Faculté des bioingénieurs

Evaluation of the effect of nitrogen fertilization and tillage on the yield and the nutritional profile of flaxseed

Master's thesis

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Année académique 2018-2019
Bioingénieur : sciences agronomiques

Acknowledgements

First of all, I would like to thank my two supervisors, Prof. Pierre Bertin and Prof. Yvan Larondelle for making this master's thesis happen. I wished to work on an experiment that combined the agronomic and the nutrition sector and I am very happy that they helped me to find such a great project. I am very grateful for their support and their commitment in this work. They guided me throughout all this year and their advices and recommendations were always very helpful.

I also would like to thank all the members of the two laboratories who helped me during the experiments. More precisely, Pierre Van Thorre from the laboratory ECAV was really involved in the cultivation of flaxseed and the soil analysis. I am very grateful that he made sure the crop was going well. I would also like to thank Gerard Collignon and the staff of the Center Alphonse de Marbaix for the great work they have done with the flaxseed cultivation. Finally, Cecile Gardin and Eric Mignolet from the laboratory BNTE showed me many laboratory manipulations. I would like to thank them for their time, their patience and their kindness.

I also thank Professors Michel Focant and Richard Lambert for accepting being part of my jury.

Finally, I must express my profound gratitude to my parents, my friends and Antoine for providing me continuous support and encouragement throughout this year of master's thesis.

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List of abbreviations

N	Nitrogen
ALA	Alpha-linolenic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
PUFAs	Polyunsaturated fatty acids
LA	Linoleic acid
ARA	Arachidonic acid
SDG	Secoisolariciresinol diglycoside
GC	Gas chromatography
HPLC	High-performance liquid chromatography
DM	Dry matter
NUE	Nitrogen use efficiency

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Context

Regenacterre is a non-profit organization that promotes a regenerative agriculture in Belgium, which means a sustainable agriculture that respects both men and environment. The organization helps farmers to implement appropriate techniques on their fields. They give advice concerning many parameters such as crop protection, biological activity, soil preparation, crop rotation and cover crops. As soil protection is a major current concern, they promote conservation agriculture and help farmers to move to reduced tillage practices (Regenacterre).

The Walloon Region is willing to promote local food production and local networks. In that context, *Regenacterre* is involved in a project carried out by the Walloon Region that aims at developing *relay halls*. *Relay halls* would provide a place to regroup and store local harvests so that they could be transformed and commercialized locally. This aims at creating a network of stakeholders and infrastructures to reduce risks and costs associated with agricultural products (Halls-relais agricoles - Portail de l'agriculture wallonne)

Regenacterre also aims at better rewarding farmers who apply sustainable agricultural techniques. Currently, farmers growing food using conservation techniques do not get more benefit than those using conventional techniques. In order to set a better reward, it would be interesting to know whether products coming from conservation techniques have a better quality than those originating from conventional farming. *Relay halls* could be a place to store products grown under conservation agriculture techniques and could help selling them at better prices.

A meeting with the managers of *Regenacterre* has focused our attention on oilseed flax. Flaxseed has many advantages for both farmers and consumers. As Belgium imports large amounts of flaxseed, it would be beneficial to increase the production of this crop in Belgium. Collecting more information about flaxseed could encourage Belgian farmers to grow it.

This master's thesis is about determining the influence of two agronomical factors on the nutritional profile of flaxseed grown in Belgium: nitrogen (N) fertilization level and application of tillage. The first chapter is a state of the art on flaxseed, the agronomical practices required to grow it and its health benefits. It will allow setting the objectives of the thesis, which are presented in the second chapter. In the third chapter, the experimental plan and the methods are going to be developed. The fourth chapter presents the results of the experiments. The fifth chapter contains the discussion of the results and their comparison with the literature. Finally, the last part gives the main conclusions that can be drawn from this work. It ends up with a set of perspectives for further investigations.

Chapter I: State of the art

1. Presentation of flaxseed

Flax (*Linum usitatissimum)* is a blue flowering annual herb that belongs to the family *Lineaceae*. It is a major oilseed crop grown for industrial, feed and food purposes. Seeds appear shiny brown, are both crispy and chewy and are said to have a nutty taste. They are called flaxseeds or linseeds. As the former term is mostly used for nutritional purposes and the latter for industrial applications, the term "flaxseed" will be preferably used in this work. Domesticated in the Middle East more than 5000 years ago, flax plant was gradually grown throughout the world for lots of different purposes (Zuk *et al.*, 2011). It used to be grown for its high-quality fibers, which were used to make textile or paper. The flax oil is incorporated in paints, coatings, inks, soaps and adjuvants (Bekhit *et al.*,2018). Shives and straws are used as thermic insulator materials and as animal litters. Finally, flaxseed raised interest after the discovery of its incredible nutritional and health potential and started to be recommended in the human diet in the early 1990's. Its various uses earned it the name *usitatissimum*, which means "most useful" in Latin. Domestication of flaxseed has led over time to the dissociation of the plant into oilseed and fibrous varieties, that have different physiologies and phenotypes. Fiber flax is largely grown in Europe and is used to make fishnets, twines, fabrics and threads whereas seed flax is grown throughout the world for its oil (Singh *et al*., 2011).

Since the emergence of cotton and synthetic fibers, flax production has decreased. Despite its relatively low current demand, there is a renewed interest for flaxseed for both its nutritional value and its raw materials. The growing demand for flaxseed oil results in an increase in the industrial activity of extraction as well as in the quantity of by-products generated (Bekhit *et al*., 2018). Only a small number of countries grow flaxseed today: Kazakhstan, Russia, Canada, China, India, USA and Ethiopia are the main producers according to FAO STAT, 2017. Belgium only grew 7722 tons of flax in 2017 (FAO STAT, 2017), which places it 15th in the world producers ranking. Most of the fax grown in Belgium is for the purpose of fiber production. This low production is surprising considering that Belgium is the largest importer of flaxseed in the world with 586,515 tons imported in 2016. Belgium is also the largest re-exporter of flax and flax oil, showing that this country is a real trade hub for flax, via the port of Antwerp. Belgium has indeed an advanced industrial sector, largely represented by the Vandeputte company. Boosting Belgian flaxseed production could therefore be beneficial for this sector by reducing the dependence on Canada and other suppliers and allowing a better traceability of its products.

2. The many uses of flax

Flax is used in many different sectors. It has the particular quality that every part of the plant can be used, directly or following a process step.

2.1 Flax as a food

The growing interest for flaxseed might be linked to the increasing concern of consumers about their health combined with the scientific validation of the implication of flaxseed compounds in preventing chronic diseases. There are three main components of flaxseed that are interesting for human health and that make it a functional food: alpha-linolenic acid (ALA), fibers and lignans (see section 5 for more details about these compounds). According to Kajla *et al.* (2015), a functional food can be defined as "the food or food ingredient that may provide physiological benefits and helps in preventing and/or curing diseases". This concept keeps gaining interest in the food science domain.

Flaxseed oil is the most common form for consuming flaxseed and it generates by-products (seedcakes, fibers and shives) that can be used for different purposes. Flaxseed oil is usually extracted by applying cold pressure on the seeds to preserve the omega-3 fatty acids that are sensitive to heat. It is not recommended to use it as a cooking oil, but it is a good food supplement that is often added in salad dressings, cooked vegetables or included in smoothies. The pressing process yields a flaxseed mass called flaxseed cake, that is used as animal feed or as fertilizer. This by-product has a huge nutritional potential but is currently underutilized as a low-value product.

Flaxseed can also be used as a supplement to fortify some food products (Bekhit *et al.*, 2018) and many are already on the market: bread, muffins, energy bars, biscuits, cakes. Incorporating functional foods such as flaxseed into staple products such as bread could have widespread impacts on public health. Flaxseed flour can also be added to pasta or bakery products (Secyk *et al.*, 2017).

Although whole flaxseed is of high quality, the form under which flaxseed is ingested will influence the bioavailability of the bioactive compounds. It is required to crack or mill the seeds to benefit from their nutritional value. Otherwise, the whole seed passes through the intestinal tract without being digested and nutrients are not available for the organism (Singh *et al*, 2011). Seeds might also be dehulled, that is to remove the outer layers, which correspond to the mucilage and the hull, to improve the availability of nutrients (Kajla *et al*, 2015). Flaxseed oil is more digestible than whole seeds, but it is more difficult to incorporate in foodstuffs because it tends to rancidity (Shim *et al.*, 2014).

2.2 Flax in animal feed

A large part of the demand for flaxseed is assigned to the animal industry, mostly under the form of extruded seed. The latter is very beneficial for health status and reproductive performances of said animals (Panaite *et al*., 2017). The high omega-3 content of flaxseed allows enriching animal products such as meat, eggs or milk in omega-3 fatty acids, which makes them even more valuable for human health. The fatty acid profile of the meat of monogastric species is directly influenced by their dietary fatty acid intake (Singh *et al.,* 2011). It is therefore possible to shift the fatty acid profile of meat toward a higher proportion of omega-3 fatty acids by feeding animals with flax oil or flaxseed. Chickens and piglets are the main targets to improve their meat content in unsaturated fatty acids (see section 5.2 for details about unsaturated fatty acids). Regarding the eggs, their omega-3 fatty acid content can be increased by feeding flax to laying hens. The milk-fat composition of cow's milk can also be improved by feeding flax to dairy cattle. Besides its fatty acid content, flaxseed is also used as a cheap protein source in animal feed (Rabetafica *et al*., 2011).

The whole seed can be used but many by-products are also very requested, which strengthens the economic potential of flax (Zuk *et al*., 2015). Flaxseed cakes are currently used a lot for horses and bovine feed. They contain high amounts of proteins (more than 30%) that have a good digestibility coefficient and are very appreciated by those animals (Singh *et al*., 2011).

In aquaculture, feeding flax to fishes is also a promising alternative source of omega-3 fatty acids. It could replace fish oil, known to be highly contaminated and non-sustainable. Flax oil is also more economical than fish oil. The downside of replacing fish oil by flax oil is the lower resulting amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the fish since the conversion efficiency of ALA into those fatty acids is limited, depending on the fish species (see section 5.2 for more details about these fatty acids) (Debier and Hantson, 2018).

2.3 Flax for industrial applications

The main outlet of flax oil remains the industry, where 70% of the global flaxseed oil production is reported to be exploited for technical purposes (Rabetafika *et al*., 2011). As examples, it can be used as a natural resource to make numerous industrial products such as varnishes, linoleum, paints and printing inks. Even though cheaper substitutes exist, paints and coatings based on flax oil are known to be of higher quality due to the high reactivity of the oil (Flénet, 2004).

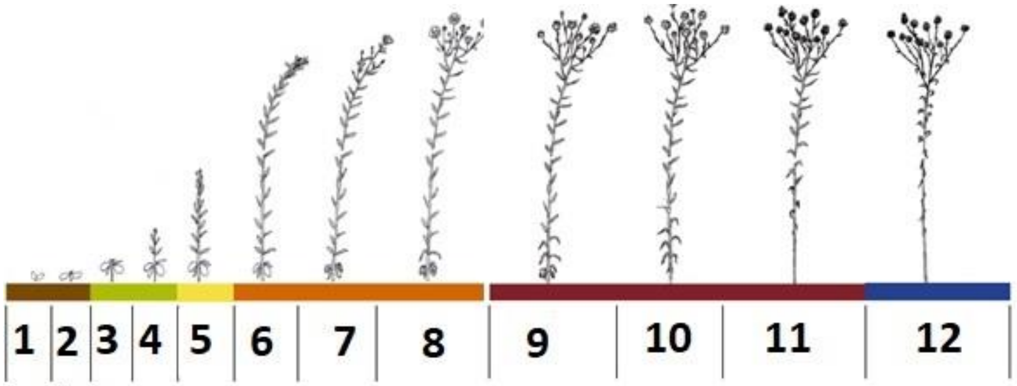
Flax straws contain high-quality fibers that can be used as isolating biomaterials, animal litters or even paper (Terres Inovia, 2017). In addition to being biodegradable, flax fibers have good mechanical properties: they are strong, flexible and soft. Even though they seem currently under-exploited, flax stem fibers have a large potential for the emerging bio-fiber industry (Flax council of Canada, 'Growing flax', 2015)

3. Agronomical characteristics of flax

3.1 Description of flaxseed

Flaxseed consists of three parts: two cotyledons, which make up the main part of the seed, a hull or a seed coat and an embryonic axis (root apical meristem, hypocotyl, shoot apical meristem) (Bekhit *et al*., 2018). The hull is made of a thin endosperm (inner hull) and a thick seed coat (outer hull). The seed is flat and oval, is 4 to 6 mm long and weights 5 mg on average.

Figure 1 summarizes the 12 growth stages that can be distinguished during flax development. One can see that two cotyledons emerge after the seed has germinated. After the first true leaf emergence, some pairs of leaves unfold, and the stem elongates. The flax plant measures 40 to 90 cm (Flax council of Canada, 'Growing flax', 2015). Later, a bud develops at the top of the plant. This terminal bud undergoes a floral transition phase and two new auxiliary buds develop, giving rise to more stems. The flowering period usually starts in May for winter flax and in June for spring flax and typically lasts 15 to 25 days. After the pollination and the fall of the petals, the ovary swells, and the seeds start to develop inside. The boll starts to ripen 20 to 25 days after flowering, leading to a mature fruit called capsule.



Growth stages

1. Cotyledon
2. Growing point emerged
3. First pair of true leaves unfolded
4. Third pair of true leaves unfolded; start of leaf spiral
5. Stem extension
6. Buds visible
7. First flower; early branching
8. Full flower; capsule start forming; continuation of branching
9. Late flower; most branches and capsules formed
10. Green capsule; seed white; lower leaves yellow
11. Brown capsule; seeds light brown, plump and pliable; maximum dry matter; branches, stem and upper leaves green/yellow; middle leaves partly senescent and lower leaves shrivelled or dropped
12. Seed ripe; seeds brown and rattle in capsules; branches and upper leaves senescent but stem still green/yellow

Figure 1: Growth stages of flaxseed (Source: adapted from Flax Council of Canada, 'Growing flax', 2015)

3.2 Growing flaxseed

Flax life cycle can be divided into 3 parts: a vegetative period, a flowering period and a maturation period (Zuk *et al.*, 2015). The spring oilseed flax cycle lasts about 140 days and that of winter oilseed flax lasts about 190 days (Parmentier).

The decision of growing a spring or a winter variety of flaxseed relies on many criteria. Winter flax is seeded around September and is harvested from early July to mid-August whereas spring flax is seeded in March and is harvested from early August to mid-September (Terres Inovia, 2017). In Belgium, since late frosts are quite frequent, spring flaxseed is often preferred and that is the type of flax that was grown for this study. Many varieties are available on the market and are classified according to their susceptibility to lodging, cold and diseases.

Flax is very demanding in terms of water and is therefore very susceptible to drought, mainly during the flowering period. Water shortage might have terrible consequences on the plant and is one of the main factors reducing yields. The demand for water is approximately 400-450 mm during the whole cycle of spring flax (Klein *et al.*, 2017). In terms of inputs, spring flax requires less fungicides, insecticides and anti-slug than any other crop in Europe (except maize), but herbicides are often applied in higher quantities (Flénet, 2004).

3.2.1 Soil preparation

In terms of cultivation practices, the best preceding crop seems to be a cereal. It is prudent to avoid crops of the *Brassicaceae* family before growing flaxseed to avoid flea beetles' attacks and legume crops to avoid excess of residual soil nitrogen (Gaumé and Coulombel, 2009). Next, flaxseed requires a good soil structure and depth to allow root development, which is important for the water uptake and hence for the resistance to drought periods (Terres Inovia, 2017). Flaxseed is relatively small, which means it has few reserves for germination and soil preparation is therefore important. The best soils to grow flaxseed are deep with a good water reserve and a pH between 6 and 6.5 is favorable (Gaumé and Coulombel, 2009). In order to have a high emergence rate, the seedbed should be firm, well prepared, moist and relatively even (Flax council of Canada, 'Growing flax', 2015).

3.2.2 Seeding

Spring flax is usually seeded between the end of February and the beginning of April in the Walloon region (Parmentier). It is recommended to sow as soon as possible after the risk of late frosts has disappeared to avoid high temperatures and water shortages during the flowering period. Early seeding usually provides higher and more stable yields, reduces the risks of diseases and insects and makes weed control easier (Flax council of Canada, 'Growing flax', 2015). The beginning of March is a good compromise between guaranteeing a long cycle and limiting the risk of late frosts. Flaxseed can be seeded with a cereal seeder at 1-2 cm deep. Row spacing should be between 15 and 20 cm. Seeding density depends on the targeted yield but is around 550-650 seeds/m² in order to reach 450-550 emerged plants/m² (Parmentier).

3.2.3 Fertilization

Flaxseed has low requirements for phosphorus, potassium and nitrogen compared with most common crops, but those minerals need to be available as soon as flaxseed is sown. It is therefore recommended to fertilize at the seeding stage or right after if conditions are favorable. Specifically, the plant needs between 0 and 50 U (1 U=1 kg/ha) of phosphorus and between 0 and 30 U of potassium (Terres Inovia, 2017).

It is important to provide the crop with nitrogen so that it can build its biomass. The exact dose of nitrogen must be determined considering plant requirements, post-harvest residues, residues at the end of winter, mineralization and targeted yield. Lodging is also an important factor to consider in the nitrogen management as it can reduce yields by up to 50% (Parmentier). Even though lodging is less likely for spring flaxseed, some regulators are sometimes applied to limit risks. Many different recommendations about nitrogen inputs can be found in the literature, ranging from 0 to 110 U. Terres Inovia (2017) states that nitrogen requirements are about 4.5 kg of N/quintal of seeds. This must be converted into a fertilization dose after calculating the soil nitrogen.

Zinc must be taken into account in the fertilization, in particular in cold and humid soils with a pH above 7.5. This is due to high exportations of zinc by the plant (350 g/ha). A zinc deficiency slows growth and shortens the internodes (Gaumé and Coulombel, 2009).

3.2.4 Weed control

As flaxseed does not largely cover the soil, weeding is particularly important. An excessive weed development might reduce flax yield and quality (Flax council of Canada, 'Growing flax', 2015). It also causes difficulties with harvesting and could damage seeds. Mechanical weeding can be applied at the beginning of the growing period so that it does not damage young plants (Terres Inovia, 2017). Chemical treatment is difficult since flax can be damaged by numerous herbicides. This sensitivity to herbicides varies a lot with the variety. However, chemical treatments remain the most common weed management option. Due to this sensitivity to phytotoxicity, chemicals application should be limited or at least split. It is however recommended to adopt an integrated strategy, which means trying to make the crop as competitive as possible. Many practices can be combined: a long rotation, a false seedbed, the variety choice, the choice of the preceding crop, a narrow row-spacing or a higher seed rate, an adequate fertilization level, an early seeding or an early weed management. Mixt weeding is another option which includes both chemical and mechanical weeding (Flax council of Canada, 'Growing flax', 2015).

3.2.5 Insect control

Thrips (*Thrips spp.*) and flea beetles (*Alticinae spp.*) are the main insects threatening flaxseed. Flea beetles are the most frequent and must be monitored as soon as the plant emerges. They might proliferate very fast and affect cotyledons and young leaves. Once the plant reaches 10 cm, the risk of flea beetles damages decreases. On the other hand, thrips start attacking flaxseed when it is higher than 10 cm, from the emergence to the end of the flowering period. Many insecticides are available on the market, but it is important to use them in a thoughtful way. An attack of those insect can be detected by observing the crop. There are systems of monitoring and alarming that farmers can use to respond adequately and moderately to those threats (Terres Inovia, 2017).

3.2.6 Disease control

Flax is prone to a few diseases whose magnitude varies a lot from year to year (Terres Inovia, 2017). Pasmo is the most frequent disease for flaxseed and is caused by *Septoria linicola*. *Fusarium oxysporum f. sp. lini* is a seedborne or soilborne fungus that causes fusarium, which is another very common disease. Next, *Kabatiella lini* causes the eye spot disease and *Oidium lini* causes powdery mildew but both only concern winter flax. Again, many measures can be taken to prevent these diseases: a good crop rotation, some resistant varieties, an early seeding, a good soil structure, a good destruction of the harvest plant residues and a good seed placement. Fungicides can also be applied (Gaumé and Coulombel, 2009).

3.2.7 Harvest

The right timing for harvesting is when seeds are free in the capsule and when 95% of them are brown. An early harvest results in an incomplete seed development and hence in a lower seed weight (Flax council of Canada, 'Growing flax', 2015). For spring flaxseed, harvest usually takes place at the end of August or early September in the Walloon region (Parmentier). It is better to harvest when the weather is dry. Harvesting flaxseed does not require any specific material. A combined harvester can be used with some adjustments due to the fibrous nature of flax straws.

3.2.8 Post-harvest treatment

Seeds usually pass through sieves of different mesh sizes to get rid of undesired residues (Morris, 2007). Flax storage requires more attention than most cereal crops. Moisture migration during storage can lead to moisture spots. If weeds are present, the moisture may increase and cause heating and molding. Storage insects usually do not represent a threat for flaxseed (Flax council of Canada, 'Growing flax', 2015).

3.3 Advantages of growing flaxseed

Growing flaxseed has many advantages for farmers. As flaxseed is from another family (i.e. *Linaceae*) than most common crops, it has a good value in crop rotations. It allows to diversify the rotation, makes it longer and redistributes the labor. Indeed, seeding and harvest of spring flax occur in February-March and August-September, respectively, which are not busy periods for farmers and allows to spread out workloads over time (Terres Inovia, 2017). A better rotation helps to manage diseases and weeds. Flax is not susceptible to the same diseases as cereals and other popular crops (Parmentier). Therefore, insects and pathogens cycles are broken up by inserting flax in the rotation. As flax requires low inputs, the labor is reduced and the return on investment is interesting. The low consumption of inputs also reduces the fossil fuel consumption. Growing flaxseed does not require any heavy investment either. In addition to that, flaxseed is a very good preceding crop to wheat. It reduces the labor and increases the yield of the next wheat. No or very little soil labor is required for the crop following flaxseed because the soil structure left over is usually very good. Weeding practices leave the soil clean for the next crop.

As a new family in the rotation, flaxseed contributes to a better biodiversity of the ecosystem. A diversified ecosystem allows a better balance for the soil and the different species. The blue flax flower is also part of the beauty of the scenery (Flénet, 2004).

3.4 Yield and profitability of growing flaxseed in Belgium

In France, Terres Inovia (2017) stated that spring oilseed flax can yield 20 q/ha in deep soils. Flénet (2004) claimed that it is possible to reach yields above 40 q/ha, but the average yield remains 20 q/ha in France. However, Gaumé and Coulombel (2009) stated that spring flax yield is usually lower than that of winter flax: 8-15 q/ha for spring flaxseed vs. 10-20 q/ha for winter flaxseed in France, in the department Mayenne, which should be close to Belgian conditions. These values show how yields tend to be very unstable. Terres Inovia (2017) roughly estimated operational loads around 350 €/ha and gross margin around 500 €/ha. Those calculations are based on many hypotheses: a fertilization NPK of 120, a certain number of herbicides, fungicides and insecticides, a yield of 17 q/ha and a selling price of flaxseed of 500 €/t. By comparison, gross margins of oilseed rape and sunflower are estimated around 750 and 500 €/ha, respectively (Chambre d'agriculture Mayenne, 'Marges Brutes des cultures de vente', Récolte 2015 & 2016). This shows that flax can compete with these two common crops, mostly with sunflower.

