass was 413% higher in the ORG-oat field. It was 31% lower and 33% higher in the ORG carrot and potato fields, compared with the CON-fields. Fungi seem to profit more from the ORG-system in the oat and potato field then the bacteria and they especially thrive in the ORG-oat field, as has been discussed before. The results of the total fungal biomass (SF and AMF combined) compared to bacterial biomass is in agreement with results of Emmerling et al. (2003) and Kuntz et al. (2013). Besides SF also the mycorrhizal fungi (AMF) thrive in the ORG-oat and potato fields, which especially accounts for AMF storage lipids and spores and in lesser amount for AMF hyphae. The ORG-carrot field contained 13% and 25% less AMF hyphae and AMF spores compared to the CON-carrot field. Why AMF hyphae and spores are reduced in the ORG-carrot field compared to the CON-carrot field is not clear and in contrast with earlier hypotheses of elevated AMF levels in the ORG-fields.

Fungi:bacteria (F/B) ratios were higher in the ORG-oat and potato fields compared to the CON-fields but similar in the carrot fields. This is due to the relatively large contribution of fungal concentrations compared to bacterial biomass concentrations. This result is according to earlier hypotheses and findings in literature where especially in strict no-till systems higher F/B ratios are found and in reduced tillage (and non-inversion tillage) systems slightly higher F/B ratios are found (Frey et al. 1999; Kuntz et al. 2013). The reduced disturbance in the ORG-farming system seems to benefit both free-living SF fungi as well as symbiotic AMF fungi. SF fungi were significantly negatively correlated with available P and the available P levels in the ORG-carrot field were high. The ORG-oat and potato field had lower levels of available P, which might partially explain the raised SF biomass levels. Besides the effects of tillage on fungal biomass and F/B ratios there is also an expected effect of fertilization regimes, whereby the ORG-systems green manure fertilization benefits fungi over bacteria. Fungi are superior in surviving in N limited conditions and can mine the soil for recalcitrant soil C while bacteria excel in utilizing inorganic and simple organic materials

(Frey et al. 2004; Knorr et al. 2005). Although the ORG-fields showed higher available and total N levels, it is expected that by the start of the growing season and during the growing season N levels were significantly higher in the CON-fields due to the inorganic N fertilization practices on the CON-fields. This remains speculation, as N levels were not measured at the start of the growing season and during the growing season. This is however advisable in future studies, in order to draw well-founded conclusions on the causes of SF and AMF abundance.

Gram+ and Gram- bacteria were measured in the PLFA analysis. In this classification type bacteria are divided based on their cell wall structure (Huang et al. 2002). Gram- bacteria are the smallest in size, have a relative thin cell wall and are most sensitive to water stress. Gram+ bacteria are larger in size, with thicker cell walls and a negative charge on their cell wall surface, creating the high metal ion binding capacity of Gram positive bacteria (Hoorman, 2011). Gram+/Gram- ratios were greater in all ORG-fields when compared to the CON-fields, in agreement with findings of Bernard et al. (2012).

In the ORG-oat and ORG-potato field Gram+ bacteria were more abundant by 365% and 4% respectively whilst in the ORG-carrot field they were reduced by 41% when comparing them with counts in the CON-fields. In the ORG-oat Gram- bacteria were increased by 145% whilst in the ORG-carrot and potato field they were reduced by 47% and 17%, respectively, when comparing them with counts in the CON-fields. Griffith et al. (1999) reported the effects of C addition to the soil, which favoured the Gram- bacterial biomass. Buyer et al. (2010) also reported of higher Gram- bacteria in high C input cover crop systems compared with bare, low C input cropping systems. These results are in contrast with our current data as Gram- bacteria counts are highest in the CON-carrot and potato field (low C input and SOM status), compared with the ORG-fields (relatively higher C input and SOM status). The two mentioned articles are the few reporting on management effects on soil Gram+ and Gram- bacteria and the function of these two soil bacterial classes, which decreases the possibilities of reviewing current results in the context of other literature.

4.2 EFFECT OF FARMING SYSTEM PRACTICES ON SOIL CHEMICAL PROPERTIES

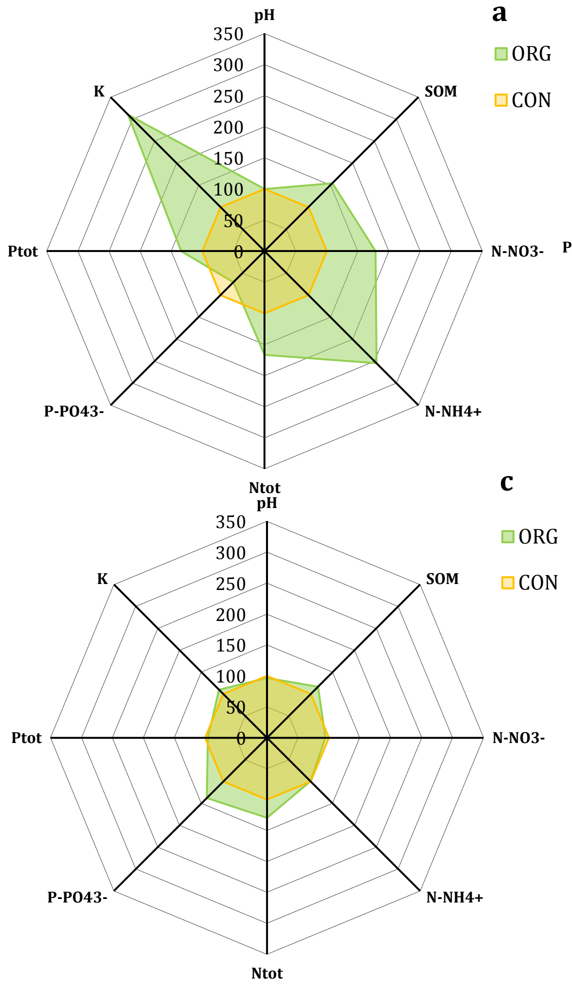


Figure 4.2
Relative contents of several chemical soil properties, presented relative to the conventional system (=100%).

a) oat
b) carrot
c) potato

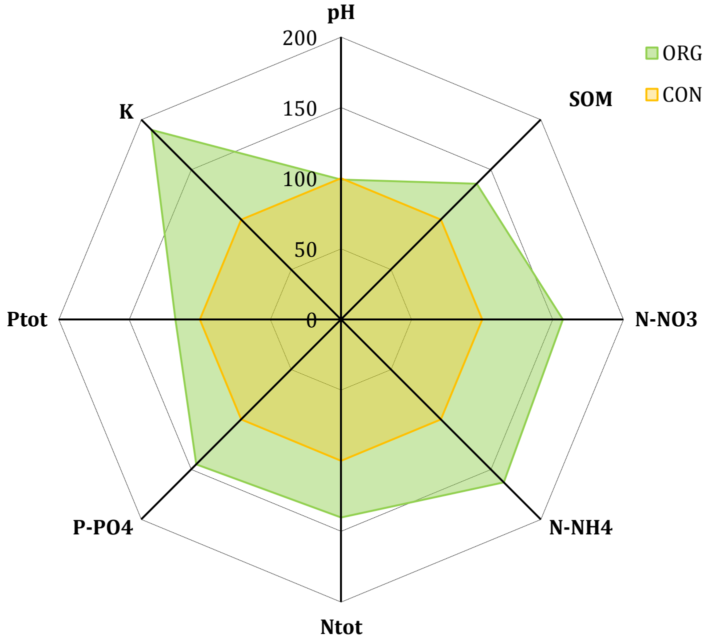


Figure 4.3
Relative contents of several chemical soil properties, presented relative to the conventional system (=100%) whereby the soil properties in the organic system are higher/lower than the CON-fields with:
- average over three crops -

pH: -1%;
SOM: +36%;
N-NO3: +57%;
N-NH4: +63%;
Ntot: +40%;
P-PO4: +45%;
Ptot: +17%;
K: +90%.

4.2.1 pH

Soil pH (KCl) was overall 0.9% lower in the ORG-fields compared to the CON-fields. The difference was most clearly represented in the potato fields, when soil layers were combined there was a reduction in pH of 3% in the ORG-potato field compared to the CON-potato field. Some authors reported no differences in soil pH comparing no-till and mouldboard ploughing (Motta et al. 2002; Rasmussen, 1999) however others reported a decreasing pH in no-till systems (Rahman et al. 2008). The latter authors attributed the lower pH to the build-up of SOM in the top in a NT system similar to SOM results in the current study which according to the authors, results in an increase of electrolytes and a reduction of pH. In these papers the effects of tillage have been the focus whereas in the current study the effects of both tillage and fertilization are the focus. In the 21-year comparison trial 'DOK' a slightly higher pH was found in the fields fertilized with farmyard manure compared with the fields amended with mineral fertilizer exclusively (Mäder et al. 2002). The application of fertilizers with an acidification potential (ammonium based fertilizers) would theoretically enhance a pH reduction. Therefore, it was expected to find reduced pH levels in the CON-fields, however no clear differences were found except for the potato field, where higher pH levels were found in the CON-field which is thus in contrast with earlier assumptions.

The latest soil analysis of the organic fields dates from around 1999-2000, which gives a chance to look at the pH trend over time. Overall, we saw a pH decrease of -0.1 in the oat field, -0.1 in the carrot field and no difference in the potato field in these 5 years.

4.2.2 Soil organic matter

On average, soil organic matter was 36% higher in the ORG-fields compared to the CON-fields, to which the ORG-oat field contributes most (54%). Within the ORG-farm there is differentiation in SOM contents between fields. It is likely that this results from inherent soil properties, field-specific tillage practices and the cultivated crops during the last years. From autumn 2012 on the ORG-oat field has been cultivated with GM crops and therefore it is likely that SOM content could further increase to the current average 5%. The ORG-carrot and –potato field showed strong similarities in SOM values and trend lines with 3.9% and 3.8% SOM (average of 3 soil layers), respectively. The following table (Table 4.1) shows the SOM inputs during the researched season; starting with the crop and/or GM residues from the 2014 crop followed by possible organic amendments. The amount of effective organic matter (EOM=part of SOM which remains present in the soil one year after application) applied is lowest in the ORG-oat field (0.4 t ha) whilst this soil scores highest in SOM content in the soil analysis and contrastingly, the CON-potato field scores lowest in SOM content (2.7%) however, had the highest EOM input.

