Eesti Maaülikool
Estonian University of Life Sciences

SOIL PROPERTIES AFFECTED BY COVER CROPS AND FERTILIZATION IN A CROP ROTATION EXPERIMENT

VAHEKULTUURIDE JA VÄETAMISE MÕJU MULLA OMADUSTELE KÜLVIKORRAKATSES

DIEGO SÁNCHEZ DE CIMA

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2016

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LIST OF ORIGINAL PUBLICATIONS

This thesis based on the following papers, which are referred to by their Roman numbers in the text. All papers are reproduced with due permission from the publishers.

I **Sánchez de Cima, D.**, Reintam, E., Tein, B., Eremeev, V., Luik, A. 2015. Soil nutrient evolution during the first rotation in organic and conventional farming systems. *Communication in Soil Sciences and Plant Analysis*, 00: 1–13.

II **Sánchez de Cima, D.**, Luik, A., Reintam, E. 2015. Organic farming and cover crops as an alternative to mineral fertilizers to improve soil physical properties. *International Agrophysics*, 29: 405–412.

III **Sánchez de Cima, D.**, Tein, B., Eremeev, V., Luik, A., Karin, K., Reintam, E., Kahu, G. 2015. Winter cover crops effect on soil structural stability and microbiological activity in organic farming. *Biological Agriculture & Horticulture* (accepted).

IV Kauer, K., Tein, B., **Sánchez de Cima, D.**, Talgre, L., Eremeev, V., Loit, E., Luik A. 2015. Soil carbon dynamics estimation and dependence on farming system in a temperate climate. *Soil & Tillage Research*, 154: 53–63.

The contributions from the authors to the papers were the following:

	I	**II**	**III**	**IV**
Idea and design	AL, ER	AL, ER, **DSdC**	AL, ER, **DSdC**	AL, KK
Field work	BT, VE	**DSdC**, ER	**DSdC**, ER, BT, VE	**DSdC,** BT, LL, VE
Data collection	BT, VE	**DSdC**, ER	**DSdC**, ER, BT, VE, GK	**DSdC**, BT, LL, VE
Data analysis	DSdC	DSdC	**DSdC**	KK
Preparation of the manuscript	**DSdC**, ER	**DSdC**, ER	**DSdC**, ER	**DSdC**, AL, BT, EL, KK

DSdC – Diego Sánchez de Cima; ER – Endla Reintam; AL – Anne Luik; BT – Berit Tein; KK – Karin Kauer; VE – Vyacheslav Eremeev; LL – Liina Talgre; EL – Evelin Loit; GK – Greete Kahu.

1. INTRODUCTION

The current problems associated with the intensive conventional management of agriculture, such as the decline of organic matter, degradation of the soil structure, nitrate leaching (Herzog & Konrad, 1992), and groundwater pollution (Poudel *et al.*, 2002), have promoted the interest in and study of organic farming (van Diepeningen *et al.*, 2006). Furthermore, the interest in organic farming has also been stimulated by the financial support of the European Union through the Rural Development Programme (Cesevičienė *et al.*, 2009; Sacco *et al.*, 2015) and the increasing consumer awareness of food safety.

In 2011, the total area of organically farmed land in the world was 37.2 million hectares and there were more than 1.8 million organic farmers. In Europe, in the same year, there were 280,000 organic farmers and the total area of organically farmed land was 10.6 million hectares. In Estonia, after the re-establishment of independence in 1991, several governmental actions promoted a number of environmentally friendly agricultural practices across the country, including organic farming. In 2014, the Estonian Farming Action Plan 2014–2020 was launched to support the development of organic farming and increase the consumption of local, organic food (Estonian Minister of Agriculture, 2014).

Organic farming is an agricultural management practice that leads to the improvement of soil quality and fertility by maximizing the use of resources that are already present in the agro-ecosystem and excluding or limiting the use of chemical fertilizers, pesticides and genetically modified organisms (Shannon *et al.*, 2002; Steinshamn *et al.*, 2004). Among these management practices, crop rotation and cover crops have been incorporated into many farming systems, and their use is especially common in organic farming. The benefits of both practices on soil fertility, plant nutrition and productivity have been previously explored (Berzsenyi *et al.*, 2000; Hao *et al.*, 2002; Aziz *et al.*, 2011). The adoption of crop rotation and/or cover cropping commonly leads to an increase in soil organic carbon (SOC) (Hao *et al.*, 2002; West & Post, 2002; Aziz *et al.*, 2011) and a decrease in nitrogen (N) leaching (Hooker *et al.,* 2008). However, less known are the effects of these practices on soil microbial biomass activity and soil biota as well as on the soil physical properties, such as soil porosity and aggregate stability (Haruna *et al.*, 2015).

The management of soil fertility in stockless farms is not always sustainable, and in many cases, the use of rotations or cover crops do not exclude the need for additional fertilizers. Animal manure is a good source of nutrients (Rodrigues *et al.*, 2006; Guo *et al.*, 2016) and may be a feasible way of creating sustainably fertile soils; unfortunately, in Estonia, manure is not sufficiently available in organic arable farming (Edesi *et al.*, 2012). This is a problem, as reduced soil nutrient availability is regarded as the main factor that is responsible for low organic system productivity (Berry *et al.*, 2002; Seufert *et al.*, 2012). Therefore, with the current emphasis in agriculture on maximizing yields in the short term, many producers still prefer mineral fertilizers of conventional farming because of their fast assimilation by plants, solubility and relatively inexpensive price (Bokhtiar & Sakurai, 2005). However, many farmers forget that mineral fertilizers not only affect the soil nutrient content but also the soil physical properties, as well as the population and activity of the biota. The direct impact of mineral fertilizers on the soil physical properties is difficult to demonstrate (Głąb, 2014). As a result, there is a large variability in the effects of mineral fertilizers on SOC, aggregate stability and microbiological activity. For that reason, as well as the lack of studies that examined the physical properties of soil, particularly its porosity, water holding capacity, water permeability and structural stability, in different fertilization and management systems, this study was initiated.

In many Eastern European countries, such as Estonia, where the nutrient balance is negative (Astover *et al.*, 2006), cover crops and crop rotations play an important role in soil fertility in both conventional and organic farming. As a result, many countries have recently started to investigate the possibility of reducing soil nutrient loss in the plant-free period (Talgre *et al.*, 2009). However, one question remains: are cover crops reliable for maintaining soil fertility in the mid-term?

The effects of cover crops on the physical, chemical and microbiological properties of soil depend on the species, fertilization, tillage operations, climate and local conditions. The research on cover crops has been mostly confined to their use as nitrogen-binding agents and their effects on succeeding crops (Sainju *et al.*, 2003; Talgre *et al.*, 2009). However, the direct impact of cover crops on soil characteristics, such as aggregate stability and microbial activity, and their ability to limit phosphorus (P) and potassium (K) losses are still unquantified. In addition, relatively few studies on the effect of cover crops and crop rotation on soil physical and biological properties have been conducted under Nordic climate conditions.

The current study is focused on assessing the impact of cover crops that are used alone and combined with cattle manure on the soil chemical properties (pH, SOC, N, P, K, Mg, Ca), physical properties (bulk density, porosity, water permeability, penetration resistance, soil aggregate stability), earthworm population and microbial activity compared with the impact of mineral fertilizers in conventional systems using a five-year crop rotation of winter wheat (*Triticum aestivum* L.), pea (*Pisum saetivum* L.), potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.) under-sown with red clover (*Trifolium pratense* L.) and red clover.

2. LITERATURE REVIEW

2.1. Crop rotations in northern climate conditions

The objectives of crop rotation are to maintain soil fertility, minimize the risk of phyto-sanitary problems by breaking the cycles of certain pests or diseases (Peng *et al.*, 2015), and allow natural processes in the soil to take place (Martin Rueda *et al.,* 2007). The effects of crop rotation on soil parameters, such as enhancing the SOC content, N stock and soil microbial activity, have been largely documented (Snapp *et al.*, 2005; Edesi *et al.*, 2012). Less attention has been paid to the effects on soil porosity and aggregate stability. In any case, the effect of rotation on soil conditions is not permanent and mainly depends on tillage, as well as the local soil and weather conditions (Głąb *et al.,* 2013).

Northern European countries are generally considered to be less favourable growing areas in the European Union (EU), with average cropland areas ranging from 0 to 25% of the total land area (Rounsevell *et al.*, 2005). The growing season in these latitudes is characterized by a relatively low number of effective growing days, harsh winters, long days during the summer months, and high risk of frost and early summer drought (Peltonen-Sainio *et al.*, 2009). Most of the crop rotation experiments conducted in Estonia counted similar crop sequences, where high demanding N crops with high biomass production were followed by legumes, which were directly sown or under-sown with a cereal and alternating deep-rooted species (Ilumäe *et al.*, 2009; Edesi *et al.*, 2012; Tein *et al.*, 2014). Barley, red clover, winter wheat, potato and pea are the most common crops in Estonian agriculture, covering 41% of the total cultivated area (Statistika, 2014). These crops are both commonly used as well as combined in crop rotations. Some examples of local experiments in which crop rotations were used are the Ilumäe *et al.*, (2009) experiment, which consisted of a rotation of spring wheat, spring barley with pre-sown clover, clover, potato, oat (*Avena sativa* L.), pea, spring barley with pre-sown clover, clover, and rape (*Brassica napus* L.). The Edesi *et al.* (2012) experiment was based on a five-year crop rotation of potato, oat, barley under-sown with red clover, red clover, and winter rye (*Secale cereale* L.). Finally, Tein *et al.* (2014) conducted their investigation on the same experimental rotation as the present study: winter wheat, pea, potato, and barley under-sown with red clover.

To ensure the preservation and improvement of both the crop yield and soil fertility, the crop sequence must be planned, taking into account local climate conditions and estimates of the nutrient balance, as well as what nutrients will the crop take from the soil and how much will remain in the soil after harvest (Ilumäe *et al.*, 2009). Legumes are usually a part of all crop rotations and represent a low energy cost and sustainable source of N (Crews & Peoples, 2004). There is considerable research on the significant impact of legumes, such as red clover, alfalfa (*Medicago sativa* L.) or soybean (*Glycine max* L. Merr.), on the soil nitrogen content and dynamics, independent of the farming system (Li *et al.*, 2016). Researchers estimate that from 40 to 75% of the total nitrogen contained in a legume cover crop is available in the soil for subsequent crops, depending on the plant species, the amount of biomass formed, the Rhizobia-plant symbiosis, and the soil properties (Mohammadi1 *et al.*, 2012). For example, cereals grown after legumes usually take up approximately 15 to 20% of legume N (Crews & Peoples, 2005). Increasing amounts of N that are available from organic or mineral fertilizers and deficits of K and P as well as low temperatures can lead to a decrease in the proportion of N derived from atmospheric fixation (Hųgh-Jensen, 2003; Riesinger & Herzon, 2010). The influence of legumes on soil structure has been less studied. Whitbread *et al.* (2000) found that legumes influenced the size and stability of the soil aggregates collected; meanwhile, Dapaah & Vyn (1998) found a significant positive impact of red clover on soil aggregate stability, although not as positive as ryegrass.

Cereals in the rotation contribute to increases in the SOC from straw incorporation after harvest. Wang *et al.* (2015) showed an increase in the topsoil (0–20 cm) SOC stock of 3.98 kg C m^{-2} in a long-term field experiment in the North China Plain using an input of wheat crop residues. Moreover, cereal residues have a positive impact on soil porosity, soil structure, microbial population (Bulluck *et al.,* 2002; Liu *et al.,* 2007; Doan *et al.*, 2013; Sudhakaran *et al.,* 2013) and earthworms. Scullion (2007) concluded that during arable phases, earthworm populations tended to be larger in organic systems where cereal residues were incorporated. Gaudin *et al.* (2015) reported an increase in maize and soybean yields (depending on the tillage) in a crop rotation experiment in Canada where winter wheat was included. This yield increase was explained by a decrease in the maize and soybean N requirement when winter wheat was present in the rotation.

2.2. Cover crops

Cover crops are defined as annual, biennial, or perennial grasses and/or legumes that provide an off-seasonal soil cover and a wealth of potential benefits. These benefits include protection from soil erosion and compaction (Lal *et al.*, 1991; Williams & Weil, 2004), scavenging of N and decreasing leaching during the winter period, ultimately releasing it for subsequent crops (Sainju *et al.*, 2003; Hooker *et al.,* 2008; Messiga *et al.*, 2015; Plaza-Bonilla *et al.,* 2015) and building soil organic matter when the cover crop is tilled into the soil (green manure) (Lal, 2004; Talgre *et al.*, 2009; Frųseth *et al.*, 2014). However, less information has been found regarding their potential for diminishing losses of other nutrients in the soil as well as their effect on the soil physical properties, microbial activity and earthworms.

The effectiveness of cover crops depends on the choice of species, sowing time, main crop harvesting time, weather and local conditions. In northern latitudes, the growing season is short and low temperatures as well as sunlight are the limiting factors for plant growth in autumn. In Europe, the most common cover crops are white mustard (*Sinapis alba*, L.), westerwold ryegrass (*Lolium multiflorum* Lam.), fodder radish (*Raphanus sativus* var. *oleiformis*) and phacelia (*Phacelia tanacetifolia* Benth.). Among the legumes used as cover crops, pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.) are also used in soils with low N levels (Talgre *et al.*, 2011).

In this study, winter rye, winter oilseed rape and ryegrass (*Lolium perenne* L.) were used as cover crops.

2.2.1. Winter rye (*Secale cereale* L.)

Among cereals, winter rye is considered to be the most tolerant to low temperatures, short day lengths, low fertility and broad pH ranges (Oljača *et al.*, 2010; Cougnon *et al.*, 2015). Its use as a cover crop is extensive in northern Europe (Cougnon *et al.*, 2015). Characterized by a high biomass production, rye represents an important source of SOC. In the long term, Moore (2012) observed a 15 to 44% greater organic matter content in winter rye cover crop treatments compared with no cover crops. However, after the incorporation of winter rye in the soil and as the high C:N ratio (>30) residues decay, immobilization may reduce N availability to the main crop (Allison, 1966).

The structure of the rye plant enables it to capture and hold protective snow cover and is also expected to decrease the bulk density and enhance water availability (Welch *et al.*, 2016), which are beneficial in areas with a dry winter season. The extensive fibrous root system (that can cover a 0.3 m radius and a depth of 1.5 m, depending on the soil texture and water and nutrient availability) (Weaver, 1926) helps to alleviate compaction and mobilize nutrients from deeper soil layers. Müller *et al.* (2006) showed that rye is the best winter cereal cover for reducing N leaching.

2.2.2. Winter oilseed rape (*Brassica napus* L.)

Brassicas can provide more than 80% of coverage when used as cover crops. Depending on the location, planting date and soil fertility, they produce up to 9,000 kg dry biomass ha^{-1} (Haramoto & Galland, 2004). Winter oil seed rape produces large taproots that can penetrate up to 2 m, alleviating soil compaction and enhancing water infiltration and porosity to a greater or lesser extent, depending on the soil moisture and weather conditions (Chen & Weil, 2009). Due to the deeper root system, oil seed rape has been identified as superior to rye at scavenging N (Thorup-Kristensen, 2001; auf'm Erley *et al.*, 2011). Justes *et al.*, (1999) found that Brassicas can reduce nitrate leaching by >50% in northern climates. However, to achieve optimum N uptake, brassicas generally require sufficient sulphur (S) nutrition (Schnug, 1997). In addition, brassica cover crops mineralize nitrogen at a faster rate than cereals (because the C:N ratio is lower), but slower than legumes (Poudel *et al.,* 2001). Additionally, brassica species are noted for achieving high calcium (Ca) concentrations during their growth (White & Broadley, 2003). Despite the fact that more research is needed in this field, brassicas can be good cover options for soils that are deficient in Ca or naturally acidic after liming.

2.2.3. Ryegrass (*Lolium perenne* L.)

Perennial ryegrass is a potential cover crop species with a slow development in the early stages and a very linear response in dry matter production, up to nitrogen levels of 500 kg ha^{-1} (Aavola & Kärner, 2008). Ryegrass is very tolerant to a wide range of soil and climate conditions, adapting to temperatures below zero once established in both heavy clays and sandy soils. However, it poorly adapts to growing roots in compacted soil because of the relatively fine nature of its roots (Crush & Thom, 2011).

Ryegrass is also used for binding N left in the soil by seasonal crops (Müller *et al.*, 2006). Reviewing the data from 35 different field experiments in northern Europe, Valkama *et al.* (2015) concluded that compared to soil control groups with no cover crops, ryegrass used as a cover crop reduced N leaching loss by 50%. Regarding biomass production, Poeplau *et al.* (2015) defined ryegrass as an effective, multi-beneficial measure for increasing SOC stocks in a temperate humid region. On the other hand, the results from Talgre *et al.* (2009) showed that the biomass productivity and nutrient binding ability of ryegrass under Estonian conditions were low and negatively affected succeeding crop yields.

Few studies have focused on the effect of ryegrass on soil physical properties. Among these studies, Stone & Buttery (1989) and Zhou & Shangguan (2007) reported an increase in water stable soil aggregates with ryegrass alone or with grasses combined with legumes. Dapaah & Vyn (1998) also found, on different soil types, that the aggregate stability of ryegrass plots was significantly higher compared with plots with different cover crop treatments after three years.

2.3. Fertilization

Fertilization is a common management practice for maintaining soil fertility and crop productivity. The selection of fertilizer affects not only the nutrient concentration and availability in the soil but also the soil physical properties as well as the biota population and activity. Many studies have focused on N as a limiting factor affecting crop growth and yield. Nowadays, however, the use of synthetic fertilizers has eliminated a major elemental constraint and the effects on soil nutrient (NPK) content and yield are well known (Wang *et al.,* 2015a). However, the direct impact of mineral fertilizers on soil physical properties is difficult to assess (Głąb, 2014). As a result, there are many contradictory findings regarding the effects of mineral fertilizers on the SOC and soil physical properties. On one hand, a number of studies concluded that the SOC and soil structure are negatively affected by the continuous application of inorganic fertilizers without any organic inputs. In a field experiment lasting nine years in India, Sarkar *et al*. (2003) reported a decrease in the stability of soil macro aggregates and moisture retention capacity and an increase in the bulk density when mineral fertilizers were applied alone. In India, Hati *et al.* (2008) reported a decrease of 28.3% in the SOC when inorganic N fertilizers were applied in the long term. However, other studies did not find any deleterious effects of mineral fertilization on the SOC or soil physical properties. Rasmunssen & Rohde (1988) reported a linear increase in the SOC with applied N fertilizers and noted that the crop residue also has a positive impact on the SOC pool. Similarly, Yu *et al.* (2012) found that soil amendment with mineral fertilizers (NPK) mainly increased organic C concentrations in the soil macroaggregates. How mineral fertilizers affect the SOC will also determine the effect on soil bulk density and porosity.

The number of studies that have focused on the effects of mineral fertilizers on the soil structure is limited and the results are conflicted. Some authors reported that the application of mineral fertilizers promoted macroaggregation (Rasool *et al.*, 2008). In contrast, Sarkar *et al.* (2003) found that the application of inorganic fertilizers in the mid-term (9 years) decreased the stability of soil aggregates. Similar to the farming practices previously mentioned, the different effects of mineral fertilizers on the SOC, soil aggregation and soil porosity depends on the specific soil characteristics and climate conditions. Therefore, additional experiments are needed.

Many studies have found a negative effect of nitrogen-based fertilizers on soil microbial communities. Repeated application of inorganic fertilizers (especially N based fertilizers) decreases the soil pH (Imtiaz Rashid *et al.*, 2013; Geisseler & Scow, 2014), which, in turn, can reduce the nutrient availability and soil microbial biomass (Bardgett *et al.*, 1999) and can also change the microbial community composition (de Vries *et al.*, 2006). Liu & Greaver (2010) found that N addition reduced the microbial biomass by 20% across 57 studies. Lu *et al.* (2011) reported that the application of mineral N fertilizers significantly decreased the microbial biomass by 5.8%, with the effect being greatest in studies lasting five to ten years. However, Geisseler & Scow (2014) concluded that soils fertilized with N had a 15.1% higher microbial population than the same soils without fertilizer addition based on 64 long-term trials around the world. The effects of mineral fertilizers on earthworms are contradictory. At moderate levels of mineral fertilization, earthworms can benefit from the higher plant biomass and residues, but higher levels of nitrogen can inhibit their activity (Edwards, 2004).

An organic input, such as manure, has been shown by many studies to improve the soil physical properties by reducing the bulk density, increasing the water holding capacity, and improving the soil structure and infiltration rates (Sarkar *et al.*, 2003; Rasool *et al.*, 2008; Gopinath *et al.*, 2009; Guo *et al.*, 2016). Some examples include the studies carried out by Sarkar *et al.* (2003), who found that the incorporation of straw or manure in soil annually for nine years stabilized the 0.25 to 8 mm size category of aggregates under wet conditions. In a study by Rasool *et al.* (2008), the application of farmyard manure increased the percentage of soil stable aggregates as well as the soil porosity by 79% compared with systems receiving no inputs of manure, during the first year of study. Affecting the organic carbon input, manure amendments promote higher microbiological and earthworm activity than the same soils under conventional fertilization (Castillo & Joergensen, 2001; Fließbach *et al.*, 2007; Edesi *et al.*, 2012). However, while it is generally true that higher microbial activity is linked to a high soil organic matter content, the impact of these fertilizers on the soil microbial community, structure and activity as well as on soil nutrient availability can vary widely (Lazcano & Gómez-Brandón, 2013). Similarly, the effect of organic amendments on the soil nutrient content is heterogeneous and it is harder to predict their impact on soil properties and crops compared with mineral fertilizers (Rodrigues *et al.*, 2006). Furthermore, how effective organic amendments are for supplying sufficient nutrients for plant growth is far less clear (Lehman *et al.*, 2003). Some studies have shown that organic fertilizers, such as composted manures, can provide adequate levels of N in soil (Sullivan *et al.*, 1991; Steffen *et al.*, 1995; Sacco *et al.*, 2015). For example, Sacco *et al.* (2015) concluded that in the long term, green manuring in combination with commercial organic N can maintain SOC and increase the total N in soil. Bulluk *et al.* (2001) compared the effects of organic and synthetic soil fertility amendments on soil microbial communities and soil physical and chemical properties at six different farms in the USA for two years. The results showed that in both years, the concentrations of Ca, K, Mg, and Mn were higher in soils amended with organic fertility amendments than with synthetic fertility amendments.

2.4. Soil management and tillage

The type of management system, such as organic or conventional, affects the soil chemical, physical and biological properties due to the different crop rotation, fertilization and tillage practices. The crop rotation and fertilization effects were discussed in previous paragraphs.

Due to the limits on the use of plant protective substances, organic farming depends on a higher tillage intensity to destroy weeds and pests.

However, how tillage affects the soil structure depends on the local and climate conditions, soil texture, amount of organic matter, and crop rotation (Paustian *et al.*, 1997; Puget & Lal, 2005). The effects of tillage on the soil bulk density, soil water retention, compaction, aerobic condition, SOC and N have been largely studied (Martín-Rueda *et al.,* 2007). In general, any tillage based land use, irrespective of the type of land, caused a decline in the SOM content in the tilled soil layer, even in the short term (Basch *et al.*, 2012). Especially in soils with a low organic matter content, the main factor affecting the soil physical properties is mechanical soil tillage (Głąb & Kulig, 2008). This is the result of the physical alteration of the soil profile, which exposes aggregate protected C to microbial attack and soil particles to the effect of drying/rewetting and freezing/thawing cycles, accelerating SOM decomposition and decreasing soil particle aggregation (Plaza-Bonilla *et al.,* 2016).

The impact of tillage on the overall soil condition will depend on the intensity and type of operation. Gadja *et al.* (2012) found in less disturbed tillage systems a 15 to 40% higher enzymatic activity, on average, compared with the same soils under a conventional tillage system. Intensive tillage in agricultural systems can lead to soil compaction and a significant decline in the SOC (Basch *et al.*, 2012) and total nitrogen concentrations (Dikgwatlhe *et al.*, 2014) due to degradation of the soil structure. Martín-Rueda *et al.* (2007) also found P, K, Fe, Mn, Cu and Zn depletion in the soil upper layers under intensive tillage. As was previously mentioned, Plaza-Bonilla *et al.* (2016) showed that even in the short term, intensive tillage significantly decreases the soil organic carbon (SOC) and nitrogen (SON), even though the rotation counted two legumes and cover crops. Many stockless organic farming systems rely on the ploughing of plant residues to maintain soil fertility. However, in some cases, this practice might have a negative effect on the soil fertility, requiring tillage operations to be carefully planned. For example, ploughing creates unfavourable soil environmental conditions and disrupts earthworm populations (Chan, 2011; Crittenden *et al.*, 2014). Boström (1995) estimated that tillage practices can kill approximately 75% of the earthworm population.

In addition to tillage, the soil biological properties are also affected by the use of pesticides. The use of chemical pesticides in conventional farming can kill earthworms directly or have long-term toxic impacts on their growth and reproduction (Paoletti, 1999; Riley *et al.*, 2008).

3. HYPOTHESIS AND AIMS OF THE STUDY

Cover crops are commonly used to increase N recycling by reducing leaching; however, their capacity for preventing other nutrient losses and their effect on the soil physical structure and condition is still unquantified. Within the frame work of northern European climate conditions, the current research is focused on evaluating the effects of cover crops and manure on soil physical, chemical and biological properties in a five-year crop rotation compared with mineral fertilizers in conventional farming.

Hypothesis of the present study:

- In crop rotation, cover crops alone or with cattle manure will be sufficient for maintaining a constant nutrient (N, P, K, C, Mg and Ca) concentration in the soil compared with conventional systems using mineral fertilization and no cover crops (I, IV).

- In organic systems, cover crops and their combination with cattle manure will have a positive impact on soil physical parameters. These systems will have a higher aggregate stability, lower bulk density, and higher percentage of available water compared with conventional farming, with or without fertilization (II, III).

- The organic systems with cover crops alone or with cattle manure will have larger numbers of earthworms and higher microbiological activity compared with organic systems without cover crops and fertilization (II, III).

- Crop rotation is expected to have a positive yearly effect on the overall soil physical condition. At the end of the five-year rotation, all of the systems, organic and conventional alike, will have a lower bulk density, higher plant water availability and higher percentage of air filled pores compared with the starting stage (II).

To confirm or disprove the hypotheses the following aims were established:

- To study the changes in the soil reaction (pH_{KCl}), organic carbon (C_{org}), total nitrogen (N_{tot}), available phosphorus (P), potassium (K), magnesium (Mg) and calcium (Ca) content in the soil due to the use of cover crops (rye, rye grass, oilseed rape)and cattle manure in organic farming and mineral fertilizers in conventional farming in a winter wheat – pea – potato – barley under-sown with red clover – red clover crop rotation (I, IV).

- To study the changes in the soil physical properties, such as the bulk density, penetration resistance, porosity and water permeability due to the use of cover crops and cattle manure in organic farming and mineral fertilizers in conventional farming in a five-year crop rotation (II, III).

- To study the effects of cover crops and their combination with cattle manure on the soil water stable structure aggregates (WSA), number and biomass of the earthworm population, and microbial activity, as determined by the fluorescein diacetate hydrolysis activity (FDA), under organic management in a five-year crop rotation (III).

4. MATERIALS AND METHODS

4.1. Field experiment

Data were collected from the research field situated at the experimental station of the Estonian University of Life Sciences (EMÜ) in Eerika, Tartu, Estonia (58°22’N, 26°40’E). The soil was described as *Stagnic Albic Luvisol* (IUSS Working Group WRB, 2006), with 56.5% sand, 34% silt and 9.5% clay.

On this field, a five-year stockless crop rotation trial –winter wheat, pea, potato, barley, (undersown with red clover) and red clover–was started in 2008 (Table 1 in IV). This rotation was managed under different farming systems (conventional and organic), and the results were compared over a seven-year period: 2008 to 2012 (first rotation) and 2013 to 2014.

4.1.1. First rotation (2008–2012): Comparison between conventional and organic farming systems (I, II, IV)

During the first rotation, the experimental field was divided into 6 separated systems, four conventional and two organic. For each of the farming systems, four replications (of the crop rotation) were performed. Each replication consisted of five plots of approximately 60 m^{-2} in which the five rotating crops were established (Figure 1). The six fertilisation systems were as follows:

1. Conventional I: Conventional managed system with pesticides but without addition of any chemical fertilisers ($N_0P_0K_0$). This system was used as the control system.

2. Conventional II: Conventional managed system in which different concentrations of complex mineral fertiliser were added along with chemical pesticides; the additions were made once before planting and twice during the growing period. The average ratio applied was dependent on the culture: barley received $N_{120}P_{25}K_{95}$, winter wheat and potato received $N_{150}P_{25}K_{95}$, and a low N ratio ($N_{20}P_{25}K_{95}$) was used for pea because it is a leguminous crop. Red clover alone was not fertilised. Of the total amount of N added, 5 kg was provided in the composition of complex fertiliser and the rest as ammonium nitrate (NH_4NO_3).

3. Conventional III: Conventional managed system with yearly addition of chemical pesticides and mineral fertilisers in the following ratios: barley, $N_{40}P_{25}K_{95}$; winter wheat and potato, $N_{50}P_{25}K_{95}$; pea, $N_{20}P_{25}K_{95}$.

4. Conventional IV: Conventional managed system with yearly addition of chemical pesticides and mineral fertilisers in the following ratios: barley, $N_{80}P_{25}K_{95}$; winter wheat and potato, $N_{100}P_{25}K_{95}$; pea, $N_{20}P_{25}K_{95}$.

5. Organic I: Organic managed system including winter oilseed-rape after pea, winter rye after potato and ryegrass after winter wheat as winter cover crops (catch crops) lately ploughed and used as green manure.

6. Organic II: Conventional management system including the same cover crops as Organic I with the addition of 40 t ha^{-1} of composted cattle manure in autumn (2009) or in spring (2010–2012) in plots in which potato was cultivated. On average, the composted cattle manure contained: total nitrogen (N): 9.7 g kg^{-1}; total phosphorus (P_{tot}): 4.6 g kg^{-1}; total potassium (K_{tot}): 8.6 g kg^{-1}; total carbon (C_{tot}): 138 g kg^{-1}; total calcium (Ca_{tot}): 11.7 g kg^{-1}; total magnesium (Mg_{tot}): 3.4 g kg^{-1} and 44.8% dry matter (DM).

Note that the present study as well as the original publications (I, II and III) primarily focus on the first two conventional systems (Conventional I and Conventional II) and on the organic systems described above; only in the case of IV are the Conventional III and IV systems taken into consideration.

Organic and conventional plots were separated with an 18-m long section of mixed grasses. This prevented organic plots from contamination with synthetic pesticides and mineral fertilisers from the conventional side. Conventional plots, in turn, were separated by 40 m; in the case of the organic systems, a 10-m long protective area prevented manure from reaching plots in which its use was not intended.