Low and unstable prices combined with low yields are the main factors impeding flaxseed development in Europe. In rich countries, farmers usually grow crops that have agronomical benefits, high yields and that are money makers. According to Flénet (2004), flaxseed grown in the 90's gave low yields and was hard to weed and to harvest. The lack of reference for growing flax also contributed to its low popularity in the 90's. Since flax was not popular, it hampered its technical and scientific development. There was neither an interest to invest in the flax industry. Moreover, the low input requirements did not attract the investors. Flax cultivation is now better understood, and a lot of documentation is available. Even though yields remain lower than most European crops, they now are satisfactory, and the crop is valued in many sectors.

Belgium is the largest importer of flaxseed in the world due to its important industrial sector. The company Vandeputte located in Mouscron is the main player: 10% of flax produced in the world is transformed in that company (Vandeputte – Notre société). Vandeputte uses flax to make diverse products: inks, linoleum, paints, soaps, putties but also oil and seed cakes. The large Belgian animal feed sector also contributes to the growing demand for flax. Russia, Kazakhstan and Canada are currently the largest suppliers. It would be interesting to break the dependence on these countries, to lower the costs and to provide a steady supply. There is a strong interest for a local supply of flaxseed for traceability and quality reasons. As a local crop, flax could also help breeders to reach food self-sufficiency (Flénet, 2004).

4. No-tillage practices

4.1 No-tillage in the context of conservation agriculture

According to Busari *et al.* (2015), tillage can be defined as "the mechanical manipulation of the soil for the purpose of crop production affecting significantly the soil characteristics such as soil water conservation, soil temperature, infiltration and evapotranspiration processes". For a long time, tillage has enhanced agricultural production by providing a warm and adequate seedbed, controlling weeds, incorporating and mixing fertilizers, manures and residues into the soil and making available nutrients deeply stored in the soil (Triplett et Dick, 2008). Tilling the soil dilutes the organic matter that is normally in the upper layers of the soil, which promotes its mineralization in the short term. However, long-term tillage depletes the soil organic matter and ruins the soil structure, decreasing soil fertility.

The reduction of tillage is part of the conservation agriculture techniques. According to Busari *et al.* (2015), conservation agriculture is "a method of managing agro-ecosystems for improved and sustained productivity, increased profits and food security while preserving and enhancing the resource base and the environment". The three guiding principles of conservation agriculture are reducing the soil disturbance (which includes reducing tillage), covering the soil permanently and diversifying the crop rotation. The positive effects of no-tillage are recognized when combined with these other agronomic practices.

4.2 The effects of reducing tillage

Reducing tillage appears to have many benefits for both the soil and environment. In terms of soil physical properties, the porosity is higher without tillage, which increases the water conductivity and availability for plants (Busari *et al*., 2015). Tillage makes the soil more compacted and a plough-pan may appear over years, which can impede root development. There is less water evaporation and erosion risks in a no-tilled topsoil, which is also good for the water and nutrient reserves. However, this reduced evaporation may lead to lower soil temperatures, which could have negative effects on crop growth.

Regarding the soil chemical properties, they are usually better in no-tilled soils. The long-term organic carbon and nitrogen soil contents are higher without tillage, possibly due to lower mineralization and leaching rates. This higher organic carbon concentration has among others, the effect of stabilizing soil aggregates. The nutrient cycling seems to be enhanced under no-tilled conditions (Pittelkow *et al*., 2015). Some studies also reported a higher CEC under no-tillage but the effect on pH is still a matter of debate (Busari *et al*., 2015).

Moreover, tillage affects the macro- and the micro-flora of the soil. Large organisms such as earthworms seem to be the organisms the most disrupted by the soil overturning. Decreasing tillage increases the population of earthworms that have many functions in soils: they increase organic matter decomposition, enhance nutrient cycling, increase soil fertility, aeration and water infiltration and promote root development. Tillage also disrupts the soil microorganisms' equilibrium by altering the microorganism species composition and favoring bacteria over fungi. The increasing bacteria biomass causes different reactions than the usual ones obtained with the fungal biomass, which disrupts the soil properties. Furthermore, tillage significantly reduces arbuscular mycorrhizal fungi population by impairing their survival and colonization of roots (Kabir *et al*, 2005). Mycorrhizal fungi are more abundant in the topsoil and are therefore particularly affected by the tillage. These mycorrhizal symbioses are beneficial for the crop production by improving the nutrient uptake for instance.

Regarding the environment, stopping tillage reduces runoff and erosion and, hence water and surface pollution. Tillage has also large impacts on greenhouse gas emission. Considering that the agricultural sector contributes to 10-12% of the global greenhouse gas emissions, it is necessary to tackle this issue and reducing tillage could be a promising alternative. Stopping tillage increases carbon sequestration, decreases availability of unmineralized organic substances to microorganisms and, hence reduces their decomposition and the CO_2 emission. As tillage promotes oxygen availability for bacteria, it results in a higher aerobic metabolism and in a higher gaseous production. Nitrous oxide emissions have also been reported as higher under tillage.

These statements continue to be debated and some recent studies even claim that environmental benefits of no-tillage have been previously underestimated (Pittelkow *et al*., 2015).

4.3 The effects of no-tillage on yields

The well-established habit of tillage combined with the high yields associated with it explain the reluctance of many farmers to end up with this practice. However, it turns out that no-tillage could give better yields than tillage. The result heavily relies on crop category, climatic conditions, residues management, no-till duration and fertilization level. Pittelkow *et al.* (2015) assessed the yield impacts of tillage through a meta-analysis. They found that overall yields were reduced by 5.1% under no-tilled conditions. The negative effects of no-tillage on yields can be attributed to lower soil temperatures that slow down crop development, rise in waterlogging under susceptible conditions and easier development of weeds.

However, oilseeds, cotton and legumes show no significant difference between till and no-till yields. Many factors could explain the good productivity of the latter crops under no-tilled practices. First, the better soil structure and the higher number of worm channels in no-tilled soils results in a greater root density and length. Next, the water and nutrient use efficiencies seem to be higher under no-till conditions. This is the main reason why no-tilled crops respond better to hot and dry weather conditions than tilled crops, leading to higher yields in these conditions. If the climatic conditions are good, the tilled crops are usually likely to give better yields.

Flax has been reported to have similar or better yields under reduced or zero tillage compared to conventional tillage practices (Flax council of Canada, ‘Growing flax’, 2015). A better soil organic matter content, a higher soil moisture, the stimulation of arbuscular mycorrhiza colonization and the root system of flaxseed might explain this result. Other studies report that the tillage system affects flax yield, but this heavily depends on soil and weather conditions (Pudelko *et al*., 2015), as mentioned before. The outcomes of studies focusing on flax under no-till are not numerous and vary a lot in the literature, which makes it hard to make an opinion.

It is important to consider crop residues in the definition of no-tillage. Residues play an important role in the erosion lowering and the water use efficiency improvement (Pittelkow *et al*., 2015). The duration of the tillage suppression is also important to consider. It usually takes five years for yields to be similar to conventional tillage and ten years for yields to be higher.

5. Nutritional composition and health potential of flaxseed

Table 1 summarizes the composition of nutrients and bioactive compounds in flaxseed. Flaxseed has a high energy value (4.5 kcal/g). Fats represent on average 41.0%, proteins 20.0%, dietary fibers 28.0% and minerals 2.4% of the seed weight (Carraro *et al.,* 2012). However, the composition of flaxseed fluctuates due to many factors such as cultivars, growth conditions, soil properties, time of harvest and analysis methods.

Table 1: Chemical composition in nutrients and phytochemicals of flaxseed (Source: Carraro et al., 2012)

Nutrients/bioactive compounds	Quantity per 100 g of seed	Nutrients/bioactive compounds	Quantity per 100 g of seed
Protein	20.0 g	Biotin (B8)	6 mg
Total carbohydrates	29.0 g	Niacin (B3)	3.21 mg
Total fats	41.0 g	Calcium	236 mg
Dietary fiber	28.0 g	Copper	1 mg
Lignans	10-2600 mg	Magnesium	431 mg
Ascorbic acid (C)	0.5 mg	Phosphorous	662 mg
Thiamine (B1)	0.53 mg	Potassium	831 mg
Riboflavin (B2)	0.23 mg	Sodium	27 mg
Pyridoxine (B6)	0.61 mg	Zinc	4 mg
Folic acid (B9)	112 mg	Energy	450 kcal

Elements in the brackets indicate the corresponding vitamins

5.1 Proteins

Flax contains between 20 and 30% of proteins (Bekhit *et al*., 2018) that are mostly concentrated in aleuronic grains in the cotyledons (Rabetafika *et al., 2011)*. When seeds are pressed, proteins mostly remain in the press cakes. Flaxseed oil therefore contains a very low level of proteins.

Besides the energy supply, proteins are the main source of nitrogen for humans. Dietary proteins provide amino acids required for the synthesis of endogenous proteins and other nitrogen-based molecules. The amino acid score of a protein reflects the protein quality, that is how the dietary protein has the adequate profile in terms of essential amino acids. To express the actual capacity of a protein to meet the amino acid requirements, this score needs to be corrected by the amino acid digestibility and availability. Table 2 compares the pattern of essential amino acids requirements to that of a few plant protein sources, including flaxseed. Green boxes indicate that the amount of essential amino acid is higher than the requirement (in blue), which is good for the body. Orange boxes indicate that the amount of essential amino acid is lower than the requirement, which means it is a limiting amino acid. Indeed, all the amino acids are required together in appropriate proportions to optimize the synthesis of endogenic proteins. If one is missing, the proteins can no longer be synthetized, which is why it is called the first limiting amino acid. The essential amino acid content of a food item is particularly important since the organism cannot synthetize these amino acids and therefore they must be supplied in the diet. Non-essential amino acids are important as a nitrogen source, but as the body can transform them into other non-essential amino acids, they are not all required in specific amounts. The recommended amino acid scoring pattern (i.e. the amino acid pattern for the reference protein) is calculated for a few age groups, as growth requires a stricter protein pattern. The amino acid profiles of eggs or milk proteins are often taken as reference to assess the quality of proteins, since they are close to human protein composition.

Flax proteins seem to raise more and more the interest of nutritionists owing to their favorable amino acid composition (Rabetafika *et al*., 2011). Indeed, Table 2 shows that flax contains most of the essential amino acids in adequate quantities, except for lysine and leucine, which are limiting. Lysine is limiting in many vegetal sources such as cereals. In flaxseed, essential and semi-essential amino acids account for 34% of total protein (Panaite *et al*., 2017), which makes flaxseed a good source of proteins.

Table 2: Essential and semi-essential amino acid score of some vegetal sources compared to the ideal essential amino acid patterns for pre-school children and adults. (Source: adapted from FAO Paper 92 (2011) and Flax council of Canada, 'Growing flax', 2015)

Essential and semi-essential amino acids (mg/g protein)	Amino acid requirements (1-2 years old child)	Amino acid requirements (adult)	Egg	Wheat	**Flaxseed (Brown flax, NorLin cultivar)**	Soy flour
Histidine	18	15	22	25	22	25
Isoleucine	31	30	54	35	40	47
Leucine	63	59	86	72	58	77
Lysine	52	45	70	31	40	58
Methionine + cysteine	25	22	57	43	26	23
Phenylalanine + tyrosine	46	38	93	80	69	85
Threonine	27	23	47	31	36	36
Tryptophan	7	6	17	12	18	10
Valine	41	39	66	47	47	52

Nutritional value and amino acid profile of flaxseed proteins are said to be similar to those of soy proteins, known for having one of the best nutritional values among plants (Rabetafika *et al*., 2011). The global amino acid profile of flax compared with that of soy flour is given in Table 3. Asparagine and glutamine are not mentioned since the analytical procedure leads to their conversion into aspartic and glutamic acids.

Many biological properties of flax proteins have been reported: anti-cholesterol, anti-tumor, anti-hypertriglyceridemic, anti-hypertensive, antioxidant, anti-inflammatory, anti-diabetic, anti-fungal, antithrombin effects as well as protection against neurodegenerative diseases (Rabetafika *et al*., 2011). These properties can be attributed to the amino acid composition, to their interaction with fibers or lignans and to the fact that flax provides bioactive peptides. Those bioactive peptides are associated with a decrease in risk factors of cardiovascular diseases (Carraro *et al.*, 2012). In addition to these physiological properties, flax proteins exhibit some interesting functional properties. As other oilseed proteins, they are emulsifying and can form a foam. As a result, they can serve as egg substitutes. The water holding capacity of flaxseed proteins is also high compared with that of other plant proteins. This gives a good texture, viscosity and elasticity to the products containing flax proteins. They can be added to many food products such as bread and other bakery products, where they can enhance the nutritional value of gluten-free products. All these techno-functional properties make flaxseed proteins good additives or food ingredients.

Table 3: Amino acid composition of flax compared to that of soy flour (Source: Flax Council of Canada, 'Growing flax', 2015) (E) stands for essential amino acids

Amino acid	Brown flax	Yellow flax	Soy flour
Alanine	4.4	4.5	4.1
Arginine	9.2	9.4	7.3
Aspartic acid	9.3	9.7	11.7
Cysteine	1.1	1.1	1.1
Glutamic acid	19.6	19.7	18.6
Glycine	5.8	5.8	4.0
Histidine (E)	2.2	2.3	2.5
Isoleucine (E)	4.0	4.0	4.7
Leucine (E)	5.8	5.9	7.7
Lysine (E)	4.0	3.9	5.8
Methionine (E)	1.5	1.4	1.2
Phenylalanine (E)	4.6	4.7	5.1
Proline	3.5	3.5	5.2
Serine	4.5	4.6	4.9
Threonine (E)	3.6	3.7	3.6
Tryptophan (E)	1.8	1.9	1.0
Tyrosine	2.3	2.3	3.4
Valine (E)	4.6	4.7	5.2

5.2 Fatty acids

As mentioned by Saini and Keum (2018), fatty acids can be categorized according to their degree of unsaturation and the position of their last double bond. Saturated fatty acids do not have any double bond. Some of them are associated with higher risks of developing diabetes, hypertension and hypercholesterolemia whereas polyunsaturated fatty acids (PUFAs), which contain more than one double bond, are known to have positive effects on health. There are also monounsaturated fatty acids that contain a single double bond. Among PUFAs, omega-3 fatty acids have their first double bond starting from the methyl end between the third and the fourth carbon of the chain, whereas the first double bond of omega-6 fatty acids is located between the sixth and the seventh carbon of the chain starting from the methyl end (Figure 2).

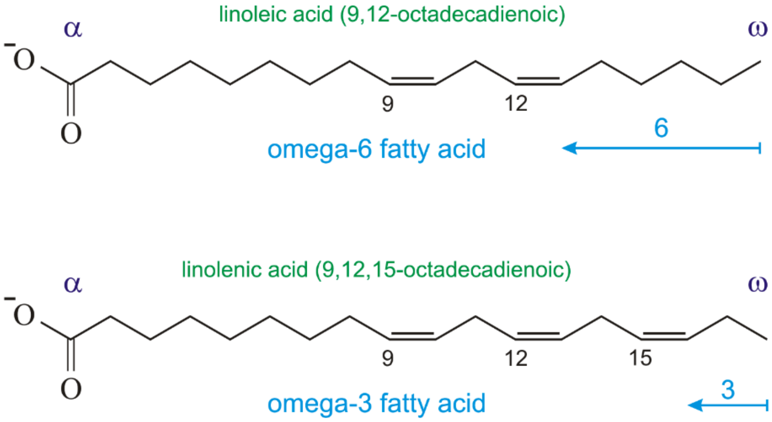


Figure 2: Difference between omega-6 and omega-3 fatty acids
(Source: https://glossary.periodni.com/glossary.php?en=omega-3+fatty+acids)

The fatty acid profile of flaxseed, outlined in Table 4, is very favorable due to the good balance between saturated and unsaturated fatty acids (Singh *et al*., 2011). Flax oil has an extremely high content in omega-3 fatty acids (about 55% of the total fatty acids), which is very interesting since most oil crops contain low levels of omega-3 fatty acids and in many cases, high levels of omega-6 fatty acids, leading to an inappropriate balance in the current Western diet. This Western diet has drastically changed during the last century. The percentage of dietary fat in the total energy intake has increased to reach about 36% of total energy intake nowadays in Belgium (De Ridder *et al*., 2016). This has been associated with an increase in saturated fatty acid intake. Among PUFAs, omega-6 fatty acids have increased whereas omega-3 fatty acids have decreased, leading to a dramatic imbalance between these two PUFA families.

Omega-3 and omega-6 fatty acids are both important for the proper functioning of the human body but play different and sometimes opposite roles. They are key factors in the regulation of many functions such as inflammation, homeostasis, vasodilatation or vasoconstriction, bronchodilatation or constriction, platelet aggregation and anti-aggregation. Omega-6 fatty acids are involved in inflammation, constriction of blood vessels and platelet aggregation whereas omega-3 fatty acids are anti-inflammatory and decrease the risk of cancer and cardiovascular diseases. Omega-3 PUFAs also contribute to a lower influence of chronic and metabolic diseases such as diabetes, obesity and osteoporosis (Saini and Keum, 2018).

Table 4: Fatty acid profile of flaxseed (Source: adapted from Panaite et al., 2017)

Fatty acids		% of total fatty acids
Palmitic acid	C16:0	5.95
Stearic acid	C18:0	4.15
Oleic acid	C18:1 (n-9)	17.38
Linoleic acid	C18:2 (n-6)	16.13
Alpha-linolenic acid	C18:3 (n-3)	54.51
Octadecatetranoic acid	C18:4 (n-3)	0.15
Arachidic acid	C20:0	0.12
Eicosadienoic acid	C20:2 (n-6)	0.11
Docosadienoic acid	C22:2 (n-6)	0.28
Docosatrienoic acid	C22:3 (n-6)	0.25
Other fatty acids		0.97
Type of fatty acids		**% of total fatty acids**
Saturated fatty acids		10.29
Monounsaturated fatty acids		17.52
Polyunsaturated fatty acids, of which		71.84
n-3		54.77
n-6		17.07

The imbalance between omega-3 and omega-6 PUFAs intake has important consequences on the metabolism. It must be clarified that plants and algae can transform oleic acid (C18:1) into linoleic acid (LA; C18:2) and ALA (C18:3). In contrast, animal cells are unable to do so because they lack the desaturases necessary to add double bonds beyond the tenth carbon of the fatty acid chain. As a result, animals cannot convert omega-6 to omega-3 fatty acids (Simopoulos, 2002). As the compounds of these two families compete for the same enzymes (specific elongases and desaturases) in the human organism and are not interconvertible, the type of fatty acid intake is going to modify the composition of human tissues and cell membranes. Membrane PUFAs are the starting point for the synthesis of many compounds involved in the organism regulation such as eicosanoids, protectins and resolvins. These compounds have different effects depending on their fatty acid of origin. For instance, eicosanoids derived from arachidonic acid (ARA; an omega-6) and from EPA (an omega-3) have quite contradictory effects (see below). Their relative production depends on the ratio omega-6/omega-3 ingested. Figure 3 shows members of the omega-3 and omega-6 families and the enzymes required for their synthesis. ALA and LA are considered as the precursors of the omega-3 and omega-6 families, respectively (Simopoulos, 2002).

LA and ALA are essential because the animal organism cannot synthetize them and because they are required for the composition of membrane polar lipids and for the production of signaling molecules such as eicosanoids. The higher amount of omega-6 fatty acids in the Western diet results in higher amounts of ARA-derived eicosanoids (Simopoulos, 2002). These ARA-derived eicosanoids increase the risk of thrombus, atheroma, allergies and cardiovascular and inflammatory disorders. Many non-infectious diseases such as cancer, obesity, diabetes, autoimmune diseases, cardiovascular disease, rheumatoid arthritis, asthma and depression are influenced by the high amounts of omega-6 derived eicosanoids, which are favored by the relative excess of dietary omega-6 fatty acids over their omega-3 counterparts. Increasing the ingestion of omega-3 fatty acids helps to limit the development of such diseases. The ratio omega-6/omega-3 is therefore recommended to be between 1:1 and 4:1. In typical Wester diets, it is often around 15:1 (Saini and Keum, 2018).

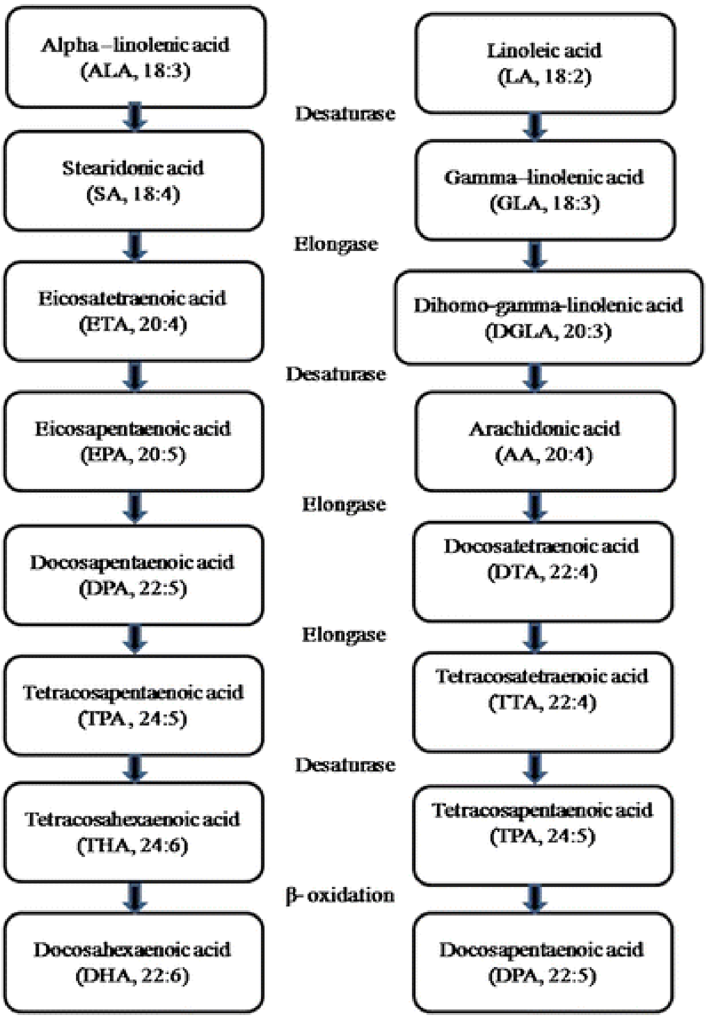


Figure 3: Flowchart for the metabolism of fatty acids from the omega-6 (left part) and omega-3 (right part) families (Source: Kajla et al., 2015)

The anabolic processing of LA and ALA to their derivatives appears to be poorly efficient in humans. Only 5 to 10% of the ingested ALA may be converted into long chain omega-3 fatty acids (Singh *et al.* 2018). Many factors affect this conversion, including the ratio of ingested omega-6/omega-3 because of the competition for the same enzymes. Among all omega-3 PUFAs, it has been demonstrated that EPA and DHA are the most important for mammals because of their health benefits and their anti-inflammatory capacity (Agazzi *et al*., 2015). DHA especially has important functions in cell membranes and is considered as a health protective agent (Singh *et al*., 2011). As DHA is the main structural fatty acid of the brain and the retina, it is required for the growth and the development in the early life. The limited conversion of omega-3 precursors into DHA combined with its important effects prompted nutritionists to consider DHA as an essential fatty acid. Thus, three fatty acids are essential to include in the diet: ALA, LA and DHA. EPA formation is higher than that of DHA, so EPA is recommended to be included in the diet but is not considered as essential. Besides their role of precursors of eicosanoids, lipids are also important constituents of membranes, modulators of transcription factors and a significant energy source.