Table 4.1. Overview of OM and effective organic matter inputs | season autumn 2014 - 2015

		In (t/ha)				
	Source	Fresh	DM	OM	EOM	EOM total
Crop						
Oat ORG	GM (*yellow mustard*)	n.d.	2[c]	n.d.	0.4	**0.4**
Oat CON	Winter wheat stover +	n.d.	n.d.	5.2+	1.6+	**3.0**
	solid chicken manure[a]	+10	+5.7	4.2	1.4	
Carrot ORG	Pumpkin crop residues +	n.d.+	n.d.	n.d.+	0.4+	**1.3**
	GM (*rye+Brassica Rapa*)	n.d.	+5[c]	3.5	0.9	
Carrot CON	Winter wheat stover	n.d.	n.d.	5.2	1.6	**1.6**
Potato ORG	GM (*leguminous mix*)	n.d.	7.5[c]	n.d.	1.8	**1.8**
Potato CON	Winter wheat stover +	n.d.+	n.d.+	5.2+	1.6+	**3.7**
	champost[b]	20	7	4.2	2.1	

[a] DM chicken manure: 573 kg t^{-1}, OM and EAM chicken manure: 416 and 137 kg t^{-1} (*source : kennisakker.nl*)

[b] DM champost: 350 kg t^{-1} (*source : eurolab.nl*), OM and EOM champost: 210 and 106 kg t^{-1} (*source : kennisakker.nl*)

[c] *source : observations by Harm Westers*

The organic farmer has been organic since 2002 and ever since amended his soils with fertilizers in organic form. Since 2010 a crop rotation dominant in green manures (GM) has been developed which has further contributed to the SOM accumulation.

All ORG-fields show a clear accumulation of SOM in the top 0-10 cm, in contrast with the CON-fields, where this accumulation did not appear. It was expected to find such a clear SOM gradient in the ORG-fields as it is reported as an effect of no-till or non-inversion tillage practices (Smith et al. 1998; West and Post, 2002; Prior et al. 2003). The majority of C inputs from root exudates and root turnover will appear in the top 10-15 cm of the soil and furthermore, the GM crops are only shallowly incorporated by the ORG-farmer. The absence of inverting tillage in the ORG-fields prevents mixing these SOM rich top layer with deeper soil layers and therefore the distinct gradient can develop – as seen in Figure 3.9.

4.2.3 Macronutrients

Effect on soil nitrogen content

The ORG-oat field had higher soil N_{min} (nitrate and ammonium combined) and N_{tot} levels compared to the CON-counterpart. Especially the top 10 cm of the soil accounted for the steep increase in N_{min} and N_{tot} in the organic fields. These results are in contrast with assumptions made prior to this study as it was expected to find elevated N levels in the conventional fields. This assumption is strengthened by looking at the input/uptake balance (Table 4.2). In the organic field residues of the previous yellow mustard crop were used as green manure and before winter the above ground biomass was estimated at 2 t DM ha^{-1}. Yellow mustard is estimated to have a C:N ratio of 18 (range 15-25) and a 2% N content resulting in 40 kg N ha^{-1} (Timmer et al. 2004). The crop took up 71.8 kg N ha^{-1} and accumulated this in the oat grains, resulting in a negative N balance of 31.8 kg N ha^{-1} whilst the CON-oat field has a positive N balance of 148.5 kg N ha^{-1}. However, this is not reflected by the N soil analysis as in the organic field more N_{min} and N_{tot} was present in the soil. Soil samples in both oat fields were taken in the week after oat harvests (mid-August). One hypothesis for the raised N levels in the organic field is the fact a green manure mixture was undersown in the oat crop. It contained several clover species, mallow and Vicia, which was in pre-flowering/flowering stage at time of soil sampling. It is assumed that these species contributed to N fixation and N supply and in significant amounts to the, relative high, N levels in the organic soil at time of soil sampling.

Table 4.2 Overview of input and uptake of macronutrients nitrogen (N) and phosphorus (P).

	N			P		
	In[a]	Out[b]	**Balance**	In	Out	**Balance**
Crop						
Oat ORG	40	71.8	**-31.8**	8	18.8	**-10.8**
Oat CON	230	81.5	**148.5**	242	22.7	**219.3**
Carrot ORG	125	45.4	**79.6**	20	15.9	**4.1**
Carrot CON	115	100	**15.0**	20.4	27.2	**-6.8**
Potato ORG	225	78.3	**146.7**	69.5	21.3	**153.7**
Potato CON	138	95.2	**42.8**	133	16.0	**117.0**

[a] In=input in kg N (P) ha^{-1} $year^{-1}$
[b] Out=uptake in kg N (P) ha^{-1} $year^{-1}$

The ORG-carrot field had higher N_{min} and N_{tot} levels compared to the CON-carrot field. Especially the top 20 cm of the soil accounted for the steep increase in N_{min} and N_{tot} in the ORG-field. The green manure mixture sown prior to the organic carrot crop consisted of turnip (*Brassica rapa* subsp. *rapa*) and rye, together forming an above ground biomass of 5 t DM ha^{-1}, with N contents of 2 and 3% respectively and C:N ratios of

18 and 15, respectively (Timmer et al. 2004). This results in an N supply of 125 kg N ha^{-1} to the organic carrot crop and an N balance of +79.6 kg N ha^{-1}. This positive balance is partly due to the relative low organic carrot yield, resulting in a low N uptake and thus low N leaving the field in crops. This low yield did not seem to be caused by low N supply, as evidenced by the high N_{min} and N_{tot} levels at time of soil sampling (early October – one week before harvest). However, the fact that there might have been a low N supply early in the season has not been researched.

The ORG-potato field had slightly reduced N_{min} and increased N_{tot} levels compared to the CON-potato field. The reduced N_{min} levels in the ORG-potato field were mainly caused by the high soil nitrate content in the 20-30 cm soil layer in the CON-potato field. The N_{tot} levels are 30% higher in the ORG-potato field compared to the CON-potato field N_{tot} levels, which follows the trend of the two other crops, which also had increased N_{tot} levels. In Table 2.6 it is indicated what the sources of N were in the different farms. The ORG-potato field is fertilized with GM crops (~ 3.5% N) which resulted in an estimated total of 7.5 t DM ha^{-1}, resulting in a total N application rate of 225 kg N ha^{-1}. Knowing that 78.3 kg N ha^{-1} is 'lost' from the field in autumn 2015 by crop removal this results in a positive balance.

Effect on soil phosphorus content
All three organic fields had been solely fertilized with GM crops for five years. This does not seem to have affected the P_{tot} levels in the ORG-fields, which is even slightly raised compared to the CON-fields (mean= +17%). The ORG-oat field had lower available P (PO_4) levels compared to the CON-oat field, with a mean reduction across soil layers of 30% compared to the CON-oat field (for detailed data see Appendix III). The ORG-oat field had elevated P_{tot} levels of 34% compared to the CON-fields. The CON-field was fertilized with chicken manure resulting in a P application of 242 kg P ha^{-1} yr^{-1} and the ORG-field had a P application of 8 kg ha^{-1}, an estimation based on calculations of Wijk et al. (2013) of P input from GM residues. In Table 4.2 it is made visible that the P application in the ORG-oat field is limiting, as more P has been taken up then has been applied (balance= -10.8 kg P ha^{-1}) which thus results in a negative balance.

Both amendments deliver organic P to the soil, which needs to be mineralized before effective plant uptake. This process seems to be enhanced in the ORG-field, as overall more P has been mineralized in the ORG-field, from a proportionally smaller amount of resources (90% less kg P ha^{-1}). The results in the oat field are similar to findings in Mäder et al. (2002) who reported lower soluble P fractions in organic soils compared to conventionally managed soil, however found a higher flux of P between the bulk soil (inorganic and organic P) and the soil solution in the organically managed soil. They related the potential of a soil to deliver inorganic P from organic P mainly to enhanced microbial functioning in the organic soil and found increased P sequestration in the microbial biomass (Mäder et al. 2002; Oehl et al. 2004). Mäder et al (2002), described this as: *"In soils of the organic systems, dehydrogenase, protease, and phosphatase activities were higher than in the conventional systems, indicating a higher overall microbial activity and a higher capacity to cleave protein and organic phosphorus".* In the Spearman's rank correlation analysis (paragraph 3.3.1) we found a significant negative correlation between total microbial biomass and available P (r=-0.886). This result was against prior expectations as it was expected that total microbial biomass would result in a positive relationship with available P. Also other PLFA biomarkers were significantly negatively correlated with available P, including total bacterial biomass (r=-0.886*), saprotrophic fungal biomass (r=-0.829*), arbuscular mycorrhizal fungal spores (r=-0.829*) and actinomycetes (r=-0.943**).

The CON-carrot crop had taken up twice as much P from the soil than the ORG-carrots, as the CON-yield was twice as high (Table 3.9). This is reflected in the P_{min} soil analysis; the ORG-carrot field has on average 12% more P_{min} and 23% more P_{tot} compared to the CON-carrot field. The CON-carrots were given 20.4 kg inorganic P fertilizer whilst P in the ORG-carrot field was supplied by use of green manure, estimated as being an organic P source of 20 kg P ha^{1}. It seems that the green manure P source was effective supplying the carrot crop of P, as high amounts of P_{min} are found in the field. Also, a significant portion of plant available P remained in the ORG-carrot field, with the potential for effective uptake if the ORG-carrot crop would have yielded more.

The ORG-potato field had more P_{min} and P_{tot} in the top 0-10 cm but less P_{min} and P_{tot} in deeper soil levels (10-30 cm (Figure 3.10). This result was against expectations as the ORG-potato field had high P inputs, due to the fact the field was under GM crops for almost 18 months. A significant amount of biomass accumulated over this period of time (estimated at 17.5 t DM ha^{-1}). With the estimations of Wijk et al. (2013) of 3 – 5 gr P kg^{-1} DM of GM one finds estimations of P uptake by the GM crop of 52 – 87 kg ha^{-1} in 18 months. Talgre et al. (2012) reported of significant portions of P accumulated in GM biomass, ranging from 17 kg P ha^{-1} by red clover up to 24 kg P ha^{-1} which will be released to the crop in the following year. These amounts of P are sufficient to cover most crop uptake rates.

Mineralisation of organic P from annual GM usage is the main P source in the ORG-farming. Another source of P for the crops in the ORG-system as the crops in the CON-system, might be the weathering of soil parent material. Several authors have made an attempt to estimate the P release to crops by natural weathering (Kahnt, 1999; Letkeman et al. 1996; Newman, 1995), their estimates range from 0.05 kg P ha^{-1} yr^{-1} up to 1 kg P ha^{-1} yr^{-1}. The real P weathering potential of a soil will depend heavily on age and inherent origin and related mineral structure of the soil parent material. It is difficult to say how much P weathering has contributed to the high P_{tot} levels in the ORG-fields in this study.

Effect on soil potassium content

The ORG-oat and ORG-carrot fields had higher available K levels in all three soil layers compared to the CON-oat and carrot fields. In the ORG-oat and carrot field we saw a strong upwards trend line, whereby available K seems to accumulate in the top 0-10 cm soil layer and both ORG-oat and carrot field had around 200 mg K kg^{-1} ha^{-1} in the top soil layer. This findings were in contrast with prior assumptions, especially when looking at the K fertilization regimes. The CON-oat field had been fertilized with chicken manure in autumn 2014 (10 t ha^{-1} – 133 kg K ha^{-1}) and the CON-carrot field had been fertilized with several inorganic K fertilizers resulting in a total K application rate of 416 kg K ha^{-1} in 2015. Contrastingly, the ORG-fields had not been fertilized, solely with green manure crops grown on site.