Prior to the current experiment management and distribution, in 2006 the whole field was amended with complex fertilisers; therefore, the period of conversion to organic farming began in 2007, and in 2011

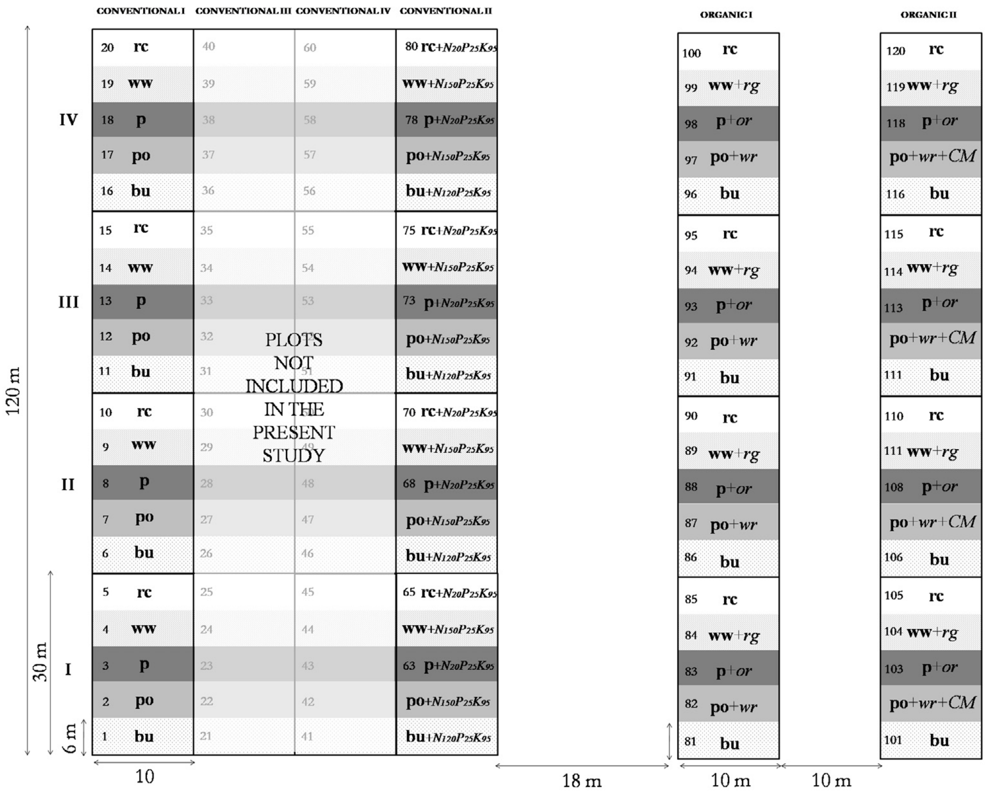

Figure 1. Crop sequence during the first rotation. Roman numerals on the left indicate the number of replications per system.
Main crops: bu – barley undersown with red clover; po – potato; p – pea; ww – winter wheat; rc – red clover.
Winter cover crops: *wr* – winter rye; *or* – oilseed-rape; *rg* – ryegrass.
Fertilisation: M – cattle manure.

the organic plots can be considered fully organic. For simplicity, in the present study the term 'Organic' has been used to refer to both organic farming systems in all years of the experiment.

4.1.2. Experimental setup from 2013 to 2014: Comparison among organic systems (III)

After the first rotation, the investigation focused on the organic farming systems. The original Organic I system was divided into two organic systems: Organic 0, without cover crops during the winter period and used as a control; and Organic I, managed under the same conditions as in previous years (Figure 1 in III). Therefore, during 2013 and 2014, the experiment consisted of three organic systems with four replications per system as in the previous period: Organic 0 (control); Organic I (with cover crops); Organic II (with cover crops and cattle manure). In these two years, composted cattle manure was not applied only in the plots in which potato was planted but was divided between potato (20 t ha^{-1}), winter wheat (10 t ha^{-1}) and barley undersown with red clover (10 t ha^{-1}). Manure was applied in spring.

As during the first period, the systems were separated by a 10-m long protective area for preventing accidental spread of cover crop residues or cattle manure among the systems.

4.2. Tillage and field operations

Table 1 in I shows the average sowing rates of both the main cultures and the cover crops. The main cereal and legume crops were sown during the last days of April, with the exception of barley undersown with red clover and pea, which was sown at the beginning of May, and winter wheat, which was sown during late August. All sowing labour was performed using a Kongskilde Combiseed N30 combi driller. Potato was planted at the beginning of May using a Juko Ekengards 4100 potato planter.

Cereal crops and pea were harvested at the beginning/middle of August using a Sampo SR2010 harvester. Potato plots were ploughed at 25 cm, harrowed at 4–5 cm depth, subsequently furrowed, hilled and finally harvested at the end of August/beginning of September depending on the growing season. After the rows were opened, the tubers were hand-picked using a German origin two-row elevator-picker machine. Red clover was cut twice a year using a Müthing MU-H7S 140 mower. The first cut was made in the second half of June, and red clover residues were left on the soil surface to decompose. During the second half of August, red clover was cut again but this time was ploughed into the soil at a depth of 27–29 cm.

In the organic systems, soil was harrowed after the main crops were harvested and before the cover crops were sown. The main crop residues in conventional systems were ploughed into the soil at the end of October. The plougher used was a Kverneland ES80. The cover crops used in the organic systems were ploughed into the soil directly one or two weeks before sowing/planting the main crops in spring. The only plant material removed from the field was grains and tubers. Other crop residues were left on the field.

The mineral fertilisers were applied in the conventional systems using a Fiona Birdie fertiliser spreader designed for field experiments; the pesticides were applied using an Amazone UF1501 sprayer. In the organic systems, cattle manure was weighed and then manually spread. Fully composted cattle manure was weighed and then spread on the potato plots manually.

It is important to remark that the same tillage operations were performed during the entire experiment for all of the systems, but in the case of the organic systems, the winter cover crops were sown after harrowing and ploughed afterwards.

4.3. Sampling, measurements and analysis

All of the analyses were carried out at the laboratories of the Department of Soil Science and Agrochemistry of the EMU.

4.3.1. Soil chemical analysis

During the spring and prior to any field operations, 8 soil samples per plot were randomly taken from 0 to 25 cm depth and combined to create one average sample. The air-dried soil samples were passed through a 2-mm sieve (I, III, IV) prior to analysis.

A solution of soil in 1M KCl (1:2.5) was used to determine the pH (I, IV). The soil organic carbon concentration (SOC) was determined by the Tjurin method (Vorobyova, 1998) (I, III, IV); total nitrogen (N) was measured after Kjeldahl digestion (van Reeuwijk, 2002). The concentrations of plant-available phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the soil were determined by the ammonium lactate (AL) method (Egnér *et al.,* 1960) (I, III). For measurement of dissolved organic carbon (DOC), 10 g of air-dried soil was shaken with 30 ml of distilled water for 1 h, followed by centrifugation and filtration through a 0.45-mm filter. The extracts were analysed for DOC (IV) using a varioMAX CNS elemental analyser (ELEMENTAR, Germany). Carbon input from the main crop residues and cover crops (kg C ha^{-1} y^{-1})

were also calculated for the first rotation. A detailed description of the formulas used for this calculation can be found in paper IV.

The same equipment and procedures were used in the analysis of the composted manure and the determination of N and SOC content. In addition, acid digestion by sulphuric acid solution (van Reeuwijk, 2002) was used to determine total phosphorus (P) and potassium (K) concentrations.

4.3.2. Plant analysis

Aboveground samples for determining biomass were gathered from an area 0.25 m^2 in size. Acid digestion by sulphuric acid solution (Method of Soil…, 1986) was used to determine the P, K, Ca and Mg content of the plant material (plant nutrient uptake) (Table 3 in I).

The carbon inputs for each system were calculated as the crop average of the amount of C measured from dry matter yield of above-ground agricultural products removed from the fields together with straw, roots and the extra C associated with rhizodeposits (IV).

4.3.3. Soil physical analysis

Soil samples were taken in autumn (II) and spring (III, IV) from the middle area of each plot prior to tillage. Four replications per plot at 5–10 cm depth (II, III, IV) and at 30–35 cm (III) were obtained using steel cores (54 mm internal diameter and 40-mm height, with wall thickness approximately 1.5 mm). Along with the steel cylinders, 250 g of fresh soil from the same depth was placed into plastic tubs for the later determination of plant-available and non-available water and percentage of stable aggregates (III).

The soil cores were first weighed under field-moist conditions and then capillary wetted for 24 hours until saturation, after which the soil water content was calculated (II, III). The cylinders were then placed on a sand bed at a water tension of 6 kPa for 12 days, followed by drying in an oven at 105°C for 24 hours. From these analyses, bulk density, saturation water content, total porosity and air-filled pores were calculated (II, III). Water permeability was measured using a Hauben permeameter (Eijkelkamp) (II, III). From the air-dried soil samples in the plastic tubs, three replications were placed in small steel cylinders (1.5 cm in diameter, 0.5 cm in height and 0.1 cm thick), put into pressure vessels at 1500 kPa for 30 days, weighed, dried at 105°C and weighed again. From these analyses, plant-available and non-available water was calculated (II, III).

Finally, from the same air-dried samples, the percentage of water-stable aggregates (WSA) (<0.05 mm) was measured using a single-sieving apparatus (Eijkelkamp sieving apparatus with 60 mesh screen cylinders) following the methodology and calculations used by Kemper & Rosenau (1986) (III).

4.3.4. Earthworms and fluorescein diacetate hydrolysis activity (FDA)

Earthworm samples were taken in autumn prior to harvesting at a depth of 0 to 20 cm within a 40×40-cm frame that was randomly placed in each of the plots of the three systems. The samples were brought to the laboratory, where they were classified according to species, counted and weighed; they were then returned to the field (III).

Fluorescein diacetate hydrolytic activity (FDA) is used for the determination of microbial activity in soil and litter. Microorganisms hydrolyse FDA, and microbial activity is evaluated by measuring the absorbance of fluorescein (Schnürer & Rosswall, 1982). For the FDA analysis, 500-g samples were taken at 5–10 cm depth; the samples were handled and stored according to ISO 10381-6, 1993. After stones and large pieces of plant material were removed by hand, approximately 200 g (fresh weight) of each sample was taken as a sub-sample and sieved through 2-mm mesh. For reagent preparation and subsequent FDA analysis, the method described by Adam & Duncan (2001) was followed, with the exception that acetone was used as the termination reagent and fluorescein instead of fluorescein sodium salt was used in the stock solution. The total microbial activity of FDA was expressed as "μg of released fluorescein per gram soil dry mass over a period of 1h" (III).

4.4. Weather conditions

Average monthly temperatures (T) and monthly precipitation were calculated based on the daily average data monitored with a Metos Compact (Pessl Instruments) electronic weather station situated in Eerika,

Table 1. Average air temperature (T) and total precipitation (P) at the experimental site during the years of study (2008–2014) and long term average values (1969–2014).

Month	Temperatures (°C)							
	2008	2009	2010	2011	2012	2013	2014	1969-2014
January	−1.3	−3.4	−5.1	−4.7	−6.1	−7.0	−8.1	−5.2
February	0.6	−4.9	−7.9	−11.2	−11.5	−3.5	−0.3	−5.7
March	0.4	−1.5	−2.1	−1.9	−0.3	−7.8	2.2	−1.6
April	7.2	5.3	6.1	6.4	5.0	3.5	6.3	4.8
May	10.7	11.5	12.6	11.0	11.8	14.8	11.6	11.4
June	14.5	13.8	14.6	17.2	13.6	18.2	13.0	15.3
July	16.1	16.9	22.2	19.9	18.0	17.7	18.7	17.5
August	15.7	15.4	18.2	15.8	15.1	16.9	16.2	16.17
September	9.8	12.8	11.1	12.3	12.2	11.0	11.7	10.8
October	8.2	4.1	4.2	6.8	5.7	6.9	5.22	5.6
November	2.3	2.3	0.3	2.9	2.6	3.9	1.4	0.5
December	−1.1	−8.6	−8.2	1.0	−6.8	1.4	−1.2	−3.2
Year	6.9	5.3	5.5	6.3	4.9	6.3	6.4	5.5

Month	Precipitations (mm)							
	2008	2009	2010	2011	2012	2013	2014	1969-2014
January	21.8	10.2	40.4	19.0	30.0	8.8	25	29.5
February	34.4	7.2	4.8	9.0	18.6	14.4	12.4	22.3
March	8.4	22.4	30.2	4.4	39.4	15.4	9	23.9
April	26.8	12.4	26.4	11.2	42.0	16.8	13.4	26.7
May	27.4	13.4	61.4	58.4	81.6	61.2	83.8	57.2
June	110.6	137.4	72.6	35.2	100.6	52.4	103.4	75.4
July	98.6	54.6	36.0	48.2	75.0	62.6	71.4	89.2
August	214.4	89.2	106.8	54.6	87.4	75.6	113	57.9
September	45.6	14.0	93.0	80.0	59.8	33	22.2	53.7
October	67.6	116.0	49.4	47.8	45.2	42.4	35.8	57.0
November	49.4	35.8	77.6	34.4	50.2	58.4	36	44.4
December	23.6	57.0	98.5	52.6	9.0	77.2	92	36.1
Year	728.6	606.4	697.1	454.8	638.8	518.2	617.4	573.3

Tartu. Weather conditions during the first rotation (2008–2012) (I, II, IV) differed (Table 1) from those during the second period of study (2013-2014) (III). Comparing the data from the growing period with the average long-term data (1969–2014), 2008 was colder, with an average temperature (T) of 13.36°C, and wetter, with a total average precipitation (P) of 496.6 mm, mostly occurring during the summer. Temperatures in 2009 were quite similar to the long-term period average (T = 14.0°C and T = 14.2°C, respectively) with the difference that 2009 was drier, with P = 308.6 mm compared with an average total precipitation of 333.4 mm for 1965–2014 during the vegetative period. During the first rotation, 2010 was the warmest year, with T = 15.7°C, more than one degree higher than the long-term average and reaching a maximum of 22°C in July. Precipitation during the growing period was basically similar to the long-term average (369.8 mm).

The driest year during the first rotation was 2011. The summer season, in particular, was considerable drier and relatively hot in comparison with the other years. The average air temperature for the vegetative period in 2011 was T = 15.2°C, and cumulative precipitation in 2011 was 276.4 mm. In 2012, the comparable values were T = 14.4°C and P = 404.4 mm.

On the other hand, 2013 and 2014 were warmer than the previous years, with mild temperatures during the growing season (between 11°C and 18.2°C and 11.6°C and 18.7°C in 2013 and 2014, respectively. However, 2014 was wetter than 2013, with rainfall mainly occurring during the summer.

4.5. Statistical analysis

To test the effect of the four fertilisation systems on soil nutrients and pH as well as to determine whether there were significant differences among the systems, one-way analysis of variance was applied, followed by the least significant difference (LSD) test at the 95% level of confidence (I).

For the other soil parameters studied, a general linear mixed model in R-Studio (R Core Team 2012) was used to test the effects of the systems on the studied soil properties. Because five different crops were grown in the same plot and system (Figure 2 in III) but the comparison of specific crop effects on soil properties was not the aim of the present study, crop effect was included and was considered a confounding effect. In addition, the random effect of plot was included to evaluate potential correlations between measurements made on the same plot. To test for significant yearly differences within each of the systems, a t–test ($P<0.05$) was run. Finally, Pearson's correlation coefficients (r) were calculated to study the statistical relationship between the number of earthworms and the total soil porosity as well as the influence of organic matter on the aggregation stability because normally these variables are correlated in agro-ecosystems. In this case, all results were considered statistically significant at $P<0.05$.

5. RESULTS

5.1. First rotation (2008–2012)

5.1.1. Soil chemical properties (2008–2012)

Different fertilisation practices resulted in significant differences in pH among the systems; however, no statistically significant differences ($P<0.05$) were found in soil acidity along the years (Table II in I). The results showed a slight decline in pH with time when mineral fertilisers were added in the Conventional II system, with average pH values decreasing from 5.77 to 5.67 after five years. On the other hand, in the Organic II system soil acidity decreased as result of the application of cattle manure, reaching higher average values in comparison with the conventional systems during the entire rotation (Table II in I).

Nutrient concentration in the soil varied irregular along the first rotation (Figure 2 in I). The lack of any extra fertilisation input resulted in lower nutrient concentration in the conventional control compared with the rest of the systems during most of the rotation. Of the nutrients analysed, the levels of K, Mg and Ca decreased irregularly in all of the systems during the first rotation. The addition of mineral fertilisers in the Conventional II system increased the levels of total nitrogen (N) and available phosphorus (P). In addition, this system also displayed the highest plant nutrient uptake during the first rotation. Especially in the case of N and P, plant uptake was double that in the other systems (Table III in I). On the other hand, the use of winter cover crops alone or in conjunction with cattle manure had no significant overall effect on the N content of the soil. Phosphorus showed a significant decrease after the first rotation in the organic systems. However, comparing the data from 2013 and with the data from the establishment stage (2008), it was observed that N content as well as N stock in the soil declined in all of the systems with the exception of the Organic II system, in which both of these values remained basically the same (Table 12 in IV). Comparing the two years, soil organic carbon concentration and stock in the ploughing layer showed a positive response to the presence of cover crops and the addition of manure (Figure 2 in I and Table 11 in IV). The use of cover crops and green manure yearly increased the SOC stock to 0.77 Mg C ha^{-1} y^{-1}, whereas the application of cattle manure increased the SOC level to 2.57 Mg C ha^{-1} y^{-1}.

5.1.2. Soil physical properties (2010–2012)

Four years after beginning the crop rotation in 2008, significant differences ($P<0.05$) were already detectable among the systems in the ploughed layer. During this period, all of the systems displayed quite similar values for bulk density, porosity, water permeability and penetration resistance. Comparing the average soil physical values from 2010 and 2012, a significant general increase in soil porosity, plant water availability and permeability is observed in all of the systems (Table 2 in II).

Variations in soil bulk density and the porosity fractions studied were related to changes in soil water content (θ) and soil organic carbon (SOC). Hence, significant variations in these parameters resulted in changes in soil density and porosity (Table 2 in II). In 2011, there was a decrease in air-filled pores and especially in plant-available water in all of the systems with the exception of the Conventional II system, in which these porosity fractions remained basically constant during the first two years (2010 and 2011).

The continuous application of mineral fertilisers resulted in significant differences in soil physical properties among the systems and as the years of the study progressed (Table 2 in II). At the end of the rotation, the Conventional II system presented a lower bulk density (1.39 Mg m^{-3}), significantly higher percentages of air-filled pores (19.11%) and plant-available water (22.82%) and lower plant non-available water (4.59%) compared with the other systems.

On the other hand, cover crops and manure did not produce any significant effect when the organic and the control systems were compared. The organic systems had a lower percentage of air-filled pores (AFP) and as a result higher bulk density. However, water permeability (k) showed a significant response to the addition of manure and the presence of winter cover crops. As result, the average k values in the two organic systems were three times higher in 2012 than in 2010 (Table 2 in II).

Like the previously discussed soil physical parameters, penetration resistance displayed quite similar values along the soil profile in all of the systems during the three years (Figure1 in II). Soil moisture content at the time of sampling is reported in Table 2 in II. The average values of penetration resistance in the ploughing layer (0 to 22 cm) ranged from

1.31–1.8 MPa in 2010 to 1.09–1.34 MPa in 2012. Penetration resistance increased linearly with depth except in 2011, where it changed abruptly at 25–30 cm depth (Figure 1 b) in II).

5.2. Second period. Organic farming systems (2013–2014)

5.2.1. Soil chemical properties

As during the first rotation, no statistically significant differences ($P<0.05$) were found in soil acidity as the years of the study progressed. In addition, no significant differences were found among the systems; the average values were very similar to those registered during the first rotation (Table 2).

In the same way, taking into consideration the results obtained during the first rotation in the Organic I and Organic II systems, the nutrient concentration and availability in the soil did not present any trend. N and P decreased significantly in all of the systems during this period, whereas the SOC concentration remained constant. The effect of cover crops and cattle manure was only significant for some of the nutrients studied and never for both years (Table 2). Comparing the average nutrient concentration values in this period with those from the first rotation (Figure 2

Table 2. Average values of soil pH_{KCl}, soil organic carbon (SOC), total nitrogen (N), plant-available phosphorus (P), plant-available potassium (K), plant-available magnesium (Mg) and plant-available calcium (Ca) in 2013 and 2014.

	ORGANIC 0		ORGANIC I		ORGANIC II	
	2013	2014	2013	2014	2013	2014
pH	5.96	5.94	6.04	6.02	6.06	6.0
SOC (%)	1.61	1.55	1.64	1.54	1.66	1.62
N (%)	0.14^{A}	0.11^{B}	0.14^{A}	0.11^{B}	0.14^{A}	0.11^{B}
P (mg kg^{-1})	138.8^{A}	111.7^{B}	128.4	115.7	124.9	117.7
K (mg kg^{-1})	134.8^{Aa}	110.9^{Bab}	131.2^{Aa}	108.5^{Bb}	142.0^{Aa}	124.0^{Ba}
Mg (mg kg^{-1})	255.6^{Aa}	124.0^{Ba}	266.2^{Aa}	137.8^{Bab}	286.3^{Aa}	154.0^{Bb}
Ca (mg kg^{-1})	1561.7^{a}	1484.8^{a}	1512.1^{a}	1643.1^{b}	1504.8^{Aa}	1672.4^{Bb}

Note: Mean values followed by different capital letter in each column indicates significant yearly differences (t-test; $P<0.05$) within the same system for the same parameter. Mean values followed by different small letter in each row indicates significant difference (linear mixed model; $P<0.05$) among the systems within the same year.

in I), it can be observed that clear increases in P, K, Mg and Ca occurred in both the Organic I and Organic II systems.

K also showed a decrease in all of the systems when 2013 and 2014 were compared, with the difference being significant for the Organic 0 and Organic II systems. Comparing the three systems, in 2014 significant differences appear between Organic I, which has the lowest P average value, and the other two systems. Finally, in the Organic I and Organic II systems, the Mg and Ca values were significantly higher in 2013 than in 2012. In the case of Mg, although all of the systems showed a significant yearly decrease, significant differences among the systems only appeared between the organic control system and the Organic II. On the other hand, Ca increased in the Organic I and Organic II systems, and both systems showed significant differences in 2014 with respect to Organic 0.

5.2.2. Soil physical properties

During this period, the bulk density decreased in all of the systems, mainly due to a significant ($P<0.05$) increase in the %AFP in the ploughing layer. On the other hand, the other two porosity fractions studied, plant-available and non-available water, varied unevenly during this period. Although both of the organic systems with cover crops presented more favourable soil physical conditions at the top layer, with lower bulk density and greater water permeability (Table 2 in III), no significant differences were found when these two systems were compared with the organic control system (Organic 0). As during the first rotation, the addition of cattle manure did not show a significant effect when Organic I and Organic II systems were compared, and only in 2014 Organic I were significant differences between these systems found (Table 2 in III).

The results of analysis of samples taken at the deeper soil layer (30–35 cm) showed a significant increase in field water content and plant-available water, whereas the plant non-available water decreased in both years in all of the systems (Table 3 & Figure 2). On the other hand, bulk density, water permeability and the percentage of air-filled pores under the ploughing layer did not vary during this period.

All of the organic systems presented similar tendencies in penetration resistance, and no significant differences were found in penetration resistance either with depth among the systems or in different years. However, comparing the penetration resistance during the last five years of the

Table 3. Field moisture content (%), bulk density (g cm^{-3}) water permeability (cm day^{-1}) and percentage of soil water-stable aggregates (% WSA) at 30–35 cm for the years 2013 and 2014.

Year	System	Field moisture content (%)	Bulk density (g cm^{-3})	Water permeability (cm day^{-1})	% WSA
2013	ORGANIC 0	13.8 A ± *0.20*	1.59 ± *0.01*	113.8 ± *45.3*	58.1^{Aa}
	ORGANIC I	13.7 A ± *0.21*	1.59 ± *0.01*	105.5 ± *53.0*	48.0^{Ab}
	ORGANIC II	13.9 A ± *0.23*	1.57 ± *0.01*	120.0 ± *45.2*	53.3^{ab}
2014	ORGANIC 0	20.6 B ± *0.28*	1.61a ± *0.01*	125.8 ± *32.5*	51.9^{Ba}
	ORGANIC I	19.1 B ± *0.24*	1.56b ± *0.01*	121.2 ± *21.5*	40.9^{Bb}
	ORGANIC II	22.5 B ± *0.23*	1.57a ± *0.02*	120.8 ± *18.0*	50.3^{a}

Note: Mean values followed by different capital letter in each column indicates significant yearly differences (t-test; $P<0.05$) within the same system for the same parameter. Mean values followed by different small letter in each column indicates significant difference (linear mixed model; $P<0.05$) among the systems within the same year.

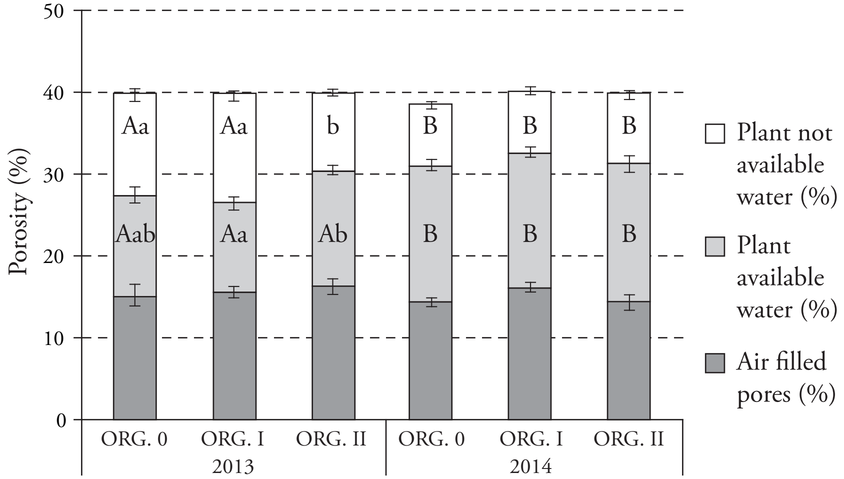

Figure 2. Average percentage of air-filled pore content and plant-available and non-available water content for the two years of study measured at 30–35 cm depth.

Note: Total porosity (%) = air-filled pores (%) + plant-available water (%) + plant non-available water (%).

The different capital letters in each bar indicate significant yearly differences (t-test; $P<0.05$) within the same system for the same parameter.

The different small letters in each bar indicate significant differences (linear mixed model; $P<0.05$) among the systems within the same year for the same parameter.

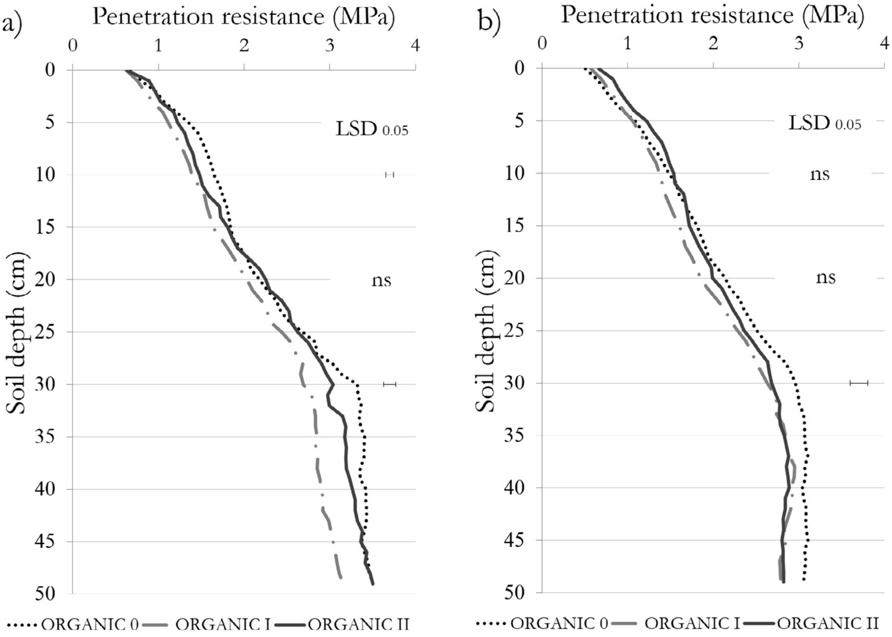

Figure 3. Average penetration resistance (MPa) with depth measured in the four systems in a) 2013 and b) 2014.
Note: LSD$_{0.05}$, least significant differences at significance *P*<0.05; ns, no significant differences among treatments. No significant yearly differences were found at any of the three depths compared (10 cm, 20 cm & 30 cm).

study (2010–2014), it is possible to observe that penetration resistance values at the plough soil layer (approximately 0–27 cm) were lower at the end of the experiment than in 2010 (Figure 1 in II & Figure 3). In 2010, the average penetration resistance values in the ploughing soil layer were 1.51 MPa in the Organic I system and 1.8 MPa in the Organic II system; in 2014 at the same depth, the average values were 1.44 MPa, 1.33 MPa and 1.49 MPa for Organic 0, Organic I and Organic II, respectively.

With respect to the percentage of soil stable aggregates at 5–10 cm, in both organic systems with cover crops, the percentage of water-stable aggregates (%WSA) decreased significantly in 2014. Only in the control system did the %WSA remain constant during this period (2013–2014) (Table 2 in III). However, at 30–35 cm depth, the Organic 0 and Organic I systems showed a significant decrease in %WSA, whereas in the Organic II system the percentage of stable soil aggregates did not change.

5.2.3. Earthworms and fluorescein diacetate hydrolysis activity (FDA)

Most of the earthworm species found were endogenic species: grey worm (*Aporrectodea caliginosa*), mucous worm (*Aporrectodea rosea*) and marsh worm (*Lumbricus rubellus*). Green worm (*Allolobophora chlorotica*) and common earthworm (*Lumbricus terrestris*) were also found at the time of sampling.

During this period (2013–2014), earthworms showed a positive response to the presence of winter cover crops and manure amendments. Therefore, Organic II contain a greater number of earthworms, followed by the Organic I system and finally the organic control system. However, significant differences in *n* and earthworm biomass were found only in 2013. In addition, Pearson's correlation coefficient (*r*) did not reveal a strong significant correlation between the SOC in the soil and the number of earthworms (Table 3 in III).

The microbiological activity measured as fluorescein diacetate hydrolysis activity (FDA) decreased in 2014 in all of the systems. Like the number of earthworms, it was higher in the systems that had a greater organic matter input (Organic II > Organic I > Organic 0). However, only the Organic II system showed significant differences in FDA compared with the other two systems (Figure 3 in III); therefore, the use of cover crops plus the addition of manure had a significant positive effect on soil microbial activity.

6. DISCUSSION

6.1. Soil chemical properties (I, III, IV)

The relatively short time course of the present experiment can explain why no statistically significant differences (*$P<0.05$*) in soil acidity were found during the course of the experiment (Table II in I). However, the measured differences among the systems are the result of the addition of mineral and organic fertilisers. On one hand, in the conventional fertilised system (Conventional II), pH decreased with time during the first rotation. As has been shown in previous studies (Magdof *et al.*, 1997; Imtiaz Rashid *et al.*, 2013), nitrogenous fertilisers, and in particular ammonium-based N fertilisers, supply N as NH^{4+}, which releases H^+ ions after oxidation decreasing the soil pH. In contrast, continuous addition of organic amendments (green matter and cattle manure) resulted in a slight increase in soil pH during the entire experiment (2008–2014) (I, IV). This can be explained by a positive effect of manure on the soil buffer capacity. Plants accumulate various organic anions that, once incorporated into the soil profile, cause a significant soil pH increase (Yan & Schubert, 2000). Similar to the results discussed by Yan & Schubert (2000), Bulluck *et al.* (2001), Liu *et al.* (2007), Gopinath *et al.* (2009) and Tein *et al.* (2014), different soils under organic management showed a general increase in pH over time in comparison with the same soils after treatment with synthetic fertilisers.

The soil organic carbon content showed a positive response to the addition of green matter and cattle manure (Figure 2 in IV) and the presence of cover crops. This is directly connected with the higher average C input that both organic systems received during the first rotation (Table 10 in IV). It is a general trend in most soils that the SOC concentration rises with increasing C input (Nyborg *et al.*, 1999; Campbell *et al.*, 2005; Bandyopadhyay *et al.*, 2010). However, although it is true that after the first rotation the organic systems displayed an increase in SOC, no significant differences in SOC were found in comparison with the conventional fertilised system (Conventional II). This suggests that other factors, such as the larger number of tillage operations that the organic systems received during the year (preparation of the soil for sowing and sowing of the cover crops), counteracted to a larger or smaller extent the positive impact of C input on SOC. Supporting this idea, it has been shown that large amounts of organic matter must be added to the soil to appreciably increase soil organic matter in the short term (Johnston, 1973). In addition, the influence of tillage on SOC has been reported in many studies (Lal *et al.*, 1994; Allmaras *et al.*, 2004; Plaza-Bonilla *et al.*, 2016). Basch *et al.* (2012) concluded that in tillage-based land use, tillage caused a considerable decline in SOM content even in the short term, irrespective of the type of land use. Tillage operations accelerate the mineralisation of SOC, exposing aggregate protected C to microbial attack and soil particles to the effects of drying/rewetting and freezing/thawing cycles (Allmaras *et al.*, 2004). Because the same intensive tillage management continued in the organic systems during 2013 and 2014, SOC was also affected during these years, in which it showed no significant increase despite the rotation, C input (manure) and use of cover crops.