ALA and other omega-3 PUFAs are reported to have a huge health potential. In addition to favoring the anti-inflammatory pathway, they prevent cardiovascular diseases, rheumatoid arthritis and asthma. They also seem to prevent cancer by reducing tumor growth in advanced stage of the cancer. EPA and DHA, that are derived from ALA, are also helpful in reducing the appearance of depressing symptoms. Omega-3 fatty acids present in flaxseed also positively impact blood triglycerides and blood cholesterol (Kajla *et al.,* 2015).

Considering the importance of omega-3 PUFAs for human health and the excess of omega-6 in the diet, it is important for humans to increase their omega-3 intake. It is recommended for males to consume 2.8g of ALA every day and 2.2g/day for women (Conseil Supérieur de la Santé, 2016). However, omega-3 fatty acids are not easy to find since most crops and food products are rich in omega-6 fatty acids. Fish oil has a good omega-3 fatty acid profile but is expensive and non-sustainable. Meat and dairy products also supply some EPA, DPA, DHA and ARA but they remain of animal origin. There are vegetal alternatives such as walnut oil, canola oil or olive oil, algae, nuts, and seeds such as seeds of chia and flaxseeds (Morris, 2007). Flaxseed appears as a promising alternative since it is the richest source of ALA compared with other common crops.

Most of the lipids (75% of the flaxseed) are located in the cotyledons, 22% in the seed coat and 3% in the embryo. Those lipids stored in microscopic oil droplets are the main energy supply for seed germination (Bekhit *et al*., 2018).

5.3 Carbohydrates

Flaxseed is composed of 29% dietary fibers, which are indigestible carbohydrates, and only 1% of simple sugars (Carraro *et al*., 2012). Most carbohydrates are located in the hull of flaxseed. Flax does not significantly contribute to the sugar intake, which is good since it is already in excess in the Western diet.

Flax fibers can be divided into two groups: soluble (one third) and insoluble (two-third) fibers (Kajla *et al*., 2015). The mucilage refers to the soluble part of flaxseed fibers, which is composed of water-soluble polysaccharides and is located in the outer layers of the seed. It has a high-water binding capacity and is responsible for many interesting health properties such as reduction of cholesterol and blood triglycerides, reduction of nutrient absorption and increase of fetal fat excretion (Shim *et al.*, 2014). It is also a prebiotic that stimulates the gut microbiome and therefore, increases the microbial biomass in the colon (Bekhit *et al.*, 2018). Soluble fibers are also reported to increase the feeling of satiety and reduce the nutrient absorption, and consequently the energy intake. Including more fibers in human diet could therefore help in preventing obesity. It could also slow diabetes development by reducing the insulin secretion after meals and hence the insulin resistance (Shim *et al.*, 2014). Therefore, soluble fibers are already used for treating constipation, gastritis, enterocolitis, irritable bowel syndrome and have potential for preventing many metabolic disorders such as obesity and cardiovascular diseases. The insoluble fibers of flaxseed are less documented. They are made of cellulose, hemicellulose and lignin. Their water-binding capacity increases the intestinal bulk. Thus, both soluble and insoluble fibers increase the weight of the colon feces by two different mechanisms. When dietary fibers reach the colon, they are fermented by the microbiota. This releases short chain fatty acids, hydrogen, carbon dioxide and methane and results in a laxative effect. The whole seed must be consumed to enjoy from benefits of fibers, as they remain in the press cake during oil production.

5.4 Phenolic compounds

Phenolic compounds are secondary plant metabolites involved in different functions such as plant defense or attraction of insects (Morris, 2007). They are associated with many health benefits such as anti-microbial, anti-inflammatory, anti-thrombotic and anti-teratogenic effects.

Flax contains three types of phenolic compounds: phenolic acids, flavonoids and lignans. Phenolic acids include mainly p-hydrobenzoic acids, chlorogenic acids, ferulic acids and coumaric acids. Flavonoids are benzo-gamma-pyrane derivatives. Lignans are one of the three compounds in flax that make it a functional food. They give flax protective properties and result in a wide range of health benefits (Singh *et al.*, 2011). Lignans are diphenolic compounds containing a 2,3-dibenzylbutane skeleton. Most importantly, lignans are phytoestrogens, which means that their structure is similar to that of human estrogens and that they are able to produce an estrogenic and/or anti-estrogenic effect in the human body, depending on the initial estrogen level. Secoisolariciresinol diglycoside (SDG) is the most prominent lignan found in flaxseed, it represents between 6.1 and 13.3 mg/g of whole flaxseed flour (Kajla *et al.*, 2015). Once ingested, SDG is transformed by the gut microbiota into mammalian lignan metabolites. These can be absorbed by the colon and be active in the body. The ability of lignans to scavenge free radicals and to balance some hormonal mechanisms explains their anti-carcinogenic properties. Lignans exert a protective effect against breast and colon cancers, reduce the risk of cardiovascular diseases, osteoporosis, hypercholesterolemia, atherosclerosis, hypertension and diabetes. Flaxseed contains more lignans than any other plant used for human consumption.

All these phenolic compounds are hydrophilic and hence, are located in the non-lipid fraction of flaxseed. Consequently, flax oil does not contain a lot of them. Flaxseed cakes, however, have high contents in phenolics, which is valuable. The antioxidant activity of these phenolic compounds is interesting for preserving elements in the seeds as well as for human health.

5.5 Vitamins and minerals

Flax contains low amounts of vitamins (Morris, 2007). Among the water-soluble vitamins, there are vitamin C and a few forms of vitamin B (see Table 1). Among the fat-soluble vitamins, there are mostly vitamin E (552 mg of gamma-tocopherol/kg of oil, 7 mg of alpha-tocopherol/kg of oil and 10 mg of delta-tocopherol/kg of oil) and a low amount of vitamin K. Tocopherols are the most potent fat-soluble antioxidants (Oomah *et al*., 1997). Gamma-tocopherol is present in quite high amounts in flaxseed and has many health benefits such as lowering the blood pressure and the risk of cardiovascular diseases as well as some types of cancer. Vitamin E is also appreciated in the oil for the protection it provides against the oxidation of omega-3 fatty acids. Regarding minerals, flax contains significant amounts of potassium, magnesium, phosphorus and calcium (see table 1). It is quite low in sodium, which is good considering its negative effects on health. It should be noted that the potassium content is high compared to that of most food products. Potassium is reported to have positive effects on blood free radicals, blood platelet aggregation and stroke incidence (Klein *et al*., 2017).

5.6 Undesired traits

Flaxseed contains a few undesired compounds that can be toxic or that negatively affect the metabolism of other nutrients. However, as the dose of flaxseed ingested and the concentration in anti-nutrients are low, it seems that they do not have adverse effects. Anyway, the safety of flaxseed remains contested, particularly when consumed regularly or in high amounts (Singh *et al*., 2011).

5.6.1 Oxidation products

A first problem of flax oil is its susceptibility to rancidity due to the instability of omega-3 fatty acids. The oil is very prone to oxidation and has therefore a very short shelf life (Zuk *et al*., 2015). In the full grain, lipid oxidation is limited by the antioxidant activity. Even a cold extraction drastically decreases this antioxidant activity. The shelf life of the oil might be extended by adding antioxidants such as vitamins A and E in the oil and by storing it properly, that is in the dark and at a temperature below 21°C (Klein *et al*., 2017). Oxidation leads to the formation of toxic aldehydes that are correlated with arteriosclerosis and cancer. Oxidation products also give flaxseed a rancid taste and odor.

5.6.2 Cyanogenic glycosides

Cyanogenic glycosides are responsible for the bitter taste of flax. Their hydrolysis yields hydrogen cyanide (HCN), which is highly toxic for the electron transport chain, and thiocyanate which is toxic for the thyroid gland (Klein *et al*., 2017). The enzymatic hydrolysis of cyanogenic glycosides in the body can lead to cyanide poisoning, with the main symptoms being blood pressure drop, headache, dizziness and confusion (Bekhit *et al*., 2018). As cyanogenic glycosides are hydrophilic, most of them remain in the press cake (Klein *et al*., 2017). Intoxication is therefore not likely by consuming flax oil.

5.6.3 Cadmium

Cadmium is a heavy metal that hyperaccumulates in flaxseed. A maximum content of 5 µg Cd/g of flaxseed is admitted by the European Commission. Cadmium causes kidney and bones failure and is also carcinogenic (Klein *et al*., 2017). Since it is not well eliminated, it builds up in the organism and causes chronic disorders. Cadmium is mostly present in seeds or cakes but should not be of concern when consuming the oil (Grant *et al*., 2010). A normal diet containing 20 g of flaxseed every day remains far below the limits recommended by the European Commission. The latter recommends ingesting maximum 2.5 µg per kilo of body weight per week.

5.6.4 Linatine

Linatine is a polar compound with an amide part that prevents vitamin B_6 absorption possibly leading to severe vitamin B_6 deficiencies and growth failures (Shim *et al*., 2014). Other vitamin B_6 deficiency symptoms are loss of appetite, nervous disorders and anemia (Bekhit *et al*., 2018). Since humans only consume low amounts of flaxseed, no vitamin B deficiencies have been reported so far.

5.6.5 Phytic acid

Phytic acid is present in many seeds and chelates mono- or divalent cations such as magnesium, iron, or zinc, preventing their absorption and leading to possible deficiencies (Bekhit *et al*., 2018).

Chapter II: Objectives

Environmental and agronomic factors are known to have large effects on the yield of any crop. The influence of such parameters on flax is not well documented. A better documentation could help farmers to increase flax yields and hence promote flaxseed production and meet the growing demand. It could also contribute to improve the nutritional profile of flaxseed, that is increasing beneficial compounds such as omega-3 fatty acids and decreasing undesired compounds. As *Regenacterre* is interested in boosting oilseed flax sector in Belgium and strongly supports conservation agriculture practices, it would be interesting to analyze the yield and the composition of flaxseed grown in Belgium under this type of practice.

This work focuses on nitrogen fertilization and tillage. On the one hand, nitrogen fertilization is the most essential input in crop production and is applied to most crops to improve yields. An adequate nitrogen management is important for both crop profitability and environment. On the other hand, an increasing number of farmers move to reduced tillage and flax seems to respond well to this practice. However, studies on the effect of tillage on flax as well as on quality parameters of crops are still lacking.

The aim of this master's thesis is to evaluate the effect of two agronomic factors on the yield as well as on the quality profile of flaxseed grown in Belgium: tillage and nitrogen fertilization level. The analysis of flaxseed quality components will mainly consist of lipid content, fatty acid profile, crude protein as well as vitamin E contents. All of these heavily contribute to the health potential of flax and are likely to be influenced by the nitrogen fertilization level.

To achieve these objectives, a field trial was implemented in a field that belongs to the "Centre Alphonse De Marbaix" in Vieux-Sart (Belgium). The field was divided into a tilled part and a no-tilled part and six different doses of nitrogen were applied. Yield parameters as well as flaxseed quality components were assessed in order to establish relationships.

Chapter III: Materials and methods

1. Growing flaxseed

The first part of the experiment was supervised by Prof. P. Bertin from the Earth and Life Institute of UCLouvain. It consisted of growing flax and applying different treatments.

1.1 Experimental design

The experiment was carried out in a field that belongs to the “Centre Alphonse De Marbaix”, which is situated in Corroy-le-Grand, close to Louvain-la-Neuve in Belgium. This center is an experimental farm where many field trials are carried out by scientists from UCLouvain. More precisely, the experimental field is situated in Vieux-Sart, in the municipality of Chaumont-Gistoux (Figure 4). The geographic coordinates are approximately the following: 50° 68’ 23’’ N. and 4° 64’ 21’’ E. The area of the experimental plot is about 0.5 ha. A part of this plot has always been in a tilled-system while the other has undergone no-tillage for about 12 years (since 2007-2008). The agricultural area of the plot is said to have sandy silty soils (Aba) but is close to the frontier with the silty area (Régions Agricoles).

Figure 4: Localization of the experimental field (Source: adapted from Google)

To analyze the effect of tillage, half of the flax was grown in the no-tilled part of the field and the other half was grown in the tilled part (Figure 5). Six different doses of nitrogen were applied to determine the effect of fertilization: 0, 25, 50, 75, 100 and 125 kg N/ha. This was determined based on what was found in the literature and was supposed to include all doses applied by farmers. The range of nitrogen applied often varies between 0 and 120 kg N/ha in the literature (Grant *et al.* (2015); Dohat *et al.* (2017); Andruszczak *et al.* (2016); Herzog *et al*. (2017)).

The tillage effect was evaluated without any repetition, which is statistically not rigorous. Flax should have been grown in more different fields to properly assess the effect of tillage. The tight timing combined with the lack of material and economic means did not make it possible. The tilled and the no-tilled parts must therefore be considered as two independent trials and no conclusion can be drawn from their comparison. In the present study, they will be compared with an ANOVA as an exercise.

For the purpose of statistic consistency, three repetitions of each nitrogen treatments were implemented in each part of the field (tilled and no-tilled). That makes 18 plots in each part of the field and 36 plots in total. A plot was 10 m long and 1.5 m large, which makes a surface area of 150 m². The experimental design is a randomized complete block, which is typical for agricultural experiments. Each part of the field was divided into 3 blocks that aimed at representing the variation in the field. Indeed, there are often heterogeneity gradients in fields due to slope, granulometry, water availability or tractor track and it is important to include them in the experimental design. The nitrogen treatments were assigned at random between the different blocks. Between the tilled and the no-tilled parts as well as all around the whole experimental plot, there were some buffer plots that consisted of flax without any treatment. Between the blocks, the buffer consisted of normal plots of 10x1.5 m. Around the blocks, there were at least 15 m of buffer flax to protect the experimental flax from potential interactions with the field nearby.

Before this experiment, barley was grown from September 2016 until July 2017 on the whole field with the same treatment. A cover crop of oat and vetch was grown between barley and flax from September to December 2017. Thus, the previous crops in the recent history of the fields were homogenous.

NO-TILLED PART

N°	Nitrogen	N°	Nitrogen	N°	Nitrogen
1	0	7	25	13	100
2	125	8	100	14	0
3	25	9	50	15	125
4	100	10	0	16	50
5	50	11	75	17	25
6	75	12	125	18	75
19	25	25	125	31	75
20	100	26	0	32	25
21	50	27	25	33	100
22	0	28	100	34	50
23	75	29	75	35	0
24	125	30	50	36	125

TILLED PART

Figure 5: Experimental design of the different treatments of the flaxseed culture (Source: adapted from Google)

1.2 Development of the experiment

As shown in Table 5, the cover crop was milled, and the soil was tilled the same day for the tillage part of the field. For the no-tilled part, the cover crop remained longer on the field and was milled a few months later. The first herbicide treatment consisted of glyphosate (3l/ha). The whole field was harrowed and sown the same day with a precision seeder pulled by a tractor. The variety was Omegalin, which is quite tolerant to lodging and fusarium (Linea, 2017). This variety is mostly renowned for its high oil and ALA contents. Flax was seeded at 46 kg/ha in order to reach 650 seeds per square meter. This was calculated based on the recommended seed rate by Terres Inovia (2017), the seed weight as well as the germination rate. Seeding depth was approximately 1-2 cm and row spacing was about 12 cm. The nitrogen fertilization was under the form of ammonium nitrate (27% N). For the three lower levels, the whole dose was applied the same day whereas for the 3 highest doses, it was split into two applications to improve the absorption rate. No fungicide treatment was required for the experiment. However, an insecticide treatment (Karate 0,62l/ha) against flea beetle and a few herbicide treatments (Allié 15g/ha, Agroxyl 0.32l/ha, Xinca 1l/ha and Fusillade 1l/ha) were needed. In addition to that, some mechanical interventions were necessary to eliminate weeds, mostly on the no-tilled part of the field.

Table 5: Schedule of the cultivation of flax

Schedule	
05/12/17	Milling of the cover crop and tillage of the tilled part
06/02/18	Milling of the cover crop on the no-tilled part
06/04/18 10/04/18	Stubble of the no-tillage part
10/04/18	Stubble of the no-tillage part and pre-culture soil analysis
16/04/18	First herbicide treatment (glyphosate 3l/ha)
17/04/18	Rotary harrow and seeding (both parts)
27/04/18	First nitrogen fertilization
04/05/18	Insecticide treatment (Karate 0,62 l/ha)
14/05/18	Herbicide and insecticide treatments (Karate 0,62 l/ha, Agroxyl 0,32 l/ha, Allié 15 g/ha)
28/05/18	Second nitrogen fertilization
04/06/18	Herbicide treatment (Xinca 1l/ha, Fusillade 1l/ha)
09/07/18	Data collection
02/08/18	Harvest
04/09/18	Post-culture soil analysis
13/11/18	Seed cleaning

The harvest took place earlier than expected given the dry and hot climatic conditions. It has been done the 2nd of August with a combine harvester designed for small seeds that belongs to Ecosem. Ecosem sprl is a very small company based in the Center Alphonse De Marbaix that provides a large range of organic and indigenous seeds. Flaxseed was cleared from the impurities based on size and density, with a device from Ecosem. It should be noted that it was the very first time that the staff of the Center Alphonse De Marbaix grew flax and therefore the soil preparation and the applied treatments may not have been perfectly appropriate. The staff has experimented no-tillage practices for a few years only, so the technique may also be not perfectly adequate. It was a tedious work for the team to find which treatments to apply and which machines to use.

1.3 Measurements

First, a soil analysis was carried out in order to assess the nitrogen status. Before the experiment, four soil cores were sampled on each part of the field (tilled and no-tilled) and were gathered in a pot. It was then homogenized and a part of it was put in a bag that was then sent to the laboratory of the Provincial Center of Agriculture and Rurality (La Hulpe, Belgium). This was done for two soil layers: 0-30 cm and 30-60 cm. This type of analysis should have been done before the onset of the experiment. However, it was not possible due to external time constraints.

The soil analyses provided information regarding the pH, the organic carbon, the total nitrogen, the C/N ratio, the dry matter content and the mineral nitrogen content. Based on the remaining nitrogen in the soil, one can calculate the quantity of applied nitrogen required in order to reach the target nitrogen level of each plot, provided that the analysis is available before fertilizing. At the end of the experiment, the same soil sampling was carried out. Again, four soil cores were sampled on each plot in order to make one homogenized samples that were then sent in the same laboratory. The same analyses were carried out.

Next, regarding the yield, some data were collected on 9th July 2018 on each experimental plot:

- The density of plants was estimated by measuring the number of plants per square meter. On a length of 1 meter, the number of plants was counted four times in different random places of the same plot. The obtained number was then multiplied by 8.67 because the plot contains on average 8.67 rows. Each plot was indeed 1.5 meter large and contained 13 rows, which makes 8.67 rows per meter. The data for the density of plants corresponds to the mean of the four measurements.
- The height of plants was measured in centimeters on 15 randomly selected plants for each plot. The mean of these 15 measurements gave the average plant height for each plot.
- The maturity of each plot was assessed based on a personal scale of value. A number from 1 to 9 was attributed to each plot based on the observed global color and maturity.

Number 1 stood for green non-mature plants whereas number 9 indicated mature and dry plants.

- After harvest, all the seeds of each plot were weighted to assess the seed yield. These seed weights were used to evaluate seed and oil yields of flax.

2. Analyzing flaxseed nutritional profile

The second part of the experiment was supervised by Prof. Y. Larondelle from the Louvain Institute of Biomolecular Science and Technology of Uclouvain. It consisted in the partial analysis of the nutritional profile of flaxseed. Before starting the experiments, a homogeneous sample of flaxseed was ground with a blender, vacuum-packed and stored in a cold room at 4°C for later use. For every experiment, a bag was taken out of the fridge and the desired amount of flaxseed powder was collected from there.

2.1 Oil extraction: Soxhlet method

The evaluation of the oil content of the seed was performed with the Soxhlet method. Each repetition of the experiment could analyze up to six samples and lasts three days. This experiment was therefore repeated six times to examine the 36 samples. As there were numerous samples, this experiment was not duplicated even though it would have been better for statistical consistency purposes. The first step of the experiment was an acid hydrolysis. Two grams of the ground flaxseed were put in a round bottom flask and heated in 100 ml of hydrochloric acid for an hour. After cooling, the samples were filtered and washed with 800 ml of distilled water. The collected material was then dried overnight on the filter. The next day, each filter with the collected dried material was inserted in a paper thimble that was then inserted in a typical Soxhlet extractor to proceed to the extraction step. To this end, 100 ml of diethyl ether, the extraction solvent, were introduced in a round bottom flask and heated for six hours. The Soxhlet device allowed the solvent to evaporate, condense and accumulate in the extractor and solubilized the lipids of the sample. Thanks to the specificity of the Soxhlet device, the lipids were transferred again in the initial round bottom flask. Extraction cycles were repeated this way for six hours. After extraction, the solvent and the oil were dried overnight, which caused the solvent to evaporate. The resulting oily liquid in the round bottom flask was then dried in a desiccator at 105°C for 2 hours and weighted.

This final weight allowed to calculate the oil yield. As the dry matter content was required in this calculation, it was determined at the same time as the Soxhlet extraction. Another 2 grams of the milled seed sample were put in the desiccator at 105°C for 24 hours. Duplicate analyses were performed on each sample. The weight difference indicated the water content of the seeds.

2.2 Protein content: Kjeldahl method

It is usually recommended to defat fatty samples before extracting nitrogen to facilitate their mineralization (Aubry, 2012). Therefore, ground flaxseed samples were defatted before starting the Kjeldahl analyses. Each sample was analyzed twice and then averaged. Two grams of the flaxseed powder were put on a nitrogen-free filter. The filter was then introduced in a paper thimble and the latter was introduced in the same Soxhlet extractor that was used for the previous oil extraction. Again, 100 ml of diethyl ether were added in a round bottom flask at the lower level of the Soxhlet device. The extraction was performed by heating it for an hour. The filter was retrieved and could be used for later Kjeldahl extraction. The filter containing the defatted flaxseed powder was introduced in a large test tube with 8 grams of selenium (the catalyst), 25 ml of hydrochloric acid (97%) and 30 drops of octanoic acid (98%) to prevent an excessive foam formation. The tube was heated, and the acidic vapors were drawn away in order to mineralize the organic matter. After a couple of hours, the tube was cooled down at room temperature for a few minutes before the distillation step. The distillation unit (Buchi K-355) added 25 ml of distilled water and some sodium hydroxide (NaOH) in excess. Then, the tube underwent 5 minutes of distillation and 150 ml of distillate were collected in an Erlenmeyer containing 10 ml of boric acid (0.8073 M) and 90 ml of distilled water. This solution was titrated with hydrochloric acid (HCl) 0.1 N. The amount of HCl required for the neutralization was noted for further calculations.