An assumption on the reasons of the high K availability in the ORG-oat field despite the absence of K fertilization is related to the crop history of this field, having a crop sequence of leguminous GM (2013) and yellow mustard (2014). Besides the seeds of the yellow mustard no K has left the field in terms of crop (residue) removal for two years. It is possible that both the leguminous GM and the yellow mustard have accumulated soil mineral K in their roots and shoots which became available to the following crop after harvest, due to processes leading to the mineralization of the organic K in roots and shoots (Askegaard & Eriksen, 2008; Eichler-Löbermann et al. 2009). In the 1940's Eve Balfour mentioned the potential of deep rooting crops for organic agriculture, as they have the ability to extract K from deeper soil layers and make them accessible for the following, often shallow rooting cash crops (Balfour, 1975). Witter & Johansson (2001) reported total K uptake rates of chicory and ryegrass of 124 and 122 kg K ha^{-1} yr^{-1}, respectively, wherein the percentage K uptake from subsoil (>25 cm) ranges from 63% (chicory) to 41% (rye grass). Talgre et al. (2012) reported of 89 – 144 kg K ha^{-1} yr^{-1} taken up by pure legume sowings of Bird's foot and Red clover, respectively. Brassicas, used as preceding crop for both ORG-oat and carrots (*Brassica hirta*: oat; *Brassica rapa L. var. rapa* (+rye): carrot) are not mentioned in Witter & Johansson (2001), however are known for their ability to scavenge nutrients from pools located in deeper soil layers. Kutschera (1960) reported that mustard roots generally to a depth of 140 cm, whereas some other brassicas like rapeseed, forage radish and turnip roots can penetrate the soil up to a depth of 180 cm (Sustainable Agriculture Network SAN, 2007). The fact brassicas and rye were used as preceding GM crop might have contributed to the high K availability in the topsoil and the downward trend of K availability in the deeper soil layers. However, for a full conclusion on this behalf, soil K analyses in deeper layers must be included as exchangeable K is present in high amounts at deeper soil depths. Witter & Johansson (2001) reported of exchangeable K amounts of 9.4, 12.4 and 25.2 mg K 100 g^{-1} at 0-30, 60-90 and 120-150 cm depth.

GM species might not only prove beneficial in acquiring nutrients from deeper soil layers but might also be useful in terms of accessing different nutrient pools. K exists in four forms in the soil: in soil solution, exchangeable, non-exchangeable and mineral (Sparks, 2001). The first two pools are easily accessible to plants where the non-exchangeable and mineral fraction are not. However, Steffens and Mengel (1979) found that rye could take up non-exchangeable K while red clover could not, which was assumed to be related to the plant's specific root mass, root length and root morphology. It was assumed in Mengel (1985) that monocots can feed better on non-exchangeable K than dicots. The organic farmer is using several monocots in his GM mixtures, such as oat, rye and sorghum which might result in a higher K uptake from a K pool unreachable for dicot root systems. He furthermore leaves the stover of his cash crop oat on the field, ensuring that all K from crop residues is returned to the soil. With a harvest index of 0.47 (own data), K return to the soil from residue accounts for a significant portion. The fact that the organic farmer has monocots in his GM mixtures and in his crop rotation (oats) and leaving the oat shoots on the field after harvest, might have had an effect at the high soil K status, due to K recycling in soil and plant. A large amount of K can be accumulated in the shoots of a crop, with known K shoot:root ratios of 50:50 in soy bean (Fageria, 2009). The conventional farmers remove the cereal shoots from the field after harvest, thus also removing considerable amounts of K from the field.

In the ORG-carrot field the available K was greater than in the CON-field, in all three soil layers. This result was against prior expectations, as the CON-carrot field had been fertilized with inorganic K fertilizers during the full cropping season. However, the CON-carrot yield was 50% greater than the ORG-carrot yield, resulting in a K uptake of 283 kg K ha^{-1} by the CON-carrots vs 187 kg by the ORG-carrots. This increased K uptake in the CON-field could have reduced K levels in the field at time of soil sampling (one week prior to harvest).

K availability levels in the potato fields show a different trend where K levels are lower in the ORG-field in soil layer 0-10 cm and 10-20 cm but higher in 20-30 cm. Potato is a high K demanding crop, having accumulated 193 kg K ha^{-1} and 220 kg K ha $^{-1}$ in the ORG- and CON-potatoes, respectively. Some more kg K will be accumulated in the potato leaves but this fraction is minimal compared to the tubers. In both the ORG- and CON-crop the 10-20 cm showed the lowest K availability, showing the potato roots have potentially taken up most of their K in these soil layer. This assumption is confirmed in Vos & Groenwold (1985) who reported of highest root length densities in the 10-20 cm soil layer (zero level is top of the ridge).

4.2.4 Mesonutrients and micronutrients

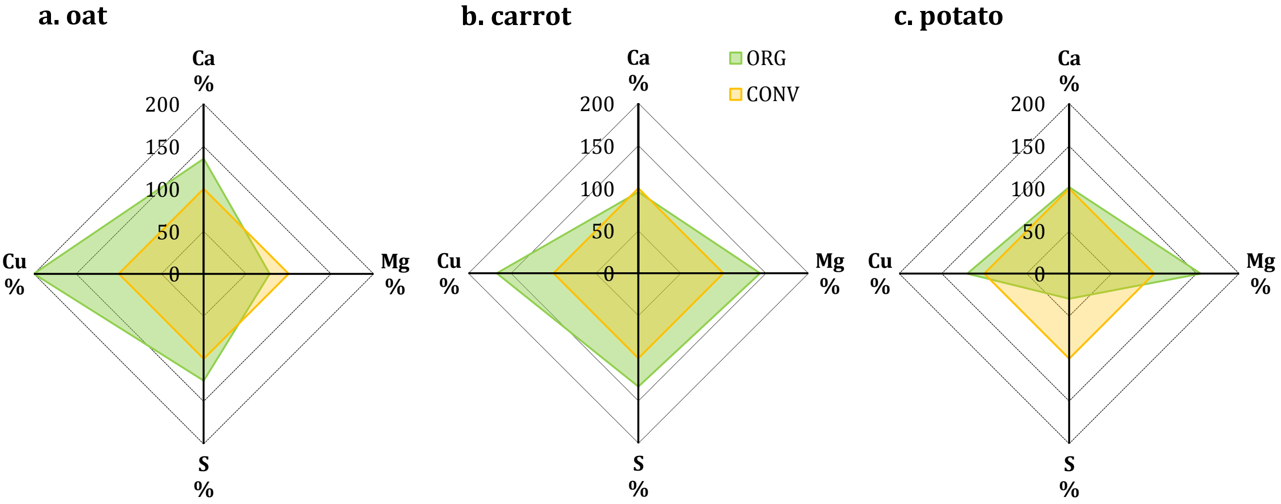


Figure 4.4 Results of several soil meso- and micronutrient analysis, presented relative to the conventional system (=100%) in the oat (a), carrot (b) and potato (c) field.

Effect on soil calcium content

On average, the soil in the ORG-fields, was 11% more abundant in available calcium (Ca^{2+}) then the analysed CON-soils (Figure 4.4). Especially the ORG-oat field (+35%) contributed heavily to this relatively high mean percent difference (MPD) in soil available Ca, followed by the ORG-potato crop (+2%) and the ORG-carrot crop, where the Ca content was lower in the ORG-fields (-5%). All soils in the current study, except the soil of the CON-oat field, are originally rich in calcium and classed as calcium rich "Polder" vague soils (Nederlands: *kalkrijke Poldervaaggronden*) (STIBOKA, 1968). Calcium carbonate ($CaCO_3$) content ranged from 0.8% - 7.7% with lowest soil calcium content measured in the CON-oat field and highest measured in the CON-potato field (see chapter 2). Aside from the parent material and the degree of weathering of this material, the addition of calcium through fertilizers or manure also contributes to the Ca content of the soil. Chicken manure and *"champost"* was applied on the CON-oat and potato field respectively, whilst the CON-carrot field received a one-time synthetic Ca application in April 2015, with a rate of 47 kg ha^{-1} (*Top Mix wortel*). The CON-carrot field had the highest soil solution Ca content of 133 mg kg^{-1} followed by the ORG-carrot field, with a soil solution Ca content of 127 mg kg $^{-1}$. So, while no Ca fertilization was performed in the ORG-carrot field, still, comparable soil solution Ca contents to the CON-carrot field were found at the end of the growing season. This can be potentially explained by the relative low yield of the ORG-carrots ensuring a reduced Ca (and other nutrient) uptake and thus reduced removal of Ca from the soil available Ca pool. Ca removed from the CON-carrot field through crop uptake and harvest is estimated at 38 kg ha^{-1} compared with 16 kg ha^{-1} on the ORG-carrot field. The Ca application rate of 47 kg ha^{-1} fits within the crop Ca removal rate. Thus, the Ca crop removal rate is 3 times lower in the ORG-field then in the CON-carrot field.

The following table presents an overview of the Ca:Mg ratios and Ca:Mg occupancy of the negative exchange sites of the clay-humus complex. The latter data is only present for two of the six fields (based on pre-existing data from farmers) however, it shows the relatively high occupancy of the Ca cation and low Mg presence. The ideal Ca:Mg balance on the clay-humus complex varies widely and is depending on soil texture (clay or sand dominated) however, the optimal range of negative exchange sites occupied by Ca^{2+} is 60% to 70% and 10% to 20% of the exchange sites should be occupied by Mg^{2+} (SoilTech Solutions, 2008). The first column in the table shows the Ca: g ratios of plant available Ca and Mg cations.

Table 4.3 Ca:Mg ratio and Ca:Mg occupation on clay humus complex in organic and conventional managed fields.

		Ca:Mg ratio	**Ca:Mg % on clay-humus complex (CEC)**
		From soil tests 2015	From soil tests farmers
Crop	**System**		
Oat	CON	0.87 : 1	n.d.
	ORG	1.51 : 1	n.d.
Carrot	CON	2.61 : 1	93% : 3.9% (2010)
	ORG	1.74 : 1	86% : 6.4% (2016)
Potato	CON	2.57 : 1	95% : 2.1% (2015)
	ORG	1.70 : 1	n.d.

