# Cover page

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# Semi-field experiment assessing the influence of compost and nitrogen fertilizer on plant growth and soil microbial life

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Master thesis

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# Semi-field experiment assessing the influence of compost and nitrogen fertilizer on plant growth and soil microbial life

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# CONTENTS

# LIST OF ABRIVIATIONS

| | |
|---|---|
| β-GA | β-glucosidase activity |
| DAP | Days after planting |
| DHA | Dehydrogenase activity |
| C | Carbon |
| MBC | Microbial biomass carbon |
| N | Nitrogen |
| $NH_4^+$ | Ammonium |
| MBN | Microbial biomass nitrogen |
| $NO_3^-$ | Nitrate |
| NUE | Nitrogen use efficiency |
| MBP | Microbial biomass phosphate |
| SOM | Soil organic matter |

## ABSTRACT

A fertile soil is the foundation of sustainable and productive agriculture. The use of compost is seen as a possible way to maintain soil productivity. Application of nitrogen (N) is important for plant growth, but excess application of N can result in pollution of the environment. Composting is a solution for waste recycling, with sanitization and stabilization of the biomaterial. Compost application is a way of incorporating soil organic matter into the soil, which might be a possible measure for mitigating climate change.

The aim of this project is studying the response of plant growth, soil microbial life and N dynamics, with the application of the soil amendment compost and different doses of N fertilizer in a horticultural cropping system. For studying this a semi-field experiment was designed with mini-plots, which were tubs inserted into the field with lettuce plants. The two treatments were (1) soil of a farmer's field with focus on compost application and (2) different N fertilizer applications. Over the experimental period plant growth were monitored by plant area, root growth and harvest yield, assays of microbial activity and biomass were conducted, and N dynamics in soil and plants were measured.

The effect of the fertilizer treatment was generally absent. The soil treatment with compost gave lower plant growth, however it increased the soil microbial activity.

Generally, for the experiment there was found an effect of the soil treatment and less so an effect of the fertilizer treatment. A major struggle with the setup was that the long- and short-term compost effect could not be separated, and the use of field soils made it difficult to conclude anything of the use of compost due to other unknow differences between the two fields.

The effect of fertilizer on plant growth was absent, which might be due to termination before full growth and the late application of fertilizer. The soil treatment with compost showed a lower crop growth than without compost, the reduce growth could be due to either a lower N availability of the SC soil or the effect of compost due to immaturity.

The experiment shows an elevated microbial activity and microbial biomass in the soil treatment with compost application, which could indicate the rate of the increase of SOM was bigger in the SC soil and that it might be explained by a long-term compost effect.

# 1. INTRODUCTION

A fertile soil is important for plant production. During recent decades cultivation of soils has intensified, resulting in soil degradation with decreasing levels of soil organic matter (SOM) both worldwide (Johnston et al., 2009; Lal, 2018) and in the north European region (Heikkinen et al., 2013; Meersmans et al., 2009; Taghizadeh-Toosi et al., 2014; Wiesmeier et al., 2012). For vegetable production the cropping systems are generally even more intense than arable cropping systems with frequent and intensive tillage to prepare beds and manage weeds stimulating loss of SOM (Bajgai et al., 2014).

Nutrient supply is vital for plant growth. The quantitatively most important plant nutrient nitrogen (N) is also a major source of pollution for the environment (Sylvestre et al., 2019; Tei et al., 2020). Therefore minimum application of N and improved fertilizer management is important for a sustainable farming practice (Gustafson, 2012).

Soil texture is an important aspect for a productive soil, the. The contents of sand, silt and clay are mostly static, whereas the soil organic matter (SOM) content is very dependent on the practice used in field management (Diacono & Montemurro, 2011; Oelofse et al., 2015). The SOM plays an role in important soil functions and is central for the soil fertility (Kumar & Karthika, 2020). A possible practice for increasing the organic matter content in the soil is the use of compost (Debosz et al., 2002; Martínez-Blanco et al., 2013).

Compost is a good way to recycle biomass and nutrients. Recycling with compost creates a product well suited as soil amendment. During the composting process pathogens and weeds are killed by the heat generated when the process is taking place and the resulting product is stable and suitable for soil amendment (Martínez-Blanco et al., 2013).

In the global carbon cycle, soils play an essential role as a major storage of carbon as part of the carbon balance. With the amount of SOM decreasing this balance is pushed and carbon (C) is released to the air, and thereby impacting negatively on the greenhouse gas effect, with global climate change happening more rapidly (Lal, 2004). Therefore, carbon sequestration might play a role in mitigating climate change.

### 1.1 Aim and hypotheses

This project is about the study of the response of plant growth, soil microbial life and N dynamics, with the application of the soil amendment compost and different doses of N fertilizer in a horticultural cropping system. A goal of the project was to design a semi-field experimental setup, with the possibility of testing soils of different origin, control of soil applied treatments under field conditions with less use of resources in relation to area, demand of labor and time compared to regular field experiments. The setup was attempted to improve imitation of conditions in outdoor production in relation to agronomic parameters compared to pot trials in greenhouse experiments. In the experiment, treatments of soil with (SC) and with no compost application (SnC), and different doses of N fertilizer (4 levels) was applied to mini-plots with 4 repetitions, the setup is further explained in section 3. Observations were made on plant growth, soil enzyme activity, soil microbial biomass, N content of plant biomass and soil mineral N.

The hypothesis of the project was (1) that crop growth, above and below ground, is increased by application of compost and fertilizer; however, it is less affected by suboptimal fertilization, if soils have received long-term compost application compared to no compost application, due to improved resilience of the cropping system with improve soil fertility. (2) Optimal N fertilizer application can sustain an optimal yield and increase the N-fertilizer recovery efficiency, by which the risk of N leaching is minimized (3) Microbial activity is increased by adding compost to the soil. (4) Compost application increases microbial activity and biomass, which are acting as an early indicator for enhanced levels of SOM. (5) N availability affects microbial activity and biomass differently in soils with or without long-term application of compost.

In addition to the hypotheses selected aspects of the methodology of the experiment will be discussed as a potential method for testing soils of different origin with compost or other biological substances stimulating crop growth or composition of soil microbiology.

## 2. BACKGROUND

### 2.1 Soil and SOM

Soil is essential for all terrestrial ecosystems and the most complex biomaterial on the planet (Young & Crawford, 2004). Plant production relies on a good soil quality, which is a

combination of chemical, physical and biological properties. Soil texture and the composition of rock minerals is defining a soil and generally unchangeable. Other properties such as SOM content and soil structure, change from one management system to another (Schjønning et al., 2009). There is a concern that if the SOM content in soil decrease too much the production capacity of agriculture will decrease due to degradation of soil physical properties and by deterioration of soil nutrient cycling mechanisms (Loveland & Webb, 2003). This might partly be overcome by use of fertilizer; however, it has been shown that there is a positive relationship between soil organic carbon and yield (Oldfield et al., 2019).

SOM affects soil biological, physical and chemical properties. In agriculture and horticulture, SOM is considered important as it can contribute in a variety of ways to improve some of the factors influencing crop yield, which makes it important to keep a certain level of organic matter in the soil in order to maintain a healthy and productive soil (Johnston et al., 2009; Lal, 2004; Loveland & Webb, 2003). Oldfield et al. (2019) found in a meta-analysis on maize and wheat that yields up until a soil organic carbon content of 2 % there was a strong potential of increasing yields, above 2 % the increase in yields leveled off. SOM is considered to have a positive effect on several conditions influencing crop yield, e.g. by improving plant nutrition by binding and exchanging nutrients, improve soil structure and tilth by promoting aggregation and improved water holding capacity (Johnston et al., 2009; Loveland & Webb, 2003).

The most important components of SOM are humic substances, both quantitatively and qualitatively (Hayes & Clapp, 2001; Senesi & Plaza, 2007). Humic substances are dark colored, heterogenous organic compounds, mainly made up of humic and fulvic acids responsible of several of the soil functions and processes (Senesi & Plaza, 2007). Humic substances are formed in the process of humification, consisting of a complex decomposition and resynthesizes process, often defined as the stabilization of organic substances against biodegradation (Hayes & Clapp, 2001; Kögel-Knabner, 2002).

### *Soil Degradation*

Degradation of soil makes it less fertile, and can be caused by several different events (Diacono & Montemurro, 2011). E.g. soil erosion, compaction, acidification, runoff, crusting, soil organic matter loss, salinization, and nutrient depletion, accumulation of heavy metals or toxins (Kumar & Karthika, 2020).

Intensive soil management is common in modern agriculture and can have a strong negative impact on soils and SOM (Schjønning et al., 2009). In vegetable systems soil management is even more intensive than in arable cropping systems (Bajgai et al., 2014; Morgan et al., 2010). Sequential cropping is common for some vegetable crops grown outdoors in Denmark, which doubles or triples the potential microbial activity in the soil and disrupts soil structure, by the repeated management operations (HortiAdvice, 2015). Intensive tillage, e.g. operations with plough, soil rock separator and bed former, improves soil aeration and soil/crop residue contact, which stimulates the loss of SOM by enhancing microbial activity and mineralization (Guérif et al., 2001). Additionally destruction of soil aggregates by mechanical operations expose SOM for microbial decomposition, which was formerly physically protected (Six et al., 2000).

*Carbon sequestration*

Increase in SOM due to change in arable management is commonly termed carbon sequestration. This is a tool for mitigating climate change (Powlson et al., 2011).

In the global carbon cycle, soil serves as a major sink for carbon, see Figure 2.1. It is roughly estimated that carbon sequestration on land, soil and wetlands can count for $2-3$ Pg (Petagram) $\mathrm{C\ yr^{-1}}$, with about one third related to cropland. This can be achieved by the introduction of best management practice in relation to sequestering carbon in the soil, e.g. management practices reducing mineralization (minimum tillage), application of compost, crop rotations with perennial crops etc. (Lal, 2018; Le Quéré et al., 2018).

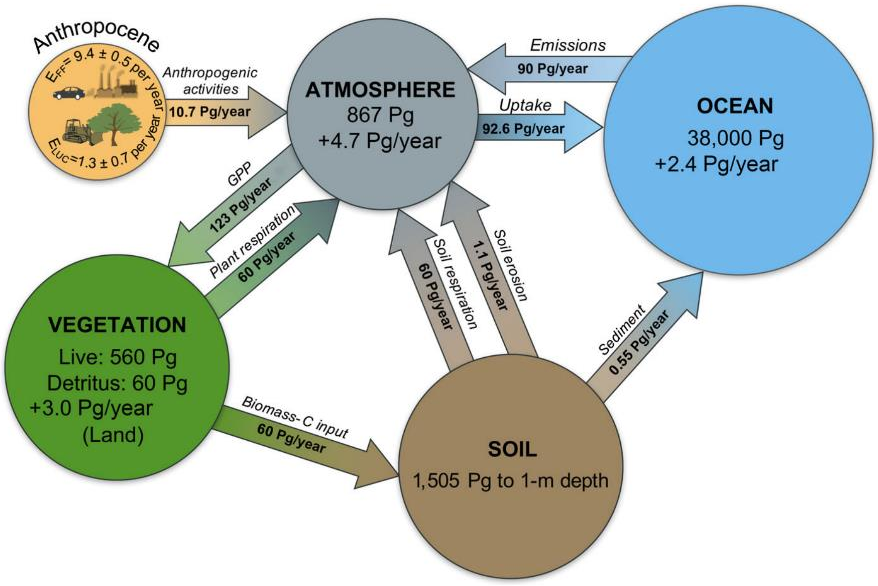


*Figure 2.1: The global carbon cycle with the major carbon pools and fluxes between them* (Lal, 2018)

The limitation of SOM capacity in soil is linked to the adsorption of organic matter to clay and silt particles (Beare et al., 2014; Hassink, 1997). Dependent of the management practice used and the soil properties, SOM content will move against a saturation point (Six et al., 2002; Stewart et al., 2007). E.g. if field management is shifted from continuous arable rotation to permanent grassland, the soil organic carbon content is expected to raise until a certain saturation point (Jensen et al., 2019).

For horticulture and similar highly intensive managed crops, the attention on C sequestration is more limited than other arable systems. Specialized field management practice, diverse rotations and timing of critical management practices to achieve optimum market timing for high value cash crops, can impact efforts to sequester soil C negatively and dampen of the effect (Morgan et al., 2010). However, research suggest promising use of cover crops in potatoes promoting increased soil organic carbon (Al-Sheikh et al., 2005) and compost has also proven to increase SOM (Fortuna et al., 2003).

There are several management practices that have effect on the amount of SOM, e.g. plant cover all year (winter crops, cover crops, catch crops or green manure), crop rotation with perennial grass, reduced tillage, leaving straw and crop residues or organic amendments as compost and manure (Chenu et al., 2019).