2.3 Fatty acid profile determination: Folch extraction and gas chromatography (GC)

The fatty acid profile and the vitamin E content were both determined on the same lipid extract that was obtained by the Folch method. First of all, 1 gram of the ground flaxseed was put in a plastic test tube. A first extraction was performed using methanol (CH_3OH) and chloroform ($CHCl_3$). To this end, 10 ml of methanol were added to the seed powder and mixed for 1 minute with an Ultra-Turrax at 9500 rpm to homogenize the mixture. Then 20 ml of chloroform were added to the mixture, mixed for 2 minutes with the Ultra-Turrax at 9500 rpm before performing a first filtration. A second extraction was performed on the seed residues. These residues were resuspended in a solution of methanol and chloroform (2:1), mixed with the Ultra-Turrax at 9500 rpm for 3 minutes, filtered and rinsed with 20 ml and chloroform and then with 10 ml of methanol. Both extracts were then pooled. The next step consisted of a decantation, which aims at removing the non-lipid residues of the filtrate. Thirty milliliters of an aqueous solution of KCl 0.88% was added to the filtrate. After mixing, the solution was left 20 minutes for decantation before removing the upper phase. Then, thirty milliliters of H_2O:MeOH (1:1) were added to the lower phase, mixed and the solution was left 20 minutes for decantation again. It was necessary to add some ethanol in the solution to break emulsions and make the liquid clearer. The lower phase of the decantation flask was collected for the next step, which consisted of evaporating the solvent under vacuum in a rotary evaporator (Rotavapor R-3000 Buchi) in order to isolate the lipids in the tube. Those pure lipids were transferred into a methylation tube with diethyl ether and stored at -20°C until the methylation step.

The methylation aimed at converting fatty acids into fatty acid methyl esters, which are more volatile and will thus be more easily analyzed in GC. First, the diethyl ether was evaporated from the samples with nitrogen. Then, 10 ml of a solution of KOH (0.1M) dissolved in MeOH were added and the sample was put in a water bath at 70°C for an hour. In this way, fatty acids were separated from glycerol. The samples were then put in the water bath for another 20 minutes after addition of 4 ml of a solution of HCl (0.1M) dissolved in MeOH, which allows methyl esters to form. Finally, 20 ml of hexane and 10 ml of water were added to extract fatty acid methyl esters. Samples were then kept in the cold room at 4°Covernight to decant.

In vials of 1000 µl, 20 µl of the lipid phase, that is upper phase of the methylation tube, containing the fatty acid methyl esters in hexane were put together with 40 µl of the injection standard (methylated C11:0) and 940 µl of hexane. The 36 vials, corresponding to the 36 plots to be analyzed, were then injected on a GC Trace 1310 (ThermoFisher scientific) to separate and identify the fatty acids. The GC is equipped with an automatic injector (TriPlus AS), a capillary column 100mx0.25mm Rt-2560 (Restek) and a flame ionization detector. It also contains an oven that follows a particular temperature program, which is shown in Table 6. The gas carrier was hydrogen, at a constant pressure of 200 kPa. The identification of the peaks associated with the fatty acids was performed by comparing them with the peaks of the external standards, of which all the fatty acids concentrations are known. ChromQuest 5.0 (ThermoFisher scientific) software was used to perform the analysis of the chromatograms.

Table 6: Temperature program of the gas chromatography procedure used to analyze the fatty acids of different samples

Rate (°C/min)	Temperature (°C)	Hold time (min)
	80.0	0.00
25.0	175.0	25.0
10.0	200.0	20.0
10.0	220.0	5.0
10.0	235.0	15.0
20.0	80.0	0.0

2.4 Vitamin E content: Folch extraction and high-performance liquid chromatography (HPLC)

The vitamin E content was determined on the lipid extract obtained with the Folch method. The lipids were stored in diethyl ether at the end of the Folch procedure and 120 mg of this oil were transferred in a vial after evaporating diethyl ether under a flux of nitrogen. The resulting oil was then diluted with 580 µl of hexane. This was filtered on a 0.45 µm filter (Chromafil Pet 45/15 MS, Macherey Nagel). Fifty µl of the solution were analyzed by HPLC with a Phenomenex column (Luna 5µ Silica (2) 100 A, 250mmx4.6mm) and a fluorescence detector (Fluo FP 2020 Plus, Jasco). Tocopherols were detected at 295 nm and separated by isocratic chromatography with a mobile phase made of 98% heptane and 2% tetrahydrofuran, at a flow rate of 1.8 ml/min. Analyses were performed at 45°C in the column and the samples were put in the tray at 15°C. Tocopherols were quantified thanks to standard calibration curves of α-, β-, γ- and δ-tocopherols ranging from 1 to 100 ppm, prepared with the adjusted weight of the standards by Eric Mignolet. ChromQuest 5.0 (ThermoFisher scientific) software was used to perform the analysis of the chromatograms.

3. Statistical analysis

The aim of the statistical analysis is to determine whether the treatments applied (i.e. nitrogen fertilization level and tillage) have significant effects on different parameters. The data were analyzed based on the analysis of variance model (ANOVA) on JMP®, Version <2014> (SAS Institute Inc.). The statistical model that was used is the shown in the equation (1):

$$Y_{ij} = \beta_0 + \beta_1 X_i + \gamma_j + \varepsilon_{ij} \tag{1}$$

with Y_{ij} the variable of interest subject to the treatment i in the block j, β_0 and β_1 two parameters, X_i the nitrogen, γ_j the block and ε_{ij} the residual error. More sophisticated models were tried, including interactions and quadratic effects but are not shown here because they were not relevant and did not provide any additional information. ANOVA uses Fisher test to determine whether the treatments are significant. The Fisher test is a hypothesis test that compares the means obtained for each treatment and determines if those means can be considered as different. If $p<0.05$, the null hypothesis (that assumes that each treatment has the same mean) was rejected and the differences between treatments were considered as significant. If $p>0.05$, treatments were considered as non-significantly different.

Chapter IV: Results

It should be recalled that, contrary to the nitrogen treatment, there was no repetition of flax cultivation for the tillage treatment. The following graphs show the results for the tilled and the no-tilled parts together to see if a trend emerges. ANOVA tests were applied considering tillage as a factor but the resulting p-values must only be considered as indications. The trends cannot be extrapolated or generalized. More trials on different fields are required to do so.

For each parameter, a graph will be presented to highlight the effect of nitrogen and tillage. For each of them, the mean of the three blocks will be indicated, accompanied by the standard error and a smooth curve through the data. For each analyzed parameter, it will be specified if the block effect was significant or not. The point of the factor block is to take a part of the variability so that the residual variance is reduced. In this way, more importance is given to the effects of nitrogen and tillage practice. Thus, it is promising when the factor block is not significant because it means that the field was homogeneous and therefore that the position in the field did not influence the different parameters. However, it is possible that the high variability hid it.

1. Growing flax

1.1 Soil analyses

The main soil characteristics before and after flax growth are summarized in Table 7 and Table 8, for the no-tilled and the tilled parts of the field, respectively. The results of the two soil layers (i.e. 0-30 and 30-60 cm) were close and only their average is presented for the sake of simplicity. The amount of the soil nitrogen content before flax growth was subtracted from the amount after harvest in order to determine the difference, presented in the last column of the Tables. Thus, the difference reflects the nitrogen that was taken up during the plant growth. The soil nitrogen content after harvest was extremely heterogeneous, indicating sampling or analysis issues. It is therefore not possible to draw any conclusion. The difference in dry matter (DM) content before and after the cultivation was also determined.

The dry matter and the nitrogen contents before flax growth were the same for each plot as no treatment was applied yet. After harvest, each plot was sampled separately and therefore has a specific value. A marked increase of the dry matter content was observed regardless of the nitrogen and the tillage treatments. This can be viewed in the column “Difference” of DM. Statistically, the tillage factor significantly influenced soil dry matter content after harvest (p=0.0018). The increase of the soil dry matter content was indeed lower under no-tillage.

The pH, the organic carbon content, the humus content and the C/N ratio (Table 9) are not of interest here since no significant change can be observed in such a small period, but rather over many years. However, these values are typical from Wallonia.

Table 7: Soil nitrogen and dry matter balance for the no-tilled part of the flax field (DM = dry matter)

No-tillage		Before flax		After flax		Difference	
N fertilization	block	DM (%)	Nitrogen (kg N/ha)	DM (%)	Nitrogen (kg N/ha)	DM (%)	N uptake (kg N/ha)
0	1	83	60	91,5	76	8,5	-16
25	1	83	60	91	180	8	-120
50	1	83	60	91	169	8	-109
75	1	83	60	92	160	9	-100
100	1	83	60	91,5	95	8.5	-35
125	1	83	60	91	71	8	-11
0	2	83	60	92	155	9	-95
25	2	83	60	92	79	9	-19
50	2	83	60	91,5	234	8.5	-174
75	2	83	60	91,5	64	8.5	-4
100	2	83	60	92,5	104	9.5	-44
125	2	83	60	91,5	234	8.5	-174
0	3	83	60	92	78	9	-18
25	3	83	60	92	59	9	1
50	3	83	60	92	76	9	-16
75	3	83	60	92	118	9	-58
100	3	83	60	92,5	40	9.5	20
125	3	83	60	91,5	106	8.5	-46

Table 8: Soil nitrogen and dry matter balance for the tilled part of the flax field (DM = dry matter)

Tillage		Before		After		Difference	
N fertilization	block	DM (%)	Nitrogen (kg N/ha)	DM (%)	Nitrogen (kg N/ha)	DM (%)	N uptake (kg N/ha)
0	1	82	45	90	98	8	-53
25	1	82	45	90,5	122	8.5	-77
50	1	82	45	91	132	9	-87
75	1	82	45	91	178	9	-133
100	1	82	45	91,5	152	9.5	-107
125	1	82	45	92	255	10	-210
0	2	82	45	92,5	108	10.5	-63
25	2	82	45	93	155	11	-110
50	2	82	45	92	149	10	-104
75	2	82	45	92	143	10	-98
100	2	82	45	92	230	10	-185
125	2	82	45	92	258	10	-213
0	3	82	45	92	121	10	-76
25	3	82	45	92	135	10	-90
50	3	82	45	92	92	10	-47
75	3	82	45	91,5	124	9.5	-79
100	3	82	45	92	116	10	-71
125	3	82	45	92,5	121	10.5	-76

Table 9: Report of pH values, organic carbon and humus contents and C/N ratio of the soil before and after harvest of flax for both tilled and no-tilled parts of the field

	Before				After			
	pH	Organic carbon (g/kg)	Humus (%)	C/N	pH	Organic carbon (g/kg)	Humus (%)	C/N
No-tillage (0-30 cm)	6	10	2	9	6.6	10.5	2.15	Not reported
Tillage (0-30 cm)	6.3	11	2.1	10	5.6	9	1.8	Not reported

Based on the soil nitrogen content before harvest, one can calculate the amount of nitrogen that was available for the crop. To this end, all the nitrogen inputs must be taken into account. Table 10 and Table 11 show all the nitrogen inputs and outputs for flax under tillage and no-tillage, respectively. The explanations are detailed below.

Regarding the inputs, the remaining mineral nitrogen corresponds to what is already in the mineral form after winter and was given in the soil analysis report: 45 and 60 kg N/ha for the tilled and the no-tilled parts, respectively. The mineralization was the humus that was expected to undergo mineralization during flax growth. As the humus content was about 2%, it was estimated that 25 kg N/ha were going to be released ('Protecteau, module ferticulture'). As the previous crop was exported, it did not contribute to nitrogen inputs. However, as the cover crop was incorporated into the soil, it brought around 30 kg N/ha, as estimated by 'Procteau, module ferticulture'. The sum yielded 100 kg N/ha for the tilled part and 115 kg N/ha for the no-tilled part. The applied fertilization level was then added to yield the total nitrogen inputs.

Considering that the needs of flax are estimated around 90 kg N/ha, the soil already contained an excess of nitrogen. This made the experimental field totally inappropriate for the present experiment. As proof, it was recommended to apply only between 0 and 30 kg N/ha in this case ('Protecteau, module ferticulture'). The soil analysis results were unfortunately made available too late to allow the selection of another field.

Regarding the outputs, the remaining soil nitrogen after harvest was given in the soil analysis report. The nitrogen content of the seeds was based on the results obtained with the Kjeldahl method. Finally, the nitrogen content of the straws was based on the findings of Herzorg *et al.* (2017) as the straws were not harvested in the present experiment. It was estimated at 50 kg N/ha for all treatments, even though it is very likely that the nitrogen in the straws vary with the applied treatment and the field. The estimation of the outputs is therefore very rough and should be taken with some precaution.

Table 10: Nitrogen soil balance for flax under no-tillage

No-tillage		Inputs (kg N/ha)					Outputs (kg N/ha)			
N fertilization (kg/ha)	block	Soil mineral N	Humus mineralization	Cover crop	Fertilization	Total N inputs	Soil N after harvest	N in seeds	N in straws	Total N outputs
0	1	60	25	30	0	115	76	52.1	50	178.1
25	1	60	25	30	25	140	180	56.6	50	286.6
50	1	60	25	30	50	165	169	60.7	50	279.7
75	1	60	25	30	75	190	160	55.9	50	265.9
100	1	60	25	30	100	215	95	60.4	50	205.4
125	1	60	25	30	125	240	71	61.7	50	182.7
0	2	60	25	30	0	115	155	50.0	50	252.0
25	2	60	25	30	25	140	79	47.3	50	176.3
50	2	60	25	30	50	165	234	54.1	50	338.2
75	2	60	25	30	75	190	64	53.9	50	167.9
100	2	60	25	30	100	215	104	57.8	50	211.8
125	2	60	25	30	125	240	234	51.1	50	335.1
0	3	60	25	30	0	115	78	55.3	50	183.3
25	3	60	25	30	25	140	59	61.7	50	170.7
50	3	60	25	30	50	165	76	68.5	50	194.5
75	3	60	25	30	75	190	118	54.1	50	222.1
100	3	60	25	30	100	215	40	60.1	50	150.1
125	3	60	25	30	125	240	106	64.9	50	220.9

Table 11: Nitrogen soil balance for flax under tillage

Tillage		Inputs (kg N/ha)					Outputs (kg N/ha)			
N fertilization (kg/ha)	block	Soil mineral N	Humus mineralization	Cover crop	Fertilization	Total N inputs	Soil N after harvest	N in seeds	N in straws	Total N outputs
0	1	45	25	30	0	100	98	49.8	50	197.8
25	1	45	25	30	25	125	122	48.3	50	220.3
50	1	45	25	30	50	150	132	61.5	50	243.5
75	1	45	25	30	75	175	178	62.5	50	290.5
100	1	45	25	30	100	200	152	57.4	50	259.4
125	1	45	25	30	125	225	255	46.4	50	351.4
0	2	45	25	30	0	100	108	49.4	50	207.4
25	2	45	25	30	25	125	155	51.5	50	256.5
50	2	45	25	30	50	150	149	45.1	50	244.1
75	2	45	25	30	75	175	143	41.7	50	234.7
100	2	45	25	30	100	200	230	48.6	50	328.6
125	2	45	25	30	125	225	258	48.2	50	356.2
0	3	45	25	30	0	100	121	52.1	50	223.1
25	3	45	25	30	25	125	135	53.8	50	238.8
50	3	45	25	30	50	150	92	58.5	50	200.5
75	3	45	25	30	75	175	124	57.9	50	231.9
100	3	45	25	30	100	200	116	62.7	50	228.7
125	3	45	25	30	125	225	121	54.1	50	225.1

Based on these tables, one can calculate the nitrogen use efficiency (NUE). The NUE estimates the efficiency of plants to use the available nitrogen and therefore indicates the nitrogen losses. Only a proportion of the nitrogen available for plants ends up in the harvested material that is, the seed in this case. Another part is used to build the plant biomass and the rest is lost through the soil, water and atmosphere. There are many different ways of measuring the NUE and one should select the most appropriate depending on the crop, the harvested product and the interest for the physiological processes (Good *et al*., 2004). Four formulae were tried to describe the nitrogen use efficiency of flax, based on the definitions of Good *et al*. (2004).

- The **uptake efficiency** (NUE_{uptake}) is the ratio between the total nitrogen [kg N/ha] in plants (that is the N in seeds and straws in this case) and the nitrogen supply [kg N/ha]. It measures the efficiency of the uptake of nitrogen by the plant and was calculated as follows:

$$NUE_{uptake} (\%) = (\text{total N in plants/N supplied}) \times 100 \tag{2}$$

- The **physiological utilization efficiency** ($NUE_{utilization}$) is the ratio between the seed weight [kg seeds/ha] and the total nitrogen [kg N/ha] in the plant. It reflects the proportion of N converted into seeds, the product of interest. It was calculated as follows:

$$NUE_{utilization} (\text{kg of seeds per kg of N absorbed}) = (\text{seed weight/total N in plants}) \times 100 \tag{3}$$

- The **agronomic efficiency** ($NUE_{agronomic}$) is the product of the uptake efficiency and the utilization efficiency. It can be written as the ratio between the seed weight [kg seeds/ha] and the nitrogen supplied [kg N/ha]. It represents the how nitrogen was efficiently converted in seed yield. The formula used was the following:

$$NUE_{agronomic} (\text{kg of seeds per kg of N supplied}) = (\text{seed weight/N supplied}) \times 100 \tag{4}$$

- The **agronomic oil efficiency** ($NUE_{agronomic\ oil}$) was also calculated as the end product is often the oil in the case of flax. It gives an idea of how nitrogen was converted into the component of interest. The seed weight was replaced by the oil weight [kg oil/ha] in the $NUE_{agronomic}$ formula, which yielded the following formula:

$$NUE_{agronomic\ oil} (\text{kg of oil per kg of N supplied}) = (\text{oil weight/N supplied}) \times 100 \tag{5}$$

In practice, the nitrogen fertilization is often used instead of the nitrogen supply because the latter requires a complete soil diagnose. It is more rigorous to consider the nitrogen supply as it accounts in the nitrogen that the plant takes up. That is what has been done here.

Table 12: Summary of the four types of NUE obtained for the tilled and the no-tilled parts of the flax field

		No-tillage				Tillage			
N fertilization (kg N/ha)	block	NUE uptake (%)	NUE utilization (%)	NUE agro. (%)	NUE agro. oil (%)	NUE uptake (%)	NUE utilization (%)	NUE agro. (%)	NUE agro. oil (%)
0	1	88.7	1339.5	1189.0	578.7	99.8	1389.8	1386.7	674.0
25	1	76.1	1204.5	917.1	432.2	78.6	1562,.6	1228.3	596.3
50	1	67.1	1513.3	1015.8	505.1	74.3	1402.5	1042.2	514.7
75	1	55.7	1575.7	877.9	417.1	64.3	1330.2	854.9	409.6
100	1	51.3	1427.1	732.7	358.9	53.7	1473.5	791.0	386.8
125	1	46.5	1108,.6	515.8	247.5	42.8	1743.4	746.7	360.9
0	2	84.3	1383.5	1167.0	560.6	99.4	1322.4	1314.6	647.5
25	2	69.5	1525.4	1060.5	503.5	81.2	1304.2	1058.9	503.8
50	2	63.1	1185.3	748.3	357.6	63.4	1549.3	981.8	470.5
75	2	54.7	1093.5	598.2	278.8	52.4	1569.4	822.1	403.3
100	2	50.1	1257.7	630.1	307.7	49.3	1540.5	759.7	364.6
125	2	42.1	1282.5	540.0	266.3	43.7	1415.2	617.8	301.1
0	3	91.5	1428.9	1307.8	641.0	102.1	1461.0	1492	694.9
25	3	79.8	1265.1	1009.1	491.9	83.1	1609.0	1336.5	653.9
50	3	71.8	1305.2	937.0	445.4	72.3	1701.0	1230.2	592.1
75	3	54.8	1518,.6	831.9	401.5	61.8	1318.8	813.3	398.3
100	3	51.2	1597.2	817.7	401.0	56.4	1446.4	815.3	385.9
125	3	47.9	1240.2	593.9	278.4	46.3	1694.4	784.4	382.2

Table 12 summarizes the NUE obtained for each experimental plot. The block factor had no influence on any of the NUE calculated. It can be seen on Figure 6 that the NUE $_{\text{uptake}}$ decreased with the increasing nitrogen fertilization ($p<0.0001$). The NUE $_{\text{uptake}}$ was also significantly higher under tillage ($p=0.0130$): the mean of NUE $_{\text{uptake}}$ was 63.7% under no-tillage and 68.0% under tillage. The NUE $_{\text{utilization}}$ was also higher under tillage than under no-tillage ($p=0.0075$) but it did not seem to be affected by the nitrogen fertilization (Figure 7). The mean of NUE $_{\text{utilization}}$ was 1347.3 kg of seeds per kg of N absorbed under no-tillage and 1490.8 kg of seeds per kg of N absorbed under tillage. Next, the NUE $_{\text{agronomic}}$ had the same pattern as the NUE $_{\text{uptake}}$ (Figure 8). The NUE $_{\text{agronomic}}$ decreased with the increasing nitrogen fertilization ($p<0.0001$) and the NUE $_{\text{agronomic}}$ was significantly higher under tillage ($p<0.0001$): 860.5 kg of seeds per kg of N supplied vs. 1004.2 kg of seeds per kg of N supplied under no-tillage and tillage, respectively. Finally, the same trends were observed for the NUE $_{\text{agronomic oil}}$ (Figure 9): it decreased with the increasing nitrogen fertilization ($p<0.0001$) and was globally higher under tillage ($p=0.0001$). The mean of the NUE $_{\text{agronomic oil}}$ was 415.2 kg of oil per kg of N supplied under no-tillage and 485.6 kg of oil per kg of N supplied under tillage.