Effect on soil magnesium content

On average, the ORG-soil was 25% more abundant in available magnesium (Mg) then the analysed CON-soils. This result is similar to the results presented by Mäder et al. (2002) where the researchers found increased soil magnesium levels on organic farms. In the current study, especially the ORG-carrot field (+43.3%) and the ORG-potato field (+54.8%) contributed heavily to this relatively high mean percent difference (MPD) in soil available Mg. The available soil Mg was lower in the ORG-oat field (-22.4%) compared to the CON-oat field. The large difference in soil available Mg in the two carrot fields is surprising. From January 2015 – September 2015 the CON-carrot field was fertilized 8 times with fertilizer containing Mg, which results in an Mg application rate of 20.3 kg ha^{-1}. The largest part of this results from a spring application of the fertilizer *Top Mix Wortel* (18 kg ha^{-1}) (Table 2.6). It is surprising that without any Mg fertilization 43.3% more available soil Mg was found in the ORG-carrot field. This result could be due to the reduced uptake by the carrots in the ORG-field, as the carrot yields are about 50% lower in the ORG-field. Also, it could be due to the higher Mg occupation on the clay-humus complex (Table 4.3). The ORG-carrot field has a higher percentage Mg base saturation then the soil of the CON-carrot field has. In this study the plant available Mg was measured (Mg in soil solution), which is in equilibrium with exchangeable Mg (the Mg held by clay particles and OM). The soil in the ORG-carrot field had a comparable clay fraction (ORG: 18% vs CON: 17%) however a significant higher OM content, compared to the CON-carrot field. These facts leads to the assumption that management on the ORG-farm led to a higher CEC, ultimately resulting in, - amongst others - a higher soil exchangeable Mg fraction leading to an increase in Mg in soil solution. A final hypothesis why soil available Mg was increased in the ORG-carrot and potato fields is the fact that no fertilizers containing other positively charged ions, such as potassium and ammonium, have been used. Several such cations behave as antagonists to Mg and too much of any other major cation will shut down the availability of Mg.

Effect on soil sulphur content

On average, the ORG-soil was 3.8% less abundant in available soil sulphur (S) then the analysed CON-soils. The soil in the ORG-oat and carrot field contained 26% and 33% more available S then in the CON-oat field, respectively. In contrast, the soil in the ORG-potato field contained 71% less available S then in the CON-potato field. The CON-oat field was fertilized with chicken manure, which might have been a valuable source for soil S. The ORG-oat field however, has had no fertilization at all, but still has a higher available soil S content, which is surprising. Similarly, the ORG-carrot field contains more soil S and has had, in contrast with the fertilized CON-carrot field, no fertilization treatments. The CON-carrot and potato fields were fertilized with several inorganic fertiliser mixes wherein also S was added, often in the form of sulphur dioxide (SO_2).

Fageria (2009) discusses the three main sources of sulphur including soil organic matter, soil minerals and sulphur gasses from the atmosphere. Due to the clean air legislation enforced in Western Europe in the end of the 19th century, the S deposition from the atmosphere declined drastically (Haneklaus et al. 2007). It is predicted that partly due to the implementation of this legislation and partly due to changing crop practices, large areas will be sulphur deficient (Fageria, 2009).

It is difficult to say anything about S decline in the fields in this research, as no monitoring has been performed during a wider time span and the soil analysis has been performed only once. However, it is interesting to notice the higher soil available S in the ORG-oat and carrot field compared with the CON-fields. It might be the case this is a result of the elevated SOM status of the ORG-fields. SOM contains around 0.5% S (Barber, 1995) and Tabatabai and Bremner (1972) estimate that 95-98% of the soil S is present in the SOM. This shows what a potential a solid SOM level has, in terms of S supply to soil microorganisms and ultimately the plant.

Effect on soil copper content

On average, the ORG-soil was 62% more abundant in bioavailable copper (Cu) than the analysed CON-soils. Especially the ORG-oat field (+100%) contributed heavily to this relatively high mean percent difference (MPD) in soil available Cu, followed by the ORG-carrot crop (+67%) and the ORG-potato crop (+20%). This result is quite surprising as sources of addition as manure or inorganic fertilizers, have not been used for over five years on the ORG-fields. Atmospheric deposition and the return of crop residues to the soil are thus the only sources of Cu in the ORG-fields. Besides external additions, the soil itself is a considerable sink of bound Cu, from where soluble and thus bioavailable Cu can emerge. Mean background values of total Cu contents in soils in Europe are estimated at 17.3 mg/kg (mean for top soils, FOREGS, 2005), a Figure relatively low when comparing it with the world-soil average of Cu of 38.9 mg/kg (Kabata-Pendias, 2011). A limited part of this total amount of Cu is available for plant uptake and the concentration in the soil solution ranges from 0.0018-0.135 mg kg^{-1} (Mengel and Kirkby, 1987; Kabata-Pendias, 2011). Barber (1995) estimates 50% of the Cu in the soil is insoluble, 30% is bound by organic sites, 15% is in the oxide form and only 5% is present in the soil solution and available for plant uptake. Ponizovsky et al. (2006) even estimates only 1% of the total Cu amount is present in the soil solution. Soil variables that control Cu bioavailability are: pH, SOM, soil texture, soil mineral composition, soil temperature, soil moisture, soil microbiology and root morphology (Fageria, 2009). The relative explanation index (RDI) of statistically significant relationships indicates that the clay fraction contributes strongly to the bioavailable Cu content (30-35%), followed by total content of Fe and Mn (20%), CEC (15%) and SOM (15%) (Kabata-Pendias, 2011). The former author does not describe the RDI of soil biological parameters (e.g. soil microbiology and root morphology) effect on soil Cu availability. On the contrary, Ponizovsky et al. (2006) reports SOM and DOM are the main soil variables affecting Cu availability.

Several of the soil variables described above might have played a role in the elevated available soil Cu levels in the ORG-fields and are made visible by the performed soil analysis. The SOM levels were significantly higher in the ORG-fields, compared to the CON-fields, which was especially evident in the ORG-oat field and to lesser account in the ORG-carrot and potato field. The free Cu^{2+} ion is strongly adsorbed by organic compounds in the soil (Hodgson et al. 1996) and Cu in solution is primarily found as organic ligand complexes. The small organic compounds (e.g. extracts of SOM, fulvic acid, root exudates) chelate the copper ions and move them into the soil solution (Hale et al. 1971). The high levels of OM in the ORG-fields might have contributed to the elevated available Cu levels in the ORG-fields. The second soil variable which might have caused the relative high Cu levels in the ORG-fields, especially in the ORG-oat field might have been the fact that a cover crop mixture was growing in the ORG-oat field at time of soil sampling. The root exudates of both the oat and the cover crop mixture might have increased soil nutrient availability (Next to Cu potentially also Zn and Fe). Root exudates that increase the availability of metallic soil micronutrients are called metal chelators. Whilst forming complexes with soil metals they release the soil bound metals and thus make them available for effective plant uptake (Bais et al. 2006). It is not expected that the clay fraction or soil pH was the main cause of the difference in available Cu amounts between the ORG- and CON-oat fields. The clay fraction (<2µm) differed by only 1% in the fields whilst pH difference was also very small (0.04).

4.3 EFFECT OF FARMING SYSTEM PRACTICES AND BIOCHEMICAL PROPERTIES ON CROP NUTRIENT DENSITIES

4.3.1 Crop DM

Crop dry matter contents did not differ significantly between the CON- and ORG-grown carrots, a finding similar to Bender et al. (2009) who also found no differences between organic and conventional carrot root DM. A larger difference in DM was expected in this study, as organically produced foods are often reported to have higher DM contents then conventional products (Bourn and Prescott, 2002; Heaton, 2001; Magkos et al. 2003; Siderer et al. 2005; Woese et al. 1997). It appears to be connected with nitrogen application levels (Evers, 1988; Lieblein, 1993) and Kaack et al. (2002) reported a significant negative correlation whereby DM content decreases linearly with increasing N_{min} at germination. The fact that in the carrot fields in the current research no difference was found between carrot DM contents, can be due to similar N rates at germination (sown at 13 and 20 May 2015) as most N was applied in June and July in the conventional field and all N in the organic field was applied in organic form, by means of the crop residues of the preceding yellow mustard crop. However, data on N_{min} rates early in the season are not present in the current study, so this remains speculation. Kaack et al. (2002) found furthermore carrot DM was significantly correlated with temperature and solar radiation.In the current study it is not expected that these two variables created significant different environmental conditions, as the sample locations were in close proximity of each other.

In the potato crop, the DM content was significantly higher (P=0.01) in the ORG-grown tubers (+18%) compared to the CON-grown tubers. These results are similar to those reported in Moschella et al. (2005), reporting of significant higher (P=0.0005) tuber DM contents of 22% vs. 20% in ORG- and CON- grown tubers respectively. The authors link the results to the “carbon/nitrogen (C:N) balance” theory (Rembialkowska, 2007). This theory states that when nitrogen availability limits plant growth, the metabolism of the plant shifts towards compounds rich in carbon, e.g. starch (carbohydrate), cellulose and non-nitrogen secondary metabolites such as phenolics and terpenoids. Sugars, deposited as starch in the tuber, make a large contribution to tuber DM (Lombardo et al. 2012). In a medium where nitrogen is in an easily available form, the plant will first accumulate compounds with high nitrogen levels, e.g. proteins and nitrogen containing secondary metabolites (Rembialkowska, 2007). The reduced DM tuber content in the CON-fields could potentially be attributed to high N availability early in the season in the CON-potato field, where champost was applied in autumn 2014 (120 kg N ha^{-1}). Champost contains 5% N_{min} and 95% N_{org} (KOCH-Eurolab, 2015) thus releasing 6 kg N_{min} ha^{-1} in October 2014 and the remaining 114 kg N in organic form in winter and following season. Ammonium polyphosphate (APP) (18 kg N ha^{-1}) application followed in early May 2015, applied two weeks after sowing the potatoes, followed by liquid UREAN (108 kg N ha^{-1}) in June. In APP, 100% of the total N consists of NH_4, while UREAN consists of 25% NH_4, 25% NO_3 and 50% NH_2 (amine N) of total N. The N application rate in the ORG-potato crop is of considerable amount, however, N was applied in organic form by means of a leguminous GM crop. This crop accumulated N during the cropping season of 2014 and it is assumed to be slowly released to the potato crop throughout the following growing season 2015, depending on temperature and microbial activity (Lambers et al. 2009).

DM data for the conventional oat crop is missing, as the conventional oat crop was harvested before sampling and a sample could only be taken in the, already dry oats. DM content in the organic oat crop is 77%.

4.3.2 Crop macronutrient density

Several single-factor studies have been conducted on whether agricultural production methods influence food nutrient compositions, showing that plants exposed to different growing conditions can significantly differ in their nutrient composition. Until now, eight meta-analyses have been accumulating the single-factor studies results to assess if there are consistent trends to be detected in differences in nutrient compositions (Brandt et al. 2013; FIBL, 2015). Seven of these reports included minerals in their analysis and are thus of special interest to the current study (Worthington, 2001; Heaton, 2001; Benbrook et al. 2008; Dangour et al. 2009; Hunter et al. 2011; Smith-Sprangler et al. 2012; Baranski et al. 2014). The three most recent meta-analyses will be used to assess and compare the crop nutrient compositions of the current study, however, caution should be in place when using the meta-analyses, as the outcome of these is heavily depending on the chosen time frames, selection and inclusion criteria, percentage of publications included after application of quality criteria and variables such as selected genotype, growing season, growing year and location (Brandt et al. 2013).

