Eesti Maaülikool
Estonian University of Life Sciences

EFFECTS OF CROPPING SYSTEMS ON SOIL FERTILITY AND WINTER WHEAT DOUGH QUALITY

VILJELUSVIISI MÕJU MULLA VILJAKUSELE JA TALINISU TAINA KVALITEEDILE

INDREK KERES

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

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2022

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CONTENTS

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II. **Keres, I**.; Alaru, M.; Talgre, L.; Luik, A.; Eremeev, V.; Sats, A.; Jõudu, I.; Riisalu, A.; Loit, E. 2020. Impact of weather conditions and farming systems on size distribution of starch granules and flour yield of winter wheat. Agriculture, *10*, 22. https://doi.org/10.3390/agriculture10010022

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The contributions from the authors to the papers were following:

	I	**II**	**III**
Idea and design	All	**IK**, MA, EL	**IK**, MA, EL
Field experiment	VE, LT, MA, **IK**	VE, LT, MA, **IK**	MA, **IK**
Data collection	MA, VE	MA, VE, AR, **IK**	RK, MA, **IK**
Data analysis	**IK**, MA, VE	MA, AS, VE, **IK**	MA, RK, **IK**
Preparation of manuscript	All	All	All

VE – Viacheslav Eremeev, MA – Maarika Alaru, AS – Andres Sats, RK – Reine Koppel, LT – Liina Talgre, AR – Anu Riisalu, **IK** – Indrek Keres, All – all authors of the paper

Abbreviations

BBCH	Scale to identify the phenological development stages of plants
BBCH65	Flowering
BU	Brabender unit
DDT	Dough development time
DM	Dry matter
DPA	Days past anthesis
DQN	Dough quality number
DS	Dough softening degree
DV	Derived diameter
GDD	Growing degree days
GI	Gluten index
K	Potassium
Mineral N	N from mineral fertilizers
N	Nitrogen
Organic N	N from organic fertilizers
P	Phosphorus
PC	Protein content
PhM	Physiological maturity
S	Dough stability time
W	Baking strenght
WAC	Water absorption capacity
WGC	Wet gluten content

1. INTRODUCTION

One of the biggest challenges in modern agriculture is to achieve the highest possible yield of crops in an environmentally friendly way (Ricroch et al. 2016). Organic farming (cropping system) is considered to be an environmentally friendly production method and its prevalence in Europe has grown rapidly in recent decades (EC, 2014; Eurostat, 2019). Additionally, the European Union has set a target to have 25% of its arable land organic by 2030 (Publication Office, 2022). The aim of organic cropping system is to produce enough high-nutrition food in a way that is sustainable for the environment. At the same time, the general disadvantage of organic cropping system is the uneven and relatively low yield of crops, mainly due to the unstable availability of nitrogen (N) from organic fertilizer during the early stages of plant growth. In particular, lack of adequate input of mineral fertilizers can lead to progressive decrease of soil fertility in organic farming with associated reduction of crop yield (Alaru et al. 2014; Article **I**). Insufficient availability of nutrients caused a decrease in the level of yield and dough quality (Draghici et al. 2011), as a result of which the popularity of organic cropping system among Estonian producers also decreased. While in the previous five years the annual increase in organic area was more than 10,000 hectares, 2020 marked the first year in which organic area decreased (Vetemaa et al. 2021). The level of soil fertility also depends to a large extent on the type of cropping system used in production, which in turn affects crop yields (Esperschütz et al. 2007; Alaru et al. 2014; Kaš et al. 2016; Article **I**). The cropping system also affects soil pH, which in turn affects the availability of nutrients to plants (Kaš et al. 2016).

From the crops in the crop rotation, wheat (*Triticum aestivum* L.) was selected for research due to its great importance in the global food and feed industry; up to 78% of total wheat production is intended for human consumption (Psaroudaki, 2007). In Estonia, winter wheat cultivation has become more popular in both organic and conventional cropping. The total area of winter wheat in Estonia is 136 000 ha, 37% of total crop production area (Statistikaamet, 2022).

The physical, chemical, and textural properties of wheat grains determine the quality of the final product (e.g., noodles, breads, cookies) obtained from wheat flour. The texture of winter wheat grains is influenced by several factors, such as cropping system, variety, region, and post-winter weather (Krejčířová, 2006). Among other factors, the N supply of the wheat plant (fertilization with organic or mineral N fertilizer) is crucial for the development of the rheological properties of the dough obtained from its flour. As wheat dough consists of several flour components (protein, starch, etc), this makes it difficult to assess their complex effect (Cao et al. 2019). While the rheological properties of dough have so far been mostly evaluated analyzing separately the effects of flour gluten or starch content, this study evaluates the interaction between starch granules and gluten on these properties. As organic production becomes more widespread (Eurostat, 2019; Publication Office, 2022), it is necessary to further clarify the extent to which organic fertilization affects the accumulation of gluten and the formation of starch granules during grain filling. Gluten-starch granules interaction is important for the quality of the dough (Gao et al. 2020).

This dissertation deals with the long-term effects of organic or mineral N fertilizers on soil fertility in a five-field crop rotation (barley with undersown red clover – red clover – winter wheat – field pea – potato) with high nutrient requirements, winter wheat yield and quality of the final product, i.e., wheat dough. Article **I** investigated whether the long-term cultivation of winter catch crops and fertilization with manure in organic farming could prevent the decrease of soil fertility (more precisely soil phosphorus (P) and potassium (K) content and pH). As stated above, one of the biggest challenges for organic farming is to maintain soil fertility and provide sufficient nutrients for plants in their early development stages to obtain higher yield level. The Article **I** also examined the effect of long-term fertilization with mineral or organic fertilizers (over 10 years) on soil pH, which in turn affects the availability of nutrients.

The Article **II** analyzed the impact of cropping system, i.e., fertilization with mineral or organic fertilizers and annual weather conditions (air temperature and precipitation) on the size/diameter distribution of starch granules. The size distribution of starch granules strongly influences the rheological properties of wheat dough (Edwards, 2010).

In Article **III** investigated the influence of mineral and organic fertilizers on the accumulation of protein and gluten in winter wheat grains, also the interaction of starch granules with different size and gluten content on the rheological properties of wheat dough. It is known that starch granules act as filler particles in the gluten-starch matrix (Cao et al. 2019) and Article **III** examined the quality of wheat dough dependence on gluten-starch network strength.

2. REVIEW OF LITERATURE

2.1. Impact of cropping systems on soil fertility and nutrient availability to plants

To ensure high yields, large amounts of nutrients are consumed by the plants from the soil, which, if plant nutrition is mismanaged, can lead to the depletion of soil reserves (Murugappan et al. 2007). To alleviate this problem and reduce the leakage of nutrients into environment, alongside so-called conventional farming organic farming has become more widespread in agriculture (Eurostat, 2019). The main difference between the cropping systems is the way in which the problems related to maintaining or increasing yields are solved. The gold standard in both crop systems is the selection of the right crops for the crop rotation and their sequence which are important in ensuring control over the circulation and retention of nutrients in the soil and the control of weeds, pests and diseases (Stockdale et al. 2001).

In conventional cropping system, the plant is fertilized to obtain the highest possible yield, but in organic farming, efforts are made to maintain and even improve soil fertility with regard to its biological, chemical and physical properties (Baldwin, 2006). Conventional farming generally uses short-term solutions (e.g. use of mineral fertilizers and herbicides), while organic farming relies on long-term solutions (preventive way, nutrient cycling through soil organic resources (Watson et al. 2002; Wander, 2015)). In organic farming, cover crops and organic fertilizers can be used in addition to proper crop rotations to improve soil biological, chemical and physical properties and maintain soil organic matter and fertility (Baldwin, 2006; Doltra and Olesen, 2013).

Two major challenges in organic farming are the arrangement of nitrogen (N) fertilization (manure application time, variability in nutrient content in organic fertilizers, etc.) and the irregular availability of N due to factors influencing mineralization in the soil (Osman et al. 2011). In conventional fertilization, mineral N is readily available to plants in their early stages of development, resulting in more tillers and longer ears, thus leading towards higher yields (Hanell et al. 2004). Xue et al. (2016) found that in the later stages of plant development, additional mineral N given as nitrate or urea increases the content and composition of cereals’ protein and increases the volume of the loaf made from wheat flour. Rossini et al. (2018) further suggested that at a given N input, use of N from mineral fertilzers (mineral N) results in higher and better-quality yield than use of N from organic fertilizers (organic N), indicating better availability of mineral N for crop growth.

However, the use of mineral N fertilizers carries a high risk of N leaching into the environment and resultant eutrophication of waterbodies (Houlton et al. 2019). Conventional mineral fertilizer application guidelines are based on an assessment of the nutrients available to plants in the soil. Crop nutrient uptake and yield are key factors in determining optimal fertilizer use (Ju and Christie, 2011). Therefore, it is important to use fertilizers in a timely manner and in optimal amounts to minimize losses and improve nutrient efficiency (Li et al. 2009).

In contrast, N is released relatively slowly from organic fertilizers, and additional carbon is provided to soil. The results of several long-term studies have shown that the addition of organic fertilizers, e.g. fully-composted cattle manure, alters the physical properties of the soil by reducing the density and increasing soil ability to retain water (Weber et al. 2007). In addition, organic fertilizer significantly increases the organic carbon content of the soil compared to mineral fertilizers and provides a wider range of plant nutrients (García-Gil et al. 2000; Bulluck et al. 2002; Nardi et al. 2004; Weber et al. 2007).

Soil phosphorus and potassium content and pH changes after long-term organic cropping have been less researched (Gosling and Shepherd, 2005; Kirchmann et al. 2007; Kaš et al. 2016). P and K occur in different fractions in the soil, some of which are more accessible to plants than others (Kulhánek et al. 2009; Vanden Nest et al. 2015; Srinivasarao and Srinivas, 2017). This dissertation deals with the long-term effects of organic and mineral fertilizers on soil, P, K and pH dynamics in conventional and organic cropping over a period of 10 years. One of the aims of this study was to assess whether organic fertilizers (cattle manure and winter catch crops) met the P and K requirements of the experimental crop rotation. Potato, which is known to be major exporter of soil K (Srinivasarao and Srinivas, 2017), was also cultivated in this crop rotation.

Soil pH also affects the availability of P and K to plants (Järvan and Vettik, 2016) and in the present work, the effect of long-term use of different amounts of different fertilizers on soil pH was evaluated.

2.2. Effect of cropping system on the quality of winter wheat dough

2.2.1. Formation of starch granules

When wheat flour is mixed with water, a dough is formed, whose quality is influenced mainly by gluten content, strenght and size distribution of starch granules. Different-sized starch granules are located in the voids of the high-molecular-mass gluten network. The quality of the dough depends to a large extent on the strength of the mutual contact between the starch granules and gluten (Gao et al. 2020).

During the filling period of cereal grains, four types of cells gradually develop within the endosperm: first the cells surrounding the embryo, then the transfer cells, the aleurone layer and finally the starch-rich endosperm cells. The latter accumulates more and more reserves (mainly proteins and starch) during the filling period and cells are filled with amyloplasts and protein bodies during the ripening period of the grains (Jane, 2004). In the endosperm of wheat, the starch granules are trimodal, i.e. they contain lenticular A-granules (10 to 50 µm in diameter), spherical B-granules (5 to 9.9 µm in diameter) and irregularly shaped C-granules (diameter <5 µm) (Bechtel and Wilson, 2003). The different size of the starch granules may be due to the different timings of their formation during the filling period of the grain (Xie et al. 2008). A-granules are formed approximately 4 to 14 days past anthesis (DPA), when the endosperm is still actively dividing (Wilson et al. 2006). The formation of B-granules begins at about 10–16 DPA, and small C-granules first appear at about 21 DPA (Bechtel and Wilson, 2003). The C-granules have irregular shape, which may be caused by the very small in diameter and tightly packed in the seed. Based on the size of the diameter, the distribution of the granules can be expressed as percentiles. For example, the 10, 50, and 90% percentiles are associated with the diameter of type C, B, and A granules, respectively (Edwards, 2010; Zi et al. 2019). Previous studies have found that variability in starch granule size/diameter correlates significantly with dough viscosity (Zeng et al.

1997), dough mixing properties (Peterson et al. 2001), and loaf structure (Park et al. 2009).

Li et al. (2013) reported that the use of N-fertilizer (together with S-fertilizer) promotes the accumulation of types A and B starch granules in the central endosperm tissue during the grain filling phase. Xiong et al. (2014) found that the increased rate of N-fertilizer mainly increased the number of small starch granules, and at the same time decreased the number of larger ones, but these results varied between different regions of the wheat endosperm.

The size of the starch granules also affects the amount of flour produced during the milling process, including the size of the fine flour yield (Edwards, 2010). MacNeill et al. (2017) found that the mobilization of starch and photosynthetic carbon from stems and leaves to reproductive tissues is affected by the fertilization regime in cropping system because different rates of N-fertilizers affect pre-flowering above-ground biomass formation and post-flowering grain filling period differently (e.g., flag leaf assimilation). In addition to cropping system, the post-flowering environment, such as water availability and temperature, strongly influences seed size, which in turn determines several physical properties of the seeds, such as screening or milling yield (Nuttall et al. 2017).

When grinding with first break roller mill, the wheat grain is crushed, resulting in the formation of a wide variety of flour particles with diameters ranging from <200 µm to > 2000 µm (Campbell et al. 2007). The first break stage of the grains is usually followed by four or more breake stages, screening, purification and eight or more reduction steps to separate the germ and bran from the endosperm. The higher the flour yield after the first breake stage, the more economically profitable is the milling process (Edwards, 2010; Fang and Campbell, 2002). Bechtel and Wilson (2003) reported that the yield of the flour depends on the proportion of endosperm in the wheat grain, which in turn depends on the cropping system.

2.2.2. Properties of wheat dough

Unlike other cereal flours, wheat flour with added water can form a dough with a unique three-dimensional structure and viscoelastic properties (Gao et al. 2020). Wheat dough has been considered a complex mixture in which starch granules act as filler components in the holes of the storage protein network (Edwards et al. 2002). High- and low-molecular mass gluten have been shown to determine dough formation and quality (Wang et al. 2018). However, the rheological properties of wheat dough are determined not only by proteins but also by other flour components (e.g., starch) and their interactions (Cao et al. 2019). Starch is an important preservative carbohydrate, accounting for about 70% of grain and 75% of flour by mass. Starch alone and also in interaction with gluten plays an important role in the development of dough quality by influencing the functional internal structure and physicochemical properties of dough (Wang et al. 2017).

The particle size of the starch granules has been reported to increase the rheological properties of the dough, with smaller granules increasing the elasticity of the dough (Edwards et al. 2002; Tao et al. 2016). Large A and smaller type B starch granules have different physical and chemical characteristics (Liu et al. 2007; Li et al. 2008). The size distribution of wheat starch granules is important for its functional properties (Chiotell and Le Meste, 2002; Park et al. 2009), resulting in different quality levels for many end products.

Although the effect of gluten or starch on dough quality has been studied (Gao et al. 2018; Roman et al. 2018), the effect of protein-starch interaction on wheat dough has rarely been studied due to its versatile nature (Yang et al. 2011). This study compares the rheological properties of winter wheat dough grown in organic and conventional systems.

3. AIMS AND HYPOTHESIS OF THE THESIS

Sustainable crop production is the most important goal of modern agriculture. There is the need to produce more by using less input, e.g., fertilizers and pesticides. However, to achieve this and to provide food security and quality, there are several gaps in knowledge that need to be filled. This research provides valuable analysis on how different mineral nitrogen rates and organic cropping affects crop yield and some soil fertility parameters. Sufficient soil nutrient content together with suitable pH is fundamental for stable crop yield and quality. Wheat quality analysis focuses on the impact of fertilization on dough quality, starch granule size distribution, and gluten-starch interaction. Latter have been very little studied, and thus current thesis provides novel and complex overview that is especially important for baking industry. The most valuable and novel part of the thesis is the comparison of conventional and organic cropping in the same field experiment using the same rotation and growing conditions. The knowledge presented in this thesis is important within the entire value chain from field to endproduct producers.

This thesis addresses the following aims:

A. To assess impact of organic and conventional cropping systems over the course of 10 years on:

1. The total yield of the five-field crop rotation, with all crops occurring each year.
2. Soil pH, the plant available P and K content of the soil, and the preservation of soil fertility via long-term use of winter catch crops and well-composted manure.

B. To investigate the effects of two cropping systems with seven fertilization treatments and weather conditions (air temperature and precipitation) on winter wheat:

3. Size distribution of starch granules.
4. Yield of whole and fine flour.
5. Gluten-starch interactions.
6. Stability and quality of the rheological properties of dough.

Hypotheses:

1. Total yield of a five-field crop rotation is close to one quarter higher in conventionally fertilised treatments than in organic treatments (Article **I**).
2. Long-term use of winter catch crops and well-composted manure in organic cropping improves soil fertility levels (Article **I**).
3. The overall diameter of the starch granules of organically grown wheat is smaller than conventionally grown wheat (Article **II**).
4. The size distribution of the starch granules depends on the length of the filling period of the grain (Article **II**).
5. The cropping system affects the yield of wheat flour (Article **III**).
6. The dough of wheat fertilized with mineral nitrogen (conventional cropping) has higher and more stable quality (Article **III**).

4. MATERIALS AND METHODS

4.1. Trial design

In 2008, a five-field crop rotation experiment was established at the Estonian University of Life Sciences (58^{o}36, N, 26^{o}66’ E; near Tartu), which compares the effects of organic and conventional cropping on crop yield, crop quality and soil properties and soil fertility (Figure 1). The crop rotation consists of the following crops: spring barley (*Hordeum vulgare* L.) with under sown red clover - red clover (*Trifolium pratense* L.) - winter wheat (*Triticum aestivum* L.) - field pea (*Pisum sativum* L.) - potato (*Solanum tuberosum* L.). The varieties used in this study were mainly local varieties bred at the Estonian Institute of Plant Breeding: potato varieties Reet (2008–2010) and Maret (2011–2017), barley varieties Leeni (2008–2010) and Anni (2011–2017), red clover varieties Jõgeva 205 (2008–2011) and Varte (2012–2017). The foreign varieties used were winter wheat varieties Portal (2008–2010), Olivin (2011) and Fredis (2012–2017), pea varieties Madonna (2008–2010) and Tudor (2011–2017). These varieties were and still are popular among Estonian farmers.

The soil is a sandy loam Stagnic Luvisol (IUSS WG WRB 2015) (WRB, Deckers et al. 2002), (at the beginning of trial for A horizon: total C content 1.38%, N content 0.13%, and $pH_{KCl} = 6.0$). The fertilizer treatments of the field experiment are arranged in systematic blocks side by side in four replications, with three organic fertilizer treatments in the organic system (10 x 6 m; Org 0, Org I and Org II; Figure 1) and four different nitrogen addition rates in the conventional crop system [N0, N1, N2, N3 (Article **I**); N0, N50, N100, N150 (Articles **II**, **III**)]. Well-composted cattle manure and winter catch crops were used as organic fertilizer; ammonium nitrate (NH_4NO_3) was used as mineral N fertilizer.

Figure 1. Trial scheme

The data for this study covered the period 2008-2017, i.e., two rotation periods.

Treatment N0 is a conventional system control without mineral fertilizers. In the other three conventional treatments: N1, N2 and N3; P and K fertilizers were applied at the rates of 25 and 95 kg ha^{-1}, respectively (P and K amounts were similar in all treatments, Kemira and Yara Mila fertilizers were used). In the conventional treatments, the mineral N fertilizer NH_4NO_3 was used during growing period (N1 = 40 – 50 kg N ha^{-1}; N2 = 80 – 100 kg N ha^{-1}; and N3=120 – 150 kg N ha^{-1}), while for the last two fertilizer treatments, N fertilizer was divided into two sections: 50 + 50 kg N ha^{-1} for the N100 treatment and 100 + 50 kg N ha^{-1} for the N150 treatment. The first time of winter wheat fertilization was in the early spring and the second in the booting phase (BBCH 47). A lower N rate was used for barley sown with red clover; red clover alone did not receive mineral fertilizers. Pea received mineral N in 20 kg N ha^{-1} N1, N2 and N3 treatments. Conventional treatments were treated with synthetic pesticides against weeds, diseases, and pests one to four times during growing period as needed.

The first organic treatment (Org 0) is a control of organic cropping without organic fertilizers. In the second organic treatment (Org I), catch crops were used as green manure in winter: after harvesting winter wheat, potato and pea, a mixture of rye (*Secale cereale* L.) and winter oilseed rape (*Brassica napus* ssp. *oleifera* var. *biennis*), rye and winter oilseed rape were sown respectively. Catch crops were plowed into the soil in spring as soon as possible after the snow had melted in April. In the third organic treatment (Org II), fully composted cattle manure was added once during the first rotation before the potato. Manure (40 t ha^{-1})

was plowed into the soil to a depth of 20–23 cm in late September/early October, before winter rape was sown as a catch crop. During the second rotation (2013–2017) the manure application regime was changed: the first application in the crop rotation was 10 t ha^{-1} before sowing barley, the second in the early spring before winter wheat started to grow at 10 t ha^{-1} and the third application of 20 t ha^{-1} before planting potato. Together with the variable content of dry matter (DM) and nutrients in the composted cattle manure, the amounts of N, P, K used with the manure also varied (Article **I**, Table 1).

All treatments were plowed 20-23 cm deep. In the organic treatments, weed control was carried out after sowing and in the winter wheat field at the end of April with spring harrowing. In all treatments, red clover was mowed, crushed, and then plowed into the soil in the second half of August.

Biomass samples of red clover were taken from a 1 m^2 quadrat before mowing. Winter wheat, barley and pea were harvested with a Sampo combine with a header width of 2 m, i.e. a test area for calculating the grain yield was 20 m^2. The samples were dried at 105 °C for 48 hours to measure DM. Measurements of potato DM have been described previously (Tein et al. 2014).

4.2. Chemical analyses and calculations

Each year in mid-April, before the start of the fieldwork, soil samples were taken from each test plot at a depth of 0–23 cm. Soil pH was determined from 2 mm sieved air-dry samples in 1 M KCl 1: 2.5 solution. Acid digestion with sulfuric acid solution was used to determine the P_{tot} and K_{tot} concentrations of cattle manure and plant samples (Methods of Soil and Plant Analysis 1986). Total nitrogen (N_{tot}) content of the oven-dried well-composted manure samples and plant samples was determined by the dry combustion method with a varioMAX CNS elemental analyzer (ELEMENTAR, Germany). Available P and K concentrations in soil samples were determined by the ammonium lactate (AL) method (Egnér et al. 1960).

Calculation of total yield per treatment is described in Article **I.** The total yield was calculated as the sum of the DM yields per ha of organic and conventional crops in each treatment (i.e., 5 crops x 1 treatment x 4 replicates).

Calculation of annual nutrient balances for organic cropping with cover crops and without organic fertilizer addition and for conventional cropping is described in Article **I.**

The amounts of P and K immobilized in cover crops and red clover biomass were not considered in the calculation of the P/K input / output balance, as they did not add PK to the system (Article **I**).

4.3. Size distribution of starch granules

The size distribution of the starch granules (Article **II**) was determined in the endosperm of winter wheat grain using a Malvern Mastersizer 3000 analyzer (Malvern Instruments Ltd., Malvern, UK). A standard protocol was used to separate starch from 100 mg of material taken from each treatment. The starch solution was used in laser diffraction analysis. Particle size analysis was performed as described by Li et al. (2016) and Tanaka et al. (2017). Approximately 0.1 mL of starch was dissolved in 1 mL of reverse osmosis water (Class 2, conductivity 5–6 μ S/cm^{-1}) in 2 mL of Eppendorf tubes and mixed briefly before adding the reverse osmosis water of the same origin to the dispersion tank of the particle size distribution analyzer. Distribution statistics were calculated from the results using the volume - derived diameter (DV), an internationally agreed method for determining the average diameter and diameter size distribution. Dv (90), Dv (50) and Dv (10) are standard percentile readings for the measured size distribution. In Articles II, III and this dissertation, the terms Dv (90), Dv (50) and Dv (10) as well as type A, B and C starch grains are used to indicate the diameter of the starch grains, where Dv (90) = type A, Dv (50) = type B and Dv (10) = type C starch grains. This is consistent with previous literature (Edwards, 2010; Zi et al. 2019).

4.4. Yield of wheat flour

Yields of wheat flour were measured over four experimental years (Article **II**). No flour yield was determined in 2016 because the winter wheat harvest (2015/2016) failed and only a small amount of grain was available for milling. Cereal samples, 1000 g per test plot, 14% moisture, were milled in a laboratory mill LM 3100 (Perten Instruments, Hägersten, Sweden), after which the flour was sieved into three fractions: bran and shorts (sieve PA-47GG, SEFAR NYTAL PA, Retsch, Haan, Germany) with a particle size of > 375 µm, coarse flour (PA-72GG sieve, SEFAR NYTAL PA) with a particle size of 224– 375 µm and fine flour with a particle size of <224 µm. The yield of whole flour was calculated as the sum of coarse and fine flour (particle size <375 µm).

4.5. Determination of dough properties

The wet gluten content (WGC; Article **III**) was determined according to ISO standard 5531 (ISO 5531) with a Glutomatic 2100 instrument (Perten Instruments AB, Huddinge, Sweden). Gluten index was measured with a Perten instrument (ICC 155; Glutomatic 2100, Centrifuge 2015; Perten Instruments). The gluten index (GI) characterizes the ratio of gliadins to glutelins. To form a dough with good strength, the GI values should be in the range of 60–95 (Borkowska et al. 1999). According to Cubadda et al. (1991), gluten can be assessed from GI indicators – whether the quality of gluten is poor (GI <30%), normal (GI = 30–80%) or strong (GI> 80%).

Protein content was calculated by multiplying total nitrogen (N_{tot}) content by 5.7.

The water absorption of the flour and the mixing properties of the dough were studied with Brabender Farinograph-TS version 2.1.0 (Brabender GmbH & Co, Duisburg, Germany) using the Brabender ICC BIPEA 50 method (Article **III**). The analyses were performed according to ISO 5530-1. The principle of using a farinograph is based on the resistance of kneading the dough. The farinogram graphs show the dough formation time, the stability time, and the degree of softening of the dough (after 10 and 12 minutes). Dough development time (DDT; min) determines the time from the start of mixing to the point of maximum viscosity, while dough stability (S) is the time (min) measured from when the upper part of the farinograph exceeds 500 Brabender units (BU) to the point at which it falls again below of this. The degree of softening (DS; FE) is the difference in height from the center of the graph at maximum agitation resistance to the center of the graph at 10 or 12 minutes later. The quality number of the dough (DQN) is the length (mm) from the water point to 30 FE below the center line of maximum consistency along the time axis. A low DQN indicates a weak dough that weakens early and rapidly, while a high DQN indicates a strong dough that weakens late and slowly (Kebede, 2019).

An alveograph (Chopin Technologies, Villeneuve la Garenne, France) was used to measure the viscoelastic properties of wheat (AACC approved method 54-30.02, ICC 121, ISO 27971: 2015). The alveograph measures the main parameters of the reaction of the dough to biaxial expansion by pumping it with air, i.e., the pressure (expressed by the variable W, 10^{-4} J) inside the dough bubble and the deformation of the dough piece until it breaks, and what is named baking strength.