The conventional systems only received C input from the main crops when they were ploughed in autumn. Despite the C input, in the long term and without any extra addition of organic matter the continuous application of nitrogenous fertilisers can lead to a potential loss of SOC in this type of system due to a positive impact of the high N availability on SOC mineralisation (Khan *et al.*, 2007).

It is also important to note that yearly variations in SOC may also be connected with changes in weather conditions. Low temperatures and drier periods negatively affect soil organic matter decomposition; hence, the SOC in the soil is expected to be higher during such periods than in warmer and wetter periods (Enwezor, 1967). As an example, during the first quarter of 2012 prior to sampling, the average temperatures were relatively low in comparison with previous years (-3.4, -4.9 and -1.5°C for the months of January, February and March, respectively) (Table 1). This may largely explain the significant increase in SOC observed in all of the systems in this year (Figure 2 in I).

Soil total N in the soil appeared to be related to external inputs, plant uptake and the binding effect of cover crops. In the organic systems, N was always slightly higher than in the conventional systems (Figure 2 in I). This can be explained by the combined effect of cover crops, legume N fixation and cattle manure but may also be due to the lower N uptake of the organic systems compared with the conventional ones (Table III in I). The use of cover crops after manure application has been shown to be a good practice for binding the N released during the decomposition of organic matter and hence preventing N losses through leaching or gaseous denitrification (Olesen *et al.*, 2007). However, in our experiment, the direct effect of organic fertilisation on N content was heterogeneous (Figure 2 in I, Table 2); hence, it is difficult to predict the effect in the long term. According to the previous literature, nitrogen can be a limiting nutrient in organic farming soils under cold climatic conditions in the long term, even after a productive green manure ley (Frųseth *et al.*, 2014).

On the other hand, under conditions of high N availability, the main crops increased their N uptake. This phenomenon was also observed for P and K. This explains why the Conventional II system, which received a yearly addition of these nutrients in the form of mineral fertilisers, also presented higher uptake (Table III in I). This higher nutrient uptake can explain the higher yield in this system during the first rotation (Alaru *et al.*, 2014; Tein *et al.*, 2014). However, it is important to note that plants only use a certain amount of the total input of nutrients provided by fertilisers. In the case of N and under Estonian pedoclimatic conditions, Astover *et al.* (2006) concluded that of the total N provided by mineral fertilisers, crops would utilise 40–50% in the first year and 50–60% in the whole crop rotation, whereas for manure, these values would be 20–30% and 40–60%, respectively. These numbers are subject to variation due to the balance between decomposition, mineralisation and immobilisation, processes that in turn are strongly influenced by environmental conditions, pH and the composition of the manure applied (Imtiaz Rashid *et al.*, 2013). According to our results (Table III in I), N, P and K were found in mineral fertilisers in forms more easily assimilated by plants than the forms of these nutrients found in cattle manure.

During the whole experiment, the concentration of plant-available phosphorous (P) in the soil varied heterogeneously with time (Figure 2 in I & Table 2). Therefore, it was difficult to associate the variability in P concentration with any effect of fertilisation. However, differences in P uptake by the main crops can partially explain yearly changes in P. As an example, the low P uptake that occurred in 2010 (Table 3 in I) can explain the increase in the P soil content observed in the next year (Figure 2 in I). Tillage can also cause changes in P content. As previously noted, tillage affects the mineralisation and decomposition of SOM, which in turn affects the soil aggregate stability and the concentration of various P forms in soil aggregates. Therefore, variability in SOC can also be related to variability in P content. This phenomenon has been extensively studied (Urioste *et al.*, 2006; Wright, 2009; Messiga *et al.*, 2011; Wang *et al.*, 2011).

During the first rotation, only the conventional system in which mineral fertilisers were applied showed an overall increase in P in the plough layer; the magnitude of this increase was 8.7 kg P ha^{-1} (Figure 2 in I). On the other hand, the concentration of P in the organic systems increased significantly in the second period (2013–2014) (Table 2). In their long-term experiment on five farms managed organically in Norway, Lųes & Ųgaard (2001) concluded that because P is relatively stable in the soil, it is very possible that the average P concentration will stabilise in the medium to long term, becoming slowly available; however, in the present study, considering the yearly variability during the first rotation and the short duration of the experiment, this level appears not to have been reached (Table 2).

Based on the results of all fertilisation experiments conducted in Estonia, Astover *et al.* (2006) concluded that for avoiding significant losses of P the average fertiliser amount should not be less than 10–15 kg P ha^{-1}. In the Organic II system, the average total amount of P in cattle manure (16.5 kg P ha^{-1} y^{-1}) slightly exceeded this amount. This suggests that most of the extra phosphorus provided via manure was found in forms that are not readily assimilated by plants or that other factors constrain the availability of this macronutrient in this system. Regarding the use of P from manure, Astover *et al.* (2006) showed that plants use 25 to 45% during the first year and 40 to 60% in the whole crop rotation of the total amount of P applied via manure.

Plant-available potassium (K) decreased in all of the systems during the whole experiment (2008–2014). The lack of any extra fertiliser input in the conventional control system (Conventional I) and in the organic system with winter cover crops (Organic I) resulted in these systems presenting the lowest concentrations of K, with decreases of 34.2 mg kg^{-1} and 35.6 mg kg^{-1}, respectively, after the first rotation (Figure 2 in I). However, as occurred with P, the Organic II system also showed a decrease in plant-available K despite the yearly addition of cattle manure at 30.8 kg K ha^{-1} y^{-1} in an average of rotation (divided among the crops at 15.4 kg K ha^{-1} y^{-1} for potato and 7.7 for barley and winter wheat). Under Estonian local and climate conditions, plants utilise 50–70% of K from manure in the first year and 70–80% in the whole rotation (Astover *et al.*, 2006). Our findings agree with those of Madaras & Lipavský (2009), who found in nine different arable soils in the Czech Republic that crops undergoing high rates of fertilisation with manure or mineral K only used approximately 22–25% of the K applied.

In our experiment, the general decrease in K can be linked to textural constraints associated with the low clay content of the soil or with K fixation into non-available forms that was favoured by the soil tillage operations (Martín-Rueda *et al.*, 2007). The ability of soil to retain applied K, as well as other nutrients, depends on the clay mineralogy and content, which influences the cation exchange capacity (CEC) and therefore the degree of K leaching. In the present experiment, the soil only contained 9.5% clay, suggesting (although it was not tested in the experiment) that K can be relatively easily leached from plant residues by moisture. Similar results were obtained by Madaras & Lipavský (2009) in the Czech Republic; in their work, K losses during the last year of the rotation were explained by leaching or K fixation into non-available forms in all of the systems. Lehmann *et al.* (2003) measured nutrient availability and leaching in two different soils in the Central Amazon. Despite the differences in the experimental conditions, the results from their study show that N and K applied in the form of manure or mineral fertilisers were mobile in the soil and that the proportion of leaching was larger in fertilised treatments than in unfertilised ones.

The decrease in K in the different years was also affected by the K uptake by plants. As an example, the increase in K uptake by the main crops in 2011and 2012 in all of the systems (Table 3 in I) enhanced this decrease in K in the soil in the last period of the first crop rotation. Lauringson *et al.* (2004) reported a large amount of K removal by potato harvest (138 kg ha^{-1} y^{-1} on average). Therefore, it is expected that under similar conditions potato contributes as well to the decrease in K concentration in the soil.

Despite these results, none of the systems presented lack of phosphorus or potassium according to the fertiliser demand classification for Estonian soils based on soil texture and organic carbon content proposed by Loide (2004); hence, P and K availability cannot be considered limiting factors for plant growth in the short term. With average soil concentration values ranging from 98.8 to 138.8 mg kg^{-1} for P and from 133 to 142.0 mg kg^{-1}

for K (Figure 2 in I), the systems presented a 'low to very low' demand for these nutrients after seven years.

Moreover, nutrient uptake by plants is generally linked to soil water content. An increase in the soil moisture content is associated with an increase in the diffusion rate of nutrients from the soil matrix to the absorbing root surface (di Bene *et al.*, 2011). The higher cumulative precipitation in 2012 in comparison with the previous year (Table 1) resulted in an increase in soil water content in all of the systems (Table 2 in II). This can explain the higher P and K uptake in 2012 (Table III in I) and hence the general decrease in the levels of these nutrients in the soil in this year (Figure 2 in I).

The concentration of available calcium (Ca) and magnesium (Mg) in the soil decreased for all of the systems (Figure 2 in I) during the first rotation. With the exception of the Organic II system (via cattle manure), none of the systems received an external input of either of these nutrients. According to previous studies, under local conditions, the soils of the experimental area are gradually becoming poorer in Ca and Mg (Loide, 2004). The same study noted that approximately 75–80% of Estonian arable soils have a deficiency in plant-available Mg and that calcium losses from the arable layer may amount to 150–500 kg ha^{-1} per year (Loide, 2004). However, comparing the average values of Ca and Mg measured during the first rotation, the organic systems showed higher concentrations of this cation than the conventional ones in most years (Figure 2 in I). In addition, the organic systems displayed a significant increase in the concentration of both nutrients at the beginning of the second rotation (2013) that may be connected with a positive effect of cover crops. Despite the fact that both nutrients are basically immobile in the soil, this can be explained due to a positive cover crop effect in these systems. Cover crops help decrease Ca losses from the soil during the autumn–winter period and even promote plant-available Ca mineralisation from soil minerals (Tein *et al.*, 2014). Cover crop species from the family *Brassicaceae*, such as winter oilseed rape, are noted for achieving high calcium (Ca) concentrations during growth (White & Broadley, 2003). Comparing conventional and organic farming systems, these findings agree with those of Kennedy & Smith (1995) and Liu *et al.* (2007), who found significantly higher levels of soil calcium, magnesium and other secondary nutrients in soils from organic and sustainable farms. In the USA, Bulluck *et al.* (2002) found an increase in Ca, K and Mg in soils that received organic amendments (including cattle manure) but not in soils receiving synthetic fertilisers; in 10 different organic soils in India, Sudhakaran *et al.* (2013) found significantly higher concentrations of several macronutrients, including N, P, K, Ca and Mg, compared with conventional and sustainable farming systems, and Liu *et al.* (2007) showed that organic managed farms in the long term (managed for over 20 years; none were in transition) presented higher concentrations of Ca and Mg in comparison with conventional farms.

Finally, the availability of both Ca and Mg is strongly influenced by climate conditions, parent mineral material, soil acidity (Potočić *et al.*, 2005; Loide, 2004), the presence of other cations (K^+, NH_4^+, Mn^{2+}) (Marschner, 2012) and soil texture. According to Marschner (2012), above a certain level of Ca in the soil there is a competition for plant uptake among Ca, K and Mg; this can explain our finding that the peak of available Ca in the soil in 2010 corresponded to a low concentration of K in the same year. Furthermore, Ca and Mg are less available in sandy soils and at low pH (Zhao *et. al*, 2011). The local condition of the soil epipedon at the experimental area is naturally acid ($pH_{KCl}<6$), which constrains the availability of these nutrients to plants (Reintam & Köster, 2006). Because the soil has not been limed for more than 10 years, re-acidification has taken place, and levels of Mg and Ca might remain low in the long term.

6.2. Soil physical properties (II, III, IV)

Comparing the initial and final average soil physical values from 2010 and 2012 for the conventional systems and those from 2010 and 2014 for the organic ones, all of the systems showed an improvement in soil physical properties in terms of bulk density, air-filled pores and plant-available water (Table 2 in II; Table I in III). Previous studies have shown a cumulative crop rotation effect of enhancing soil porosity and preventing soil organic carbon losses in similar systems (Hao *et al.*, 2002; Aziz *et al.*, 2011). However, this effect was not notable at 30–35 cm depth (Table 3), probably due to the lower amount of organic matter at this depth. According to the results obtained in 2014, the more favourable soil physical conditions did not parallel the soil particle aggregation behaviour (Table 1 in III). In this sense, an apparent decrease in bulk density in the ploughing layer did not indicate an improvement in soil general structure in the organic systems. Many researchers have presented a positive correlation between soil organic carbon content and soil aggregate stability (Haynes & Naidu, 1998; Boix-Fayos *et al.*, 2001; Bronick & Lal, 2005; Tejada *et al.*, 2008); however, the results of the present experiment suggest that other factors had a greater impact on the soil structural stability. First, because the three organic systems received the same amount of tillage, a negative tillage effect on %WSA must be considered. Tillage has been reported to decrease soil aggregation by accelerating the turnover of aggregate-associated soil organic matter (Six *et al.*, 1999). On the other hand, depending on the nature of SOC, in some cases manure can enhance the susceptibility of soil to dispersion and hence decrease its aggregate stability. The presence of certain cations in the manure, especially Na^+, contributes to the presence of repulsive charges in the soil solution that disperse soil particles. However, Na^+ content was not measured in the present study. Divalent cations, such as Ca^{2+} and Mg^{2+}, improve soil structure through cationic bridging with clay particles and SOC (Bronick & Lal, 2005). During the 2013–2014 period, there was a significant decrease in Mg in all of the systems (Table 2). At deeper soil layers, the %WSA was lower (Table 3). This may be related to the presence of less soil organic carbon and less microbial activity. As previously noted, the correlation between these variables has been largely documented (Bronick & Lal, 2005; Tejada *et al.*, 2008).

The yearly variability in soil physical properties was also related to differences in the time of sampling. On one hand, in 2010 and 2011 samples were taken in the fall. By that time, the plots had been cultivated throughout the spring, and the winter cover crops had already been harvested. On the other hand, in 2012–2014, samples were taken in the spring, when cover crops were present in the organic systems and all of the plots in the conventional systems were covered with plant residues because no tillage took place in autumn 2011. Therefore, in 2010 and 2011, a notable tillage effect on the soil physical condition was expected as a result of the operations previously carried out during the growing period, whereas in 2012–2014 the soil remained unaltered until the sampling time and was only affected by the freezing and thawing cycles that occurred during the winter-spring period; here, the Organic I and II systems benefitted from the presence of cover crops.

Weather conditions also played an important role with respect to certain soil physical conditions in the present experiment. Penetration resistance and bulk density are dependent on the soil moisture content (Molina *et al.*, 1999; Velykis *et al.*, 2014). Compared with other years of the study, 2011 was relatively drier (Table 1) and presented higher plant non-available water (PnAW), which corresponds to the portion of water stored in micropores (Table 2 in II). This can be explained by the plants' morphological response to the low precipitation. Under moderate water stress conditions, crop roots tend to increase the fine root length per unit of soil volume so as to be able to absorb nutrients; the fine roots expand through macropores or regions of weakness in the soil, compacting the surrounding soil particles and decreasing the pore size. This was more marked in the organic systems, where the availability of certain nutrients was lower. In the Conventional II system, despite the general increase in PnAW in 2011, this porosity fraction remained basically constant. This agrees with results obtained by Reintam (2006), who showed that under non-compacted soil conditions root densities were lower in plots to which mineral fertilisers had been added because here nutrients are more accessible to the plants. Beyond this, other specific positive effects of mineral fertilisers on soil bulk density and porosity are difficult to demonstrate. However, other studies, such as the study conducted by Rasool *et al.* in 2008, reported a significant increase on SOC concentration in conventional fertilised soils after application of mineral fertilisers ($N_{100}P_{50}K_{50}$) and as a result an increase in the total soil porosity and a decrease in soil bulk density compared to that in control plots.

Weather conditions can also determine the percentage of soil stable aggregates. According to various studies, aggregate stability has been found to be negatively correlated with soil moisture (Gerald, 1987; Perfect *et al.*, 1990). Perfect *et al.* (1990) observed that soil moisture at sampling explained between 20.3 and 84.9% of the seasonal variation in WSA. Therefore, the higher soil moisture at the time of sampling in 2014 compared with 2013 (Table 3), especially in the organic plots with cattle manure amendment, can also explain the higher %WSA in 2014 compared with 2013.

The positive impact of organic amendments on soil physical properties has been widely studied. An increased level of organic matter in the soil is associated with many desirable soil properties, including higher plant-available water-holding capacity and lowering of bulk density (Bulluck *et al.*, 2002; Gopinath *et al.*, 2009; Guo *et al.*, 2016). However, the results of the present experiment indicated that in the organic systems, despite the sum of crop rotation, cover crop and manure effects, the intensive tillage operations had a greater impact on SOC. This, in turn, resulted in higher bulk density and lower percentage of air-filled pores in comparison with the conventional systems (Table 2 in II). The negative tillage effect continued after the first rotation (2013–2014). Comparing the organic systems, cover crops did not have a significant effect on the soil properties studied; no significant differences were found between the control system (Organic 0) and the Organic I system (with cover crops). Similarly, no significant differences were found between the two organic systems with cover crops (Figure 3 in III).

According to the textural classification, the soil at the experimental area contained more than 50% sand. Sandy soils have been shown to be more liable to lose structural stability than clayey soils and more prone to the formation of microaggregates (Boix-Fayos *et al.*, 2001; Spaccini *et al.*, 2001; Hathaway-Jenkins *et al.*, 2011). Under these textural conditions, the tillage operations carried out in the systems could counteract a positive effect of crop rotation and manuring. By mixing the plough-layer, new soil is exposed to dry-wet cycles at the soil surface. This contributes to degradation of the soil structure and also affects the SOM dynamics (Denef *et al.*, 2001); as a result, soil aggregate stability is also affected (Greacen, 1958).

Like the rest of the soil physical properties studied, the penetration resistance was positively affected by crop rotation, showing a decreasing tendency with depth as the years of the study progressed. In 2011, the discontinuity in penetrability with depth under the ploughing layer (25–30 cm depth) may explain the evidence of a possible plough pan (Figure 1b in II). It is common at this depth (approximately 8 cm below the tillage depth) that the penetration resistance normally reaches higher average values (Tsimba *et al.*, 1999). However, the moderate weight of the tractor (5 tons), the relatively low clay content (under 10%) and the natural processes of freezing/thawing and wetting/drying do not make the soil very prone to structural problems due to soil compaction (Reintam, 2006). In fact, this soil hardening was a transient phenomenon and was not apparent in later years (Figure 1 c in II; Figure 3). Therefore, the differences in nutrient uptake are not likely to be problems derived from compaction but instead due to the availability of nutrients in the systems.

Despite the intensive tillage, especially in the organic systems, average penetration resistance values over 2 MPa at the soil root zone were only reached occasionally by some of the systems in 2010 and 2011 (Figure 1 in II). According to Whalley *et al.* (2012), in general, penetrometer values greater than 2 MPa can significantly affect crop development and biomass production. Soil compaction can have a negative effect on soil water and nutrient acquisition (Reintam, 2006). However, as previously mentioned, differences in nutrient uptake tend to be more connected with the availability of these nutrients in the soil and differences in yield (Alaru *et al.*, 2014; Tein *et al.,* 2014), and among our systems as well they seem to be more connected with the presence or absence of mineral fertilisers and not with problems caused by compaction.

6.3. Earthworms and fluorescein diacetate hydrolysis activity (FDA) (III)

Most of the earthworm species found in the field were endogenic. In general, in arable soils under intensive tillage, most of the species found are endogenic because these are more tolerant to disturbance (Ivask *et al.*, 2007). Despite the fact that the three organic systems presented an increase in the number and biomass of earthworms when the 2013 and 2014 values are compared, this increase was only significant in the case of the organic control system; therefore, the use of winter cover crops and cattle manure did not have a significant effect on these two variables (Table 3 in III). Because there are no existing data from previous years, it is difficult to determine whether this yearly variability is merely stationary. When the three organic systems were compared, the organic system with cattle manure addition (Organic II) showed the highest number and biomass of earthworms. This could be explained by the higher SOC in this system (despite the fact that no significant differences among the systems in SOC were found); however, no significant correlation between these variables was found. As with other factors, such as %WSA and SOC, certain tillage operations, especially ploughing, disrupt earthworm populations and eventually create unfavourable soil environmental conditions (Głąb *et al.*, 2013; Crittenden *et al.*, 2014). Boström (1995) reported that rotary cultivation and ploughing killed approximately 75% of the earthworms.

Annual precipitation and soil water content can also influence the quantitative characteristics of earthworm populations (abundance and biomass) (Ivask *et al.*, 2007; Birkas *et al.*, 2010; Crittenden *et al.*, 2014). In the present experiment, the cumulative precipitation (Table 1) during the summer (prior to the sampling time), as well as the soil water content

(Table 3), were higher in 2014 than in 2013. This, in addition to the mild average summer temperatures ranging from approximately 12°C to 19°C, created more favourable conditions for earthworms and therefore can also explain the increase in earthworm number and biomass in 2014 despite the tillage operations. Other chemical factors, such as pH, have also been shown to impact earthworm populations (Iordache & Borza, 2010). In the present experiment, no significant differences in pH were found between the years (2013 and 2014) or among the systems; therefore, the variability in the number of earthworms cannot be explained by changes in soil acidity. This is also supported by the fact that during the whole experiment the soil pH was >5, which is considered the limiting pH below which earthworms will not thrive in the soil (Edwards, 2004).

Due to the difference in sampling depth of the earthworm samples (0–20 cm) and the samples taken for the study of other soil properties (5–10 cm), it is difficult to establish any connection among them. This may explain why no significant correlation (all $P>0.05$) was found between the number of earthworms and the percentage of soil aggregates, total porosity or SOC (Tables 2 & 3 in III). The fact that earthworms favour soil aggregation as a result of the excretion of cast (Barois *et al.*, 1993; Bossuyt *et al.*, 2006) and enhance soil porosity by drilling and that organic matter in the soil represents a food supply for earthworms and better living conditions for microfauna (Scullion *et al.*, 2007) cannot be neglected. However, other factors, especially tillage, might have greater impact on these soil properties.

Enzymatic activity showed a significant positive response to the presence of cover crops and to the application of cattle manure in both 2013 and 2014 (Figure 4 in III). Most of the microbial populations in the soil are heterotrophs and therefore depend on the availability of organic carbon and energy (Odlare, 2005). The application of manure to soil results in a rapid soil microbial response, shown by an increase in enzyme activity (Kanchikerimath & Singh, 2001) due to the incorporation of easily degradable organic materials (green matter and cattle manure) that stimulate the activity of autochthonous microbial and also exogenous microorganisms (Tejada *et al.*, 2008). This may explain why the Organic II system with higher SOC content also presented higher FDA despite the fact that there were no significant differences in SOC during this period. The positive effects of the addition of organic amendments on soil microbial activity have been reported in several studies (Bulluck *et al.*, 2002; Odlare, 2005; Liu *et al.*, 2007; Doan *et al.*, 2013; Sudhakaran *et al.*, 2013).

No yearly significant differences in FDA were found in any of the systems. This can be explained by the fact that the experimental setup was relatively short (the division into three organic systems was made in 2013). In general, long-term crop rotation has a significant impact on microbial communities and their activities (Edesi *et al.*, 2012); however, because no yearly significant changes in SOC occurred during this period, no yearly significant differences in FDA hydrolysis were found. In addition, due to the intensive tillage, the mineralisation of organic matter in the soil occurred relatively rapidly.

Other factors, such as the soil moisture, pH, nutrient concentration and presence of earthworms, can also affect soil microbial populations and activities. Although it is true that significant variations were found in N, P and K between the years (Table 2), data from only two years are available, and no yearly significant differences in FDA were found; thus, it is difficult to establish a clear connection between these factors and soil microbiological activity.

7. CONCLUSIONS

In the present experiment, which was based on a five-year crop rotation, the effect of the use of cover crops (rye, ryegrass and oilseed rape) alone or combined with cattle manure (40 t ha^{-1}) on soil physical and chemical properties, as well as on earthworm and soil microbial populations, was studied and compared with the effect of the use of mineral fertilisers in conventional systems in the same rotation (winter wheat, pea, potato and barley undersown with red clover). According to the aims of the study, the following conclusions may be presented:

1. On average, cover crops and cattle manure showed a significant effect on soil acidity, raising the soil pH, which is naturally acid, to an average pH=6.

2. During the first rotation, the organic systems with and without cattle manure presented the highest C input, with average rotation values of 4,762 kg C ha^{-1} y^{-1} and 4,415 kg C ha^{-1}, respectively. However, these systems did not present significant differences in the soil organic carbon (SOC) concentration in comparison with the conventional fertilised systems.

3. After the first 5-year rotation, the yearly addition of mineral fertilisers at an average rate of N_{88}, P_{25}, K_{95} in the conventional system maintained an average constant level of total N and SOC and increased by 8.7 mg kg^{-1} the concentration of available phosphorus (P) in the soil. In addition, this system also presented the highest nutrient (NPK) uptake of all the systems.

4. Cover crops showed a positive effect on binding soil N in the organic systems. Despite the yearly fluctuations, during the first rotation the organic systems both with and without cattle manure showed the highest average concentration of N, an average concentration of 0.14% for both systems.

5. The continuous decrease in available potassium and magnesium in the soil during the whole experiment in all investigated treatments suggests that current soil management, whether conventional or organic, is not adequate in terms of K. After the first rotation, the

conventional fertilised system showed an average decrease in K of 5.2 mg kg^{-1} y^{-1}, whereas in the organic system the average decreases in K were 8.9 mg kg^{-1} y^{-1} and 34.2 mg kg^{-1} y^{-1} in the systems without and with cattle manure, respectively.

6. The lack of any extra source of Mg and Ca other than cattle manure in combination with the local acidic conditions and the interaction of these with other cations resulted in a decrease in both nutrients in the soil. Both nutrients were strongly affected by the local climate and soil conditions and the low clay content of the soil.

7. Without any fertiliser use, the nutrient concentration in the soil decreased over time. As result, the conventional non-fertilised control system presented significant decreases in N (0.02%), P (4.5 mg kg^{-1}), K (34.2 mg kg^{-1}), Ca (385.3 kg^{-1}) and Mg (56.1 kg^{-1}) until the end of the first rotation compared to the starting level in 2008. However, despite the lack of fertilisers, this system presented better overall soil physical condition than the organic systems, with lower bulk density (1.45 Mg m^{-3}) and higher percentages of air-filled pores (15.96%) and plant-available water (22.01%).

8. The use of mineral fertilisers in the conventional fertilised system at an average rate of N_{88}, P_{25}, K_{95} per year boosted the positive yearly effect of crop rotation on soil physical properties to a greater extent than occurred with any of the organic management systems tested. At the end of the first rotation, this system presented lower bulk density (1.39 Mg m^{-3}) and a higher percentage of air-filled pores (19.11%) and plant-available water (22.82%).

9. Intensive tillage counterbalanced the beneficial effect of cover crops and manure amendments on soil bulk density and porosity in the organic systems during the entire experiment. As a result, the organic systems presented the highest bulk density (1.47 Mg m^{-3} and 1.48 Mg m^{-3} for organic systems without and with cattle manure, respectively) and the lowest percentage of air-filled pores (14.62% and 14.07%, respectively) compared with the other systems.

10. Comparing the average values of the bulk density, porosity and SOC from 2010 and 2012, it can be concluded that all of the systems showed overall improvement in the soil physical condition and SOC

concentration in the plough layer that was connected with a cumulative yearly effect of crop rotation. In order, the conventional control system, the conventional fertilised system, organic system with cover crops and organic system with cover crops and manure presented average decreases in bulk density of 0.04 Mg m^{-3} y^{-1}, 0.07 Mg m^{-3} y^{-1}, 0.065 Mg m^{-3} and 0.055 Mg m^{-3}; increases in plant-available water of 3.85%, 1.46%, 1.98% and 1.69%; and increases in SOC of 0.12%, 0.07%, 0.12% and 0.13%.

11. The sum of various factors, such as intensive tillage, low clay content and the decrease in Mg, affected the percentage of soil stable aggregates in the organic plots in the 2013–2014 period. Both organic systems presented a decrease in the percentage of water-stable aggregates (%WSA). This decrease was 1.61% in the organic system with cover crops only and 9.47% in the organic system with cover crops and a yearly application of cattle manure.

12. The most common earthworm species found were mainly endogenic species. Among these, the grey worm (*Aporrectodea caliginosa*), mucous worm (*Aporrectodea rosea*) and marsh worm (*Lumbricus rubellus*) were the most abundant. The organic system in which cover crops were sown and cattle manure was applied yearly yielded the highest average number of earthworms in both 2013 and 2014 (104.4 and 112.5, respectively).

13. The enzymatic activity responded positively to the presence of cover crops in combination with a yearly application of cattle manure. In 2013 and 2014, the organic system in which the two practices were combined showed the highest FDA, with average values of 59.7 µg fluorescein g dry $soil^{-1}$ h^{-1} and 58.4 µg fluorescein g dry $soil^{-1}$ h^{-1}, respectively.

In summary, the use of cover crops alone or in conjunction with cattle manure cannot be considered to be sufficient for maintaining a constant concentration of nutrients in the soil after seven years of rotation under intensive tillage. The potential benefits of these practices on the soil porosity, structure and nutrient content were counteracted to a greater or lesser extent by various factors and cannot be taken for granted.

Issues requiring further research:

- The present study showed that intensive tillage counteracts the positive effect of cover crops and manure on soil structure, carbon content and soil physical properties. Reducing the number of operations or changing to another tillage system may help optimise the impact of cover crops and manure on the soil. The inclusion of cover crops may be a potential strategy for improving soil physical condition under reduced tillage.

- To gain deeper and more practical insight from the results, it would be desirable for further studies to include calculation and analysis of nutrient balances and nutrient use efficiency.

- To evaluate the combined effects of crop rotation, cover crops and manure on soil properties, it would be interesting to continue the experiment over a longer term, especially to judge the effects on microbial activity and earthworms because it is difficult to derive conclusions from data gathered over only two years.

- The presence of certain cations, especially Na^+, in the soil or manure can lead to soil particle dispersion. Analysis of the soil Na^+ content could provide a better understanding of the dynamics of soil particle aggregation and might clarify why our research results differed in part from those of previous studies in which the application of manure enhanced soil particle aggregation.

- Cover cropping is a farming practice used in organic farming and conventional farming. The N-binding capacity of cover crops, which has been widely studied, was also demonstrated in the present study. In addition, the results of the experiment showed that the presence of cover crops during the winter season can enhance soil microbial and earthworm populations. Therefore, it would be interesting to test the effect of both practices by combining the use of cover crops with that of mineral fertilisers at various concentrations.

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SUMMARY IN ESTONIAN

VAHEKULTUURIDE JA VÄETAMISE MÕJU MULLA OMADUSTELE KÜLVIKORRAKATSES

Sissejuhatus

Intensiivse põllumajandusega kaasnevad keskkonnaprobleemid ning uued suunad Euroopa Liidu poliitikas on suurendanud huvi maheviljeluse vastu (van Diepeningen *et al.*, 2006). Maheviljelus on põllumajanduspraktika, kus mullaviljakus tagatakse tänu külvikorra rakendamisele ning kompostide, sõnniku ja haljasväetiste kasutamisele, välistades sünteetiliste väetiste ja taimekaitsevahendite kasutamise.

Üheks oluliseks põhimõtteks maheviljeluses mullaviljakuse säilitamisel on olemasolevate ressursside võimalikult tõhus kasutamine (Steinshamn *et al.*, 2004). Põhilisteks praktikateks nii mahe- kui ka tavaviljeluses on tasakaalus külvikorra kasutamine ning vahe- ja järelkultuuride kasvatamine, mis viib orgaanilise aine sisalduse suurenemiseni mullas, parandades seeläbi nii mulla struktuursust, mikrobiaalset aktiivsust kui ka teisi mulla füüsikalisi ja keemilisi omadusi (Berzsenyi *et al.*, 2000; Hao *et al.*, 2002; Aziz *et al.*, 2011). Ainult külvikorra ja vahekultuuride kasutamine ei taga aga toitainete tasakaalu põllul ning vaja on anda toitaineid juurde orgaaniliste või mineraalväetistena. Paljudes Euroopa idapoolsetes riikides, sh Eestis, on põldude toitainete bilanss negatiivne (Astover, 2007) ning tasakaalus külvikord ja vahekultuuride kasvatamine on seal olulise tähtsusega mullaviljakuse säilitamisel nii tava- kui ka maheviljeluses. Senised külvikordade ning väetamisalased (mineraal- ja orgaanilised väetised) uuringud on keskendunud põhiliselt mulla orgaanilise aine või toitainete tasakaalule kas mahe- või tavaviljeluse tingimustes, kuid mulla füüsikalistele omadustele on vähe tähelepanu pööratud ning pole ka süsteeme omavahel võrreldud.