*SOM in the north of Europe*

SOM has been sampled all over Denmark in 1989, 1997 and 2009 in a 7-km grid (Taghizadeh-Toosi et al., 2014). A study on these data indicated a slight loss of carbon from 1989 to 2009. The loss was found to be $0.2\ \mathrm{Mg\ C\ ha^{-1}\ year^{-1}}$, however it was not found to be statistically significant. This loss was found in the soil profile from 0-100 cm. However, there has been observed a difference between sandy and loamy soils, which has gained and lost soil organic carbon, respectively, over the period from 1986 until 2009. The explanations for this seem to be a difference in the land use in Denmark, where sandy soils have a higher share of dairy farms with grass frequently in the rotations, and loamy sand generally has more cereal cropping systems. This difference is ascribed to be the main explanation between this variance in the change of SOM on different soil types in Denmark (Taghizadeh-Toosi et al., 2014). On average $63\ \mathrm{Mg\ C\ ha^{-1}}$ was measured in the topsoil (0-25 cm), with the highest amount of C in coarse sandy soils at $78\ Mg\ C\ ha^{-1}$, and the lowest in sandy loam soil at $54\ \mathrm{Mg\ C\ ha^{-1}}$ (Taghizadeh-Toosi et al., 2014). Similar results have been found several places in northern Europe, e.g. Finland, Belgium and southeast Germany (Heikkinen et al., 2013; Meersmans et al., 2009; Wiesmeier et al., 2012). In these countries' the management intensity, the choice of crops and crop rotations are similar. This gives an indication that even if the geological origin of soils and the climate gradient differs, similar management practice gives comparable levels of SOM (Taghizadeh-Toosi et al., 2014).

## 2.2 Soil ecology and decomposition of soil organic matter

*Soil ecology*

The turnover of organic matter in soil is the result of a great diversity of organisms.

The organisms involved in this process can be split up into three groups being macrofauna, mesofauna and micro fauna/flora. Macrofauna is in the size range from >2 mm, and includes e.g. earthworms, mammals, ants etc. Mesofauna is in the size range between 100 µm – 2 mm, and includes e.g. collembolas, mites, tardigrades etc. Micro fauna/flora is in the size range 1 - 100 µm, and includes e.g. bacteria, fungi, protozoa etc. (Jeffery et al. 2010).

Abundance and diversity of soil life is astonishing. Protozoa abundance varies greatly in soil, and it often reaches densities of $10^4 - 10^6$ in 1 g soil near the surface (Schnürer et al., 1986).

The abundance of bacteria is equally astounding with densities of $10^{10} - 10^{11}\ g^{-1}\ soil$ (Horner-Devine et al., 2004). In a handful of soil 10.000 genetically distinct prokaryotic types can be present (Torsvik et al., 2002) and 4.000.000 prokaryotic taxa are known to be apparent in soil (Curtis et al., 2002).

Generally, decomposition is mainly carried out by the micro fauna/flora, however before the microorganisms become effective the material needs be broken-down to increase the surface area, and this preliminary work is done by many organisms from the group of macrofauna and mesofauna (Brady & Weil, 2009).

*Decomposition of soil organic material*

Decomposition is an ongoing process with plant residues being broken down, thereby releasing nutrients for plant up-take and $CO_2$ to the atmosphere or stabilizing nutrients in the stable pool of carbon called humus. The rate of decomposition is related to the composition of the organic matter, some compounds being decomposed slower than others. E.g. sugars, starches, and simple proteins are rapidly decomposed, whereas cellulose is decomposed slower and lignin is decomposed very slowly. The rate of decomposition of organic compounds lasts from days to years, with two main defining factors, the soil environment and the quality of residues. Soil environment includes soil moisture, aeration, temperature and pH, and has a great effect on the rate of decomposition. The quality of residues is partly defined by the physical state of the residues, i.e. its size and chemical composition, i.e. the nutrient content of the organic matter. In general, C:N ratio is considered as the most important aspect defining for the rate of decomposition. Generally, the C:N ratio increases with increasing plant age, due to a decrease in proteins in the tissue while the proportion of cellulose and lignin increase (Brady & Weil, 2009).

Recently the classical concept of SOM formation has been re-evaluated due to new tools for analysis, which have brought new insights into the chemical structure of SOM (Lehmann & Kleber, 2015; Liang et al., 2017). Traditionally formation of SOM has primarily been seen as dependent on plant inputs and their chemistry, e.g. recalcitrant complex polymers such as lignin derivatives and long-chain lipids (Kallenbach et al., 2016; Lehmann & Kleber, 2015). The recent developing understanding is that the microbial communities and their biomass play a more important role, which has previously been largely overlooked (Paul, 2016). The

understanding of microbial communities decomposing a wide range of plant compounds and using the C to synthesize their own biomass is not new, however this pathway seen as a primary way to SOM formation is new. This emerging view is a swift from looking at SOM as carbon stocks to carbon flows (Lehmann & Kleber, 2015). A way of conceptualizing the formation of SOM with the recent evolving view is the two major pathways of soil C dynamics driven by microbial catabolism and/or anabolism: 1) ex vivo modification which happens outside of the microorganisms and implies restructuring or transformation of plant residues by extracellular enzymes, with deposition of plant-derived C which is not readily assimilated by microorganisms, 2) in vivo turnover by microorganisms assimilating organic substrates, for biosynthesis and growth, with deposition of microbial derived C when the microbes die as microbial biomass and necro mass, which can be stabilized through associations with soil minerals (Liang et al., 2017).

SOM matter can be split in two major groupings: (1) The unaltered biomaterial, containing fresh animal and plant debris and older debris not jet transformed, and (2) biomaterial that is transformed and has no morphological resemblance to the source material, which is generally referred to as humified products e.g. humic acids, fulvic acids and humin, and compounds of identifiable chemical groups e.g. carbohydrates, peptides, altered lignin's and fats (Hayes & Swift, 2018). However, looking at SOM as a "continuum of progressively decomposing organic compounds" as described in Lehmann and Kleber (2015) the stationary concepts of humified products is not ideal (Hayes & Swift, 2018). By looking at the transformation or humification as a progressive transformation of organic debris, which along the line appear as humified products. These fractions can then be further stabilized and retained through association with soil clay particles or oxide minerals e.g. iron (Hayes & Swift, 2018).

## 2.3 Compost

Composting is a way of treating and recycling biowaste or organic waste products, which ensures reduced volume, stabilization of the product and destruction of undesired content e.g. weed seeds and diseases (Andrade et al., 2018; Larney & Blackshaw, 2003). Composting is considered a successful way of utilizing and transforming organic waste into a useful product (Erhart et al., 2005; Senesi & Plaza, 2007).

In the recent decades the primary way of handling waste has gone from landfilling to recycling and incineration. In Europe municipal solid waste landfilling has gone down with 61% from 1995 to 2018, and composting has increased with 202 % in the same period (Eurostat, 2020). The amount of source separated biowaste is increasing in Denmark, see Table 2.1. This increase has mainly happened due to better sorting in the recycling system, with a bigger fraction of biowaste sorted from the residual waste. This increase has happened especially with food waste separated as an organic fraction with an increase of 112 % and a smaller increase for garden waste increasing with 11 % from 2014 until 2018. However the drop in garden waste for 2018 is properly explained by a dry summer with less growth of e.g. grass and hedges (Madsen et al., 2020).

*Table 2.1: Biowaste produced in Denmark from 2014 until 2018 adapted from Madsen et al.*(2020).
**termed organic waste up to and including 2017, then termed food waste.*

| **Biowaste** | **2014** | **2015** | **2016** | **2017** | **2018** |
|---|---|---|---|---|---|
| | **Mg (1.000)** | | | | |
| Food waste* | 207 | 245 | 282 | 327 | 439 |
| Garden waste | 832 | 906 | 946 | 991 | 929 |
| Sludge - wastewater treatment plant | 129 | 119 | 121 | 119 | 106 |
| Sludge - other | 33 | 44 | 59 | 64 | 60 |

Municipal biowaste is ideal for compost and a common management practice in Denmark and in Europe, especially with garden waste (Andersen et al., 2010; EEA, 2020). However several other sources of organic matter is used for making compost e.g. residues from vegetable/fruit production and animal manure (Larney et al., 2006; Nevens & Reheul, 2003).

### *Process of composting*

Composting is a process of microbial degradation of a wide variety of organic materials, with the presence of moist and aerobic conditions. The process goes through different phases primarily defined by the temperature, due to the fact that temperature is critical for which different microbial populations are active e.g. thermophilic, thermotolerant or mesophilic microorganisms, including a large variety of bacteria and fungi (Amir et al., 2008; Beffa et al., 1996; Teutscherova et al., 2017). The process can be split up in three stages (1) the initial stage with a rapid increase in temperature due to a rising activity and growth of mesophilic organisms

(10°C – 42°C), (2) the thermophilic stage with high temperatures and rapid degradation, with thermophilic organism taking over of non-thermotolerant organisms (45°C – 70°C), (3) the last phase includes the cooling, stabilization and maturation, which is characterized by the development of a new mesophilic populations (Amir et al., 2008; Kazemi et al., 2017).

The initial stage starts with rising temperatures due to rapid metabolism of the labile C-rich substrates. In this stage a wide variety of mesophilic microorganism contribute to the decomposition of the organic material. As temperatures increase, thermophilic conditions arise, and at 40 °C the system turns from the initial stage to the thermophilic stage. Thermophilic conditions will continue until substrates begin to decline, and gradually temperature will start to decrease. As temperature declines mesophilic microorganisms reappear in the cooling and maturing stage, especially with fungi which can decompose remaining lignin and cellulose substrates (Smith et al., 2015).

The last stage includes maturation and stabilization. Maturation is linked with plant-growth potential, and stabilization is related to the compost microbial activity. However, these is partly connected because high microbial activity in the compost can result in suboptimal plant-growth conditions (Bernal et al., 1998; Wu et al., 2000). The maturation/stabilization process of compost is similar to the process organic matter goes through in the soil called humification (Fornes et al., 2012; Senesi & Plaza, 2007).

Composting is an aerobic process, and usually it involves aeration, often accomplished by turning the pile several times (Fornes et al., 2012; Larney et al., 2006). Lack of proper aeration in the composting process can lead to immature compost, continuous decomposition and formation of phytotoxic components. Natural ventilation with PVC pipes in a static pile has proven to be insufficient compared to pile turning, when looking at several parameters for maturity of compost (Rasapoor et al., 2016). Lguirati et al. (2005) found in an experiment with composting of urban waste landfill, that aeration and watering of organic matter landfill had a major impact of maturity, measured on phytotoxicity among other parameters.

*Compost use*

Compost is generally used as a soil amendment with two main beneficial effect on crop production, (1) it improves the quality of the soil by increasing soil workability and increasing soil water and nutrients holding capacity; and (2) as a source of nutrients (Martínez-Blanco et

al., 2013). Other positive impacts of compost is carbon sequestration, weed pest and disease suppression, decreased soil erosion, soil aggregate stability, enhanced soil biological properties and biodiversity, and gain in crop nutritional quality (Martínez-Blanco et al., 2013).
However, application of immature compost can impose poor plant-growth which can be due to several reasons, (1) immobilization of N due to partially decomposed material, which is continued in the soil (Gagnon & Simard, 1999), (2) anaerobic conditions induced due to breakdown of organic material by microorganisms (Benito et al., 2003), (3) compost containing phytotoxic components due to incomplete decomposition, e.g. phenolic acids and volatile fatty acids (Benito et al., 2003; Bernal et al., 1998).

### 2.4 Plant and root growth – model crop lettuce

Lettuce is a common crop in temperate areas, and is well suited for cool weather (Yordanova et al., 2020). It is a mayor vegetable crop in Denmark, in 2018 it was the 5$^{th}$ biggest vegetable crop in acreage and the 4$^{th}$ by mass (Møllenberg & Larsen, 2019).
In the experiment of this project (described in section 3) the lettuce *Lactuca sativa* L. var. *capitate* 'Maravilla de Verano' was used, which belongs to the horticultural type Crisphead subtyped Batavia lettuces (bingenheimersaatgut, 2020; Ryder, 1999).
The growth of head forming lettuce can be divided into four phases: The seedling, the rosette/leaf appearing, heading/hearting and reproductive phase (Jenni & Bourgeois, 2008; Ryder, 1999). Lettuce is in Denmark recommended grown as seedling in a green house before transplanting (HortiAdvice, 2015), this includes the seedling and first part og the rosette phase. After transplanting the rosette phase is continued with expansion and maturation of leaves. The diameter of the plant increases substantially during this phase. Then the heading phase starts, which is the formation of cup-shaped leaves. The head form a spherical structure with earlier leaves enclosing later ones. The heading is only for some types of lettuce. When the heads reach the desired size and firmness, they are ready for harvest. The reproductive phase comes after the heading phase, which is important for seed production (Ryder, 1999).
The growth period is very dependent on temperature and solar radiation. The production cycle is estimated to be around 55-65 days (Ryder, 1999). In Denmark the recommended growth period after transplanting of 'iceberg' lettuce is 77-84 days in spring, 42-49 days in summer and about 77 days in the autumn (HortiAdvice, 2015). Some varieties have been shown to grow

under winter conditions, however the growth period becomes even longer. In Bulgaria Yordanova et al. (2020) reported possible growth of lettuce under cover in winter with harvest about 150 days after planting, with 140 days with temperatures below 5 ˚C of which 16 were below -5 ˚C.