4.6. Weather conditions

The Estonian climate at the experimental site is slightly continental. The winter period (average air temperature permanently below 0°C) lasts on average 115 days, with an average temperature of -5.5° C. The average duration of the growing season (air temperature permanently above 5°C) is 175–190 days. The average frost-free period is four months, during which the average mid-summer (July) temperature is 16–17°C. The average annual rainfall is 550–700 mm; in the wettest months (April to the end of October) the average rainfall is 350–500 mm (Keppart and Loodla, 2006).

Meteorological data were collected from Meteorological station located about 1 km from the experimental site (Figure 2 and Figure 3; Article **I,** Tables 2 and 3). Weather data are reported in Article **I** for the years 2008–2017, Articles **II** (Table 1) and **III** report the data for the period 2013–2017. The effect of weather on yield is discussed in more detail in the Results section.

The growth of winter wheat (Articles **II** and **III**) after the snowmelt started on the 16 of April 2013 and on the 1st of May 2017, which was 5 to 25 days, respectively, later than in the other trial years (2014–2016). In 2015, the average temperature in May was 1.1° C lower than the long-term average, but precipitation was sufficient, which promoted winter wheat tillering and the development of above-ground biomass. In 2015, lower temperatures and regular precipitation in the post-winter period caused a record harvest of winter wheat. The most unfavorable weather conditions for the formation of the crop were in 2016, when the amount of precipitation in May was only 2 mm and the temperature data in the post-winter vegetation period were higher than the long-term average.

The accumulation of effective temperatures (growing degree days) was considered (GDD; Article **II**) to characterize the effect of weather conditions on the size distribution of starch granules. The GDD was calculated using a sum of mean daily temperature above 5 °C during the period from flowering (BBCH65) to physiological maturity (PhM). Physiological maturity was reached at 37% of grain water content.

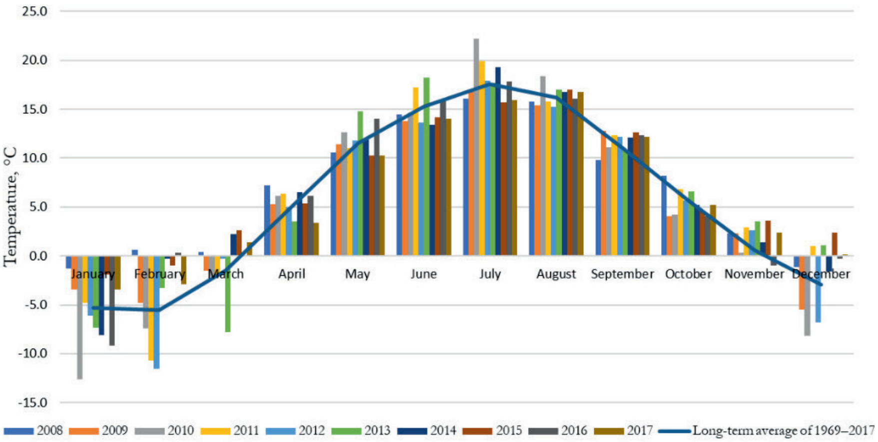

Figure 2. Average temperature (°C) in 2008–2017 compared to long-term average (1969–2017); data from Eerika meteorological station (58°37' N, 26°66'E)

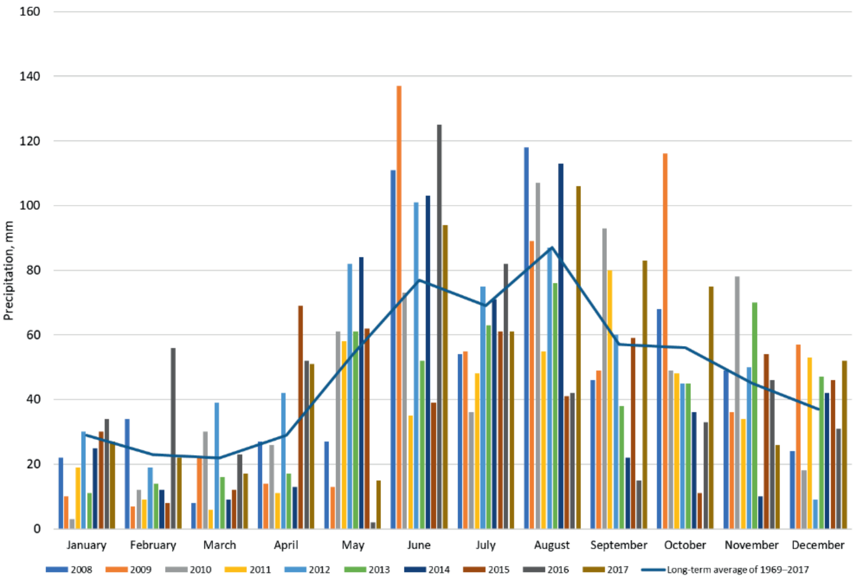

Figure 3. Precipitation (mm) in 2008–2017 compared to the long-term average (1969–2017); data from Eerika meteorological station (58°37' N, 26°66'E)

4.7. Statistical analyses

Correlation and variation analyses (two-way ANOVA) were used to test the effect of cropping system and climatic conditions on the DM yield of each crop, yield quality, winter wheat starch grain formation and dough properties. Averages of the same endpoints were assessed by ANOVA and LSD test using homogeneous groups to determine the significance of the effects of cropping system and to test weather conditions of the experimental years. Mean values are given with standard errors (± SE) (error bars in the bar graphs). Statistical significance was defined as $p < 0.05$ unless indicated otherwise.

5. RESULTS

5.1. Effects of cropping system on crop yield and soil fertility

5.1.1. Total crop yield in different types of cropping system

The total yield of the five similarly fertilized crops was significantly influenced by the cropping system ($p < 0.001$, Figure 4) and climatic conditions ($p < 0.001$, Figure 5). Two-factor (year and cropping system) analysis of variance showed that the total yield of the crops in the organic treatments was on average between 21.5–22.4 t ha^{-1} during experimental years compared to 29.0–29.8 t ha^{-1} in the conventional system depending on N rate (Figure 4). The differences between the treatments within each cropping system were not significantly different, except for treatment N0, which was similar to the organic treatments. The total yield of the organic treatments was 3–28% lower than that of the conventional treatments (Figure 4)

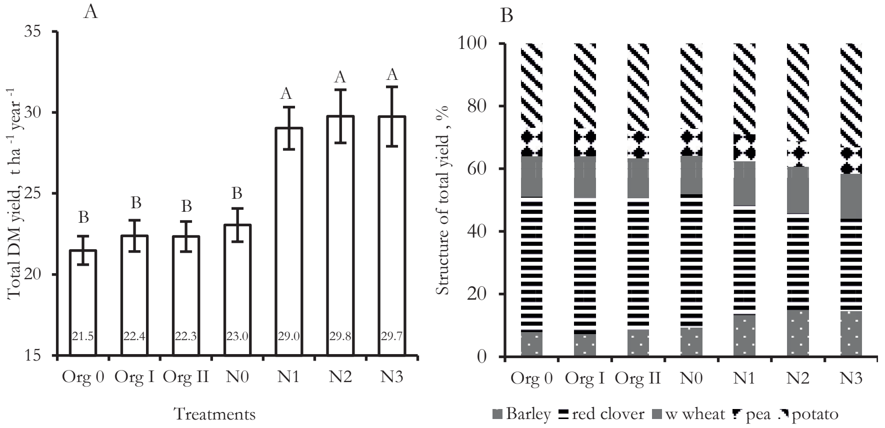

Figure 4. Total annual yield of five crops (DM t ha^{-1} year^{-1}) in different fertilization treatments (A). Proportion of crop yield (%) in the total yield of five crops in different fertilization treatments (B).
F (6, 63) = 9.271, $p < 0.001$
Bars with the same letter are not significantly different; treatments Org 0 and N0 = organic and conventional treatment control, respectively; Org I = organic with winter catch crops; Org II = well-composted cattle manure used in addition to CC; N1 = quantities of mineral NPK per ha: 40-50 kg N, 25 kg P, 95 kg K; N2 = quantities of mineral NPK per ha: 80 to 100 kg N, 25 kg P, 95 kg K; N3 = amounts of mineral NPK per ha: 120-150 kg N, 25 kg P, 95 kg K. Barley undersown with red clover was given less mineral N fertilizers. (Reproduced from Article **I**)

Over a ten-year period, the total yield of the five crops averaged between 20.3 and 31.5 t ha^{-1} (Figure 5). In 2009, 2012 and 2017, the total crop yield was 19–27% higher than in the other experimental years, mainly due to the increase in potato and winter wheat yields. Results showed that the temperatures in April and September were important for the development of the yield. The higher temperature of 1.1–2.2°C (2008, 2010, 2011,2014, 2016) in April compared to the long-term average (Figure 2; Article **I**, Table 2) resulted up to 70% lower level of winter wheat yield ($r = -0.42$, $p < 0.01$, $n = 70$), while 2.3–2.8° C higher temperatures in September resulted up to 55% higher potato yield ($r = 0.47$; $p < 0.001$; $n = 70$;)

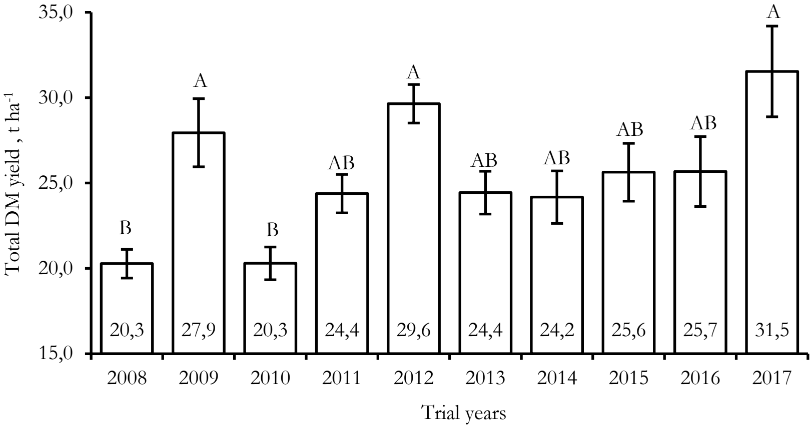

Figure 5. Average (± SE) total yield of five crops (t ha^{-1}) across treatments in different experimental years. The bars with the same letter are not significantly different ($p > 0.05$). (Reproduced from Article **I**)

The distribution of precipitation strongly varied among the years (Figure 3; Article **I**, Table 3). In 2015, the annual precipitation was 93 mm lower than the long-term average, the distribution of precipitation during the growing season was even and the total yield of the five crops did not differ from the record yields of 2009, 2012 and 2017. However, the effect of precipitation on total yield was not significant ($r = 0.2$, $p > 0.05$, $n=70$).

5.1.2. Soil pH in organic and conventional cropping

Soil pH was significantly influenced by the cropping system ($p < 0.001$), accounting for 14% of the total effect of the experiment. In the beginning of the experiment, the pH values of the treatments did not differ significantly ($p>0.05$; Figure 6); the pH values of the organic and conventional treatments were 5.93 ± 0.03 and 5.82 ± 0.03, respectively. After 10 years the pH values of the organic treatment Org II had increased on average by 0.24 units due to fertilization with cattle manure (pH values ranged from 5.95 to 6.17; Figure 6). The soil pH values of all conventional treatments had decreased on average by 0.23 units (pH value ranged from 5.55 to 5.82).

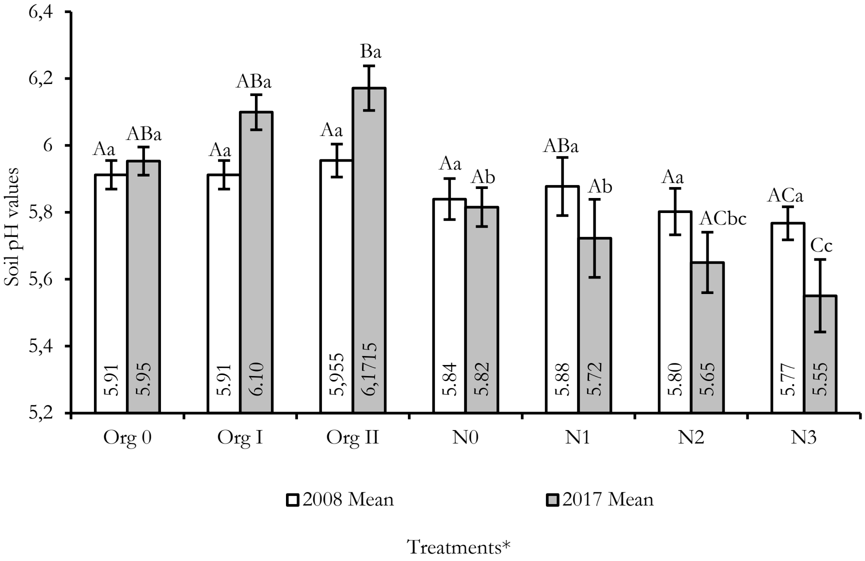

Figure 6. Soil pH values in different treatments in the beginning of the experiment and after ten years. 2008_{Mean}: F (6, 133) = 1.268, p = 0.276; 2017_{Mean}: F (6, 133) = 8.215, $p < 0.001$

Different uppercase letters indicate significant differences between years and different lowercase letters indicate differences among treatments within a given year; * See explanations in Figure 4 (Reproduced from Article **I**)

5.1.3. Content of plant available P and K in soil after 10 years in organic and conventional cropping

Plant available P content in the soil of organic treatments decreased continuously during ten years of cultivation ($r = -0.19$; $p < 0.001$). In the beginning of the experiment, it did not differ significantly between the cropping systems (varied from 90.7 to 118.7 mg P kg^{-1} in soil). However, after ten years soil P content had decreased significantly ($p<0.05$) from 18.9 to 23.6 mg P kg^{-1} in all organic and conventional control treatments

(Figure 7). Soil P content in fertilized treatments of the conventional cropping system after ten years did not differ significantly ($p>0.05$) from the data obtained in the beginning of the field experiment.

The plant available K in the soil decreased in all treatments over ten years ($p < 0.001$). At the start of the experiment, there was no significant difference between the two cropping system treatments (varied from 160.7 to 174.4 mg K kg^{-1} in soil, Figure 8). Ten years later, the largest decrease in K content was in the control treatments of both cropping systems (60 mg K kg^{-1} in soil), followed by Org I and Org II. The content of plant available K decreased less in the fertilized conventional treatments (17.1–39.5 mg K kg^{-1} in the soil).

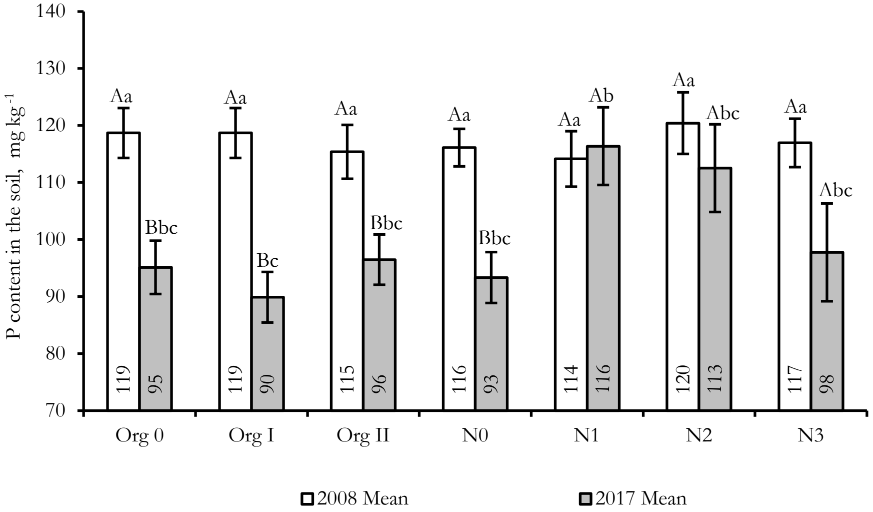

Figure 7. Content of plant available P (mg kg^{-1}) in soil in the beginning of the field trial and after ten years. 2008_{Mean}: F (6, 133) = 1.268, p = 0.276; 2017_{Mean}: F (6, 133) = 2.754, p = 0.0149
Different uppercase letters indicate significant differences between years and different lowercase letters among treatments within a given year * See explanations in Figure 4 (Reproduced from Article **I**)

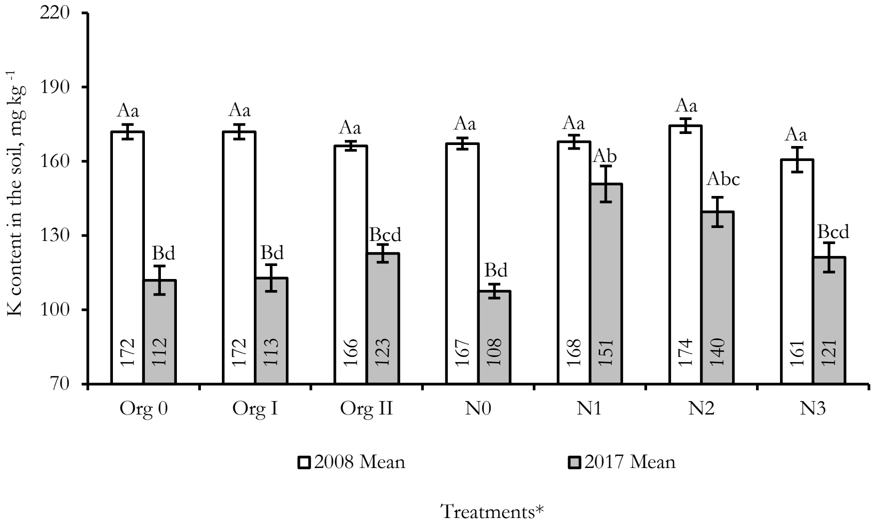

Figure 8. Content of plant available K in soil (mg kg^{-1}) in the beginning of the field trial and after ten years. 2008_{Mean}: F (6, 133) = 1.268, p = 0.276; 2017_{Mean}: F (6, 133) = 8.215, $p < 0.001$
Different capital letters indicate significant differences between years and different lowercase letters indicate differences among treatment within each year * See explanations in Figure 4 (Reproduced from Article **I**)

5.1.4. PK balance in soil in different treatments

The amount of P and K removed from the field depended on the yield of the crops in both organic and conventional treatments. The annual amount of P removed by crops from all organic treatments did not differ from the conventional control treatment (difference between 11.4 and 11.9 kg ha^{-1}); the annual amount of P removed in the fertilized treatments of the conventional cropping system was 28–35% higher (Table 1). The same data on K removal per year were 42.0–44.2 kg ha^{-1}, which was 28–40% higher than in the organic treatments (Table 2).

Table 1. Mean amount of P (kg ha^{-1}) annually applied and removed by different crop yields from different treatments and input/output balance in the soil as an average of ten years. (Reproduced from Article **I**)

Treatment	Input kg ha^{-1}	P output kg ha^{-1}				Mean output across crops*	Input - output
		Barley + red clover	Winter wheat	Pea	Potato		
Organic							
Org 0	0	6.9 ±0.3	12.2±0.6	9.9±0.6	15.6±1.2	**11±1.8 b**	-11.2
Org I	0	7.0±0.3	13.2±0.9	10.6±0.6	16.0±1.1	**12±1.9 b**	-12.2
Org II	10.0±2.5	7.9±0.5	12.2±0.9	9.5±0.6	16.2±1.0	**12±1.8 b**	-2.2
Conventional							
N0	0	8.5±0.5	11.5±0.8	9.8±0.7	17.9±1.4	**12±2.1 b**	-11.9
N1	25	14.9±0.9	17.8±1.1	11.7±0.7	21.5±1.2	**17±2.1 a**	8.5
N2	25	16.6±0.9	19.1±1.0	11.3±0.7	22.9±1.4	**18±2.4 a**	7.5
N3	25	16.0±0.9	17.9±1.2	11.6±0.9	24.5±1.7	**18±2.7 a**	7.5
Mean over treatments		**11±1.7c**	**15±1.2b**	**11±0.3 c**	**19±1.4 a**	**14.0±1.1**	

The means marked with the same letter do not differ statistically significantly from each other.

Table 2. Average amount of K (kg ha^{-1}) per year applied and removed with different crop yields in different treatment and soil input/output balance as a ten-year average. (Reproduced from Article **I**)

Treatment	Input kg ha^{-1}	K output kg ha^{-1}				Mean output across crops*	Input - output
		Barley + red clover	Winter wheat	Pea	Potato		
Organic							
Org 0	0	9.1±0.5	12.8±0.7	20.2±1.0	121±7.0	**41±26.8a**	-40.8
Org I	0	9.3±0.5	13.7±0.9	20.8±1.1	126±7.0	**43±28.0a**	-43.9
Org II	32.1±4.4	10.6±0.7	13.0±0.9	19.7±1.0	131±5.8	**44±29.2a**	-11.5
Conventional							
N0	0	11.8±0.6	12.8±1.0	20.2±1.3	135±7.7	**45±30.1a**	-45.0
N1	95	21.3±1.0	20.2±1.4	26.6±1.2	180±7.3	**62±39.3a**	33
N2	95	22.6±1.0	21.8±1.2	25.3±1.2	194±8.9	**66±42.7a**	29.1
N3	95	23.0±0.9	20.5±1.4	33.8±6.2	215±12.2	**73±47.4a**	21.9
Mean over treatments		**15±2.5c***	**16±1.6 c**	**24±2.0b**	**157±14.4a**	**53±19.9**	

The means marked with the same letter do not differ statistically significantly from each other.

The input/output balance calculated by P and K was different in the organic and conventional systems. The P balance showed that in the organic cropping system, the use of cattle manure improved the P balance by 9 kg ha^{-1} compared to the control treatment Org 0 (Table 1).

The K balance showed that it was negative in all treatments of the organic system (Table 2). The use of cattle manure improved the K balance by 29.3 kg ha^{-1} compared to the control Org 0. In the conventional cropping system, the calculated balance of P and K was positive in all fertilized treatments (Tables 1 and 2). Both the P and K balances were the most negative in the control treatments of different cropping system, while the reduction in these elements was higher in the conventional control due to the use of pesticides and up to 7% higher total yields. In all years, the highest removal of P and K was with potato, which was also expected due to the much higher yields of tubers. The P and K removed by potatoes were up to 1.8 and 10.2 times higher, respectively, than the nutrients removed by other crops.

5.2. Factors affecting the size of starch granules

The size distribution of starch granules was significantly related to several factors studied, such as fertilization with organic or mineral N fertilizers ($p < 0.001$), weather conditions ($p < 0.001$), biomass yield in the wheat flowering development stage (BBCH65; $p < 0.01$) and length of time period from BBCH65 to physiological maturity (PhM; $p < 0.001$; Table 3).

The proportion of the effect of weather conditions and cropping system on the total effect of the experiment on Dv (10), Dv (50) and Dv (90) was 38 and 18%, 24 and 24%, 18 and 18%, respectively. The same data for the combined effect of these two factors was 44, 24 and 63%, respectively (data not shown).

Table 3. Correlation between the size distribution of starch granules and several factors examined in this experiment. (Modified from Article **II**)

Factors	**Dv(10)**	**Dv(50)**	**Dv(90)**
Temperature*[a]	-0.39***	-0.37***	-0,26**
Precipitation*[a]	ns	ns	-0.26**
Length of period BBCH65–PhM[b]	-0.54***	-0.52***	-0.40***
N amount (treatment)[a]	0.20*	0.19*	0.19*
Farming system[a]	ns	ns	ns
Biomass of wheat at BBCH65[b]	-0.27**	-0.26**	ns
Fine flour yield[c]	-0.40**	-0.27*	-0.27*
Whole flour yield[c]	-0.31*	-0.31*	-0.29*

* The average temperature and the amount of precipitation is for the grain filling period.
*, **, *** - the statistical significance at p <0.05, 0.01, and 0.001 levels. [a] – n=105; [b] – n=112; [c] – n=56

5.2.1. Effect of weather conditions on the size distribution of starch granules

The size distribution of starch was most affected by the length of the filling period of the cereal grains, i.e., the BBCH65–PhM period, which in turn was positively correlated with the mean temperature and growing degree day (GDD) for this period ($r = 0.84$; $p < 0.001$). In 2013 and 2017, the BBCH65–PhM period was 3-5 days longer and therefore GDD was 40–168° C higher than in the other experimental years (Table 4). In 2013 and 2017, the average temperature during grain filling were higher (between 16.2 and 18°C), while in other years they fluctuated between 14.5 and 15.7°C.

Table 4. Accumulated growing degree days in 2013–2017 and length of grain filling period (from flowering to physiological maturity). (Modified from Article **II**)

Variable	Years				
	2013	2014	2015	2016	2017
	Dates of flowering and physiological maturity				
BBCH65	05.June	10.June	15.June	04.June	29.June
PhM	15.July	16.July	20.July	11.July	07.August
BBCH65–PhM, days	40	36	35	37	40
GDD for period of BBCH65–PhM, °C	519	351	358	408	448

BBCH65 – flowering; PhM - physiological maturity; GDD - growing degree days

In 2016, the value of GDD was affected by a much higher precipitation, which was 197 mm (in other years it ranged from 67 to 115 mm). In 2013 and 2017, the diameter of the starch granule for Dv (10), Dv (50) and Dv (90) was up to 17, 29 and 33% smaller, respectively, than in 2014–2016, except for DV (90) 2016 vs. 2013 (Table 5).

Table 5. Mean values (±SE) of starch granule size distribution over farming system treatments and experimental years. (Reproduced from Article **II**)

Year	Dv(10), µm*	Dv(50), µm	Dv(90), µm
2013	2.85 ± 0.05 b	6.63 ± 0.29 bc	17.80 ± 1.54 ab
2014	3.13 ± 0.06 a	7.86 ± 0.30 ab	20.40 ± 1.04 ab
2015	3.05 ± 0.03 a	8.15 ± 0.35 a	22.61 ± 0.88 a
2016	3.12 ± 0.04 a	7.08 ± 0.24 abc	17.01 ± 0.67 b
2017	2.70 ± 0.05 b	6.25 ± 0.20 c	16.77 ± 0.99 b

Different letters within the same column indicate significant differences (Fisher LSD test, $p < 0.05$). * Dv (10), Dv (50), Dv (90) - Particle diameters below which 10, 50 and 90% of the starch granules are located, respectively.

5.2.2. Effect of organic and mineral N on the size distribution of starch granules

Higher amounts of organic and mineral N given in the organic and conventional system treatments increased the diameter of the starch granule. In the organic farming system, the use of composted cattle manure (Org II) increased the values of Dv (10), DV (50) and Dv (90) by up to 15, 23 and 31%, respectively, compared to other organic treatments (Table 6). In the conventional system, higher amounts of mineral N also increased the diameter of the starch granules and the values of Dv (10), Dv (50) and Dv (90). The increase compared to conventional control was 8, 30 and 33%, respectively. The diameter of the starch granules as an average in the organic system treatments was similar to the average diameter in the conventional farming treatments ($p > 0.05$ for the comparison of the means).