Senised vahekultuuride uuringud piirduvad enamasti nende kasutamisega lämmastiku sidumise eesmärgil ja käsitlevad mõju järgnevate kultuuride saagile (Sainju *et al.*, 2003; Talgre *et al.*, 2009). Otsene mõju mulla omadustele, näiteks orgaanilise aine sisaldusele, struktuuriagregaatide stabiilsusele ja mikroobide tegevusele ning nende võimele vähendada P ja K kadu on aga seni teadmata (Talgre, 2013). Lisaks sellele on suhteliselt vähe uuringuid vahekultuuride mõju kohta mulla füüsikalistele ja bioloogilistele omadustele Põhjamaade klimaatilistes tingimustes.

Käesolevas doktoritöös keskendutakse mulla keemiliste, füüsikaliste ja bioloogiliste omaduste muutuste uurimisele viieväljalises külvikorras tava- ja maheviljeluse tingimustes, kasutades eri väetuspraktikaid, nagu vahekultuurid, sõnnik ja mineraalväetised.

Töö hüpoteesid on:

- Vahekultuuride kasvatamine üksi või koos taheda veisesõnniku kasutamisega on piisav, et säilitada mulla toitainete (N, P, K, C, Mg ja Ca) tasakaal maheviljeluses võrreldes mineraalväetiste kasutamise ja vahekultuuride mittekasutamisega tavaviljeluses (I ja IV).

- Vahekultuuride kasvatamine üksi või koos sõnniku kasutamisega tagab mulla struktuuriagregaatide suurema stabiilsuse, madalama lasuvustiheduse ja kõrgema taimedele omastatava vee sisalduse mullas võrreldes nende mittekasutamisega maheviljeluses ja ainult mineraalväetiste kasutamisega tavaviljeluses (II, III).

- Vahekultuuride kasvatamine üksi ja koos sõnniku kasutamisega suurendab vihmausside arvukust ja mulla mikrobiaalset aktiivsust maheviljeluses võrreldes variandiga, kus vahekultuure ja väetisi ei kasutata (III).

- Külvikorra rakendamisel on positiivne mõju mulla füüsikalistele omadustele, sõltumata viljelusviisist ja väetusrežiimist. Külvikorra lõpuks on kõikides süsteemides madalam lasuvustihedus, kõrgem taimedele omastatava vee sisaldus ja aeratsioonipoorsus võrreldes algusaastaga (II).

Tulenevalt püstitatud hüpoteesidest oli uurimistöö eesmärkideks:

1. Uurida mulla happesuse (pH), orgaanilise süsiniku (SOC), üldise lämmastiku (N_{tot}), liikuvate fosfori (P), kaaliumi (K), kaltsiumi (Ca) ja magneesiumi (Mg) sisalduse muutusi mullas sõltuvalt vahekultuuride ja sõnniku kasutamisest maheviljeluse ning mineraalväetiste kasutamisest tavaviljeluse tingimustes viieväljalises külvikorras (I ja IV).

2. Uurida mulla füüsikaliste omaduste, nagu lasuvustiheduse, poorsuse, veeläbilaskvuse ja penetromeetrilise takistuse muutusi mullas sõltuvalt vahekultuuride ja sõnniku kasutamisest maheviljeluse ning mineraalväetiste kasutamisest tavaviljeluse tingimustes viieväljalises külvikorras (II ja III).

3. Uurida mulla vees stabiilsete struktuuriagregaatide stabiilsuse, vihmausside populatsiooni ning mikrobiaalse aktiivsuse (FDA) muutusi sõltuvalt vahekultuuride ja sõnniku kasutamisest maheviljeluse tingimustes viieväljalises külvikorras (III).

Materjal ja metoodika

Katseandmed on kogutud 2008. aastal Eesti Maaülikooli maadele Eerikale Tartumaale, Eestisse (58°22'N, 26°40'E) rajatud 5-väljalise külvikorra viljelussüsteemide võrdluskatsest. Külvikorras olid talinisu (*Triticum aestivum* L.), hernes (*Pisum sativum* L.), kartul (*Solanum tuberosum* L.), oder (*Hordeum vulgare* L.) punase ristiku (*Trifolium pratense* L.) allakülviga ja punane ristik. Katseala mullaks on kerge liivsavilõimisega näivleetunud (LP) muld, milles on 56,5% liiva, 34% tolmu ja 9,5% savi ning huumushorisondi tüsedus varieerub 20 kuni 30 cm-ni (Reintam & Köster, 2006).

Esimese rotatsiooni ajal oli katse jaotatud 6 eraldi süsteemiks, milles igas oli esindatud kõik viis külvikorra kultuuri, ning katse oli neljas korduses. (Joonis 1). Käesolevas uurimistöös keskenduti põhiliselt neljale süsteemile: Tava I: tavaviljeluse kontrollvariant, kus mineraalväetisi ei antud, kuid kasutati taimekaitsevahendeid; Tava II: tavaviljeluse variant, kus lisaks taimekaitsevahenditele kasutati mineraalväetisi arvutatuna toiteelementidena järgmiselt: odral $N_{120}P_{25}K_{95}$, talinisul ja kartulil $N_{150}P_{25}K_{95}$ ja hernel kui liblikõielisel kultuuril $N_{20}P_{25}K_{95}$, punast ristikut ei väetatud; Mahe I: sünteetiliste taimekaitsevahenditeta süsteem, kus vahekultuuridena kasutati talirapsi (*Brassica napus ssp. oleifera* var. *biennis*) pärast hernest, rukist (*Secale cereale* L.) pärast kartulit ja raiheina (*Lolium perenne* L.) pärast talinisu; Mahe II: lisaks vahekultuuridele kasutati 40 t ha^{-1} kompostitud veisesõnnikut. Keskmiselt oli sõnniku kuivaines (44,8%) lämmastikku (N) 9,7 g kg^{-1}, fosforit (P) 4,6 g kg^{-1}, kaaliumit (K) 8,6 g kg^{-1}, kaltsiumit (Ca) 11,7 g kg^{-1}, magneesiumit (Mg) 3,4 g kg^{-1} ja süsinikku (C) 138 g kg^{-1}.

Pärast esimest rotatsiooni keskenduti maheviljeluslikele süsteemidele (2013–2014). Esialgne Mahe I süsteem jagati kaheks ning loodi juurde kontrollvariant, Mahe 0, kus vahekultuure ja sõnnikut ei kasutatud. (Joonis 1, III). Vahekultuurides asendati raihein rukki ja rapsi seguga.

Kevadel lisati katselappidele sõnnik: talinisule 10 t ha^{-1} ning odrale 10 t ha^{-1} ja kartulile 20 t ha^{-1}. Vahekultuurid külvati sügisel augustis pärast põhikultuuri koristust ning künti mulda kevadel aprillis 22 cm sügavuselt enne põhikultuuri külvi. Külviks ettevalmistamiseks kasutati kultiveerimist ning umbrohutõrjeks teraviljadel ja hernel äestamist 2 korda ning kartulil 3 korda vaheltharimist kasvuperioodi jooksul. Tavaviljeluses kasutati taimekaitsevahendeid sõltuvalt kultuuride vajadusest. Kõikides väetussüsteemides eemaldati põllult ainult kaubanduslik saak, ülejäänu (põhk ja juured) künti mulda.

Mullaproovid nii keemiliste, füüsikaliste kui ka mikrobiaalse aktiivsuse näitajate kohta koguti kuni 2011. aastani sügisel pärast põhikultuuri koristust, kuid alates 2012. aastast kevadel enne harimistööde algust künnikihist. Alates 2013. aastast koguti mulla füüsikaliste näitajate proovid ka künnikihi alusest kihist (30–35 cm). Mulla füüsikaliste parameetrite määramisega alustati 2010. aastast. Mulla keemilistest näitajatest määrati happesus (pH_{KCl}), üldine lämmastik, orgaaniline süsinik, liikuvad fosfor, kaalium, kaltsium ja magneesium (I ja IV). Mulla füüsikalistest näitajatest määrati alates 2010. aastast kõikidel uuritud variantidel penetromeetriline takistus, lasuvustihedus, poorsus (aeratsioonipoorsus pF1,8 juures, taimedele omastatava veega täidetud poorsus ja taimedele omastamatu veega täidetud poorsus pF4,2 juures) ja veejuhtivus (II). Alates 2013. aastast määrati ainult maheviljeluslikel variantidel lisaks nimetatutele ka vees stabiilsete struktuuriagregaatide sisaldus, vihmausside liigiline koosseis, arvukus ja mass ning mulla mikrobiaalne aktiivsus (FDA) (III).

Mulla toitainetesisalduse muutuste hindamiseks kasutati ühefaktorilist dispersioonanalüüsi 95% usutavuse juures. Ülejäänud mullaparameetrite hindamisel rakendati lineaarset segamudelit R-studios (R Core Team 2012) ning leiti korrelatsioon mulla eriparameetrite vahel.

Tulemused ja arutelu

Rakendatud erinev väetamine põhjustas usutavaid erinevusi ($P<0,05$) mulla happesuses, kus mineraalväetiste kasutamisel pH vähenes (I). Põhjuseks võis olla mineraalväetistega $NH4^+$ kujul antav lämmastik, mis vabastab pärast oksüdatsiooni mulda H^+ ioone ning alandab sellega pH-d. Teisest küljest põhjustas pidev vahekultuuride sissekünd ja sõnniku kasutamine mõningast pH tõusu terve uuritava aja vältel (2008–2014).

Ka mõjusid vahekultuurid ja sõnnik positiivselt mulla orgaanilise aine sisaldusele, põhjustades selle sisalduse tõusu võrreldes teiste uuritud väetussüsteemidega (Joonis 2, IV), kuid see muutus ei olnud võrreldes tavaviljeluslike süsteemidega (Tava II) statistiliselt usutav. See viib järelduseni, et muud faktorid, nagu sagedasem harimine maheviljeluses, võisid ära neutraliseerida lisanduva orgaanilise aine positiivse mõju. Samas, võrreldes intensiivselt väetatava tavaviljeluse variandiga ei olnud erinevused mulda tagastatava orgaanilise aine osas suured, sest intensiivne väetamine põhjustas ka suurema taimemassi ning hiljem mulda küntava taimejäänuste (põhk, juured) koguse.

Muutused mulla lämmastikusisalduses olid seotud nii väetistega juurde antava lämmastiku kui ka rotatsioonis olevate herne ja ristiku ning vahekultuuride poolt kinni hoitavaga. Maheviljeluses oli mulla N sisaldus alati veidi kõrgem kui tavaviljeluses (Joonis 2, I), mida võib põhjendada nii vahekultuuride poolt paigal hoitud kui ka sõnnikuga lisanduva lämmastikuga. Lämmastikusisaldus intensiivselt väetatud tavaviljeluslikus süsteemis püsis esimeses rotatsioonis suhteliselt stabiilsena, kuigi seal oli taimede lämmastiku (ka P ja K) omastamise tase kõrgeim.

Enim varieerus kogu katseperioodi jooksul mulla omastatava fosfori sisaldus (Joonis 2, I & Tabel 2), mistõttu oli varieeruvust raske seostada uuritud väetussüsteemidega. Aastased kõikumised olid seotud nii taimede ebaregulaarse toitainete omastamise kui ka katseaastate erinevate ilmastikutingimustega, mis põhjustasid fosfori ebaühtlast sidumist ja vabanemist. Vahekultuuride mõju ei olnud eristatav. Ka liikuva kaaliumi sisalduse muutus mullas oli ebaregulaarne, kuid alates katse rajamisest 2008. aastal võib täheldada sisalduse järjepidevat vähenemist (2008–2014) kõikides uuritud süsteemides. Üldine kaaliumisisalduse vähenemine mullas võib olla põhjustatud mulla väiksest savisisaldusest (9,5%), mistõttu mulla kaaliumihoiuvõime on väike, kuid ka liblikõieliste poolt seotud kaaliumiga. Vaatamata sellele, et liikuva kaaliumi ja fosfori sisaldus aastatega vähenes, on antud mulla vajadus lähtuvalt selle lõimisest ja orgaanilise aine sisaldusest nende toitainete järele väike kõikides uuritud väetussüsteemides (Loide, 2004).

Kaltsiumi ja magneesiumi sisaldus vähenes kõikides uuritud süsteemides esimeses rotatsioonis (Joonis 2, I), sest ainult Mahe II süsteemis anti sõnnikuga kaltsiumit ja magneesiumit juurde, kuid see ei olnud piisav eelneva taseme säilitamiseks mullas. Alates teisest rotatsioonist saavutati maheviljeluslikes süsteemides mullas tasakaal ning vahekultuuride ning sõnniku mõjul Ca ja Mg sisaldus mullas enam ei vähenenud.

Rakendatud külvikorral oli positiivne mõju mulla füüsikalistele omadustele kõikides uuritud väetussüsteemides, kui võrrelda mulla näitajaid 2010. ja 2012. aastal tavaviljeluses ning 2010. ja 2014. aastal maheviljeluses. Vähenes mulla lasuvustihedus ning seoses sellega suurenes üldine poorsus, muu hulgas aeratsiooni- ja omastatava veega täidetud poorsus (Tabel 2, II; Tabel 1, III). Vahekultuuridel ning vahekultuuride ja sõnniku kombineerimisel on mulla veejuhtivusele oluliselt suurem mõju kui tavaviljeluslike süsteemide puhul, kui võrrelda muutust lähteaastaga.

Mulla poorsuse, veehoiuvõime, veejuhtivuse ja penetromeetrilise takistuse paranemine jätkus ka teises rotatsioonis (2013–2014) maheviljeluslikes süsteemides (Tabel 1, Joonis 3, III, Joonis 3), kuid see ei olnud seotud mulla suurema vees stabiilsete struktuuriagregaatide sisaldusega (Tabel 2, III). Nii vahekultuuride kasutamine eraldi kui ka kombinatsioonis sõnnikuga vähendas struktuuriagregaatide stabiilsust võrreldes kontrollvariandiga. See võis olla põhjustatud nii intensiivsemast harimisest seoses vahekultuuride külviga kui ka mulla magneesiumisisalduse usutavast vähenemisest. Katioonide, eriti kahevalentsete katioonide muutus mullas võib oluliselt mõjutada struktuuriagregaatide stabiilsust. Ka on täheldatud kergesti lagundatava orgaanilise materjali (lehed) negatiivset mõju struktuuriagregaatide stabiilsusele võrreldes raskesti lagundatava materjaliga (põhk).

Katsetulemustest selgus, et vihmaussiliikidest domineerisid uuritud väetussüsteemides harilik mullauss (*Aporrectodea caliginosa* L.) ja punane vihmauss (*Lumbricus rubellus* L.), kes moodustasid enamuse leitud liikide arvukusest. Väetatud maheviljeluse variantides leidus ka roosat mullaussi (*Aporrectodea rosea* L.) ja harilikku vihmaussi (*Lumbricus terrestris* L.), samas leidus ainult üksikuid rohelise mullaussi (*Allolobophora chlorotica* L.) isendeid. Vihmausside arvukus ja mass olid suurimad sõnniku ja vahekultuuride koos kasutamisel, kuid ka ainult vahekultuure kasutades oli nende arvukus ja mass sõltuvalt aastast kuni kaks korda suurem kui kontrollvariandis (Tabel 3, III). Samas ei olnud need erinevused väetussüsteemide vahel statistiliselt usutavad, kuna kultuuride mõju väetussüsteemi sees oli suur.

Vahekultuuride kasvatamisel oli statistiliselt usutav positiivne mõju ka mulla ensümaatilisele aktiivsusele (FDA), mille kaudu hinnati mulla mikrobiaalset aktiivsust. See positiivne mõju avaldus nii vahekultuuride eraldi kui ka koos sõnnikuga kasutamisel mõlemal uuritud aastal (2013 ja 2014) (Joonis 4, III) tänu mulda viidud kergesti lagunevale orgaanilisele ainele (taimelehed, sõnnik), kus lämmastiku ja süsiniku vahekord on bakteritele soodne (ca 1:10).

Kokkuvõte

Lähtuvalt püstitatud eesmärkidest võib teha järgmised järeldused:

1. Katseaastate keskmisena avaldas vahekultuuride (raps, raihein, rukis) eraldi ning koos sõnnikuga kasutamine statistiliselt usutavat mõju mulla happesusele (pH_{KCl}), suurendades naturaalselt happelise mulla pH 6-ni.

2. Suurim aastane orgaanilise süsiniku (C) juurdetulek mulda leiti maheviljeluslike süsteemide puhul, kus põhikultuuride põhule ja juurtele lisandus vahekultuuridest ja sõnnikust tulev orgaaniline aine.

3. Vahekultuuride ja sõnniku kasutamine suurendas küll mulla orgaanilise süsiniku ja üldise lämmastiku sisaldust, kuid ei taganud liikuvate toitainete, nagu fosfori, kaaliumi, kaltsiumi ja magneesiumi tasakaalu esimeses rotatsioonis.

4. Mineraalväetiste kasutamisel N_{88}, P_{25}, K_{95} rotatsiooni keskmisena pärast esimest viieaastast rotatsiooni püsis mulla üldise lämmastiku ja orgaanilise süsiniku sisaldus muutumatuna ning suurenes mulla liikuva fosfori sisaldus 8,7 mg kg^{-1}. Nimetatud süsteemis oli ka taimede toitainete omastamise tase kõrgeim võrreldes kontrollvariandi ja maheviljeluslike süsteemidega, kus kasutati vahekultuure ja sõnnikut.

5. Mulla liikuva kaaliumi ja magneesiumi sisalduse pidev vähenemine kõikides uuritud väetussüsteemides katseperioodil (2008–2014)

lubab järeldada, et mitte ükski rakendatud süsteemidest ei ole jätkusuutlik nende elementide taseme säilitamiseks mullas.

6. Rakendatud külvikorral – talinisu, hernes, kartul, oder punase ristiku allakülviga, punane ristik – oli kõikides uuritud väetussüsteemides positiivne mõju mulla füüsikalistele omadustele, nagu poorsus, veejuhtivus ja penetromeetriline takistus, kui võrrelda mulla näitajaid 2010. ja 2012. aastal tavaviljeluses ning 2010. ja 2014. aastal maheviljeluses.

7. Mineraalväetiste kasutamine tavaviljeluses suurendas külvikorra mõju mulla füüsikalistele omadustele rohkem kui vahekultuuride ja sõnniku kasutamine maheviljeluses. Esimese rotatsiooni lõpuks oli antud süsteemis väikseim lasuvustihedus, penetromeetriline takistus ning suurim aeratsiooni- ja taimedele omastatava veega täidetud poorsus.

8. Intensiivne harimine, mis kaasnes umbrohtude hävitamise ja vahekultuuride külviga maheviljeluses, vähendas vahekultuuride ja sõnnikuga mulda viidava suurema koguse orgaanilise aine positiivset mõju mulla lasuvustihedusele ja poorsusele terve katseperioodi jooksul.

9. Vahekultuuride kasvatamine ja sõnniku kasutamine avaldasid vastuolulist mõju mulla vees stabiilsete struktuuriagregaatide sisaldusele – 2013. aastal suurendas, kuid 2014. aastal vähendas nende sisaldust. Struktuuriagregaatide stabiilsuse vähenemine võis olla põhjustatud nii intensiivsest harimisest kui ka mulla magneesiumisisalduse vähenemisest.

10. Domineerivateks vihmaussiliikideks nii väetamata kui ka vahekultuure ja sõnnikut kasutades olid katsealal harilik mullauss (*Aporrectodea caliginosa* L.) ja punane vihmauss (*Lumbricus rubellus* L.), sõnniku kasutamisel leiti ka roosat mullaussi (*Aporrectodea rosea* L.). Sõltuvalt katseaastast oli vihmausside arvukus vahekultuure ja sõnnikut kasutades kuni kaks korda suurem kui väetamata mullas.

11. Mulla mikrobiaalne aktiivsus, mida hinnati ensümaatilise aktiivsuse põhjal, reageeris positiivselt vahekultuuride ja sõnniku näol lisandunud kergesti lagundatavale orgaanilisele ainele, olles neis väetussüsteemides statistiliselt usutavalt suurem kui väetamata mullas.

Edasist uurimist vajavad teemad:

- Täpsema tulemuse vahekultuuride mõju kohta mulla füüsikalistele omadustele saaks väiksema harimise intensiivsuse juures kui läbi viidud uuringus.

- Et välja selgitada, milline külvikorra, vahekultuuride ja sõnniku kombineeritud kasutusviis mõjutab mulla omadusi kõige soodsamalt, tuleks jätkata katsega ning pöörata rohkem tähelepanu mikrobiaalsele aktiivsusele ja vihmaussidele.

- Teatud katioonide, eriti Na^+ olemasolu mullas või sõnnikus võib viia mulla struktuuriagregaatide lagunemiseni, mistõttu nende uurimine annaks parema arusaamise struktuuriagregaatide stabiilsusest (sh vahekultuuride ja sõnniku vahelduvast mõjust).

Käesolev doktoritöö näitas vahekultuuride positiivset mõju mulla elustiku aktiivsusele, kuid negatiivset mõju struktuuriagregaatide stabiilsusele, mistõttu ei saa antud töö põhjal anda soovitusi vahekultuuride praktikas kasutamiseks. Vajalik on pikemaajalisem uuring leitud tulemuste kinnitamiseks või ümberlükkamiseks, et vältida lühiajalistest muudatustest tingitud tulemuste põhjal väärade järelduste tegemist. Lisaks on vaja läbi viia majandusliku tasuvuse uuring.

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I

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Soil Nutrient Evolution during the First Rotation in Organic and Conventional Farming Systems

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Since 2008, a 5-year crop rotation experiment (winter wheat, pea, potato, barley undersown with red clover, and red clover) has been run in Tartu, Estonia, to evaluate the changes in soil chemical parameters under four fertilizer managements: (1) unfertilized conventional plots (conventional I), (2) conventional plots with addition of mineral fertilizers (conventional II), (3) organic plots with cover crops during the winter period (organic I), and (4) organic plots with the same cover crops plus a yearly amendment of 40 t ha^{-1} of cattle manure (organic II). After the first rotation, results showed significant differences ($P < 0.05$) in soil acidity dependent on the system with mean values ranging between 5.67 (conventional II) and 6.10 (organic II). In the organic II system, manure had a significant effect on the system, increasing the organic carbon (C) content by 0.34%, but in both organic systems, both cover crops and cattle manure were insufficient for maintaining a constant level of plant-available phosphorus (P) or potassium (K) in the soil. In the conventional II system, mineral fertilizers provided a sufficient amount of nitrogen (N) to the system and increased the concentration of P to 8.7 mg per kg. The yearly mineral or organic amendments did not counteract the significant decrease in soil-available K after the first rotation. Lastly, calcium (Ca) and magnesium (Mg) availability, strongly influenced by the soil pH local conditions, decreased with time for all systems even though organic ones presented greater concentrations of both compounds. In conclusion, the four fertilization systems managed independently would not guarantee a constant soil nutrient concentration after the first rotation.

Keywords Cattle manure, cover crops, crop rotation, farming system, green manure, soil nutrients

Introduction

Organic farming is presented nowadays as an alternative agricultural strategy to conventional farming. Relaying on maximizing the use of the resources present in the agroecosystem, this farming practice uses crop rotation, legumes, compost, and

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manure as the main pillars to improve soil fertility (Talgre et al. 2009), excluding or strictly limiting the use of chemical fertilizers and pesticides (Zehnder et al. 2007). As evidenced by a recently reviewed European policy on organic agriculture to develop more environmentally sensitive farming practices (European Commission 2013), the increasing demand on ecofriendly products and the concern for better economically balanced agricultural practices (especially in the terms energy resources) have boosted the interest and investigation of organic farming (van Diepeningen et al. 2006).

However, meanwhile, many conventional farming systems need to deal with soil degradation problems derived from the intensive agriculture, including the decline of organic matter, degradation of soil structure, nitrate leaching (Herzog and Konrad 1992), and groundwater pollution (Poudel et al. 2002); one of the challenges that dairy organic farming systems face since this limitation in the use of synthetic fertilizers is to maintain the productivity and soil nutrient pool (Steinshamn et al. 2004), making questionable in some cases its sustainability in time. This situation is notably unfavorable in northern Europe soils, where the availability of nutrients is very limited, especially of nitrogen (N) (Brozyna et al. 2013).

In Estonia's small farms, where the use of mineral fertilizers and cattle manure is relatively low, cover crops and green manure play important roles improving the nutrient supply for succeeding crops (Talgre et al. 2009).

Among the different crops, legumes are the most widely used in both rotations and green manure because of their N-fixing capacity. Rasmussen et al. (2012) found that different combination of legumes can provide annual N yields ranging between 300 and 400 kg N per ha, which can cover the N needs of the nonleguminous crops included in the rotation.

To reduce the use of fertilizers and prevent soil erosion, nutrient leaching, and depletion, catch crops can be included in the farming system. The term *catch crop* is used for those cover crops included in the system for preventing nutrient losses after the harvest of the main crop (Talgre et al. 2009). Their use after harvesting is common in southern regions of Europe (Constantin et al. 2012; Mancinelli et al. 2013) where bare soil is more likely to get eroded and suffer nutrient loss, as well as in northern countries (Talgre et al. 2009), where soil remains mostly frozen during the winter. It is under these conditions, where the soil is tilled mostly in autumn and may get exposed to extreme weather events such as storms, heavy rains, and melting-freezing cycles during the winter period, where cover crops play an important role.

On the other hand, the benefits of cover crops and green manure to the crop nutrient supply depend on the composition of the crop residues, the field management, and the pedoclimatic conditions (Brozyna et al. 2013).

Several long-term experiments have compared organic and conventional systems under different conditions (Bulluck et al. 2002; Poudel et al. 2002; Chirinda et al. 2010; Doltra, Lægdsmand, and Olesen 2011) and have focused on the catch crops and their capacity for preventing N losses through leaching or denitrification. However, less researched is the capacity of these crops to fix phosphorus (P), potassium (K), calcium (Ca), and other nutrients (Talgre et al. 2009).

The aim of this study was to contribute to this field by testing the capacity of catch crops and manure to counteract the soil nutrient depletion in organic farming in counterpoint with the extra addition of mineral fertilizers in conventional farming. It was hypothesized that catch crops alone or with cattle manure would be sufficient for preserving a constant nutrient concentration in the soil after the first rotation.

Materials and Methods

Field Experiment

The present study was based on a 5-year stockless crop rotation trial situated at the experimental station of the Estonian University of Life Sciences (EMÜ) in Eerika, Tartu, Estonia (58° 22′ N, 26° 40′ E). The soil was described as Stagnic Albic Luvisol (IUSS Working Group WRB 2006), with 56.5% sand, 34% silt, and 9.5% clay and 20- to 30-cm-deep plowing layer (Reintam and Köster 2006).

The experiment had a total of four separated groups of 20 plots according to the farming system (conventional and organic) and the fertilization management, where each culture corresponds with a different plot, resulted in a total of four replications for every crop in each of the systems. The succession of cultures in the rotation was winter wheat (*Triticum aestivum* L.), pea (*Pisum saetivum* L.), potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.) undersown with red clover (*Trifolium pratense* L.), and red clover. The four fertilization systems are described as follows:

1. Conventional I: 20 plots under conventional management, with pesticides but without any addition of chemical fertilisers ($N_0P_0K_0$).
2. Conventional II: 20 plots under conventional management, where different concentrations of complex mineral fertilizers along with chemical pesticides were added, once before planting and twice during the growing period. The average ratio applied was dependent on the culture: barley received $N_{120}P_{25}K_{95}$, winter wheat and potato received $N_{150}P_{25}K_{95}$, and a low N rate ($N_{20}P_{25}K_{95}$) was used for pea because it is a leguminous crop (red clover alone was not fertilized). In the case of N, from the total amount added, 5 kg were given in the composition of complex fertilizer and the rest as ammonium nitrate (NH_4NO_3).
3. Organic I: 20 plots under organic farming conditions with the same rotating crops but including winter oilseed rape (*Brassica napus* ssp. oleifera var. biennis) after pea, winter rye (*Secale cereale* L.) after potato, and ryegrass (*Lolium perenne* L.) after winter wheat as winter cover crops (catch crops) lately plowed and used as green manure.
4. Organic II: 20 plots under organic farming conditions including the same crop rotation and the same cover crops, with the addition of 40 t ha^{-1} of composted cattle manure in autumn (2009) or in spring (2010–2012) in those plots where potato was cultivated.

Organic and conventional plots were separated with an 18-m-long section of mixed grasses. This prevents organic plots from be contaminated with synthetic pesticides and mineral fertilizers from the conventional side. Conventional plots were separated by 20 m and in the case of organic systems, a 10-m-long protective area prevented that manure was spread over those plots were it should not be used.

All cultures were sown during the last days of April and the beginning of May, except winter wheat, which was sown in late August. Table 1 shows the different sowing rates of the cultures and cover crops involved in the rotation. One week before sowing, the organic and red clover plots were plowed to 22 cm deep, incorporating the cover crops in the soil. During the growing season, and depending on the culture, soil was cultivated at 10–12 cm and harrowed at 1 cm deep. In particular, potato plots were cultivated at 25 cm, harrowed at 4–5 cm deep, and subsequently furrowed and hilled. Finally, all plots except those where barley was undersown with red clover were plowed in autumn, and in the case of

Table 1

Sowing rates of the cultures and cover crops in the rotation

Culture	Seeds per m^2	Cover crop	kg ha^{-1}
Winter wheat	450	Winter rye	220
Pea	100	Ryegrass	25
Potato	5.3[a]	Oilseed rape	6
Barley	375	Red clover (us.)	9
Red clover	280		

[a] Planting rates of potato in tubers per m^2.

organic plots, cover crops were sown after plowing. All the operations were made in opposite directions in consecutive years and separately for each of the systems.

Prior to the current experiment, in 2006, spring wheat was sown. In 2007, the field was divided already into the four blocks (systems) and barley along with red clover were sown in four plots per system (as one of the crops of the experimental rotation). In the same year and only in both conventional systems, 50 kg ha^{-1} of mineral N were added. According to this, years 2009 and 2010 were the second and third of the conversion to organic farming, and 2011 was the year when the organic plots can be considered fully organic. In any case, for a better understanding of the present study, the term *organic* has been used for all the years of the experiment for both organic farming systems.

Laboratory Analysis

Before any field operation, eight replications per plot were randomly taken from 0 to 25 cm deep and combined to make one average sample. Every sample was air dried and passed through a 2-mm sieve.

A solution of soil in 1 M potassium chloride (KCl) (1:2.5) was used for determining the pH. The organic carbon concentration (C_{org}) was determined by the Tjurin method (Vorobyova 1998); meanwhile the total nitrogen (N_{tot}) was measured after Kjeldahl digestion (van Reeuwijk 2002). The concentrations of the plant-available phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) in the soil were determined by the ammonium lactate (AL) method (Egnér, Riehm, and Domingo 1960).

For the analysis of the composted manure nutrient content, N_{tot} and C_{org} from four samples were measured by the dry combustion method in a Vario Max CNS elemental analyser (ELEMENTAR, Germany). In addition, acid digestion by sulfuric acid solution (van Reeuwijk 2002) was used to determine total phosphorus (P_{tot}) and potassium (K_{tot}) concentrations. Average values corresponding to these analyses were as follows: C_{tot}, 138 g kg^{-1}; N_{tot}, 9.7 g kg^{-1}; P_{tot}, 4.6 g kg^{-1}; K_{tot}, 8.6 g kg^{-1}; Ca, 11.7 g kg^{-1}; Mg, 3.4 g kg^{-1}; and dry-matter content, 44.8%. The same methodology was used for determining the nutrient content in plants.

All the laboratory analyses were carried out at the Department of Soil Sciences and Agrochemistry.