Table 4.4 Mean differences[a] in nutrient content of organic versus conventional crops.

	Studies	**Pairs***	**Significant higher in**		**Meta-analysis****				
	n	*n*	ORG	CON	Unweighted		Weighted		
					n	MPD	*n*	MPD	SMD≠
DM[a]	85	130	8	2	130	2.99%	24	2.46%	1.31
Nitrogen[a]	55	88	2	11	88	-6.75%	35	-9.77%	-0.88
Vegetables[a]	-	-	-	-	42	-10.26%	20	-5.82%	-
Cereals[a]	-	-	-	-	14	-14.31%	7	-21.92%	-
Phosphorus[b]	30	82	24	12	-	-	-	-	0.82
Phosphorus[c]	30	141	-	-	141	5.1%	96	6.6%	-
Potassium[b]	37	108	18	18	-	-	-	-	0.45
Potassium[c]	36	160	-	-	160	1.8%	98	5.0%	-
Zinc[a]	-	-	-	-	88	12.03%	37	4.65%	0.20
Zinc[c]	27	131	-	-	131	6.4%	73	7.9%	-
Iron[b]	24	77	10	12	-	-	-	-	0.30
Iron[c]	27	126	-	-	126	-1.0%	77	3.3%	-
Copper[c]	25	113	-	-	113	14.6%	73	9.5%	-

[a] Baranski et al. 2014
[b] Smith-Spangler et al. 2012
[c] Hunter et al. 2011
* n: number of data-pairs (comparisons) included in the meta-analysis ~ significant higher in: the number of comparisons in which a statistically difference was identified with higher levels in the ORG or CON food
** meta-analysis unweighted: papers did not provide information on # replicates, SD, SE ~ meta-analysis weighted: papers did provide information on # replicates, SD, SE ~ n: number of data points included in the comparison ~ MPD: mean percent differences with value <0 indicate higher concentration in CON, value >0 indicate higher concentration in ORG.
≠ SMD = standardized mean difference: The difference between mean nutrient level in organic minus that in conventional divided by the pooled SD; thus, a positive (negative) number indicates higher (lower)
nutrient levels in organic

Effect on crop nitrogen content

Nitrogen ($\mu g\ kg^{-1}$ dry weight) was elevated in the organic oat and carrot crop with 7% and 9% compared to the conventional crops, respectively. In the ORG-potato crop nitrogen content was 16% lower than the CON-potato crop. It was expected that nitrogen levels would be reduced in all organic crops, due to previous studies showing reduced nitrogen levels in organic produce (Baranski et al. 2014). The significant elevated

DM levels and decreased N levels in the ORG-grown potatoes might be connected with the C:N balance theory described in 4.4.1, explaining the reaction of plants in N limited situations, wherein the plant accumulates more C rich compounds (carbohydrates and cellulose) and less compounds rich in N (proteins). This theory is confirmed with the data of the meta-analysis of Baranski et al. (2014) who found significant (P=0.008) raised levels of carbohydrate compounds (SMD=1.54) in ORG-vegetables and decreased levels of proteins (SMD=-3.01). A second theory to add to the reduced N content in the ORG-potatoes are the potential raised levels of molybdenum (Mo) in the organic fields. The main form Mo is present is molybdate (MoO_4^{2-}), a highly soluble anion. In the topsoil Mo forms complexes with humic materials and tannin compounds present in OM (Wichard et al. 2009) and in the subsoil Mo forms complexes with iron oxides and OM (Reddy and Gloss, 1993; Stiefel, 2002). With the significantly higher SOM content in the organic soils in the current research one would expect a reduced level of Mo susceptible to leaching and reduced Mo iron oxide complexation. Furthermore, it is expected to observe a raised available Mo in the SOM, which is a fraction bound to the tannins present in the OM and available for complexing agents released by free-living N_2 fixing bacteria (Wichard et al. 2009). The effect of this raised soil Mo might be translated in raised Mo levels in plants and Baranski et al. (2014) found significant raised levels of Mo (65%; *P*=0.002) in organic crops (barley, faba bean, onion, pea, potato) in the weighted meta-analysis. The elevated N levels in the ORG-oat and carrot crop are against expectations and earlier research on N levels in cereals and vegetables show contrasting results. Baranski et al. (2014) studied nutrient composition studies (period 1992-2011) and found a mean percentage difference (MPD) of -22% and 6% in cereals and vegetables respectively, when comparing N levels of ORG- and CON-crops.

Effect on crop phosphorus content

Phosphorus (μg kg^{-1} dry weight) was elevated in the organic oat, carrot and potato crop with 0.1%, 41% and 35% respectively, compared to the conventional crops, creating an MPD of +25%. Hunter et al. (2011) concluded that organic vegetables, fruits, legumes and grains were significantly higher in phosphorus with 6.6% (96 comparisons), Dangour et al. (2009) found phosphorus increases of 8.1% whilst Worthington (2001) found a significant phosphorus increase of 13.6% in organic fruits, vegetables and grains. Compared to those MPD's the elevated P levels in the ORG-grown carrot and potato crop from this study are relatively high.

The high P status of the ORG-grown potatoes might be explained by increased arbuscular mycorrhizal fungi (AMF) colonization of their root systems and by the enhanced microbial life in general, as soil microbes are known to solubilize and mineralize P from plant-unavailable pools (Richardson, 2001). The increased AMF density benefits the plant by the increased soil volume explored, especially beneficial in the case of the immobile P nutrient (Clark and Zeto, 2000). In the microscopic analyses of the potato's root systems it was found that the ORG-potatoes contained 3% more AMF colonization in the 0-10 cm soil layer and 2% in the 10-20% soil layer, when compared to the CON-potato root system. Similar results come from the PLFA analyses, where both AMF hyphae and AMF spore density was increased in the ORG-potato root system. In the Spearman's rank correlation matrix, crop P content was moderately positive correlated with AMF hyphae and spores (r=0.600 and 0.657). However, the fungal density in the ORG-carrot crop is reduced compared to the CON-carrot crop yet the crop P content is increased by 41% in the ORG-carrot crop. Also, the fungal density was exponentially increased in the ORG-oat crop however, no increases in crop P content were visible in the ORG-oat crop. To say that the fungal density – and specifically AMF density - has an effect on crop P content is therefore speculation, at least with the results of the current study. There is however an extensive body of literature reporting on the beneficial effects of fungi and specifically AM fungi on the P availability in the soil (Smith et al. 2011).

It was expected to find higher crop P contents in the ORG-oat crop because of the presence of a very diverse green manure crop during the growing season of the oat. It was hypothesized that the increase in belowground biomass in terms of root volume would enhance root exudation and by this process, have an impact on the P availability in the root rhizosphere and P uptake by the plant. However, this is not seen in the results of the current study.

It is important to note that no severe P deficiencies were detected in the ORG-crops, whilst already for 5 years no P has been applied to the fields (in terms of organic amendments), which is in contrast with the CON-fields, where high P input levels were present in the 2015 growing seasons. In the ORG-fields, total P stocks are similar or higher in most soil layers, compared to the CON-managed soils and crop P levels are similar and higher in the ORG-crops.

To conclude, it is expected that there are effects of farming systems practices on crop P content and furthermore, that there is an effect of soil biological properties on the crop P content.

Effect on crop potassium content

Potassium (µg kg^{-1} dry weight) was elevated in the ORG-oat and carrot crop with 357% and 59% respectively, and reduced in the ORG-potato crop with 11% compared to the conventional crops creating an MPD of 135% in favor of the ORG-products. In the meta-analysis of Hunter et al. (2011) potassium was researched in 36 studies resulting in 160 unweighted comparisons and an MPD of +1.8%. In the weighted analysis (*n* comparisons=98) it was found that potassium had an MPD of +5.0% in favor of the organic products. Similar to what has been described in the paragraph above, all ORG-products which have been used for the comparisons have been acquired from organic farms using manure as main source of fertilization. The ORG-fields in this study have not been fertilized with manure for the last 5 years and it is therefore difficult to compare the current results with findings from the meta-analysis and relate it to literature.

Until now, it is a mystery why the available K content in the grain of the ORG-oat crop is so much elevated compared to the CON-oat (+357%). It might be related to the high available K content in the ORG-oat field. In the Spearman's rank correlation matrix (Table 3.12) soil available K was showing a significant positive correlation with K content of the crops (r=0.829*).

4.3.3 Crop micronutrient density

Effect on crop copper content

Copper (µg kg^{-1} dry weight) was elevated in the ORG-oat and potato crop with 5% and 57% respectively, and reduced in the ORG-carrot crop with 16% compared to the conventional creating an MPD of 16% in favor of the ORG-products. In the meta-analysis of Hunter et al. (2011) copper was researched in 25 studies resulting in 113 unweighted comparisons and an MPD of +14.6%. In the weighted analysis (*n* comparisons=73; *n* studies=14) it was found copper had an MPD of 9.5% in favor of the organic products. In these 14 studies the effects of mineral fertilizers (solely NPK) on nutrient density were compared with fertilization regimes consisting of combinations of manure, compost, sewage sludge and/or blood meal. In these cases, the elevated Cu levels in the ORG crops were explained by the often natural presence of Cu and other trace elements in the former mentioned amendments. In the current study however, no amendments were used on the ORG fields. It is therefore highly curious to find that on the ORG fields – without external amendments one can produce crops higher in nutrient density then on fields with external amendments, being either artificial fertilizers or organic amendments. It is expected that there is a relationship between the elevated crop Cu contents and bioavailable soil Cu levels, as all ORG soil showed a higher bioavailable soil Cu compared to the CON fields. This is however not traceable in the Spearman's rank analysis (soil Cu x crop Cu; r=-0.120). Interestingly, crop Cu contents have a significant relation (r=0.886*) with soil Ca levels. Which is surprising as copper availability normally increases with a reduced pH thus in a slightly acidic soil (Sims, 1986).

Effect on crop iron content

Iron (µg kg^{-1} dry weight) was elevated in the ORG-carrot crop with 78% and reduced in the ORG-oat and potato crop with 26% and 31% respectively compared to the conventional crops creating an MPD of 7% in favor of the ORG-products. In the meta-analysis of Hunter et al. (2011) iron was researched in 27 studies resulting in 126 unweighted comparisons and an MPD of -1.0%. In the weighted analysis (*n* comparisons=77; *n* studies=16) it was found iron had an MPD of 3.3% in favor of the organic products.