Table 6. Mean values (± SE) of the size distribution of starch granules compared to experimental years in the treatment of agricultural systems. (Reproduced from Article **II**)

Treatment	Dv(10), μm*	Dv(50), μm	Dv(90), μm
		Organic	
Org 0	2.85 ± 0.07 b*	6.76 ± 0.36 abc	18.15 ± 1.59 ab
Org I	2.96 ± 0.05 b	7.14 ± 0.38 abc	17.83 ± 1.50 ab
Org II	3.22 ± 0.06 a	8.03 ± 0.28 a	22.77 ± 1.04 a
		Conventional	
N0	2.91 ± 0.05 b	6.26 ± 0.11 c	15.94 ± 0.82 b
N50	2.86 ± 0.05 b	6.43 ± 0.18 cb	16.90 ± 0.70 b
N100	2.97 ± 0.07 ab	7.49 ± 0.40 ab	19.93 ± 1.22 ab
N150	3.04 ± 0.07 ab	8.23 ± 0.49 a	20.94 ± 1.70 ab

Different letters within the same column indicate significant differences (Fisher LSD test, $p < 0.05$). * Dv (10), Dv (50), Dv (90) – Threshold particle diameters for 10, 50 and 90% of the starch granules, respectively. Org 0 - organic control system; Org I - organic treatment with catch crops; Org II - organic treatment with catch crops and composted cattle manure; N0 - conventional system control treatment; N50, N100 and N150 - conventional system fertilizer treatment with mineral nitrogen additions of 50, 100 and 150 kg N ha^{-1} respectively.

Fertilization with organic and mineral N affected winter wheat biomass, which in turn affected the diameter of the starch granules. The relationship between starch granule diameter and above-ground winter wheat biomass yield in the BBCH65 stage was negative (Article **II**, Table 2). The average biomass yield in the experimental years for the N-fertilized treatments of the conventional system ranged from 10.6 to 12.6 t ha^{-1}, which was 19–39% higher than for the organic treatments (data not shown).

5.3. Factors affecting flour yield and dough quality

5.3.1. Effect of cropping system on wheat flour yield

According to the ANOVA analyses, the yield of winter wheat cultivar Fredis whole (flour particle size 0–375) was significantly ($p<0.001$) affected by weather conditions (65% of the total effect) and the cropping system (9% of the total effect). The effect of the same factors on the yield of fine flour (particle size <224 μm) was 69% and 7%, respectively. However, the average yields of whole and fine flour in the organic system (averages of the treatments) were not significantly different from the average flour yields of the conventional system ($p>0.05$). In 2013 and 2017, the average yield of whole and fine flour in all treatments was 3–6% and 8–23% higher, respectively, than in 2014–2015 (Table 7). The ratio of the yield of whole flour to bran and shorts was significantly influenced by the weather conditions of the test year (the proportion of variation was 57%).

Table 7. Mean values of winter wheat flour yield (± SE) during the test period. (Reproduced from Article **II**)

Year	Fine flour yield, ($g\ kg^{-1}$)	Whole flour yield, ($g\ kg^{-1}$)	Bran and shorts, ($g\ kg^{-1}$)	Ratio of whole flour yield to bran
2013	483 ± 10 bc	758 ± 3 a	204 ± 2 b	3.72 ± 0.06 a
2014	440 ± 6 d	725 ± 3 b	229 ± 3 a	3.18 ± 0.05 b
2015	459 ± 9 cd	729 ± 3 b	229 ± 2 a	3.19 ± 0.05 b
2017	554 ± 7 a	760 ± 4 a	213 ± 8 b	3.59 ± 0.08 a

Different letters within the same column indicate significant differences (Fisher's LSD test, $p < 0.05$).

Although significant ($p<0.01$), the effect of the cropping system on the yield of fine flour was small (only 7%). In conventional system, the effect of mineral N fertilizer on fine flour yield was non-significant ($p>0.05$). The fine flour yield in the organic treatments ranged from 410 to 590 g per kg^{-1} of grain and from 398 to 584 g per 1 kg^{-1} of grain in the conventional treatments, and higher values were observed in the treatments without N or less N. As the yield of cereals in the conventional system (an average of the treatments and test years) was 26–36% higher in this experiment than in the treatments of the organic cropping system (data not shown), the yield of fine flour per hectare was also higher in the conventional system ($p<0.05$) The yield of fine flour in the organic and conventional treatments ranged from 1507–2206 to 1873–2757 kg ha^{-1}, respectively. The yield of whole and fine flour was significantly affected by the size of the starch granules while the diameter of the starch granules was negatively correlated with the yield of the flour (Table 3).

5.3.2. Effects of cropping system practices and climatic conditions on starch grain size and gluten content

The size distribution of the starch granules and the amount of protein and gluten accumulated during the grain filling period depended on the length of the grain filling period and the availability of N from organic or mineral N fertilizers.

The size distribution of starch granules depended on weather conditions during the grain filling period after winter wheat flowering (the share of weather and fertilizer treatment in the total effect was 38% and 17%, respectively). The diameter of type A starch granules was significantly smaller in 2013, 2016 and 2017 ($p < 0.01$), due to the longer grain filling period of 3–5 days. The proportion of type C starch granules in the grain endosperm was also higher in these years. Due to the effect of N fertilizer, the diameter of type A starch granules was larger in Org II (winter catch crops and manure) and N150 treatments, where the average diameter of granules was 22.8 ± 1.9 and 20.9 ± 3.2 µm, respectively (Table 6).

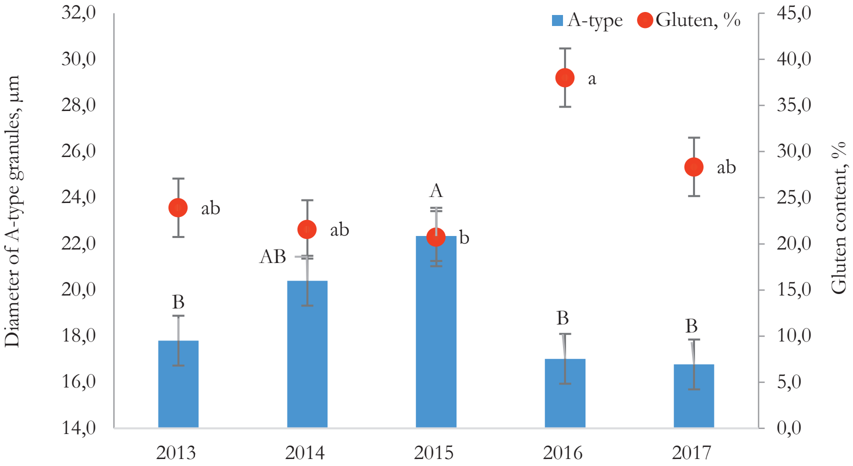

Figure 9. Average (± SE) diameter (µm) of type A starch granules and gluten content (%) across the treatments in different trial years. Values marked with the same letter do not differ significantly. (Reproduced from Article **III**)

The gluten content of the flour was most likely affected by climatic conditions, which in turn affected the availability of N during the grain filling period (the proportion of variation for weather was up to 83%).

Gluten levels were the highest in the N150 treatment, ranging from 24 to 43% in the study years. In those experimental years when the diameter of the type A starch grains was significantly smaller, the gluten content of the wheat grains was higher (Figure 9), relationship between gluten content and A-type starch grains' diameter was negative ($r = -0.34$; $p < 0.05$).

N fertilizer had a strong positive effect on protein content ($r = 0.70$, $p < 0.001$; Table 8). Protein content (PC) in winter wheat did not differ significantly ($p > 0.05$) between all organic and conventional treatments with lower N fertilizer amounts. The average PC was highest in the N150 treatment, up to 3.9% higher than the average PC in all other treatments. PC was affected by weather conditions in each growing year, while higher values were obtained in years with lower grain yields. Higher average PC concentrations over treatments were 12.4±0.30 and 14.6±0.48% in 2014 and 2016, which was 2.3 and 4.5% higher than in other years. PC correlates positively with the dough quality number ($r = 0.70$, $p < 0.001$).

Wet gluten content (WGC) correlated strongly with PC ($r = 0.77$, $p < 0.001$). Significantly higher gluten content were obtained for N150 treatment grains (Table 8) in 2016 and 2017 (mean WGC values over N treatments were 38 ± 0.7 and 28 ± 0.6%, respectively). According to the protein and gluten values, only flour obtained from treatment N150 is in a higher quality class. The flour obtained from the N100 treatment belonged to class A and the flour of all other treatments was of low quality (Klingler, 2010). WGC was positively correlated with water absorption and dough formation time ($r = 0.57$ and 0.51, respectively; $p < 0.001$ for both values).

The gluten index (GI) characterizes the strength of the dough. The mean GI value for the treatments and years was 83 ± 1.3%, whereas the GI varied between 69 and 96% in different experimental years (Figure 10). GI values were mostly influenced by the N-treatment ($r = 0.43$, $p < 0.01$) and weather conditions had little to no effect ($p > 0.05$). The share of weather and fertilizer treatment in the total effect on GI values was 36% and 14%, respectively. The availability of N in different treatments had a strong effect on GI ($r = 0.56$, $p < 0.001$); lower values were measured in flours from N100 and N150 treatments, these treatments were fertilized twice after wintering (Table 8). The mean GI values in the organic and conventional cropping systems ranged from 83 to 87%, which were up to 5–15% higher than in the N100 and N150 treatments, respectively.

Table 8. Rheological values of doughs obtained from different N-treatments. (Reproduced from Article **III**)

Indicator	Org 0 *	Org I	Org II	N0	N50	N100	N150
PC, %	11.2 ± 0.7 b	11.5 ± 0.6 b	11.3 ± 0.9 b	11.3 ± 0.6 b	11.6 ± 1.1 b	12.9 ± 1.1ab	13.6 ± 0.7 a
WGC, %	25 ± 2.6 b	25 ± 2.8 ab	25 ± 4.2 ab	25 ± 2.8 ab	25 ± 3.8 ab	29 ± 3.4 ab	31 ± 3.3 a
GI,%	86 ± 2.2 a	84 ± 2.2 a	87 ± 1.9 a	87 ± 2.7 a	83 ± 4.9 a	82 ± 3.3 a	73 ± 3.5 b
WAC,%	57 ± 0.8 b	57 ± 0.7 b	57 ± 0.8 b	58 ± 0.6 b	58 ± 0.7 b	60 ± 0.9 a	61 ± 0.4 a
DDT, min	2.08 ± 0.2 b	2.02 ± 0.2 b	2.02 ± 0.2 b	2.24 ± 0.3ab	2.16 ± 0.2ab	3.00 ± 0.3ab	3.54 ± 0.2 a
S, min	4.20 ± 0.55 a	4.17 ± 0.42a	4.07 ± 0.50 a	4.39 ± 0.43a	4.28 ± 0.55a	5.08 ± 0.40a	6.20 ± 0.38a
DS, FE	67 ± 12.5 a	65 ± 5.9 a	68 ± 11.4 a	67 ± 9.0 a	66 ± 9.4 a	56 ± 8.1 a	44 ± 5.4 a
W, 10^{-4} J	204 ± 18 a	204 ± 7 a	213 ± 11 a	233 ± 11 a	217 ± 11 a	234 ± 10 a	255 ± 17 a
DQN, mm	49 ± 11.2 a	48 ± 7.3 a	47 ± 9.8 a	50 ± 8.5 a	53 ± 9.5 a	65 ± 9.7 a	81 ± 7.4 a

Different letters indicate a significant difference; * PC - protein content, WGC - wet gluten content, GI - gluten index, WAC – water absorption capacity, DDT - dough development time, S - dough stability time, DS - dough softening degree, W – baking strenght, DQN - dough quality number; * Org 0 and N 0 = control treatments for organic and conventional farming respectively. Org I = organic winter catch crops (CC); Org II = application of cattle manure in addition to CC; N50, N100 and N150 = amounts of mineral N50, 100 and 150 kg N ha^{-1} respectively.

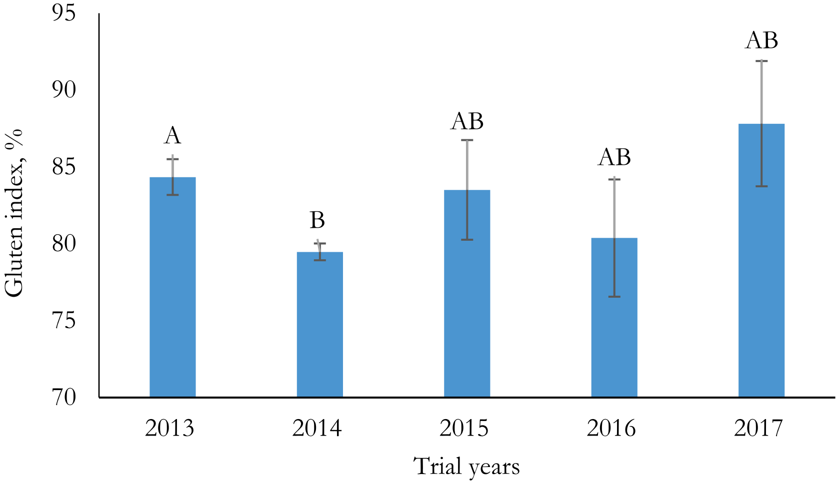

Figure 10. Gluten index values in different experimental years. The means with the same letter do not differ significantly from each other (p<0.05). (Reproduced from Article **III**)

The GI value was negatively correlated with dough formation time (r = 0.53, p <0.001), water absorption capacity (r = 0.49, p <0.01), dough stability (r = 0.49, p <0.01) and finally with dough quality number (r = 0.56, p <0.001). The diameter of the smaller starch granules was negatively correlated with GI values (r values for the C and B granules were -0.32 and -0.34, respectively).

5.3.3. Influence of cropping system on rheological properties of wheat dough and starch-gluten interaction

When water is added to wheat flour, it forms a dough. The water absorption capacity (WAC) of flour is one of the indicators of dough quality. WAC values were influenced by both study factors – weather conditions during the test years and N fertilizer; the effect calculated proportion of variance was 36 and 46%, respectively. The WAC of the flour correlated positively with PC and WGC (r = 0.83, p <0.001 and 0.57, p <0.001) and positively with small type B and C starch granules (r = 0.32 and 0.34, respectively). The differences in WAC values between the treatments were not significant, except for the N100 and N150 treatments, where the WAC was up to 61% (Table 8). The diameter of smaller starch granules was larger in 2014 and 2016 (Article **II**) and the WGC was higher in 2016 and 2017 (Figure 9); WAC averages ranged from 58% to 60% in 2014, 2016 and 2017, up to 3 percentage points higher than in other years.

Of the factors studied (year, i.e. weather conditions in trial years and treatment), only the treatment had a significant effect on dough development time (DDT) (r = 0.52, p <0.001) and it also correlated positively with PC and WGC (r = 0.83, p <0.001; r = 0.51, p <0.001, respectively). The DDT ranged from 1.46 to 4.09 min, while only the average DDT of the N150 treatment was significantly longer than the organic treatments, i.e., up to 2.23 minutes longer (Table 8).

Dough stability (S) was most affected by PC (r = 0.48, p <0.01). The stability of the dough was better when the diameter of the smaller starch granules (types C and B) was larger (r = 0.32). The most stable dough was made from N150 flour (Table 8). The average values of dough stability during the experimental years did not differ significantly for any of the treatments, it varied between 3.33 and 6.58 min.

The degree of softening (DS) of the dough (10 min after the start of mixing and also 12 min after reaching the maximum peak of the farinogram curve) was significantly affected by both study factors. The overall effect for the test year and the N treatment was 63 and 17%, respectively

Mean DS value for fertilizer treatments over the experimental years ranged from 39 to 76 BU. Lower softening values were measured in the dough obtained from the flour of the N100 and N150 conventional treatments (Table 8). Because the differences between trial years were large, the mean effect of treatment on trial years was not significant. The coefficient of variation was generally higher in the organic system than in the conventional system (range 20–42%; for conventional treatments 27–32%, Table 9).

Table 9. Coefficient of variation (%) of different dough quality characteristics averaged for the experimental years (2013–2017) in different fertilizer treatments. (Reproduced from Article **III**)

Quality characteristic	Org 0 *	Org I	Org II	N0	N50	N100	N150
WAC, %	3.2	2.6	3.2	2.2	2.6	3.3	1.5
DDT, min	40.1	30.7	42.5	44.6	36.7	41.3	15.6
S, min	47.6	36.8	45.9	34.7	46.1	29.5	22.4
DS, BU	41.9	20.2	37.4	29.9	31.8	32.5	27.4
DQN, mm	51.1	33.9	47.1	37.9	40.2	33.4	20.4
Average	33.0	22.8	32.2	26.8	29.8	26.6	16.6

WAC - water absorption capacity, DDT - dough development time, S - dough stability time, DS - dough softening degree, PC - protein content, DQN - dough quality number; * See explanation in Table 8.

DS values were positively correlated with GI ($r = 54$, $p < 0.001$), negatively correlated with grain PC ($r = -0.55$, $p < 0.001$), and with the smallest type C starch granules ($r = -0.32$, $p < 0.05$). The larger granules (types A and B) did not significantly affect the degree of softening of the dough.

According to the results of the alveograph, the baking strength (W), characterized by the force required to create a bubble was mostly influenced by the N treatment ($r = 0.50$, $p < 0.001$), followed by the weather of the experimental year ($r = 0.38$, $p < 0.05$). The force values required to create the bubbles in the organic and conventional systems ranged from 204–213 and 217–255 x 10^{-4} J, respectively, while the differences between the treatments were not significant. Higher numbers of this value were obtained in the years when the grain yield was higher, and the protein content was lower (2015 and 2017).

The dough quality number (DQN) was influenced by both study factors (cropping systems and weather conditions), while the annual weather conditions had a much stronger effect than the treatment (the proportion of the variation was 46 and 29%, respectively). In 2014, improved DQN values were obtained, with quality values ranging from 64 to 108 mm, followed by 2016, when quality values ranged from 59 to 77 mm. The lowest dough quality values were obtained in 2017, when these varied between 21 and 31 mm for all values, except for N150 (Figure 11). DQN was significantly dependent on N availability during the grain filling period and correlated positively with grain PC (r = 0.70, p <0.001; Table 8). The average dough quality value did not differ among treatments due to the large variability between the years (Table 10). The DQN value was found to be the result of a combination of several factors, and Table 9 shows the correlation coefficients between the DQN and the characteristic that significantly affected its value. In particular, the flour PC was crucial in the development of the DQN value, as these parameters significantly affected the properties of the dough. Type B and C starch granules affected DQN values in interaction with WAC and GI (Table 10).

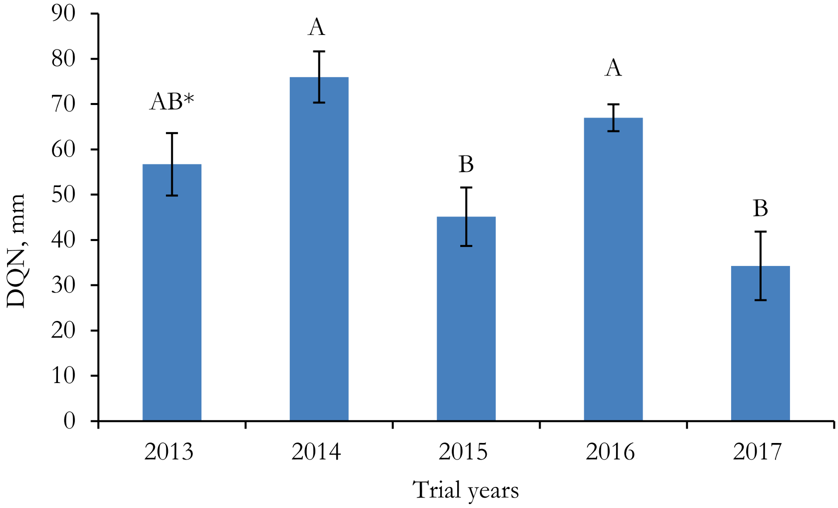

Figure 11. Average values of winter wheat (Triticum aestivum cv. Fredis) dough quality numbe r (DQN; mm) over fertilizer treatment. * Different letters indicate significant difference among years. (Reproduced from Article **III**)

Table 10. Correlation of dough quality number (DQN) with key dough characteristics. (Reproduced from Article **III**)

Factors	r	p
Dough stability (S)	0.94	$p < 0.001$
Degree of dough softening (DS)	−0.89	$p < 0.001$
Dough development time (DDT)	0.86	$p < 0.001$
Water absorption capacity (WAC)	0.73	$p < 0.001$
Grain protein content (PC)	0.70	$p < 0.001$
Gluten index (GI)	−0.56	$p < 0.001$
WAC x diameter of B- and C-type starch granules	0.33	$p < 0.05$
GI x diameter of B- and C-type starch granules	−0.32	$p < 0.05$

r - Pearson correlation coefficient; p- statistical significance.

5.3.4. Stability of the dough quality number

The results of this field study show that the rheological properties of the dough and its quality were significantly influenced by both study factors. The proportion of variation in DQN due to weather conditions and the effect of the fertilizer treatment was 46% and 29%, respectively. Both factors in turn affected the accumulation of protein and gluten in winter wheat grains and the size distribution of starch granules. Due to the significant variation in the rheological properties and quality values of the dough over the years, no significant differences were often found between the fertilizer treatments. The coefficient of variation for the different dough properties (Table 10) showed that the smallest variability between these values was for the N150 treatment of the conventional system, where WGC was highest, and GI was lowest (Table 8).

6. DISCUSSION

6.1. Nutrient balance and preservation of soil fertility

The aim of the experiment was to compare the efficiency of long-term use of composted manure and catch crops with the use of mineral fertilizers (with different N application rates) to maintain soil fertility during a high nutrient demand crop rotation. Changes in soil plant available P and K content over 10 years were considered.

The availability of N in the early growth phase of plant development is very important for the growth and development of crops and the level of yield. Earlier results from our long-term field experiment showed that N deficiency limited the yield of the organic system (Alaru et al, 2014). Two legumes in crop rotation, winter catch crops and well-composted cattle manure addition did not meet the N requirements of a five-field crop rotation in this organic system, as the availability of organic N was not always in line with the needs of the fast-growing crops (Alaru et al. 2014). Higher yields remove more nutrients from the soil. In this field study, crop yields were 25% higher in the conventional cropping than in organic cropping. Watson et al. (2002) reported that in organic farming systems, higher amounts organic fertilizers have increased soil microbial biomass and activity but have not necessarily resulted in higher yields.

Due to the higher yield the annual amounts of P and K removed from the soil of the conventional fertilized treatments were 28–35% and 28–40% higher, respectively, than for the organic treatments. Fertilization with mineral N resulted in a higher use of P and K, which is also in agreement with Káš et al. (2016). This can lead to a decrease in nutrient content in soils (Bhattacharyya et al. 2015). Over a ten-year period, P and K levels in the organic cropping system decreased by up to 24 mg kg^{-1} (72 kg P ha^{-1}) and 60 mg kg^{-1} (180 kg K ha$^{-1)}$ respectively. Although P and K outputs were lower in the organic system due to lower yields, the use of organic fertilizers did not increase the amount of (mobile) P and K available to plants in the soil. The largest annual consumer of P and K in this crop rotation was potato, followed by winter wheat. The amount of P and K removed by the potato tubers was 35% and 74% of the total amount of P and K removed during the crop rotation, respectively. Srinivasarao and Srinivas (2017) reported that tuber crops can remove up to 1000 kg K ha^{-1}. The choice of crops in rotation is very important to maintain soil fertility. In our field experiment, replacing potatoes with another crop would promote a less negative K-balance in organic cropping system. If soil fertility declines, the crop rotation should be reviewed at regular intervals. Srinivasarao and Srinivas (2017) reported that due to the high removal of nutrients from the soil with high yield and fertilizer-requiring varieties (potato in this study, for example), the P and K content of soils change rapidly.

The balance (input/output) of P and K was negative in all treatments in the organic system. The compilation of nutrient balances in organic farms often shows P and K deficits (Gosling and Shepherd 2005, Kirchmann et al. 2007). The PK balance for the conventional system showed that all fertilized treatments should have reserved enough P and K in the soil, however the actual K contents in the soil did not confirm this. Our results showed that during the 10-year experimental period, K content in the conventional treatments decreased up to 40 mg K kg^{-1} in the soil. Yadav et al. (2000) found that despite the annual addition of K to the recommended rate, the K content still decreased. This difference in the results may be because part of the K applied to the fertilized plants was either chemically immobilized by the soil particles or leached and, as a result, the K uptake by the plant decreased (Srinivasarao and Srinivas, 2017). The P balance showed that the amount of P should have increased from 7.5 to 8.5 kg ha^{-1} every year, but the actual results showed that after ten years of cultivation, soil P content in the soil of fertilized treatments of conventional copping system was similar to that in the beginning (Figure 7). P is a particularly problematic element in Estonian mineral soils, because considering the pH of our soils, the optimal pH range of P absorbed by plants is narrow (Roostalu 2012). According to Berry et al. (2003), the P deficit is small if it is <10 kg ha^{-1} per year. A K deficit is large if it exceeds 50 kg ha^{-1} per year. In the beginning of the experiment, the need for soil P fertilizer was average and did not change over the ten years of cultivation, but the demand for K fertilizer increased to above the average (Kanger, 2017). It is important to monitor these changes so that P and K do not become a limitation on crop production.

Káš et al. (2016) found that long-term fertilization with mineral and organic fertilizers affected yield and overall nutrient removal from the field and led to many changes in the soil. After 10 years of using mineral fertilizers in a conventional cropping system, the soil became more acidic in all treatments, i.e., the pH values decreased by up to 0.5 units. In Estonian climatic conditions, where precipitation exceeds evaporation, many soil types are characterized by cation leaching and acidification over time (Järvan and Vettik, 2016). After 10 years of using cattle manure in the organic version, the soil pH became less acidic, which was also expected because the pH values of the used manure ranged from 6.6 to 8.3. Gosling and Shepherd (2005) and Kirchmann et al. (2007) found that crop rotations that relied heavily on legumes in organic farming acidified the soil faster than crop rotations with fewer legumes. Our crop rotation included only two legumes (red clover and field pea). It would be interesting to conduct another field trial with more legumes in the rotation to see their effect on pH.

6.2. Size of starch grains

In the changing weather conditions of Estonia (alongside the Baltic Sea), the yield and quality of winter wheat is variable among years, as has been the case in other wheat-producing countries (Rossini et al., 2018; IPCC, 2012; Campbell et al., 1981; Garrido-Lestache et al., 2005; Barraclough et al. 2010).

The baking quality of wheat flour is better if the number of large starch granules (type A) is higher than that of the smaller ones (types B and C), resulting in a larger volume of loaf. It is influenced by the structure of the starch granule, i.e., the size and ratio of amylose and amylopectin molecules in the starch granules (Kihlberg et al. 2004).

The results of our study showed that the size distribution of starch granules was significantly affected by weather conditions during the grain filling period after winter wheat flowering. The average diameters of type C, B and A starch granules varied from 2.7 to 3.2, 6.1 to 8.2 and 15.8 to 23.5 µm, respectively, in different experimental years. The size of type C starch granules was significantly smaller in 2013 and 2017 ($p < 0.01$) (Table 5), because the average air temperature from the wheat growth phase BBCH65 (i.e. flowering) to PhM (physiological maturity) was 0.5–3.5° C higher and 3 – 5 days longer than in other test years. The lower diameter of the C-type granules in these years was probably due to their higher abundance in starch, as they are formed later in the filling period of the grain. An increase in the number of starch grains is usually accompanied by a decrease in their diameter (Edwards, 2010). Hurkman et al. (2003) reported that a shorter grain filling period reduces the levels of enzymes involved in starch biosynthesis. Our results showed that the length of the grain filling period was negatively correlated with the mean air temperature and GDD values during this period. Acevedo et al. (1991) found that the average air temperature did not significantly affect the filling time of the grain. Heat stress during the filling period of cereals mainly affects the mobility of assimilates, the translocation of photosynthetic products into the grain and the synthesis and accumulation of starch in the grain. The effect of weather conditions on the length of the filling period of the grain needs further investigation. The effect of precipitation was not significant.