Climate Conditions

Climatic conditions at the experimental site are typical of the Baltic States, with temperatures below zero from the second half of November until the end of March, rainfall

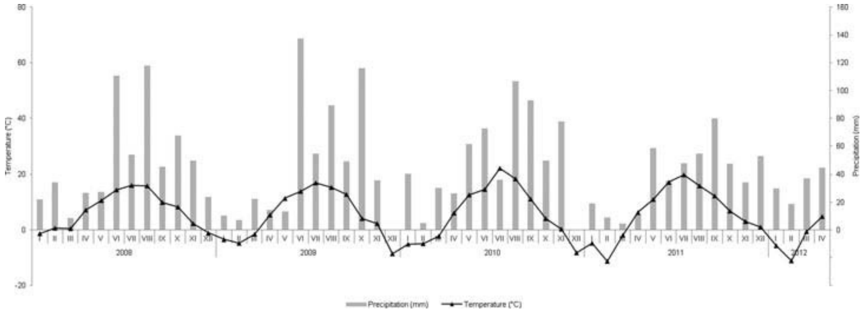

Figure 1. Ombrotermic diagram (2008–2012). Data for the rest of the months of 2012 have been omitted because the last sampling took place in April.

concentrated in spring and early autumn, and continuous snow during the winter period. Consistent with the data gathered for the 5 years of the experiment, the cumulative annual rainfall at the area was 568.6 mm, with a mean temperature during the year of 5.9 °C (Figure 1). The period of active plant growth (number of days with a continuous temperature above 10 °C) ranged between 115 and 135 days (Tarand 2003). The driest year of this period was 2011, with only 454.8 mm of precipitation. Long-term (1969–2012) precipitation at the site is 593.2 mm per year, with an average temperature of 5.5 ºC.

Statistical Analysis

To test the effect of the four fertilization systems with time on the different soil nutrients as well as the significant differences among the systems, one-way analysis of variance (ANOVA) was applied, followed by the least significant difference (LSD) test at the 95% level of confidence. The R-Studio (R Core Team 2012) statistical package was used to analyze all of the experimental data.

Results and Discussion

The relatively short time course of the present experiment and the fertility level prior to the experiment started can explain why no statistically significant differences ($P < 0.05$) were found in terms of soil acidity along the years. Our results showed a slightly decline of pH with time when mineral fertilizers were added in the conventional II plots, with average values decreasing from 5.77 to 5.67 after 5 years. This can be explained because nitrogenous fertilizers supply N as ammonium (NH^{4+}), which releases hydrogen (H^+) ions after oxidation and hence decreases the soil pH (Magdof, Lanyon, and Liebhardt 1997; Imtiaz Rashid et al. 2013).

On the other hand, the different fertilization managements resulted in significant differences in pH among the systems (Table 2). The greater pH presented by both organic systems in comparison with the conventional systems is consistent with other studies. Bulluck et al. (2002) and Liu et al. (2007) in their respective long-term experiments on different soil types found that soils under continuous organic amendments presented a greater buffering capacity, increasing the pH to a greater level than the same soils where mineral fertilizers were applied.

Table 2

Soil pH_{KCl} in the different farming systems

Year	System Conventional I	 Conventional II	 Organic I	 Organic II
2008	5.84ABa ± 0.061	5.77Aa ± 0.049	5.91Ba ± 0.043	5.96Ba ± 0.049
2009	5.97ABa ± 0.059	5.83Aa ± 0.065	6.02Ba ± 0.046	6.03Ba ± 0.052
2010	5.86ABa ± 0.085	5.76Aa ± 0.061	5.97Ba ± 0.037	5.95Ba ± 0.068
2011	5.83ABa ± 0.058	5.73Aa ± 0.072	6.00BCa ± 0.042	6.12Ca ± 0.071
2012	5.79ABa ± 0.060	5.67Aa ± 0.076	5.95BCa ± 0.048	6.10Ca ± 0.074

Note. Means followed by different capital letters within each row indicate significant influence (Fisher's LSD; $P < 0.05$) of system; means followed by different small letters within each column indicate significant influence (Fisher's LSD; $P < 0.05$) of year; ± value represents standard error of the mean (SE); n = 20 (one year); n = 100 (average of 2008–2012).

Cover crops, cattle manure, or the inclusion of red clover in the rotation were not sufficient for maintaining a constant level of N in the soil after first rotation in the organic systems (Figure 2). This contradicts some previous studies, which have shown that an adequate level of N can be provided by including cover crops, legumes, and/or compost (Stivers and Shennan 1991; Sullivan, Parrish, and Luna 1991; Steffen et al. 1995) in the soil. However, only in the case of the conventional I plots did the concentration of N in the soil decrease significantly ($P < 0.05$) from 1.33% (2008) to 1.15% (2012), due to the lack of fertilization.

It is necessary to remark that plants only use a certain amount of the total input of nutrients provided. Astover et al. (2006) estimated that in the pedoclimatic conditions of Estonia, crops would utilize 40–50% in the first year and 50–60% in the whole crop rotation of the total N provided via mineral fertilizers, and from manure, plants utilize 20–30% and 40–60% of N, respectively. However, these numbers are dependent on the balance among decomposition, mineralization, and immobilization, which are processes strongly influenced by environmental conditions, pH, and composition of the manure applied (Imtiaz Rashid et al. 2013). In our experiment, we observed an increase of N uptake by plants after the first year for all the systems. In addition, the conventional II system, where the availability of N is greater due to the yearly addition of mineral fertilizers, also presented greater nutrient uptake not only for the N but P and K as well (Table 3). This might explain why this system, despite receiving N amendments one year before the start of the experiment, presented the lowest N soil concentration in 2008.

The use of cover crops after manure application binds the N released in the decomposition of organic matter (Olesen et al. 2007) and prevents the losses through leaching or gaseous denitrification. However, organic amendments have a heterogeneous effect on the N content and usually it is hard to predict their long-term effect (Rodrigues et al. 2006). Hence it would be recommended to include an extra source of organic N such as compost or another legume crop in the rotation to ensure a proper balance of this nutrient in long term in the organic I system. Lastly, regarding the use of legumes in the rotation, factors such as low temperature, pH, or low nutrient availability negatively affect the symbiotic fixation of N (Peoples, Landha, and Herridge 1995). In our experiment, the low temperatures registered during almost 5 months per year can negatively affect the N fixation by the

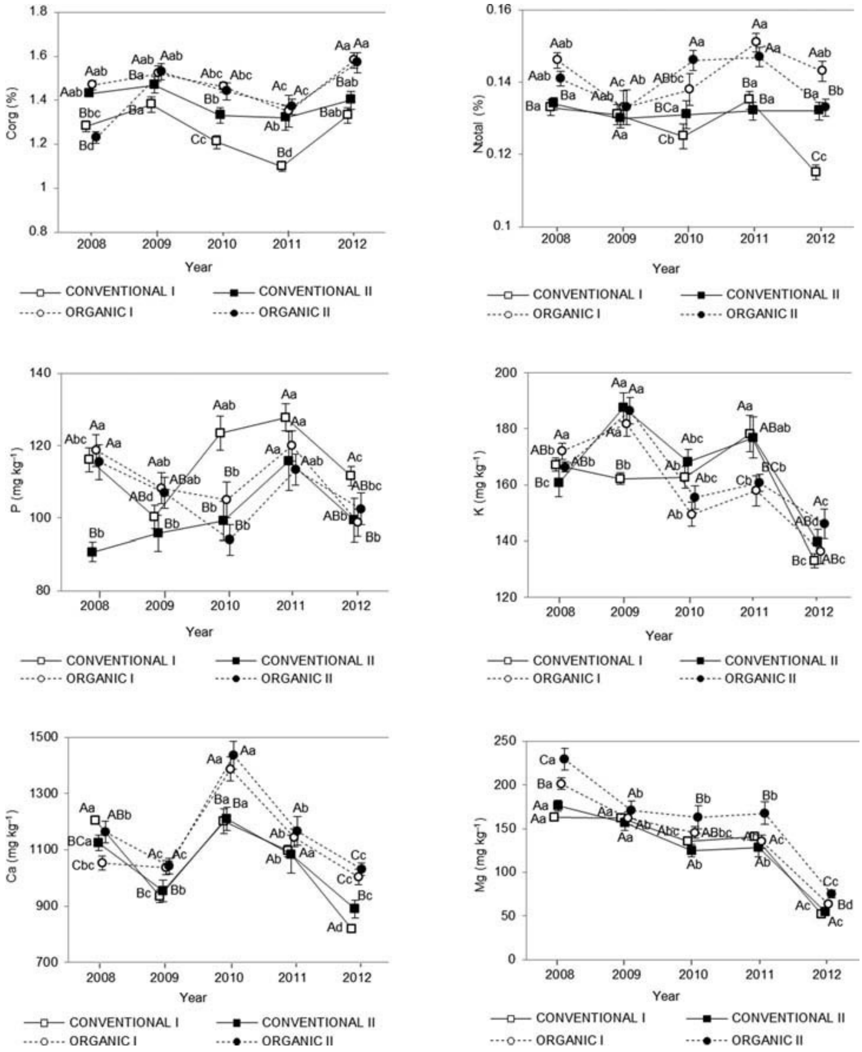

Figure 2. Mean soil nutrient concentration in the five years of study (2008–2012). Different capital letters indicate significant influence (Fisher's LSD; P<0.05) of system. Different small letters indicate significant influence (Fisher's LSD; P<0.05) of year. n=20 (one year), n=100 (5 years). Means followed by different small letters indicate significant influence (Fisher's LSD; P < 0.05) of year.

red clover. This might be a bigger disadvantage in the case of the conventional I system, where there is no extra source of N or catch crops that bind the N present already in the soil.

Enhancements in C_{org} were expected in the organic plots. The cattle manure amendments significantly increased the C_{org} content in the organic II plots to a much greater extent than the organic I ones did, with values rising from 1.23% in 2008 to 1.57% in 2012 (Figure 2). A long list of researchers, including Hoyt and Rice (1977), Wong et al. (1998),

Table 3

Crop nutrient uptake (kg ha^{-1}) in the different farming systems[a]

		System			
	Year	Conventional I	Conventional II	Organic I	Organic II
N	2008	30.52Ab ± 4.77	54.68Ab ± 7.96	28.46Ab ± 4.81	28.12Ab ± 4.57
	2009	49.13Aab ± 7.17	95.94Aa ± 14.2	44.80Aab ± 6.82	48.69Aa ± 7.15
	2010	36.76Aa ± 6.25	70.83Aab ± 8.30	37.14Aab ± 6.07	39.30Aab ± 6.01
	2011	47.22Aab ± 6.81	74.53Aab ± 11.40	41.23Aab ± 5.23	43.53Aab ± 6.02
	2012	58.00Aa ± 8.64	97.56Aa ± 12.83	46.62Aa ± 7.77	47.16Aa ± 7.59
P	2008	6.33Ac ± 0.93	9.31Bc ± 1.32	6.00Ab ± 0.89	5.98Ab ± 0.87
	2009	11.38ABab ± 1.63	16.4Aab ± 2.23	9.87Bab ± 1.38	11.39ABa ± 1.61
	2010	6.01Ac ± 0.87	10.83Bc ± 1.41	6.72Ab ± 0.91	6.84Ab ± 0.89
	2011	9.34Abc ± 1.23	12.00Abc ± 1.60	9.18Aab ± 1.11	9.66Aab ± 1.23
	2012	14.65Ba ± 2.09	20.43Aa ± 2.55	12.27Ba ± 2.38	12.58Ba ± 2.17
K	2008	24.94Aa ± 7.94	35.07Aa ± 10.47	21.94Aa ± 6.53	22.40Aa ± 6.68
	2009	35.30Aa ± 10.64	63.28Aa ± 20.98	27.68Aa ± 7.82	33.99Aa ± 10.30
	2010	22.34Aa ± 5.41	41.88Aa ± 10.16	23.48Aa ± 5.54	28.05Aa ± 7.62
	2011	41.64Aa ± 13.43	65.77Aa ± 23.45	30.64Aa ± 8.26	40.81Aa ± 12.67
	2012	45.19Aa ± 13.00	59.64Aa ± 16.06	48.79Aa ± 17.28	43.78Aa ± 14.25

Note. Different capital letters within each row indicate significant influence (Fisher's LSD; $P < 0.05$) of system; different small letters within each column indicate significant influence (Fisher's LSD; $P < 0.05$) of year; ±, standard error of the mean (SE); n = 20 (one year); n = 100 (average of 2008–2012).

[a] Calculation was made based on the following formula: Nutrient uptake = yield (kg ha^{-1}) × nutrient concentration (%) / 100.

Chirinda et al. (2010), and others, have reported similar results in different soils where manure has been applied after several years. This greater C_{org} content resulted in a greater C/N ratio in the organic systems ($C/N_{Org.\ I}$ 11:1 and $C/N_{Org.\ II}$ 11.8:1) in comparison with the conventional II ($C/N_{Conv.\ II}$: 10.6:1), suggesting that the incorporation of manure has a greater effect on increasing the C_{org} than has the N fixed by the legumes in the rotation.

The only source of organic C in conventional plots is the incorporation of plant residues once the soil is plowed in autumn, which does not suppose an influx but merely a recycling of the carbon initially present in the soil. Furthermore, over the long term and without any organic amendment, continuous addition of synthetic N fertilizers can result in a potential loss of soil C because greater application of synthetic nitrogenous fertilizers leads to larger loss of soil C as carbon dioxide (CO_2) (Khan et al. 2007).

The P content in the arable horizon varied irregularly with time except in the conventional II plots. This system presented a positive respond to the mineral fertilization with a constant increase of P concentration in the soil for the first 4 years, showing a global increase of 8.7 mg kg^{-1}after the first rotation (Figure 2). Generally, Estonian soils are poor in nutrient content. In the case of P, Astover et al. (2006) concluded that for avoiding significant decrease in P levels in the soil, the average fertilizer amount should not be less than 10–15 kg P ha^{-1}. This explains why the conventional II system was the only one presenting a progressive improvement in terms of soil P content after successive fertilization amendments. In contrast, organic plots showed a significant

($P < 0.05$) decrease of 12.9 mg kg^{-1} (organic I) and 19.9 mg kg^{-1} (organic II), suggesting that most of the extra P provided by the cattle manure (P_{tot}) in the organic II was not found in forms readily assimilated by plants. This does not agreed with Astover et al. (2006), who showed that the use of P by plants provided in the form of cattle manure was 20% bigger than the one from mineral fertilizers, which may contribute to the depletion of this nutrient in the organic plots. On the other hand, other experiments like the one carried out by Løes and Øgaard (2001) in five farms located in different parts of Norway reported lower concentrations of P and long-term losses as well (8 to 27 mg kg^{-1} per year) in organically fertilized soils compared with conventionally managed ones.

Conventional I and organic I systems presented the lowest concentration of K with an average decrease of 34.2 mg kg^{-1} and 35.6 mg kg^{-1}, respectively, after 5 years (Figure 2). This decrease was more significant in 2012 due to the relatively high nutrient uptake that occurred in 2011 (Table 3). Some experiments carried out in organic farmed soils have shown negative K balances or depletion of the soil exchangeable K, if the amount of K that is harvested exceeds the K released from soil minerals (Simonsso et al. 2007). In our case, not only the organic but all of the fertilization systems were insufficient for maintaining a constant concentration of K in the soil. This phenomenon may be enhanced by the presence of two groups of legumes that were in the rotation. Legumes are considered as greater P- and K-demanding crops and hence this may favor the depletion of these nutrients in the soil.

However, according to the fertilizer demand classification for Estonian soils proposed by Loide et al. (2004), depending on the texture and organic C content, any of the systems presented lack of P and K. Average soil concentration values ranged between 98.8 and 111.6 mg kg^{-1} for P and between 133 and 141.1 mg kg^{-1} for K (Figure 2). Based on this classification, the systems presented low to very low demand of these nutrients after 5 years.

Finally, the availability of calcium (Ca) and magnesium (Mg) decreased for all the systems after 5 years since there were not any external inputs of any of these two elements, except in the case of organic II system via manure. In particular, Mg shows a clear decreasing tendency over time (Figure 2). However, despite the general decline, both organic systems present greater concentrations not only of Ca but also of Mg, consistent with the findings made by Liu et al. (2007). In their short-term experiment in ten different agricultural soils in North Carolina, where organic plots (with cover crops, legumes, and different composted manure amendments) showed greater levels of phosphates, Ca, Mg, and other minerals in comparison with the conventional plots, where mineral fertilizers were applied.

The availability of Ca and Mg nutrients is strongly influenced by climate conditions, soil acidity (Potočić, Ćosić, and Pilaš 2005), presence of other cations [K^+, NH_4^+, manganese (Mn^{2+})] (Marschner 2012), and soil texture. According to Marschner (2012), over a certain level of Ca in the soil there is an antagonism cation competition for plant uptake. This results in a reduction of K and Mg uptake. This phenomenon might explain that the peak of available Ca in the soil occurred in 2010 corresponds with a low concentration of K for the same year.

Furthermore, Ca and Mg are less available in sandy soils and low pH (Zhao et al. 2011). The local condition of the soil epipedon at the experimental area is naturally acid ($pH_{KCl} < 6$), which constrains the availability of these nutrients for the plants (Reintam and Köster 2006); therefore, these soils should be limed for correcting the low values of Ca and Mg. Because the soils of the experimental area have not been limed for more than 10 years, reacidification has taken place, and levels of Mg and Ca remain low.

Conclusions

The selection of one or another fertilization system has repercussions on the soil nutrient content and hence in the soil fertility. After 5 years, once the first crop rotation has been completed, none of the four fertilization systems proposed was sufficient for preventing the yearly nutrient decrease. In the conventional II system, mineral fertilizers provided adequate levels of N and P to the soil; meanwhile in the organic II system cattle manure supposed a notable source of organic C. However, both fertilized conventional and organic systems presented a decreased availability of K in the soil, and even though the local soil conditions showed a low demand of K and P for all the systems, under these fertilization managements there might be a the depletion of these nutrients in the long term, especially in the organic systems, which have been shown by previous studies to be more sensible to suffer depletion of these nutrients.

Cover crops alone did not maintain a constant nutrient level in the soil in the organic I system, but they presented a positive effect on binding the N fixed by the legumes included in the rotation. On the other hand, from the low crop uptake in the organic II system in contrast to the high nutrient concentration present in the cattle manure, we can conclude that most of these nutrients present in the manure were not in forms available for the plants.

Finally, the local acidic soil conditions and the absence of any addition of Mg and Ca can explain the decrease of these nutrients after the first rotation.

Consequently, the present experiment confirms that the potential benefits of organic farming practices on soil fertility should not be taken for granted but also that conventionally managed soils exacerbate the lack of organic carbon over time and reduce soil pH, affecting the availability of various nutrients. Therefore, mineral nutrient amendments combined with organic farming practices may help to maintain a constant concentration of nutrients in the soil over time. However, continuing with the present experiment for a longer period would be necessary, especially for the evaluation of soil quality in organic plots, because soils require several years from conversion to reach a new equilibrium.

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II

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Organic farming and cover crops as an alternative to mineral fertilizers to improve soil physical properties**

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Abstract. For testing how cover crops and different fertilization managements affect the soil physical properties in a plough based tillage system, a five-year crop rotation experiment (field pea, white potato, common barley undersown with red clover, red clover, and winter wheat) was set. The rotation was managed under four different farming systems: two conventional: with and without mineral fertilizers and two organic, both with winter cover crops (later ploughed and used as green manure) and one where cattle manure was added yearly. The measurements conducted were penetration resistance, soil water content, porosity, water permeability, and organic carbon. Yearly variations were linked to the number of tillage operations, and a cumulative effect of soil organic carbon in the soil as a result of the different fertilization amendments, organic or mineral. All the systems showed similar tendencies along the three years of study and differences were only found between the control and the other systems. Mineral fertilizers enhanced the overall physical soil conditions due to the higher yield in the system. In the organic systems, cover crops and cattle manure did not have a significant effect on soil physical properties in comparison with the conventional ones, which were kept bare during the winter period. The extra organic matter boosted the positive effect of crop rotation, but the higher number of tillage operations in both organic systems counteracted this effect to a greater or lesser extent.

Keywords: bulk density, compaction, manure, penetration resistance, porosity

INTRODUCTION

In the last 20 years, agriculture cannot be understood without the use of mechanization and fertilizers. The increasing number of operations and the use of larger equipment, in addition to the continuous and intensive cropping systems, have led to soil degradation as a common problem on most of the farms that till the soil (Liu *et al*., 2007). In Estonia, one-third of the arable land is affected by some kind of degradation such as erosion, compaction, soil acidification, and other phenomena that reduce the soil fertility and crop productivity (Astover *et al*., 2006). In 2008, research carried out by the Agricultural Research Centre in collaboration with the Estonian University of Life Sciences defined soil compaction as one of the biggest problems affecting the soil not only at the regional level (Reintam *et al.,* 2009a), but also as a current issue in most of the Eastern European soils (European Commission, 2006).

Compaction is defined as a process of densification, in which porosity and permeability are affected. This reduces water drainage and air exchange between the soil and the atmosphere (Weisskopf *et al*., 2010), hampers the root penetration, and diminishes the nutrient uptake, finally affecting the crop yield (Reintam *et al*., 2009a; 2009b).

In the past, nitrogen fertilizer amendments were considered as a compensatory measure for the negative effects of soil compaction. These fertilizers indirectly influenced the soil organic matter content by increasing crop productivity and root density (Edgerton, 2009) and the amount of soil organic carbon (SOC) when the crop residues were incorporated later into the soil. However, this costly practice is not environmentally sustainable since it carries a severe risk of nitrate contamination of soil and water (Ju *et al*., 2006).

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This contributed to increased research on different sustainable soil management strategies. During the last decades, the decline in SOC in most of agricultural systems and the awareness towards the importance of global C budgets have boosted the interest in organic farming as an alternative agricultural practice to enhance crop production and maintain soil quality under increasing world population and climate change conditions (Lal, 2009; van Diepeningen *et al*., 2006).

Organic farming relies on the use of cover crops, rotations, and residue management as some of the key practices for enhancing the organic matter content in the soil and hence improving the soil quality (Olesen *et al*., 2007). Several studies have shown that continuous applications via manure significantly reduce the values of compaction indicators such as bulk density and penetration resistance (Chen *et al*., 2013), stimulate the microbial and fauna activity of the soil, and enhance soil aggregate structure (Treonis *et al*., 2010). The use of crop rotations favours a more efficient use of soil nutrients by plants. Crops with different root lengths and densities are able to mobilize and extract nutrients and water from deeper layers. However, these roots also create biopores in the soil profile and can be used, especially plants with vigorous roots, to prevent and potentially alleviate problems derived from compaction as well (Chen and Wail, 2011). Finally, maintaining vegetation on crop fields during the off-season through cover cropping has been shown as a good measure to improve overall soil condition. Traditionally used for preventing erosion and nitrogen losses (Sainju *et al.*, 2003), some winter cover crop species, like those from the genus Brassica, are used to potentially alleviate soil compaction problems due to their root system morphology (Hamza and Anderson 2005).

However, the positive effects of these practices are not permanent and changes during the whole rotation (Głąb *et al*., 2013) linked to the local soil and weather conditions, the crop species, the fertilization rates, tillage practices *etc*. can occur (Głąb and Kulig, 2008).

Despite the number of studies focused on the effect of organic and conventional farming practices on the chemical and biological properties, there is still no comparative research on soil physical properties under organic and conventional farming management (Papadopoulos *et al*., 2013). Similarly, research on cover crops has been confined to their use as nitrogen-binding agents and their effect on succeeding crops (Sainju *et al*., 2003), while relatively few studies on the effect of winter cover crops on soil physical properties under northern climate conditions have been conducted (Talgre *et al*., 2011).

The aim of this research is to contribute to this field of study by comparing the effect on soil physical properties –focusing on dry bulk density, porosity, penetrability, water retention capacity, and water availability– of four different fertilization managements under the basis of organic and conventional farming in a plough based tillage system. Although physical changes in soil are expected in a long-term period, it was hypothesized that organic systems, due to the higher content of organic matter (green and cattle manure) and the presence of cover crops during the winter period, would offer more favourable soil physical conditions (lower bulk density, higher plant water availability, *etc*.), than the conventional systems within 4 years (2012) after the rotation was started (in 2008), even in an intensively tilled soil.

MATERIAL AND METHODS

In a field experiment set up at the experimental station of the Estonian University of Life Sciences in Eerika, Tartu, Estonia (58°22′ N, 26°40′ E), soil physical properties under different farming systems were compared during the years 2010-2012. On a soil described as an Albic Stagnic Luvisol in the World Reference Base for soil resources classification (IUSS Working Group WRB, 2006), five-year crop rotation: winter wheat (*Triticum aestivum* L.), field pea (*Pisum sativum* L.), white potato (*Solanum tuberosum* L.), common barley (*Hordeum vulgare* L.) undersown with red clover (*Trifolium pratense* L.), and red clover – was established in 2008. The soil texture fractions were 56.5% sand, 34% silt, and 9.5% clay and the humus layer had a depth between 27 and 29 cm.

The experimental design consisted of eighty plots of approximately 66 m^2, divided into four groups (farming systems). Each system was divided into four subgroups (replications) of five plots, where every plot corresponded to a different crop. The farming systems are described as follows:

- conventional I: used as a control, consisted of 20 plots under conventional management without any incorporation of chemical fertilizers ($N_0P_0K_0$) but with a yearly addition of different pesticides;
- conventional II: 20 plots under conventional management where different concentrations of complex mineral fertilizers were added depending on the culture (common barley received $N_{120}P_{25}K_{95}$, winter wheat and white potato received $N_{150}P_{25}K_{95}$, low-N-rate $N_{20}P_{25}K_{95}$ was used for field pea, as it is a leguminous crop, and red clover alone was not fertilized). In addition, these plots were treated with different pesticides as well;
- organic I: 20 plots under organic farming conditions with the same crop rotation but including winter oilseed-rape (*Brassica napus* ssp. *oleifera* var. *biennis*) after pea, rye (*Secale cereale* L.) after potato, and perennial ryegrass (*Lolium perenne* L.) after winter wheat, as winter cover crops. In these plots weeds were removed by harrowing and no chemical plant protection was used;
- organic II: 20 plots under the same conditions than the previous system (organic I), plus manual addition, in autumn 2009 and every spring since 2010, of 40 t ha^{-1} of composted cattle manure in those plots where potato

was cultivated. On average, the composted cattle manure contained: total nitrogen (N) – 9.7 g kg^{-1}, total phosphorus (P) – 4.6 g kg^{-1}, total potassium (K) – 8.6 g kg^{-1}, total carbon (C – 138 g kg^{-1}, total calcium (Ca) – 11.7 g kg^{-1}, total magnesium (Mg) – 3.4 g kg^{-1}, and 44.8% of dry matter (DM).

Organic and conventional plots were separated with an 18-meter long section of mixed grasses. This prevented the organic plots from being contaminated with synthetic pesticides and mineral fertilizers from the conventional side. In the case of organic systems, a 10-meter long protective area prevented manure spreading over those plots were it should not be used.

Both conventional systems were treated twice per year with herbicides, two to three times with different insecticides; meanwhile, fungicides were applied three to four times.

From 2008 to 2010, after the harvesting of field pea, white potato, and winter wheat in the conventional plots in the early autumn, no more operations took place on the soil until the end of October when the plots were ploughed at 22 cm depth. The plots where common barley was undersown with red clover remained unaltered during the winter period. No ploughing was done in autumn in 2011. Later, since 2012, the soil ploughing was done in spring at the same time as in the organic plots. In the case of the organic systems, winter cover crops were sown after harvesting the main crops and ploughed at the same depth (22 cm) into the soil the next year at the end of April. In May, barley with red clover and pea were sown in all systems and, finally, red clover was ploughed in July. During the spring, the plots were cultivated several times at 10-12 cm depending on the culture and the year and harrowed at 1 cm depth to destroy the weeds. The plots where potato was planted were cultivated at 25 cm, harrowed at 4-5 cm depth, and subsequently furrowed and hilled.

It is important to remark that 2010 and 2011 were the third and fourth year, respectively, of conversion to organic farming. Hence, by 2011, the organic farming systems had already been fully organic. For a better understanding, in the present paper the notation ‘organic’ is used for defining both organic systems independently of the year.

The weather conditions differed slightly between the three years of study. In 2010, the average temperature (T) was 5.73ºC with temperatures below zero already in the second half of November. The total precipitation (P) was 598.6 mm, concentrated mainly during the summer period. On the other hand, 2011 was warmer (T = 6.28ºC) and drier, with total precipitation P = 454.8 mm. Finally, 2012 was characterized by high precipitation occurring in the first half of the year in comparison with the two previous years, with P = 584.6 (Table 1), and an average temperature of 5.35ºC.

The sampling and measurements were done in autumn after harvesting the main crops from the medium section of each plot in 2010 and 2011. In 2012, samples were taken in spring before ploughing the soil.

A cone penetrometer (Eijkelkamp Penetrologger with 60 degree 1 cm^2 cones) was used for measuring the penetration resistance down to 50 cm depth. For the rest of the physical properties studied, steel cores (54 mm internal diameter and 40 mm height, with wall thickness approximately 1.5 mm) were used at 5 to 10 cm depth, taking four replications of undisturbed soil per plot; this makes a total of 320 samples per year. In addition, for every plot, approximately 250 grams of soil were placed into plastic tubs for further analysis and determination of plant available/not available water content. For the soil organic carbon (SOC) analysis, 8 replications per plot were randomly taken at the same time from 0 to 25 cm depth and later combined to make one average sample. Every sample was air dried and passed through a 2 mm sieve.

At the Department of Soil Sciences and Agrochemistry of the Estonian University of Life Sciences, the soil cores were weighted (at field-moist condition) and capillary wetted for 24 h on a plate until saturation. Total porosity (TP), air filled pores (AFP), and dry bulk density (p_d) were determined by subjecting the cylinders to constant drainage over a sand bed to a water tension of 6 kPa for 12 days and later drying in the oven at 105°C for 24 h (Angers and Larney, 2007). Water permeability (k) was measured using a Hauben permeameter (Eijkelkamp, The Netherlands). For the calculation of the plant available (PAW) and not available water (PnAW), three air dried soil replications

T a b l e 1. Average quarter (three months) temperature (T) and total precipitation (P) values at the experimental site during the period 2010-2012

Year	January-March		April-June		July-September		October-December	
	T (ºC)	P (mm)	T (ºC)	P (mm)	T (ºC)	P (mm)	T (ºC)	P (mm)
2010	-4.10	75.4	11.09	160.4	17.20	235.8	-1.25	127.0
2011	-5.94	32.4	11.52	104.8	15.99	182.8	3.57	134.8
2012	-5.77	85.8	9.93	221.4	14.93	205.0	2.95	113.0

Table 2. Mean values of the soil physical parameters and organic carbon, in the three years of study at the top layer (5–10 cm)

Parameter	Year	Conventional I	Conventional II	Organic I	Organic II
θ (%)	2010	16.79Aa	16.03Aab	15.26Ab	16.11Aab
	2011	16.33Aa	16.48Aa	18.12Bb	18.03Bb
	2012	19.75Ba	19.13Ba	18.84Ba	19.41Ba
p_d (Mg m^{-3})	2010	1.53Aa	1.53Aa	1.60Ab	1.59Ab
	2011	1.54Aa	1.46Bb	1.49Bab	1.44Bb
	2012	1.45Bab	1.39Cb	1.47Ba	1.48Ba
TP (%)	2010	41.34Aa	41.20Aa	38.69Ab	39.24Ab
	2011	40.88Aa	44.14Bb	42.86Bab	44.68Bb
	2012	44.53Bab	46.52Cb	43.53Ba	43.21Ba
AFP (%)	2010	16.70Aa	14.11Aab	11.78Ab	12.49Ab
	2011	12.58Ba	15.36Ab	13.10Aa	15.52Bb
	2012	15.96Aa	19.11Bb	14.62Aa	14.07ABa
PAW (%)	2010	18.16Aa	21.36Ab	20.05Abc	19.73Aac
	2011	16.66Aa	20.83Ab	15.81Ba	16.48Ba
	2012	22.01Ba	22.82Ba	22.03Ca	21.42Ca
PnAW (%)	2010	6.48Aa	5.73Ab	6.86Aac	7.02Ac
	2011	11.63Ba	7.93Ac	13.95Bb	12.75Bab
	2012	6.63Aa	4.59Bb	6.90Aac	7.71Ac
k (cm d^{-1})	2010	87.74Aa	96.50Aa	19.88Ab	26.72Ab
	2011	46.79Ba	81.81Ab	48.20Ba	68.82Ba
	2012	103.8Aa	82.32Aa	94.68Ca	91.32Ca
SOC (%)	2010	1.21Aa	1.33Ab	1.46Ac	1.44Ac
	2011	1.10Ba	1.32Ab	1.35Ab	1.37Ab
	2012	1.33Ca	1.40Ba	1.58Bb	1.57Bb

θ – soil water content, p_d – dry bulk density, TP – total porosity, AFP – air filled pores, PAW – plant available water, PnAW – plant not available water, k – soil water permeability, SOC – soil organic carbon content. Mean values followed by a different capital letter within each column indicates significant yearly differences (*t*-test, $p<0.05$) within the same system. Mean values followed by a different small letter within each row indicate significant difference (linear mixed model, $p<0.05$) among the systems within the same year.

from the tubs were placed in small steel cylinders (1.5 cm diameter, 0.5 cm height, and 0.1 cm thick) and placed into pressure vessels at 1 500 kPa during 30 days, weighted, dried at 105°C, and weighted again. Finally, soil organic carbon (SOC) was determined by the Tiurin method (Vorobyova, 1998).