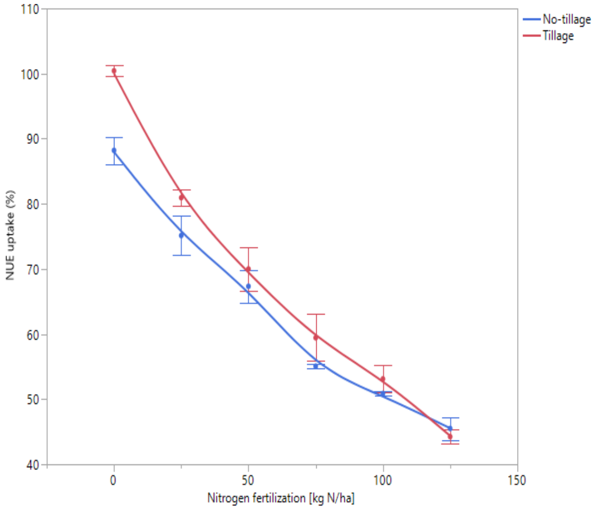


Figure 6: Uptake efficiency (NUE uptake) of flax under tillage and no-tillage vs. Nitrogen fertilization

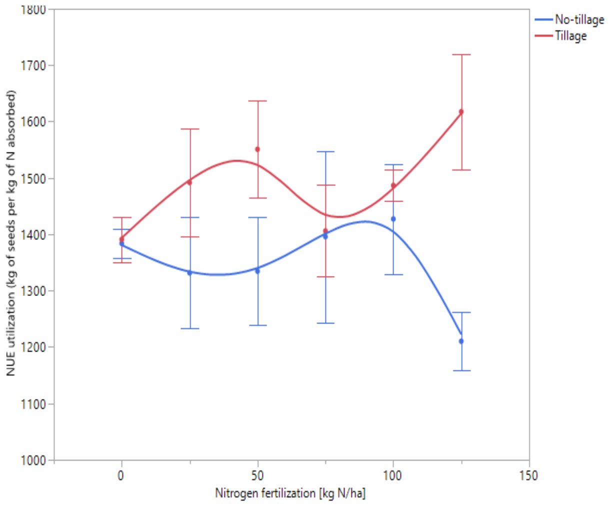


Figure 7: Physiological utilization efficiency (NUE utilization) of flax plants under tillage and no-tillage vs. Nitrogen fertilization

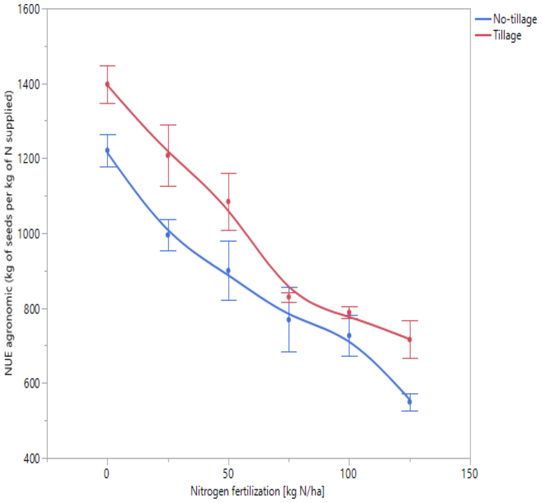


Figure 8: Agronomic efficiency (NUE agronomic) of flax plants under tillage and no-tillage vs. Nitrogen fertilization

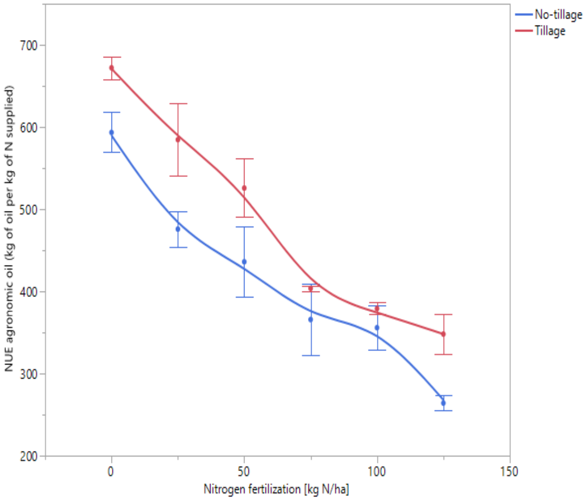


Figure 9: Agronomic oil efficiency (NUE agronomic oil) of flax plants under tillage and no-tillage vs. Nitrogen fertilization

The soil analyses reported that the pre-harvest nitrogen content was significantly higher under no-tillage (p=0.0104) but no effect of tillage on the soil nitrogen content after harvest was perceived. Figure 10 shows the soil nitrogen content after harvest for both parts. The factor block was significant (p=0.0004). The blue curve, which represents the no-tilled part, has a very strange pattern, indicating an analysis or a sampling issue. For the tilled part only, there was a significant effect of the nitrogen fertilization level (p=0.0041). One can observe that the soil residual nitrogen was higher when higher doses of nitrogen fertilization were applied.

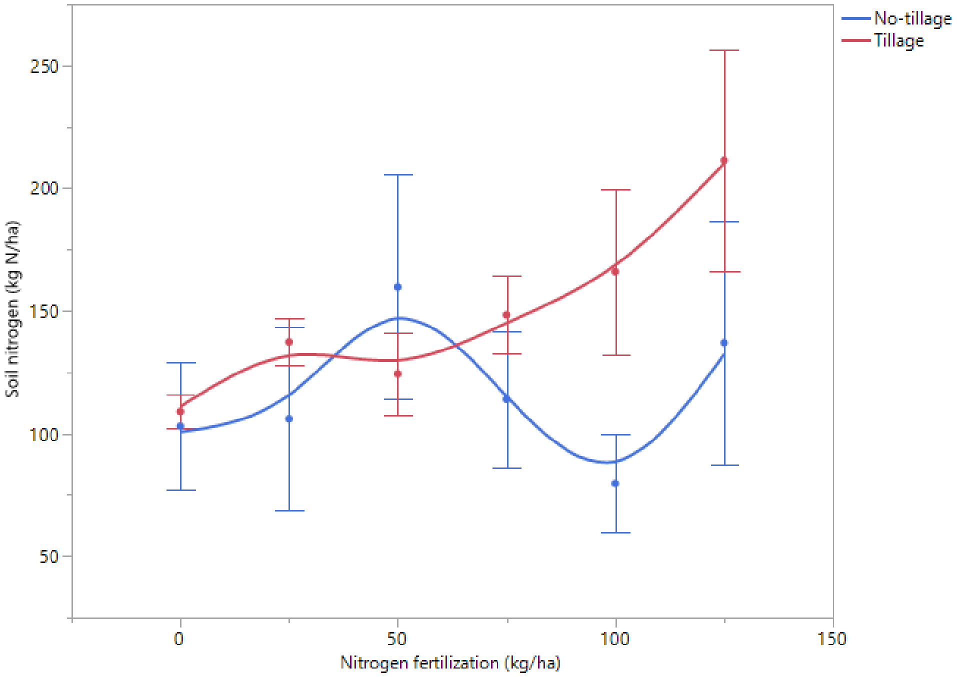


Figure 10: Post-harvest soil nitrogen content for the tilled and the no-tilled parts of the flax field vs. Nitrogen fertilization

1.2 Plant density

The average plant density was 403 and 352 plants/m² for the tilled and the no-tilled part, respectively. The analysis of variance showed that the block had no significant effect. Figure 11 displays the results of the density of flaxseed as a function of the nitrogen fertilization level for both soil practices. It can be inferred that the density of plants tended to increase with the nitrogen fertilization for lower levels but quickly started to decrease when the nitrogen fertilization further increased. However, even though this trend seemed consistent, the statistical analysis revealed that nitrogen fertilization level was not significant, which suggests that there is no relationship between nitrogen inputs and density of plants. On the other hand, the analysis of variance showed a significant difference in plant density between the tilled and the no-tilled parts of the field (p=0.0011), which means that the tillage practice influenced the plant density. Plants were denser under tilled conditions.

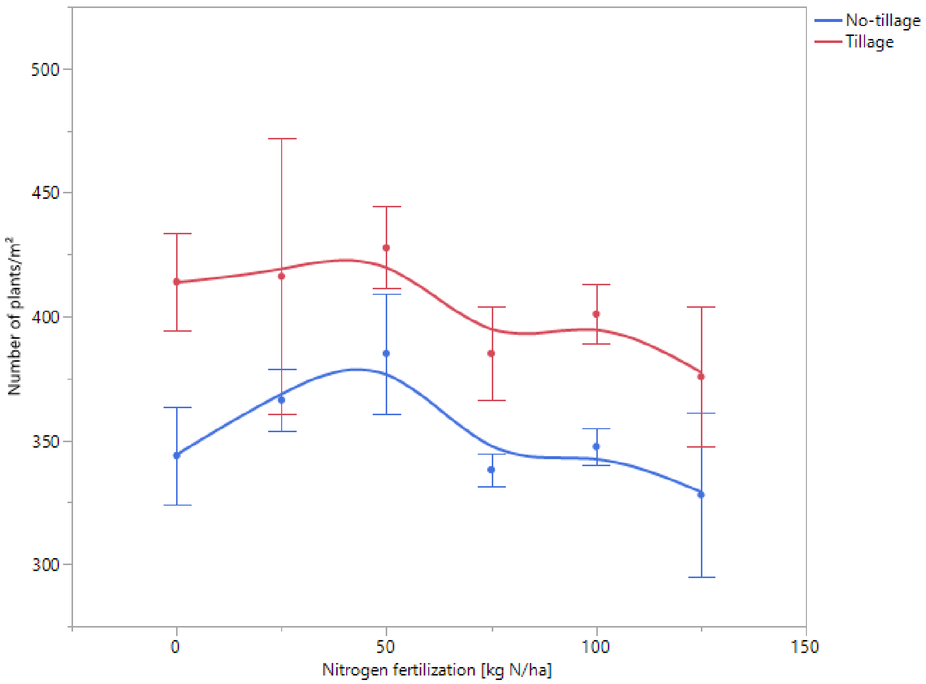


Figure 11: Density of plants during the growth trial of flax under tillage and no-tillage conditions vs. Nitrogen fertilization

1.3 Plant height

The average flax plants height was 59.98 cm on the tilled part and 58.27 cm on the no-tilled part of the field. A significant block effect was present (p=0.0142).

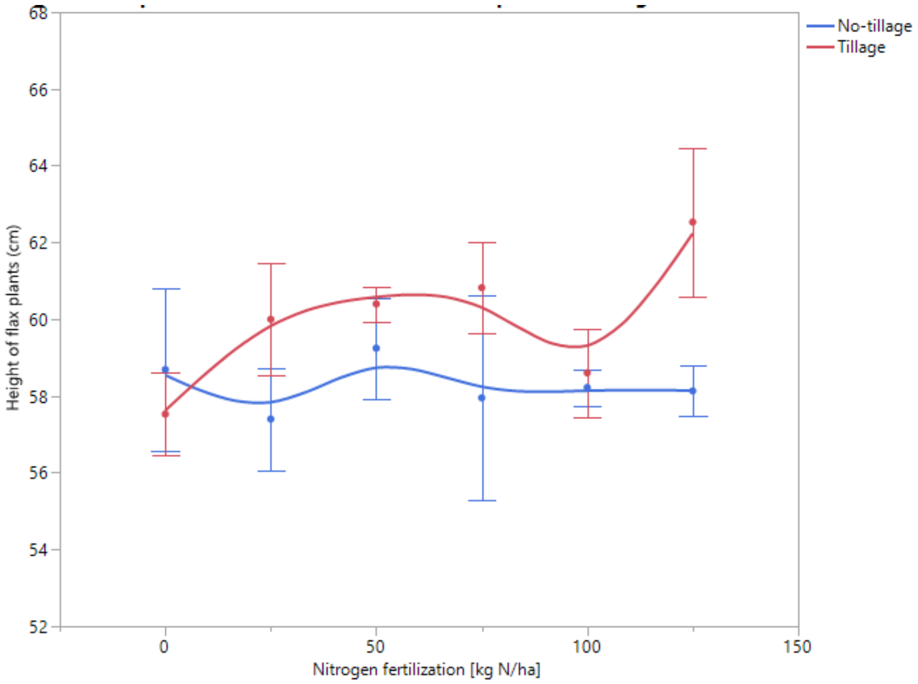


Figure 12: Height of flax plants at the end of the growth under tillage and no-tillage vs. Nitrogen fertilization

The tillage significantly influenced plant height: plants were on average taller under tillage (p=0.0347). Moreover, the tilled part of the field clearly showed an increase of the plant height when the fertilization increased (Figure 12) and the analysis of variance confirmed that the nitrogen fertilization level significantly influenced the height of plants for the tilled part only (p=0.0240). No trend was observed for the no-tilled part.

1.4 Plant maturity

The block had a significant effect on the maturity stage (p=0.0264) but neither the tillage nor the nitrogen factors influenced this parameter (Figure 13). However, a slight trend could be observed: the more nitrogen the plant received, the less mature it seemed to be. Flax also looked more mature when grown under tillage. However, as these trends were not significant, no conclusion could be drawn.

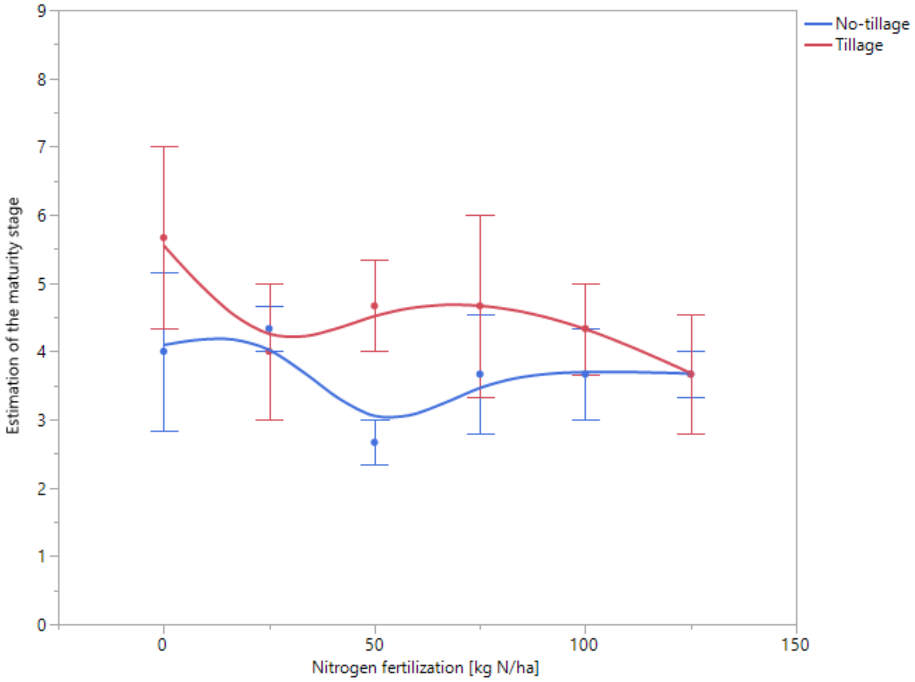


Figure 13: Estimation of the maturity stage of flax plants grown under tillage and no-tillage vs. Nitrogen fertilization

1.5 Estimation of seed and oil yields

The weight of the harvested seeds of each plot (kg/150m² of land) was converted in yield (q/ha). The oil content obtained with the Soxhlet method was then used to express the oil yield of each plot. The average yield of flax was 14.38 q/ha under no-tillage and 15.29 q/ha under tillage. The average oil yield was 697.3 kg/ha under no-tillage and 739.9 kg/ha under tillage.

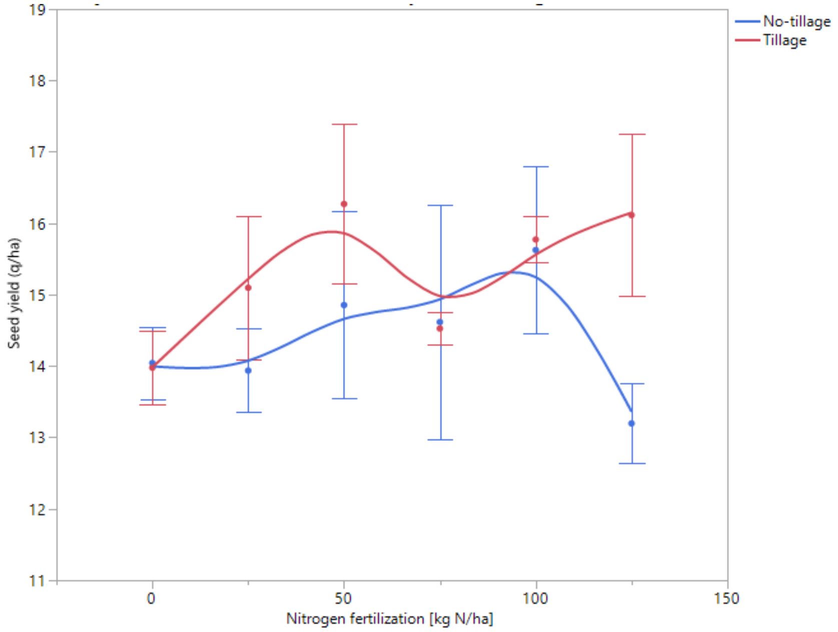


Figure 14: Seed yield of flax grown under tillage and no-tillage vs. Nitrogen fertilization

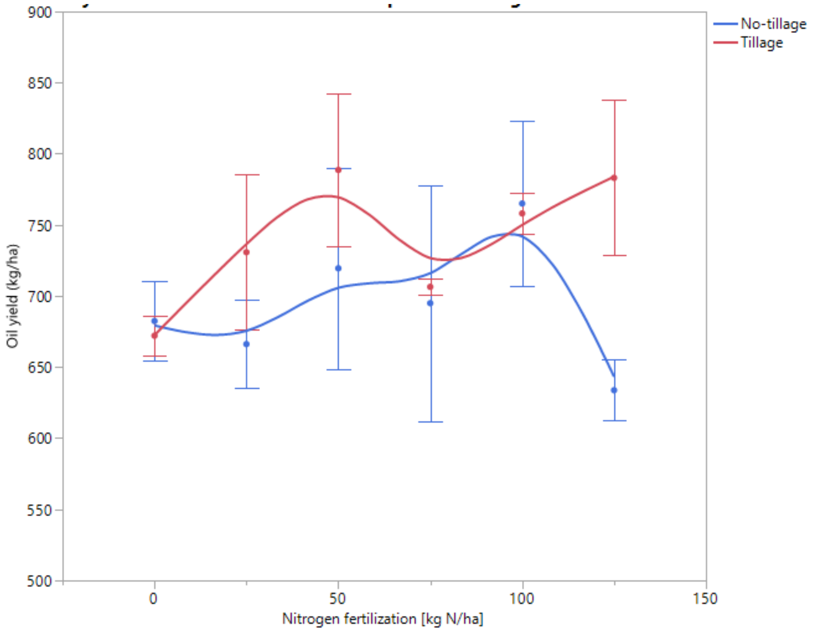


Figure 15: Oil yield of flax grown under tillage and no-tillage vs. Nitrogen fertilization

Flax grown under tillage seemed to perform slightly better than flax grown under no-tillage (Figure 14 and Figure 15). However, this trend was not statistically significant. The standard errors of the measurements were also extremely high, making the result interpretation difficult.

Even though it is not always significant, flax grown under tillage appears to present higher values for all the previously analyzed parameters (i.e. plant density, plant height, and seed and oil yields).

2. Composition of flaxseed

2.1 Lipid content

The lipid content varied between 47.33 and 49.73% of the flaxseed dry matter (in weight). No clear trend appeared on Figure 16, showing the oil content vs. the nitrogen fertilization level and nothing was significant. The data showed that neither tillage nor nitrogen fertilization level statistically influenced the lipid content of flaxseed in the present experiment. However, the high standard errors indicate that the sample might not be very representative of the overall data. It can also suggest a too small sample size.

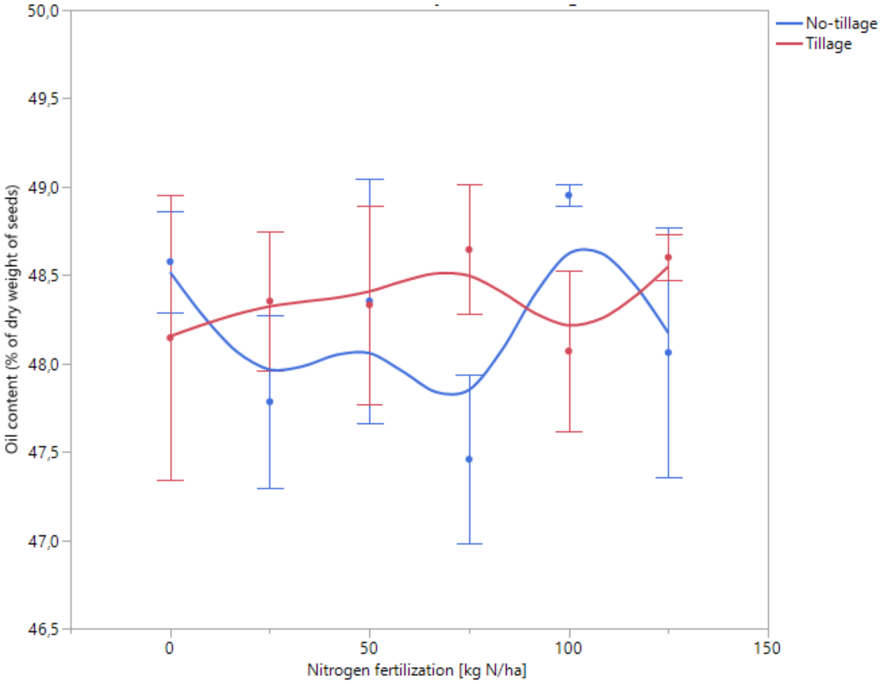


Figure 16: Lipid content of flaxseed harvested from plants grown under tillage and no-tillage vs. Nitrogen fertilization

2.1 Fatty acid profile

The fatty acid profile of flaxseed was determined by GC analysis. The peaks of the chromatograms were attributed their corresponding fatty acid, based on the retention time and thanks to the use of a fatty acid standard mixture analyzed in parallel. The surface of each peak was then converted into the concentration of the fatty acids. The concentration is expressed as the weight percentage of the sum of all the identified fatty acids. As no extraction standard was added, the precise amount of fatty acids in flax oil could not be determined in this experiment. Out of the 20 detected fatty acids, the 9 more represented were selected and are shown below. The other fatty acids represented less than 0.02% of the identified fatty acids and were therefore not analyzed for simplicity reasons.

Figure 17 and Figure 18 show the proportion of the nine major fatty acids in the oil of flax grown under tillage and no-tillage, respectively. Alpha-linolenic acid was clearly the major fatty acid in both conditions. The results were similar for the tilled and the no-tilled parts of the field. The flat curves indicate that the nitrogen fertilization level did not impact on the fatty acid profile. To provide a better overview for each fatty acid, a zoomed graph dedicated to each of them will be presented below.