Fertilization with different amounts of N in different cropping systems significantly affected the diameter of the starch granule, which is also in accordance with Li et al. (2013). Larger diameter values were obtained from Org II, N100 and N150. In the Org II treatment, well-composted cattle manure was given to winter wheat during the tillering phase (first decade of May), and it is likely that N mineralization and further uptake by plants took place in June and July (winter wheat flowering and grain filling period). Conventional treatments of N100 and N150 were fertilized twice with mineral N (a second time in BBCH 47), which was likely to increase the diameter of the starch granules.

In this study, the size of the C- and B-type granules of starch were negatively affected by the above-ground biomass yield of winter wheat (Table 3). In conventional cropping system, fertilization with mineral N yielded up to 1.6 times higher biomass during flowering (BBCH65) than in organic cropping system. The stems and leaves of the plant are temporary sites of carbon storage that can be mobilized into reproductive tissues, contributing to grain filling in later growth phases (MacNeill et al. 2017). Smidansky et al. (2003) found that for cereals, higher biomass increases grain yield, which in turn increases seed survival rate (i.e., decreased embryo abortion) rather than starch content per seed.

The yield and quality of wheat flour depends on the size of the starch granules (Edwards, 2010), the type of cropping system and the proportion of endosperm in the grain (Bechtel, 2003; Dziki et al. 2014). This study showed that the total flour and fine flour yields were mainly influenced by weather conditions and the combined effect of weather conditions and farming practices. The higher ratio of the yield of whole flour to bran and shorts was due to the smaller diameter of the starch granules, i.e., the longer filling time of the grains in years.

The yield of fine flour during milling was higher in years when the filling period of winter wheat grain was longer (2013 and 2017), and the yield of fine flour was negatively correlated with the size/diameter of starch granules. The smaller diameter of the starch granules also indicates a higher number of smaller starch granules, and those findings are similar to Edwards (2010).

6.3. Properties of the dough

When wheat flour is mixed with water, the natural hydrocolloid gluten reacts with water molecules to form a wheat dough, whose gaps in the three-dimensional gluten network are filled with starch granules (Gao et al. 2020). Gluten traps the gases generated during the fermentation process, which causes the dough to rise, while starch-gluten interaction has a significant effect on the rheological properties of the dough. It has been shown that the specific interaction of gluten and starch in the microstructure of wheat dough affects the processes that take place in the dough (Jekle et al. 2016). Therefore, separate determination of the properties of separated gluten and starch may not fully explain the properties of wheat dough (Gao et al. 2020). The present study evaluated the effects of mineral and organic nitrogen fertilizers on the gluten content of wheat dough, the size of starch granules and the complex effect of starch-gluten on the rheological properties of the dough. The effect of weather conditions on the stability of dough quality was also assessed.

The quality of the dough was assessed on the basis of its rheological properties and the results showed that the quality depended on a combination of factors but mainly on protein and gluten quantity and quality, which in turn depended on both test factors (weather conditions during the test years and the availability of treatment, i.e., availability of mineral N or organic N during the filling period of the cereals). The protein and gluten content and subsequently the dough quality was better in 2014 and 2016, when the grain yield was lower, which is also in line with the findings of Giancaspro et al. (2019). The average protein and gluten content in the experimental years was highest in the flours obtained from the N-treatment N150. Gluten plays a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity and elasticity on dough (Wieser 2007).

Water absorption capacity (WAC) is one of the indicators of higher quality dough, which also refers to smaller diameter starch granules in flours (Cao et al. 2019; Gao et al. 2020). Previous studies have shown that smaller diameter starch granules have a higher surface area/volume ratio and are able to bind more water than larger granules and swell the dough more efficiently (Chiotelli and Le Meste, 2002; Vasanthan and Bhatty, 1996). Therefore, the increase in the number of small granules should increase the water binding capacity of the flour (Li et al. 2013; Soh et al. 2006). In our experiment, however, the WAC values correlated positively with the diameter of the type B and C starch granules (larger diameter indicates a smaller number of granules; Edwards, 2010). The highest WAC values were obtained in this experiment when the diameter of the smaller types of granules was between 3.2 and 9.0 µm, i.e., the WAC was larger when the diameter of the smaller starch grains was slightly higher than the average of each (measured for types C and B; the diameter of the starch granules ranged from 2.45 to 9.84 µm). WAC values were much greater for N100 and N150, mainly due to higher protein and gluten levels. At higher gluten contents, its network gaps are smaller and the contact between gluten and starch grains is closer (Zi et al., 2019). At lower gluten contents, the gaps in the gluten network are larger, in which case the gluten-starch contacts rely mainly on the starch granules with a larger diameter. In this field experiment, the calculated variance showed that N treatments had a stronger effect on WAC values as compared to weather conditions. Wheat flour with a WAC value of more than 58% was classified as strong flour (Kassomeh, 2019).

Dough formation and stability lasted longer*,* and the degree of dough softening was lower in the N100 and N150 treatments due to higher protein content (PC) and wet glutein content (WGC). The stability time of the dough was about 2 minutes longer in these treatments than in the other treatments. These results are consistent with the results of Gao et al. (2020) because the microstructure of dough, which has higher PC and WGC, has smaller holes and fewer gaps between starch and gluten. Gao et al. (2020) found that wheat flours with low PC and WGC may contain larger holes of irregular shape. Zi et al. (2019), discussing the effect of starch granules on dough stability, found that the larger diameter and smaller number of starch granules indicate poorer processing properties of the dough, which in turn is due to the heterogeneity of the gluten network voids. In this experiment, the stability of the dough was shorter in the organic and N0 and N50 treatments, because the WGC was lower in these treatments; this was probably due to the larger gaps in the irregular shape of the gluten network, which makes such dough unstable. According to Kulhomäki and Salovaara (1985), the dough stability time of 4–12 min shows good dough quality. In our experiment, the dough stability times in the organic treatments and in the N0 and N50 treatments were close to the lower stability range, probably due to the irregular and insufficient accumulation of nitrogen in the grains during the different grain filling periods of the experimental years. This resulted in lower protein and gluten levels, which was often lower than required. The unstable availability of N in these treatments also caused great variability in the quality of the dough.

According to Kassomeh (2019), the dough is weak if DDT < than 2.5 min, medium (2.5–4.0 min), and strong (4.0–8.0 min). DDT was positively correlated with dough quality number ($r = 0.86$, $p < 0.001$).

The gluten index (GI) characterizes the ratio of gliadins to glutelins. GI, dough formation time, and stability are all critical parameters that reflect dough strength, and higher values of these parameters characterize stronger dough (Uthayakumaran et al. 2013; Yegin et al. 2018). It has been suggested that the content of gliadins affects the viscosity of the dough and the content of glutelins affects the elasticity of the dough (Khatkat and Schofield, 1997; Szafranska and Stepniewska, 2021). Oikonomou et al. (2015) found that GI values are positively correlated with protein content, which in turn is affected by the amount of N fertilizer. Our experiment showed the opposite results - GI values were negatively correlated with the protein content and the amounts of N fertilizers used, which is in line with the results reported by Borkowska et al. (1999); higher GI values were associated with lower PC values in most treatments (above 80% and 11.2–11.6%, respectively; Table 8), except for N150, where the mean GI was 73 ± 3.5% and the PC was up to 2.4% higher than in other treatments. GI values above 80% indicate an increase in the proportion of glutenins, i.e., an increase in the stretch resistance of the dough (Uthayakumaran et al. 2013). Too strong wheat dough does not rise well and does not become airy. Due to higher PC and WGC, GI average values in 2014 and 2016 were up to 9% lower than in other years.

The quality of the dough was affected by several factors, with the N fertilization regime having the greatest effect. Our results confirmed results of Draghici et al. (2011) that organic dough has lower quality. In our experiment, dough with a higher DQN was obtained from flours with lower GI values (N150, GI = 73%; Table 8) and medium-diameter type B and C starch granules. As said before and reported in the literature (Cao et al., 2019) that closer contact between gluten and starch grains occurs when type B and type C granules are smaller in diameter and larger in number; in general, this pattern works for higher PC and WGC flours with fewer gaps and smaller gaps in the gluten network. In this field experiment, the levels of PC and WGC in the flours were relatively low (Table 8), indicating larger gaps in the gluten network and therefore closer contact between gluten and starch could occur with larger diameter starch granules. With a constant protein content and an optimal gluten-gliadin ratio, the dough was found to have a longer stability time, a lower level of softening and a higher dough quality (Table 8).

The experimental results showed that the quality of the dough varied more in the organic treatments, which is in line with Oikonomou et al. (2015) results. The amount of 150 kg ha^{-1} of mineral N applied in two sections before flowering ensured stable plant growth and protein and gluten content in different years. Xue et al. (2016) reporter that N splitting changed grain protein composition by enhancing the percentages of gliadins and glutenins as well as certain high molecular weight glutenin subunits (HMW-GS), which led to an improved baking quality of wheat flour. In this trial the treatments fertilized with smaller amounts of N and organic treatments (fertilization with manure or use of winter cover crops) were more sensitive to changing weather conditions than N150. The amount of organic N available to plants was significantly affected by the distribution of precipitation and temperatures during the growing season, which caused greater variability in results over the years. In the organic treatments the average variability of different dough properties during the experimental years (Org 0, Org I and Org II) was 1.4–2.0 times higher than in the N150 treatment. The different weather conditions in the experimental years (2013–2017) were also reflected in the proportion of experimental factors and changing weather conditions resulted in highly variable results in winter wheat yield, protein, and gluten content

(Article **II**) and thus dough rheological properties. Oikonomou et al. (2015) reported that higher values of the coefficient of variation indicate stronger relationships between the treatment and weather conditions. The high variability of the N50 treatment protein content indicates that this amount of mineral N was not always sufficient for complete or optimal protein accumulation during the grain filling period throughout the experimental years.

CONCLUSIONS

This 10-year comparison between organic (winter cover crops and with and without well-composted manure) and conventional (with different mineral fertilizer rates) systems showed that: 1) the total yield of a five-field crop rotation was 25% higher for conventional fertilizers than for organic treatments; 2) the soil became more acidic in the conventional cropping system due to the long-term use of mineral fertilizers, the use of liming can be a good solution for raising the pH; 3) during the 10-year conventional cropping system experiment, the P content in the soil remained unchanged and the K content decreased by up to 40 mg K kg^{-1}. In the conventional cropping system treatments, the annual amounts of P and K removed were 12–18 kg P ha^{-1} and 45–73 kg K ha^{-1}, which were 28–35% and 28–40% higher, respectively, than in the organic treatments due to higher yields; 4) the use of winter crops and well-composted cattle manure did not maintain the basic levels of P and K in the soil, thus it is important to monitor the nutrient balance, especially in organic farming, so that soil fertility does not decrease. One way to prevent the depletion of soil nutrient reserves is regular adjusting of crop rotation considering nutrient requirements of different crops; 5) the weather conditions during the grain filling period of winter wheat cv. Fredis had a strong effect ($p < 0.001$) on the grain size distribution of starch granules; 6) the size of the starch granules was not affected by the cropping system; 7) fertilization with manure and twice with higher mineral N rate during the growing season increased the average diameter of the starch granules. The overall average diameter of the starch granules was smaller in years when grain filling period was longer, due to a higher proportion of type C granules in those years; 8) the flour yield of winter wheat grains was affected by the size distribution of starch granules. The increased proportion of smaller diameter granules (type C granules) increased the yield of fine flour. The effect of cropping systems on the yield of fine flour was not significant.

Taken together, these analytical results for winter wheat flour dough show that of the seven treatments tested, fertilization with the highest mineral N level (150 kg/ha^{-1}) resulted in the highest quality dough. On the other hand, organic cropping, even with added manure, is still more vulnerable and this sensitivity is reflected in the quality of the dough. Future research on different crop rotations and different organic fertilizers should focus on soil nutrient content to investigate how soil/root uptake mechanisms could be influenced to stabilize plant growth over the years and to improve the yield quality. The interaction between gluten and starch granules in wheat dough grown in organic and conventional systems also needs to be further investigated. In addition, in the context of the increased organic production, it is important to explore other solutions in the bread making value chain, that could compensate for the variability in the quality of cereals and improve the quality of wheat dough.

In addition to the fertilization regime, local weather conditions significantly affected the N availability and protein content of thewheat grains.

This knowledge is valuable for farmers and industry alike. Protein is important for endproduct quality, however, the challenge remains, how to achieve high protein content, when the prices of mineral fertilizers have increased, and the overall availability is limited.

Future research should study the effect of liming on increasing the pH and how this, in turn, will affect the soil quality and crop yield and dough quality. As it was shown that organic cropping did not quarantee enough protein for good quality dough, then there is the need to study different options to increase protein content in organic cropping system. Wheat quality analysis could focus on nitrogen rate and cropping system impact on other parameters, such as beta-glucan and arabinoxylan. Furthermore, gluten-starch interaction should be studied in different spring-and winter wheat varieties.

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SUMMARY IN ESTONIAN

KOKKUVÕTE EESTI KEELES

Sissejuhatus

Tänapäeva põllumajanduse üks suurimaid väljakutseid on lahendada probleem, kuidas loodussäästlikult tootes tagada kultuuride võimalikult suur saagikus (Ricroch et al. 2016). Loodussäästlikuks tootmisviisiks peetakse maheviljelust ja viimastel aastakümnetel on selle levik Euroopas kiiresti kasvanud (EC 2014; Eurostat, 2019).

Mahepõllumajanduse eesmärk on toota keskkonnasõbralikult piisavas koguses kõrge toiteväärtusega toitu. Samas loetakse mahepõllunduse üldiseks puuduseks põllukultuuride ebaühtlast ja suhteliselt madalat saagikust, mida põhjustab eelkõige lämmastiku (N) ebastabiilne kättesaadavus orgaanilisest lämmastikväetisest taimede varastes kasvufaasides. Tootmises kasutatavast viljelusviisist sõltub suurel määral ka mullaviljakuse tase, mis omakorda mõjutab põllukultuuride saagikust. Antud töös uuriti, kas maheviljeluses pikaajaline talviste vahekultuuride kasvatamine ja väetamine sõnnikuga on suutnud vältida mulla viljakuse langust.

Põllukultuuridest valiti uurimisobjektiks nisu (*Triticum aestivum* L.), sest nisu on üks tähtsamaid teravilju maailma toidu- ja söödatööstuses ning 75–78% tema kogutoodangust on mõeldud toiduks inimestele (Psaroudaki, 2007). Nisu terade füüsikalised, keemilised ja tekstuurilised omadused määravad nisujahust saadava lõpptoote kvaliteedi. Talinisu terade tekstuuri mõjutavad mitmed tegurid nagu viljelusviis, sort, kasvukoht ja ilm talvitumisjärgsel perioodil (Krejčířová, 2006). Eestis on aastatega talinisu kasvatamine muutunud populaarsemaks nii mahe- kui ka tavaviljeluses.

Nisutaime N toitumine (väetamine orgaanilise või mineraalse N-väetisega) on tema jahust saadava taina reoloogiliste omaduste kujunemisel ülioluline. Kuna nisu tainas koosneb mitmest jahukomponendist (peamiselt gluteen ja tärklis), siis teeb see nende kompleksmõju hindamise keeruliseks (Cao et al. 2019). Kui taina reoloogilisi omadusi on hinnatud seni enamasti eraldi kas tema gluteeni- või tärklise sisalduse kaudu, siis antud uurimustöös hinnatakse tärklise graanulite ja gluteeni vastastikust koostoimet nendele omadustele. Uurimustöös selgitatakse, mis mõjutab tärklise graanulite suurust/läbimõõtu ja milline on madala gluteeni sisalduse tingimustes tärklise-gluteeni võrgustikus nende koostoime nisu taina kvaliteedile.

Kuna mahepõllumajanduslik tootmine muutub laialdasemaks (Eurostat, 2019), on vaja täpsemalt selgitada, mil määral mõjutab orgaaniline väetamine gluteeni kogunemist ja tärklise graanulite moodustumist tera täitumise ajal.

Antud doktoritöös (uurimuses) käsitletakse orgaaniliste või mineraalsete N-väetiste pikaajalist mõju mulla viljakusele üsna suure toitainete vajadusega viieväljalises külvikorras (täpsemalt mulla fosfori- ja kaaliumisisaldusele ning pH tasemele), talinisu terasaagi kujunemisele ning saadava lõpp-produkti, st nisutaina kvaliteedile.

Uurimustöö eesmärgid ja hüpoteesid olid järgmised:
A. Hinnata mahe- ja tavaviljeluse pikaajalist mõju:
1) viieväljalise külvikorra kogusaagile;
2) mulla pH-le, mulla liikuva P ja K sisaldusele, mullaviljakuse säilimisele (läbi talviste vahekultuuride ja hästi komposteeritud sõnniku pikaajalise kasutamise)

B. Erinevate viljelusviiside ja ilmaolude (õhutemperatuur ja sademed) mõju:
3) tärklise graanulite suuruse jaotusele;
4) täisterajahu ja peenjahu saagile;
5) gluteeni-tärklise koostoimele nisutaina omaduste kujunemisel;
6) talinisu taina reoloogiliste omaduste stabiilsusele ja kvaliteedile.

Hüpoteesid:

1. Kogusaak tavapõllumajanduslikus viieväljalises külvikorras on ligikaudu veerandi võrra kõrgem kui mahepõllumajanduslikus külvikorras (Artikkel **I**)
2. Talviste vahekultuuride ja hästi kompostitud sõnniku pikaajaline kasutamine maheviljeluses parandab mullaviljakuse taset (Artikkel **I**)
2. Mahenisu tärklise graanulite üldine läbimõõt on väiksem kui tavaviljeluse nisul (Artikkel **II**)

3. Tärklise graanulite suuruse jaotus sõltub tera täitumisperioodi pikkusest (Artikkel **II**)
4. Viljelusviis mõjutab jahu saagikust (Artikkel **III**).
5. Mineraallämmastikuga väetatud (tavaviljelus) variantidelt saadud nisu tainas on kõrgema ja stabiilsema kvaliteediga (Artikkel **III**).

Katseskeem

2008. aastal rajati Eesti Maaülikoolis (58°36’ N, 26°66’ E; Tartu lähedal) viieväljaline külvikorrakatse (Joonis 1), milles võrreldakse mahe- ja tavaviljeluse mõju põllukultuuride saagikusele, saagi kvaliteedile ja ka mulla omadustele ning mulla viljakuse säilimisele (Joonis 1). Külvikord koosneb põllukultuuridest: suvioder (*Hordeum vulgare* L.) punase ristiku allakülviga – punane ristik (*Trifolium pratense* L.) – talinisu (*Triticum aestivum* L.) – põldhernes (*Pisum sativum* L.) – kartul (*Solanum tuberosum* L.). Muld on näivleetunud (Stagnic Luvisol; IUSS WG WRB 2015) (WRB, Deckers jt 2002), (kerge liivsavi, C 1,38% ja N 0,13%, pHKCl 6,0). Põldkatse väetisvariandid paiknevad süstemaatiliste plokkidena üksteise kõrval neljas korduses, kusjuures mahesüsteemis on kolm varianti (10 x 6 m; Org 0, Org I ja Org II Joonis 1; artikkel **I**) ja tavaviljeluse süsteemis on nelja erineva lämmastiku normiga [N0, N1, N2, N3 (artikkel **I**); N0, N50, N100, N150 (artikkel **II**, **III**)] väetisvarianti. Orgaanilise väetisena kasutati hästi komposteeritud veisesõnnikut ja talviseid vahekultuure, mineraalse lämmastikväetisena kasutati ammooniumnitraati - NH_4NO_3). Variant N0 on tavasüsteemi kontroll ilma mineraalväetisteta, kuid pestitsiididega. Ülejäänud kolmel tavaviljeluse variandil N1, N2 ja N3 kasutati külvil P- ja K-väetisi vastavalt normiga 25 ja 95 kg ha^{-1} (P ja K kogused olid kõikides variantides sarnased, kasutati Kemira ja Yara Mila väetisi). Artiklis **I** käsitletakse külvikorra kogusaaki ja seal on tavasüsteemi väetisvariantide märgistus järgmine: N1 = 40–50 kg N ha^{-1}; N2 = 80–100 kg N ha^{-1}; ja N3 = 120–150 kg N ha^{-1}, kusjuures madalamat N-normi kasutati odra puhul, millel oli punase ristiku allakülv; punane ristik üksi ei saanud mineraalväetisi. Hernes sai mineraalset N-i 20 kg N ha^{-1} N1, N2 ja N3 variandis.

Tavaviljeluse väetatud variantides N100 ja N150 anti N-väetis kahes jaos: N100 variandi puhul anti 50+50 kg N ha^{-1} ja N150 variandi puhul 100+50 kg N ha^{-1}. Esimene andmisaeg oli teravilja võrsumisfaasis varakevadel ja teine kord loomiseelses faasis (BBCH 47). Mahesüsteemi kontrollvariant oli Org 0 oli ilma orgaaniliste väetisteta, variandis Org

I kasutati talvel haljasväetisena vahekultuure: pärast talinisu, kartuli ja hernesaagi koristamist külvati vastavalt rukki (*Secale cereale* L.) ja talirapsi (*Brassica napus* ssp. *oleifera* var. *biennis*) segu, rukist ja talirapsi. Vahekultuurid künti mulda nii kiiresti kui võimalik pärast lume sulamist, tavaliselt aprillis. Kolmandas mahevariandis Org II lisati külvikorra esimese rotatsiooni jooksul üks kord hästi komposteeritud veisesõnnik (40 t ha^{-1}) enne kartulit. Sõnnik künti pinnasesse 20–23 cm sügavusele septembri lõpus või oktoobri alguses, enne kui külvati vahekultuurina taliraps. Külvikorra teise rotatsiooni ajal (2013–2017) muudeti sõnniku andmise režiimi: esimene andmine külvikorras oli enne odra külvamist normiga 10 t ha^{-1}, teine kord toimus varakevadel enne talinisu kasvu algust normiga 10 t ha^{-1} ja kolmas kord enne kartuli mahapanemist normiga 20 t ha–1. Sõnnikuga mulda viidud N, P, K kogused on toodud artiklis **I** tabelis 1.

Muutusi mulla toitainetes vaadeldakse ajavahemikus 2008–2017, st kahe rotatsiooniperioodi jooksul.

Tulemused ja kokkuvõte

Antud uurimus baseerus kahefaktorilisel (viljelusviis – mahe- ja tavaviljelus ning aasta) pika-ajalisel katsel, kus võrreldi viljelusviiside mõju mullaviljakusele, talinisu saagikusele, talinisu terades tärkliseterade kujunemisele ja proteiini akumuleerumisele, lisaks uuriti tekkinud gluteeni-tärklise võrgustiku koostoimet taina kvaliteedile. Võrdlustulemused viljelusviiside vahel (maheviljeluses kasutatud talvised vahekultuurid ja hästi komposteerunud sõnnik ning tavaviljeluses mineraalväetised ja pestitsiidid) näitasid, et: 1) suure toitainetevajadusega viieväljalise külvikorra kogusaak oli mineraalväetiste toimel keskmiselt 25% kõrgem kui maheviljeluses; 2) muld muutus tavaviljeluses happelisemaks pikaajalise mineraalväetiste kasutamise tõttu. Lupjamise kasutamine võib olla hea lahendus pH tõstmiseks; 3) 10-aastase katseperioodi jooksul tavaviljeluse variantide mullas ei muutunud taimele kättesaadava P sisaldus, samas K sisaldus vähenes kuni 40 mg K kg kuiva mulla kohta. Tavasüsteemi variantidel olid sõltuvalt kultuuride saagikusest eemaldatud P ja K aastased kogused 12–18 kg P ha^{-1} ja 45–73 kg K ha^{-1}, mis olid vastavalt 28–35% ja 28–40% suuremad kui mahevariantides. Suuremad eemaldatava P ja K kogused olid põhjustatud suuremast saagikusest; 4) talivahekultuuride ja hästi kompostitud veisesõnniku kasutamine ei säilitanud mullas P ja K baastasemeid, mistõttu on oluline eriti maheviljeluses jälgida toitainete bilanssi, et mullaviljakus ei langeks. Üheks võimaluseks, et vältida mulla toitainetevarude ammendumist, on aeg-ajalt külvikorda muuta.

Viljelusviisi mõju talinisu cv Fredise tärkliseterade suurusele/diameetrile ei olnud märkimisväärne, küll aga mõjutasid seda usutaval määral ($p < 0,001$) ilmastikutingimused katseaastatel. Samas kummagi viljelusviisi/viljelussüsteemi üksikud väetisvariandid, st väetamine mahesõnnikuga (Org II) ja väetamine mineraalse lämmastikväetisega nisutaimede kasvuperioodil kaks korda (N100+50) suurendas oluliselt tärklise graanulite keskmise läbimõõdu väärtust. Lämmastikväetise teistkordne andmine talinisu loomiseelses faasis (BBCH 47) suurendab toitainete liikumist taimede maapealsest biomassist teradesse, millega kaasneb nii proteiini kui ka tärklise koguse suurenemine terades ja A- ning B-tüüpi tärklise graanulite läbimõõt. Ilmaoludest tingituna oli tärklisegraanulite üldine keskmine läbimõõt oli väiksem nendel aastatel, kui terade täitumisperiood oli pikem, kuna neil aastatel oli suurem C-tüüpi graanulite osakaal. Talinisu terade jahu saagikust mõjutas tärklise graanulite suuruse jaotus, samas kui väiksema läbimõõduga graanulite (C-tüüpi graanulite) suurenenud osakaal suurendas oluliselt peene jahu saagikust. Viljelussüsteemide mõju peenjahu saagikusele ei olnud märkimisväärne.

Taina kvaliteeti mõjutasid mitmed tegurid, kusjuures lämmastiku väetamise režiimil oli määrav mõju. Antud eksperimendis saadi kõrgema DQN-iga tainas jahudest, millel olid madalamad GI väärtused (N150, GI = 73%; tabel 8) ja keskmise läbimõõduga B- ja C-tüüpi tärklise graanulid. Kirjanduses (Cao et al., 2019) on väidetud, et tihedam kontakt gluteeni ja tärkliseterade vahel tekib juhul, kui B- ja C-tüüpi graanulite läbimõõt on väiksem ja nende arv suurem; üldiselt toimib see seaduspärasus jahu kõrgema PC ja WGC puhul, kui gluteeni võrgustikus on tühikuid vähem ja nad on väiksemad. Antud põldkatses oli jahude PC ja WGC tase suhteliselt madal (Tabel 8), mis viitab suurematele tühikutele gluteeni võrgustikus ja seetõttu võis tihedam gluteeni- ja tärklisevaheline kontakt tekkida suurema läbimõõduga tärkliseterade puhul. Piisava proteiinisisalduse (>13%) ja optimaalse gluteniini-gliadiini suhte korral (73%) on taina stabiilsusaeg on pikem, pehmenemise tase madalam ja taina kvaliteet kõrgem.