Modern lettuce is yielding optimally under high input systems of nutrients and irrigation (Johnson et al., 2000; Ryder, 1999), which makes the plants sensitive to scarcity of nutrients. Lettuce forms a tap root which usually grows to about 60 cm, but can also grow longer (Jackson, 1995). Along the tap root it forms lateral roots, most densely at the top, and decreasing with increasing depth (Mou, 2008; J. E. Weaver & Bruner, 1927). Through history, breeding lettuce for high input systems, it has developed a shallow root system focused on the topsoil, with less roots further down. Cultivated crisphead lettuce (*L. sativa*) is found to have 78% of its total root length in the upper layer (0-20 cm), compared to only 50 % for wild Lettuce (*L. serriola*) (Gallardo et al., 1996; Johnson et al., 2000).

Batavia type lettuce weighs about 500 g when ready for harvest and is harvested at a smaller weight than the head lettuce commonly known as “iceberg” which weighs between 700 and 1000 g when harvested. It is also less dense and softer than “iceberg” (Mou, 2008).

### *Biometric observations on plants - Shoot and Root*

Above ground plant parts of lettuce is commonly observed as above ground biomass, but as a non-destructive method it can also be observed through its growth by measuring the plant leaf area or rosette diameter (Trupiano et al., 2017; Viger et al., 2015), however the plant area generally increase most during the rosette phase (Ryder, 1999).

Observations on roots are challenging, especially in a non-destructive way. Most methods are often time consuming, tedious and the accuracy is not very great (Böhm, 1979; Judd et al., 2015). The available methods for field experiments, which allows assessment of changes with time is limited. One method is the use of transparent material were observations of root growth are made through a transparent interface with soil, e.g. as mini-rhizotrons or root windows. This makes it possible to study in situ root growth of plants over time with minimum disturbance (Atkinson, 2000; Fukuzawa et al., 2012). The mini-rhizotron method is widely used, whereas the root window is less common (Smit et al., 2000).

Mini-rhizotrons is tubes (glass or plastic) installed in the soil temporarily or in a permanent setup, through which root observations can be made (Hefner et al., 2019; Parker et al., 1991; Svane et al., 2019). Root windows, which is the method used in this experiment, is essentially a transparent plane surface which makes it possible to observe roots over time. The setup is quite variable in matter of how it is constructed, and the method is used both in field (Tscherning et al., 1995) and greenhouse (Silva & Beeson, 2011). Often root windows are mostly possible for the upper soil layer, however setups has been made for down to 4 meters depth (Thorup-Kristensen et al., 2020).

The minirhizotron and the root window technique enables easy and repeated measurements on specific roots, however it surveys only on a static and limited two dimensional area, and there is a risk of aberrant growth along the installed window (Judd et al., 2015).

## 2.5 Nitrogen as fertilizer

Several conditions are essential for plant growth including nutrients, water, radiation and suitable temperatures. Liebig's law says that the growth is not dictated by the total amount of resources available, but the scarcest resource, becoming the limiting factor.

In plants N is the fourth most abundant element after hydrogen, carbon and oxygen (Hawkesford et al., 2012). N is often the most limiting nutrient, the one applied at the highest dose and often the nutrient in focus in matters of environmental problems due to leaching (Gaskell & Smith, 2007; Tei et al., 2020). In this experiment the setup was made in such way that N becomes the limiting factor by reducing N application in different levels. However, this is only the case if no other resources are limiting, with reference to Liebig's law of minimum.

Increasing use of N fertilization is part of the reason why a doubling of food production in the past four decades is achieved (Tilman, 1999), and to a great extent because of the use of mineral fertilizer, which is the largest source of anthropogenic reactive N worldwide (Fowler et al., 2013). However over the past 4-5 decades the pressure on avoiding pollution of environment has been rising, which has increased the focus on reducing leaching of N by limiting the amount of N applied and utilizing what is applied (Gustafson, 2012).

N is vital for plant growth and metabolism, as it is a part of molecular compounds, e.g. proteins, enzymes, and nucleic acids (RNA, DNA) (Hawkesford et al., 2012). A direct effect of N is rapid growth with higher rates of photosynthesis, other more indirect effects is modulation of the

hormone balance e.g. by stimulation of the synthesis of cytokinins (Gu et al., 2018). N is generally taken up as either nitrate ($NO_3^-$) or ammonium ($NH_4^+$), however at some occasions a considerable part is taken up as organic N e.g. as amino acids or amino sugars (Lipson & Näsholm, 2001; Paungfoo-Lonhienne et al., 2012) however this assimilation is limited due to the low diffusivity of organic N molecules (Jacoby et al., 2017).

Evaluating efficiency of N application, N use efficiency (NUE) is a useful measure. The NUE of the cropping system can be considered a measure of applied N, which is not lost out the system either by N leaching or losses of gaseous N forms. Looking at plants the N-fertilizer recovery efficiency or $NUE_{crop}$ is also often used, which is the percentage of N fertilizer applied that is recovered by the above ground biomass during the growing season (Cassman et al., 2002; Martinez-Feria et al., 2018).

Mineral fertilizer is directly added to the available N pool in the soil. Which makes it ready for plant uptake, leaching or microbial uptake, and the apparent availability makes the NUE very dependent on the application method, rate, timing and type (Snyder, 2017).

Organic matter contains a large pool of N and if it is released when plants do not need it, it becomes a risk for leaching. The process of N mineralization-immobilization is resulting in either N released or assimilated by microorganisms. If the net N mineralization is positive the available N pool increase (Tei et al., 2020). When N is applied as organic matter the availability is influenced by several factors, one of the most important being the C:N ratio of the material. In general, if the added organic material has a C:N ratio above 25 the short-term net N mineralization is negative and if its below 25 it is unchanged or positive. On the long-term the net N mineralization will eventually become positive; however, the timing is vital for plant growth. When organic matter is applied the soil microorganisms starts the mineralization process, if the material lack N, the microorganisms take up N from the soil N pool, decreasing N availability for the plants thereby immobilizing N, which however eventually will be released (Brady & Weil, 2009).

# 3. METHODS, DATA PROSSEING AND STATISTICS

The semi-field experiment was set up with two factors, a soil treatment and a fertilizer treatment (with two and four levels) and four repetitions in a complete randomized block design

(Oehlert, 2010). An overview of the experiment can be seen on Figure 3.1. Each mini-plot was a tub dug into the soil at the test site, a field at Aarhus University Årslev, Denmark (55°18'33.1"N 10°26'37.7"E), and filled with soil gathered in two different fields. In each mini-plot lettuce where used as model crop and the sample size was 8 plants. This method enables control of the soil from different localities and fertilizer treatments in a randomized design, at equal climate conditions and control of agronomic factors in an open field setup.

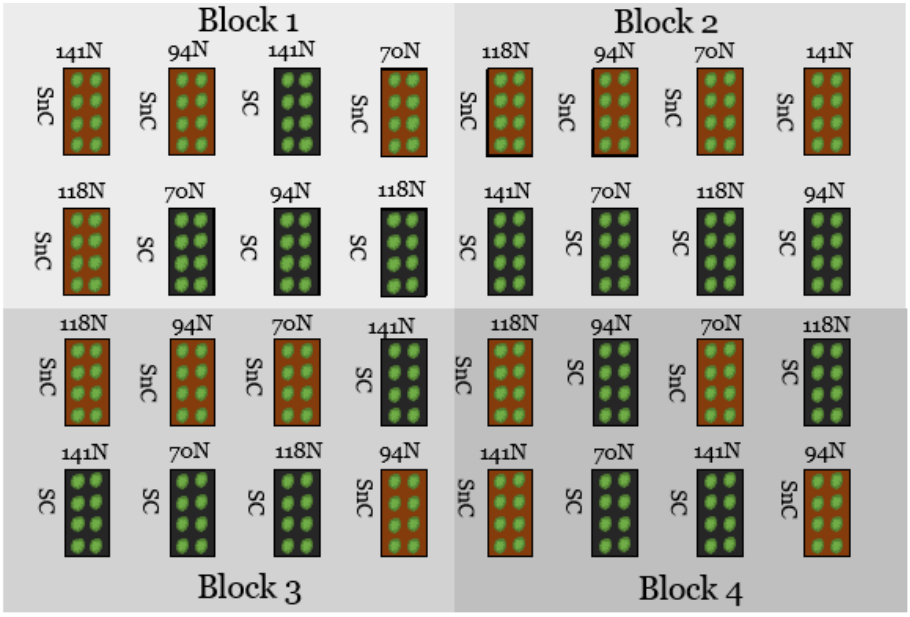


*Figure 3.1: An overview of the experimental setup*

The soil used in the experiment was collected from two fields from the farmer Erik Andersen of Eastern Funen, Denmark (Figure 3.2). The soil was chosen because one had received compost for a considerable period and the other had not. The field with compost application (SC) had received compost amendment every 2nd/3rd year since 2007. By most other parameters the two fields were managed in a similar way, e.g. in relation to tillage and fertilizer application. The only difference known was the crop rotation (Table 3.1).

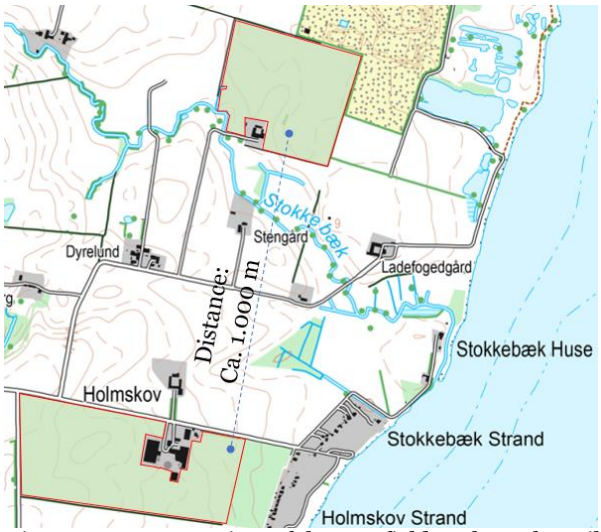


*Figure 3.2: An overview of the two fields, where the soil for the experiment was collected. The southern one with no compost application (SnC, 55°10'08.1"N 10°47'22.6"E), and the northern one with compost application (SC, 55°10'39.5"N 10°47'33.8"E). The blue dots mark the place in the two field where the soil was collected, with ca. 1.000 m between them.*

*Table 3.1: The crop history of the fields, where the soil was collected for the experiment (Data received from* (The Danish Agricultural Agency, 2020)*)*

| | ***Field with no compost application (SnC)*** | ***Field with compost application (SC)*** |
|---|---|---|
| ***2012*** | Grass seed | Winter wheat |
| ***2013*** | Grass seed | Winter wheat |
| ***2014*** | Grass seed | Winter barley |
| ***2015*** | Spring barley | Spring barley |
| ***2016*** | Winter rape seed | Spring barley |
| ***2017*** | Winter wheat | Winter barley |
| ***2018*** | Spring barley | Winter wheat |
| ***2019*** | Spring barley | Winter wheat |

The two factors were:

1. Soil treatment: Field soil gathered from two different fields, with compost application as a major difference:
   - SnC: Soil from a field with no compost application.
   - SC: Soil from a field with compost application every 2nd/3rd year over the last 10 years. Additionally, compost was applied ($20\ \mathrm{Mg\ compost\ ha^{-1}}$, $3.2\ \mathrm{kg\ N\ (NO_3^-/NH_4^+)\ ha^{-1}}$) to this treatment at the start of the experiment.
2. Fertilizer treatment: The N fertilization level recommended in commercial lettuce production is 140 $\mathrm{kg\ N\ ha^{-1}}$ (de Visser, 2019; HortiAdvice, 2015). The mineral N ($\mathrm{NO_3^-}$ and $\mathrm{NH_4^+}$) present in the two soil treatments was 11.65 (SnC) and 14.1 (SC) $\mathrm{mg\ N\ kg^{-1}}$dry weight soil equivalent to 42 (SnC) and 51 $\mathrm{kg\ N\ ha^{-1}}$ (SC) in the added soil layer in the semi-field setup of the two field soils used. This was taken into account for, resulting in a total mineral N in the topsoil of ca. 70, 94, 118 and 141 $\mathrm{kg\ N\ ha^{-1}}$ after addition of fertilizer for the four treatments, see Table 3.2.