To test the effect of the four fertilization systems on the soil penetration resistance at three depths, one-way analysis of variance was applied, followed by the least significant difference (LSD) test at the 95% level of confidence. The system effect on the different soil parameters was tested according to a general linear mixed model in R-Studio (R Core Team, 2012), considering also the effect of crop and repeated measurements made on the same plot, where $p<0.05$ was considered as statistically significant. For testing the significant differences among the three years of study, the *t*-test was used.

RESULTS AND DISCUSSION

Even though bigger changes in physical soil properties may be expected in a longer term, within four years after the crop rotation started (in 2008), significant differences ($p<0.05$) were already found among the systems.

Making an overall comparison of the soil physical properties in 2010 and 2012, despite the continuous tillage operations carried out in the systems, a general improvement in terms of soil porosity, water availability, permeability, and organic carbon content in the plough layer can be observed in all the systems (Table 2). Without taking into account other factors affecting soil structure analyzed later, this can be associated with a positive crop rotation effect (higher organic matter content and crop roots effect). Similarly to other studies (Carter *et al*., 2003; Hao *et al*., 2002), crop rotation showed a cumulative positive effect (Hao *et al*., 2002) on soil structural stability (higher porosity) and prevented soil organic carbon losses in the systems (Table 2).

The variability in the results along the years was also connected with the soil conditions at the time of sampling. In 2010 and 2011, soil samples were taken during the autumn period. By that time, the plots had been cultivated during the spring and main crops had already been harvested. On the other hand, in 2012, soil sampling took place in spring. Winter cover crops were present in the organic systems, in the conventional system only common barley undersown with red clover remained in the soil, while the other plots were just covered by plant residues (without tillage). Therefore, in 2010 and 2011, a notable tillage effect on the soil physical condition was expected as result of the operations previously carried out during the growing period. In turn, in 2012, the soil was kept unaltered until the sampling time and was only affected by the freezing and thawing cycles during the winter period and the organic systems benefited from the presence of the cover crops.

On the other hand, climate conditions during the whole year play an important role with regard to certain soil physical conditions. The weather conditions in 2011 were relatively drier in comparison with the other two years. In general, plant roots under moderate water stress conditions tend to increase the fine root length per unit soil volume, even in deeper soil layers (Chaves *et al*., 2002). Plant roots extend easily through macropores or regions of weakness in the soil, compacting the surrounding soil particles and decreasing the pore size. This is connected with the increase in PnAW (Table 2), which corresponds to the portion of water stored in the micropores. Reintam (2006) showed that under non-compacted soil conditions, root densities were lower in those plots where mineral fertilizers were added, since nutrients are found more accesible for plants. This can explain why despite the general increase in PnAW in 2011, the porosity fraction remained basically unvariable in the Conventional II system. Lower root density resulted in lower compaction and hence the different porosity fractions remained the same.

The yearly variability as well as the differences among the systems can be the result of a cumulative fertilizer and SOC effect. On the one hand, the lack of any fertilization, mineral or organic, and/or cover crops, negatively affected the control system (Table 2). In addition, the relative low crop yield recorded in the system during the period from 2009 to 2011 (Tein *et al*., 2014) poorly contributed to maintenance of the SOC pool in the soil. Other short-term experiments similarly showed a quick decline in soil organic carbon after a few years without any extra organic matter amendments (Liu *et al*., 2007).

On the other hand, the systems where fertilizers were applied showed an improvement in some of the soil physical parameters studied. Among the porosity fractions, AFP exhibited a positive response to the addition of mineral fertilizers (Table 2). The direct impact on soil physical properties derived from NPK fertilizers is hard to demonstrate (Głąb, 2014); however, previous studies have reported a positive response of SOC to the addition of chemical fertilizers in combination with crop rotation (Liu *et al*., 2007), mostly connected with higher crop yields. In the conventional II system during the period 2009-2011, Tein *et al.* (2014) found 45 to 55% higher potato yields than in the same plots under organic management, and on average, conventional systems exhibited a 80% higher yield than the organic ones. This corresponds to a bigger amount of crop residues incorporated into the soil, which in turn suggests a SOC source in the system that remains unaltered for a longer period due to the lower number of tillage operations carried out in the conventional systems.

In the organic systems, cover crops alone or with cattle manure had a significant effect on water permeability (k) connected with the significant increase in SOC (Table 2). The average SOC gains (2010-2012) from winter cover crops in these systems were by 0.24-0.25 and 0.17-0.18%

higher in than the conventional I and conventional II system, respectively (Table 2). This suggests that the possible SOC stock losses from tillage, water erosion, and mineralization were lower than the SOC sequestered and maintained from the cover crops. Organic matter represents a food supply for earthworms and provides better living condition for the microfauna (Scullion *et al*., 2007). Organic I contained twice and organic II 2.6 times more earthworms, compared to the control system (unpublished data). Earthworms contribute to an increase in the number of medium and larger pores and hence an increase in water permeability.

However, the positive effect of cover crops and manure was counteracted by the higher number of tillage operations carried out in the organic systems. Tillage operations accelerated the mineralization of SOC, exposing aggregate protected C to microbial attack and soil particles to the effect of drying/rewetting and freezing/thawing cycles. In the same way, the conventional I system, compared with the rest of the systems, was more sensible to the effect of tillage operations due to the low organic matter in the system.

In the same way as the physical parameters previously discussed, all the systems showed similar decreasing tendencies with time in terms of penetration resistance (Fig. 1) as the balanced bulk density decreased and porosity increased (Table 2). At the ploughing depth (22 cm), the average values in 2010 ranged between 2.54 and 3.09 MPa for the Conventional I and Organic I systems, respectively (lowest and highest values); in 2012, these values ranged between 1.92 and 2.14 MPa for the same systems. This might correspond to the crop rotation and SOC effect already explained and to differences in the soil water content at the time of sampling (Table 2) since these factors are directly correlated with the penetration resistance (Hamza and Anderson, 2005). It is important to note that even though the rest of the data collected derive from the analysis of the soil top layer (5-10 cm), looking at the penetration resistance diagrams, no big differences among the systems are expected at the ploughing depth (Fig. 1).

Despite the small number of tillage operations and the moderate weight of the tractor used (5 t), the discontinuity in penetrability with depth under the ploughing layer (25-30 cm depth) observed in 2011 may explain the evidence of a possible plough pan. At this depth, around 8 cm below the tillage depth, penetration resistance normally reaches higher average values (Tsimba *et al*., 1999). However, the relatively low clay content (under 10%), the small number of operations, and the natural processes of freezing/thawing and wetting/drying, do not make the soil very prone to structural problems due to soil compaction (Reintam, 2006). In fact, this soil hardening was a transient phenomenon and was not shown in 2012 (Fig. 1).

In the existing literature about penetration resistance, several authors including Whalley *et al*. (2012) concluded that, in general, penetrometer values greater than 2 MPa can significantly affect the correct crop development and

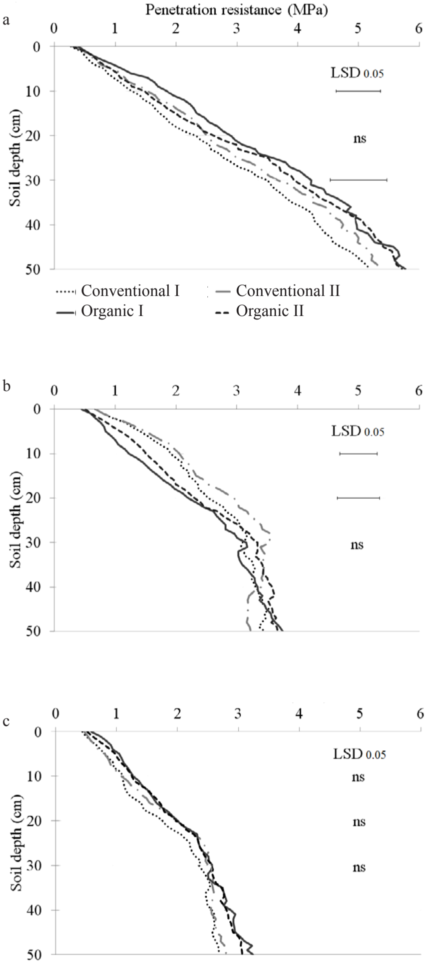

Fig. 1. Average penetration resistance (MPa) with depth measured in the four systems in: a – 2010, b – 2011 and c – 2012 year. $LSD_{0.05}$ – least significant differences at significance p<0.05, ns – not significant differences among treatments. Different capital letter indicates significant yearly (2010, 2011, 2012) differences (*t*-test, p < 0.05) within the same system (conventional I, conventional II, organic I, organic II) at the three studied depths: 10 cm (A,B,A; A,B,C; A,B,B; A,A,A), 20 cm (A,A,B; A,B,C; A,B,B; A,AB,B), 30 cm (A,A,B; A,A,B, A,B,C; A,B,C).

the yield. These values were reached in 2010 and 2011 at 10-15 cm depth in some of the systems (Fig. 1). However, in the same field experiment, Tein *et al*. (2014) studied the crop yield response to four fertilization levels. The differences in yield found among the systems seem to be connected with the presence/absence of mineral fertilizers and not with problems derived from compaction.

The results derived from the present experiment do not confirm so far the initial hypothesis. Despite the higher amount of SOC, neither organic plot exhibited more favourable physical properties than in the conventional fertilized systems in the ploughing layer. However, many studies have presented a significant effect of cattle manure enhancing soil particle aggregation, reducing bulk density, and increasing water holding capacity (Hati and Bandyoopadhay, 2011) and hence bigger differences might be expected in a longer term.

CONCLUSIONS

1. Crop rotation and fertilization were the main factors affecting soil physical properties and soil organic carbon. Within 4 years after the crop rotation started, all the systems showed a general improvement in their soil physical condition in the plough layer: lower bulk density, higher plant available water and water permeability, and soil organic carbon), which is connected with a positive crop rotation effect.

2. The presence of winter cover crops, the return of the crop residues, and the application of cattle manure contributed to an increase in soil organic carbon and water permeability. However, in a short term, this effect was not statistically significant probably because the higher number of tillage operations counteracted this effect.

3. The lack of any fertilization treatment in the control system resulted in higher bulk density, lower percentage of air filled pores, plant available water and water permeability, and soil organic carbon compared with the other systems.

4. Mineral fertilizers boosted the positive effect of crop rotation to a greater degree than any of the organic managements proposed.

5. Taking into account that 2011 was the first year when organic systems could be considered fully organic and not all the plots had received cattle manure, bigger differences are expected in a long term linked to the disparity in the amount of organic matter among the systems.

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Winter cover crop effects on soil structural stability and microbiological activity in organic farming

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ABSTRACT

In a field experiment based on a five-year crop rotation (pea, potato, barley undersown with red clover, red clover and winter wheat), several soil parameters, porosity, number and biomass of earthworms, total nitrogen, organic carbon, percentage of water stable aggregates and enzymatic activity, were studied during 2013 and 2014, the first and second year, respectively, since the first rotation concluded. This rotation was managed under three organic farming systems: Organic 0 (control), Organic I (with winter cover crops lately incorporated into the soil as green manure) and Organic II (with the same cover crops plus a yearly amendment of 40 t ha^{-1} of cattle manure). Crop rotation had a yearly positive effect on the soil bulk density, and enhanced the percentage of air filled pores; nonetheless, despite the leguminous crops in the rotation, all the systems presented a yearly decrease in total nitrogen in 2014. Cover crops along with manure only had a significant effect on enzymatic activity; however no significant effect was found in soil organic carbon content, soil particle aggregability or number and biomass of earthworms. This was connected with the intensive tillage carried out in the systems, the weather conditions and the characteristics of the organic amendments. However according to other studies these results could be transient and further long-term investigations will be needed.

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Introduction

Maintaining vegetation on farm fields during the off-season through cover cropping and the subsequent recycling of crop residues has been shown as a good measure to improve overall soil conditions. This practice is commonly associated with organic farming; however it is considered suitable not only in organic farming but in any agricultural production system. Winter cover crops have traditionally been used to prevent erosion and *N* losses by the use of the residual NO_3^- that may leach into ground water after crop harvest (Sainju et al. 2003). Winter cover crops also increase the above- and below-ground biomass in the system, which maintains and in some cases improves the concentration of organic carbon in the soil (Sainju et al. 2002). This in turn benefits the soil microbial activity as well as the

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aggregation of soil particles by the enmeshing action of roots (Tisdall & Oades 1979) and polymers, binding agents in the aggregation processes (Roberson et al. 1991). Fließbach et al. (2007) found dehydrogenase activities 3.8–6.4 times higher on organic farms where cover crops were present than on conventional ones.

The selected species must be biologically suited to the rotation to break up pest and diseases cycles as well as control weed growth (Newenhouse & Dana 1989). Species, including those from the genus *Brassica*, are used as cover crops potentially to alleviate soil compaction, due to their root system morphology (Hamza & Anderson 2005), at the same time improving the soil biological activity after their incorporation into the soil as green manure (Tejada et al. 2008). The non-leguminous cover crops most commonly used in Europe are white mustard (*Sinapis alba* L.), westerwold ryegrass (*Lolium multiflorum* L.), fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) and phacelia (*Phaceliatana cetifolia* Benth.; Thorup-Kristensen et al. 2003).

However, sometimes cover crops are not enough to maintain productivity in organic farming systems which basically depend on maximizing the use of resources already present in the agro-ecosystem (Steinshamn et al. 2004). Therefore, a large portion of organic soils suffer from low nutrient availability, in particular nitrogen, and depend to a greater or lesser extent on external manure inputs. This is the case of most of the soils cultivated under Nordic climatic conditions (Brozyna et al. 2013). In northern latitudes, the plant species used as cover crops must be adapted to low temperatures, be resistant to frost and have rapid tillering and growing periods. A good example is winter rye (*Secale cereale* L.) which has fast root growth and a great potential to scavenge residual soil NO_3^- (Isse et al. 1999). In the case of Estonia, white mustard, fodder radish, winter oilseed rape (*Brassica napus* L. var. *oleifera*) and winter oil turnip (*B. rapa* L. var. *silvestris*) have shown good results in terms of nutrient binding (Talgre et al. 2011).

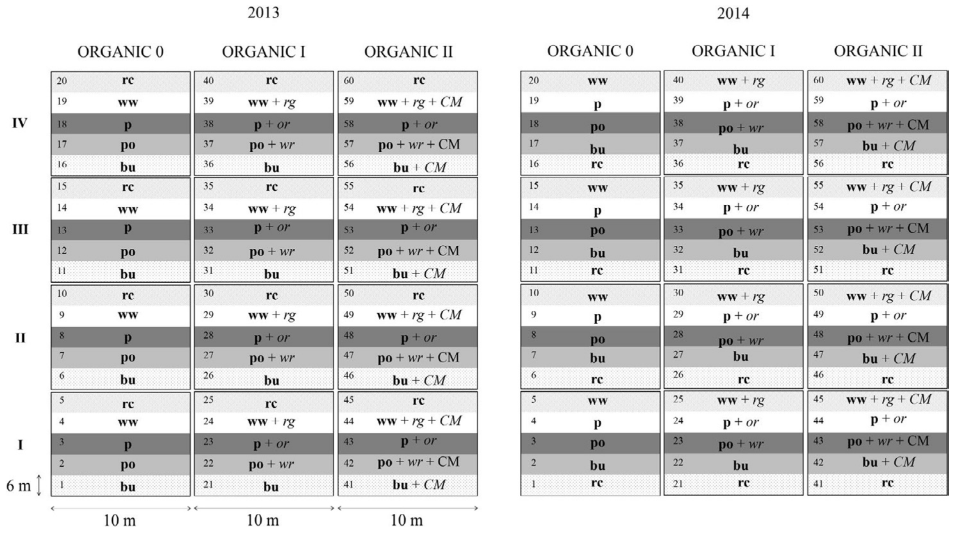
Winter cover crop effects on soil physical, chemical and biological properties depend on the species, climate and soil conditions. Research on winter cover crops has, to a large extent, been confined to their use as nitrogen-binding agents and their effect on the succeeding crops (Sainju & Singh 1997). However, relatively few studies on the effect of winter cover crops on soil physical and biological properties have been reported. The aim of this work was to evaluate the effect of winter cover crops and different organic matter amendments (green manure and green manure plus cattle manure) on soil aggregate stability and biological activity under organic farming conditions under northern European climate conditions. It was hypothesized that those systems where cover crops were present during the winter season would show an improvement in soil physical properties and structure connected with a higher amount of organic matter. This in turn would increase the microbiological activity and the soil particle aggregability in these systems.

Materials and methods

Field experiment

From 2008, a five-year crop rotation; winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.) undersown with red clover (*Trifolium pratense* L.) and red clover was established on the experimental station of the Estonian University of Life Sciences located in Eerika, Tartu County, Estonia (58°22'N, 26°40'E). The soil was described as an Albic Stagnic Luvisol in the World Reference Base for soil resources (IUSS Working Group WRB 2006) classification with 56.5% sand, 34% silt and 9.5% clay, and 20 to 30 cm depth of the ploughing layer (Reintam & Köster 2006).

The experimental field had three organic blocks (systems) (Figure 1). Organic 0: organically managed plots without addition of any fertilizers and used as the control; Organic I: organically managed plots with winter oilseed-rape (*B. napus* ssp. *oleifera* var. *biennis*) after pea, winter rye (*S. cereale*) after potato and ryegrass (*L. perenne*) after winter wheat, as winter cover crops. The residues of these crops were lately incorporated in the soil as green manure; Organic II: under the same conditions as Organic



	2013						2014					
	ORGANIC 0		ORGANIC I		ORGANIC II		ORGANIC 0		ORGANIC I		ORGANIC II	
IV	20	**rc**	40	**rc**	60	**rc**	20	**ww**	40	**ww** + *rg*	60	**ww** + *rg* + *CM*
	19	**ww**	39	**ww** + *rg*	59	**ww** + *rg* + *CM*	19	**p**	39	**p** + *or*	59	**p** + *or*
	18	**p**	38	**p** + *or*	58	**p** + *or*	18	**po**	38	**po** + *wr*	58	**po** + *wr* + CM
	17	**po**	37	**po** + *wr*	57	**po** + *wr* + CM	17	**bu**	37	**bu**	57	**bu** + *CM*
	16	**bu**	36	**bu**	56	**bu** + *CM*	16	**rc**	36	**rc**	56	**rc**
III	15	**rc**	35	**rc**	55	**rc**	15	**ww**	35	**ww** + *rg*	55	**ww** + *rg* + *CM*
	14	**ww**	34	**ww** + *rg*	54	**ww** + *rg* + *CM*	14	**p**	34	**p** + *or*	54	**p** + *or*
	13	**p**	33	**p** + *or*	53	**p** + *or*	13	**po**	33	**po** + *wr*	53	**po** + *wr* + CM
	12	**po**	32	**po** + *wr*	52	**po** + *wr* + CM	12	**bu**	32	**bu**	52	**bu** + *CM*
	11	**bu**	31	**bu**	51	**bu** + *CM*	11	**rc**	31	**rc**	51	**rc**
II	10	**rc**	30	**rc**	50	**rc**	10	**ww**	30	**ww** + *rg*	50	**ww** + *rg* + *CM*
	9	**ww**	29	**ww** + *rg*	49	**ww** + *rg* + *CM*	9	**p**	29	**p** + *or*	49	**p** + *or*
	8	**p**	28	**p** + *or*	48	**p** + *or*	8	**po**	28	**po** + *wr*	48	**po** + *wr* + CM
	7	**po**	27	**po** + *wr*	47	**po** + *wr* + CM	7	**bu**	27	**bu**	47	**bu** + *CM*
	6	**bu**	26	**bu**	46	**bu** + *CM*	6	**rc**	26	**rc**	46	**rc**
I	5	**rc**	25	**rc**	45	**rc**	5	**ww**	25	**ww** + *rg*	45	**ww** + *rg* + *CM*
	4	**ww**	24	**ww** + *rg*	44	**ww** + *rg* + *CM*	4	**p**	24	**p** + *or*	44	**p** + *or*
	3	**p**	23	**p** + *or*	43	**p** + *or*	3	**po**	23	**po** + *wr*	43	**po** + *wr* + CM
	2	**po**	22	**po** + *wr*	42	**po** + *wr* + CM	2	**bu**	22	**bu**	42	**bu** + *CM*
6 m	1	**bu**	21	**bu**	41	**bu** + *CM*	1	**rc**	21	**rc**	41	**rc**
	10 m		10 m		10 m							

Figure 1. Crop sequence in the rotation in 2013 and 2014. Roman numbers on the left indicate the replications per systems.

Notes: Main crops: bu: barley undersown with red clover; po: potato; p: pea; ww: winter wheat; rc: red clover. Winter cover crops: *wr*: winter rye; *or*: oilseed-rape; *rg*: ryegrass. Fertilization: *CM*: manure.

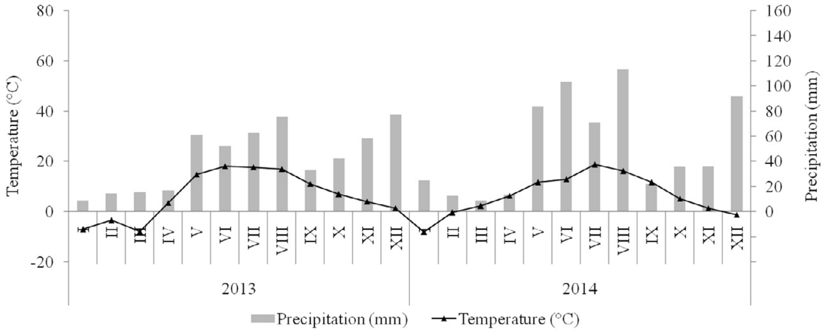


Figure 2. Ombrotermic diagram (2013–2014).

I plus the yearly manually addition of 40 t ha^{-1} of composted cattle manure from 2009 divided among potato (20 t ha^{-1}), winter wheat (10 t ha^{-1}) and barley undersown with red clover (10 t ha^{-1}). Therefore, by 2014 all the plots from this system had received cattle manure. On average, the composted cattle manure contained total nitrogen (N_{tot}) 9.7 g kg^{-1}, total phosphorus (P_{tot}) 4.6 g kg^{-1}, total potassium (K_{tot}) 8.6 g kg^{-1}, total carbon (C_{tot}) 138 g kg^{-1}, total calcium (Ca) 11.7 g kg^{-1}, total magnesium (Mg) 3.4 g kg^{-1} and 44.8% of dry matter (DM). Each block contained a total of 20 plots of 60 m^2, so each of the five years of the rotation was represented by four replications in each of the three systems.

After the harvesting of pea, potato and winter wheat in the early autumn, the plots were ploughed at 22 cm depth. Plots with barley undersown with red clover remained unaltered during the winter period. In the case of the Organic I and Organic II systems, cover crops were sown after harvesting the main crops and incorporated in the soil during spring. Finally in May, barley undersown with red clover and pea were sown. In the first half of June clover was cut, crushed and spread on the soil; in July a second cut took place and red clover was ploughed and incorporated into the soil. During the growing season, plots were cultivated at 10–12 cm and harrowed at 1 cm depth. In the case of potato, the tubers were planted, cultivated at 25 cm, harrowed at 4–5 cm depth, furrowed and hilled.

The management of the three systems did not include any use of chemical fertilizers, herbicides or pesticides. Weeds were removed by hand several times during the growing season.

Weather conditions

Climatic conditions of the two years of study were slightly mild compared with the average temperatures registered during the previous years (Sánchez de Cima et al. 2015). The year 2013 was distinguished by a colder winter, with temperatures below zero from January to March. According to the Estonian Weather Service database (Estonian Environment Agency 2015) the first quarter of 2013 was colder and drier than in previous years. The average temperature (T) in 2013 was 6.35 °C and the total precipitation (P) was 518.2 mm, concentrated in the summer period and early winter. On the other hand, in 2014 temperatures were similar to the previous year ($T = 6.39$ °C) and with $p = 617.4$ mm occurring mostly during the summer. However, the first quarter of the year was warmer and drier than the same period in 2013 (Figure 2).

Sampling and laboratory analysis

Earthworm samples were taken in autumn before harvesting from 0 to 20 cm depth within a frame of 40 cm × 40 cm randomly placed in each of the plots for the three systems. They were then classified, counted and weighed in a laboratory.

Soil samples were taken in spring before tillage from the middle area of each plot. Four replications per plot at 5–10 cm depth were taken using a steel corer (54 mm internal diameter and 40 mm height, with wall thickness approximately 1.5 mm). This made a total of 480 samples per year. Along with the steel cylinders, 250 g samples of fresh soil from the same depth were placed into plastic tubs for later determination of plant available and non-available water, percentage of stable aggregates and organic carbon content.

All soil analysis took place at the Institute of Agricultural and Environmental Sciences at the Estonian University of Life Sciences. The soil cores were firstly weighed at field-moist condition and capillary wetted for 24 h until saturation. Then cylinders were placed on a sand bed at 6 kPa of water tension for 12 days and finally dried in an oven at 105 °C for 24 h. From these analyses, the field moisture content, bulk density, maximum water holding capacity, total porosity and air filled pores were calculated. Water permeability was measured using a Hauben permeameter (Eijkelkam, Giesbeek, The Netherlands). From the air dried soil samples in the plastic tubs, three replications were placed in small steel cylinders (1.5 cm diameter, 0.5 cm height and 0.1 cm thick), put into pressure vessels at 1500 kPa for 30 days, weighted, dried at 105 °C and weighed again. Plant available and non-available water was calculated from these analyses. Finally from the same air dried samples, the percentage of water stable aggregates (WSA) was measured using a single-sieving apparatus from the <2 mm fraction (Eijkelkamp sieving apparatus with 60 mesh screen cylinders), following the methodology of Kemper and Rosenau (1986).

The soil organic carbon concentration (SOC) was determined by the Tjurin method (Vorobyova 1998) and the total nitrogen (N_{tot}) was measured after Kjeldahl digestion (van Reeuwijk 2002).

For the fluorescein diacetate hydrolysis activity (FDA) analysis, 500 g samples were taken at 5–10 cm depth, handled and stored according to ISO 10381-6 (1993). After the large plant material and stones were picked out by hand, about 200 g (fresh weight) was taken as a sub-sample and sieved through a 2 mm sieve. For the preparation of the reagents and the subsequent FDA analysis, the method described by Adam and Duncan (2001) was followed but using acetone as termination reagent and fluorescein instead of fluorescein sodium salt in the stock solution. Total microbial activity of FDA was expressed as μg of fluorescein released per gram soil dry mass over 1 h.

Statistical analysis

A general linear mixed model in R-Studio (R Core Team 2012) was used to test the system effect on the different soil properties studied. As there were five different crops grown in the same plot and system (Figure 2), but the comparison of specific crop effects on the soil properties was not the aim of the present study, the crop effect was included and considered as a confounding effect. In addition, the random effect of plot was included to consider the potential correlation between measurements made on the same plot. As the two study years were very different, the analyses were performed separately for 2013 and 2014. Additionally, a t-test was run to test the statistical significance of the differences between the two years of study. Finally, Pearson's correlation coefficients (r) were calculated to study the relationship between the number of earthworms (n) and the total porosity, as well as the influence of organic matter on the aggregation stability. All results were considered statistically significant at $p < 0.05$.

Results and discussion

Soil physical properties

In addition to the significant differences in soil water content at the time of sampling, yearly variations in the soil physical properties analysed were connected with a positive crop rotation effect and the accumulation of organic matter in the soil along the years. As a result, bulk density significantly

Table 1. Maximum water holding capacity, bulk density and water permeability at 5–10 cm for the two years of study.

Year	System	Field moisture content (%)	Maximum water holding capacity (%)	Bulk density (g cm^{-3})	Water permeability (cm day^{-1})
2013	ORG. 0	19.12 Aa ± 0.20	27.47 Aa ± 0.31	1.51 Aa ± 0.01	96.8 Aa ± 45.3
	ORG. I	19.72 Aa ± 0.21	28.59 Aab ± 0.40	1.47 Aab ± 0.01	187.0 Aa ± 53.0
	ORG. II	19.95 Aa ± 0.23	29.06 Ab ± 0.38	1.46 Ab ± 0.01	192.0 Aa ± 45.2
2014	ORG. 0	17.0 Ba ± 0.28	30.54 Ba ± 0.43	1.43 Ba ± 0.01	140.2 Aa ± 29.5
	ORG. I	16.81 Ba ± 0.24	30.80 Ba ± 0.40	1.41 Ba ± 0.01	167.0 Aa ± 28.6
	ORG. II	15.05 Ba ± 0.23	30.48 Ba ± 0.49	1.44 Aa ± 0.02	211.0 Aa ± 39.7

Notes: Mean values followed by different capital letter in each column indicates significant yearly differences (*t*-test; $p < 0.05$) within the same system. Mean values followed by different small letter in each column indicates significant difference (linear mixed model; $p < 0.05$) among the systems within the same year.

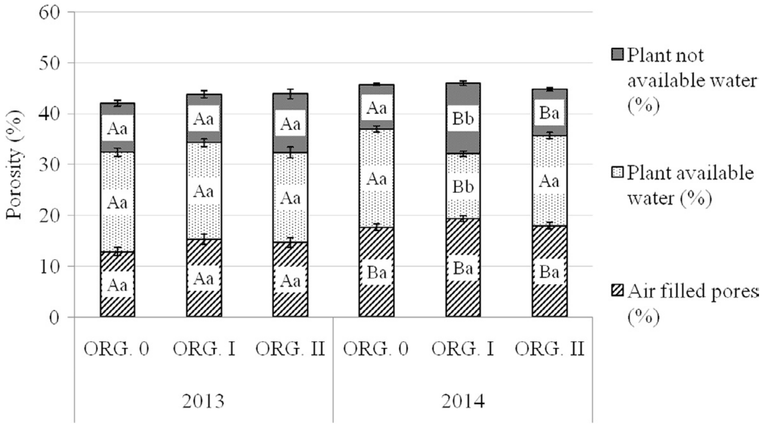


Figure 3. Average percentage of air filled pores content, plant available and not available water content for the two years of study. Notes: Total porosity (%) = air filled pores (%) + plant available water (%) + plant not available water (%). Different capital letter in each bar indicates significant yearly differences (*t*-test; $p < 0.05$) within the same system for the same parameter. Different small letter in each bar indicates significant difference (linear mixed model; $p < 0.05$) among the systems within the same year for the same parameter.

decreased ($p < 0.05$) in all the systems, with exception of the Organic II, where values reminded basically the same in both years of study (Table 1).

Contrary to other studies where the physical action of the cover crops roots had a positive impact on the soil physical conditions, decreasing bulk density and increasing soil porosity (Figure 3; Steele et al. 2012), in the present study no significant difference have been found between the system with cover crops alone (Organic I) and the control (Organic 0). This lack of significance can be explained by the short duration of the experiment. This can explain as well why cattle manure did not show any significant effect when results from the Organic I and Organic II systems were compared. Normally changes in physical soil properties are difficult to detect in the short term (Trabaquini et al. 2015). In addition, as already occurred during the first rotation, cover crop and manure effects were counteracted by the negative impact of the tillage operations carried out in these systems (Sánchez de Cima et al. 2015). Nevertheless, many studies have shown the positive effect of manure maintaining a better soil structure, increasing water holding capacity, aeration and pore continuity, and hence reducing bulk density (Chen et al. 2013). Therefore, significant differences may be expected in long term among the systems connected with the amount of organic matter.

Table 2. Average percentage of soil water stable aggregates, soil organic carbon content, total nitrogen and Pearson's correlation coefficient between both variables.