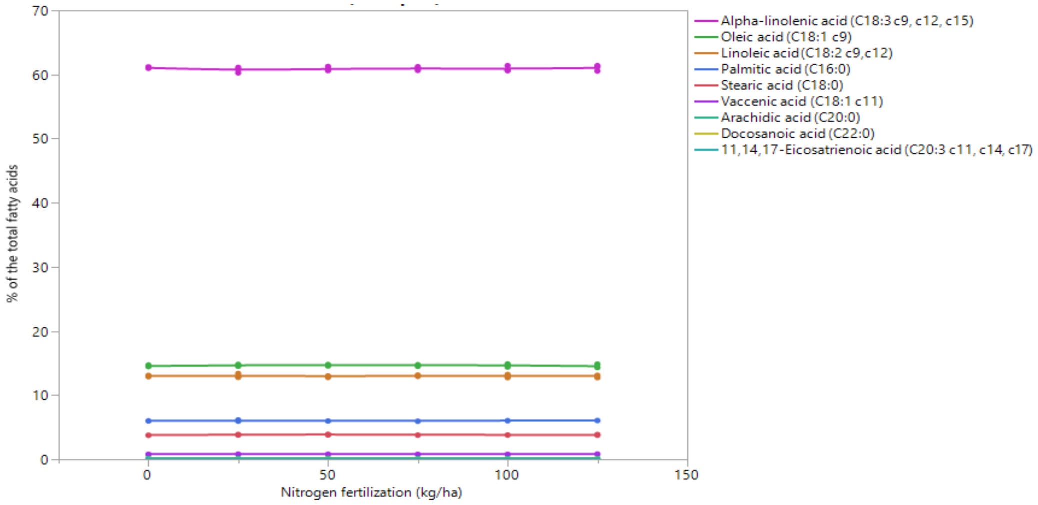


Figure 17: Proportion of the 9 more represented fatty acids in the oil of flax grown under tillage vs. Nitrogen fertilization

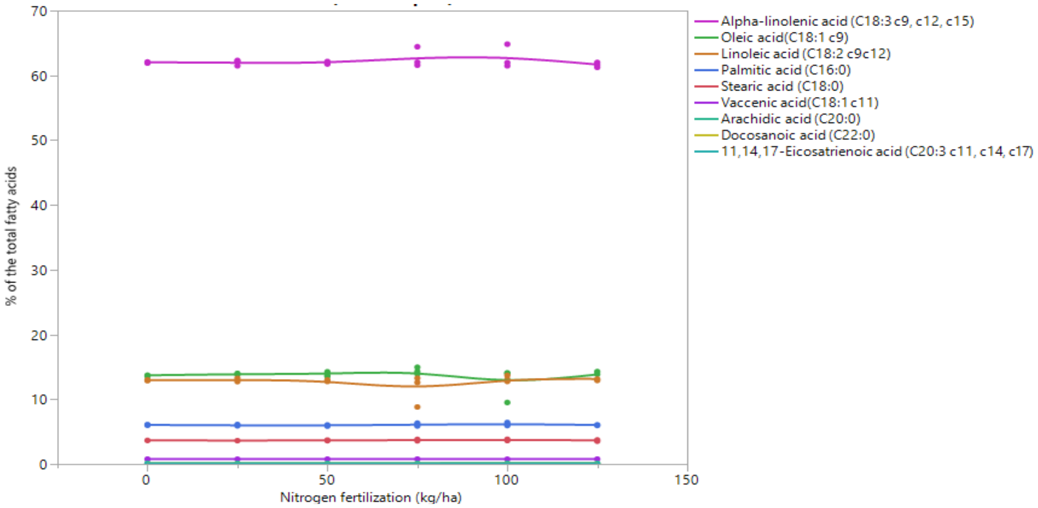


Figure 18: Proportion of the 9 more represented fatty acids in the oil of flax grown under no-tillage vs. Nitrogen fertilization

First of all, palmitic acid (C16:0) did not seem to be influenced by the nitrogen fertilization level or by the tillage practice. Analysis of variance revealed that none of these factors were significant. Indeed, no inference could be made based on the Figure 19.

As can be seen in Figure 20, the stearic acid (C18:0) proportion was significantly higher for seeds grown under tillage than for those grown under no-tillage ($p<0.0001$). However, this difference was only of about 0.2%, which is a minuscule scale and is not likely to have impacts on human nutrition. On the other hand, nitrogen fertilization level had no effect on it.

The same results were obtained for the oleic acid proportion (C18:1 cis 9). Tillage significantly influenced the proportion of oleic acid, which was higher under tillage conditions ($p=0.0022$). Oleic acid proportion differed here by about 1%, which is still a tiny change that is not impactful. It was however not sensitive to the nitrogen fertilization level (Figure 21). One point seems to deviate from the others, driving down the blue curve (no-tillage). This distant point is the average of three data that represented the proportion of oleic acid in three experimental plots that received the same treatment (i.e. 100 kg N/ha under no-tillage). One of these three points was very different from the two others. It can mean that the oleic acid proportion was very different in this plot but it is more likely that an experimental error was made somewhere in the whole sampling or analysis process. As a consequence, the standard error of this point is extremely high.

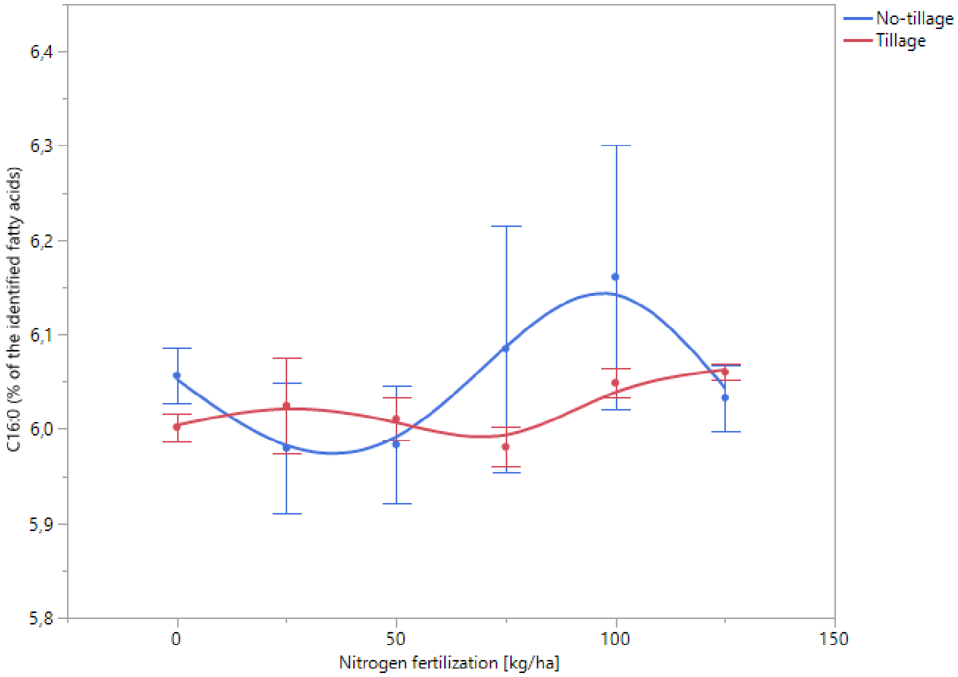


Figure 19: Proportion of palmitic acid (C16:0) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

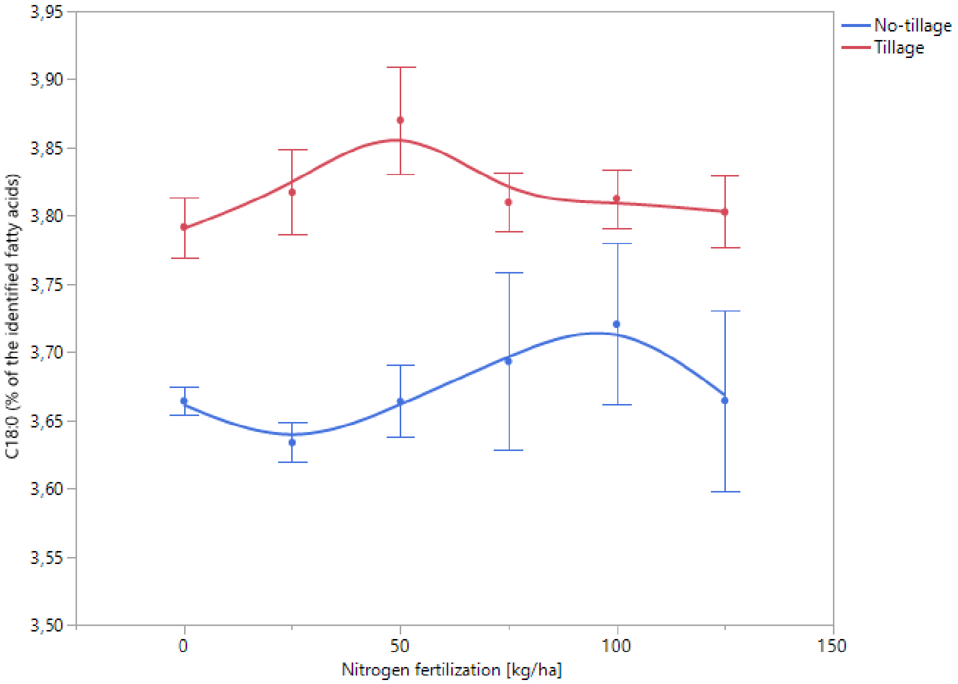


Figure 20: Proportion of stearic acid (C18:0) in total fatty acids of the flax oil, under tillage and no-tillage practices parts vs. Nitrogen fertilization

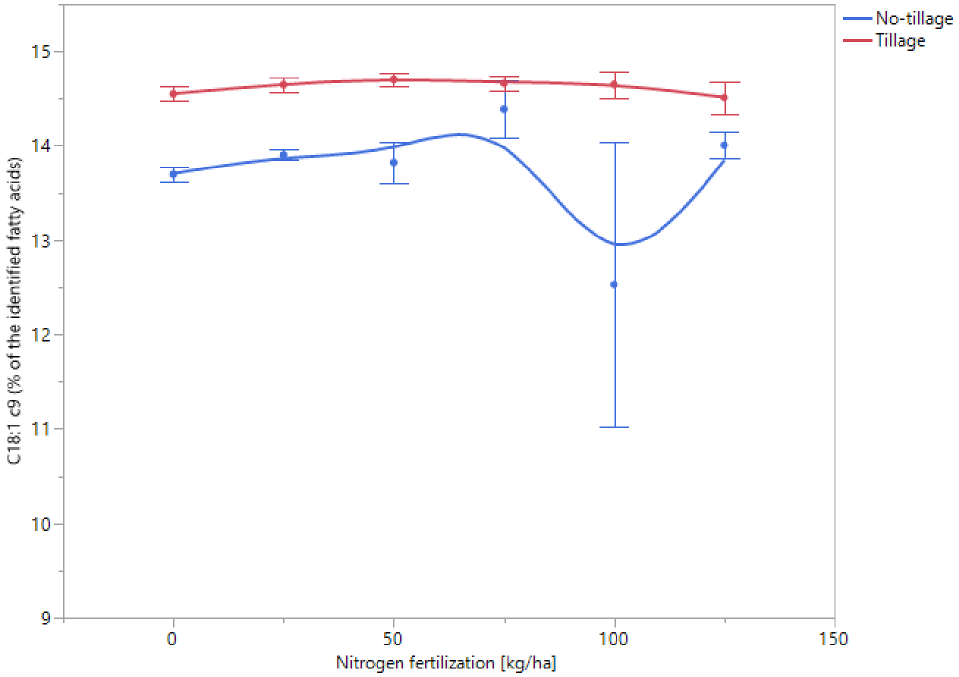


Figure 21: Proportion of oleic acid (C18:1 c9) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

Vaccenic acid (C18:1 cis 11) was not influenced at all by the nitrogen fertilization level. However, tillage significantly influenced its level (p<0.0001), which was again significantly higher for flax grown under tillage. The difference was however limited to about 0.1% and thus does not have a large impact on the general fatty acid profile (Figure 22). The block effect was also significant (p=0.0033).

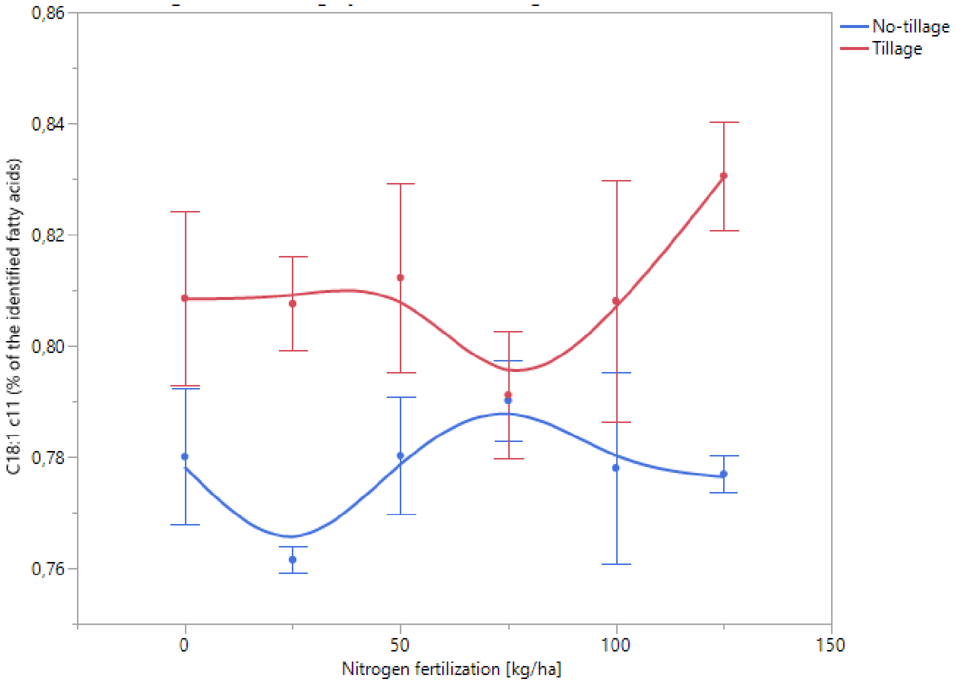


Figure 22: Proportion of vaccenic acid (C18:1 c11) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

Neither the tillage nor the fertilization level influenced the linoleic acid (C18:2 cis 9, cis 12) proportion (Figure 23). It should be noted that one point also seemed suspiciously distant from the others, driving the blue curve down.

Neither the tillage nor the fertilization level influenced the proportion of arachidic acid (C20:0) (Figure 24). Once again, one point was suspicious compared to others, driving down the blue curve.

The tillage significantly influenced the proportion of alpha-linolenic acid (C18:3 cis 9, cis 12, cis 15) (p<0.0001), but the nitrogen fertilization level did not (Figure 25). One can observe that the tillage gave lower proportion of alpha-linolenic acid. So far, tillage was giving higher proportion of the previous fatty acids (C18:0, C18:1 c9, C18:1 c11) or similar (C18:2 c9 c12, C20:0) than no-tillage and it seems that the trend reverses.

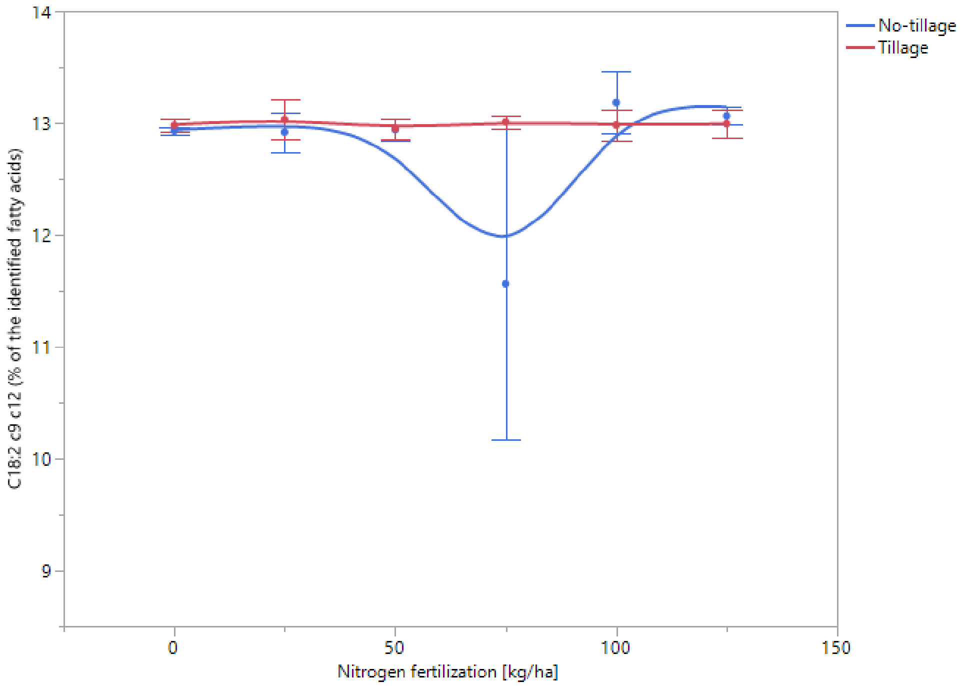


Figure 23: Proportion of linoleic acid (C18:2 c9 c12) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

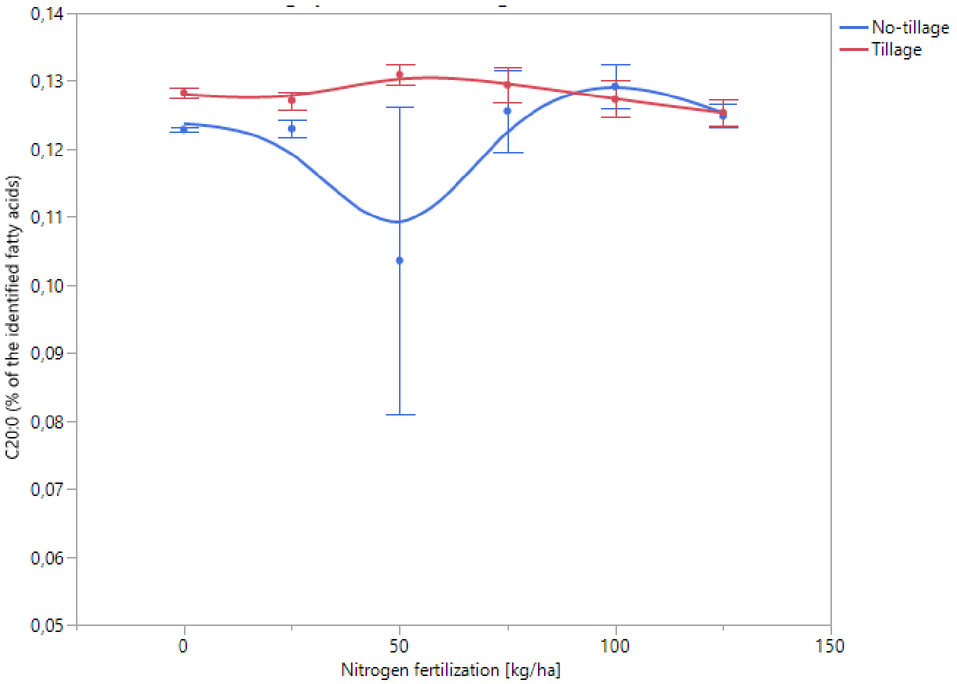


Figure 24: Proportion of arachidic acid (C20:0) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

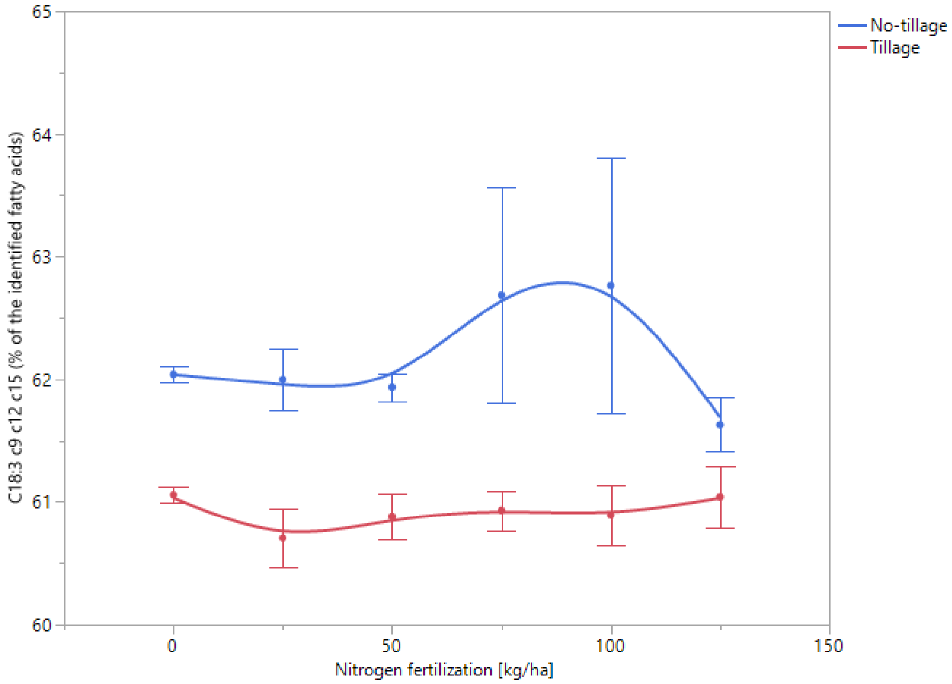


Figure 25: Proportion of alpha-linolenic acid (C18:3 c9, c12, c15) in total fatty acids of the flax oil, under tillage and no-tillage practices parts vs. Nitrogen fertilization

The docosanoïc acid (C22:0) level was neither influenced by the tillage nor by the nitrogen fertilization level. The two curves even intersect a few times. Once again, two points seemed quite aberrant, probably resulting from experimental errors (Figure 26).

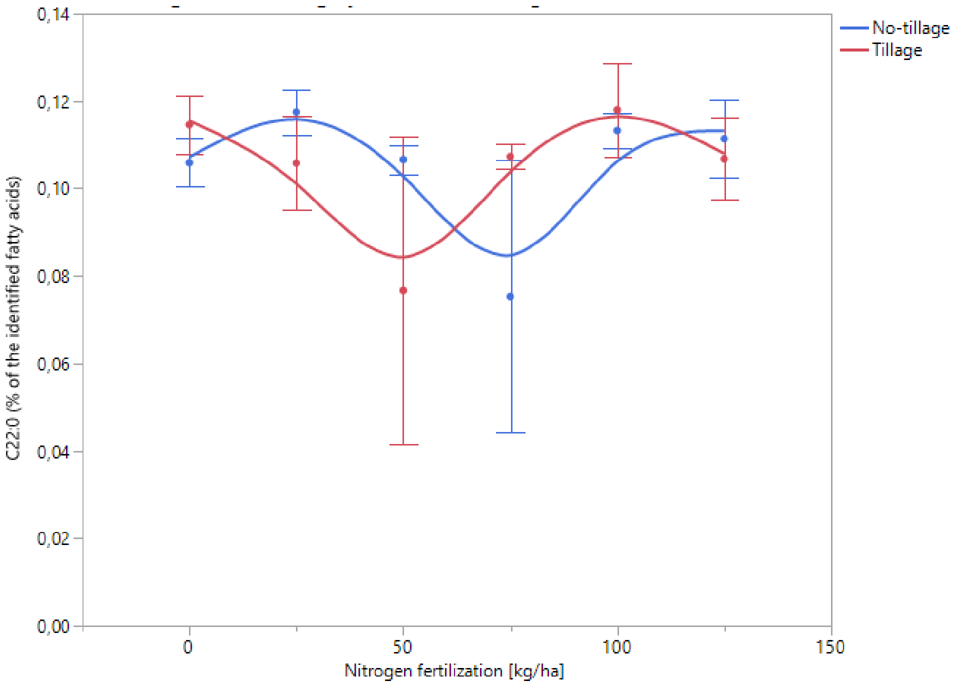


Figure 26: Proportion of docosanoic acid (C22:0) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

As for alpha-linolenic acid, no-tillage seemed to give higher proportions of 11,14,17-eicosatrienoic acid (C20:3 c11, c14, c17) than tillage ($p<0.0001$) (Figure 27). Once again, no effect of the nitrogen fertilization level was detected.