Katsetulemused näitasid, et taina kvaliteet varieerus enam mahevariantides. Mineraalse N-i 150 kg ha^{-1} kogus, mida anti kahes jaos enne õitsemist, tagas taimede stabiilse kasvu ning valgu- ja gluteenisisalduse erinevatel aastatel. Väiksemate N kogustega väetatud variandid ning mahevariandid (väetamine sõnnikuga või talviste kattekultuuride kasutamine) olid muutuvate ilmastikutingimuste suhtes tundlikumad kui variant N150. Taimedele kättesaadava orgaanilise N-i kogust mõjutas oluliselt sademete ja temperatuuride jaotumine kasvuperioodil, mis põhjustas tulemuste suurema kõikumise aastate jooksul; mahevariantides oli erinevate tainaomaduste keskmine varieeruvus katseaastate jooksul (Org 0, Org I ja Org II) 1,4–2,0 korda kõrgem kui N150 variandis. Katseaastate (2013–2017) erinevad ilmaolud peegeldusid ka katsefaktorite mõju osakaalus ja muutlikud ilmastiku tingimused põhjustasid aastati väga varieeruvaid tulemusi talinisu teraviljasaagis, proteiini- ja gluteenisisalduses (vt täpsemalt artikkel **III**) ja seega ka taigna reoloogilistes omadustes. Oikonomou jt (2015) leidsid, et variatsioonikoefitsiendi kõrgemad väärtused näitavad tugevamaid seoseid variandi ja ilmastikutingimuste vahel. Variandi N50 proteiinisisalduse suur varieeruvus näitab, et selline mineraalse N-i kogus ei olnud alati piisav proteiini täielikuks või optimaalseks kogunemiseks tera täitumise perioodil kõigi katseaastate jooksul.

Kokkuvõtvalt näitavad talinisu jahust tehtud taina analüütilised tulemused, et seitsmest testitud variandist on kõrgeima mineraalse lämmastikunormiga (150 kg/ha^{-1}) väetamine kvaliteetse taigna saavutamiseks oluline. Teisest küljest on maheviljelus, isegi lisatud sõnnikuga, endiselt haavatavam ja see tundlikkus peegeldub taina kvaliteedis. Tulevased uuringud erinevate külvikordade ja erinevate orgaaniliste väetiste kohta peaksid keskenduma mulla toitainete sisaldusele, et uurida, kuidas mulla/juure omastamise mehhanisme saaks mõjutada, et muuta taimede kasv ühtlasemaks ja aastati stabiilsemaks ja et see kajastuks ka proteiini-tärklise koosmõjus. Lisaks on mahepõllumajandusliku tootmise suurenemise kontekstis oluline uurida saiavalmistamise väärtusahelas muid lahendusi, mis võivad seletada teravilja kvaliteedi varieeruvust ja parandada nisu taina kvaliteeti.

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I

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Long-term effect of farming systems on the yield of crop rotation and soil nutrient content

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The effects of organic (manure, cover crop) and mineral fertilisers on total yield, soil phosphorus (P) and potassium (K) dynamics and soil pH changes were studied over 10 years. Five field crops (spring barley, red clover, winter wheat, field pea, potato) were grown organically and conventionally in rotation. The total yield of the five crops fertilized similarly was 24–25% higher in conventionally fertilised treatments than in organic treatments. The higher yielding conventionally fertilised treatments (annual total yield 29.0–29.8 t ha^{-1}) removed 12–18 kg ha^{-1} P and 45–73 kg ha^{-1} K per year, which was respectively 28–35% and 28–40% higher than organic treatments. The soil became more acidic in the conventional system (pH 5.4–5.9 versus 5.9-6.3). The highest annual P and K uptake was by potato, followed by winter wheat. Use of winter cover crops and composted cattle manure in the organic system did not maintain the levels of P and K in the soil at baseline.

Key words: total yield, farming system, organic, conventional, manure, cover crop

Introduction

Agriculture faces many challenges if it is to maximize yields while operating in an environmentally sustainable manner (Ricroch et al. 2016). One of the key challenges is maintaining soil fertility which is fundamental in determining the productivity of all farming systems. Optimisation of the nutrient cycling of agro-ecosystems and development of a suitable fertility strategy is a serious challenge for farming systems. High yields and intensive cropping make significant demands for nutrients from the soil, which leads to depletion of reserves (Murugappan et al. 2007). To improve the biological, chemical and physical properties of the soil, crop rotation, winter cover crops and composted manure can be used to maintain soil organic matter and fertility (Baldwin 2006, Doltra and Olesen 2013).

In past decades, organic farming has increased rapidly in Europe (EC 2014). The aim of organic agriculture is to produce food of high nutritional quality, in sufficient quantity and in an environmentally friendly way. Comparing organic with conventional farming, a fundamental difference between their management is the way in which challenges are addressed. Organic farming systems are designed with the aim of maintaining nutrients in organic reservoirs or in bioavailable mineral forms instead of supplying nutrients through frequent fertiliser additions. This is achieved by cycling nutrients through organic reservoirs (Wander 2015**).** The results of several long-term studies have shown that the addition of compost improves soil physical properties by decreasing bulk density and increasing the soil water holding capacity (Weber et al. 2007). To improve the biological, chemical and physical properties of the soil, crop rotation, winter cover crops and composted manure are used to maintain soil organic matter and fertility (Baldwin 2006, Doltra and Olesen 2013). Moreover, in comparison with mineral fertilisers, compost produces significantly greater increases in soil organic carbon and delivers a wider range of plant nutrients (García-Gil et al. 2000, Bulluck et al. 2002, Nardi et al. 2004, Weber et al. 2007). Long-term beneficial effects of composted materials are also observed in soil humic substances, as well as in soil sorption properties (Weber et al. 2007). Conventional fertiliser management guidelines are based on assessments of plant-available nutrients in the soil. Crop nutrient uptake and crop yields are the principal factors that determine optimal fertilisation practices (Ju and Christie 2011). Therefore, it is very important to apply fertilisers in an efficient way to minimize loss and to improve the efficiency of nutrient use (Li et al. 2009).

Several articles have dealt with the long-term effects of organic and conventional cultivation on soil microbiological activity (Fliessbach et al. 2000, Mäder et al. 2000, Oehl et al. 2003, Esperschütz et al. 2007), but less research has been published on changes in soil phosphorus (P) and potassium (K) content and pH after long-term organic cultivation (Gosling and Shepherd 2005, Kirchmann et al. 2007, Kaš et al. 2016). This article discusses the long-term effects of organic and mineral fertilisers on soil P, K and pH level dynamics in organic and conventional farming systems over 10 years. We investigated whether long-term winter cover crop cultivation and fertilisation with manure

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have been able to prevent a decrease in soil P and K levels and to improve the soil pH in a crop rotation that has quite a high nutrient requirement. P and K in soils are present in different fractions some of which are more available to plants than others (Kulhánek et al. 2009, Vanden Nest et al. 2015, Srinivasarao and Srinivas 2017). Soil pH has also an effect on the availability of P and K to plants. The most mobile and plant-available fraction of P and K is soil solution P and K, followed by exchangeable P and K, fast release fixed P and K, and slow release fixed P and K.

Long-term field trials are important for the study of soil processes under natural conditions. The aim of this study was to compare the long-term effects of organic and mineral fertilisers on (i) total yield of a five course crop rotation, with all crops present every year, (ii) soil pH changes and (iii) soil plant available P and K dynamics in organic and conventional farming systems over 10 years and iv) whether long-term use of winter cover crops and well composted manure ensured the maintenance of soil fertility.

Materials and methods

Experiment set up

A rotational experiment comparing the effect of organic and conventional management on the yield of field crops and soil properties was established at the Estonian University of Life Sciences (58°22′ N, 26°40′ E; near Tartu) in 2008. The rotation consists of spring barley (*Hordeum vulgare* L.) undersown with red clover– red clover (*Trifolium pratense* L.)– winter wheat (*Triticum aestivum* L.)– field pea (*Pisum sativum* L.)– potato (*Solanum tuberosum* L.) rotation. The soil is a Stagnic Luvisol (IUSS WG WRB 2015) (WRB, Deckers et al. 2002), (sandy loam surface texture, C 1.38%, and N 0.13%, pH_{KCl} 6.0). The field experiment has a systematic block design with four replicates that included the following treatments: organic fertilisation and mineral fertilisation. In the conventional system there were four subplots (10 x 6 m) with different fertiliser (pure ammonium nitrate, NH_4NO_3) application rates (N0, N1, N2, N3). The organic system was divided into 3 fertility building treatments: Org 0, Org I and Org II Fig S1). The experimental design was described by Alaru et al. (2014). The data in the present study concerned the period 2008–2017, i.e. two rotation periods, lasting ten years.

The treatment N0 was the control treatment for the conventional system, without mineral fertilisers, but with pesticides. Plant protection with pesticides can potentially increase yield compared to Org 0. This may affect the P and K balances as well. The other three conventional treatments N1, N2 and N3, had P and K fertilisers applied at sowing at the rate of 25 and 95 kg ha^{-1}, respectively (amounts of P and K were similar in all treatments, Kemira and Yara Mila commercial fertilisers were used). In conventional treatments the mineral N fertiliser NH_4NO_3 was applied once/or twice during growth (N1 = 40–50 kg N ha^{-1}; N2 = 80–100 kg N ha^{-1} and N3 = 120–150 kg N ha^{-1}). A lower N application rate was used for the barley crop with undersown red clover; red clover alone did not receive any mineral fertilisers. Peas received mineral N at 20 kg N ha^{-1} in N1, N2 and N3 treatments.

The first organic treatment (Org 0) was a control for the organic system, without organic fertilisers. In the second organic treatment (Org I) cover crops were used as a green manure in winter: after crops of winter wheat, potato and pea, the cover crops winter rye (*Secale cereale* L.) + winter oilseed rape (*Brassica napus ssp. oleifera var. biennis*) mixture, winter rye and winter oilseed rape, respectively, were sown. Cover crops were ploughed into the soil as soon as possible after the snow melted in April. In the third organic treatment (Org II), fully composted cattle manure was added once during the first crop cycle, before potato. Manure (40 t ha^{-1}) was ploughed into the soil to a depth of 20–23 cm at the end of September or beginning of October before sowing winter oilseed rape as a cover crop. In the second crop cycle period (2013–2017) the timing and rate of manure application was changed: the first application was in early spring before winter wheat re-growth at a rate of 10 t ha^{-1}, the second application before barley sowing at a rate of 10 t ha^{-1} and the third application before potato sowing at a rate of 20 t ha^{-1}. As the content of dry matter (DM) and nutrients in the composted cattle manure were variable, the N, P, K amounts applied with manure also varied (Table 1).

Table 1. N, P and K applied to the organic system in manure (2008–2017)

Crop rotation	Crop	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
I / 2008–2012	Potato	165–179	75–90	130–145
II/ 2013–2017	Winter wheat	44–54	8–18	17–43
	Barley with red clover	44–54	8–18	17–43
	Potato	88–108	16–32	34–86

The tillage method in all treatments was mouldboard ploughing to a depth of 20–23 cm. The conventional systems were treated with several synthetic pesticides against weeds, diseases and pests one to four times during growth as required. In the organic systems, weed control after sowing and in the winter wheat field at the end of April was carried out by spring tine harrowing. The cultivars used in this trial were mostly local cultivars bred at the Estonian Plant Breeding Institute: the potato cultivars Reet (2008–2010) and Maret (2011–2017), the barley cultivars Leeni (2008–2010) and Anni (2011–2017), the red clover cultivars Jõgeva 205 (2008–2011) and Varte (2012–2017). The foreign varieties used: winter wheat cultivars Portal (2008–2010), Olivin (2011) and Fredis (2012–2017), the pea cultivars Madonna (2008–2010) and Tudor (2011–2017). Those varieties are popular among Estonian farmers. In all treatments, the red clover was cut and ploughed into the soil in mid to late August.

Above-ground biomass samples of the red clover crop were taken from a sample size of 1 m^2 before harvest. Winter wheat, barley and pea were harvested with a Sampo harvester with header width of 2 m, i.e. the test area for grain yield calculation was 20 m^2. The samples were dried for 48 h at 105 °C for biomass DM measurement. Potato DM measurements are previously described (Tein et al. 2014).

Chemical analyses

Once a year in mid-April before the start of field operations, soil samples were taken from each plot to a depth of 0–23 cm. Eight samples were taken from each plot and combined to provide one composite sample for analysis. Soil pH was determined on 2mm sieved, air dry samples in 1M KCl 1:2.5. Acid digestion by sulphuric acid solution was used to determine P_{tot} and K_{tot} concentrations of cattle manure and plant samples. Total nitrogen (N_{tot}) content of oven-dried well composted manure samples was determined by dry combustion method on a varioMAX CNS elemental analyzer (ELEMENTAR, Germany) (Methods of Soil and Plant Analysis 1986). Plant available P and K concentrations in the soil samples were determined by the ammonium lactate (AL) method (Egnér et al. 1960).

The P and K amount ploughed into the soil with cover crop biomass (P and K input into the soil) was calculated using total P and K uptake by cover crops (P or K concentration multiplied by above-ground biomass DM yield).

Calculation of total yield per treatment

The number of indicators used in statistical analysis was 280 for each crop (7 treatments × 4 replication × 10 years).

Total yield was calculated as the sum of the DM yields of organic and conventional crops in each fertiliser treatment (i.e. 5 crops × 1 treatment × 4 replication):

$$Total\ Y(1..7) = G\ Y_{barley(1..7)} + Biom\ Y_{clover(1..7)} + G\ Y_{ww(1..7)} + G\ Y_{pea(1..7)} + T\ Y_{potato(1..7)} \qquad (1),$$

where (1…7) = fertilising treatments Org 0(1), Org I(2), Org II(3), N0(4), N1(5), N2(6), N3(7), respectively; $GY_{barley(1..7)}$, $GY_{ww(1..7)}$, $GY_{pea(1..7)}$ = grain yield of barley, winter wheat and pea for respective fertilising treatment; $BiomY_{clover(1..7)}$ = biomass yield of red clover crop for respective fertilising treatment; $TY_{potato(1..7)}$ = tuber yield of potato for respective fertilising treatment.

Total DM yield of fertilising treatment as an average of 10 years for each fertilising treatment was calculated as follows:

$$Annual\ total\ \ Y(1..7) = \frac{Total\ Y_{2008(1..7)} + \ldots + Total\ Y_{2017(1..7)}}{10} \qquad (2),$$

where Total $Y_{2008\,(1..7)}$ = total yield of five field crops in respective treatment in 2008. Total yield (the sum of the DM yields of the five crops receiving the same fertiliser treatment) was calculated for each year (i.e. Total $Y_{2008\ldots2017}$). Total database for statistical analysis was n=1400 (5 crops × 7 treatments × 4 replication × 10 years).

Calculation of PK-balance

The plant available P and K amounts immobilised by winter cover crops and red clover biomass were not taken into account in calculation of input/output balance of P and K as they did not add any PK into the system and were ploughed back into the soil.

$$Annual\ P,K\ balance\ (Org0, OrgI, N0) = 0 - (\frac{P,Kbarley}{10} + \frac{\mathrm{P,Kww}}{10} + \frac{P,Kpea}{10} + \frac{P,Kpot}{10} \quad (3),$$

$$Annual\ P,K\ balance\ (OrgII) = \frac{Pm,Km}{10} - (\frac{P,Kbarley}{10} + \frac{\mathrm{P,Kww}}{10} + \frac{P,Kpea}{10} + \frac{P,Kpotato}{10})/4 \quad (4),$$

$$Annual\ P,K\ balance\ (conv) = (P,Krate) - (\frac{Pbarley}{10} + \frac{\mathrm{Pww}}{10} + \frac{Ppea}{10} + \frac{Ppotato}{10})/4 \quad (5),$$

where P_m, K_m = input of P and K with cattle manure; $\left(\frac{P,Kbarley}{10} + \frac{\mathrm{P,Kww}}{10} + \frac{P,Kpea}{10} + \frac{P,Kpotato}{10}\right)/4$ = mean output of P and K over 4 crops (barley, winter wheat, pea and potato, respectively) as an average of ten years; conv = P, K balance in the soil of conventional treatments; P, K rate = annually applied mineral P and K in conventional treatments (25 and 95 kg ha^{-1}, respectively).

Weather conditions

The climate of Estonia is slightly continental at the experimental site. The winter period (average air temperature permanently below 0 °C) lasts on average 115 days with an average mean temperature of the coldest months of −5.5 °C. The average duration of the vegetation period (air temperature permanently above 5 °C) is 175–190 days. The average period without night frosts is four months, during which time the average midsummer (July) temperature is 16–17 °C. Mean annual precipitation is 550–700 mm; the average precipitation in the wettest months (April to the end of October) is 350–500 mm (Keppart and Loodla 2006).

Meteorological data were collected from a meteorological station approximately 2 km from the trial site (Tables 2 and 3). The effect of weather on yields is discussed in more detail in the results section.

Table 2. Mean temperature (°C) in 2008–2017 compared with the long-term average (1969–2017) data

Month	Trial years										Long-term average 1969–2017
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	
January	-1.3*	-3.4	-12.6	-4,8	-6.1	-7.3	-8.1	-1.9	-9.2	-3.4	-5.3
February	0.6	-4.8	-7.4	-10.7	-11.5	-3.3	-0.3	-1.0	0.3	-2.9	-5.5
March	0.4	-1.5	-2.1	-1.9	-0.3	-7.8	2.2	2.6	0.0	1.4	-1.5
April	7.2	5.3	6.1	6.4	5.0	3.5	6.5	5.4	6.1	3.4	5.0
May	10.6	11.4	12.6	11.0	11.8	14.8	11.9	10.3	14.0	10.3	11.5
June	14.5	13.8	14.6	17.2	13.6	18.2	13.4	14.2	15.9	14.0	15.3
July	16.1	16.9	22.2	19.9	17.9	17.7	19.3	15.7	17.8	15.9	17.6
August	15.8	15.4	18.4	15.8	15.2	17.0	16.8	17.0	16.1	16.8	16.2
September	9.8	12.8	11.1	12.3	12.2	10.8	12.1	12.6	12.3	12.2	11.1
October	8.2	4.1	4.2	6.8	5.7	6.6	5.2	4.6	4.1	5.2	5.6
November	2.3	2.3	0.3	2.9	2.6	3.5	1.4	3.6	-1.0	2.4	0.6
December	-1.1	-5.5	-8.2	1.0	-6.8	1.1	-1.6	2.4	-0.3	0.2	-2.9
Average of year	6.9	5.6	4.9	6.3	5.0	6.2	6.6	7.2	6.3	6.3	5.6

*data from Eerika meteorological station

Table 3. Sum of precipitation (mm) in 2008–2017 compared with the long-term average (1969–2017) data

Month	Trial years										Long-term average 1969–2017
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	
January	22*	10	3	19	30	11	25	30	34	27	29
February	34	7	12	9	19	14	12	8	56	22	23
March	8	22	30	6	39	16	9	12	23	17	22
April	27	14	26	11	42	17	13	69	52	51	29
May	27	13	61	58	82	61	84	62	2	15	54
June	111	137	73	35	101	52	103	39	125	94	77
July	54	55	36	48	75	63	71	61	82	61	69
August	118	89	107	55	87	76	113	41	42	106	87
September	46	49	93	80	60	38	22	59	15	83	57
October	68	116	49	48	45	45	36	11	33	75	56
November	49	36	78	34	50	70	10	54	46	26	45
December	24	57	18	53	9	47	42	46	31	52	37
SUM Σ	588	605	586	456	639	510	540	492	539	629	585

*data from Eerika meteorological station

Statistical analyses.

Correlation, factorial analyses of variance (ANOVA) and two-factor ANOVA were used to test the effect of farming systems and climatic conditions on each crop's DM yield. Descriptive analysis and Fisher's least significant difference test for homogenous groups were used for testing significance differences between farming systems, experimental year and crop mean DM yields. The means are presented with their standard errors (±SE) (bars in the figures). The level of statistical significance was set at $p < 0.05$ if not indicated otherwise.

Results

Total yield and amount of P and K removed in different farming systems

The total yield of the five crops fertilized similarly was significantly influenced by farming system ($p < 0.001$, Fig. 1) and weather conditions ($p < 0.001$, Fig 2). The proportion of variation for farming system and weather conditions was quite similar, 47% and 43%, respectively. The total yield of crops as an average of trial years in the organic treatments ranged between 21.5–22.4 t ha^{-1} compared with 29.0–29.8 t ha^{-1} in the conventional system (Fig. 1). Differences between the treatments within each system were not significant, except conventional 0, which was similar to organic treatments. Total yields of organic treatments were 3–28% lower than those of conventional treatments.

Over the ten years, the total yield of the five crops averaged across treatments ranged between 20.3–31.5 t ha^{-1} (Fig. 2). A 19–27% higher total yield of crops was obtained in 2009, 2012 and 2017, caused mostly by an increase in potato and winter wheat yields. Our results showed that temperature in April and September was important in terms of total yield formation (Table 3). A 1.4–2.2 °C higher temperature than the long-term average in April resulted in 9–72% lower yield level of winter wheat ($r= -0.42$, $p<0.01$, $n=70$) while a temperature 2.3–2.8 °C higher than the long-term average in September resulted in 26–57% higher yield level of potato ($r= 0.47$, $p<0.001$, $n=70$). A higher potato tuber yield was obtained in years when the mean temperature in September was higher than the long-term average. The influence of precipitation on total crop yield was not significant, but the distribution of precipitation was very important. In 2015 the sum of precipitation per year was 93 mm lower than that of the long-term average, but the total yield of the five crops was not different from the record yields in 2009, 2012 and 2017.

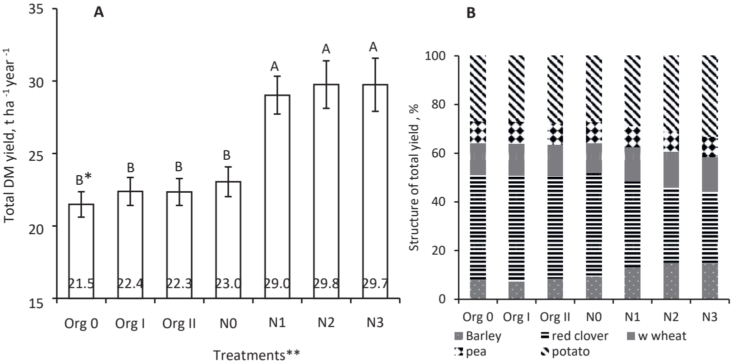

Fig. 1. Annual total yield of five crops (t ha^{-1} per year) in different treatments (A). Composition (%) of total yield of five crops in different treatments (B). $F_{(6, 63)}$ =9.271, p<0.001. * The means marked with the same letter do not differ statistically significantly from each other; **Org0 and N0 = control treatments of organic and conventional farming, respectively; OrgI = organic treatment with winter cover crops CC; OrgII = additionally to CC the well composted cattle manure applied; N1 = amounts of mineral NPK per ha: 40–50 kg N, 25 kg P, 95 kg K; N2 = amounts of mineral NPK per ha: 80–100 kg N, 25 kg P, 95 kg K; N3 = amounts of mineral NPK per ha: 120–150 kg N, 25 kg P, 95 kg K. Less mineral N fertilisers were applied to barley undersown with red clover.

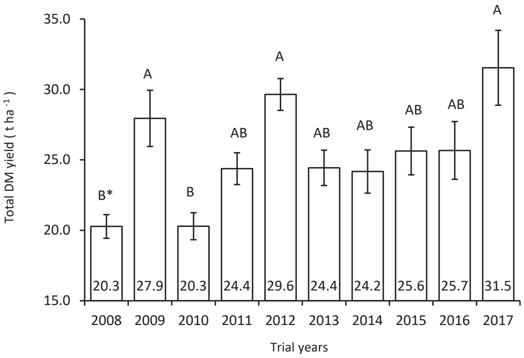

Fig. 2. Total yield of five crops (t ha^{-1}) in different trial years as an average of treatments.*the means marked with the same letter do not differ statistically significantly from each other

The amount of P and K removed from the field depended on crop yield in both organic and conventional treatments. Annual amounts of P removed with field crops in all organic treatments did not differ statistically from the control treatment of the conventional system (variation between 11.4–11.9 kg ha^{-1}); annual amounts of P removed from fertilised treatments of the conventional system were 28–35% higher. The same data for annually K removal were 42.0–44.2 kg ha^{-1}, which was 28–40% higher than that of the organic treatments.

Soil pH in the organic and conventional systems

The soil pH was significantly influenced by farming system (r=0.31, p<0.001) and weather conditions (r=0.07, p<0.01); the proportion of variation for farming treatments and weather conditions were 14% and 5%, respectively (Fig. 3).

Descriptive analysis showed that at the beginning of the field trial the pH values of all treatments did not differ statistically; mean pH values in organic and conventional treatments were 5.93±0.03 and 5.82±0.03, respectively. After 10 years of field experiment the pH values in organic treatments had increased by 0.24 units on average (pH values ranged between 5.9–6.3) after fertilisation with cattle manure. The soil pH values of conventional treatments had decreased by 0.23 units on average (pH value ranged between 5.4–5.9).

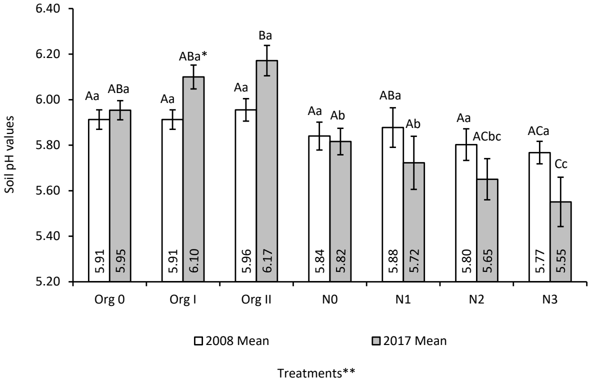

Fig. 3. Soil pH values under different treatments at the beginning of the field experiment and after ten years. 2008 Mean: $F_{(6, 133)}=1.268$, $p=0.276$; 2017 Mean: $F_{(6, 133)}=8.215$, $p<0.001$; *different upper case letters indicate a significant difference between years, and different lower case letters indicate the difference between treatments in a given year; **See explanations under Figure 1

PAL and KAL contents in the soil (mg kg^{-1}) after long-term organic and conventional farming

The correlation analysis showed that plant available P in the soil of organic treatments declined significantly after ten years (r=-0.19, *p*<0.001). At the beginning of the experiment it did not differ significantly between the farming system treatments (variation was 90.7–118.7 mg P kg^{-1} soil^{-1}). However, by descriptive analysis plant available P had decreased in all organic treatments after 10 years and in the control treatment of conventional farming by 18.9–23.6 mg P kg^{-1} soil^{-1} (Fig. 4); after 10 years the P content of fertilised treatments of the conventional farming system did not differed statistically from the data from the beginning of the field trial.

The plant available K in soil decreased over ten years in all treatments (r=0.88, *p*<0.001). At the beginning of the experiment there was no statistical difference between the two farming system treatments (variation was 160.7–174.4 mg K kg^{-1} soil^{-1}, Fig. 5). After ten years the greatest decrease in K content was in control variants of both farming systems (60 mg K kg^{-1} soil^{-1}), followed by Org I and Org II. The amount of available K in the soil decreased less in the fertilised conventional treatments (17.1–39.5 mg K kg^{-1} soil^{-1}, see chapter Discussion).