*Table 3.2: Total N (kg N $ha^{-1}$) for each fertilizer treatment. Soil N at the start of the experiment at 42 (SnC) and 51 kg N $ha^{-1}$ (SC) plus applied mineral N fertilizer added in different doses.*

| | **70N** | **94N** | **118N** | **141N** |
|---|---|---|---|---|
| **SnC** | 42+25=67 | 42+50=92 | 42+75=117 | 42+100=142 |
| **SC** | 51+22.5=73.5 | 51+45=96 | 51+67.5=118.5 | 51+90=141 |

For both fields where soil was gathered for treatment SnC and SC the soil type was a sandy loam, see The compost used for the experiment was from the municipal owned waste company Klintholm I/S, see Table 3.3 for selected information of the compost. The composted organic material was municipal garden and pruning waste (grass clippings, leaves and shrubs). First the material was shredded and screened and placed in a pile of about 4-5 meters height and +30 m wide and long. The material laid for 4-6 months and was left without any handling or control. Before use it was screened again (22 mm). The compost used by the farmer through years from 2007 was of the same origin and similar properties.

Table 3.3 for selected information. The soil was gathered with a tipper grab from the 0-0.25 m soil layer at one location in the field see Figure 3.2.

The compost used for the experiment was from the municipal owned waste company Klintholm I/S, see Table 3.3 for selected information of the compost. The composted organic material was municipal garden and pruning waste (grass clippings, leaves and shrubs). First the material was shredded and screened and placed in a pile of about 4-5 meters height and +30 m wide and long. The material laid for 4-6 months and was left without any handling or control. Before use it was screened again (22 mm). The compost used by the farmer through years from 2007 was of the same origin and similar properties.

*Table 3.3: Selected properties of soils (0-0.25 m) and compost.*

| | **Soil with no compost application (SnC)** | **Soil with compost application (SC)** | **Compost (C)** |
|---|---|---|---|
| **Dry matter (%)** | 88.35 | 87.25 | 70.45 |
| **$NH_4^+$-N (mg $kg^{-1}$)** | 2.10 | 2.75 | 18.00 |
| **$NO_3^-$-N (mg $kg^{-1}$)** | 9.55 | 11.35 | 147.95 |
| **$NH_4^+$: $NO_3^-$ ratio** | | | 0.12 |
| **$pH_{CaCl_2}$** | 6.50 | 6.30 | 7.45 |
| **Phosphorus (mg 100 $g^{-1}$)** | 4.35 | 4.70 | 25.00 |

| | | | |
|---|---|---|---|
| **Potassium (mg 100 $g^{-1}$)** | 21.00 | 8.30 | 320.00 |
| **Magnesium (mg 100 $g^{-1}$)** | 8.10 | 6.10 | 46.00 |
| **Organic C, total (%)** | 1.62 | 1.29 | 10.71 |
| **Nitrogen, total (%)** | 0.15 | 0.13 | 0.71 |
| **C: N ratio** | 10.83 | 9.59 | 14.95 |
| **Humus (%)** | 2.10 | 2.15 | |
| **Clay (%)** | 11.70 | 12.30 | |
| **Silt (%)** | 8.05 | 9.40 | |
| **Fine sand (%)** | 47.85 | 47.30 | |
| **Coarse sand (%)** | 30.40 | 28.85 | |

The experiment was conducted in the autumn 2019. The soil type in the field where the experiment was conducted was a sandy loam (Typic Agrudalf). Accumulated precipitation during the growth period (2nd of September until the 28st of October) was 223 mm, for overview of temperature and precipitation see Figure 3.3. The sunshine hours of September and October were 151 and 88, compared to >200 hours in each of July and August.

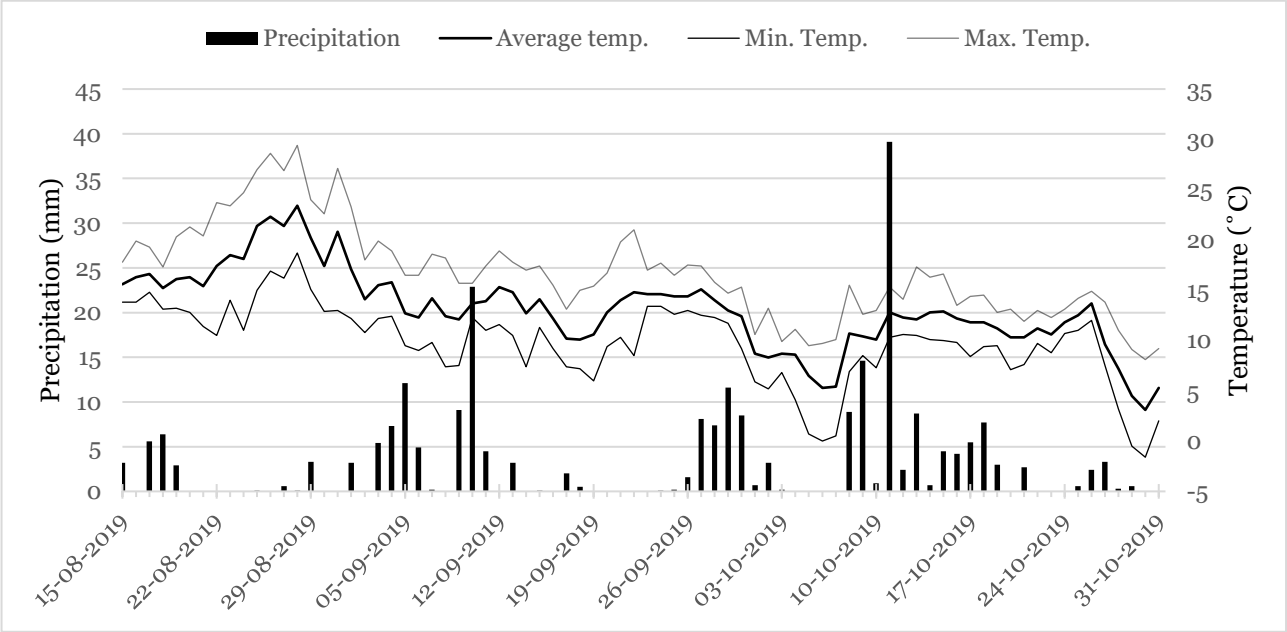


*Figure 3.3: Weather data from the experimental site. Temperature on the right axis and precipitation on the left axis.*

## 3.1 Establishment of the experiment

Each mini-plot was a truncated pyramid shaped polyethylene tub (Top:(W42 cm x L72 cm); Bottom:(W34.5 cm x L64 cm) x H30 cm), with an installed window (25 cm x 21 cm for root

observations and with the bottom cut out, see Figure 3.4. The tubs were dug into the soil and then filled with one of the two soils used.

*Figure 3.4: The tubs before and after filled with soil.*

The tubs were installed and filled with fresh soil between the 23rd and 26th of august. Soil was not weight when applied but filled to the rim at the same bulk density, a full tub holds 115 kg of fresh soil. The soil was sieved through a 2 cm sieve. 800 g compost (20 Mg Compost $ha^{-1}$) was applied to the SC mini-plots on the 27th of august. Additional ca. 9 kg soil was added to all mini-plots prior to planting to fill them to the rim, because in the meantime the soil had settled. The root window was covered with a sheet of black plastic and soil to ensure no light to pass through the window. When pictures were taken of the window, a pit was dug in front of each window, and filled afterwards.

On the 2nd of September 8 lettuce plants (*Lactuca sativa* L. var. *capitate* 'Maravilla de Verano') were transplanted into the mini-plots. The fertilization was split in two, half was given on the 17th of September and the other half was given on the 25th of September (15 and 23 days after planting (DAP)). The mineral fertilizer was mixed up in water in different doses and applied as top dressing on the mini-plots by hand with a container. N was given as described above in different doses, in addition an equal amount of potassium, phosphorus and sulfur was given to each mini-plot due to recommendations for lettuce in Denmark (de Visser, 2019; HortiAdvice, 2015).

The transplants had been sown in greenhouse on the 5th of August and grew under natural light and a base temperature at 20˚C reaching higher when the outside temperature surpassed this.

The growth medium was a sphagnum based mixture with added clay and nutrients (Pindstrup, 2020). After 3 weeks the plants were put outside of the greenhouse to become acclimatized.
The first week after transplanting 6 plants died, and these were replaced at 14 DAP. However, at the end of the experiment 11 plants were missing and 13 had severe stunted growth. Of the 32 mini-plots 17 mini-plots were intact at harvest with all 8 plants of uniform growth, in 9 mini-plots 1 plant, in 3 mini-plots 2 plants and in 3 -mini-plots 3 plants were either missing or stunted.

## 3.2 Data collection and analyses

*Biometric data on the plants*

On the 28th of October the plants were harvested, and the fresh weight of each plant was measured.
A dry matter analysis was also carried out, one for each mini-plot. For each mini-plot 2 plants were cut into pieces and the fresh weight were scaled, then the plant material was dried for 20 h at 80°C and weighed again. An average dry matter content of each mini-plot was calculated, which was used to calculate a dry matter weight for each plant. The dried plant samples were further analyzed for content of N. The total plant N content was analyzed by the VDLUFA method (VDLUFA, 1991). The plant samples were first burnt at 900˚C and then molecular N was determined by use of LECO TruSpec CN (St. Joseph, Michigan).
During the experiment pictures of the plants were taken from above and pictures of the roots were taken through the window in the end of each tub, images were taken 8 times, on the 12th, 18th, 23rd, 26th of September, 1st, 7th, 15th and 28th of October (10, 16, 21, 24, 29, 35, 43 and 56 DAP). Images were taken with the camera on a Huawei P20 Pro.
Heads that were missing at harvest the 28th of October was excluded at all observation dates.

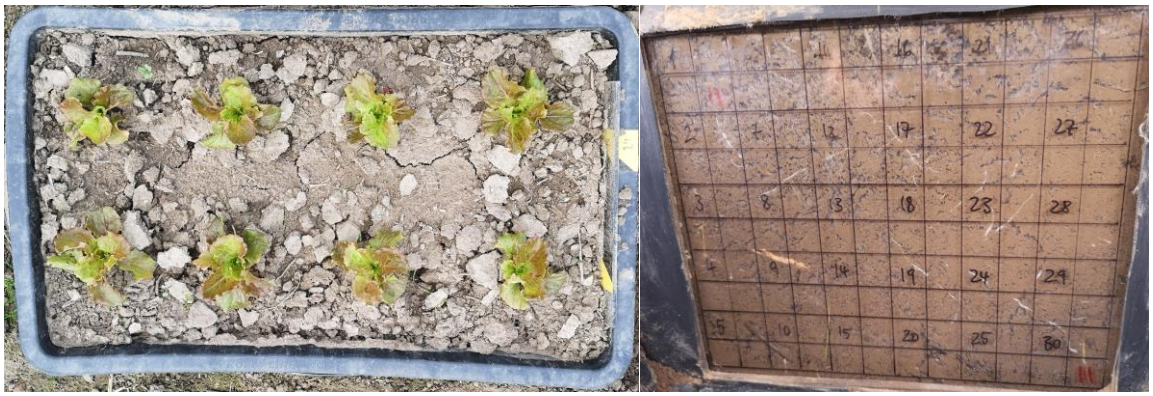

*Figure 3.5: left) above ground, right) root observation window images taken of mini-plot 11 on the 23rd of September (21 DAP)*

The images of the plants were processed using a software ImageJ 1.52 (Rasband, 2020), calculating the area covered of the soil by each plant in the mini-plots. For each plant the outline was drawn on the photo to fit the plant perimeter, and the number of pixels within the enclosed area was determined and converted to $cm^2$.

For the roots there were made a count of intensity of roots. The intensity was found by counting roots in each of the 30 numbered square window with a line intersection, one root count is a root that cross the grid (vertical and horizontal lines) of each numbered square window (Figure 3.5), either from the top or from the right side equivalent to the root registration in field minirhizotrons described in Kristensen & Thorup-Kristensen (2004).

*Soil N*

The content of mineral N in soil was analyzed at the start of the experiment and at shortly after harvest. Soil samples were taken at 4 depths from 0–0.25 m, 0.25–0.5 m, 0.5–0.75 m, and 0.75–1 m layers.

The start soil sample was taken separately first directly from the two fields where soil was collected SnC and SC, and second in 0.25-0.5, 0.5-0.75 and 0.75-1 m depth at the experimental site. First at the 21st of august 4 samples were taken from each of the two fields where soil was collected (SnC and SC) representing the 0-0.25 m soil layer. Then soil samples were taken in the field at two different spots between the mini-plots at the 9th of September by a machine-driven soil piston auger with a 14mm inner-diameter from 0.25–0.5 m, 0.50–0.75 m, and 0.75–1 m layer.