Similar to what has been described in the paragraph above, studies in which ORG crops have an elevated Fe content explain this effect by the natural presence of Fe in organic amendments.

Unfortunately, bioavailable soil Fe was not measurable in this study and therefore it is difficult to discuss the differences in uptake between the ORG and CON-crops, from the perspective of the soil.

The results of the current study are against former hypothesis. It was expected to find elevated levels of Fe in the ORG crops, because of the increased SOM contents in the ORG fields. A variety of humic organic compounds are involved in Fe mobility in soils and it is expected the level of humic compounds are linked to the level of SOM (Schulte, 2004; Kabata-Pendias, 2011). Furthermore, it was expected to see an effect of the elevated levels of microbial biomass in the ORG oat crop. Microorganisms have a key role to play in the Fe soil cycle and at least 18 Fe minerals and carbonates are biologically induced (Bazylinsk and Frankel, 2003). Also, microorganisms decompose OM resulting in a release of plant available Fe which was previously tied up (Schulte, 2004). However, the crop Fe content was lower in the ORG oat crop when compared to the CON-oat crop. A management practice that could have influenced the reduced crop Fe content in the ORG oat and potato crop, might have been the year-round presence of a cover crop which is only removed in late spring, just before the sowing of the cash crop. This results in a cold soil in spring, unable to warm up by the sun due to the layer of cover crops on top of the soil. Cold root-zone temperatures can induce low Fe availability (Chaney, 1984). An additional crop management practice potentially responsible for reduced Fe contents in the ORG-oat and potato crop is crop maturity. Fageria (2009) found that variations in Fe concentrations in plant tissue could be explained for 91% by plant age. All organic crops in this study were harvested at an earlier age compared to the conventional crops (ORG-oat: 121 DAS vs CON-oat: 146 DAS, ORG-potato: 114 DAS vs CON-potato: 159 DAS, ORG-carrot: 136 DAS vs CON-carrot: 154 DAS). The harvesting date could be partly responsible for the reduced Fe content in the ORG-oat and potato crop, however this remains speculation.

Effect on crop zinc content

Zinc (µg kg^{-1} dry weight) was elevated in the ORG-oat and carrot crop with 26% and 13% respectively, and similar in the ORG- and CON-potato crop creating an MPD of 13% in favor of the ORG-products. In the meta-analysis of Hunter et al. (2011) zinc was researched in 131 unweighted comparisons resulting in a MPD of +6.4%. In the weighted analysis (*n* comparisons=73) it was found zinc had a MPD of 7.9% in favor of the organic products. In the meta-analysis of Baranski et al. (2014) zinc was researched in 88 unweighted comparisons with a MPD of 12.03% in favor of the ORG products and in weighted comparisons (n=37) the MPD was found to be 4.65% in favor of the ORG products. In the current research the MPD of 12.9% can be seen as relatively high, in comparison with the results from the two meta-analysis.

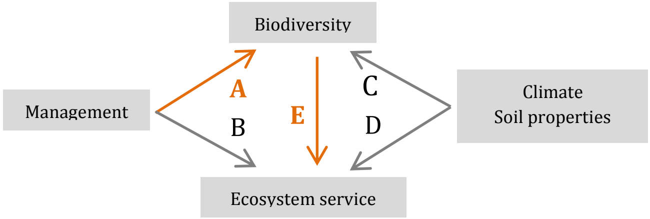
Unfortunately, bioavailable soil Zn was not measurable in this study and therefore it is difficult to discuss the differences in uptake between the ORG and CON-crops. It is expected however, that the elevated crop Zn contents are linked to several management practices, which potentially have effect on the Zn content and availability in the soil. Zn sorption in soils is influenced by soil pH, clay minerals, OM content, Fe content and $CaCO_3$ content (Fageria, 2009). Soil pH and texture (clay content) are relatively similar between the farms in the current study however OM content is severely impacted by the management practices of the farmers participating in this research. Alloway (2004) reported significant positive correlations between soil extractable zinc and OM content. In the Spearman's rank correlation matrix (Table 3.12) the relationship between OM content and crop Zn content is to be found positive yet non-significant (r=0.552). The negative relation described in Fageria, 2009, between crop Zn content and carbonate levels cannot be traced back in the current research. In the ORG-oat and carrot soil the carbonate contents ($CaCO_3$ %) are higher than in the corresponding CON-soils, however the ORG-oat and carrot crop have elevated crop Zn levels.

Besides soil chemical properties having an effect on crop nutritional value it was also expected to find an effect of soil biological properties on crop Zn content. In the Spearman's rank correlation matrix several strong positive relations were found between soil biological properties and crop Zn content. Earthworm density was significantly related to crop Zn (r=0.986***) and AMF spores was significantly positive related to crop Zn (r=0.812*). Furthermore, the total PLFA, total bacterial biomass and the presence of actinomycetes were all positively related to crop Zn, with r=0.638, r=0.638 and r=0.638 respectively. Stevenson (1986) reports of the importance of soil microorganisms in the soil Zn cycle, as the chelating agents produced by the microorganisms play an important role in the transport of the relative immobile Zn, to the plant roots.

5. Conclusions

The overall objective of this thesis was to explore the outcomes of alternative farm management practices (reduced tillage and usage green manure crops) on the functioning of soil biota as governors of ecosystems functioning and micronutrient cycling. The study aimed to explore the relationships ‘*management – soil biodiversity – ecosystem service*’, ultimately exploring ecosystem service outputs in terms of food nutritional value; in this case the micronutrient output (Fe, Cu, Zn) of an alternative farming system compared to a conventional farming system. In the following chapter the findings will be summarised, starting with linking the initial hypotheses with the results. Several general reflections on the thesis topic and its context will follow in paragraph 5.2 whereafter paragraph 5.3 reviews on the implications for further research.

5.1 REFLECTION ON HYPOTHESIS



The scope of this study was to explore the potential of farm management practices to influence crop micronutrient concentrations, with specific attention to the role of soil biological properties in soil micronutrient availability and mobility. The first relation assessed (A) was the effect of farm management, specifically fertilization and tillage practices, on soil biota and specifically those functional groups which activities show a strong relationship with the ecosystem service nutrient cycling. The second relationship assessed (E) was the effect of soil biota on ecosystem service (micro-) nutrient cycling, availability and uptake.

5.1.1 Hypotheses on relation A: management – biodiversity effects

Table 5.1 Overview of the effects of farm management practices on soil biological properties related to initial hypotheses.

Hypotheses	Results
▪ **Reduced tillage and high OM application will** (from now on called **ORG** fields):	
Increase earthworm density and biomass	Earthworm density and biomass was higher in ORG fields, largest effect was on the endogeic adults. Adult biomass (average weight/earthworm) increased in ORG fields
Increase microbial community abundance	Enhanced microbial community abundance in ORG oat and potato field, abundance reduced in ORG carrot field
Increase microbial community diversity	Diversity was not included in analysis due to financial constraints
Increase VAM colonization	VAM colonization was higher in ORG fields, the effect was especially visible in the oat crop and the 0-10 cm soil layer

5.1.2 Hypotheses on relation E: biodiversity – ecosystem service

Table 5.2 Overview of the effects of farm management practices on micronutrient availability and crop micronutrient density related to initial hypotheses.

Hypotheses	Results
▪ **Increased earthworm density will:**	
Increase OM breakdown	…
Increase mineralization rates of macro and micronutrients and ++ availability	…
▪ **Increased microbial activity will:**	
Increase mineralization rates of micronutrients and ++ availability	…
▪ **Increased VAM colonization will:**	
Increase uptake of poor mobile nutrients	…
▪ **Increased OM breakdown, mineralization rates, micronutrient availability and uptake will:**	
Increase crop mineral density (Cu, Zn, Fe)	…

It is not easy to say concluding words about the above stated hypotheses in Table 5.2. Due to the chosen soil and crop analysis it was possible to compare the effects of two farming systems on soil biological and chemical indicators as well as on crop quality indicators. However, as soil and crop analysis were only performed once, it was not possible to assess the effects of the two farming systems in time. It is thus difficult to say something about changing rates. Furthermore, it is also difficult to say something about which soil biological or chemical indicator caused which effect, as the research was limited in time and finances. In the following section a reflection will be given on the effect of the ORG farming system on the chosen soil biochemical indicators and on crop quality.

Table 5.3 Overview of the percent differences in soil and crop parameters with CON-crops as baseline for comparisons

	Oat	Carrot	Potato	Mean
Soil N	+117	+67	-3	+60
Soil P	+34	+23	-4	+17
Soil K	+211	+49	+10	+90
Soil Ca	+35	-5	+2	+11
Soil Mg	-22	+43	+55	+25
Soil S	+26	+33	-71	-4
Soil Cu	+100	-67	+20	+62
DM	n.d.	0	+18	0
Crop N	+7	+9	-16	-1
Crop P	+0.1	+41	+35	+25
Crop K	+357	+59	-11	+135
Crop Cu	+5	-16	+57	+16
Crop Fe	-26	+78	-31	+7
Crop Zn	+26	+13	0	+13

In Table 5.3 it is made visible what kind of effect the ORG-farming system has on soil biochemical indicators and on crop quality. Overall, it can be concluded that there were more available soil macro- and mesonutrients in the ORG-fields. Soil available Cu was present in larger amounts in two out of the three ORG-fields. The ORG-farming system furthermore had an overall positive impact on crop nutrient density, where the ORG-crops contained more macro and micronutrients then the CON-crops and also contained more macro and micronutrients then the ORG-crops from literature.

5.2 GENERAL REFLECTION

Many studies show the decline of nutrients in crops during the last fifty years (Davis et al, 2004; Mayer, 1997; White and Broadly, 2005) however, this research shows promising possibilities of reversing the trend. The potential of farm management practices that culture healthy soils are expected to be great. In a time with more and more appreciation for preventative health care, nutrition and food quality it is expected that this farming system, which has possible beneficial effects on the nutrient density of common crops, has great potential to be expanded in research and practice.

5.3 IMPLICATIONS FOR FURTHER RESEARCH

During the study it became clear that the attention for the relation between soils and human health has been relatively limited in science so far, in both soil science as with medical professionals (Deckers and Steinnes 2004). It is acknowledged that veterinary science has been aware of this relation in a much wider extent resulting in extensive literature on the relation between soil (deficiencies) and animal health (Lewis and Anderson 1983; Mills 1983; Frøslie 1990). Several soil and agricultural scientists acknowledge the limited research done on this topic and confirm the increase of interest in - and knowledge about the topic and emphasize the necessity of performing more research on the topic. Dr. Kristine Nichols, chief scientist at the Rodale Institute said in 2014 that science of soil and nutrition remains in the early stages (Arnason, 2014): "*We're recognizing more of the complex bio-molecules that are important in the soil and how that can get translated into the food products and into our bodies*". Dr. Jill Clapperton, former rhizosphere ecologist at the Lethbridge Research Centre in Alberta, Canada, said there isn't an abundance of research linking enhanced soil health to more nutritious food. "*If we're doing things right and we're measuring some soil health indicators, are we actually seeing more protein in the grain, for example? Are we seeing more micronutrients in the grain? That's (the) data that we're seeing now. It's coming out,*" Clapperton said at the World Congress of Conservation Agriculture in Winnipeg in June 2014 (Arnason, 2014). Also in the Netherlands scientists are acknowledging the necessity and growing interest for research on this topic. Wijnand Sukkel, research coordinator organic plant production and soil management for the Applied Plant Research science group at the WUR, confirmed early 2016 the growing interest in the group for the relation of soil management and food quality and considered a shift in emphasis from food quantity to food quality (e.g. flavour and nutrients).