PK-balance in the soil of different treatments

Since cover crops and red clover biomass and straw of other crops were not removed from the field, the values of these data were not accounted for in the balance calculation of soil P and K (Table 4 and 5).

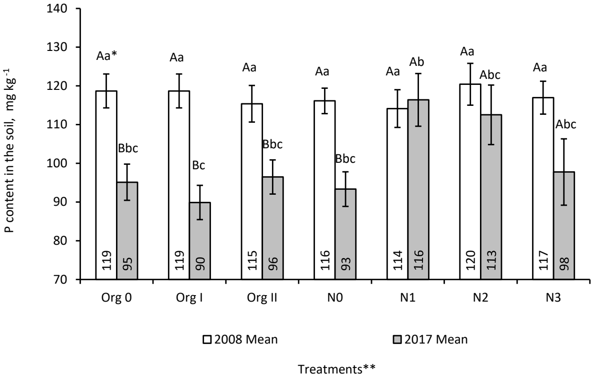

Fig. 4. Plant available P content (mg kg^{-1}) in the soil at the beginning of the field trial and after ten years. 2008 Mean: $\mathrm{F}_{(6,\ 133)}$=1.268, *p*=0.276; 2017 Mean: $\mathrm{F}_{(6,\ 133)}$=2.754, *p*=0.0149; *different large letters indicate a significant difference between years, and different small letters indicate the difference between treatments in a given year; **See explanations under Figure 1

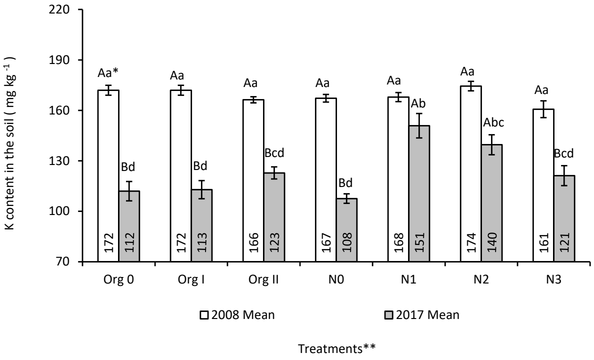

Fig. 5. Plant available K content (mg kg^{-1}) in the soil at the beginning of the field trial and after ten years. 2008 Mean: $\mathrm{F}_{(6,\ 133)}$=1.268, *p*=0.276; 2017 Mean: $\mathrm{F}_{(6,\ 133)}$=8.215, *p*<0.001; *different large letters indicate a significant difference between years, and different small letters indicate the difference between treatments in a given year; ** See explanations under Figure 1

The calculated input/output balance of P and K was quite different in the organic and the conventional system. The P balance showed that in the organic system the cultivation of winter cover crops did not decrease the annual loss of P, whereas the application of cattle manure decreased the loss of P by 9 kg ha^{-1} compared with control treatment Org 0 (Table 4). The K balance showed that in the organic system all treatments had a negative balance (Table 5). Only the cattle manure application decreased the annual loss of K by 29.3 kg ha^{-1} compared with control treatment Org 0. In the conventional system the calculated balance of P and K was positive in all fertilised treatments (Table 4 and 5). The input/output balance in both control treatments for P and K amounts was the most negative, whereas the decrease of these elements was higher in the control treatment of the conventional system because of use of pesticides and up to 7% higher total yield level. Most of the plant available P and K was removed by the potato crop each year, which was also expected because of the much higher yield of tubers. P and K removed by the potato crop were up to 1.8 and 10.2 times higher than removal by other crops, respectively.

Table 4. Mean amount of P (kg ha^{-1}) annually applied and removed by different crop yields from different treatments and input/output balance in the soil as an average of ten years

Treatment	Input kg ha^{-1}	P output kg ha^{-1}				Mean output across crops*	Input - output
		Barley + red clover	Winter wheat	Pea	Potato		
Organic							
Org 0		6.9 ±0.3	12.2±0.6	9.9±0.6	15.6±1.2	11±1·8 b	-11.2
Org I	0	7.0±0.3	13.2±0.9	10.6±0.6	16.0±1.1	12±1·9 b	-12.2
Org II	10.0±2.5	7.9±0.5	12.2±0.9	9.5±0.6	16.2±1.0	12±1·8 b	-2.2
Conventional							
N0	0	8.5±0.5	11.5±0.8	9.8±0.7	17.9±1.4	12±2·1 b	-11.9
N1	25	14.9±0.9	17.8±1.1	11.7±0.7	21.5±1.2	17±2·1 a	8.5
N2	25	16.6±0.9	19.1±1.0	11.3±0.7	22.9±1.4	18±2·4 a	7.5
N3	25	16.0±0.9	17.9±1.2	11.6±0.9	24.5±1.7	18±2·7 a	7.5
Mean over treatments		11±1·7c*	15±1·2b	11±0·3 c	19±1·4 a	14.0±1.1	

*the means marked with the same letter do not differ statistically significantly from each other

Table 5. Mean amount of K (kg ha^{-1}) applied and removed by different crop yields from different treatments annually and input/output balance in the soil as an average of ten years

Treatment	Input kg ha^{-1}	K output kg ha^{-1}				Mean output across crops*	Input - output
		Barley + red clover	Winter wheat	Pea	Potato		
Organic							
Org 0	0	9.1±0.5	12.8±0.7	20.2±1.0	121±7.0	41±26·8a	-40.8
Org I	0	9.3±0.5	13.7±0.9	20.8±1.1	126±7.0	43±28·0a	-43.9
Org II	32.1±4·4	10.6±0.7	13.0±0.9	19.7±1.0	131±5.8	44±29·2a	-11.5
Conventional							
N0	0	11.8±0.6	12.8±1.0	20.2±1.3	135±7.7	45±30.1a	-45.0
N1	95	21.3±1.0	20.2±1.4	26.6±1.2	180±7.3	62±39.3a	33
N2	95	22.6±1.0	21.8±1.2	25.3±1.2	194±8.9	66±42·7a	29.1
N3	95	23.0±0.9	20.5±1.4	33.8±6.2	215±12.2	73±47.4a	21.9
Mean over treatments		15±2·5c*	16±1·6 c	24±2·0b	157±14.4a	53±19·9	

*the means marked with the same letter do not differ statistically significantly from each other.

Discussion

The purpose of this experiment was to compare the effectiveness of long-term use of composted manure and winter cover crops and the use of mineral fertilisers (with different N norms) on the maintenance of soil fertility in a five course crop rotation, with a high requirement for nutrients. This article discusses the changes in soil P and K content and pH values during 10 years. Káš et al. (2016) found that long-term fertilisation with mineral and organic fertilisers affected yields and overall export of nutrients from the field and brought about many changes in the soil. After 10 years of mineral fertiliser use in the conventional farming system, the soil became more acidic in all treatments, i.e. the pH values decreased up to 0.5 units. In Estonian climatic conditions, where precipitation exceeds evaporation, many soil types are characterized by acidification over time (Järvan and Vettik 2016). After 10 years of cattle manure use in the organic farming system, soil pH became less acidic, which was expected, because the pH values of applied cattle manure ranged between 6.6–8.3. Gosling and Shepherd (2005) and Kirchmann et al. (2007) found that organic cropping systems, which rely heavily on legumes in the rotation, will acidify soils faster than systems with less legumes in the rotation. Our five field-crop rotation includes only two legume crops (red clover and field pea). Watson et al. (2002) reported that higher amounts of organic fertilisers in organic farming systems have increased soil microbial biomass and activity but have not necessarily ensured higher yields.

The availability of N in the early stages of plant development is very important in terms of crop formation and yield level. Earlier results of our long-term field experiment showed that N limited the yield level in organic system, and two legumes, winter cover crops and manure in the five-field crop rotation did not meet the N requirements of crops, because it was not always in line with N availability (Alaru et al. 2014). More nutrients are removed from the soil with higher yields. In this field trial the annual amounts of P and K removed in conventionally fertilised treatments were 28–35% and 28–40%, respectively, higher than in organic treatments because of 24–25% higher yields. Mineral N fertilisation caused a significant increase of overall P and K uptake in experimental plots, which is consistent with Káš et al. (2016). This can lead to a decrease of nutrient reserve in soils (Bhattacharyya et al. 2015). During 10 years experimentation the amounts of P and K in the organic farming system decreased by up to 24 mg and 60 mg kg^{-1} soil^{-1}, respectively. Although P and K outputs were smaller in the organic system due to lower yields, the use of organic fertilisers did not prevent the decrease of plant available P and K amounts in the soil. The highest annual uptake of P and K from soil was by the potato crop, followed by winter wheat; the amount of P and K removed with potato yield was 35% and 74%, respectively, from the total annually removed P and K in this crop rotation experiment. Srinivasarao and Srinivas (2017) reported that tuber crops can remove as much as 1000 kg K ha^{-1}. The choice of crops for crop rotation is very important to preserve soil fertility. In our field experiment the replacement of potato by another crop would be conducive to less negative K balance (for example by buckwheat). If soil fertility deteriorates, crop rotation should be reviewed at certain intervals.

Nutrient budgeting on organic farms often shows a deficit of P and K (Gosling and Shepherd 2005, Kirchmann et al. 2007). The input/output balance of P and K was negative in all treatments of the organic system.

P and K balances showed that in the conventional system the soil of all fertilised variants should have had sufficient amounts of P and K, while actual K data of soil did not confirm this. Our results showed that over the 10 year trial period plant available K content in the conventional treatments decreased up to 40 mg K kg^{-1} soil (Fig. 5). Yadav et al. (2000) found that despite annual K additions, at recommended rates through fertilisers, available K content decreased due to continuous cropping. Such a contradiction in results may be due to the fact that part of the K given to the plants with fertilisers was chemically immobilised by soil particles or leached and consequently the amounts of plant available K were reduced (Srinivasarao and Srinivas 2017). The input/output balance for P showed that P content in the fertilised treatments of the conventional system should have increased annually by 7.5–8.5 kg ha^{-1}, but the actual results showed that after 10 years the plant available P content was statistically the same as at the beginning (Fig. 4). In Estonian mineral soils, P is a particularly problematic element because, given the pH of our soils, the range of optimal response to P absorption is narrow (Roostalu 2012). According to Berry et al. (2003) the budget deficit of P is small when it is <10 kg ha^{-1} per year; deficit of K is large when it exceeds 50 kg ha^{-1} per year. In conditions of K shortage, crop plants draw from the soil reserve K to meet their nutritional requirements (Sardans and Penuelas 2015). At the beginning of our experiment the soil P fertiliser requirement was average and it did not change in 10 years, but the K fertiliser demand increased from medium to high.

Srinivasarao and Srinivas (2017) reported that because of heavy removal of nutrients from soil under multiple cropping systems with high yielding and fertiliser-responsive varieties, the P and K status of soils is changing rapidly. It is of great importance to keep a close watch on such depletion through regular monitoring to ensure that P and K do not become limiting factors in crop production and to commence their application in appropriate doses so that deficiency does not occur.

Conclusions

This 10 year comparison between organic (winter cover crops and well composted manure) and conventional (mineral fertilisers) systems showed that: i) total yield of a five course crop rotation was 24–25% higher in conventionally fertilised treatments than in organic treatments; ii) the soil became more acidic in the conventional system due to the long-term use of mineral fertilisers, the use of liming may be a good solution to increase pH; iii) during the 10 year conventional farming system experiment the plant available P content remained statistically unchanged and the K content decreased by up to 40 mg K kg^{-1} soil^{-1}. In the conventionally fertilised treatments the annual amounts of removed P and K were12–18 kg P ha^{-1} and 45–73 kg K ha^{-1}, which was respectively 28–35% and 28–40% higher than in the organic treatments because of higher yields; iv) the use of winter cover crops and well composted cattle manure did not maintain the baseline levels of P and K in the soil, which require the greatest attention and must be viewed from the perspective of maintaining the soil fertility especially in organic system. In conclusion, to prevent the depletion of nutrient reserves in the soil, crop rotation should be changed from time to time.

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Article

Impact of Weather Conditions and Farming Systems on Size Distribution of Starch Granules and Flour Yield of Winter Wheat

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Abstract: The size distribution of wheat-grain starch granules has an impact on the yield of fine flour. The aim of the study was to compare the impact of conventional (mineral fertilizers, pesticides) and organic farming treatments (cover crops, composted cattle manure) on (i) the size distribution of starch granules, (ii) the level of the first break whole and fine flour yield. The grain samples of winter wheat cv Fredis were taken from a long-term field crop rotation experiment established in 2008 at the Estonian University of Life Sciences in Tartu County (58°22′ N, 26°40′ E) on *Stagnic Luvisol* soil. The weather conditions during the grain filling period of winter wheat had a strong impact ($p < 0.001$) on the grain starch granule size distribution. The proportion of starch granules with a smaller diameter (C-type granules) was higher in years with a longer grain filling period. The size distribution of starch granules was not influenced by farming system. The increased proportion of C-type granules increased the fine flour yield significantly. Fertilisation with organic manure and twice with mineral nitrogen increased significantly the mean diameter value of different starch granules.

Keywords: organic; conventional farming; whole; fine flour yield

1. Introduction

Wheat is one of the most important cereals for human and livestock consumption, with 75–78% of the total production being consumed by humans [1]. The physical and compositional properties of wheat grains determine the quality of the end product. Wheat has two textural classes i.e., soft and hard [2] and in the Baltic Sea region it is soft wheat that is mostly cultivated. The texture of winter wheat grains is affected by several factors, such as the farming system, variety, locality and the weather conditions in the post-hibernation period [3]. Growing winter wheat has become more popular among farmers with different crop production systems in Estonia (organic and conventional). The organic farming system has been adapted for many climate zones and local conditions. However, nitrogen (N) fertilisation management and irregular availability of N due to factors influencing mineralisation in the soil, are two of the biggest challenges for organic farming [4]. In the conventional farming system, the N from mineral fertilizers is easily available to plants in their early stages of development, which results in the much higher grain yield [5]. Xue et al. [6] found that late mineral N fertilizers as additional N or split from the basal N at late boot stage or heading in the form of nitrate-N or urea improved loaf volume of wheat flour by increasing grain protein concentration and altering its composition. Rossini et al. [7] said that at the same N rate, grain yield and quality were markedly higher using mineral N as opposed to organic N.

During grain development, the differentiated endosperm contains four major cell types: the cells of the region surrounding the embryo, transfer cells, aleurone layer, and starchy endosperm. The starchy endosperm cells gradually accumulate reserves (mainly proteins and starch) during development, and are filled with amyloplasts and protein bodies at maturity. Jane [8]. Previous investigations have found that variations in starch granule distribution are significantly correlated with changes in starch pasting viscosity [9], dough mixing properties [10] and bread crumb structure [11]. Wheat endosperm has a trimodal distribution of starch granules, i.e., A-granules with lenticular shape (diameter 10–50 µm), B-granules with spherical shape (diameter 5–9.9 µm) and C-granules with irregular shape (diameter < 5 µm); [12]. The different sizes of the starch granules might be attributed to their time of formation during grain development [13]. A-granules are formed around 4–14 days post anthesis (DPA) when the endosperm is still actively dividing [14]. B-granules are initiated at about 10–16 DPA in stromules, and the small C-granules first appear about 21 DPA [12]. The irregular shape of C-granules might be due to their small size and tight packing in the seed. The results can be expressed as percentiles of diameter value of size distribution. For example, percentiles of 10, 50 and 90% are associated with the size of C-, B- and A-type granule diameter, respectively [15,16].

Li et al. [17] reported that N fertiliser application (combined with S fertiliser) is a good way to improve A- and B-type starch granule accumulation in the central endosperm tissue sections during the grain-filling stage. Xiong et al. [18] found that increased N fertilizer application mainly increased the numbers of small and decreased the numbers of large starch granules, but that the results varied in different regions of the wheat endosperm. Farming systems with different N management influence pre-anthesis above ground biomass formation and the post-anthesis grain filling period, i.e., the starch and photosynthetic carbon mobilisation from stems and leaf sheaths to developing reproductive tissues [19]. Besides the farming system, the post-anthesis environment such as water availability and temperature strongly influence seed size, thus it is important in defining physical properties such as screening and milling yield [20]. Tambussi et al. [21] said, that in C3 cereals, ear photosynthesis has been reported to provide photoassimilates especially during adverse environmental conditions.

The break flour yield from first-break roller milling was measured in this study. In modern flour milling wheat kernels are broken open using first-break roller mills [22]. This produces a wide range of particles from <200 µm to >2000 µm. After the first break stage, subsequent processes, typically four or more break passages, grading, purification, and eight or more reduction passages, mill and separate the endosperm and bran further. The higher the flour yield after the first break stage, the more economical the milling process [15,23]. Bechtel and Wilson [12] reported that flour yield is dependent on the proportion of endosperm in the wheat grain, which in turn is dependent on the farming system.

There is little research that explores the influence of farming systems and variable weather conditions on the size distribution of starch granules. The aim of the present study is to compare the impact of organic systems (cover crops, composted cattle manure) and conventional farming systems (mineral fertilizers, pesticides) on (i) the size distribution of starch granules, (ii) the level of the first break whole and fine flour yield. The hypotheses of this paper were: (1) the diameter of starch granules of winter wheat grains grown in organic conditions are smaller than that of granules grown in conventional conditions; (2) the starch granules size distribution depends on the length of grain filling period; (3) the farming system has an impact on first break fine flour yield.

2. Materials and Methods

2.1. Experiment Setup

The grain samples of winter wheat cv Fredis were taken from a long-term field crop rotation experiment to study the effects of organic and conventional systems on the size distribution of starch granules in grain and its impact on flour yield. The field experiment was established in 2008 at the Estonian University of Life Sciences Farm at Eerika, Tartu County (58°22′ N, 26°40′ E) on *Stagnic Luvisol* soil (sandy loam surface texture, C 1.38%, and N 0.13%, pH_{KCl} 6.0). During the rotation cycle five different crops followed each other in the order: barley (*Hordeum vulgare* L.) with undersown red clover, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.) and potato (*Solanum tuberosum* L.). The experiment was set up in a systematic block design with four replicates of each treatment and a plot size of 60 m^2 [24]. In the present study the data on winter wheat concerns the period 2013–2017.

These crops were treated using different farming systems: three treatments of organic and four treatments of conventional. The first organic treatment (Org 0) was a control, with symbiotically fixed atmospheric N_2 the only source of N, ploughed into the soil with the legume above-ground biomass (red clover and pea, i.e., twice during the crop cycle period). In the second organic treatment (Org I), in addition to legumes, cover crops were used as green manure in winter: after crops of winter wheat, potato and pea, the cover crops were a winter rye (*Secale cereale* L.) and winter oilseed rape (*Brassica napus* ssp. *oleifera var. biennis*) mixture, winter rye and winter oilseed rape were sown. Cover crops were ploughed into the soil as soon as possible after the snow melted in April. In the third organic treatment (Org II), fully composted cattle manure was added in early spring before winter wheat re-growth at a rate of 10 t ha^{-1}. The organic N amounts applied in 2013, 2014, 2015, 2016 and 2017 with manure were 54, 47, 46, 46 and 44 kg N ha^{-1}, respectively. The same data for phosphorus were 18, 14, 8, 11 and 13 kg P ha^{-1}, respectively, and for potassium 43, 42, 32, 17 and 29 kg K ha^{-1}, respectively.

The first conventional control treatment (N0) was the same as for the organic control treatment (Org 0). The other three conventional treatments had P–K fertilizers applied at sowing time at the rate of 25–95 kg P–K ha^{-1}. Amounts of P and K were similar in all treatments. The conventional treatment N50 had the mineral nitrogen fertilizer (NH_4NO_3) applied in early spring at the tillering phase of winter wheat, treatments N100 and N150 had N fertilizer twice—at tillering stage 50 and 100 kg N ha^{-1}, respectively, and at booting stage BBCH47 additionally 50 and 50 kg N ha^{-1}, respectively.

The tillage method in all treatments was mouldboard ploughing to a depth of 20 cm. The conventional systems were treated with several synthetic pesticides against weeds, diseases and pests one to four times during growth as required. In the organic systems, weed control after sowing and in the winter wheat field at the end of April was carried out by spring-tine harrowing. Development stages of winter wheat were determined every week by observation and using of BBCH-scale for cereals. Determination of the physiological maturity (PhM) of winter wheat was based on kernel water and dry matter [25]. The above ground biomass samples of wheat were taken from an area of 1 m^2. Samples were taken before harvest every year, from which the above ground biomass in dry matter (DM) were determined. Winter wheat was harvested with a Sampo combine on 12 August 2013, 4 August 2014, 12 August 2015, 26 July 2016 and 28 August 2017 (moisture content of kernels was 20–28%).

2.2. Chemical Analysis

Well composted manure was used in the trial. The total nitrogen (N_{tot}) content of oven-dried manure and grain samples were determined by the dry combustion method on a varioMAX CNS elemental analyzer (ELEMENTAR, Langenselbold, Germany) [26]. Acid digestion by sulphuric acid solution was used to determine cattle manure P_{tot} and K_{tot} concentrations.

2.3. The Size Distribution of Starch Granules

The size distribution of starch granules was determined in winter wheat grain endosperm using a Malvern Mastersuzer 3000 analyzer (Malvern Instruments Ltd., Malvern, UK). A standard protocol was used [27] to separate the starch from 100 mg of material, which was taken from each treatment. The starch suspension was used for laser diffraction analysis. Particle size analysis was conducted as described by Li et al. [28] and Tanaka et al. [29]. About 0.1 mL of starch was suspended with 1 mL of reverse osmosis water (Grade 2, conductivity 5-6 µS/cm) in 2 mL Eppendorf tubes and briefly vortexed prior to transfer into the particle size distribution analyser's dispersion tank containing the same origin reverse osmosis water. The statistics of the distribution are calculated from the results using the volume derived diameters Dv—an internationally agreed method of defining the mean and other measurments of particle size. Dv(50), Dv(10) and Dv(90) are standard percentile readings from the measured size distribution.

2.4. Yield of Wheat Flour

The yield of wheat flour was measured in four trial years. In 2016 the flour yield was not determined because the winter wheat crop (2015/2016) failed and only a small amount of grain was available for milling. The grain samples of 1000 g per plot were tempered at 140 g kg^{-1} moisture and were milled with a laboratory mill LM 3100 (Perten Instruments, Hägersten, Sweden, 2018), after which the flour was sieved into three fractions: bran and shorts (sieve PA-47GG, SEFAR NYTAL PA, Retsch, Haan, Germany) particle size over 375 µm, coarse flour (sieve PA-72GG, SEFAR NYTAL PA) with particle size of 224–375 µm and fine flour with particle size below 224 µm. Whole flour yield was calculated as the sum of coarse and fine flour (particle size < 375 µm).

2.5. Meteorological Data

Meteorological data of the post-hibernation vegetation period in 2013-2017 were collected from a meteorological station approximately 1 km from the trial site (Table 1).

Table 1. Monthly average temperature (°C) and total precipitation (mm) in 2013–2017 compared with the long-term average (1969–2017).

Month	**Temperature, °C ***					
	2013	**2014**	**2015**	**2016**	**2017**	**1969–2017 ****
April	3.5	6.5	5.4	6.1	3.4	4.8
May	14.8	11.9	10.3	14.0	10.2	11.4
June	18.2	13.4	14.3	15.9	14	15.4
July	17.8	19.9	15.7	17.8	15.9	17.5
August	16.9	16.8	17.0	16.1	16.8	16.2
April–August	14.2	13.7	12.5	14.0	12.1	13.1
	Precipitation, mm *					
April	17	13	51	50	52	29
May	61	84	60	2	16	56
June	52	104	40	125	94	78
July	63	71	62	82	61	70
August	75	113	42	42	106	88
April–August	268	384	251	301	329	321

* data from Eerika meteorological station; ** long term average of 1969–2017.

Weather conditions during the post-hibernation period had a strong effect on the above ground biomass and grain yield quality formation of winter wheat. The length of the post-hibernation vegetation period depended on the date that snow melting in April. Later snowfall in 2013 and 2017 and lower temperature values during the vegetation period in 2017 delayed harvest of winter wheat in that year. The regrowth of winter wheat, after the snow melted, started in 2013 and 2017 at 16 of April and 1 of May, respectively, which was 5–25 days later than of other trial years (2014–2016). In 2015 the mean temperature of May was 1.1 °C lower than the long-term average, but the precipitation was sufficient, which facilitated the tillering and growth of above ground biomass of winter wheat. In 2015 lower temperatures and regular precipitation during the post-hibernation period resulted in a record grain yield of winter wheat. The most unfavorable weather conditions for grain yield formation were in 2016, when the amount of precipitation in May was only 2 mm and temperature data during the post-hibernation vegetation period were higher than the long-term average (Table 1). However, this article does not deal with the grain yield of winter wheat.

Growing degree days (GDD) were used to characterise the impact of weather conditions on starch granule size distribution. GDD is given as the mean daily temperature above a 5 °C base temperature accumulated on a daily basis over a period of flowering-physiological maturity (BBCH65-PhM).

2.6. Statisticsal Analysis

Correlation, factorial analyses of variance (ANOVA), descriptive analysis and two-factor ANOVA were used to test the effect of farming systems and experimental year on granules size of distribution and flour yield of winter wheat. The means are presented with their standard errors (±SE). The level of statistical significance was set at $p < 0.05$ if not indicated otherwise.

3. Results

3.1. The Factors Influencing the Size of Starch Granules

The size distribution of starch granules was significantly related to several factors studied in this field trial, such as fertilisation with organic or mineral N fertilisers ($p < 0.001$), weather conditions ($p < 0.001$), biomass yield at flowering stage of wheat (BBCH65; $p < 0.01$) and length of period from BBCH65 up to physiological maturity (PhM; $p < 0.001$; Table 2).

Table 2. Correlation between starch granules size distribution and several factors studied in this trial.

Factors	Dv(10)	Dv(50)	Dv(90)
Temperature *; $n = 105$	−0.39 ***	−0.37 ***	−0.26 **
Precipitation *; $n = 105$	ns	ns	−0.26 **
Length of period BBCH65-PhM; $n = 112$	−0.54 ***	−0.52 ***	−0.40 ***
N amount (treatment); $n = 105$	0.20 *	0.19 *	0.19 *
Farming system; $n = 105$	ns	ns	ns
Biomass of wheat at BBCH65; $n = 112$	−0.27 **	−0.26 **	ns
Fine flour yield; $n = 56$	−0.40 **	−0.27 *	−0.27 *
Whole flour yield; $n = 56$	−0.31 *	−0.31 *	−0.29 *

* Mean temperature and sum of precipitation is for grain filling period; *, **, ***—these signs indicate the statistical significance at $p < 0.05$, 0.01 and 0.001 level.

The proportion of variation of Dv(10), Dv(50) and Dv(90) were for weather conditions and farming system 38 and 18%, 24 and 24%, 18 and 18%, respectively. The same data for interaction between these two factors were 44, 24 and 63%, respectively (data not shown).

3.1.1. Impact of Weather Conditions on Starch Granule Size Distribution

The size distribution of starch granules was most influenced by the length of the grain filling period, i.e., the period of BBCH65-PhM, which in turn was positively correlated with the mean temperature and GDD values in this period ($r = 0.84$; $p < 0.001$). In 2013 and 2017 the period of BBCH65-PhM was 3-5 days longer and therefore the GDD values were 40-168 °C higher than in other trial years (Table 3). In 2013 and 2017 the mean temperature values in the grain filling period were higher (ranged between 16.2–18 °C), whereas in other years the fluctuation was between 14.5–15.7 °C.