Year	System	WSA (%)	SOC (%)	N_{tot} (%)	C:N ratio	$r_{SOC\text{-}\%WSA}$
2013	ORG. 0	63.07 Aa	1.61 Aa	0.14 Aa	11.5:0	−0.15
	ORG. I	62.58 Aa	1.64 Aa	0.14 Aa	11.7:0	−0.31
	ORG. II	63.91 Aa	1.67 Aa	0.14 Aa	11.9:0	−0.04
2014	ORG. 0	65.01 Aa	1.55 Aa	0.11 Ba	14.0:0	−0.10
	ORG. I	60.67 Ab	1.54 Aa	0.11 Ba	14.0:0	+0.19
	ORG. II	54.44 Bc	1.67 Aa	0.11 Ba	15.2:0	+0.15

Notes: WSA: water stable aggregates; SOC: soil organic carbon; N: total nitrogen; C:N: carbon-nitrogen ratio; R: Pearson's correlation coefficient. Mean values followed by different capital letter in each column indicates significant yearly differences (t-test; $p < 0.05$) within the same system. Mean values followed by different small letter in each column indicates significant difference (linear mixed model; $p < 0.05$) among the systems within the same year. *No statistically significant correlations were found (all $p > 0.05$).

Soil aggregate stability

Effect of the three different managements on the SOC, total nitrogen (N_{tot}), C:N ratio and the percentage of WSA are presented in Table 2. Crop rotation had no significant yearly effect on SOC. Similarly, neither cover crops nor continuous application of manure had a significant effect when the three systems were compared. As already occurred during the first rotation (Sánchez de Cima et al. 2015), the intensive tillage in the organic systems counteracted the positive effect of the crop rotation and organic matter amendments in the plough layer. Tillage operations accelerate the mineralization of the SOC, causing carbon loss as carbon dioxide and exposing aggregate protected C to microbial attack and soil particles to the effect of drying/rewetting and freezing/thawing cycles. This agreed with the results found by Basch et al. 2012 where tillage based land use, irrespective of the type of land use, caused a considerable decline in SOM content in the tilled soil layer even in short term.

The significant yearly decrease of total nitrogen (N_{tot}) might result from a lack of plant available nitrogen forms in the soil or to the immobilization of the existing N due to the incorporation of the crop residues. In general, high 'C:N ratio cover crop' residues immobilize soil N and hence increase the amount of fertilizer N required for the subsequent crops (Dabney et al. 2001). This decrease occurred despite the presence of two leguminous crops, red clover and pea, in the rotation, the N binding action of cover crops in the decomposition of organic matter (Olesen et al. 2007) and the addition of cattle manure in the Organic II system. Already during the first rotation, organic plots showed a yearly decrease of soil N due to the low temperatures which significantly affected the N fixation by legumes (Sánchez de Cima et al. 2015). Results agreed with Frøseth et al. (2014) where nitrogen was shown as a limiting nutrient in organic soils under cold climatic conditions even after a productive green manure ley.

Cover crops and the application of cattle manure had no positive yearly effect on the %WSA in Organic I and Organic II systems and hence the initial hypothesis must be rejected. In contrast, the percentage of WSA significantly decreased in both systems in 2014 with cover crop and manure application. Therefore, soil aggregates data recorded in 2014 did not support the initial hypothesis. The addition of manure is generally associated with an increase in SOC and hence with an increase in aggregate stability (Bronick & Lal 2005). However, in the present experiment there was no significant correlation ($p > 0.05$) between the SOC and the %WSA (Table 2). Depending on the nature of SOC, in some cases manure can enhance the susceptibility to dispersion and hence decrease the aggregate stability. The presence of certain cations in the manure, especially Na^+, contributes to repulsive charges in the soil solution that disperse soil particles. On the other hand, bivalent cations such as Ca^{2+} and Mg^{2+} improve soil structure through cationic bridging with clay particles and SOC (Bronick & Lal 2005). Although neither electric conductivity nor the Na^+ concentration was measured in the present study, during the first rotation a continuous decrease of Ca^{2+} and Mg^{2+} in the Organic I and Organic II systems was shown (Sánchez de Cima et al. 2015). Since the experiment conditions did not change after the first rotation, it is expected that the concentration of these cations, despite the manure amendments, continued to decrease in 2013–2014.

The more favourable soil physical conditions in the second year (Table 1) did not run in parallel with the soil particle aggregation behaviour. In this sense, apparently a decrease in bulk density did not indicate an improvement in soil global structure. Moreover, many researchers have presented a positive correlation between soil organic content and soil aggregate stability (Haynes & Naidu 1998; Boix-Fayos et al. 2001; Tejada et al. 2008); however, results of the present experiment suggest that other factors have a bigger impact on the soil structural stability.

The experimental soil contained >50% sand. Sandy soils have been shown to be more liable to lose structural stability than clayey soils more prone to the formation of microaggregates (Boix-Fayos et al. 2001; Spaccini et al. 2001). Under these textural conditions, the tillage operations carried out in the systems could counteract a positive effect of crop rotation and manuring. By mixing the plough-layer, new soil is exposed to dry-wet cycles at the soil surface. This contributes to degrading the soil structure and also affects the SOM dynamics (Denef et al. 2001) and as result the soil aggregate stability is also affected (Greacen 1958).

Weather conditions also can determine the percentage of soil stable aggregates. According to different studies, annual rainfall positively affects soil aggregability (Molina et al. 1999; Velykis et al. 2014). Therefore, in the present experiment the higher cumulative precipitation during 2012 (638.8 mm) (Sánchez de Cima et al. 2015) than in 2013 (518.2 mm) might also explain the higher %WSA in 2013. Also the physical processes of freezing-thawing and drying-wetting have a negative impact on the aggregate stability and biotic activity of soil (Six et al. 2004). Finally, a low lignin content in the tissues from the crop residues incorporated into the soil makes them easily decomposable (Carvalho et al. 2009), providing only a transient effect on soil stability (Oades 1984; Piccolo & Mbagwu 1999). This agrees with Spaccini and Piccolo (2013), who found a slight deterioration of soil stability in some experimental fields under different compost amendments for the first year.

Earthworms

The following earthworm species were found: green worm (*Allolobophora chlorotica*), common earthworm (*Lumbricus terrestris*), grey worm (*Aporrectodea caliginosa*), mucous worm (*Aporrectodea rosea*) and marsh worm (*Lumbricus rubellus*). The last three species are classified as endogenic species. They are characterized by living deep into the organic-mineral soil layer, feeding on both soil and organic matter and they are considered as important agents of aggregation and organic matter stabilization (Lavelle & Spain 2001). Despite the relative abundance of the endogenic species, due to the difference in sampling depths between the earthworms (0–20 cm) and the rest of the soil properties studied (5–10 cm) it is difficult to establish any kind of connection among them. This can explain why no strong significant correlation was found between the number of earthworms and the percentage of soil aggregates, total porosity or SOC (Tables 2 and 3). Earthworms enhance soil porosity by drilling and forming macroaggregates (>2000 μm) as result of the excretion of casts and within these macroaggregates, microaggregates are stabilized (Barois et al. 1993; Bossuyt et al. 2006). However, the present results showed that variations in %WSA seem to be more connected with the tillage operations, soil texture and climate conditions. In the same way, while it is true that SOC represents a food supply for earthworms and better living conditions for the microfauna (Scullion et al. 2007), in the present experiment no evidence of a causal relationship was found between the SOC and the number of earthworms (Table 3). Pearson's correlation coefficient showed no significant correlation in any the systems in either years of study.

Only the control system showed a significant yearly increase in the number (*n*) and biomass of earthworms (Table 3). Therefore, cover crops or cattle manure had no significant effect on these two variables in 2014. Since there is no existing data from previous years it is difficult to conclude whether the yearly variability in the number of earthworms is merely stationary. However, as with other factors

Table 3. Average number and average biomass of earthworms, total porosity in the three systems for the two years of study and Pearson's correlation coefficient between the number of earthworms and the total porosity and number of earthworms and soil organic carbon content.

Indicator	Year	System		
		ORG. 0	ORG. I	ORG. II
n	2013	40 Aa	82.8 Ab	104.4 Ab
	2014	80 Ba	93.8 Aa	112.5 Aa
Biomass (g)	2013	22.6 Aa	48.4 Ab	51.4 Ab
	2014	53.9 Ba	58.4 Aa	70.8 Aa
Total porosity (%)	2013	42.02 Aa	43.74 Aa	43.87 Aa
	2014	45.67 Ba	45.96 Ba	44.79 Aa
$r_{n\,-\,\text{Total porosity (\%)}}$	2013	+0.05	−0.19	+0.09
	2014	+0.25	−0.17	−0.24
$r_{\text{SOC (\%)}\,-\,n}$	2013	+0.03	+0.43	−0.12
	2014	−0.24	−0.05	−0.45

Notes: *n*: average number of earthworms; r: Pearson's correlation coefficient; SOC: soil organic carbon. Mean values followed by different capital letter in each column indicates significant yearly differences (t-test; $p < 0.05$) within the same system at the same depth for the same parameter. Mean values followed by different small letter in each row indicates significant difference (linear mixed model; $p < 0.05$) among the systems within the same year. *No statistically significant correlations were found (all $p > 0.05$).

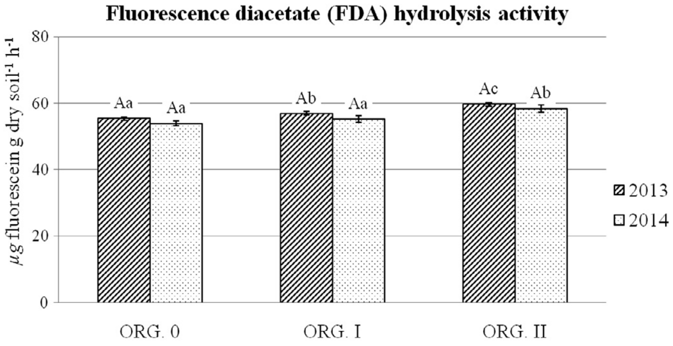


Figure 4. Average μg of released fluorescein per gram soil dry mass over 1 h for the three systems in 2013 and 2014. Notes: Different capital letter on each bar indicates significant yearly differences (*t*-test; $p < 0.05$) for the same system. Different small letter on each bar indicates significant difference (linear mixed model; $p < 0.05$) among the systems for the same year.

such as soil aggregability or SOC, certain tillage operations, especially ploughing, disrupt earthworm population and eventually create unfavourable soil environmental conditions (Springett et al. 1992).

Microbiological activity

Figure 4 shows the FDA in the three systems for the years 2013 and 2014. Cover crops along with cattle manure showed a significant effect on FDA hydrolysis in 2014, resulting in a higher microbial activity in the Organic II system. These results agree with several studies which have pointed out a higher microbial activity in organically amended soils (Bulluck et al. 2002; Edesi et al. 2012). The addition of manure to soil results in a rapid soil microbial response by increasing of enzyme activity (Kanchikerimath & Singh 2001) due to the incorporation of easily degradable organic materials (green and cattle manure) which stimulate the activity of autochthonous microbes, incorporating exogenous microorganism as well (Tejada et al. 2008).

No yearly significant differences were found in any of the systems. This can be explained by the relatively short experiment. In general, long term crop rotations have a significant impact on microbial communities and their activity (Edesi et al. 2012). Furthermore, microbiological activity is highly dependent on the organic matter content in the soil (Gadja et al. 2012). Since no yearly significant changes in SOC took place at the experiment (Table 2), no significant differences in FDA were found either.

Enzymatic activity is dependent as well on other factors such as soil moisture, which in turn affects pH, redox potential, organic matter decomposition, soil nutrient concentration and other processes linked to the microbial population and activity (Wu et al. 2010); and nitrogen which is used for the synthesis of proteins, amino acids and DNA (Gadja et al. 2012). Contrary to these earlier results, in the present experiment these factors seem to have no significant influence in the microbiological activity, since the significant yearly differences in soil moisture (Table 1) and total nitrogen (Table 2) did not result in significant variations in FDA (Figure 4).

Conclusions

Cover crops showed a positive effect on the bulk density and air filled pores fraction. In addition, cover crops along with cattle manure enhanced the microbiological activity in the system where it was yearly applied. The effect of cover crops and manure on the soil particle aggregability and SOC was counteracted by the amount of tillage operations, the soil texture, water content and the characteristics of the crop residues added. Most of the earthworms species found were endogenic. No strong correlation was found between the SOC and the number of earthworms or the porosity and earthworms. The short time since the experiment was set up can explain the lack of significant differences among systems in most of the soil parameters studied. Different results are expected in long term, especially regarding to the aggregate stability. Therefore we consider necessary to continue with the present experiment during future years and extent the sampling to two different depths.

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IV

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Soil carbon dynamics estimation and dependence on farming system in a temperate climate

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A B S T R A C T

Maintaining or enhancing the stock of soil organic carbon (SOC) is a key factor in sustaining the soil resources of the world. The objective of this research was to study the effect of different farming systems (conventional farming with mineral fertilizers and crop specific fertilization vs. organic farming with organic fertilizers (catch crops and composted manure)) under the same 5-crop rotation (red clover, winter wheat, pea, potato, barley undersown with red clover) system on the SOC stock and the stability of SOC. The second aim was to quantify plant C inputs to the soil and to identify the relationship between C sequestration rate and C input. Data presented in this paper concerned the first rotation during 2008–2012. The main factors were farming systems: conventional and organic. Four conventional farming systems differed in the mineral nitrogen application rates used. In two organic farming systems catch crops were used with or without composted solid cattle manure. The SOC stock was determined before experiment establishment and after the first rotation. The C input into the soil was calculated based on the main product yield. The stock of SOC increased (2.57 Mg ha^{-1} y^{-1}) considerably after the first rotation only in the organic farming systems, where the total C inputs were 1368 kg C ha^{-1} y^{-1} higher compared to the average C inputs in conventional systems. The mineral N rate did not influence the C-input but it had an effect on the properties and mineralisation of soil organic matter. The stable C fraction of SOC proportion increased in the system in which the highest rate of mineral N (20–150 kg N ha^{-1}y^{-1} depending on crop) was used and its proportion in the soil was comparable with the results obtained from organic farming systems. Thus, the intensive management with high N rates may benefit to a formation of more stabile SOC if the crop rotation used is properly elaborated.

1. Introduction

Soil organic carbon (SOC) plays an important role in nutrient cycling and improving soil physical, chemical and biological properties (Manna et al., 2007). In addition to promoting the ability of soil to produce food it reduces the concentration of CO_2 in the atmosphere by increasing SOC stock (Lal, 2011) and thereby stabilizes and enhances natural ecosystems (Gregorich et al., 1994). The sustainability of agricultural systems with reduced emissions of greenhouse gases has become an important issue all over the world. Depending upon the agricultural management practices applied, the soil can serve both as a source or sink for atmospheric CO_2 (Lal, 2004; Wilson and Al-Kaisi, 2008). The stock of SOC reflects the net balance between ongoing accumulation and

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Abbreviations: C, carbon; BD, bulk density; BD_{calc}, calculated bulk density; BD_{meas}, measured bulk density; CC, catch crop; DM, dry matter; DOC, dissolved organic carbon; DOCp, the proportion of soil dissolved organic carbon of the total organic carbon; HI, harvest index; N0, control system (with no additional fertilizers used); N1N2N3, systems with different N rates used depending on crop; NPP, net primary productivity; N_{tot}, total nitrogen; O + CC, farming system with catch crops; O + CC + M, farming systems with catch crops and composted manure; SOC, soil organic carbon; SOM, soil organic matter; C_P, carbon of the agricultural product removed from the field; C_S, carbon in shoot; C_R, carbon in root; C_E, carbon in rhizodeposition; S/R, shoot/root ratio; Y_P, DM yield of the agricultural products.

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decomposition processes, which are influenced by both crop productivity and field management (Carter et al., 1997). In any system where C input to the soil exceeds the C output from the soil, a positive imbalance occurs, which subsequently results in sequestration of C in the soil (Jastrow et al., 2007). Estimation of soil C sequestration requires quantification of the rates of C inputs and releases under changed soil management.

In general, agricultural utilization of soils has been found to decrease their SOC content (Dalal and Mayer, 1986; Saviozzi et al., 1994; Blair, 2000) through disruption of the equilibrium between the competing processes of humus formation and mineralization. Management practices like proper cropping systems and balanced fertilization are believed to offer the greatest potential for increasing SOC stock in agricultural soil (Paustian et al., 1997; West and Post, 2002; Lal, 2004). To characterize, predict and manage soil C dynamics, we need precise and accurate estimates of C inputs to the soil (Bolinder et al., 2007). Predicting the changes in soil C stocks depends on net primary productivity (NPP) and the proportion of the NPP returned to the soil (Paustian et al., 1997; Bolinder et al., 2006; Koga and Tsuji, 2009). Thus crop residues, plant roots and also organic amendments represent a significant source of inputs for soil C sequestration (Koga and Tsuji, 2009). Changes in inputs, such as fertilizers and residues (Janzen 1987; Campbell et al., 1991), which regulate soil microbial activity and mineralization rates, will ultimately be reflected in the SOC content (Gregorich et al., 1994). Generally, it is found that addition of organic fertilizers with or without mineral fertilizer increases the SOC content (Blair, 2000; Blair et al., 2006). Inorganic fertilizers influence SOC concentration indirectly by increasing crop yields and thereby increasing the return of crop residues to the soil (Liang et al., 2012). It is assumed that crop productivity is positively related to C amount released into the soil (Wilson and Al-Kaisi, 2008). The effects of the application of mineral fertilizers on SOC content have been contradictory: some have found SOC content increase (Campbell and Zentner, 1993; Gong et al., 2009), but others no influence on SOC content (Halvorson et al., 2002).

Previous and present soil and crop management practices determine existing SOC levels to a large extent and will also influence future SOC stock (Kätterer et al., 2008). Changes in SOC due to management practices are difficult to quantify as they occur slowly (Paustian et al., 1997) due to the high background levels of SOC and natural soil variability (Haynes, 2005). Thus, SOC is not sensitive to short-term soil quality changes with different soil or crop management practices. The use of soil organic matter (SOM) chemical fractions to evaluate changes in soil C dynamics due to the agricultural use is more effective than the determination of the total SOM (Guimaraes et al., 2013). According to Ruhlmann (1999) SOM is divided into two pools with different resistance to decomposition: (i) a small C pool with rapid (few decades) turnover time (dynamic, active, labile pool), which includes easily decomposable organic matter (e.g., microbial biomass) and (ii) a large C pool with slow (centuries to millennia) turnover time (stable, passive pool). The turnover of labile C pools influences crop productivity by regulating nutrient availability and cycling in the soil (Janzen, 2004). It is a primary source of mineralisable N, S, and P (Haynes, 2000). The content of dissolved organic C (DOC) is much more sensitive to change in soil management practices (Saviozzi et al., 2001; Xu et al., 2011), due to its rapid response to changes in soil C supply. Its use as an early indicator of the impact of land use on SOC quality has been suggested (Gregorich et al., 1994). The DOC fraction is a suitable soil quality indicator for describing the balance between the amount of labile C input into the soil and its durability and decomposition in the soil (Gregorich and Janzen, 1995). The content of DOC in the soils depend on both cropping intensity, which influences the quantity and quality of crop residues, and on tillage, which impacts residue placement in the soils. In the long term, vegetation type and the quantity of organic residues have been shown to be the primary factors influencing the amount and composition of DOC (Chantigny, 2003). Lundquist et al. (1999) found twice as much soil DOC under crops that produce more residues. He also showed that the labile C fraction is greater with organic farming than with conventional farming.

Although the major goal of any agricultural management strategy is to enhance crop yield, environmental sustainability must be major issue for the long-term stability of agroecosystems (Singh et al., 2009). Thus, improved agronomic practices that could lead to reduced carbon losses or even increased SOC storage are highly desirable (Gattinger et al., 2012).

As crop type and agronomic practices such as tillage, fertilization and application of organic amendments influence plant biomass production (Kundu et al., 2007), it is essential to understand how crop residue biomass production is influenced by different field management practices, which vary with soil type, climate, and crop rotation and how this impacts soil C sequestration (Koga and Tsuji, 2009). Therefore, it is important to assess C sequestration potential for specific climate/soil/crop systems in order to draw site-specific conclusions.

The aim of this research was to study the effect of different farming systems (conventional farming with mineral fertilizers vs. organic farming with organic fertilizers (catch crop (CC) and composted manure)) under the same crop rotation on the SOC stock. The second aim of the study was to quantify crop-specific C inputs and to identify the relationship between C sequestration and C input into the soil. We hypothesised that it is not possible to predict SOC dynamics based on the amount of C input. Different management techniques impact the properties of C input and the conditions of its mineralisation. Therefore, higher C input into the soil may not necessarily lead to higher SOC accumulation and cause higher SOC stability.

2. Material and methods

2.1. Field experiment

The field experiment was situated at the experimental station of the Estonian University of Life Sciences in Eerika, Tartu, Estonia (58° 22′N, 26° 40′E). In 2008 a crop rotation experiment with two organic and four conventional farming systems was established. The experiment was set up in four replications with each plot ($60\,m^{-2}$) in a systematic block design. Randomisation was fixed in every year in all farming systems and replications. Each plot was 6 m wide and 10 m long. Organic and conventional plots were separated with a 18 m long section of mixed grasses to avoid contamination with synthetic pesticides, mineral fertilizers and CC. Between organic systems there was also a 10 m long protective area for preventing the spread of cattle manure to plots where manure was not used. Conventional systems and all four replications were next to each other without separation. Between the conventional systems there was a transition area (1 m long and 6 m wide), where no samples were taken. The soil of the experimental field is *Stagnic Luvisol* according to the World Reference Base classification (FAO, 2006). The texture of the soil is sandy loam (56.5% sand, 34% silt and 9.5% clay) for the epipedon with a humus layer of 20–30 cm (Reintam and Köster, 2006). At the beginning of the experiment the soil humus layer characteristics were as follows: pH_{KCl} 5.9, 13.8 mg $C_{org}\,g^{-1}$, 1.4 mg $N_{tot}\,g^{-1}$, plant available P, K, Ca and Mg contents were 112.6 mg P kg^{-1}, 168.1 mg K kg^{-1}, 1185 mg Ca kg^{-1}, 188.7 Mg mg kg^{-1}, respectively. Plant available nutrients were determined by the ammonium lactate (AL) method (Egnér et al., 1960). A more detailed description of the experiment is presented in Tein et al. (2014).

2.2. Crop rotation

Over the 5-year period of 2008–2012, the sequence of crops in the rotation were: winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.), barley (*Hordeum vulgare* L.) undersown with red clover (*Trifolium pratense* L.) and red clover. On different plots the crop rotation began with different crops (Table 1).

2.3. Farming systems

In each crop sequence, the main plot factors were farming systems: conventional vs. organic. Conventional farming systems differed in terms of application rates of mineral nitrogen (N) fertilizer. The systems were: (N0) control (with no additional fertilizers used) and N1, N2, N3 in which mineral fertilizers were applied with different N rates used depending on crop (Table 2). NPK fertilizers were added during sowing/planting at the rate of 20:25:95 kg ha^{-1}. One and/or two subsequent mineral N supplements were added during growth. Winter wheat and potato received mineral fertilizers at the same rates in all conventional systems (50 (N1), 100 (N2) and 150 (N3) kg N ha^{-1} y^{-1}). Barley undersown with red clover received mineral N fertilizers as 40 (N1), 80 (N2) or 120 (N3) kg N ha^{-1} y^{-1}. Red clover alone did not receive any mineral fertilizers. For pea as a leguminous crop, the N rate was the same in systems N1-N3 (20 kg N ha^{-1} y^{-1}).

There were two organic farming systems: first (i) with catch crops (O + CC) and second (ii) with catch crops and solid cattle manure (O + CC + M). The catch crops used were winter oilseed rape (*Brassica napus* ssp. *oleifera* var. *biennis*) after pea, winter rye (*Secale cereale* L.) after potato and ryegrass (*Lolium perenne* L.) after winter wheat as a winter cover crop. In the O + CC + M system, fully composted cattle manure with the C/N ratio of 14.2 was added to the soil in the autumn (2009) or in the spring (2010–2012) at a rate of 40 t ha^{-1} in the crop rotation before potato. Manure was added only once for the rotation during the five year rotation period. On average, the composted cattle manure contained 138 g C kg^{-1}, 9.7 g N kg^{-1}, 4.6 g P kg^{-1}, 8.6 g K kg^{-1}.

Conventional systems were treated with several synthetic pesticides during the vegetation period depending on crop. In the organic systems, weeds were removed by hand, and no chemical plant protection was used.

2.4. The management of farming systems

The main cereal and legume crops were sown using the Kongskilde Combiseed N30 combi driller. Barley undersown with red clover and pea were sown at the beginning of May and winter wheat in the first half of September. Potato was planted at the beginning of May using Juko Ekengards 4100 potato planter. The mineral fertilizers were applied using Fiona Birdie fertilizer spreader that is designed for field experiments. Fully composted cattle manure was weighted and then spread to the potato plots manually. All pesticides used in conventional system were applied using Amazone UF1501 sprayer. Cereal crops and pea were harvested at the beginning or in the middle of August using Sampo SR2010 harvester. The potato was harvested in the end of August or at the beginning of September depending on the growing season. The tubers were hand-picked after the rows were opened using German origin two-row elevator-picker machine. The red clover was first cut in the second half of June and the second cut was made in the second half of August using Müthing MU-H7S 140 mower. After the first cut clover was left on the surface of the soil to decompose, but after the second cut clover was ploughed into soil. The ploughing depth was between 27 and 29 cm. After the potato harvest winter wheat and pea, as the following CC, were sown in organic systems. The main crop residues in conventional systems were ploughed into the soil in the end of October. The plougher used was Kverneland ES80. CC and manure in organic systems were ploughed into the soil directly before sowing/planting the main crops. The only plant material removed from the field was grains and tubers. Other crop residues were left on a field.

2.5. Estimating annual C inputs from the main crops

For each crop, crop-specific NNP (kg C ha^{-1} y^{-1}) and C inputs from plant residues into the soil (kg C ha^{-1} y^{-1}) were calculated. C inputs for each crop were calculated as the amount of C measured from dry matter (DM) yield of above-ground agricultural products (Y_P) removed from the fields (C_P) together with straw (C_S), roots (C_R) and the extra C associated with rhizodeposits (C_E). A conservative estimate of C_E in rhizodeposition (i.e., root exudates, root hairs, sloughed root material) for annual crops was 65% of measured root biomass C_R (Bolinder et al., 2007; Gan et al., 2009). Using the Y_P (tuber, grain) and the relative plant C allocation coefficients based on Bolinder et al. (2007) (Table 3) we calculated NPP and C allocation of barley, winter wheat and clover within different parts of the plant. The relative plant C allocation coefficients within different plant parts are expressed as the proportions of NPP:

$$R_P = C_P/\mathrm{NPP} \tag{1}$$

$$R_S = C_S/\mathrm{NPP} \tag{2}$$

$$R_R = C_R/\mathrm{NPP} \tag{3}$$

$$R_E = C_E/\mathrm{NPP} \tag{4}$$

By definition $R_P + R_S + R_R + R_E = 1$. The C of agricultural product (C_P) removed from the field was calculated using the following equation:

$$C_P = Y_p \times C \tag{5}$$

Table 1
The crop sequences in the first crop rotation during 2008–2012.

Year	Crop sequences				
	A	B	C	D	E
2008	Pea	Potato	Barley[a]	Red clover	Winter wheat
2009	Potato	Barley	Red clover	Winter wheat	Pea
2010	Barley	Red clover	Winter wheat	Pea	Potato
2011	Red clover	Winter wheat	Pea	Potato	Barley
2012	Winter wheat	Pea	Potato	Barley	Red clover

[a] Barley undersown with red clover.

Table 2
Annual application of inorganic N, P and K fertilizers (kg ha^{-1})

Farming systems	Potato and winter wheat			Barley undersown with red clover			Pea		
	N	P	K	N	P	K	N	P	K
N0	0	0	0	0	0	0	0	0	0
N1	20[a] + 30[b]	25	95	20 + 20	25	95	20	25	95
N2	20 + 60 + 20[c]	25	95	20 + 60	25	95	20	25	95
N3	20 + 90 + 40	25	95	20 + 90 + 10	25	95	20	25	95

[a] NPK fertilizer added during sowing/planting the crops.
[b] One subsequent supplement of inorganic N fertilizer.
[c] Two subsequent supplements of inorganic N fertilizers.

Table 3
Relative annual plant C allocation coefficients for barley, wheat, red clover and pea used to estimate NPP and C input into the soil.

Crop	Relative plant C allocation coefficients			
	R_P	R_S	R_R	R_E
Barley	0.451	0.400	0.090	0.059
Wheat	0.322	0.482	0.118	0.078
Red clover	0.571	0.000	0.260	0.169
Pea	0.233	0.577	0.115	0.075

where Y_p is the dry matter yield of the above-ground product (kg DM ha^{-1} y^{-1}) and C is the carbon concentration in all plant parts (in this study we assumed that the C concentration was 0.45 g g^{-1}).

For pea, relative plant C allocation coefficients were calculated using equations based on Bolinder et al. (2007):

$$C_s = \frac{Y_p \times (1 - \mathrm{HI})}{\mathrm{HI} \times C} \qquad (6)$$

$$C_s = \frac{Y_p}{(S/R \times \mathrm{HI}) \times C} \qquad (7)$$

where Y_P is the dry matter yield of above-ground product (kg DM ha^{-1} y^{-1}), HI is the harvest index, S/R is the shoot/root ratio and C is the carbon concentration in all plant parts. Harvest indices of 0.36 for pea (Nisar et al., 2011) and 3.24 for shoot/root ratio (Kumar and Goh, 2000) were used. The relative plant C allocation coefficients for pea calculated in this study were similar to the coefficients found by Gan et al. (2009). For potato, constants based on Bolinder et al. (2012) and Carter et al. (2003) were used: 890 kg above-ground and 1060 kg below-ground (roots) biomass DM ha^{-1} y^{-1}.

We considered that, with the crops used in this experiment, C was taken away from the field only as the C of the main yield. In 2008–2012, dry-weight basis crop yields of potato, winter wheat, barley and pea were recorded. The plots with red clover were cut once (in mid-June) during the growing period. The red clover biomass was ploughed in at the end of August after determining its biomass. Biomass from the center of each plot was determined using a 0.5 × 0.5 m frame. Subsamples were taken for the determination of dry matter and DM yield calculation. For the red clover C input calculations, we assumed that all of its biomass was added to the soil (the sum of the biomass measured in June and August).

2.6. Estimating annual C inputs from catch crops, weed and solid manure

In the organic farming systems the following catch crops were grown: winter oilseed rape (after pea), winter rye (after potato) and ryegrass (after winter wheat). They were sown immediately after harvest of the main crops and were ploughed into the soil in spring before the seeding of the main crops. Between 2008–2012, catch crops biomasses were not determined and the C input from the catch crops was calculated based on data from an experiment conducted on the same experimental station. In that experiment, below-ground (roots) biomass was determined in addition to above-ground biomass (Talgre et al., 2011) (Table 4). Based on Talgre et al. (2011) results the C input from catch crops (including C_R) was 562 kg C ha^{-1} annually.

In the organic systems, the weed species biomass was studied before the catch crops were ploughed in (end of April), before the first harrowing of the main following crops (end of April (winter wheat) end of May (potato)) and 3 weeks before crop harvest. In red clover, weed studies were carried out before the first cutting (within the first 10 days of June) and before aftermath incorporation (at the beginning of August). All assessments in 4 replicates per plot were done using a 0.5 × 0.5 m^2 frame. Only above-ground biomass was collected and weighed for total biomass. The total C input (including C_R) was calculated assuming that the shoot/root ratio of weeds was 0.81 based on Gavazzi (1998). In the conventional farming systems the biomasses of weeds were not determined because the systems were intensively treated with herbicides.

Carbon input from manure was calculated based on information that during the crop rotation 40 Mg ha^{-1}of manure was applied with the average DM content of 44.8%.

2.7. Soil sampling and laboratory analysis

Soil samples were collected before trial establishment in April 2008 and in 2013 before any field operations. For one average sample eight subsamples were taken from one plot from the depth of 0–25 cm. Soil samples were taken from each replication from all farming systems. Every sample was air dried and passed through a 2 mm sieve.