Besides the growing interest of science, examples from the field also show how food producers are actively practicing alternative forms of soil management, with the objective to create nutrient dense food. *Shepherd's grain,* an Oregon based co-op of 42 growers practicing cover cropping, no-till, direct seeding techniques, advertises with nutrient density and puts the data on the back of the packages (Clapperton, 2014). Dutch (conventional) farmer Arnold van Woerkom practices already 16 year the Kinsey-Albrecht system of soil fertility management. This led to an overall 30% higher mineral density in his potatoes, when compared to potatoes from conventionally managed fields (Hanse, 2016). Currently he supplies several hospitals with his potatoes, as also the management of the hospitals realise the potential of mineral dense food for the patients. These experiences from science and practice show the recently growing awareness and knowledge present on the relationship soil management – crop nutrient density, however, also plea for more attention and research on this relationship.

The current study can confirm the previous experiences. The study was set up as explorative case-study and due to the research timeframe, budget and explorative design it is difficult to state firm conclusions.

However, the research shows large differences in crop mineral densities between the farms and the trend is that the organic crops have an overall higher mineral density per unit of dry weight. The climate, crop varieties and inherent soil properties of all farms were comparable and it is therefore expected that farm management is the strongest variable which determined crop mineral density. The research strongly confirmed the initial assumption that farm management is a strong variable determining crop mineral density.

More fundamental and applied research on this topic is urgently needed but also a different research approach might be necessary. Envisioned is a holistic approach towards the topic, wherein system analysis and design are used to understand the complex relationships between soil, plant and human vitality. Furthermore, it is advised to approach the topic in an interdisciplinary way, with researchers from soil biology, soil physics, soil chemistry, plant science and human nutrition.

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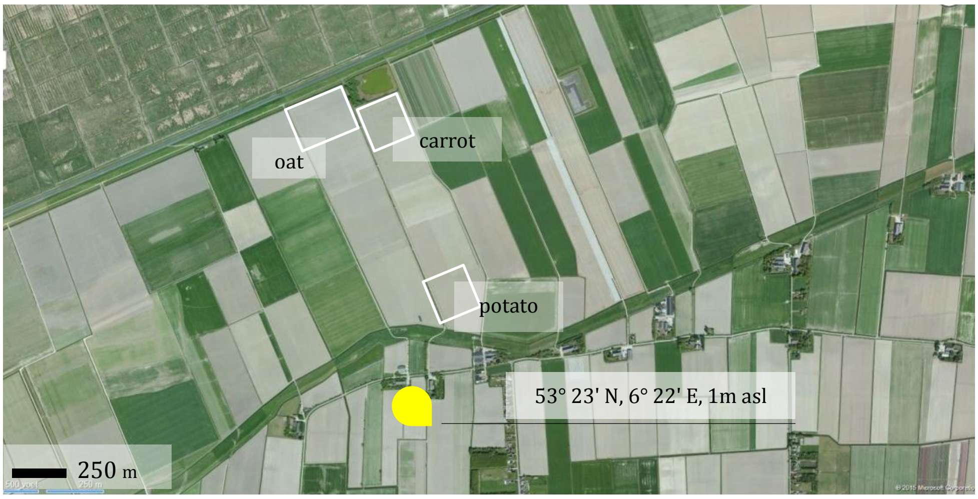
Appendix

I Location maps of case study farms and fields
II Soil maps of case study farms and fields
III Soil macro-, meso- and micronutrients, crop macro- and micronutrients
IV Nutrient balance calculation

I Location maps of case study farms and fields

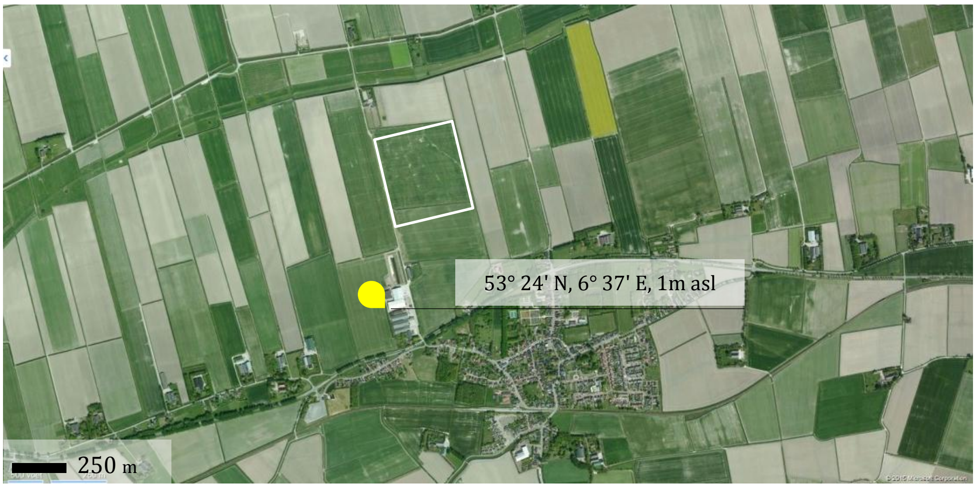


(a) farm of H. Westers and location oat, carrot and potato field (organic)

(b.1) farm of D. Wijk and location oat field (conventional)

(b.2) oat field

(c) farm of C. Gaaikema and location of potato field (conventional)

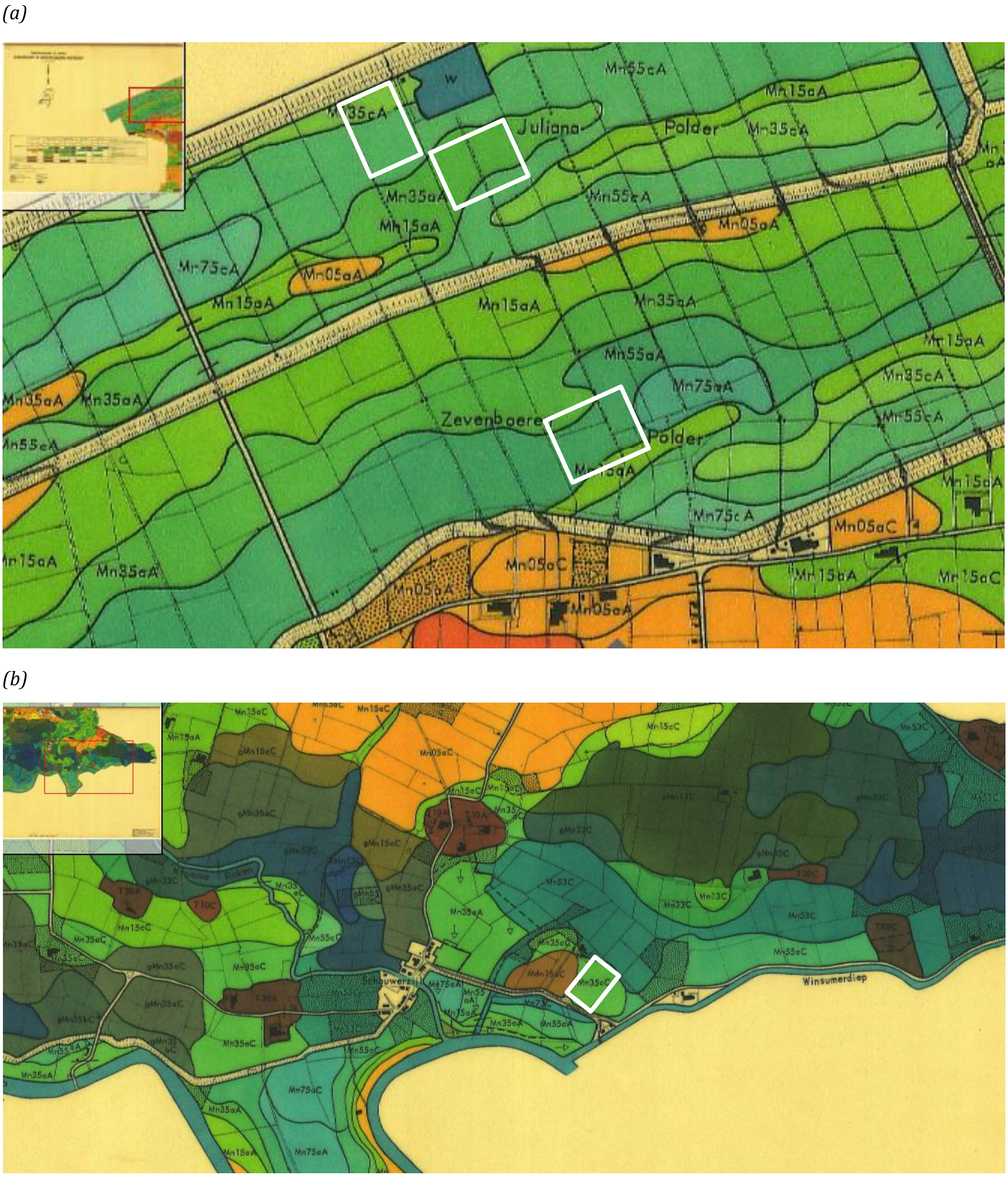


(d) farm of H. Knook and location of carrot field (conventional)

Map of location of case study *farms and fields of H. Westers (a), D. Wijk (b), C. Gaaikema (c) and H. Knook (d)*