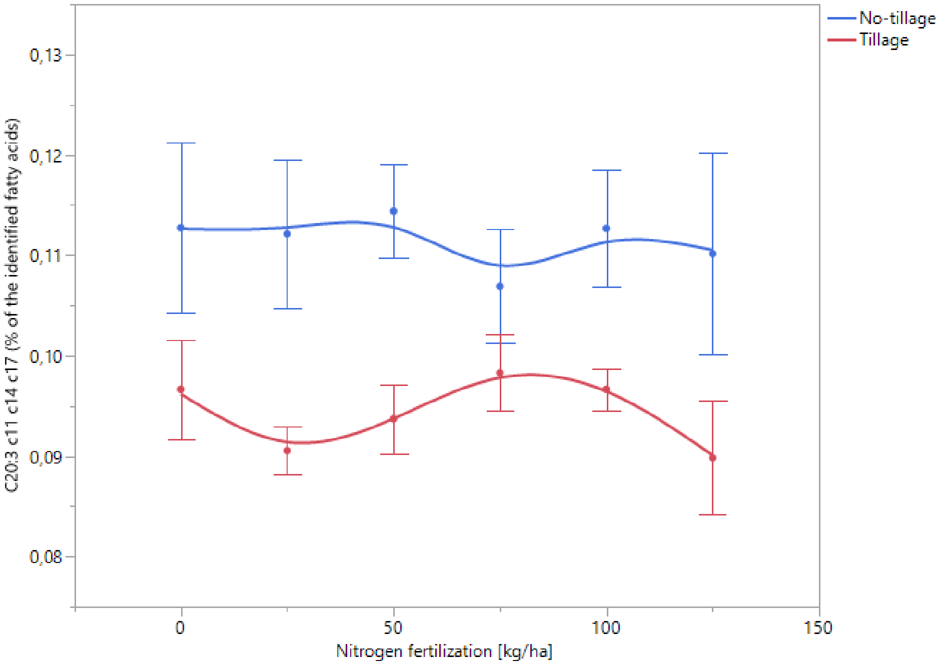


Figure 27: Proportion of 11, 14, 17-eicosatrienoic acid (C20:3 c11, c14, c17) in total fatty acids of the flax oil, under tillage and no-tillage practices vs. Nitrogen fertilization

To highlight the effect of the tillage practice on the fatty acid profile of flax oil, the 9 previous fatty acids were gathered in two distinct ways: according to the unsaturation degree and the carbon chain length. During the biosynthesis of fatty acids in plants, long chain fatty acids are synthetized from small precursors. Then, long chain fatty acids are subject to two main processes: elongation and desaturation, which are catalyzed by two distinct kinds of enzymes (elongases and desaturases). These modifications allow to form the diversity of fatty acids found in plants (Thelen and Ohlrogge, 2002). Thus, a modification of the fatty acid profile could be the consequence of a change in one of these two processes.

In Figure 28, fatty acids were grouped according to their unsaturation degree. Three groups are shown: saturated fatty acids (palmitic acid, stearic acid, arachidic acid, docosanoic acid), monounsaturated fatty acids (oleic acid, vaccenic acid) and polyunsaturated fatty acids (linoleic acid, alpha-linolenic acid, 11,14,17 eicosatrienoic acid). This figure highlights that tillage favored saturated ($p=0.0340$) and monounsaturated ($p=0.0015$) fatty acids whereas no-tillage favored polyunsaturated fatty acids ($p<0.0001$). In Figure 29, fatty acids were grouped according to their chain length. Three groups were made: fatty acids with 16 carbons or less, with 18 carbons and with 18 carbons or more. The curves of tillage and no-tillage are overlapping, which indicates that there was no influence of the tillage practice on the fatty acid carbon chain length.

2.2 Vitamin E content

The four forms of tocopherols were analyzed in flaxseed. Gamma-tocopherol was by far the most important form, as it represented 97-98% of the total tocopherol content. Alpha- and delta-tocopherols were also detected in limited amounts (1-2%) but no beta-tocopherol was present. The following results are therefore only going to show the total tocopherol content and the gamma-tocopherol proportion in the total tocopherol content. The tocopherol analyses were performed in the lipid extract obtained with the Folch method and therefore, the tocopherol content is expressed in mg/kg of oil.

As with the previously analyzed components, the block factor did not influence the tocopherol content and the gamma-tocopherol proportion. It should be noted that the profile of the plot N°23 was not interpretable and was therefore not taken into account for the following results.

The tocopherol content averaged 289.05 and 295.8 mg of tocopherol/kg of oil (ppm) for the plants grown under tillage and no-tillage practices, respectively. It was significantly influenced by the nitrogen fertilization level (p=0.0462). Indeed, Figure 30 shows that the total tocopherol content seemed to decrease when the nitrogen fertilization applied increased.

The proportion of gamma-tocopherol in the total tocopherol content (Figure 31) was not influenced by the tillage or the fertilization level.

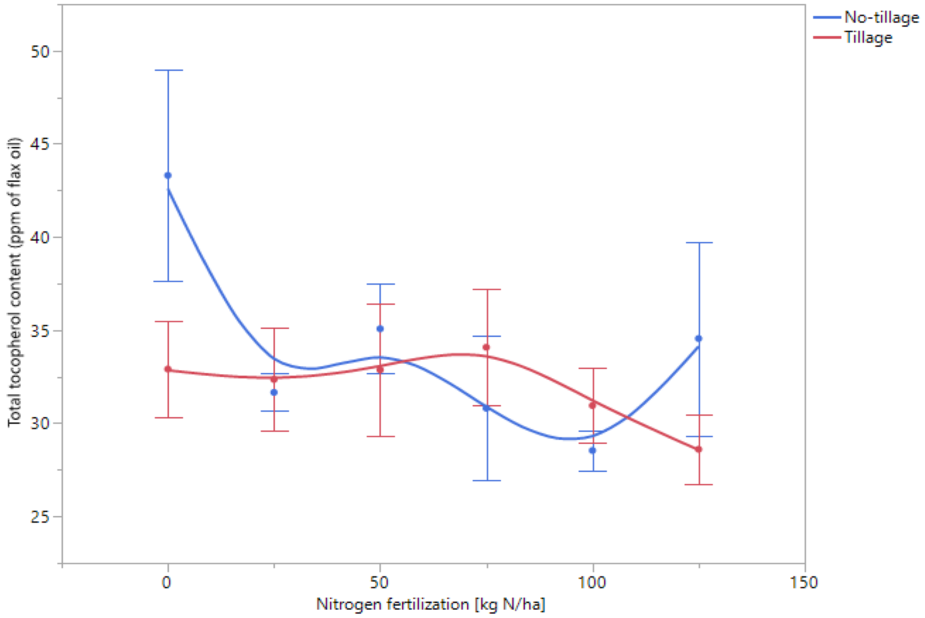


Figure 30: Total tocopherol content in flaxseed grown under tillage and no-tillage vs. Nitrogen fertilization

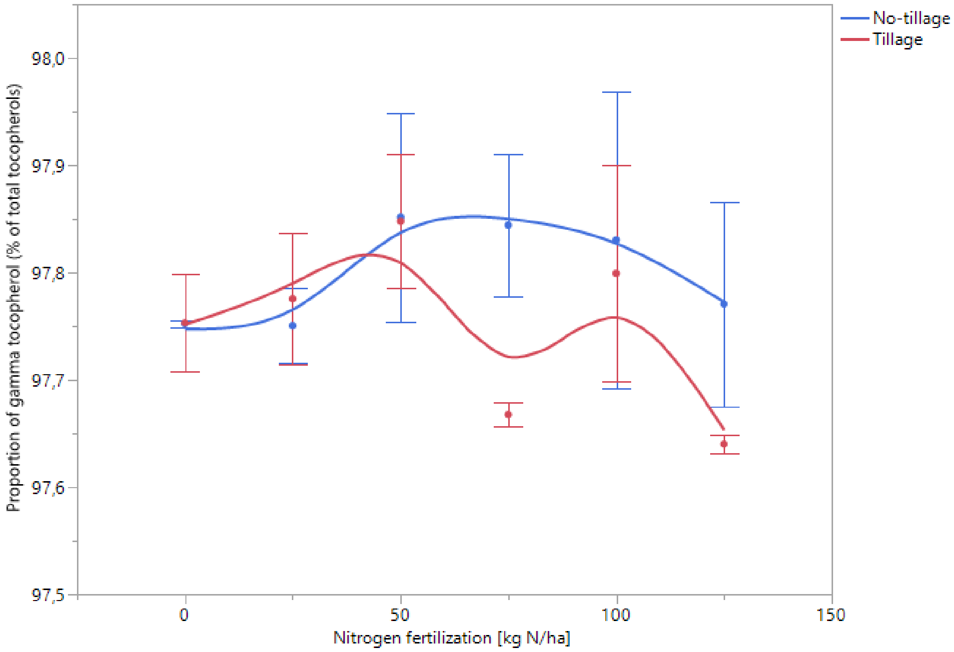


Figure 31: Proportion of gamma-tocopherol in the total tocopherol present in flaxseed grown under tillage and no-tillage vs. Nitrogen fertilization

2.3 Crude protein content

The crude protein content (Figure 32) evaluated by the Kjeldahl method was not influenced by the block factor. In addition, it was neither impacted by the nitrogen fertilization level nor by the tillage practice. The crude protein content of flaxseed under tillage was on average 23.22% of the seed's dry matter under tillage and 22.92% of the seed's dry matter under no-tillage.

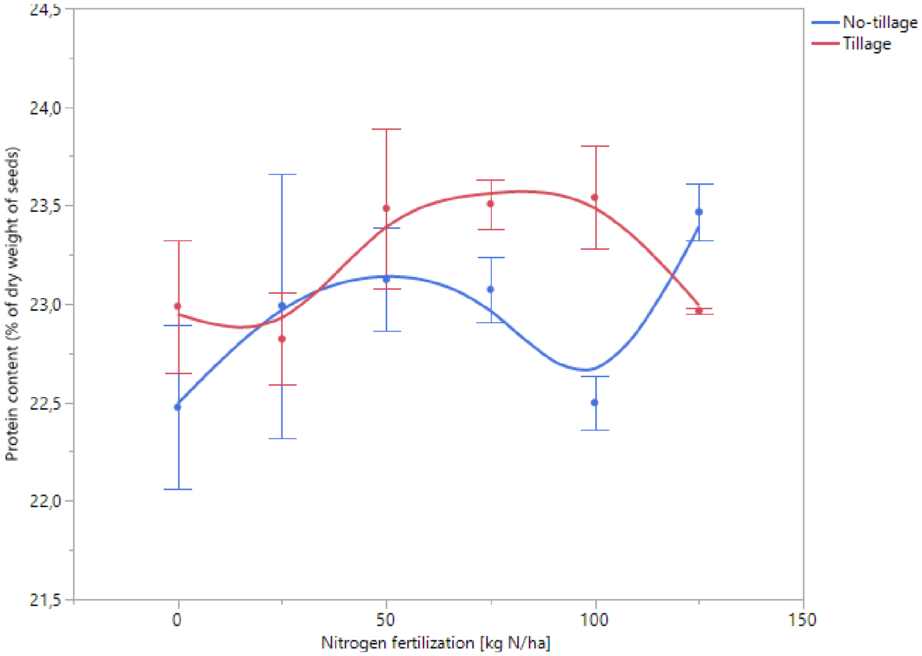


Figure 32: Crude protein content of flaxseed grown under tillage and no-tillage vs. Nitrogen fertilization

Chapter V: Discussion

1. Context of the experiment

As flaxseed was grown in only one field during one season, the results are specific to the characteristics of this field as well as to the climatic conditions of this season. Lafond *et al.* (2008) pointed out the importance of the soil type and climatic conditions on flax performance. They carried out many experiments and noticed an extremely high variability between years and sites. In the present case, it is therefore difficult to draw general conclusions.

The climatic conditions of spring and summer 2018 were very unusual in Belgium (Table 13), which had large impacts on all crops (Météo Belgique, 2019). March was cooler than the average and had lower solar radiation. The weather was too cold in March to secure the development of flax. On top of that, March was very rainy, which is why the right time for seeding was not found before early April. The temperatures in April and mostly in May were a lot higher than usual. Regarding the precipitations, they were less frequent and less intense compared to Belgian average. Globally, spring 2018 was exceptionally warm and dry. This trend went on during summer 2018, which was hit by one of the longest and hardest heatwave that Belgium has ever known, and it had dramatic consequences on many crops. The frequency as well as the intensity of precipitations were exceptionally low, and the solar radiation was one of the highest recorded over the last 30 years. During spring and summer 2018, 285.4 mm of precipitation fell in Belgium (Météo Belgique, 2019) whereas flax needs are estimated around 400-450 mm (Klein *et al.*, 2017). This shows that flax plants suffered from an important water shortage that probably caused many damages.

Table 13: Summary of the weather conditions of spring and summer 2018 (Source: Météo en Belgique, 2019)

Data	Spring 2018	Mean spring	Summer 2018	Mean summer
Temperature (°C)	11.6	10.1	19.8	17.5
Insolation (hours)	507.6	463.9	629.7	578.3
Rainfall (mm)	150.7	187.8	134.7	224.6
Precipitation (days)	42	49.0	20	43.9

Regarding the soil, characteristics were mostly normal for Wallonia. The pH of the soil was around 6, the organic carbon content was around 10-11 g/kg and the humus content around 2.0%. However, the mineral nitrogen content was about 45-60 kg/ha, which is exceptionally high, as mentioned previously. It means that, even if plants received a low nitrogen dose, they did not experience a lack of nitrogen. Plants were also rapidly saturated with nitrogen. The soil analysis results were unfortunately made available too late to allow us finding another experimental field.

2. Soil analyses

The pre-harvest soil analyses revealed that the soil nitrogen content was significantly higher under no-tillage (60 vs. 45 kg N/ha). This could be due to a lower loss of nitrate through leaching in the no-tilled field, as suggested by Soane *et al*. (2012). As this part of the field has been under no-till conditions for more than 10 years, it could confirm the higher long-term nitrogen sequestration in the soil. However, the long-term history of the two parts of the field is unknown. These two parts might differ from each other in more points than only in the tillage practice. As they belonged to different farmers, different inputs and cultivation methods might have been applied and might still influence the current soil composition. The difference in soil composition between the two parts, which was highlighted in the soil analyses report, clearly indicates that differences registered between the two parts cannot be totally attributed to the tillage itself. Many factors other than tillage could be responsible for the different outputs in the two parts of the field.

The post-harvest soil analyses did not conclude a significant difference in soil nitrogen content between both parts of the field. It is likely that this difference was hidden by the high standard error. In view of Figure 10, it is probable that an experimental error was made for in the no-tilled part of the field. Both effects of nitrogen fertilization level and tillage practice might be masked by this error. Dohat *et al.* (2017) stated that nitrogen fertilization level significantly influences soil nitrogen status after harvest. What was found for the tilled part confirms this statement. Indeed, a higher post-harvest soil nitrogen content under higher fertilization levels was observed (Figure 10).

The increase of the soil dry matter content can be attributed to the dry climatic conditions (Table 7 and Table 8). The lower increase in the no-tilled part supports what Pittelkow *et al*., (2015) said regarding the better water use efficiency of no-tilled systems. However, it must be kept in mind that other factors such as the difference in soil composition might also play a role.

The NUE_{uptake} was rather low in this experiment (63.7% under no-tillage and 68.0% under tillage). Evans *et al*. (2016) mentioned that a NUE_{uptake} below 60-70% (for the same NUE formula) can be considered as low, depending on the crop type. A low NUE_{uptake} indicates that the quantity of nitrogen applied was higher than the quantity that was removed. It suggests an overuse of fertilizers or a problem with the crop health. In this case, the initial soil nitrogen was already high, and the fertilization was excessive. The drought may also have prevented flax from growing as much as usual and therefore the crop used less nitrogen than normal. As the maturity was quickly reached, plants grew less and for a shorter period, resulting in a lower nitrogen uptake. This could be related to the low seed yield observed for both parts. As discussed in the section 3.1.4, seed yield was about 75% lower than the expected yield.

The higher NUE under tillage observed for the four NUEs might be explained by the lower initial soil nitrogen in the tilled part of the field. The trends of the NUE $_{\text{uptake, agronomic and agronomic oil}}$ seem coherent but the trend for the NUE $_{\text{utilization}}$ does not. The observed tendency of the NUE $_{\text{uptake, agronomic and agronomic oil}}$ to decrease with respect to the nitrogen fertilization level is due to the fact that the more nitrogen the plant received, the more it was in excess and the less efficiently the plant used it. Thus, an increasing amount of nitrogen was kept in the soil. The strange curves of the NUE $_{\text{utilization}}$ can be explained by the components of its formula. Indeed, the NUE $_{\text{utilization}}$ is calculated based on the seed weight and the nitrogen in the seeds. It will be explained below that the seed weight as well as the seed nitrogen content results were inconclusive. The bad performance of these two parameters affected the NUE $_{\text{utilization}}$. This also explains the very high standard error observed on Figure 7. No conclusion could be drawn from this graph.

3. Analysis of the agronomical parameters

3.1 Effect of the nitrogen fertilization level on yield parameters

3.1.1 Plant density

The average density of plants (403 and 352 plants/m² for the tilled and the no-tilled parts, respectively) was included in the upper part of the recommended range mentioned by Grant *et al.* (2016) that are 300 to 400 plants/m². The relatively high density obtained here might result from the late seeding date, as mentioned by Lafond *et al.* (2008) or from favorable environmental conditions during the germination period.

Plant density is an important factor affecting yields. On the one hand, a high plant density gives more seeds, increases the competitivity with weeds and can advance maturity. On the other hand, if the density is too high, it reduces seed size and might alter seed quality. Plants also overshadow when seeded at high densities, which decreases the leaf area efficiency.

The nitrogen fertilization level did not significantly impact on the plant density in this experiment. Some studies found significant effects of nitrogen fertilization on plant density, such as Grant *et al.* (2016) but the extent of this effect depends heavily on the growing season. However, Lafond *et al*. (1993) found the effect of nitrogen inconsistent with respect to the plant density. Thus, the results obtained in the present experiment are plausible, but it is likely that the high initial soil nitrogen content, the exceptional climatic conditions of spring and summer 2018 and the high variability of the field experiment introduced bias to the results. When one considers the Figure 8, the different values seem extremely scattered, which is confirmed by the relatively high mean square error.

3.1.2 Plant height

The height of plants was consistent with what was found in the literature. First of all, Omegalin is considered as a tall variety. Terres Inovia (2017) stated that this variety is around 49 cm high when mature. The experiment yielded higher averages: 59.98 cm for the tilled conditions and 58.27 cm for the no-tilled conditions. The high nitrogen availability could be responsible for the taller plants. Other factors might affect the plant height such as the row spacing (Lafond *et al.*, 1993) but the latter were controlled in this experiment. It was also reported that water deficiency can give taller plants (Pudelko *et al.*, 2015), especially in case of no weeding. In this experiment, plants suffered from an extremely dry period, which potentially stimulated further plant growth.

The positive response of flax height to the nitrogen fertilization level for the tillage conditions makes sense. A high nitrogen availability allows a higher biomass growth rate, resulting in taller plants. For the no-tilled conditions, the nitrogen fertilization level did not significantly influence the height of flax plants. The soil preparation is not supposed to affect the response of plants to the nitrogen fertilization level. This different response suggests an experimental error. It is also possible that the higher initial soil nitrogen in the no-tilled part made the plants more quickly saturated with nitrogen.

3.1.3 Plant maturity

While setting up the trial, it was expected that the more nitrogen the plants will receive, the slower they will reach the maturity stage. According to Dordas (2010), increasing the nitrogen fertilization level delays the flowering period and extends the seed filling period. Physiologically, a nutrient shortage usually reduces the vegetative growth of plants so that plants devote their resources to complete their cycle and hence, to flower. The trend on the figure 13 confirms this tendency, even though it is not statistically significant. The high-nitrogen treated plants seemed to have a delayed maturity whereas the low-nitrogen treated plants seemed have an advanced maturity. However, no reliable conclusion could be drawn.

Even though nitrogen seems to be an important factor affecting the maturity stage, the plant density also plays a role (Dordas, 2010). Denser plants usually flower for a shorter period and reach maturity faster (Lafond, 1993). This could be attributed to the competition for light, space and nutrient that stimulates flowering. In the Figure 13, plants under tillage are slightly, but not significantly more mature than plants under no-tillage. Plants under tillage were also significantly denser (Figure 11), which could confirm the relationship between nitrogen inputs and the maturity stage.

In this experiment, flax completed its full cycle in 107 days (between 17 April and 2 August) whereas a typical spring flax cycle lasts about 140 days. Such a short cycle illustrates how plants were affected by the drought. Most importantly, it shows that flax reached the maturity stage earlier than usual. The maturity stage of flax plants was therefore heavily impacted by many different factors, which could explains the non-significance of the data. The collection of this kind of data is also very subjective since it relies on the personal perception of the color and the maturity stage of the plants, based on a personal scale.

Finally, the maturity stage can be related to the seed yield. Indeed, the seed filling period of flax is very critical for the yield determination and usually happens in June and July, which is a very dry and hot period. Advancing the flowering date would provide more favorable conditions for the seed filling (i.e. cooler temperatures and higher humidity) and would also extend the seed filling period. Thus, plants reaching maturity faster are likely to undergo a better seed filling and hence, to provide higher seed yields. It is another way in which the nitrogen fertilization level is supposed to increase the yield.

3.1.4 Estimation of seed and oil yield

The average yields of seeds in the experiment were 14.38 q/ha for flax under no-tillage and of 15.29 q/ha for flax under tillage. This is consistent with usual yields for flax. In a peer-review, Klein *et al.* (2017) reported that farm yields ranged from 9.4 to 21.13 q/ha. Terres Inova (2017) mentioned that yields are often about 20 q/ha. The lower results here could be largely attributed to the dry climatic conditions. Dordas (2010) compared two growing seasons and found that dry climatic conditions dramatically affected flaxseed yields, as flaxseed is very susceptible to water shortages. It is important to mention that the yield of flax is far below that of other oilseed crops grown in Europe. For instance, oilseed rape yield is about 43-52 q/ha and sunflower yield is about 36-53 q/ha (Klein *et al.*, 2017).

The oil yield was estimated by the quantity of lipids extracted from the seed powder by the Soxhlet method. It averaged 739.9 kg/ha for the tilled conditions and 693.7 kg/ha for the no-tilled conditions. These values are included in the varietal range of oil yields mentioned by Klein *et al.* (2017), which was 570-1060 kg of oil/ha. Not surprisingly, the curves for oil yields followed a similar pattern to those for seed yields (Figure 14 and 15).

The nitrogen fertilization was supposed to affect yield by altering different yield components: number of capsules per plant, number of seeds per plant, length of the seed filling period, seed growth rate and seed weight (Dordas, 2010). However, in the present work, the nitrogen fertilization level did not significantly influence the seed yield and the oil yield. No clear trend was detected on Figures 14 and 15 and the variability seemed very high. The effect of nitrogen fertilization level was likely hidden by the high amounts of residual nitrogen in the soil. There was enough nitrogen available in the soil for every plant to grow and the threshold for the nitrogen toxicity was rapidly reached. The dry climatic conditions might also have played a role in hiding the nitrogen fertilization effect. Many other problems may have interfered with the effect of the nitrogen fertilization level on seed yield, such as nutrients shortages, weed proliferations or insect attacks, but as they were controlled in this trial, they are very unlikely.