The value of GDD in 2016 was influenced by much higher amounts of precipitation, which was 197 mm (in other years it ranged between 67–115 mm). In 2013 and 2017 the starch granule diameter of Dv(10), Dv(50) and Dv(90) was up to 17, 29 and 33% smaller than in 2014–2016, except for Dv(90) 2016 vs. 2013 (Table 4).

Table 3. Accumulation of growing degree days (GDD; °C) in 2013–2017 and length of grain filling period (from flowering up to physiological maturity).

Parameter	Years				
	2013	2014	2015	2016	2017
	Dates of Flowering (BBCH65) and Physiological Maturity (PhM)				
BBCH65	5.06	10.06	15.06	4.06	29.06
PhM	15.07	16.07	20.07	11.07	7.08
BBCH65-PhM, days	40	36	35	37	40
GDD for period of BBCH65-PhM, °C	519	351	358	408	448

Table 4. Mean values (±SE) of starch granule size distribution over farming system treatments and experimental years.

Year	Dv(10), μm **	Dv(50), μm	Dv(90), μm
2013	2.85 ± 0.05 b *	6.63 ± 0.29 bc	17.80 ± 1.54 ab
2014	3.13 ± 0.06 a	7.86 ± 0.30 ab	20.40 ± 1.04 ab
2015	3.05 ± 0.03 a	8.15 ± 0.35 a	22.61 ± 0.88 a
2016	3.12 ± 0.04 a	7.08 ± 0.24 abc	17.01 ± 0.67 b
2017	2.70 ± 0.05 b	6.25 ± 0.20 c	16.77 ± 0.99 b

* different letters in the same column denote a significant difference (Fisher LSD test, $p < 0.05$). ** Dv(10), Dv(50), Dv(90)—the size of particle in microns below which 10, 50 and 90% of the sample, respectively, lies.

3.1.2. Impact of Organic and Mineral N on Starch Granule Size Distribution

The starch granule diameter was significantly influenced by different doses of organic and mineral N applied in organic and conventional treatments, respectively. In the organic system the use of composted cattle manure (treatment Org II) increased the values of Dv(10), Dv(50) and Dv(90) up to 15, 23 and 31%, respectively, in comparison with other organic treatments (Table 5).

Table 5. Mean values (±SE) of starch granule size distribution over experimental years in the comparison of farming system treatments.

Treatment	Dv(10), μm **	Dv(50), μm	Dv(90), μm
Organic			
Org 0	2.85 ± 0.07 b *	6.76 ± 0.36 abc	18.15 ± 1.59 ab
Org I	2.96 ± 0.05 b	7.14 ± 0.38 abc	17.83 ± 1.50 ab
Org II	3.22 ± 0.06 a	8.03 ± 0.28 a	22.77 ± 1.04 a
Conventional			
N0	2.91 ± 0.05 b	6.26 ± 0.11 c	15.94 ± 0.82 b
N50	2.86 ± 0.05 b	6.43 ± 0.18 cb	16.90 ± 0.70 b
N100	2.97 ± 0.07 ab	7.49 ± 0.40 ab	19.93 ± 1.22 ab
N150	3.04 ± 0.07 ab	8.23 ± 0.49 a	20.94 ± 1.70 ab

* Different letters in the same column denote a significant difference (Fisher LSD test, $p < 0.05$). ** Dv(10), Dv(50), Dv(90)—the diameter of particle in microns below which 10, 50 and 90% of the sample, respectively, lies. Org 0—control treatment of organic crop production system; Org I—organic treatment with cover crops; Org II—organic treatment with cover crops and composted cattle manure; N0—control treatment of conventional system; N50, N100 and N150—conventional treatment with mineral nitrogen applied 50, 100 and 150 kg N ha^{-1}, respectively.

In the conventional system the higher amounts of mineral N also increased the starch granule diameter and values of Dv(10), Dv(50) and Dv(90). The increase was up to 8, 30 and 33%, respectively, compared to the control of the conventional system although only Dv(50) statistically significant. However, the mean diameter of starch granules for the organic system was statistically equal with the mean diameter of the conventional system.

Fertilising with organic and mineral N influenced the biomass yield formation of winter wheat, which in turn influenced the diameter of starch granules. The correlation between starch granule diameter and above ground biomass yield of winter wheat at BBCH65 stage was negative (Table 2). Mean biomass yield over trial years obtained from fertilised treatments of the conventional system ranged between 10.6-12.6 t ha^{-1}, which was 19-39% higher than the organic treatments (data not shown).

3.2. The Flour Yield

According to the ANOVA, the whole flour yield of winter wheat cv Fredis (flour particle size 0-375 and 0-224 µm, respectively) was significantly influenced by weather conditions (65%) and farming system treatments (9%). The influence of the same factors on the fine flour yield was 69% and 7%, respectively. But, the mean whole and fine flour yield over treatments of the organic system was statistically equal with the mean flour yield of the conventional system. In 2013 and 2017 the mean yield of whole and fine flour over treatments was 3-6% and 8-23% higher, respectively, than in 2014-2015 (Table 6). The ratio of whole flour yield to bran and shorts was significantly affected by trial year weather conditions (the proportion of variation was 57%).

Table 6. Mean values (±SE) of winter wheat flour yield over farming system treatments and experimental years.

Year	Fine Flour Yield (g kg^{-1})	Whole Flour Yield (g kg^{-1})	Bran and Shorts (g kg^{-1})	Ratio of Whole Flour Yield to Bran
2013	483 ± 10 bc *	758 ± 3 a	204 ± 2 b	3.72 ± 0.06 a
2014	440 ± 6 d	725 ± 3 b	229 ± 3 a	3.18 ± 0.05 b
2015	459 ± 9 cd	729 ± 3 b	229 ± 2 a	3.19 ± 0.05 b
2017	554 ± 7 a	760 ± 4 a	213 ± 8 b	3.59 ± 0.08 a

* Different letters in the same column denote a significant difference (Fisher LSD test, $p < 0.05$).

The influence of farming system treatments on the fine flour yield was significant according to ANOVA ($p < 0.01$), but the effect was small (the proportion of variation was only 7%) and according to descriptive statistics the effect of treatments was insignificant. Fine flour yield obtained from organic system treatments was between 410-590 g per 1 kg of kernels and from conventional system treatments between 398-584 g per 1 kg of kernels, whereas higher values were obtained from treatments without N or with smaller amounts of N. As mean grain yield over treatments and trial years in this field experiment was 26–36% higher in conventional than that in the organic system (data not shown), the fine flour yield per hectare was also higher in the conventional system. Fine flour yield in organic and conventional treatments ranged between 1507-2206 and 1873-2757 kg ha^{-1}, respectively. The yield of whole and fine flour was significantly influenced by the size of starch granules, and the diameter of starch granules was negatively correlated with flour yield (Table 2).

4. Discussion

In Estonia (by the Baltic Sea), wheat is subjected to ever-changing weather conditions, so the grain yield and quality is seasonally variable as already demonstrated in other wheat producing countries [7,30–33].

The baking quality of wheat flour is better when the number of large starch granules (A-type) is higher than of small (B- and C-type) resulting in higher loaf volumes. This is influenced by the composition of starch granule structure, i.e., the size and ratio of amylose and amylopectine molecules in starch granules [34].

The results of our study showed that the size distribution of starch granules was significantly influenced by weather conditions during the post-anthesis grain filling period of winter wheat. The diameter of starch granules within which 10, 50 and 90% of samples lay, ranged in different trial years between 2.7-3.2, 6.1-8.2 and 15.8-23.5 µm, respectively. The size of starch granules below which 10% of sample lay Dv(10) was significantly smaller ($p < 0.01$) in 2013 and 2017 (Table 2), when the mean temperature development stage of BBCH65 up to PhM was 0.5-3.5 °C higher and 3–5 days longer than in other trial years. Lower values of Dv(10) were probably caused by a greater proportion of small (C-type) granules, which are formed during a later phase of the grain filling period. These findings are in agreement with previous studies [35,36]. Hurkman et al. [35] reported that a shortened grain filling period decreases the levels of enzymes involved in starch biosynthesis. Our results showed that the length of the grain filling period was negatively correlated with mean temperature and GDD values in this period. Acevedo et al. [36] found that mean temperature did not influence significantly the grain filling duration. Heat stress during the grain filling period mainly affects assimilate availability, translocation of photo-synthates to the grain and starch synthesis and deposition in the developing grain. The influence of weather conditions on the length of the grain filling period needs further investigation. The influence of precipitation was insignificant.

Fertilising with different doses of N from different farming systems had a significant effect on starch granule diameter, which is in agreement with Li et al. [17] findings. Higher diameter values of Dv(10), Dv(50) and Dv(90) were obtained from treatments Org II, N100 and N150. In Org II treatments the well composted cattle manure was applied at the tillering stage for winter wheat (in Estonian conditions, the first decade of May) and it’s likely that mineralization of N and subsequent uptake by plants took place in June and July (flowering and grain filling period of winter wheat). The conventional farming system treatments N100 and N150 were fertilized with mineral N twice (second time at BBCH 47 stage of winter wheat), which probably results in higher diameter values of starch granules.

In this study the size of starch granules below which 10 and 50% of sample lay was significantly influenced by above ground biomass yield of winter wheat (negative correlation; Table 2). In conventional farming system the fertilising with mineral N resulted in up to 1.6 times higher biomass yield of winter wheat at anthesis (BBCH65) than that in the organic system. Plant stems and leaf sheaths are sites of temporary carbon storage that can be remobilized to reproductive tissues, significantly contributing to grain filling in later developmental stages [19]. However, Smidansky et al. [37] found that in the case of cereals, higher biomass yield increases grain yield due to enhanced seed number and survival of the seeds (i.e., reduced embryo abortion) rather than to increased starch per seed.

The flour yield and quality obtained from wheat grain depends on the size of starch granules [15], crop production system and proportion of grain endosperm [12,38]. In this study the yield of whole and fine flour obtained after the first break roller milling was mostly influenced by the weather conditions of the trial years and interaction between weather conditions and farming systems. Flour extraction was higher in years with longer post-anthesis grain filling period of winter wheat (in 2013 and 2017) and negatively correlated with starch granule size. A smaller diameter of starch granules indicates a larger proportion of smaller types of granules, higher ratio of whole flour yield to bran and higher flour yield. These conclusions are similar to the results of Edwards [15]. It may be noted that meteorologists predict the prolongation of plant growth period in the Baltic Sea region.

5. Conclusions

The weather conditions during the grain filling period of winter wheat cv Fredis had a strong impact ($p < 0.001$) on grain starch granule size distribution. The size of starch granules was not influenced by farming system (first hypothesis). Fertilization with organic manure and twice with mineral nitrogen during the growing season increased significantly the mean diameter value of different starch granules. The mean diameter of starch granules was smaller in trial years with a longer grain filling period because of higher proportion of C-type granules in these years (second hypothesis). The flour yield of winter wheat cv Fredis kernels was influenced by the size distribution of starch granules, whereas the increased proportion of granules with a smaller diameter (C-type granules) increased the fine flour yield significantly. The impact of farming systems on fine flour yield was not significant (the third hypothesis).

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III

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Article

The Combined Effect of Nitrogen Treatment and Weather Conditions on Wheat Protein-Starch Interaction and Dough Quality

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Abstract: The objective of this field crop study was to compare the effect of organic (cattle manure, off-season cover crop) and mineral N (NH_4NO_3; 0, 50, 100° 150 kg N ha^{-1}) fertilizers on (i) gluten-starch interaction, and (ii) rheological properties of winter wheat dough. Data were collected from the long-term field experiment located in the Baltic Sea region (58°22′ N, 26°40′ E) in years 2013–2017. The amount of minuppueral N 150 kg ha^{-1} applied in two parts before flowering ensured higher gluten content (31 ± 3.3%) and dough quality (81 ± 7.4 mm) due to more positive interactions between gluten proteins and starch granules. The quality of dough was more variable in organic treatments (ranged up to 33%) because the availability of organic N was more variable and sensitivity to the weather conditions was higher. The mean variability of different dough properties over trial years under organic treatments was 1.4–2.0 times higher than in the treatment with 150 kg N ha^{-1}.

Keywords: starch; gluten; nitrogen; mineral; organic; dough rheology

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1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most crucial crops for human sustenance and wellbeing. Unlike other grain flours, wheat flour can generate dough exhibiting a unique three-dimensional structure and viscoelasticity with added water [1]. Wheat dough is a complex mixture, where proteins link together to form the continuous reticular skeleton and in which starch granules act as filling components [2]. High- and low-molecular–weight glutenin subunits have been demonstrated as determinant factors for dough formation and quality [3]. The rheological properties of wheat dough are not only determined by proteins but also by other flour components and their interactions [4]. Approximately 70% of grain and 75% of flour weight is composed of starch. Further to its pivotal role in grain quality and dough functionality through its internal structure and physicochemical properties, it also contributes via starch-gluten interactions [5].

Wheat starch granules have been reported to have a trimodal size distribution [6,7]. The large A-granules (generally larger than 10 µm in diameter) are formed first in developing endosperm, whereas the small B-granules (smaller than 10 µm in diameter) are formed later during kernel development. The formation of very small C-type granules (less than 5 µm) is initiated very late in grain filling [8,9]. Starch granule particle size has been reported to affect dough rheological properties, wherein smaller granules increased dough's elastic characteristics [2,10]. The large A- and smaller B-type starch granules have significant differences in their physical and chemical properties [11,12]. The granule size distribution of wheat starch affects its functionality [13,14], resulting in identifiable quality levels of many final baking, pasta and other industrial products.

While several studies have reported the influence of either gluten or starch alone on the rheological properties of wheat dough quality [15,16], the protein and starch interaction effect on wheat dough properties has been seldom studied because of its complicated multifaceted nature [17,18], thus forming a major gap in knowledge important to the baking industry.

The nitrogenous nutrition of cereals (fertilizing with organic or mineral N fertilizer) is crucial for the development of grain yield, as well as the rheological properties of wheat dough. As organic production becomes more widespread [19], and farmers are urged to decrease mineral nitrogen input, it is important to clarify the extent to which organic fertilization affects gluten accumulation and starch-granule formation during grain filling, whilst the interaction of gluten and starch granules on the dough properties of cereals grown in organic or conventional systems invites further exploration. Earlier, we demonstrated that in addition to the fertilizing regime, local weather conditions significantly influence N availability and the protein content of grains [20].

The objective of this study was to compare the effect of different nitrogen fertilizers (mineral or organic N) and post-hibernation weather conditions on (i) gluten-starch interaction, and (ii) stability of dough rheological properties of winter wheat flours.

Hypotheses 1 (H1). *Wheat grown under a mineral nitrogen background has dough of higher quality.*

Hypotheses 2 (H2). *The quality of the dough is more variable in organic treatments.*

2. Materials and Methods

Grain samples of winter wheat cv Fredis were harvested from a long-term crop rotation experiment, established in 2008 at the Estonian University of Life Sciences (58°22′ N, 26°40′ E). Field experimental conditions followed the previously described methodology. The experimental design, setup, chemical analyses, weather conditions (temperatures and precipitation during growing period) and measurement of starch-granule distribution are detailed in Alaru et al. [21], Keres et al. [20,22].

2.1. Experimental Setup

The rotation consisted of five field crops that followed each other in this order: barley (*Hordeum vulgare* L.) with undersown red clover, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), and potato (*Solanum tuberosum* L.). All crops were grown every year. The field experiment had a systematic block design with four replicates that included the treatments of organic and mineral fertilization. The treatments of mineral fertilization in the conventional system was further divided into four subplots (10 × 6 m) corresponding to the mineral fertilizer (ammonium nitrate) rates of 0, 50, 100 and 150 kg N ha^{-1}. Three organic fertilization subplots were labelled Org 0, Org I and Org II. The data regarding winter wheat from this second rotation period were gathered during crop years 2013–2017.

Zero nitrogen (N0) was the control treatment for the conventional system, without mineral fertilizers, but with pesticides. The conventional treatment N50 had the mineral fertilizer ammonium nitrate (34,4N) applied in early spring at the tillering phase of winter wheat, while treatments for N100 and N150 had N fertilizer applied twice: (1) N100—50 + 50 kg N ha^{-1} at tillering and booting stage BBCH47, respectively; (2) data for treatment of N150 were 100 + 50 kg N ha^{-1}. The three conventional treatments (N50, N100 and N150) had phosphorous (P) and potassium (K) fertilizers applied at sowing at the rate of 25 kg P and 95 kg K ha^{-1} (Yara Mila Cropcare 3–11–24 was used). Amounts of P and K were similar in all treatments.

The organic treatment Org 0 was used as a control, with symbiotically fixed atmospheric N_2 by red clover and pea in the rotation as the only source of N, ploughed into the soil with the above-ground biomass (i.e., twice during the crop cycle period; red clover being pre-crop for winter wheat). In the organic treatment of Org I, in addition to legumes in the rotation, cover crops were used as green manure in winter (after winter wheat, potato and pea). Cover crops were ploughed into the soil as soon as possible after the snow melted in April. In the organic treatment Org II the cattle manure was added in early spring before winter wheat re-growth at a rate of 10 t ha^{-1}, i.e., 50 ± 4 kg N ha^{-1}. Winter wheat was harvested with a Sampo combine on 12 August 2013, 4 August 2014, 12 August 2015, 26 July 2016 and 28 August 2017 (moisture content of kernels ranged from 20 to 28 percent).

2.2. Chemical Analyses

The total nitrogen (Ntot) content of the grain samples was determined by the dry combustion method on a varioMAX CNS elemental analyzer (ELEMENTAR, Hanau, Germany). Wet gluten content (WGC) was determined according to ISO standard 5531 (ISO 5531) by a Glutomatic 2100 apparatus (Perten Instruments AB, Huddinge, Sweden). Gluten index was measured with Perten's apparatus (ICC 155; Glutomatic 2100, Centrifuge 2015; Perten Instruments (now PerkinElmer, Waltham, MA, USA).

2.3. Determination of Dough Properties

Water absorption of flour and dough mixing properties were examined by the Brabender Farinograph–TS Version 2.1.0 (Brabender GmbH & Co, Duisburg, Germany) using the Brabender ICC BIPEA 50 method. Analyses were performed in accordance with ISO 5530-1 standard. The principle of farinograph operation is based on the resistance of dough to kneading. Farinograph curves show the time of formation, i.e., development of the dough, time of stability, and the degree of softening of the dough (after 10 and 12 min). Dough development time (DDT; min) defines the duration from the start of mixing to the point of maximum viscosity, while dough stability (S) is the time (min) when top of the farinograph crosses the 500 Brabender Units (BU) line to the point when it drops below it. The degree of softening (DS; FE) is the difference in height between the centre of the graph at maximum resistance to mixing and the centre of the graph at a point 10 or 12 min later. Dough quality number (DQN) is the length (mm) from the water point to a point 30 FE below the centre line of greatest consistency along the time axis. Low DQN indicates weak flour that weakens early and quickly while high DQN indicates strong flour that weakens late and slowly [23].

The alveograph (Chopin Technologies, Villeneuve la Garenne, France) was used to measure wheat viscoelastic properties (AACC Approved Method 54–30.02, ICC 121, ISO 27971:2015) [24]. The alveograph measures the main parameters of dough response to biaxial extension by inflating it with air, ie., the pressure (expressed by parameter W) generated inside the dough bubble and the deformation of the dough piece until it ruptures.

The size distribution of starch granules from winter wheat endosperm was determined using a Malvern Mastersizer 3000 analyzer (Malvern Instruments Ltd., Malvern, UK). The trimodal size distribution of starch granules was used, i.e., the diameter of large A-granules was >10 µm, B-granules 5–10 µm and for C-granules < 5 µm.

2.4. Meteorological Data

The climate of Estonia was found to be slightly continental at the experimental site. Meteorological data of the post-hibernation vegetation period in 2013–2017 were collected from a meteorological station approximately 2 km from the trial site [20]. The length of the post-hibernation vegetation period depended on the snow melting time in April. The average duration of the vegetation period (air temperature permanently above 5 °C) was 175–190 days. Later snowfall in 2013 and 2017 and lower temperature values during the vegetation period in 2017 caused a later harvesting time of winter wheat. In 2015, lower temperatures and regular precipitation during the post-hibernation period resulted in record grain yields of winter wheat. The most unfavorable season for grain yield formation were the weather conditions in 2016, when the amount of precipitation in May was only 2 mm and temperature data during the post-hibernation vegetation period were higher than the long-term average. In addition, the wintering of wheat failed in 2016, where the number of plants per unit area decreased two-fold, resulting in a grain yield 3.4 times lower than the recorded yield in 2015.

2.5. Statistical Analysis

A statistical analysis of the collected data was performed with the software Statistica 13 (Quest Software Inc., Aliso Viejo, CA, USA). Factorial analyses of variance (ANOVA) and two-factor ANOVA were used to test the effect of cropping systems and the experimental year on granule-size distribution and flour yield. Fisher's least significant difference test for homogenous groups was used for testing significant differences between treatments and between years. The means are presented with their standard errors (±SE). The level of statistical significance was set at $p < 0.05$, if not indicated otherwise.

3. Results

3.1. Effect of N Treatment and Weather Conditions on Starch-Gluten Interaction

The size distribution of starch granules and the amount of protein and gluten accumulated during the filling period of kernels depended on the length of the grain-filling period and availability of N from organic or mineral N fertilizers.

The size distribution of starch granules was significantly influenced by weather conditions during the post-anthesis grain-filling period of winter wheat (calculated proportion of variance for weather conditions and N treatment was 38 and 17%, respectively). The diameter of A-type starch granules was significantly smaller ($p < 0.01$) in 2013, 2016 and 2017, when the proportion of C-type starch granules in kernels was higher due to 3–5 days longer grain filling period (from flowering up to physiological maturity stage) than in other trial years (Figure 1). The diameter of the A-type starch granules was larger in trial treatments of Org II (winter cover crops and manure) and N150, where the mean diameter of granules was 22.8 ± 1.9 and 20.9 ± 3.2 μm, respectively.

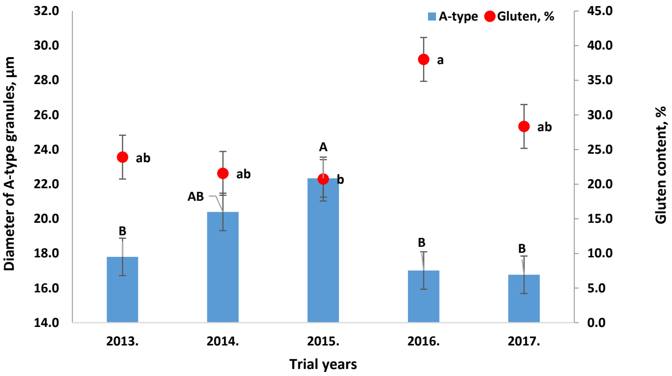

Figure 1. Mean diameter of A-type starch granules (μm) and gluten content (%) values over treatments during different trial years. The means marked with the same letter do not differ significantly from each other.

The gluten content of flour was most significantly influenced by the weather conditions, which in turn influenced the nitrogen availability during the grain-filling period (proportion of variance for weather conditions was 83%); the gluten content was the highest in the N150 treatment varying between 24–43% in the trial years. Higher amounts of gluten in wheat kernels were measured in the trial years with significantly smaller A-type starch granules (Figure 1), whereas there was no significant correlation between gluten content and starch-granule diameter.

N treatments had a strong effect on the protein content (R = 0.70, $p < 0.001$; Table 1). The protein content (PC) of winter wheat did not vary significantly between organic and conventional fertilizing treatments. Mean PC was highest in conventional treatment N150, of up to 3.9 percentage points higher than mean PC values of all other treatments. The PC was influenced by the weather conditions of each growing year, while higher values were obtained in years with a lower level of grain yield; the higher mean PC values over treatments were 12.4 ± 0.30 and 14.6 ± 0.48% in 2014 and 2016, respectively, which proved to be up to 2.3 and 4.5 percentage points higher than that of other years, respectively. PC correlated positively with dough quality number (R = 0.70, $p < 0.001$).

Table 1. Rheological values from doughs obtained from different N treatments.

Indicator.	Org 0 ***	Org I	Org II	N0	N50	N100	N150
PC, % **	11.2 ± 0.7 b	11.5 ± 0.6 b	11.3 ± 0.9 b	11.3 ± 0.6 b	11.6 ± 1.1 b	12.9 ± 1.1ab	13.6 ± 0.7 a
WGC, %	25 ± 2.6 b *	25 ± 2.8 ab	25 ± 4.2 ab	25 ± 2.8 ab	25 ± 3.8 ab	29 ± 3.4 ab	31 ± 3.3 a
GI,%	86 ± 2.2 a	84 ± 2.2 a	87 ± 1.9 a	87 ± 2.7 a	83 ± 4.9 a	82 ± 3.3 a	73 ± 3.5 b
WAC,%	57 ± 0.8 b	57 ± 0.7 b	57 ± 0.8 b	58 ± 0.6 b	58 ± 0.7 b	60 ± 0.9 a	61 ± 0.4 a
DDT, min	2.08 ± 0.2 b	2.02 ± 0.2 b	2.02 ± 0.2 b	2.24 ± 0.3 ab	2.16 ± 0.2 ab	3.00 ± 0.3 ab	3.54 ± 0.2 a
S, min	4.20 ± 0.55 a	4.17 ± 0.42 a	4.07 ± 0.50 a	4.39 ± 0.43 a	4.28 ± 0.55 a	5.08 ± 0.40 a	6.20 ± 0.38 a
DS, FE	67 ± 12.5 a	65 ± 5.9 a	68 ± 11.4 a	67 ± 9.0 a	66 ± 9.4 a	56 ± 8.1 a	44 ± 5.4 a
W, 10e-4 J	204 ± 18 a	204 ± 7 a	213 ± 11 a	233 ± 11 a	217 ± 11 a	234 ± 10 a	255 ± 17 a
DQN, mm	49 ± 11.2 a	48 ± 7.3 a	47 ± 9.8 a	50 ± 8.5 a	53 ± 9.5 a	65 ± 9.7 a	81 ± 7.4 a

* Different letters denote significant difference; ** PC—protein content, WGC—wet gluten content, GI—gluten index, WAC—water absorption capacity, DDT—dough development time, S—dough stability time, DS—degree of dough softening, W—force required to create a bubble, DQN—dough quality number; *** Org0 and N0 = control treatments of organic and conventional farming, respectively; Org I = organic treatment with winter cover crops (CC); Org II = in addition to CC the cattle manure application; N50, N100 and N150 = amounts of mineral N50, 100 and 150 kg N ha^{-1}, respectively.

Wet gluten content (WGC) correlated strongly with PC (R = 0.77, $p < 0.001$). Significantly higher values of gluten were obtained from kernels of treatment N150 (Table 1) and in the trial years of 2016 and 2017 (mean WGC values over N treatments were 38 ± 0.7 and 28 ± 0.6%, respectively). According to the protein and gluten content values, only the flour obtained from the N150 treatment belonged to the elite class; flour from the N100 treatment belonged to A class and the flours of all other variants were of low quality [25]. The WGC correlated positively with water absorption capacity and dough development time (R = 0.57 and 0.51, respectively; $p < 0.001$ for both values).