The end soil samples were taken at the 30th of October in each mini-plot from 0–0.25 m, 0.25–0.5 m, 0.50–0.75 m, and 0.75–1 m soil layer.

Potential mineralized N was determined same way as Hefner et al. (2019) according to Hart et al. (1994) using field-moist soil from the 0-0.25 m layer. Soil samples were sieved (5 mm) and put into 500 ml containers covered with perforated polyethylene (30 μm) wrap to allow gas exchange but minimize water loss. The containers were incubated for 28 days at 25°C and kept moist during the incubation by spraying samples with deionized water.

Soil samples both with and without incubation were frozen down, after they were taken or after incubation respectively, and transferred to an external laboratory, and kept frozen until analysis. Analysis of soil Nmin was completed according to the Plant Directorate of the Danish Ministry of Agriculture (1994). Samples were defrosted and two 100 g fresh weight soil subsamples were extracted in 1M KCl for 1 h. The soil extract was centrifuged, and the supernatant was analyzed for $NH_4^+$ and $NO_3^-$ by standard colorimetric methods using AutoAnalyzer 3 (Bran+Luebbe GmbH).

### *Soil microbial activity and microbial biomass*

#### *Enzyme activity*

There is a lot of methods to study microbial life in the soil. Enzyme activity assays on the enzyme's dehydrogenase and ß-glucosidase provide a way of quantifying activity of certain processes in the soil, but it should be interpreted with care in relation to conclusions on overall microbial activity (Nannipieri et al., 2018). However, dehydrogenase (DHA) and β-glucosidase enzyme activity (β-GA) is considered appropriate indicators for soil microbial activity and for the effect on microbial activity of altered management practice such as compost application (Chang et al., 2007; Moeskops et al., 2010).

Dehydrogenase (EC 1.1.1.) is an important group of enzymes in the oxidation of organic substrates under aerobic conditions in the oxidative phosphorylation or in general the respiratory chain in soil (Wolinska & Stepniewsk, 2012). Dehydrogenase activity (DHA) is generally accepted as an indication of overall microbial activity (K. Alef & Nannipieri, 1995) and used as an indicator for microbial activity (Benito et al., 2003; Hefner et al., 2020; Moeskops et al., 2010; Serra-Wittling et al., 1995).

ß-glucosidase (ß-D-glucoside glucohydrolase, EC 3.2.1.21) is categorized as a glucosidase and it hydrolyze disaccharides. It is a major catalyst in the microbial degradation or hydrolysis of cellulose to glucose, and it hugely impacts the rate of the degradation (K. Alef & Nannipieri, 1995). It has been shown that there is a significant correlation with SOM and ß-glucosidase assay (Eivazi & Tabatabai, 1988).

*Microbial biomass*

Assays of soil microbial biomass is often used, when studying dynamics of SOM (Miltner et al., 2012). The present microbial biomass is a key definable indication for studies of the formation and turnover of SOM, therefore measurements of the microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP) contained in the soil microbial biomass provide a basis for assessing these matters (Voroney et al., 2008).

*Soil sampling*

Soil samples for enzyme activity assay and microbial biomass were taken two times. The first samples were the same as the start mineral N soil samples from 0-0.25 m soil layer collected in the two fields on 21st of August and the second samples were taken the 11st of October (39 DAP) in each mini-plot with a hand-driven soil piston auger (15-mm inner diameter) in the 0-0.25 m. The first soil samples in August were taken before establishing the experiment, directly from the two fields, with four samples of each soil. The addition of compost prior to planting and the fertilizer treatment is not included in this sample.

Before the soil was used, visual animal and plant residues were removed, and soils were sieved at 5 mm. The soil samples for enzyme activity and microbial biomass analysis were stored in a refrigerator (1 °C) before the analysis took place.

For the calculations of enzyme activity rates, the dry matter and moisture content of the soil samples was measured by drying ca. 30 g field moist soil to 80 °C for 24 h and weighing the soil before and after.

*Procedure for enzyme activity assays*

The assay of DHA and β-GA in this experiment, is the same as used in Hefner et al. (2020). The assay of DHA (The TTC method (Thalmann, 1968)) and the assay of β-GA (Assay of the ß-

glucosidase enzyme activity (Eivazi & Tabatabai, 1988; Tabatabai, 1994)) generally follows the methods, both described in Alef & Nannipieri (1995).

Due to laboratory capacity, it was chosen not to conduct the assays on the fertilizer treatments N75.

In the assay of DHA, Triphenyl tetrazolium chloride (TTC) is used as substrate which is oxidized to triphenyl formazan (TPF). The method is based on the determination of released TPF per time. TTC and TPF is light sensitive, therefore the analysis was performed under diffused light. The analysis was carried out with no technical replicates but with one blank with soil and without addition of TTC. Two vials without soil and with chemicals was used to account for any background effects of the chemicals.

First 5 g field moist soil were placed in Erlenmeyer flasks (50 ml) for both samples and blanks. For the samples 2 ml tris buffer (12.1 g of Tris (tris(hydroxy methyl)-aminomethane) dissolved in distilled water, adjusted to pH 7.6 with HCl, diluted to 1000 ml) and 2 ml TTC solution (7.5 g TTC dissolved in Tris buffer diluted to 250 ml) was added. For the blanks 4 ml of Tris-buffer were added. The vials were then closed and mixed thoroughly and incubated for 24 h at 37°C in darkness.

After incubation 20 ml methanol was added to each vial and shaken, with a linear shaker (125 RPM) for 2 h in darkness. Then the suspension was transferred to 50 ml volumetric flasks through a Whatman filter paper (no. 5) pre-wetted with methanol. To ensure all TPF was out, the vials were washed twice with methanol and the filter paper were flushed twice with methanol. Finally, the 50 ml volumetric flasks were filled up to volume with methanol. The optical density of the filtrates was then measured on a Varian Cary 50 spectrophotometer at 485 nm. Then the results from the blanks were subtracted from the results of the samples.

For calculating the TPF concentration from the optical density a calibration curve was made. 0.5 ml TPF standard solution (50 mg TPF dissolved in methanol, to a concentration of 500 μg TPF ml -1) was mixed with methanol in a 100 ml volumetric flask to a concentration of 2.5 μg TPF $ml^{-1}$. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml TPF standard solution is added to 50 ml volumetric flask and methanol is added to the mark on the flask, and the following concentrations are obtained: 5, 10, 15, 20, 25 and 50 μg TPF $ml^{-1}$.

The results were corrected for the blanks without soil, and TPF ($\mu g\, g^{-1} soil\, day^{-1}$) rate were calculated by reference to the calibration curve.

In the assay of β-GA p-Nitrophenyl-β-D-glucoside (PNG) is used as the substrate. The method is based on the determination of released p-nitrophenol (PNP) after the incubation of soil with PNG solution for 1 h at 37°C.

The assay was carried out with two technical replicates and one blank with soil, as well as two vials as blanks without soil for background effects of the chemicals.

First 1 g field moist soil was placed in glass vials for both samples and blanks. Samples were added 4 ml MUB solution (Modified universal buffer solution, pH 6.0: 12.1 g of Tris, 11.6 g of maleic acid, 14 g of citric acid and 6.3 g of boric acid dissolved in 500 ml of NaOH (1 M), diluted to 1000 ml with distilled water) and 1 ml PNG solution (p-Nitrophenyl-β-D-glucoside solution (25 mM): 0.377 g of PNG dissolved in MUB buffer and diluted to 50 ml with the same buffer). The vials were closed, and the contents mixed thoroughly and incubated for 1 h at 37°C. After the incubation, 1 ml of $CaCl_2$ solution ($CaCl_2$ (0.5 M): Dissolve 73.5 g of $CaCl_2.H_2O$ in distilled water and dilute with distilled water to 1000 ml) and 4 ml of Tris buffer (0.1 M, pH 12 : 12.1 g of Tris dissolved in distilled water, adjusted pH to 12 with NaOH (0.5 M) and diluted to 1000 ml with distilled water) were added and the flasks were swirled, and immediately the soil suspensions were filtered through a Whatman filter paper (no. 5). Blanks were prepared following the same pattern as the samples, but with addition of the substrate PNG after the incubation and before adding the $CaCl_2$ and Tris buffer. Color intensity was measured on a Varian Cary 50 spectrophotometer at 400 nm.

For calculating the PNP concentration from the optical density a calibration curve was made. 1 ml of standard PNP solution (p-Nitrophenol standatd solution: 1 g p-nitrophenol dissolved in distilled water and diluted to 1000 ml with distilled water) was added to 50 ml MUB in a volumetric flask. Then 0, 1, 2, 3, 4 and 5 ml of this diluted standard solution was adjusted to 5 ml by addition of MUB.

The results were corrected for the blanks, and the rate of PNP ($\mu g\, g^{-1} soil\, hour^{-1}$)of the filtrate were calculated by reference to the calibration curve.

*Procedure for microbial biomass assays*

Assays on microbial biomass C (MBC), N (MBN) and P (MBP) was carried out following the chloroform fumigation-extraction method described in Voroney et al. (2008) method originally by (Vance et al., 1987).

MBC, MBN and MBP are calculated from the difference between the amount of total C, N and P extracted from fresh soil fumigated with chloroform and the amount extracted from the fresh unfumigated control soil. The fumigation and extraction took place over two days. Samples of

30 g fresh soil in 25 ml glass beakers was placed in a desiccator with 50 ml ethanol-free chloroform with boiling chips and moistened tissue paper. Air was evacuated until the chloroform was boiling vigorously. Then the vacuum was stabilized, and the desiccator was covered. The samples were left for 24 hours. Then the chloroform was removed and the chloroform vapor was removed by evacuating air for 3 min repeated 3 times. Non-fumigated and fumigated samples were transferred to Erlenmeyer flasks and added 60 ml 0.5 $K_2SO_4$. The samples were shaken for 1 hour, with a linear shaker (125 RPM). The extracts were filtered with Whatman filter paper (no. 5). The extraction of the non-fumigated samples was done the first day prior to the fumigation. Then the extractions were frozen (-18 °C) until analysis. The analysis of C and N of the extracts were conducted following the method of C/N analyzer (INBO) and the analysis for P was conducted following the method ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy).

The fumigation and extraction were done at Årslev a few days after the soil samples were taken in August and October. After extraction the extracts were frozen (-18 °C) and in February sent to ILVO (Instituut voor Landbouw-, Visserij- en Voedingsonderzoek Plant Teelt en omgeving) and analyzed for C, N and P on 26$^{th}$ of March.

### 3.3 Statistical analysis

Statistical analysis of the data was performed with the software R (R Core Team, 2020).

The experiment was designed with a block effect. However, this block effect did not show in the results. A one-way ANOVA was used to determine whether this block effect was significant. This test shows in most cases that there was no significant block effect, therefore it was chosen to use the simpler generalized linear model instead of the generalized linear mixed model. The few exceptions where the generalized linear mixed model is used due to a block effect are mentioned in the result section.

To ensure the data met parametric assumptions the data was visually inspected for normality of residuals and homogeneity of the variance, additionally a Shapiro-Wilk test for normality of residuals and the Bartlett's test for homogeneity of variance was conducted.

The data was analyzed in two parts. First a two-way analysis of variance (two-way ANOVA), was run to determine the effect of the categorical factors soil (SnC/SC) and fertilizer (141N/118N/94N/70N) on the response variables (yield, plant area, root intensity, enzyme

activity, microbial biomass and soil N) by considering the 8 different combinations and their interaction. With stepwise model reduction carried out from the full model with interactions. First eliminating a non-significant interaction then the non-significant main effects (Crawley, 2013). For microbial assays and soil mineral N of soil samples taken in August and September, only the soil treatment was present, and a one-way ANOVA was run in these cases.

Second a post hoc analysis was conducted for mean comparison for determining differences between individual treatments, with the R-package 'pairwiseComparisons' (available at https://users-math.au.dk/rodrigo/astatlab/software/pairwisecomparisons/).

The relation between the observations on plant area at different dates was tested against observations on yield with Pearson correlation analyses.

The differences were considered significant at $P \leq 0.05$. Differences between treatments are indicated with lower-case letters at figures and tables. The model estimates are reported with 95% confidence intervals.

# 4. RESULTS

## 4.1 Data on plant growth – Weight, area, and root intensity

The soil treatment significantly (P=0.00756) affected yield of harvested head weight (g $head^{-1}$), the fertilizer treatment (P=0.961) and interaction (P=0. 225) showed no significant effect. The effect on dry matter content was also tested but no significant effect of either treatments (Soil: P=0.293/Fertilizer: P=0.792) or the interaction between them (P=0.868) was found. For plant N content there was an effect of the soil treatment (P=0.00587) and no effect of the fertilizer treatment (P=0.729) or the interaction of the two (P=0.668).