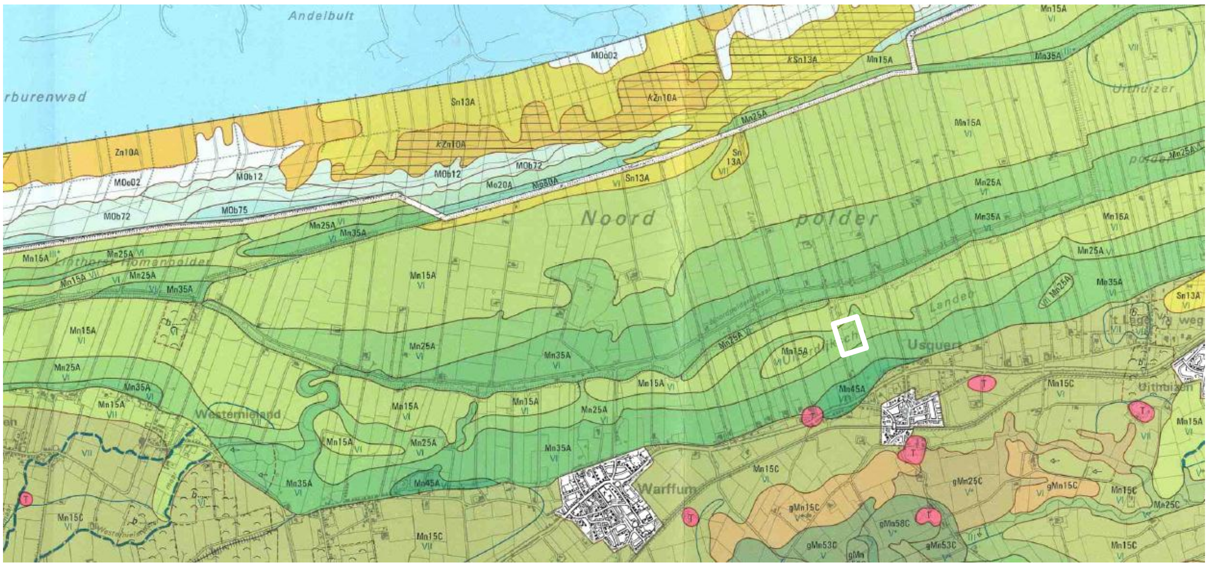
Source: bing.com/maps/

II Soil maps of case study farms and fields



Soil map municipality De Marne and location oat, carrot and potato field H. Westers (a) and oat field D. Wijk (b) (original scale 1:50000)
source: STIBOKA (Stichting voor Bodemkartering), 1968

Soil map municipality De Marne and location potato field C. Gaaikema (a) (original scale 1:50000) and legend corresponding to above mentioned maps (b)
source: STIBOKA (Stichting voor Bodemkartering), 1968



Soil map municipality Winsum and location carrot field H. Knook (original scale 1:50000)
source: STIBOKA (Stichting voor Bodemkartering), 1987

Field	Code	Zwaarteklasse **Bovenste 30 cm**	Kalkverloop **A: overwegend kalkrijk** **C: overwegend kalkarm**	Aard van klei onder de bovengrond **Normaal/knippig**	Profielverloop
Haver Westers (Noord)	Mn35cA	Zware zavel: 17,5-25%	A	Normaal	Aflopend, met een lichtere klei tussenlaag of ondergrond beginnend tussen 40 en 80 cm
Haver Westers (Zuid)	Mn55cA	Lichte klei: 25-35%	A	Normaal	Aflopend, met een lichtere klei tussenlaag of ondergrond beginnend tussen 40 en 80 cm
Wortel Westers	Mn35aA	Zware zavel: 17,5-25%	A	Normaal	Homogeen
Aardappel Westers	Mn55aA	Lichte klei: 25-35%	A	Normaal	Homogeen
Haver Wijk	Mn35aC	Zware zavel: 17,5-25%	C	Normaal	Homogeen
Aardappel Gaaikema	Mn15aA	Matig lichte zavel 12-17,5%	A	Normaal	Homogeen
Wortel Knook	Mn25A	Zware zavel: 17,5-25%	A	Normaal	Homogeen

III Soil macro-, meso- and micronutrients, crop macro- and micronutrients

Nitrate (N-NO_3 mg/kg), ammonium (N-NH_4 mg/kg), Nmin (N-NH_4 + N-NO_3 mg/kg), Ntot (g/kg) and the percentage N available measured in soil layers 0-10 cm (1), 10-20 cm (2), and 20-30 cm (3) in the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

		N-NO_3^-			**N-NH_4^+**			**N_{min}**			**N_{tot}**			**N_{min} / N_{tot}**		
		mg/kg			**mg/kg**			**mg/kg**			**g/kg**			**%**		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Crop	**System**															
Oat	CON	2.3	3.9	2.3	0.9	0.6	0.8	3.1	4.1	3.1	0.9	0.9	0.9	0.4	0.5	0.3
	ORG	6.8	5.3	2.2	3.2	1.3	1.3	9.9	6.6	3.5	1.7	1.5	1.3	0.6	0.4	0.3
Carrot	CON	4.2	3.4	4.9	1.8	2.1	2.1	5.9	5.6	7	1	1.2	1.2	0.6	0.5	0.6
	ORG	10.2	8.5	5.1	2.3	3.4	2.4	12.5	11.9	7.4	1.4	1.4	1.3	0.9	0.9	0.6
Potato	CON	7.2	10.5	9.2	1.6	2.1	1.5	8.8	12.6	10.7	0.9	0.9	0.9	0.9	1.4	1.1
	ORG	8.8	10.1	5.9	2.3	1.4	1.3	11.2	11.5	7.2	1.3	1.3	1.0	0.9	0.9	0.7

Phosphate (P-PO_4 mg/kg), total phosphorus (Ptot g/kg), the percentage P available in the soil and available potassium (K available mg/kg) measured in soil layers 0-10 cm (1), 10-20 cm (2), and 20-30 cm (3) in the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

		P-PO_4^{3-}			**P_{tot}**			**P- PO_4^{3-}/ P_{tot}**			**$K_{available}$**		
		mg/kg			**g/kg**			**%**			**mg/kg**		
		1	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Crop	**System**												
Oat	CON	1.64	1.51	2.00	0.54	0.52	0.53	0.30	0.29	0.38	42.40	33.59	34.70
	ORG	1.77	0.95	0.83	0.76	0.68	0.69	0.23	0.14	0.12	197.64	97.40	60.92
Carrot	CON	1.66	1.47	1.39	0.62	0.60	0.60	0.27	0.24	0.23	140.41	90.11	67.56
	ORG	4.55	3.24	2.59	0.78	0.73	0.72	0.58	0.44	0.36	196.81	140.35	102.49
Potato	CON	0.82	3.47	1.12	0.61	0.74	0.65	0.13	0.47	0.17	155.84	77.35	84.58
	ORG	2.59	0.81	0.81	0.67	0.63	0.59	0.38	0.13	0.14	134.90	66.03	133.23

Soil - calcium (Ca mg/kg), magnesium (Mg mg/kg), sulphur (S mg/kg), copper (mg/kg), iron (Fe mg/kg) and zinc (Zn mg/kg) measured in soil layer 0-20 cm in the oat, carrot and potato crop in the organic (ORG) and conventional (CON) farming system.

		Ca H_20	**Mg $CaCl_2$**	**S $CaCl_2$**	**Cu $CaCl_2$**	**Fe $CaCl_2$**	**Zn $CaCl_2$**
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
		0-20	0-20	0-20	0-20	0-20	0-20
Crop	**System**						
Oat	CON	86.5	100	3.5	0.03	-	-
	ORG	117	77.6	4.4	0.06	0	0
Carrot	CON	133	51.0	4.5	0.03	0	0
	ORG	127	73.1	6.0	0.05	0	0
Potato	CON	113	43.8	11.2	0.05	0	0
	ORG	115	67.8	3.3	0.06	0	0

Crop - macro- and micronutrient content (mg g) of the edible parts of the oat, carrot and potato crop (measured in dry matter) in an organic (ORG) and conventional (CON) farming system.

		N	**P**	**K**	**Cu**	**Fe**	**Zn**
		g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg
Crop	**System**						
Oat	CON	14.49	4.03	4.74	3.7	50	19
	ORG	15.47	4.04	21.67	3.9	37	24
Carrot	CON	7.75	2.11	21.97	9.6	126	16
	ORG	8.44	2.96	34.84	8.1	224	18
Potato	CON	9.63	1.62	22.34	2.8	118	9
	ORG	8.08	2.20	20.01	4.4	81	9

Mean percent differences (MPD)[a] in pH, soil organic matter and nutrient content in organic and conventional managed fields.

	pH	**SOM**	**N-NO_3^-**	**N-NH_4^+**	**N_{tot}**	**P-PO_4^{3-}**	**Pt_{ot}**	**K**
			%	%	%	%	%	%
Crop								
Oat	-0.04	+54.4	+78.4	+154.6	+66.7	-29.3	+33.9	+210.5
Carrot	+0.5	+36.7	+98.3	+34.9	+24.1	+126.6	+22.5	+49.2
Potato	-3.3	+16.7	-5.5	-0.6	+29.4	+37.7	-4.4	+9.8
Mean +/-	**-0.9**	**+35.9**	**+57.1**	**+63.0**	**+40.1**	**+45.0**	**+17.3**	**+89.8**

[a] Plus and minus signs refer to conventional crops as the baseline for comparison. e.g., mean phosphate is 45% more abundant in the soil of the organic potato crop (conventional 100%, organic 145%).

VI Nutrient balance calculation

		Inputs										Outputs		Balance	
	Farm; field	Fertilisers	Crop residues	Ammendments	TOTAL	yield	DM (%)		yield DM	Yield DM	Nutrients	Uptake	Leaching		
		kg/N,P,K/ha	kg/N,P,K/ha	kg/N,P,K/ha	kg/N,P,K/ha	(t/ha)	(%)		(T/DM/ha)	(kg/DM/ha)	gr/kg	kg/ha/yr		kg/ha/yr	
N	CONoat	30		200	**230**	7.5	75	0.75	5.625	5625	14.49	81.5		**148.5**	CONoat
	ORGoata		40		**40**	6	77.4	0.774	4.644	4644	15.47	71.8		**-31.8**	ORGoat
	CONcarrot	115			**115**	116.2	11.1	0.111	12.8982	12898.2	7.75	100.0		**15.0**	CONcarrot
	ORGcarrotc		125		**125**	48.9	11	0.11	5.379	5379	8.44	45.4		**79.6**	ORGcarrot
	CONpotato	18		120	**138**	51.2	19.3	0.193	9.8816	9881.6	9.63	95.2		**42.8**	CONpotato
	ORGpotato		568		**568**	42.5	22.8	0.228	9.69	9690	8.08	78.3		**489.7**	ORGpotato
P	CONoat			242	**242**	7.5	75	0.75	5.625	5625	4.03	22.7		**219.3**	CONoat
	ORGoatb		8		**8**	6	77.4	0.774	4.644	4644	4.04	18.8		**-10.8**	ORGoat
	CONcarrot	20.4			**20.4**	116.2	11.1	0.111	12.8982	12898.2	2.11	27.2		**-6.8**	CONcarrot
	ORGcarrotd		20		**20**	48.9	11	0.11	5.379	5379	2.96	15.9		**4.1**	ORGcarrot
	CONpotato	61		72	**133**	51.2	19.3	0.193	9.8816	9881.6	1.62	16.0		**117.0**	CONpotato
	ORGpotatoe		175		**175**	42.5	22.8	0.228	9.69	9690	2.2	21.3		**153.7**	ORGpotato