The bulk density was measured in spring 2013. For the bulk density the steel cores (54 mm internal diameter and 40 mm height, with wall thickness approximately 1.5 mm) were used at 5 to 10 cm depth, taking four replications of undisturbed soil per plot. Later in the laboratory they were weighted (at field-moist condition) and capillary wetted for 24 h on a plate until saturation. Dry bulk density was determined by subjecting the cylinders to constant drainage over a sand bed to a water tension of 6 kPa for 12 days and later drying in the oven at 105 °C for 24 h.

The SOC and nitrogen (N_{tot}) concentrations in soil were determined by the dry combustion method in a varioMax CNS elemental analyzer (ELEMENTAR, Germany). For dissolved organic

Table 4
Average above and below-ground biomass of catch crops (kg DM ha^{-1}) (Talgre et al., 2011)

Catch crop	Year	Aboveground biomass	Roots
Winter oilseed rape	Average 2008 and 2010	872	557
Rye	2010	518	325
Italian ryegrass	Average 2008 and 2010	464	267

carbon (DOC) 10 g of air dried soil was shaken with 30 mL of distilled water for 1 h, followed by centrifugation and filtration through a 0.45 μm filter. Extracts were analyzed for DOC using a varioMAX CNS analyzer (ELEMENTAR, Germany).

2.8. Calculations

The SOC stock (Mg ha^{-1}) was calculated (for a depth of 0–25 cm) as follows:

$$\text{SOC stock} = \text{BD} \times \text{SOC} \times \text{D} \tag{8}$$

where BD is soil bulk density (g cm^{-3}), SOC is organic carbon content (mg g^{-1}); and D is soil sampling depth (m). For this study, BD in 2008 was not measured and was calculated (BD$_{calc}$) according to Post and Kwon (2000) using the equation:

$$\text{BD}_{calc} = \frac{100}{\{(\text{SOM}/10/0.244) + [(100 - (\text{SOM}/10))/1.64]\}} \tag{9}$$

where SOM is the soil organic matter content (mg g^{-1}), 0.244 is bulk density of SOM, 1.64 is the bulk density of soil mineral matter. We assumed that SOM contains 58% SOC (Mann, 1986). In 2013 for the calculation of SOC stock the BD$_{calc}$ was used. Also the SOC stock calculated with measured BD (BD$_{meas}$) in 2013 were presented to estimate the difference between the use of BD$_{calc}$ and BD$_{meas}$.

The percentage of DOC (DOCp) (%) in SOC was calculated according to the equation:

$$\text{DOCp} = \frac{100 \times \text{DOC}}{\text{SOC}} \tag{10}$$

where DOC is the soil dissolved organic carbon (mg g^{-1}), SOC is the soil total organic carbon (mg g^{-1}).

2.9. Climate

The weather during the period of the experiment was monitored with a Metos Compact (Pessl Instruments) electronic weather station, which automatically calculates the average daily temperatures and the sum of precipitation. To obtain the monthly average of daily average temperatures at the weather station, the daily temperatures were averaged over each month. Weather conditions during the study period (2008–2012) differed substantially (Table 5), especially the total precipitation amounts during the growing period. In 2009, the precipitation sum during the growth period was quite similar to the long-term average (1969–2011). By contrast, the precipitation in 2008, 2010 and 2011 differed considerably from the long-term average as follows: 2011 was arid with 134.0 mm y^{-1} less rainfall than the long-term average; and 2008 as well as 2010 were unusually wet with 728.6 and 697.1 mm y^{-1} of rainfall, respectively. Average temperatures showed the expected progression from low in November to April, higher in May to August and lower again in September. For the month of May the temperatures were lowest in 2008 and highest in 2010. Over the entire season, 2008 was generally the warmest, and 2012 was the coolest and had lower temperatures than the long-term average in June and August.

2.10. Statistical analysis

The Statistica version 11.0 (Statsoft Inc.) software package was used for all statistical analyses. Factorial analysis of variance (ANOVA) and one-way ANOVA were applied to test the effect of farming systems on NPP and C inputs into the soil. Fisher's least significant difference test for homogenous groups was used for testing significant differences between farming systems and between experimental years. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Annual C inputs from main crop

Main NPP, C input and the amount of C removed with the yield depended, in addition to crop and farming system, also on crop rotation sequences and on the interaction of various factors (Table 6). The relationship between crop rotation sequences and farming systems did not affect the C input from crop, but influenced the amount of C removed with the yield.

Crop rotation average crop-specific NPP and C inputs are presented in Table 7. The crop-specific NPP was highest for red clover (6627 kg C ha^{-1} y^{-1}) and lowest for barley (2902 kg C ha^{-1} y^{-1}). Carbon inputs from different crops varied between 1188–6627 kg C ha^{-1} y^{-1}. Red clover has the highest C input in

Table 6
ANOVA results for NPP, C inputs and C removed.

	NPP	C input	C removed
Crop (C)	***	***	***
Crop rotation (CR)	***	***	***
Farming system (FS)	***	***	***
C x CR	***	***	***
C x FS	*	***	***
CR x FS	***	NS	***
C x CR x FS	***	NS	***

NS, not significant.
* $P < 0.05$.
*** $P < 0.001$.

Table 5
Weather conditions during the first rotation period (2008–2012) compared to long-term average of 1969–2013.

Month	Temperatures (°C)						Precipitation (mm)					
	2008	2009	2010	2011	2012	1969–2013	2008	2009	2010	2011	2012	1969–2013
January	−1.3	−3.4	−5.1	−4.7	−6.1	−5.2	21.8	10.2	40.4	19.0	30.0	29.6
February	0.6	−4.9	−7.9	−11.2	−11.5	−5.8	34.4	7.2	4.8	9.0	18.6	22.5
March	0.4	−1.5	−2.1	−1.9	−0.3	−1.7	8.4	22.4	30.2	4.4	39.4	23.2
April	7.2	5.3	6.1	6.4	5.0	4.7	26.8	14.2	26.4	11.2	42.0	26.5
May	10.7	11.5	12.6	11.0	11.8	11.4	27.4	13.4	61.4	58.4	81.6	57.6
June	14.5	13.8	14.6	17.2	13.6	15.4	110.6	137.4	72.6	35.2	100.6	75.9
July	16.1	16.9	22.2	19.9	18.0	17.5	98.6	54.6	36.0	48.2	75.0	70.4
August	15.7	15.4	18.2	15.8	15.1	16.1	214.4	89.2	106.8	54.6	87.4	89.2
September	9.8	12.8	11.1	12.3	12.2	10.8	45.6	49.0	93.0	80.0	59.8	57.1
October	8.2	4.1	4.2	6.8	5.7	5.6	67.6	116.0	49.4	47.8	45.2	57.2
November	2.3	2.3	0.3	2.9	2.6	0.4	49.4	35.8	77.6	34.4	50.2	45.5
December	−1.1	−8.6	−8.2	1.0	−6.8	−3.4	23.6	57.0	98.5	52.6	9.0	34.1
January–December	6.9	5.3	5.5	6.3	4.9	5.5	728.6	606.4	697.1	454.8	638.8	588.8

Table 7
Crop rotation average annual crop-specific NPP and C input (kg ha^{-1} y^{-1})

Crop	NPP	C input	C removed
Barley	2902^a	1593^b	1309^b
Red clover	6627^d	6627^e	–
Pea	4374^b	3331^c	1043^a
Potato	4628^b	1187^a	3440^d
Wheat	5323^c	3609^d	1714^c

Average followed by a different small letter within each column indicate significant influence ($P < 0.05$) of the crop.
Note: –, total red clover biomass was left on the field.

the crop rotation because it was left in the field to decompose after cutting. The lowest C inputs were for potato and barley. The highest amount of C removed with the main crop was with potato (3440 kg C ha^{-1} y^{-1}); with other crops the amount of C removed was between 1043–1714 kg C ha^{-1} y^{-1} (Table 7).

When growing barley as the first crop in the crop rotation the average NPP was the lowest after the crop rotation (4341C ha^{-1} y^{-1}, Table 8). Also, the crop rotation average C input of barley (1593 kg C ha^{-1} y^{-1}, Table 7) was one of the lowest. Crop rotation average NPP and C input were significantly ($P < 0.05$) higher when the crop rotation started with pea or red clover. NPP and C input of the crop rotation starting with potato were comparable with the crop rotation starting with red clover. The C input calculated for potato was similar to that for barley (1187 kg C ha^{-1} y^{-1}, Table 7). The cereals C inputs from the first year (2008) of the crop rotation differed from the crop rotation average C input: 979 kg C ha^{-1} y^{-1} for barley and 2293 kg C ha^{-1} y^{-1} for winter wheat (Fig. 1). In the following years the C input of the cereals increased (Fig. 1).

From the compared farming systems the highest ($P < 0.05$) NPPs were in the systems fertilized with mineral N (N1, N2, N3) (5179–5145 kg C ha^{-1} y^{-1}) (Table 9). In the control (N0), which did not receive any fertilizers, but which was treated with pesticides, the NPP was lower (4467 kg C ha^{-1} y^{-1}). In the organic farming systems the NPP varied from 4130 to 4235 kg C ha^{-1} y^{-1}.

The C input varied between farming systems from 2935 to 3697 kg C ha^{-1} y^{-1}. Farming systems had a significant ($P < 0.05$) influence on C input, but the differences between systems were not substantial. In conventional farming systems the C input was higher ($P < 0.05$). Crop rotation average above- and below-ground C input distribution did not depend on farming system. Below-ground C input was 58–62% of total C input depending on farming system.

N fertilization affected significantly ($P < 0.05$) the C input of the barley (the higher the amount of N added the higher the C input). For winter wheat N fertilization increased C input, but it did not depend on the N rate used. The C input of red clover decreased with increasing N rate (Fig. 2). The potato had the lowest (401 kg C ha^{-1} y^{-1}) and red clover the highest (3784 kg C ha^{-1} y^{-1}) above-ground C input. Below-ground C input was lowest for barley (432 kg C ha^{-1} y^{-1}) and highest for red clover (2843 kg C ha^{-1} y^{-1}).

Table 8
Crop rotation specific average annual NPP and C input (kg C ha^{-1} y^{-1}) depending on the crop sequences in the crop rotation.

Crop rotation	NPP	C input
A	5540^c	3672^c
B	4761^b	3356^b
C	4341^a	2858^a
D	4880^b	3299^b
E	4331^a	3162^b

Average followed by a different small letters within each column indicate significant influence ($P < 0.05$) of the crop rotation.
Note: Letters A, B, C, D and E note different crop sequences during the first rotation (see also Table 1).

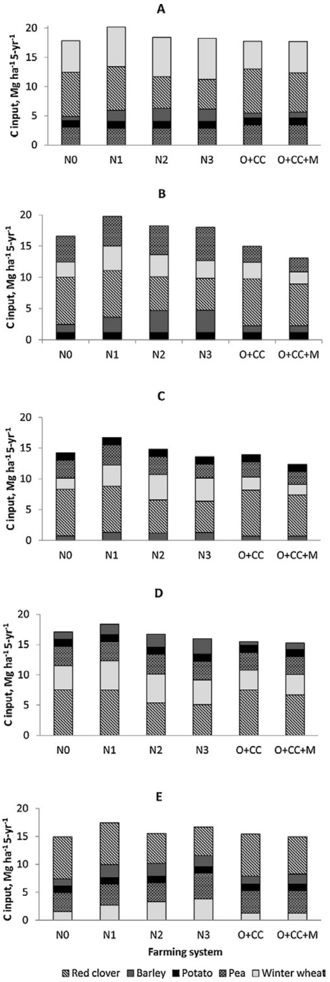

Fig. 1. The five-year total crop-specific C inputs in crop rotations with different crop sequences (A—pea, potato, barley, red clover, winter wheat; B—potato, barley, clover, winter wheat, pea; C—barley, red clover, winter wheat, pea, potato; D—red clover, winter wheat, pea, potato, barley; E—winter wheat, pea, potato, barley, red clover).

Farming systems had greatest impact on the amount of C removed with the yield; it varied between 1129 and 1877 kg C ha^{-1} y^{-1} (Fig. 3). The amount of removed C depended on N

Table 9
Average farming systems specific NPP, below- and above-ground C input ($kg\,C\,ha^{-1}\,y^{-1}$).

Farming system	NPP	Below-ground C input	Above-ground C input
N0	4467^{b}	1894^{bc}	1332^{b}
N1	5415^{c}	2227^{e}	1470^{c}
N2	5198^{c}	2045^{d}	1305^{ab}
N3	5179^{c}	2012^{cd}	1289^{ab}
O + CC	4235^{ab}	1817^{ab}	1288^{ab}
O + CC + M	4130^{a}	1723^{a}	1213^{a}

Averages followed by different small letters within each column indicate significant influences ($P < 0.05$) of the farming systems.

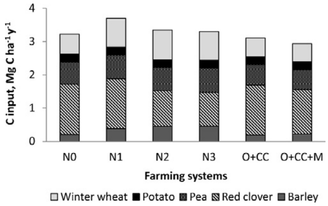

Fig. 2. Crop rotation average crop specific C inputs in different farming systems.

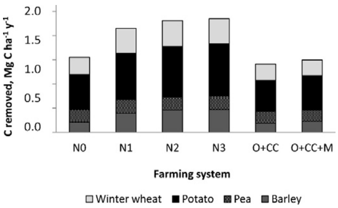

Fig. 3. The average amount of C removed with different crops depending on farming system.

fertilization and N rates used. Most C was removed with potato ($3440\,kg\,C\,ha^{-1}\,y^{-1}$).

3.2. Total annual C input from crops, catch crops, manure and weeds

In this experiment, the total amount of C input to the soil originated from the main crops (potato, wheat, barley, red clover, pea), catch crops, manure (at a rate of $40\,t\,ha^{-1}$ in the crop rotation) and weeds in systems O + CC and O + CC + M. The total annual C inputs from main crop, catch crop, manure and weeds are summarized in Table 10. The highest total C input was in system O + CC + M ($4762\,kg\,C\,ha^{-1}\,y^{-1}$), where the composted dairy cattle manure was applied annually. The calculated C input from catch crops was $562\,kg\,C\,ha^{-1}\,y^{-1}$, which did not increase the total C input substantially (Table 10). The C derived from catch crops accounted for a small part of the total C input (15% for O + CC and 9% for O + CC + M). The rate of direct annual C input from manure, expressed on a C basis, was equivalent to $495\,kg\,C\,ha^{-1}\,y^{-1}$. C input of the weeds was 748 and $769\,kg\,C\,ha^{-1}\,y^{-1}$ for O + CC and O + CC + M, respectively.

Table 10
Average annual C inputs ($kg\,C\,ha^{-1}\,y^{-1}$) from main crops, catch crops, manure and weeds in different farming systems.

C input					
Farming system	Main crop	Catch crop	Weeds	Manure	Total
N0	3226^{ab}	0	–	0	3226^{a}
N1	3697^{b}	0	–	0	3697^{a}
N2	3350^{ab}	0	–	0	3350^{a}
N3	3301^{ab}	0	–	0	3302^{a}
O + CC	3105^{ab}	562	748	0	4415^{b}
O + CC + M	2936^{a}	562	769	495	4762^{c}

Averages followed by a different small letters within each column indicate a significant influence ($P < 0.05$) of the farming systems.
Note: –, not determined.

The lowest C input from the main crop was in the organic farming systems (Table 10). When compared to conventional systems it was due to the lower NPP in organic systems ($4235\,kg\,C\,ha^{-1}\,y^{-1}$ for O + CC and $4130\,kg\,C\,ha^{-1}\,y^{-1}$ for O + CC + M) (Table 9). While mineral N fertilization increased NPP substantially (average $5264\,kg\,C\,ha^{-1}\,y^{-1}$).

3.3. Changes in SOC and N_{tot} stock

At the beginning of the experiment (establishment stage) soil SOC content varied between $12.3–14.4\,mg\,g^{-1}$ (Table 11). Content of SOC and its stock increased after the crop rotation in control and organic systems. In the system O + CC the average annual SOC stock increase was $0.77\,Mg\,C\,ha^{-1}\,y^{-1}$, application of manure changed it by $2.57\,Mg\,C\,ha^{-1}\,y^{-1}$. The SOC stock annual increase is larger if the BD_{meas} to calculate SOC stock is used, 1.26 and $3.06\,Mg\,C\,ha^{-1}\,y^{-}1$, respectively. In the systems with mineral N fertilizer the changes in SOC stock varied from 390 to $150\,kg\,C\,ha^{-1}\,y^{-1}$. The initial soil N_{tot} at the beginning of the experiment varied between $1.33–1.46\,mg\,N\,g^{-1}$. Soil N_{tot} content and the stock declined during the first rotation (Table 12). Only in the system O + CC + M there was no change in the soil N_{tot} content and stock. In other systems the stock of N_{tot} decreased between $0.04–013\,Mg\,N\,ha^{-1}\,y^{-1}$.

3.4. Soil DOC content and C/N ratio

Both DOC and DOCp contents in the soils of the systems O + CC and O + CC + M decreased after the crop rotation (Table 13). The contents of DOC and DOCp decreased even in the system with high N application rate (N3), while in the system with low N application rates the contents of DOC and DOCp increased. There was a tendency that if soil C/N ratio expanded after the crop rotation compared to year 2008, then the soil DOC and DOCp content decreased. If the C/N ratio after the crop rotation did not change, then DOC and DOCp contents increased.

In N1 and N2 DOCp increased, but it decreased in N3 and in organic systems. The DOCp did not change in the control system. The highest DOCp were in systems N0, N1 and N2.

4. Discussion

Crop rotation average main crop specific C input amounts did not differ between conventional systems. Although the SOC stock increased in the control, it did not change or even decreased in the mineral fertilized systems. It is a general trend for the SOC concentration in most soils to increase with increasing C inputs (Duiker and Lal, 1999; Nyborg et al., 1999; Campbell et al., 2005). But also some results have indicated that fertilization does not increase SOC stock (Wilson and Al-Kaisi, 2008; Kauer et al., 2013). Based on Fontaine et al. (2004) in the fertilized systems the returning plant residues did not have any impact on the soil SOC

Table 11
The changes in SOC content and SOC stock (0–25 cm soil layer) during the first crop rotation.

Farming system	SOC ($mg\,C\,g^{-1}$)		SOC stock ($Mg\,C\,ha^{-1}$)			SOC stock change ($Mg\,C\,ha^{-1}\,y^{-1}$)	
	2008	2013	2008 BD_{calc}	2013 BD_{calc}	2013 BD_{meas}	BD_{calc}	BD_{meas}
N0	12.8^{a}	13.4^{a}	46.7^{a}	48.4^{a*}	48.5^{a*}	0.34	0.36
N1	14.2^{b}	13.8^{ab}	51.1^{b}	50.5^{ab}	–	−0.12	–
N2	14.4^{b}	13.6^{ab}	51.6^{b}	49.6^{ab}	–	−0.39	–
N3	14.3^{b}	14.6^{b}	51.5^{b}	52.2^{b}	50.9^{a}	0.15	−0.12
O + CC	14.7^{b}	16.1^{c*}	52.7^{b}	56.5^{c*}	59.0^{b*}	0.77	1.26
O + CC + M	12.3^{a}	16.7^{c*}	45.3^{a}	58.2^{c*}	60.7^{b*}	2.57	3.06

Averages followed by different small letters within each column indicate a significant influence ($P < 0.05$) of the farming systems.
Note: BD_{calc}, SOC stock is calculated using calculated bulk density; BD_{meas}, SOC stock is calculated using measured bulk density; *, indicates a significant influence of the year on the change of SOC stock; –, not determined.

Table 12
The changes in N_{tot} content and N_{tot} stock (0–25 cm soil layer) during the first crop rotation.

Farming system	N_{tot} ($mg\,N\,g^{-1}$)		N_{tot} stock ($Mg\,N\,ha^{-1}$)			N_{tot} stock change ($Mg\,N\,ha^{-1}\,y^{-1}$)	
	2008	2013	2008 BD_{calc}	2013 BD_{calc}	2013 BD_{meas}	BD_{calc}	BD_{meas}
N0	1.33^{a}	1.14^{a*}	4.78^{a}	4.09^{a*}	4.12^{a*}	−0.14	−0.13
N1	1.34^{a}	1.30^{b*}	4.79^{a}	4.64^{bc*}	–	−0.03	–
N2	1.43^{bc}	1.28^{b*}	5.08^{b}	4.59^{bc*}	–	−0.10	–
N3	1.34^{a}	1.27^{b*}	4.78^{a}	4.52^{b*}	4.43^{b*}	−0.05	−0.07
O + CC	1.46^{c}	1.36^{bc*}	5.19^{b}	4.75^{cd*}	4.98^{c*}	−0.09	−0.04
O + CC + M	1.41^{b}	1.41^{c}	5.13^{b}	4.92^{dc}	5.22^{d}	−0.04	0.02

Averages followed by different small letters within each column indicate a significant influence ($P < 0.05$) of the farming systems.
Note: BD_{calc}, Ntot stock is calculated using calculated bulk density; BD_{meas}, Ntot stock is calculated using measured bulk density; *, indicates a significant influence of the year on the change of SOC stock; –, not determined.

Table 13
Soil dissolved organic carbon (DOC), the proportion of soil dissolved organic carbon of the total organic carbon (DOCp) and DOC changes during the first crop rotation.

Farming system	C/N		DOC ($mg\,g^{-1}$)		DOCp (%)	
	2008	2013	2008	2013	2008	2013
N0	9.7^{ab}	11.7^{a*}	0.33^{a}	0.36^{ab}	2.6^{ab}	2.7^{b}
N1	10.6^{b}	10.7^{a}	0.35^{a}	0.54^{c*}	2.5^{a}	4.0^{c*}
N2	10.1^{b}	10.6^{a}	0.38^{b}	0.47^{d*}	2.7^{ab}	3.5^{d*}
N3	10.7^{b}	11.5^{a}	0.37^{b}	0.32^{a*}	2.6^{ab}	2.2^{a*}
O + CC	10.0^{b}	11.9^{a*}	0.40^{c}	0.34^{ab*}	2.7^{b}	2.1^{a*}
O + CC + M	8.7^{a}	11.8^{a*}	0.40^{c}	0.38^{b*}	3.3^{c}	2.3^{a*}

Averages followed by different small letters within each column indicate a significant influence ($P < 0.05$) of the farming systems.
Note: *, indicates significant influence of the year on the soil C/N ratio, DOC and DOCp.

content. The reason why the increased C input from fertilization did not influence the SOC content may have been that the higher N availability may have a positive impact on the SOC mineralization in conventional farming systems. Also N effect on mineralization depends on the chemical composition of organic matter (Sinsabaugh et al., 2002).

The total annual C input was the highest under organic farming systems, where additional C input, besides of that originating from the main crops, came from catch crops, manure and weeds. Farming systems affected also the amount of C in the 0–25 cm soil layer that contributed to the SOM from total annual C input. From total annual C input 10.5, 18 and 39% from control, O + CC and O + CC + M contributed into the SOM, respectively. The properties of C input impacts its accumulation into the soil (Singh et al., 2009), probably because exogenous organic C inputs strongly influence the seasonal dynamics of soil microbial biomass (Singh et al., 2007). For example, roots generally appear to be of lower quality than shoots and thereby represent a more recalcitrant carbon pool (Tjoelker et al., 2005). Therefore, narrower shoot/root ratios may indicate increased soil C sequestration potential (De Deyn et al., 2008). Higher lignin content of the roots, which may be twice as high when compared to its content in the above-ground biomass, makes them more resistant to decay (Rasse et al., 2005). Based on Rasse et al. (2005), the contribution of C originating from roots to the soil organic matter is 1.5–3.7 (average 2.4) times higher when compared to C input from above-ground biomass. High quality plant residues (high N, low lignin concentrations) mineralize rapidly, but may not contribute much to the maintenance of SOM (Handayanto et al., 1997). Crop rotation average main crops above-ground and roots biomass C inputs ratios were similar between farming systems (Table 9). In organic systems, where the C input from the main crop was comparable to conventional systems and where one of the inputs came from catch crops and weeds (they constituted 30% of the total C input (Table 10)), the SOC stock increased. The ratio of catch crop below- and above-ground biomass differed from main crops, therefore also the properties of catch crops C inputs differed. In this study the calculated shoot/root ratio of the main crops in different farming systems was 2.4 as on average. Based on Talgre et al. (2011) the shoot/root ratio was on average 1.6–1.7 for the studied CC (winter oilseed rape, rye and Italian ryegrass) at the end of the growing season in late autumn (Table 4). This shows that the percentage of roots was higher for CC than for main crops. Therefore, the impact of catch crops on C input increase was also higher when compared to the main crops and SOC stock increased significantly in O + CC system. Composted manure had the greatest positive effect on SOC stock. The SOC stock increased in O + CC + M system 2.57 $Mg\,ha^{-1}y^{-1}$ despite that only 10% of C came from manure. It has been found that in composted manure the amount of easily degradable compounds is low, therefore the large part of C is in a form that is more difficult to decompose which consititutes to a formation of stabile fraction of

C. Thus due to a large stable carbon input into the soil the SOC stock increased considerably as well.

Growing at the beginning of the crop rotation crops with low C input have a negative impact on the following crops. Barley, potato and winter wheat were the crops grown in the experiment with lower below-ground input. Average NPP and C input of the crop rotations starting with cereals were lower when compared with crop rotations starting with red clover or pea. Potato also had low below-ground biomass, but growing potato at the beginning of the crop rotation did not cause low NPP and C input. In this case, the properties of C input probably had greater impact. For cereals an important input is straw. In our study, straw constituted 71% (winter wheat) and 73% (barley) of total C input. Inclusion of cereals (wheat and barley) in crop rotations often increases soil C and N sequestration (Wright and Hons, 2005), because the straw as cereal residues is a material, rich in carbon with a wide C/N ratio (Franzluebbers et al., 1995). Also it breaks down slowly and, during decomposition, N originating from microorganisms is bound to the SOM (immobilisation). Due to the slow rate of wheat straw C decomposition and other nutrients remained immobilised in undecomposed or partially decomposed portions of the wheat straw (Singh et al., 2007), perhaps making them unavailable immediately after application. The properties of potato C input differed from the properties of the straw. Firstly, it can be presumed that the biomass of potato has a narrower C/N ratio and therefore is more easily degradable. In this case, N immobilisation does not occur, SOM mineralization is not inhibited and NPP and C input are not adversely impacted. The organic and conventional systems differed also in terms that the C inputs from main crops were ploughed into the soil in different times. In conventional systems the straw material and other crop residues were ploughed into the soil in the end of October, but in organic systems they were incorporated into the soil already after the main crops were harvested and before sowing the CC. Therefore it must be considered that some of the C input already mineralized after it was incorporated into the soil because in organic systems the soil tillage operations (including sowing the CC) were made in the end of August and at the beginning of September when there was no frost, thus the climatic conditions did not inhibit the mineralization. The mineralization was also promoted because the decomposition of buried residues is generally faster than of surface-placed plant residues (Seneviratne et al., 1998; Coppens et al., 2006). This is due to a moisture because which is moisture is best stored in soil plant residues (Parr and Papendick, 1978). When the crop residues are incorporated into the soil then it will have a much better contact with the microbes (Douglas et al., 1980; Ambus and Jensen, 1997). Therefore it can be assumed that in organic systems the effect of C inputs from main crops to the SOC stock was lower compared to the conventional systems.decreasing C inputs from main crops affected the SOC stocks in O + CC and O + CC + M systems.

After the first rotation, soil DOC increased in the conventional systems fertilized with lower N rate and decreased in N3 and in the organic farming systems. This result is similar to that of Liang et al. (2012), who found higher DOC in conventional systems. Based on Lundquist et al. (1999) the labile C fraction is higher under organic farming than conventional farming and it was due to the more rapid changes in active/labile SOM concentrations than in total SOM concentrations (Janzen et al., 1992; Biederbeck et al., 1994). Active SOM as a proportion of total SOM should increase when agricultural practices which increase total SOM have been initiated. Conversely, active SOM as a proportion of total SOM should be low in soils, where organic matter content have declined according to the same reasoning. In our study we cannot conclude this, because in the organic farming system in which composted manure was applied, soil SOC content and stock increased, but DOC content and DOCp decreased. These decreases indicate that in that system more stable OM was formed. Thus the increase in the proportion of a more stable fraction may be related to the characteristics of soil ingoing organic matter. If the soil ingoing organic material contains large amounts of easily degradable compounds then the DOC concentration in soil increases (Liang et al., 1998; Gong et al., 2009; Banger et al., 2009). In our study, DOCp decreased in a system in which manure was applied because of a lower labile fraction (Gómez-Brandón et al., 2008). Also the use of mineral N fertilizers influences the decomposition of SOM. When using higher rates of mineral N more stable organic matter decomposition products were formed, because the mineralization and turnover of organic matter occurs faster. In the systems fertilized with lower N rates, DOC and DOCp increased, indicating that the percentage of labile C in the soil also increased. DOC dynamics in soil was also related to soil C:/N ratio. If it increased after the crop rotation, then DOC content decreased. The C/N ratio increased significantly after the crop rotation in organic farming systems. A larger C/N ratio indicates that the conditions for OM mineralisation were worse (Mary et al., 1996) resulting in higher soil SOC stock. In our study the N_{tot} stock decreased almost in all systems except in a system in which composted manure was used. According to the study by Alaru et al. (2014) (which is based on the same experiment as this study is) the total annual N input from different sources (main crop, CC, manure, N_2-fixation, N fertilization) varied between 80 and 150 kg N ha y^{-1}. The N input was the highest in N3 system and the lowest in N1 system. In a system in which manure was used had a total N input 118 kg N ha y^{-1} and about 30% (on average 35 kg N ha y^{-1}) of it came from manure. Despite that the total N input into the soil in the O + CC + M system was not the highest amongst the systems; it still indicates that the composted cattle manure has a major positive effect on SOC and N_{tot} stocks.

5. Conclusions

Different farming systems affected the dynamics of the stock of SOC during the first crop rotation in the sandy loam soil. Annual NPP depended on farming system, but as a crop rotation average different farming systems did not affect soil crop-specific C input. The C stock increased the most (2.57 Mg ha^{-1} y^{-1}) in a system in which the total annual C input to the soil was the highest; this was in a system in which no mineral fertilizers were used, but catch crops were grown and composted cattle manure was used once during the crop rotation at a rate of 40 Mg ha^{-1}. The use of mineral N fertilizers in conventional systems had no effect on crop-specific C inputs and the dynamics of soil SOC stock were affected more by SOM mineralization than by the amount of C input. This research revealed that, compared to the other studied systems, in a system in which the highest rate of mineral N was used the more stable C fraction of SOM proportion increased. Thus the intensive management with high N rates may benefit to a formation of more stabile SOC if the crop rotation used is properly elaborated.

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CURRICULUM VITAE

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LIST OF PUBLICATIONS

1.1. Publications indexed in the ISI Web of Science database:

Sánchez de Cima, D., Reintam, E., Tein, B., Eremeev, V., Luik, A. 2015. Soil Nutrient Evolution during the First Rotation in Organic and Conventional Farming Systems. *Communications in Soil Science and Plant Analysis*, 00, 1–13.

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VIIS VIIMAST KAITSMIST

MARET SAAR

ELECTRICAL CHARGE OF BASIDOSPORES OF HYMENOMYCETES (FUNGI) AND ITS BIOLOGICAL SIGNIFICANCE

EOSLAVASEENTE KANDEOSTE ELEKTRILAENG JA SELLE BIOLOOGILINE TÄHENDUS
Professor **Tiiu Kull**
17. september 2015

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ROD-THROUGH-PLATE FIXATOR IN THE TREATMENT OF LONG BONE FACTURES OF SMALL ANIMALS

KOMBINEERITUD METALLOOSTEOSÜNTEES PIKKADE TORULUUDE MURDUDE RAVIKS VÄIKELOOMADEL
Professor **Vladimir Andrianov**, professor **Marina Aunapuu**
22. oktoober 2015

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THE EFFECT OF DIFFERENT FARMING SYSTEMS ON POTATO TUBER YIELD AND QUALITY

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4. detsember 2015

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RESPONSES OF RESPIRATORY AND PHOTORESPIRATORY DECARBOXYLATIONS TO INTERNAL AND EXTERNAL FACTORS IN C_3 PLANTS

RESPIRATOORSE JA FOTORESPIRATOORSE DEKARBOKSÜLEERIMISE VASTUSED SISEMISTE JA VÄLISTE FAKTORITE TOIMELE C_3 TAIMEDES
Professor **Ülo Niinemets**, vanemteadur **Olav Keerberg**, vanemteadur **Tiit Pärnik**
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