Average yields were higher for the soil treatment SnC than SC, see Table 4.1**.** The dry matter content was about 5.3 %, without any significance difference between the treatments. For N content (g N $kg^{-1}$ dry mater) plants of the SC treatment had higher levels of N, compared with plants of the SnC treatment.

*Table 4.1: Model estimates of lettuce yield, dry matter content and N content, superscript letters indicate significant differences between treatments according to a post hoc analysis, ± confidence interval (n=16).*

| | **Yield** (g head$^{-1}$) | **Dry matter** (%) | **N content** (g N kg$^{-1}$ dry matter) |
|---|---|---|---|
| **SnC** | $93.37^{a} \pm 1.70$ | $5.35^{ns} \pm 0.115$ | $31.92^{b} \pm 1.24$ |
| **SC** | $86.07^{b} \pm 1.70$ | $5.26^{ns} \pm 0.115$ | $34.57^{a} \pm 1.24$ |

For plant area there was a significant effect of compost and no effect of fertilizer for all 8 dates of observations, see Table 4.2 . For observations at the 26th (24 DAP) of September, and the 1st (29 DAP) and 7th (35 DAP) of October there was an effect of the interaction between fertilizer and soil treatments, see Table 4.2. For the observation at 28th of October there was a significant block effect (P=0.00495), therefor block was taken into account as a random effect for this model. With visual inspection of the data at a Q-Q plot and the result of Shapiro-Wilk test, the data for 1st (29 DAP) (P=0.0456) and 7th (35 DAP) (P=0.0422) of October is not normal distributed and results should be interpreted with caution.

*Table 4.2: P-values of two-way ANOVA on plant area on the different dates of observations. Numbers in bold indicates a significant effect with a P-value lower than 0.05.*

| **DAP** | **Interaction of soil and fertilizer** | **Soil effect** | **Fertilizer effect** |
|---|---|---|---|
| 10 | 0.108 | $\mathbf{7.88 * 10^{-5}}$ | 0.98 |
| 16 | 0.0619 | $\mathbf{1.09 * 10^{-6}}$ | 0.755 |
| 21 | 0.0589 | $\mathbf{1.01 * 10^{-5}}$ | 0.938 |
| 24 | **0.0324** | $\mathbf{2.33 * 10^{-5}}$ | 0.923 |
| 29 | **0.0409** | $\mathbf{6.67 * 10^{-6}}$ | 0.735 |
| 35 | **0.0276** | $\mathbf{1.65 * 10^{-4}}$ | 0.676 |
| 43 | 0.187 | $\mathbf{1 * 10^{-4}}$ | 0.706 |
| 56 | 0.0728 | **0.00514** | 0.806 |

The effect of the soil treatment showed a higher plant area for the SnC treatment, see Figure 4.1.

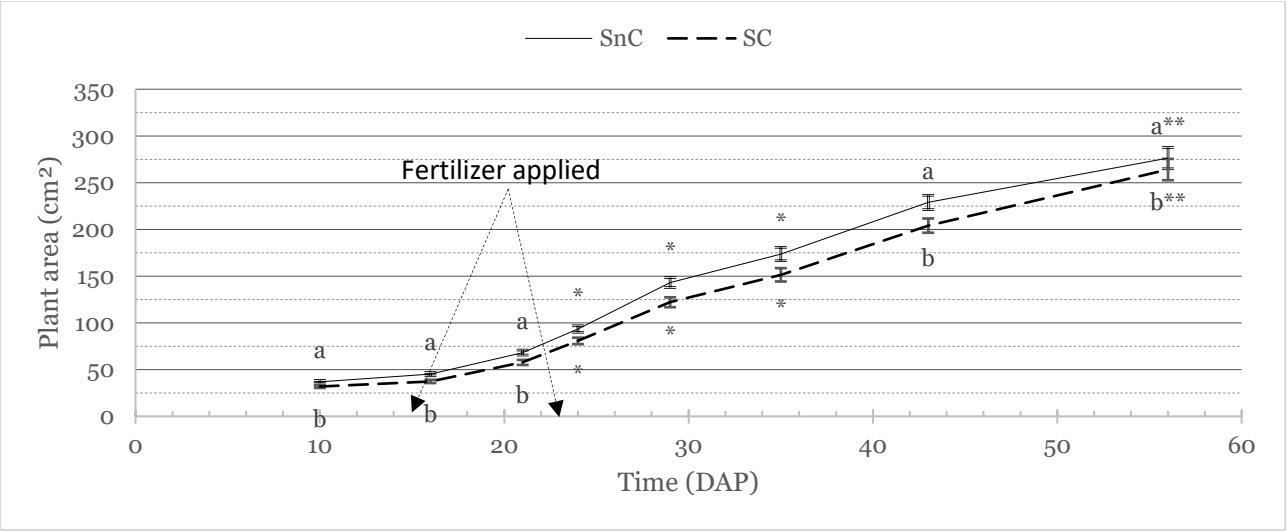


*Figure 4.1: Model estimates of plant area ($cm^2$) ± confidence interval (n=16), letters indicate significant differences between treatments according to post hoc analysis.. *there is an interactive effect of soil and fertilizer, see Figure 4.2, **there is an effect of block.*

At Figure 4.2 the estimates of the interaction between the soil and the fertilizer treatment is shown. There is a tendency for larger plant area observations for SnC than SC treatment, as seen for the other observation dates. However, there is not seen a pattern for the interaction

between soil and fertilizer. In general, there is no differences between levels of fertilizer of the same soil treatment.

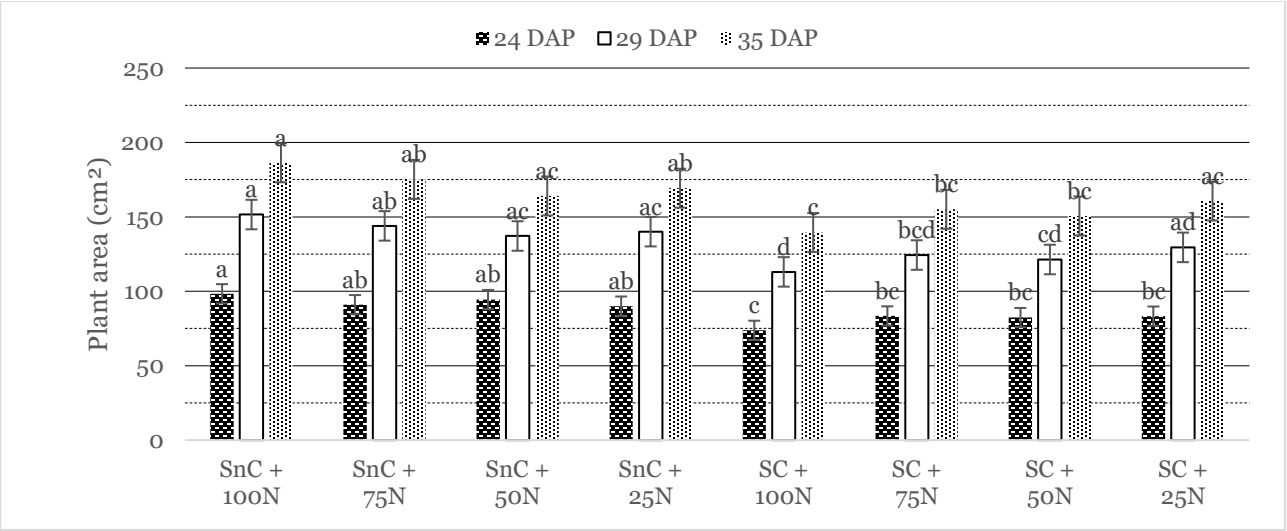


*Figure 4.2: Observed area of the plants in the mini-plots the 26th (24 DAP) of September, and the 1st (29 DAP) and 7th (35 DAP) of October, in the two soil treatments (SnC and SC), and the 4 treatments of N (141N, 118N, 94N and 70N). Values are the estimated mean of the model ± confidence interval (n=4) and the letters indicate differences according to the post hoc analysis.*

Pearson correlation analyses was carried out on plant area observed at different dates and yield observations, see Table 4.3. The analysis showed significant correlations at all dates.

*Table 4.3: Pearson correlation coefficient of the correlation between yield and plant area at different dates, * indicates significant correlation between the two variables p<0.001.*

| **Correlation between plant area and yield:** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **12th Sep.** | **18th Sep.** | **23rd Sep.** | **26th Sep.** | **1st Oct.** | **7th Oct.** | **15th Oct.** | **28th Oct.** |
| 0.74* | 0.8* | 0.83* | 0.88* | 0.87* | 0.89* | 0.86* | 0.72* |

The results of the root observations showed no effect of the soil and fertilizer treatment nor the interaction between the two for 6 of 8 observation dates, see Table 4.4. For observations the 28th of October (56 DAP) there was a significant effect of the fertilizer treatment. For observations the 12th of September (10 DAP) an interactive effect was shown; however, the visual inspection and the Shapiro-Wilk test (P=0.0012) indicates that the data is not normal distributed, therefor this is not presented.

*Table 4.4: P-values of two-way ANOVA on root counts on the different dates of observations. Numbers in bold indicates a significant effect with a P-value lower than 0.05.*

| DAP | Interaction of soil and fertilizer | Soil effect | Fertilizer effect |
|---|---|---|---|
| **10** | **0.0469** | 0.0611 | 0.683 |
| **16** | 0.198 | 0.153 | 0.887 |
| **21** | 0.188 | 0.135 | 0.513 |
| **24** | 0.266 | 0.408 | 0.367 |
| **29** | 0.143 | 0.906 | 0.0694 |
| **35** | 0.774 | 0.256 | 0.704 |
| **43** | 0.549 | 0.679 | 0.465 |
| **56** | 0.435 | 0.556 | **0.0413** |

There was not found any significant difference between the different levels of N fertilization of root counts at the 28th of October. However, the result seems to show a higher intensity of roots in the 118N fertilizer treatment, see Table 4.5.

*Table 4.5:Estiamtes of root crossings per window (25 x 21 cm) from the model ± confidence interval (n=8). Ns indicates not significant.*

| | **28th of October** |
|---|---|
| **141N** | $72^{ns\pm13}$ |
| **118N** | $86^{ns\pm13}$ |
| **94N** | $71^{ns\pm14}$ |
| **70N** | $70^{ns\pm14}$ |

Figure 4.3 shows the intensity of root crossings on the different observation dates. It shows a considerable drop in root crossings the 15th of October (43 DAP). This observation was made the 15th of October just a few days after a heavy rainfall on 11th of October, Figure 3.3.

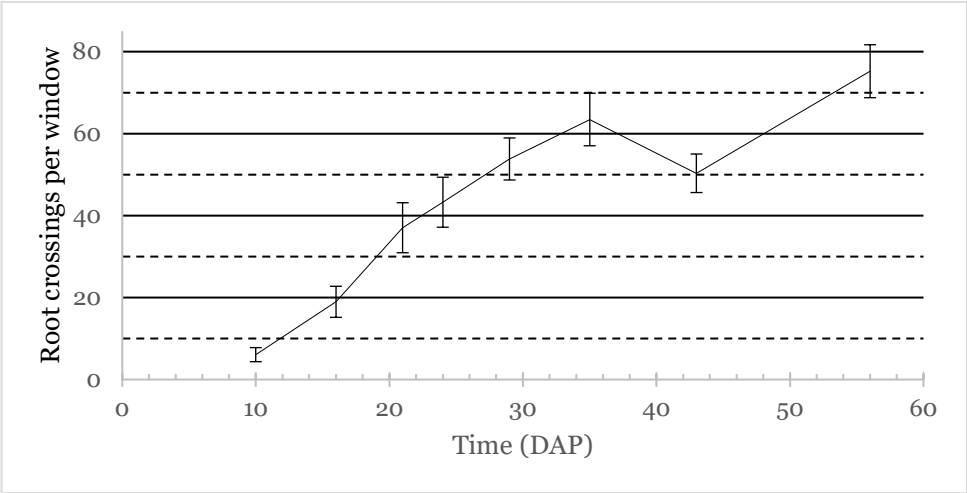


*Figure 4.3:Root intensity development of the 8 observation dates 10, 16, 21, 24, 29, 35, 43 and 56 DAP. Estimates of root crossings per window (25 x 21 cm) from the model ± confidence interval (n=32).*

## 4.2 Soil N

In august for the topsoil layer 0-0.25 m there was a significant effect of soil both for initial mineral N ($P=6.13 * 10^{-6}$) and mineral N after 28 days of incubation ($P=9.31 * 10^{-6}$) with soil samples taken in the two fields of the farmer. In October 0-0.25 m layer soil samples were taken in the mini-plots, for mineral N there was an effect of the soil treatment (P=0.0306), whereas for the fertilizer treatment (P=0.850) and the interaction between the two treatments (P=0.132) no effect was found. There was an effect of block (P=0.0271), therefor this was taken into account for the model. For mineral N after 28 days of incubation there was no effect of the soil treatment (P=0.759) nor the interaction between treatments (P=0.0975), for the fertilizer treatment there was almost shown a significant effect (P=0.0546). Block was insignificant (P=0.174) for samples in October after 28 days of incubation and not include in the model.