However, the possibility that the nitrogen fertilization level did not influence flaxseed yield should not be totally excluded but remains less likely. Even though most studies claim that the nitrogen availability is one of the most important factors affecting crop yields in general, there are some studies that contest this statement, such as Andruszczak *et al.* (2016) in the specific case of flaxseed. Hocking *et al*. (1997) notably concluded that there was no significant effect of the increasing nitrogen doses on flaxseed yields. It is possible that nitrogen inputs influenced plant biomass but not so much flaxseed yield. In the present case, plants grown under tillage were higher when a larger nitrogen dose was applied, reflecting the larger quantity of biomass synthetized under high nitrogen doses. Dordas also (2010) confirmed that flax was less respondent to additional nitrogen than most common crops such as wheat, barley or rape. However, as most results go in the other direction and as the effect of nitrogen on crop yield is widely recognized, the results obtained here should be taken with caution. The variability of the results was too high, and the environmental and agronomic conditions were not appropriate to give reliable results.

Some studies tried to determine an optimal fertilization level for flax. Herzorg *et al.* (2017) obtained the highest yield (21q/ha) by fertilizing 103 kg N/ha. When considering the price of the fertilizer and the remuneration, the economic optimum was going down to 63 kg N/ha. According to Klein *et al*. (2017), the best fertilization level for both yield and quality was about 110 kg/ha. However, the residual soil nitrogen was not taken into account in these calculations. In the present study, as the yield was not affected by the fertilization level, the curve of Figure 14 and 15 did not allow to determine an optimal fertilization level. Anyway, the optimal nitrogen application of flax is much lower than that of most common crops such as oilseed rape, which is beneficial for both the farmers and the environment (Klein *et al*., 2017).

3.2 Effect of tillage on yield

3.2.1 Effect of tillage on plant density

The lower plant density observed in the no-tilled part compared with the tilled part might reflect lower germination or survival rates. Soane *et al*. (2012) listed several crop establishment problems during very dry periods, which could have happened here. The lower plant density could also be explained by an improper soil preparation of the no-tilled part. Indeed, it was observed that the no-tilled soil contained more clods than the tilled soil. These clods may have prevented a proper seed germination. The loose contact between the soil and the seed due to these clods is not favorable to the seed development. In addition, flaxseed is relatively small, which makes the soil preparation even more important. Small seeds only contains limited reserves and therefore rely more on the structure and the quality of the soil than large seeds. Clods can also prevent young seedlings from reaching the surface. It is also possible that the larger weed establishment in the no-tilled part impeded flax growth.

3.2.2 Effect of tillage on plant height

Plants were significantly taller under tillage conditions. This is likely due to differences in soil compositions between the two parts of the field, as the tillage practice is usually not reported to affect plant height. It could also be due to the larger weed invasion in the no-tilled part of the field. More weeds compete more with flax plants, which could result in shorter plants. This underlines the presence of other factors than the tillage practices influencing the present results.

3.2.3 Effect of tillage on plant maturity

Tillage did not significantly influence the maturity process of flax plants in the present experiment (Figure 13). This suggests that external conditions such as climatic parameters may have more impacts on flax maturity time than factors such as soil composition or soil tillage.

3.2.4 Effect of tillage on yield

Most crops undergo a yield reduction when stopping tillage, usually of a magnitude about 5% (Soane *et al*., 2012). However, the effect of tillage on flax yield remains highly contested in the literature. Grant *et al.* (2009) did not report a significant effect of tillage on flax yield. On the other hand, some studies noticed an increase of the average flax yield under zero-tillage. Lafond *et al.* (1992) reported a yield of flaxseed 23% higher under zero-tillage than under conventional tillage. Halde *et al.* (2014) also reported a significantly higher yield under no-tillage, depending on the place where flax is grown and the weather conditions.

Regarding the height, the density and the yield of flax plants in this experiment, it seemed that flax under tillage globally gave slightly higher results than flax under no-tillage. Even if some curves were intersecting, the red curve (illustrating flax grown under tillage) was most of the time above the blue curve (illustrating flax under no-tillage). However, this trend was only significant for the plant density.

One of the possible reasons for the tendency of flax to have slightly higher yields under tillage is that the no-tilled part of the field seemed more invaded by weeds than the tilled part. Weed controls are known to be more challenging under no-tillage. In addition, as plants were significantly less dense under no-tillage, a lower seed yield would not be surprising, even though plants usually compensate when they are less dense, which softens the yield reduction. As mentioned previously, the poor soil preparation under no-tillage is very likely to have had a strong impact on plant density and on yield.

One could have expected that the dry weather conditions of spring and summer 2018 would have reversed this tendency of flax to have higher yields under tillage. Indeed, many crops including flaxseed are reported to have higher yields under no-tillage when exposed to unfavorable climatic conditions (Pudelko *et al.*, 2015). This can be explained by the greater soil moisture balance under no-tillage. This better water balance should have resulted in a higher emergence rate and density of plants, a higher growth rate and a better seed filling. This hypothesis was not verified in this trial. It must not be forgotten that many other factors that have nothing to do with the tillage practices might have differed between the tilled and the no-tilled parts.

Anyway, as no significant difference was observed between yields under tillage and no-tillage, it could encourage farmers to grow flaxseed under no-tillage. Moreover, lower yields could probably be tolerated if the reduction of the production costs associated with tillage compensate it. Lately, no-tillage practice has become economically more attractive due to the relative costs of fuel and herbicides (Soane *et al.*, 2012). The costs of herbicides have considerably decreased whereas those of fuel will probably continue to increase over time.

4. Effect on quality parameters

4.1 Effect of nitrogen fertilization level on quality parameters

4.1.1 Effect of nitrogen on the lipid content

The lipid content of flaxseed varied here between 47.33 and 49.73%. These values are among the highest found in the literature. Indeed, the oil content of flaxseed usually ranges from 33 to 48% (Herzorg *et al.,* 2017; Luginbulh *et al*., 2015; Dohat *et al.,* 2017; Klein *et al.,* 2017). This oil content is known to vary a lot with variety, site and years. The high lipid content obtained here could be partly explained by the variety, since Omegalin is renowned for its high oil content. Moreover, the Soxhlet method tends to overestimate the oil content, as it extracts all the compounds that are soluble in diethyl ether, which means more than what may be extracted in a classical process of flaxseed oil extraction.

In the present trial, low oil yields were more expected than high oil yields. Indeed, dry years usually result in much lower oil contents than moist years (Pudelko *et al*., 2015; Klein *et al*.; 2017). Dohat *et al.* (2017) also pointed out that irrigation level and hence water availability positively influenced many quality traits of flaxseed such as oil and protein contents. Furthermore, late seedings (which happened in this trial because of the unfavorable spring climatic conditions) are reported to decrease the oil content of seeds (Lafond *et al*., 2008). Finally, high nitrogen inputs are reported to reduce the oil content (Klein *et al*., 2017).

The curve of Figure 15 gives the impression that oil yield is relatively stable, regardless of the tillage and the nitrogen treatments. In many studies, the oil content decreases with nitrogen application while the protein content increases (Grant *et al*. 2016; Dohat *et al.* 2017; Klein *et al.* 2017). However, some studies such as Herzorg *et al*. (2017), did not observe this relationship between nitrogen inputs and oil content. Andruszczak *et al.* (2015) also found that seed protein and seed oil contents were only dependent on the cultivar and not on agronomic factors such as nitrogen application. Even though the majority of studies conclude a significant effect of nitrogen on oil content, care has to be taken in drawing general conclusions. Indeed, the effects might be largely dependent on field or climatic conditions that can be very different between the studies and that make the comparison challenging. More numerous studies including many years, sites, cultivars, seeding rates and other agronomical practices are required to clarify this question.

The management of the flax cultivation should be guided by its application. A high content in oil is usually desired to improve oil extraction whereas a high protein content is better for livestock meals.

4.1.2 Effect of nitrogen on the fatty acid profile

According to Grant *et al*. (2016) and Herzorg *et al*. (2017), oleic acid is supposed to increase when the nitrogen inputs increase, at the expense of alpha-linolenic acid that decreases. Linoleic acid also seems to be negatively correlated to oleic acid and hence decreases with the increasing nitrogen fertilization. The negative correlation between oleic acid and both linoleic and alpha-linolenic acids makes sense since the former is an intermediate in the synthesis of the latter (Grant *et al*., 2016). The action of two desaturases is required to transform oleic acid into alpha-linolenic acid. The higher nitrogen inputs may result in a lower activity of these desaturases, reducing the conversion of oleic acid into alpha-linolenic acid. In addition to that, palmitic acid content usually decreases with the increasing nitrogen application. Other fatty acids are not reported to undergo any significant change with respect to the nitrogen fertilization.

In the present study, the nitrogen input did not significantly influence any fatty acid proportion, which is in contrast with the data in the literature. The high residual nitrogen present in the soil at the start of the trial may be responsible for this. The plants were probably already saturated with nitrogen. The low water availability resulting from the dry climatic conditions may also have played a role. It might have decreased the absorption of nutrients and influenced the composition of flaxseed. Thus, no conclusion can be drawn regarding the effect of nitrogen fertilization level on the fatty acid profile of flaxseed in the local context of Belgium.

The content of alpha-linolenic acid is reported to range from 47.7% to 55.5% and 50% is ideal to produce oil for human consumption (Klein *et al*., 2017). Here, it ranged from 60.25% and 64.83%, which seems high. However, it is not abnormal, as Andruszack *et al*. (2016) also obtained values of alpha-linolenic acid close to those proportions (61.1-62.9%). Excessive proportions of that fatty acid are not desired because of its high susceptibility to oxidation, leading to oil rancidity (Klein *et al*., 2017).

Linoleic acid accounted for 12-13% of total fatty acids in this study whereas it was estimated at 15-20% in most of the studies, for many different flax varieties (Klein *et al*. (2017); Morris (2007); Kajla *et al*. (2015); Carraro *et al*. (2012)). Oleic acid is usually reported to be around 18% (Morris, (2007); Zuk *et al*., (2015), Kajla *et al*., (2015)) of total fatty acids whereas it was around 13-14% in this work.

Overall, the alpha-linolenic acid content was higher in this study than in several other studies and both linoleic acid and oleic acid contents were lower. An impact of the variety should not be excluded to explain these differences. In addition, some environmental factors might have influenced the activity of the desaturases responsible for the synthesis of alpha-linolenic acid.

Based on the fatty acid profile, one can calculate the omega-6/omega-3 ratio. In this experiment, the ratio is 0.2:1. Morris (2007) obtained a ratio of 0.3:1, which is close to our finding. The same author compared the ratio in flax oil to that of corn oil (58:1), soybean oil (7:1) and canola oil (2:1) to support that flax is a great dietary source of omega-3 fatty acids. Thus, including flax in the diet helps to increase the omega-3 intake and hence, the omega-3 content in the tissues and the blood.

4.1.3 Effect of nitrogen on the vitamin E content

The content in antioxidants of flaxseed depends on many factors: the variety, the storage, the environmental conditions and the processing methods. Only the vitamin E content was analyzed in this work. As a reminder, it is among the most important antioxidant in flaxseed, with lignans, phenolic compounds and flavonoids.

The average tocopherol content was 289.1 and 295.8 mg of tocopherol/kg of oil (ppm) for the tilled and no-tilled conditions, respectively. These results are in the lower part of the range compared with the results found in the literature. Indeed, tocopherol content is reported to range from 154 to 934 mg/kg of oil (Obranovic *et al.*, 2015) with an average of 522 mg/kg of oil. The large range results from the variation between sites, growing environmental conditions, varieties and extraction and determination methods (Oomah *et al*., 1997). The flax oil storage also appears to have an impact. In fact, the tocopherol content of the specific variety used in this trial (i.e. Omegalin) was not known and it is possible that the tocopherol content of this variety was a bit different from that of other varieties.

The nitrogen fertilization did not influence the tocopherol content in the present experiment, which is in opposition with the results of Klein *et al*. (2017). According to them, tocopherol content heavily reduces when nitrogen application increases. However, their experiment was performed in pots with only four varieties. In the present experiment, external conditions such as soil composition and climatic conditions might have hid a potential nitrogen effect. The variety used here might also have a more stable tocopherol content. Klein *et al*. (2017) also report that tocopherol content is positively correlated with the oil content, which was not noticed in the present experiment.

Gamma-tocopherol was the dominant form, representing 97.75% in tilled conditions and 97.80% in no-tilled conditions of the total tocopherol amount. According to Oomah *et al.* (1997), gamma-tocopherol usually represents 96-98% of total tocopherol, which is perfectly in agreement with our findings. The proportions of the four forms of tocopherol were very stable regardless of all treatments.

4.1.4 Effect of nitrogen on the crude protein content

The crude protein content of flaxseed was on average 23.22% of the seed's dry matter under tillage and 22.92% under no-tillage, which is very similar. This is in agreement with the protein content described by Bekhit *et al*. (2018) that mentioned 20-30%. It should be noted that this is based on the crude protein analysis, which means that the non-protein nitrogen is also included. As flaxseed is reported to contain quite high amounts of non-protein nitrogen, the actual protein content is probably a little lower than 20% (Bekhit *et al*., 2018).

As nitrogen is a constituent of proteins, protein content was expected to increase with the fertilization level (Grant *et al*., 2016). The protein content was also supposed to be inversely proportional to the oil content of the seed. This negative relationship results from the allocation of the resources of the plant. However, the present results were not in agreement with this theory. Neither tillage nor nitrogen fertilization level impacted on crude protein content. Dohat *et al.* (2017) pointed out that irrigation level and hence water availability, influenced many quality traits of flax such as oil and protein contents. Thus, the unusual climatic conditions combined with the high residual nitrogen in the soil might have hidden the effect of the nitrogen fertilization level. It is also possible that specific environmental conditions encountered in the present trial stimulated a higher allocation of the nitrogen resources to other parts of the plant.

4.2 Effect of tillage on the quality parameters

The effect of tillage on the quality parameters of flaxseed have not been investigated in the literature so far. In this trial, some trends can be observed but cannot be generalized as the tillage treatment was not repeated and as many other factors in the experimental field might have influenced the results. As there was no repetition, it is not possible to determine whether the trend is due to the tillage itself or to other components.

4.2.1 Effect of tillage on the lipid content

The average content in the extracted lipids from the seeds was 48.4% for flax under tillage and 48.2% for flax under no-tillage. The two curves of Figure 16 are close and overlapping, indicating that there was no effect of tillage on the lipid content of flaxseed.

4.2.2 Effect of tillage on the fatty acid profile

Some fatty acid proportions were significantly influenced by the tillage. On the one hand, tilled conditions gave more stearic acid, oleic acid and vaccenic acid than no-tilled conditions. On the other hand, tilled conditions led to less alpha-linolenic acid than its no-tilled counterpart. Tillage had no significant effect on palmitic acid, linoleic acid and docosanoic acid levels. One can notice that saturated and monounsaturated fatty acids were more represented in the tilled field whereas polyunsaturated fatty acids tended to be more represented in the no-tilled field (Figure 28). Even though they were significant, the differences obtained were tiny and probably not influential. However, owing to the beneficial effects on health of polyunsaturated fatty, it is interesting to notice that flax grown under no-tillage conditions could be of higher quality that that grown under tillage. This difference could be due to a factor present in the soil that might have altered the global metabolic pathway of fatty acids in the plant. Many enzymes are involved in the synthesis of fatty acids in plants, including several elongases and desaturases (Thelen and Ohlrogge, 2002). Their activity might be increased or decreased by some components that differed in the two parts of the field such as the moisture content, the temperature or the nitrogen content. In the present case, as the proportion of alpha-linolenic acid was higher, it is likely that two desaturases involved in the conversion of oleic acid into alpha-linolenic acid were promoted. Further research is necessary to understand this phenomenon.

4.2.3 Effect of tillage on the vitamin E content

The tillage did not influence the vitamin E content at all. This suggests that the tocopherol content as well as the proportion of its different forms are rather stable, regardless of any external conditions.

4.2.4 Effect of tillage on the crude protein content

Tillage did not influence protein content in this experiment. An effect of tillage could have been expected due to the difference in the nitrogen content in the two parts of the field before the experiment.

Conclusion and perspectives

This master's thesis investigated the effect of nitrogen fertilization and tillage on yield and nutritional profile of flaxseed. On the one hand, the demand for flaxseed has recently increased due to its numerous health benefits. Even though Belgium is the largest importer in the world, the local production of oilseed flax remains very low. Boosting Belgian flax production would be to its advantage. On the other hand, an increasing popularity of reduced-tillage systems and sustainable fertilization practices is widely observed. A better input management is subject to many researches and nitrogen fertilization is among the most important factors influencing yields. However, limited research focuses on the influence of such parameters on flax nutritional profile.

In spring and summer 2018, the Omegalin cultivar of flax was grown under tilled and no-tilled conditions with six different doses of nitrogen ranging from 0 to 125 kg N/ha, in Vieux-Sart (Belgium). After harvest, yield components were assessed and seeds were analyzed to figure out their partial nutritional profile with respect to the different treatments. It is important to point out that the climatic conditions were a lot drier and hotter than usual, which impacted on the results. In addition to that, the soil where flax was grown was exceptionally rich in nitrogen, which probably biased the results.

Flax requires less nitrogen inputs than most common crops, which is economically attractive and environmentally beneficial. The present work supports that nitrogen fertilization impacts on plant height under tillage but does not impact on plant density, plant maturity time or seed yield under any tillage condition. However, most studies found that flax yield increases with the increasing nitrogen fertilization level to some extent. In the present case, the effect of the nitrogen fertilization level might have been hidden by the high initial residual nitrogen in the soil. The possibility that nitrogen influences more flax plant biomass than seed yield is not excluded but remains less likely. Flax yield was about 15 q/ha, which is lower than the average yield of 20 q/ha in the area, possibly due to unfavorable external conditions.

In terms of nutritional profile, the nitrogen inputs did not influence lipid content, fatty acid profile and vitamin E and crude protein contents of flaxseed. As most studies head in the opposite direction, care must be taken when drawing conclusion with the latter results. Indeed, the increasing dose of nitrogen fertilization was expected to increase the protein content and decrease the oil content. It should also have affected the fatty acid profile: oleic acid is believed to increase at the expense of alpha-linolenic acid and linoleic acid when increasing doses of nitrogen fertilization are applied. All this was not verified in the present experiment. Some parameters such as variety, agronomic factors and climatic conditions may play a large part in flax composition and hide the effect of the nitrogen fertilization level.

Regarding the yield, tillage had no influence in this trial. Even though no solid conclusion could be drawn due to the fact that tillage was not repeated, this could stimulate farmers to move to reduced-tillage. Low yields are an important obstacle preventing farmers from growing certain crops or trying certain practices. The literature supports that flax responds well to no-tillage and that yields do not excessively decrease in these conditions. However, weed management turned out to be more difficult without tillage, which is negative considering that flax is already poorly competitive with weeds. Improving weed management under no-tillage and without excessive herbicide application is an important challenge for the agriculture of tomorrow.

According to the present trial, changes in tillage systems are not likely to influence lipid, protein or vitamin E contents of flaxseed. However, it seems that it significantly but very slightly impacted on the fatty acid profile. Indeed, saturated and monounsaturated fatty acids tended to be more represented in tilled conditions whereas polyunsaturated fatty acids were found in larger quantities in no-tilled conditions. As polyunsaturated fatty acids are recognized for their health benefits, it seems that this slight advantage adds up to the long list of no-tillage benefits. This finding requires further investigations to confirm the trend and widen the scope of possibilities, but also to understand what mechanisms are behind these changes. The activity of some enzymes such as elongases and desaturases may be affected by some components in the soil and this could result in a change in the fatty acid profile. However, the difference in the fatty acid profile of flaxseed might result from different characteristics between both parts of the field and not from the tillage practice itself. Anyway, it proves that soil quality may influence food product quality and suggests that more importance should be given to agricultural practices. If tillage is really responsible for this positive change in the fatty acid profile, it could be a possible alternative route to improve food quality while maintaining the health of the land and environment.

The present study suggests that oilseed spring flax and more precisely the Omegalin variety, is relatively well adapted to Belgian climatic conditions. As it is likely that the exceptional climatic conditions affected both yield and quality parameters of flaxseed, it would be interesting to conduct further research on this topic. Flax grown in this study contained a high oil content (about 48%) and a high proportion of alpha-linolenic acid (about 62% of the oil). Such high amounts of omega-3 fatty acids make flaxseed an excellent source of omega-3 fatty acids that could reverse the high omega-6/omega-3 ratio in the Western diet. Furthermore, its high protein content makes flax an interesting and sustainable vegetal source of proteins. The demand for flax is likely to keep increasing in the coming years, which should encourage farmers to grow it. Hopefully, the latter will be convinced by the convenience of growing flax as well as by its agronomical and environmental benefits. In this way, Belgian flax could replace imported flax on the local market. The low nitrogen requirements also suit a sustainable agriculture. As organic farming aims at reducing inputs, flaxseed could be a good candidate for organic crops in Belgium.

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Evaluation of the effect of nitrogen fertilization and tillage on the yield and the nutritional profile of flaxseed

Master's thesis presented by Louise Brison

Oilseed flax is not commonly grown in Belgium, despite its various advantages and its increasing demand. From an agronomic perspective, it is an interesting plant to insert in the crop rotation and it is relatively easy to grow. From a nutritional point of view, it is rich in omega-3 fatty acids, essential amino acids, dietary fibers and antioxidants such as lignans. This makes flaxseed a functional food, that is a foodstuff that prevents many chronic diseases and provides numerous health benefits.

More documentation regarding agronomical practices of flax cultivation and their effects on yield as well as on the nutritional profile of the seeds is needed. The nitrogen input is believed to be an important driver determining yield and quality parameters such as oil yield, fatty acid profile, vitamin E and protein contents of flaxseed. As more and more farmers move to reduced-tillage practices, it would be interesting to determine the effect of such practices on flax yield and quality.

The aim of this master's thesis was therefore to determine the effect of nitrogen fertilization level and tillage practice on the yield of flaxseed, but also on its nutritional profile. To this end, flax was grown in a field in Vieux-Sart (Belgium) with six different levels of nitrogen and either under tillage or no-tillage conditions. After harvest, seeds were analyzed to determine oil yield, fatty acid profile, vitamin E profile and protein content.

Many results were not significant, which can be partly attributed to the exceptionally dry and hot climatic conditions of spring and summer 2018 and to the high residual soil nitrogen content before the onset of the experiment.

On the one hand, nitrogen fertilization level positively influenced flax height under tillage, but did not influence flax yield, contrary to what had been expected. Neither did it influence oil content, fatty acid profile, vitamin E profile or protein content.

On the other hand, plant density was higher under tillage but yields were not significantly greater. This could encourage farmers to grow flax under no-tillage. Tillage had no effect on oil content or vitamin E content but significantly influenced the fatty acid profile, even though the effect was tiny.

From this research, it emerges that more work about the effect of soil nitrogen on flax yield and quality is needed to properly manage nitrogen fertilization. Tillage also seems to affect different yield and quality parameters, which is of interest for improving flax nutritional potential. More studies on the effect of tillage are however needed to draw more reliable conclusions.

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