The gluten index (GI) characterizes the strength of the dough. GI values were mostly influenced by N treatment and the second factor, i.e., weather conditions during each trial year had little or no effect. The calculated proportion of variance for treatment and weather conditions was 36 and 14%, respectively. N availability in different treatments had a strong effect on GI (R= −0.56, $p < 0.001$); lower values were measured in wheat flours obtained from treatments N100 and N150 that were fertilized twice after wintering (Table 1). The mean values of GI in organic treatments and conventional treatments with lower amounts of N ranged between 83–87%, which was up to 5 and 15% higher than that of N100 and N150 value, respectively. For forming dough with good strength, GI values between 60–95% are considered acceptable [26]. According to Cubadda et al. [27], GI may describe whether gluten quality is weak (GI < 30%), normal (GI = 30–80%), or strong (GI > 80%).

The GI values of this field trial were negatively correlated with dough-development time (R = −0.53, $p < 0.001$), water absorption capacity (R = −0.49, $p < 0.01$), dough stability (R = −0.49, $p < 0.01$) and finally with dough-quality number (R = −0.56, $p < 0.001$). The correlation between GI and diameter values of smaller starch granules was significantly negative (coefficient R for C- and B-type granules was −0.32 and −0.34, respectively). In general, the mean value of GI across treatments and trial years was 83 ± 1.3%, whereas GI varied under different trial years by 69–96% (Figure 2).

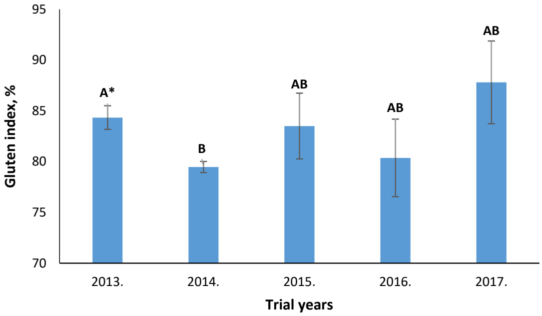

Figure 2. Gluten index values under different trial years. * The means marked with the same letter do not differ significantly from each other.

3.2. Rheological Properties of Wheat Dough

When water is mixed into wheat flour, it forms dough. The water absorption capacity (WAC) of flour is one of the indicators of the quality of dough. WAC values were influenced by both trial factors—by weather conditions in trial years and N treatments; the calculated proportion of variance was 36 and 46%, respectively. WAC of flour correlated positively with PC and WGC (R = 0.83, $p < 0.001$ and 0.57, $p < 0.001$, respectively) and positively with diameter values of small B- and C-type starch granules (R = 0.32 and 0.34, respectively). The differences in WAC values between treatments were not significant, except for the N100 and N150 treatment with a WAC of up to 61% (Table 1). The diameter of smaller starch granules was larger in 2014 and 2016 [21], and WGC was higher in 2016 and 2017 (Figure 1); mean values of WAC ranged between 58–60%in 2014, 2016 and 2017, which was up to 3 percentage points higher than that of other trial years. Wheat flours with WAC values greater than 58% were classified as strong flours [28].

Of the factors studied, the dough development time (DDT) was only significantly influenced by N treatment (R = 0.52, $p < 0.001$) and it correlated positively with PC and WGC (R = 0.83, $p < 0.001$; R = 0.51, $p < 0.001$, respectively). DDT of treatments ranged between 1.46–4.09 min, whereas only the N150 treatment had a mean value of DDT significantly longer than that of organic treatments, of up to 2.23 min longer (Table 1). According to Kassomeh [28], DDT may describe whether dough is weak (DDT is less than 2.5 min), medium (2.5–4.0 min) or strong (4.0–8.0 min). DDT was positively correlated with dough quality number (R = 0.86, $p < 0.001$).

The stability of dough (S) was most affected by PC (R = 0.48, $p < 0.01$). The correlation analysis also demonstrated that dough stability was better when the diameter of smaller starch granules (C- and B-type) was larger (R = 0.32). The most stable dough was made from N150 flour. The mean values of dough stability over trial years did not differ significantly between any of the treatments (Table 1), varying between 3.33–6.58 min. This particular time of S is classified as the medium stability [28,29].

The degree of dough softening (DS) 10 min after initiating mixing and also 12 min after attaining the maximal peak was significantly influenced by both trial factors (calculated proportion of variance for trial year and N treatment was 63 and 17%, respectively). The softening degree was significantly low in 2014 (46 ± 3.9%), followed by 2013 and 2016 (up to 10% higher than that of 2014). Mean DS values (10 min after beginning) of N treatments as an average of trial years ranged between 39–76 FE, which is classified as medium [28]. Lower softening degree values were measured in the doughs derived from flour of conventional treatments of N100 and N150. As the differences between trial years were significant, the mean effect of treatments over trial years was found to be non-significant (Table 1). The coefficient of variation was higher for organic treatments (ranged between 20–42%; for conventional treatments 27–32% as represented in Table 2).

Table 2. Coefficient of variation (%) of different dough quality parameters over trial years (2013–2017) for all trial treatments.

Indicator	**Org 0 ****	**Org I**	**Org II**	**N0**	**N50**	**N100**	**N150**
WAC, % *	3.2	2.6	3.2	2.2	2.6	3.3	1.5
DDT, min	40.1	30.7	42.5	44.6	36.7	41.3	15.6
S, min	47.6	36.8	45.9	34.7	46.1	29.5	22.4
DS, FE	41.9	20.2	37.4	29.9	31.8	32.5	27.4
FQN, mm	51.1	33.9	47.1	37.9	40.2	33.4	20.4
AVERAGE	33.0	22.8	32.2	26.8	29.8	26.6	16.6

* WAC—water absorption capacity, DDT—dough development time, S—dough stability time, DS—degree of dough softening, PC—protein content, DQN—dough quality number; ** See explanation under Table 1.

DS values were positively correlated with GI ($R = 54$, $p < 0.001$), negatively with grain PC ($R_{10min} = -0.55$, $p < 0.001$) and with diameter values of the smallest C-type starch granules ($R = -0.32$). The larger granules (A and B types) did not significantly affect the softening degree of dough.

According to the alveograph results, the force required to create a bubble (W) was mostly affected by N treatment ($R = 0.50$, $p < 0.001$), followed by the weather conditions of the trial year ($R = 0.38$; Table 1). The values of force required for bubbles ranged, in organic and conventional systems, between 204–213 and 217–255 $\times 10^{-4}$ J, respectively, while differences between all treatments were not significant. Lower values of this force were obtained in years with a higher level of grain yield and lower content of protein (in 2015 and 2017).

The dough quality number (DQN) was influenced by both trial factors, whereas the annual weather conditions had a much stronger effect than N treatment (calculated proportion of variance was 46 and 29%, respectively). Improved values of DQN were obtained in 2014, in which the quality values ranged between 64–108 mm, followed by 2016, when quality values ranged from 59–77 mm. The lowest values of dough quality were obtained in 2017, when quality values ranged in all treatments, except for N150, between 21–31 mm (Figure 3). DQN depended significantly on the availability of nitrogen during the grain-filling period, and it correlated positively with kernels PC ($R = 0.70$, $p < 0.001$; Table 1). The mean dough quality values of the treatments over the trial years did not differ significantly because of their great variability.

The final DQN value was found to be the result of an interaction of several factors and Table 2 shows the correlation coefficients between the DQN and the indicators that influenced its value more significantly. In particular, the PC and WGC of the flours were decisive in the formation of the DQN value, as these parameters greatly influenced the properties of the dough. B- and C-type starch granules indirectly affected the values of DQN in the interaction with WAC and GI (Table 3).

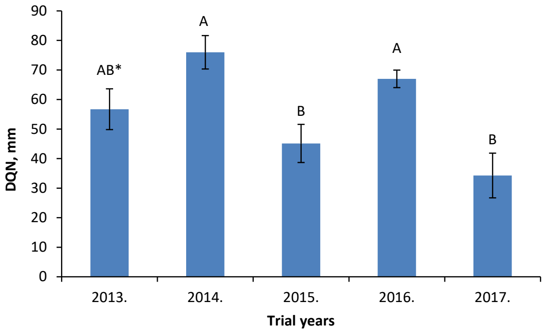

Figure 3. Mean values of dough quality number (DQN; mm) of winter wheat Fredis over N treatments. * Different letters denote significant difference.

Table 3. Factors that most affected value of dough quality number (DQN) expressed as a correlation coefficient (R).

Factors	R *	*p* **
1. Dough stability (S)	0.94	$p < 0.001$
2. Degree of dough softening (DS)	−0.89	$p < 0.001$
3. Dough development time (DDT)	0.86	$p < 0.001$
4. Water absorption capacity (WAC)	0.73	$p < 0.001$
5. Grain protein content (PC)	0.70	$p < 0.001$
6. Gluten index (GI)	−0.56	$p < 0.001$
7. WAC x diameter of B- and C-type starch granules	0.33	$p < 0.05$
8. GI x diameter of B- and C-type starch granules	−0.32	$p < 0.05$

* Correlation coefficient; ** *p* value indicates significance at 95, 99 and 99,9%.

3.3. Variation of Dough Quality Number

The results of this field trial demonstrate that dough rheological properties and its quality were significantly influenced by both trial factors; the proportion of variation for year and treatment were 46 and 29%, respectively. Both of these factors in turn influenced the accumulation of protein and gluten in grains of winter wheat and the size distribution of starch granules. Due to the significant variation in values of dough rheological properties and quality over the years, there were often no significant differences found between the treatments. The coefficient of variation for different dough properties (Table 3) indicated that the smallest variability between these values was for the N150 treatment, where WGC was the highest and the GI was the lowest (Table 1).

4. Discussion

When wheat flour is mixed with water, natural hydrocolloid gluten interacts with water molecules to create wheat dough, embracing a three-dimensional network, filled with starch granules [18]. Gluten traps the gases generated during the fermentation process, while starch significantly affects the rheological properties of the dough. The specific interaction between gluten and starch in the microstructure of wheat dough has been shown to influence dough behaviour [30]. Therefore, determining the properties of separated gluten and starch independently may not fully explain the behaviour of wheat dough [18]. The topic of this article is the study of the effect of mineral and organic nitrogen fertilizers on the gluten content of wheat dough, the combined effect of starch granule size and gluten on the rheological properties of the dough, and the effect of weather conditions on the stability of dough quality.

The first hypothesis was that wheat grown under a mineral nitrogen background has dough of a higher quality, which was mostly confirmed by our results. The quality of the dough was assessed through its rheological properties and the results showed that quality depended on a combination of several factors, but in particular on the quantity and quality of protein and gluten, which in turn depended on both of the studied trial factors (weather conditions in trial years and N treatment, i.e., mineral or organic N availability during grain filling period). The protein and gluten content and therefore, the wheat-dough quality, were better in 2014 and 2016, when the grain yield level was lower [20], which is also in line with the findings of Giancaspro et al. [31]. The mean protein and gluten content over the trial years were highest in flours obtained from N treatment N150.

The quality of dough also depended on N availability as well as on the size distribution of the starch granules formed during the grain filling period.

The water absorption capacity (WAC) is one of the indicators of higher dough quality in flours with a smaller diameter of starch granules [4,18]. Previous studies found that smaller granules have a higher surface-to-volume ratio and are able to hydrate and swell more efficiently and bind more water than larger granules [13,32]. Therefore, an increased content of small granules should increase the flour water absorption capacity [33,34]. In our experiment, the WAC values also correlated positively with B- and C-type starch granule diameters, whereas the highest values of WAC were obtained when the diameter of smaller granules ranged between 3.2–9.0 µm, i.e., the WAC was higher when the diameter of smaller starch granules was larger (the diameter of the measured C- and B-type starch granules ranged between 2.45–9.84 µm). The WAC values were much better in treatments N100 and N150, which was caused mostly by higher contents of protein and gluten (Table 1). In this field experiment, the calculated proportion of variance showed that N treatment had a stronger effect on WAC values than the weather conditions.

The development time and stability of dough in this trial were longer and the degree of dough softening was lower in treatments of N100 and N150 due to a higher PC and WGC; the stability time of dough was ca 2 min longer in these treatments than that of other treatments. These results are consistent with those of Gao et al. [18], because the micro-structure of dough with higher PC and WGC contains fewer gaps between gluten and starch. The dough for which PC and WGC are fully integrated with each other in their sizes was found to be more stable. Gao et al. [18] found that wheat flours with low PC and WGC may contain irregular and larger holes. Zi et al. [35], discussing the starch granule effects on the stability of dough, found that large starch granules or fewer small starch granules are both associated with weak dough processing properties because of heterogeneity of the gaps and holes in the gluten network. Cao et al. [4] reported that starch granules act as the filling particles in a protein–starch matrix. In this experiment, the dough stability due to WAC was lower in organic and N0 and N50 treatments, probably due to irregular and larger holes in the gluten network with a lower filling degree and less interaction between small starch granules and gluten; such dough is unstable. According to Kulhomäki and Salovaara [29] the dough stability time of 4–12 min indicates a good quality of dough. In our experiment, the dough stability times in the organic treatments and in the N0 and N50 treatments were close to the lower limit, probably because of the irregular and insufficient accumulation of nitrogen in grains during the grain filling periods of different trial years, which resulted in a lower protein and gluten content. The unstable availability of N in these variants also caused great variability in the quality of the dough (Table 3).

The gluten index (GI) characterizes the ratio of gliadins to glutelins. GI, dough development time and stability are all critical parameters reflecting dough strength, and higher values of these parameters indicate stronger dough [36,37]. It has been suggested that gliadins generally contribute to dough viscosity and glutelins contribute to dough elasticity [38,39]. Oikonomou et al. [40] found that GI values are positively correlated with protein content and the level of fertilization with nitrogen. In our experiment, we found the opposite to be true—GI values were negatively correlated with protein content and amounts of nitrogen fertilizer applied, which is in line with the results reported by

Borkowski et al. [26]; higher values of GI were found to be associated with lower values of protein in most treatments (over 80% and 11.2–11.6%, respectively), except N150, where the mean value of GI was 73 ± 3.5% and PC was up to 2.4% higher than that of other treatments. GI values over 80% indicate an increased proportion of glutenins, i.e., increased resistance to extension of dough [36]. Too strong wheat dough does not rise well and does not produce an airy loaf. Due to higher PC and WGC the mean values of GI were up to 9% lower in 2014 and 2016 than those of other years.

Dough quality was influenced by several factors, with the nitrogen fertilization regime having the greatest impact. In our experiment, the dough with a higher DQN was obtained from flours with lower values of GI (treatment N150, GI = 73%) and with a medium diameter of B- and C-type starch granules. At a constant protein content and optimal glutenin-to-gliadin ratio, the dough stability time was found to be longer, the degree of softening lower and dough quality higher.

The second hypothesis posited that the quality of the dough would be more variable in organic treatments. The results of this trial showed that the amount of mineral N 150 kg ha^{-1}, applied in two parts before flowering, ensured stable plant growth and protein and gluten content in different years. Conventional treatments with lower mineral N amounts and organic treatments (fertilizing with manure or using winter cover crops as catch crops) were more sensitive to variable weather conditions than N150. The amount of organic N available to plants was more significantly affected by the distribution of precipitation and temperatures during the growing season, which resulted in the greater variability of values over years; the mean variability of different dough properties over trial years was observed in the organic treatment trials (Org 0, Org I and Org II) at 1.4–2.0 times higher than that of the N150 treatment. A high variability in weather conditions during this study period (2013–2017 years) caused the higher value for the proportion of variation for the year; this resulted in variable values of winter wheat grain yield, protein and gluten content (see more detail in [20]) and therefore, in variable dough rheological properties. Oikonomou et al. [40] reported that higher values of the coefficient of variation indicate stronger relationships between treatments and weather conditions. The high variability in the protein content of the N50 treatment indicates that this specific amount of mineral N was not always sufficient for a full or optimal accumulation of protein during the grain-filling period across all trial years.

5. Conclusions

Taken together, these analytical results of winter wheat dough strongly indicate that, of the seven treatments tested, mineral fertilization with the highest nitrogen rate (150 kg/ha) is crucial for achieving high quality dough. On the other hand, organic cropping systems, even with added manure, are still more vulnerable to the vagaries of the air–soil microclimate, and this sensitivity is reflected in dough quality. Future studies on different crop rotations and different organic fertilizers should focus on soil nutrient density, to investigate ways in which the soil/root uptake mechanisms can be made more harmonious for plant growth and the plant's own holistic health as reflected in its protein–starch interplay. Furthermore, in the context of increasing organic production, it is important to scrutinize other solutions in the value chain of bread-making that can account for the variability in grain quality and can improve wheat-dough quality.

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- 8-2/T10038PKTM „Alternatiivsete väetusainete keskkonnahoidliku kasutuse võimalused ja efektiivsus tava- ja maheviljeluses võrdlevalt traditsiooniliste orgaaniliste ja mineraalväetistega” (3.03.2010−1.12.2014); Vastutav täitja: Henn Raave; Eesti
- 8-2/T8015PKPK „Vedelsõnniku (läga) kasutamine rohumaade ja põllukultuuride väetisena ning mõju keskkonnale ja saagi kvaliteedile” (24.01.2008−1.12.2010); Vastutav täitja: Rein Viiralt; Eesti Maaülikool, Eesti Maaülikool
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- 8-2/T6184PKPK06 „Erinevate bioloogiliste silokindlustuslisandite mõju rohu-, maisi- ja teraviljasilo kvaliteedile ning tulususele” (20.12.2006−5.12.2008); Vastutav täitja: Are Selge; Eesti Maaülikool
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Curriculum vitae

Indrek Keres

Date of birth	21.04.1974
E-mail	indrek.keres@emu.ee

Institutions and positions

01.01.2017–...	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Crop Science and Plant Biology, Lecturer (1,00)
2005–...	Pokaveski OÜ, board member, apiarist (0,50)
2012–31.12.2016	Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Department of Field Crop Husbandry, Researcher (1,00)
2006–2012	Estonian University of Life Sciences, Researcher (0,50)
2006–2012	Estonian University of Life Sciences, Lecturer (0,50)
2004–2005	AS Andressel, crop production manager (1,00)
2001–2002	Eesti Murud OÜ, sales manager (1,00)
2000–2006	Eesti Maaülikool, researcher (1,00)
1999–2000	Baltic Farming OÜ, manager (1,00)

Education

2012–2013	Apiarist, Olustvere School of Rural Economics and Service Industry
1995–1999	Higher education, Estonian Agricultural University

Academic degrees

Indrek Keres, Research Master's Degree, 2001, (sup) Are Selge, „Kura ristiku agrobioloogiline iseloomustus, soovitatavad külvisenormid ja Eesti pedoökoloogilistes oludes kasvatamiseks sobiva Rhizobium-bakteri tüve välja selgitamine/Kura clover agrobiological characteristics, seeding rates and suitable Rhizobium bacteria", Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Department of Field Crop Husbandry, Department of Grassland Science.

Honours & awards

De Laval award, 2002

Projects in progress

- RESTA28 „Valorization of cereal and oil seed crops" (1.02.2021−31.07.2023); Principal Investigator: Evelin Loit; Estonian University of Life Sciences
- L200017PKTT (ERA-Net Cofund FACCE SURPLUS rahastamistaotlus nr 45) „Biofortified and climate-resilient food and fodder production on marginal soils" (1.01.2020−31.12.2022); Principal Investigator: Evelin Loit; Estonian University of Life Sciences

Completed projects

- PRG1260 „Correlation of farming systems and gene expression in crops" (1.01.2021−31.12.2021); Principal Investigator: Evelin Loit; Estonian University of Life Sciences
- L200054MSMJ (Hankeleping nr 224510) „Riskijuhtimine nõustajatele „ (1.09.2020−30.11.2021); Principal Investigator: Rando Värnik; Estonian University of Life Sciences
- 6TX160025PKTM (27-5/72/2016/) „Kõrgkooliõpik „Taimekasvatus" „ (31.05.2016−31.12.2020); Principal Investigator: Evelin Loit; Estonian University of Life Sciences
- 8L160202PKTM „Korjetaimede seemnesegude väljatöötamine" (15.11.2016−1.11.2019); Principal Investigator: Liina Talgre; Estonian University of Life Sciences
- P170261PKTT „Tärkliseterade geneetiline ja morfoloogiline iseloomustus eri viljelusviisides „ (23.11.2017−31.08.2018); Principal Investigator: Evelin Loit; Estonian University of Life Sciences
- 8-2/T15072PKTM „The implementation of a sustainable dairy cattle managing model that is competitive and environmentally friendly in the farming enterprises of Chernihiv oblast" (15.07.2015−14.07.2017); Principal Investigator: Kalle Kask; Estonian University of Life Sciences
- 8-2/T13055PKTK „Presence of pesticide residues in honey and pollen gathered from Estonia: effect on honey bees" (1.01.2013−1.12.2014); Principal Investigator: Reet Karise; Estonian University of Life Sciences

- 8-2/T11028PKTM „Effective use of local fertilizers and economic analysis of grassland utilization in farm-based feed production” (26.01.2011−1.12.2014); Principal Investigator: Rein Viiralt; Estonian University of Life Sciences.
- 8-2/T10038PKTM „Alternative fertilizers environment-saving utilization opportunities and efficiency in conventional and organic farming in comparison with traditional organic and mineral fertilizers” (3.03.2010−1.12.2014); Principal Investigator: Henn Raave; Estonian University of Life Sciences
- 8-2/T8015PKPK „The use of liquid manure (slurry) as fetilizer for grassland and field crops and its impact on the environment and yield” (24.01.2008−1.12.2010); Principal Investigator: Rein Viiralt; Estonian University of Life Sciences, Estonian University of Life Sciences
- 8-2/T9064PKPK „Demonstration trials of fertilizers” (12.05.2009−15.12.2009); Principal Investigator: Indrek Keres; Estonian University of Life Sciences, Estonian University of Life Sciences
- 8-2/T6184PKPK06 „Erinevate bioloogiliste silokindlustuslisandite mõju rohu-, maisi- ja teraviljasilo kvaliteedile ning tulususele” (20.12.2006−5.12.2008); Principal Investigator: Are Selge; Estonian University of Life Sciences
- ETF5751 „The relationships between nutrient cycling and grassland phytoproductivity depending on stand composition, defoliation frequency and fertilizer application” (1.01.2004−31.12.2007); Principal Investigator: Rein Viiralt; Estonian University of Life Sciences.
- SF0172615s03 „The functioning of grassland communities, their species diversity, productivity and efficient management in various environmental conditions” (1.01.2003−31.12.2007); Principal Investigator: Rein Viiralt; Estonian University of Life Sciences.

Supervised dissertations

- Kristjan Tiideberg, Master’s Degree, 2020, (sup) Indrek Keres; Erkki Mäeorg „Suvirapsi seemnesaagikus ja -kvaliteet sõltuvalt taimede reavahede laiusest (Effects of row spacing on seed yield and quality of spring canola Brassica napus L.)“, Estonian University of Life Sciences.

- Kevin Laande, Master's Degree, 2020, (sup) Indrek Keres; Erkki Mäeorg „Rapsitaime väliskuju ja saagikuse vahelised seosed, tulenevalt reavahest (Relationships between rapseed canopy architecture and seed yield, depending on row spacing)", Estonian University of Life Sciences.
- Annika Puumets, Master's Degree, 2017, (sup) Are Selge; Andres Olt; Indrek Keres „Bioloogiliste ja keemiliste kindlustuslisandite mõju silo fermentatsioonile ja kvaliteedile (The effect of biological and chemical additives on fermentation and quality of silage)", Estonian University of Life Sciences.
- Heili Kaasiku, Master's Degree, 2014, (sup) Indrek Keres; Reet Karise „Varroalesta esinemine erineva intensiivsusega põllumajandusmaastikes: pestitisiidi jääkide mõju (Varroatosis in beehives from different agricultural areas: effect of pesticide residues)", Estonian University of Life Sciences.
- Mari Riisenberg, Master's Degree, 2012, (sup) Are Selge; Indrek Keres „Maisi (Zea mays) kasvatamise võimalused Eestis", Estonian University of Life Sciences.

Publications

2022

1.1. Esmaeilzadeh-Salestani, Keyvan; Samandari_Bahraseman, Mohammad Rasoul; Tohidfar, Masoud; Khaleghdoust, Banafsheh; Keres, Indrek; Mõttus, Aleks; Loit, Evelin (2022). Expression of AMT1;1 and AMT2;1 is stimulated by mineral nitrogen and reproductive growth stage in barley under field conditions. Journal of Plant Nutrition, 1−13. DOI: 10.1080/01904167.2022.2067764.

3.4. Korge, Mailiis; Khalegh Doust, Banafsheh; Alaru, Maarika; Keres, Indrek; Kurg, Max; Loit, Evelin (2022). Beetaglükaani sisaldus odra ja nisu terades sõltuvalt lämmastikväetise normist ja viljelusviisist. Maarika Alaru (Toim.). Agronoomia 2022 (224−230). Tartu: Vali Press.

2021

1.1. Keres, Indrek; Alaru, Maarika; Koppel, Reine; Altosaar, Illimar; Tosens, Tiina; Loit, Evelin (2021). The Combined Effect of Nitrogen

Treatment and Weather Conditions on Wheat Protein-Starch Interaction and Dough Quality. Agriculture, 11 (12), 1232. DOI: 10.3390/agriculture11121232.

3.5. Margus, Kalle; Eremeev, Viacheslav; Keres, Indrek; Keres, Mia; Talgre, Liina; Luik, Anne (2021). Viljelusviisi ja kartulisordi mõju mugulate toorlõikude tumenemisele. Tupits, Ilmre; Tamm, Ülle; Tamma, Sirje; Toe, Anu; Vanamb, Evelyn (Toim.). Agronoomia 2021 (129−136). Vali Press.

2020

1.1. Raimets, Risto; Bontšutšnaja, Anna; Bartkevics, Vadims; Pugajeva, Iveta; Kaart, Tanel; Puusepp, Liisa; Pihlik, Priit; Keres, Indrek; Viinalass, Haldja; Mänd, Marika; Karise, Reet (2020). Pesticide residues in beehive matrices are dependent on collection time and matrix type but independent of proportion of foraged oilseed rape and agricultural land in foraging territory. Chemosphere, 238, 1−9. DOI: 10.1016/j.chemosphere.2019.124555.

1.1. Keres, I.; Alaru, M.; Talgre, L.; Luik, A.; Eremeev, V.; Sats, A.; Jõudu, I.; Riisalu, A.; Loit, E. (2020). Impact of Weather Conditions and Farming Systems on Size Distribution of Starch Granules and Flour Yield of Winter Wheat. Agriculture, 10 (1), ARTN 22. DOI: 10.3390/agriculture10010022.

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1.1. Karise, Reet; Raimets, Risto; Bartkevics, Vadims; Pugajeva, Iveta; Pihlik, Priit; Keres, Indrek; Williams, Ingrid H.; Viinalass, Haldja; Mänd, Marika (2017). Are pesticide residues in honey related to oilseed rape treatments? Chemosphere, 188, 389−396. DOI: 10.1016/j.chemosphere.2017.09.013.

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VIIS VIIMAST KAITSMIST

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IN DIFFERENT TYPES OF WASTEWATER
BIOSENSOR-RIVI ERINEVATE REOVETE BIOKEEMILISE
HAPNIKUTARBE UURIMISEKS
Kaasprofessor Merlin Raud, kaasprofessor Jaak Nerut, professor Timo Kikas
29. august 2022

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