A statistical analysis for mineral N in August for soil layers 0.25-0.5, 0.5-075 and 0.75-1 m could not be made because only two samples were taken at the experimental site between the mini-plots. In October for layers 0.25-0.5 and 0.5-0.75 m there was found no effect of the soil (P=0.152 and P=0.710) and fertilizer treatments (P=0.600 and P=0.173) nor the interaction between them (P=0.0790 and P=0.0792). For the 0.75-1 m soil layer there was an effect of the fertilizer treatment (P=0.0170), but no effect of soil treatment (P=0.610) nor the interaction between treatments (P=0.230). For this layer there was a block effect (P=0.00117) which was included in the model.

The SC upper soil layer 0-0.25 m had initially higher level of N, but after 28 days of incubation SnC soils had the highest level of N (kg N $ha^{-1}$), see Table 4.6. In October the effect was less apparent, but also highest for the SC treatment for the initial content of N. For the soil mineral N after 28 days of incubation there was an indication of an effect of the fertilizer treatment, with the 118N treatment as the highest and 141N as the lowest, however this was not found significant.

For the deeper soil layers there was found an difference for the fertilizer treatment at 0.75-1 m, with the highest N level for the 94N treatment and lowest for the 70N treatment, see Table 4.6. From start of the experiment until harvest there was substantially lower levels of N, especially in the deeper soil layers 0.25-0.5, 0.5-0.75 and 0.75-1 m.

*Table 4.6: Model estimates of soil mineral N for initial soil samples and after 28 days of incubation, superscript letters indicate significant differences between treatments according to post hoc analysis, ± confidence interval (n=16 for the soil treatment and n=8 for the fertilizer treatment).*
**The first soil samples for the 0-0.25 layer was taken directly in the farmers fields 21st of august, and the deeper soil layers 0.25-0.5, 0.5-0.75 and 0.75-1 m was taken at the experimental site the 9th of September.*

| | **Soil mineral N ($kg\ N\ ha^{-1}$)** | | | | |
|---|---|---|---|---|---|
| | **0-0.25 m** | **0-0.25 m (incubation)** | **0.25-0.5 m** | **0.5-0.75 m** | **0.75-1 m** |
| ***Soil samples from the 21st of August and the 9th of September**** | | | | | |
| SnC | $35.83^{b} \pm 1.89$ | $148.9^{a} \pm 5.55$ | - | - | - |
| SC | $51.91^{a} \pm 1.89$ | $104.9^{b} \pm 5.55$ | - | - | - |
| Between mini-plots | - | - | 110.9 | 50.92 | 28.07 |
| ***Samples from experiment 30th of October*** | | | | | |
| SnC | $17.82^{b} \pm 3.17$ | - | $8.86^{ns} \pm 1.60$ | $4.19^{ns} \pm 1.15$ | - |
| SC | $20.85^{a} \pm 3.17$ | - | $7.78^{ns} \pm 1.60$ | $4.48^{ns} \pm 1.15$ | - |
| 141N | - | $93.59^{ns} \pm 5.19$ | - | - | $5.76^{ab} \pm 2.67$ |
| 118N | - | $102.6^{ns} \pm 4.95$ | - | - | $5.23^{ab} \pm 2.67$ |
| 94N | - | $95.04^{ns} \pm 4.95$ | - | - | $7.35^{a} \pm 2.67$ |
| 70N | - | $97.26^{ns} \pm 5.24$ | - | - | $3.40^{b} \pm 2.67$ |

## 4.3 Soil enzyme activity and microbial biomass

P-values of the ANOVA test for testing effect of the treatments on enzyme activity and microbial biomass (C, N and P), can be found in Table 4.7. For soil samples of august, there was an effect of soil for β-GA and all microbial biomass assays. For the DHA assay there was found no significant effect of soil.

Homogeneity of variances for data of MBN was not approved by the Bartlett test (P=0.0169). Looking at the data the four measured MBN data points of the SC soil treatment, are 60.43, 62.85, 60.05 and 68.97 $mg\,kg^{-1}$. During the sampling and analysis nothing unusual was reported, however since 3 of the data points are close around 61 the datapoint at 68.97 could be a possible outlier. If 68.97 is marked as an outlier variance homogeneity is visually obtained, the Bartlett test could not run with uneven number of replicates. With the outlier there is still an effect of the soil treatment.

For the samples in October there was an effect of soil for both enzyme activity and microbial biomass. For DHA there was a block effect (P=0.00305) and an effect of both soil and fertilizer treatment.

*Table 4.7: P-values of two-way ANOVA, Shapiro Wilk and Bartlett test on dehydrogenase activity(DHA), β-glucosidase activity(β-GA) and microbial biomass C, N, P (MBC, MBN, MBP). Numbers in bold indicates a significant effect with a P-value lower than 0.05. For MBN* the datapoint 68.97 for the SC treatment is marked as an outlier.*

| | Interaction of soil and fertilizer | Soil effect | Fertilizer effect |
|---|---|---|---|
| **Soil samples from fields 21st of august** | | | |
| **DHA** | - | 0.162 | - |
| **β-GA** | - | **0.00136** | - |
| **MBC** | - | **0.0217** | - |
| **MBN** | - | **0.00100** | - |
| **MBN*** | - | $\mathbf{1.25 * 10^{-5}}$ | - |
| **MBP** | - | **0.0211** | - |
| **Soil samples from experiment 11th of October** | | | |
| **DHA** | 0.529 | $\mathbf{3.65 * 10^{-4}}$ | **0.00623** |
| **β-GA** | 0.570 | **0.00327** | 0.631 |
| **MBC** | 0.720 | $\mathbf{4.75 * 10^{-4}}$ | 0.609 |
| **MBN** | 0.342 | $\mathbf{7.29 * 10^{-4}}$ | 0.0823 |
| **MBP** | 0.0897 | **0.00562** | 0.505 |

Estimated values of the enzyme activity and microbial biomass can be found in Table 4.8. The pattern was the same both for the assays in August and October. β-GA was higher for the soil treatment SnC in both assays. DHA was not significantly affected by the soil treatment when the assay was conducted in august, which might be partly due to a large variation in the data

indicated with a confidence interval of ±21.65, and the data was without any indication of single outliers but generally with a large variability for all data points. However, there seems to be a tendency for a higher enzyme activity in the soil treatment SC. For the assay in October the there was a significantly higher activity in the SC treatment. For the fertilizer treatment there was a significantly higher activity in the 70N treatment compared to the 141N treatment. For the microbial biomass the soil treatment SC resulted in a significantly higher biomass for all three.

*Table 4.8: Model estimates for enzyme activity (β-GA and DHA) and microbial biomass (MBC, MBN and MBP), superscript letters indicate significant differences between treatments according to post hoc analysis, ± confidence interval (n=16 (soil treatment)/8 (fertilizer treatment)). For MBN* the datapoint 68.97 for the SC treatment is marked as an outlier.*

| | **β-GA** | **DHA** | **MBC** | **MBN*** | **MBP** |
|---|---|---|---|---|---|
| **Soil samples from fields 21st of august** | | | | | |
| **SnC** | $116.95^{a\pm7.80}$ | $130.31^{ns\pm21.6}$ | $266.36^{b\pm29.2}$ | $61.11^{b\pm1.64}$ | $3.53^{b\pm0.429}$ |
| **SC** | $91.65^{b\pm7.80}$ | $150.24^{ns\pm21.6}$ | $318.30^{a\pm29.2}$ | $75.52^{a\pm1.42}$ | $4.30^{a\pm0.429}$ |
| **Soil samples from experiment 11th of October** | | | | | |
| **SnC** | $154.85^{a\pm13.2}$ | $92.75^{b\pm7.71}$ | $316.76^{b\pm11.3}$ | $58.98^{b\pm4.66}$ | $4.89^{b\pm0.842}$ |
| **SC** | $115.01^{b\pm13.2}$ | $104.42^{a\pm7.71}$ | $349.71^{a\pm11.3}$ | $70.70^{a\pm4.66}$ | $6.69^{a\pm0.842}$ |
| | | | | | |
| **141N** | - | $92.02^{b\pm8.45}$ | - | - | - |
| **94N** | - | $99.47^{ab\pm8.45}$ | - | - | - |
| **70N** | - | $104.27^{a\pm8.45}$ | - | - | - |

# 5. DISCUSSION

The field experiment was initially established for the purpose of testing three factors: the effect of compost (1) in the long-term and (2) short-term, and (3) the response to different N fertilization doses. However, when the experiment was conducted, compost application (short-term effect) was only given to the mini-plots with soil from a field that had been given compost for several years (SC) (long-term effect), and not to those mini-plots with soil from a field that had not been given compost (SnC). This meant that short-term compost effect was not an individual factor in the experimental design as originally planned, and instead two extra levels of N were included. Therefore, it is a mixed soil effect, with both long- and short-term compost

effect. Additionally, other aspects of the two farmer soils e.g. cropping history has influence of the soil treatment effects, which therefore restricts the conclusion of the experiment on compost application.

## 5.1 Plant growth and N dynamics

*Effect of soil and compost*

The soil treatment SnC showed increased growth compared to the SC soil treatment. There was a significantly higher harvest yield (Table 4.1), and the plant area observations shows a greater growth, see Figure 4.1. Observations the 12th of September (10 DAP) shows that the plant area was higher for the treatment SnC than SC, and fertilizer was not applied until the 17th of September (15 DAP) this indicate that the nutrient availability or other growth factors of the two soils or the applied compost had an effect on the growth.

Other studies have shown variable results of compost application on plant growth ranging from decreases of 495% to increases of 52%, with application rate, number of applications over time and management factors such as supplementing with inorganic fertilizers largely impacting on plant growth (Martínez-Blanco et al., 2013). Often results depend on whether compost application is compared to controls with no treatment or compared to a mineral fertilizer treatment of the same nutrient content as the compost. Higher yields are often found when compost application is compared with no treatment, while a comparison with a fertilizer treatment similar to the compost contents has most often no effect or a negative effect of compost application (Martínez-Blanco et al., 2013). In this experiment compost N content was low and the applied compost accounted for 3.2 kg N $ha^{-1}$. Initial soil N was determined at 42 (SnC) and 51 kg N $ha^{-1}$ (SC) see Table 3.3, which was accounted for in the N application rates see Table 3.2. However, any initial fertilization effect of compost was in this experiment eliminated, and the tested effect was compost effects on soil properties and potential N mineralization.

Initially readily available N was higher in the SC soil than the SnC see Table 3.3 and Table 4.6, however the assay of mineral N after 4 weeks of incubation shows that 44 kg N $ha^{-1}$ was mineralized for the SnC more than the SC soil see Table 4.6. The mineralization in the assay at 25°C and fully moist all the time does not fully reflect the mineralization taking place in the soil

in the experiment, however it gives an indication that more N was available in the SnC mini-plots. The mini-plots got fertilization at the 17th of September, and initial soil samples was taken the 21st of august, after which soils were left on a truck bed outside until it was screened at applied to the tubs. This means soil mineralization could take place for 27 days before additional fertilizer was given. This means that plants in the SnC treatment possibly had more plant available N at the start of the experiment and in the first 15 days of growth. This difference of plant available N might explain why growth for SnC was greater than SC.

The compost added to the experiment was seemingly mature. $NH_4^+$ is a metabolic product and an indicator for maturity (Benito et al., 2003), and a maximum $NH_4^+$:$NO_3^-$ –ratio of 0.16 is suggested as a indicator for mature compost (Bernal et al., 1998). The compost used had a $NH_4^+$:$NO_3^-$ –ratio at 0.12 see Table 3.3, indicating the compost was mature. Another indicator the C:N ratio at 14.95 was above the suggested maximum at 12 (Bernal et al., 1998), however not substantially higher. The composting method applied for the compost, that was used in this experiment, was very simple and without any control throughout the composting process. The biomaterial was placed in a large pile and was without any active aeration conducted, e.g. by turning the pile at certain time intervals. The lack of control of the composting process and indications above mentioned, makes it uncertain whether the compost was fully mature.

A negative effect of compost can be immobilization of available N. If compost is immature, it is capable of leading to N immobilization which result in less N available for the crops, due to N uptake of microorganisms decomposing organic matter (Gagnon & Simard, 1999). A study on cabbage showed lower yields due to immobilization of N in a field experiment with compost of different maturity (Mourão et al., 2012). Another aspect of immature compost is that it has been proven to have a negative effect on plant growth due to phytotoxic elements (Griffin & Hutchinson, 2007; Wilkinson et al., 2009). Additionally the base material was pruning waste (leaves and branches) and grass cuttings, containing a high share of woody material, which has been shown to be appearing mature due to general appearance and absence of bad odor, however requiring a longer maturing period (Gagnon & Simard, 1999).

These above-mentioned aspects of the soils and compost used might explain the decreased growth of the SC soil treatment, however the way the experiment was conducted does not make

it possible to separate the effect from the soil from the fields and the effect of the compost applied.

*Root growth*

The root observation was insignificant. The lack of significant difference on root observations, could be an indication that there is not a difference on lettuce roots with these treatments or that the methods is not delicate enough to see these differences. The last observation at 56 DAP there was a significant effect of the fertilizer treatment, however there was not found any differences between the 4 fertilizer treatments, see Table 4.5.

It has been found that 78% of the total root growth of lettuce is in the upper soil layer (0-20 cm) (Gallardo et al., 1996; Johnson et al., 2000), which means by observing through the windows at about that depth should be at optimal.

Other studies on lettuce has shown an effect of different soil types on root growth (Neumann et al., 2014) and Solaiman et al. (2019) found an effect of compost on root growth, however these effects was found after roots were washed.

For a further evaluation of the root window method used in this experiment, a comparation with root analysis after root washing of total root fresh weight, root length etc. would have been useful to support the insignificant results found.

In general, the data of root are counts, and is not expected to be normal distributed due to the fact that it is counting's, and these is per definition not normal distributed. However, summing counts from each window, the data becomes closer to being normal distributed, and 7 of 8 observation dates passed the Shapiro-Wilk test and visual inspection of the data, therefor the linear model was still used for the ANOVA. However, this might be the explanation of the root counting's of the first date 10 DAP failed the Shapiro-Wilk test, where the counts were positively skewed, due to very low root counting's in several root windows, due to limited root growth. A log transformation did not correct the data; however, it became closer to being normal distributed with a Shapiro-Wilk test at P=0.0206 compared to 0.0012 for the data without log transformation.

*Plant area as a yield predictor*

For the plant area observations there is seen a tendency for a larger difference between plant area for the two soil treatments at the observations the 1st (29 DAP), 7th (35 DAP) and 15th (43 DAP) of October, see Figure 4.1. These dates and the 26th of September (24 DA) the highest increase in plant area is observed , which might indicate this is the rosette phase of the plant development and this is the point in the plant development where plant area or plant diameter is increasing the most and is most effective for showing differences between different treatments due to the fact that differences of plant area is most visible in this period. It should also be emphasized that there is seen a trend of higher correlation for observations of yield and plant area the 26th of September, 1st, 7th and 15th of October, see Table 4.3. The correlation at the last observation date 56 DAP at 0.72 is seemingly lower than the previous 4 dates with correlations between 0.86 and 0.89. This might give an indication that the plants were in the heading phase of the plant development at the last observation, where the increase in area or diameter is smaller than in the rosette phase. At the first date the correlation is also low at 0.74. This might indicate that the growth is still in the seedling phase. These considerations could point out that the method of plant area or diameter is mostly effective in the rosette phase of lettuce where the increase mainly occurred. The use of plant area to predict yield has been shown for cabbage by Yang et al. (2008). However the use of precision agriculture technologies is not yet very widespread either in research nor in the production (Suarez et al., 2018).

*N dynamics*

The fertilizer treatment does not show an overall significant effect on plant growth in the experiment, although N fertilizer would be expected to have a mayor effect on lettuce yield (Sylvestre et al., 2019). There were a few observations with an effect of the fertilizer treatment for the plant area observations see Figure 4.2, however there was not found any pattern of this effect.

The lacking effect due to fertilization treatment, might be explained by the termination of the growth. Harvest of the lettuce was conducted due to low temperatures and risk of frost in the end of October; therefore, the lettuce was not grown to full size or the recommended 11 weeks when grown in summer/autumn in Denmark (HortiAdvice, 2015). At harvest this meant that the weight of the heads was notable lower than what would be expected, with a size 20-50 % of

what is found in other experiments on comparable Batavia type lettuce on the fresh weight (Missio et al., 2018; Nicoletto et al., 2014; Yordanova et al., 2020). The fact that the experiment was terminated before full growth, makes it possible that none of the four doses was limiting growth substantially, and therefore the impact of the fertilizer treatment is not noticeable.

In the soil used in the experiment there was initially 42 (SnC) and 51 kg N $ha^{-1}$ (SC) and additionally the assay of mineral N after 4 weeks incubation showed that potentially 149 (SnC) and 105 kg N $ha^{-1}$ (SC) was available from the soil after the first 4 weeks. The fertilizer was applied 15 DAP at four rates from 25/22.5 up to 100/90 kg N $ha^{-1}$ (SnC/SC). The relatively high soil N, the late application of mineral fertilizer and the early termination, might make the impact of fertilizer treatments restricted.

Calculating the crop N-use efficiency ($NUE_{crop}$) as described in (Martinez-Feria et al., 2018) for the soils with different N fertilizer doses, from scaling up the results per head in the mini-plots (0.3024 $m^2$) with 8 heads. Gives a plant N content of 42.18 (SnC) and 41.40 $kg\ N\ ha^{-1}$ (SC), and a $NUE_{crop}$ for SnC at 1.68, 0.84, 0.56, 0.42 and for SC 1.84, 0.92, 0.61, 0.46 for the 70N, 94N 118N and 141N fertilizer treatments with 25/22.5, 50/45, 75/67.5 and 100/90 mineral N $ha^{-1}$ applied (SnC/SC) respectively. These indicate that soil N was taken up by the plants in the 70N fertilizer treatment, whereas more N was applied than taken up by above ground plant biomass in the others, the amount of N in the roots were not measured. This calculation however is linked with uncertainty due to the fact that only 17 mini-plots had 8 plants at harvest. Therefor the mini-plots with fewer than 8 plants had more N available.

The $NUE_{crop}$ and the fact that the plants did not grown until maturity indicates that the lack of a fertilizer effect could be explained by lack of growth limiting effects of the doses chosen in experiment.

Observations of N in the soil layers show a substantial decrease of N in all layers from August till October (Table 4.6). This decrease can either be taken up by plants or microorganisms in the soil, or it is leached out of the root zone. Microbial biomass N has apparently dropped from August till October, indicating the microorganisms has not taken up N, see Table 4.8. Due to the $NUE_{crop}$ calculations there is an indication that the N in the root zone was lost to leaching, however the leachates of the root zone were not studied.

## 5.2 Soil microbial life and SOM

The pattern of microbial activity and microbial biomass was the same for the assays conducted in August and October, see Table 4.8. It should be emphasized that the sample of august was without additional compost applied, which was not applied until the start of the experiment. This indicate that the effect is mostly found in the soil, which might be explained by the long-term compost effect of the SC soil, however other differences of the two soils cannot be eliminated as the source of the soil treatment effect.

DHA was higher for the SC treatment whereas β-GA was higher for the SnC treatment. The fertilizer treatment also had a significant effect on DHA, with the highest activity for the 70N treatment and lowest for the 141N treatment, see Table 4.8. For microbial biomass (MBC, MBN and MBP) there was higher contents in the SC soil.

The fertilizer effect on DHA showed that the lowest application of N gave the highest activity in mid-October. However the reason for this is unknown, it could be a lower activity due to limitations of N for the decomposing organisms extending the period of decomposition further, so that earlier on the activity was higher for the 141N, but at this point the stock readily available C is used resulting in a lower activity. However, the effect was only seen for DHA, and not found in the literature.

The diverse effect on DHA and β-GA with the two soils unusual, other studies show equal effect for both enzymes when testing effects of compost application or other methods for increasing soil fertility (Bastida et al., 2008; Chang et al., 2007; Tejada & Gonzalez, 2007). Elevated enzyme activity for both enzymes and increased microbial biomass was found by Chang et al. (2007) with application of organic fertilizers compared to chemical fertilizer treatments.

The conflicting effect on the two enzymes tested on the two soil treatments SnC and SC, could not be identified by looking into the literature. However, the fact that the difference was apparent before compost was applied, indicates the cause should be found in the soil properties of the two soils used. This conflict blurs the picture, but the microbial biomass and the DHA indicates that the SOM matter content is increasing in the SC soil, due to recent emerging consensus that microbial materials is an important constituent of stable SOM (Kallenbach et al., 2016). Ndiaye et al. (2000) also found both microbial biomass and enzyme activity as a good indicator for soil quality with altered management practice of using cover crops. This potential

indication of increasing SOM content could be due to the many years of compost application of the SC field.

Initial samples of the two soils showed a higher content of organic C in the soil from the field that did not get compost (SnC), see Table 3.3. However, the increased microbial activity and increased microbial biomass could be an indication of a higher present increase in the SC soil due to compost application and will potentially reach the same level of organic C as the SnC. A survey of several fields with compost application in California showed both increased SOM and increased microbial activity, when compared to control fields (Brown & Cotton, 2011).

## 5.3 Methodological considerations

The method of the experiment was generally developed as part of the project. One of the main aims of the project was to test soils of different origin with focus on compost history. The following is a discussion of different aspects that was observed as possible concerns of the setup.

### *The semi-field experimental approach*

The semi-field experiment is an attempt to test soils of different origin and soil treatments with vegetable crops in a statistical sound design and with a high degree of control with conditions comparable with those found in the field. Experiments with vegetable crops are generally more complex compared to arable crops (cereals and similar crops), due to several aspect, such as intensive management, high labor requirements to carry out the experiment etc. Which makes it favorable to limit the number of plants in relation to limited the costs (Lúcio & Sari, 2017).

The semi-field approach is an attempt to get a higher degree of control than field experiments and improved reproducibility of soil treatments, but which resembles field conditions better than pot experiment. For instance, in this setup, we have control of the topsoil layer, and it is possible to test soils that have different history and origin, and different applications of treatments. By enclosing the plot, it is assumed that the plants access nutrients or growth stimulating substrates inside the installed tubs, and with the choice of lettuce as model crop with its main root mass in the 0-20 cm soil layer (Gallardo et al., 1996; Johnson et al., 2000), the plants are mostly restricted to the treatment applied in the tub. In that way it is possible to make a statistically sound design even with a small number of plants. The reduction of variability would be bigger for pot experiments in a greenhouse, however the open field setup

better reflects the circumstances in the production with natural levels of light, air and soil temperature (Füller et al., 2012).

The semi-field setup is a midway of the field and pot experiment. The use of the term semi-field is not uniform, however in general semi-field or mini-plot experimental setups are an intermediate solution between pot and field experiments. Li et al. (2016, 2017) conducted a mini-plot field experiment, with similarities to the semi-field experimental setup in this project, in these studies on phosphorus availability in biochar and different biomass ashes.

Pot and field experiments are the common methods doing experiments with plants and soil, which holds different advances. Both pot experiments (Papafilippaki et al., 2015; Vaverková et al., 2020) and field experiments (Lucas et al., 2018; Tits et al., 2014) are common, when testing effects of compost.

With pot experiments in greenhouse there is a high degree of control, e.g. climatic conditions can be controlled, contents of the pots (e.g. soil, treatments etc..) can be exactly determined and mixed thoroughly to form a homogeneous growth medium, measurements can be made regardless of the weather, they are often less labor intense with regard to crop management operations such as weeding, harvest etc. Pot experiments has some evident advances in matter of control and they are less laborious (de Vries, 1980). However sometimes they give erroneous results in relation to field experiments (de Vries & Tiller, 1978) and might show effects that cannot be found in the field.

Field experiments on the other hand generally lack control. Designing these for testing can be challenging and for vegetable its heavily labor intense, however these conditions are much more representative of the reality of the growers and farmers, which makes the results more applicable. The uncontrollable environmental disturbance in field experiments compared to greenhouses is much bigger, which gives a large unexplained variability in the data. This unexplained variance could to some degree be overcome by larger area, more repetitions and a design taking block effect into account. However, in horticulture the cost to maintain crops in a vegetable field demand intensive management and high labor requirements (Lúcio & Sari, 2017), which makes it a challenge to do experiments on large areas.

*Sample size and variability*

The cropping system has a huge impact on the variability of the response variables e.g. of fresh biomass production, Lúcio et al. (2016) found a higher variability of lettuce grown at field conditions than grown in a greenhouse. The reason for the different variability in the cropping system could be due to the variable gradients of moisture, physical and chemical characteristics, which is found in soil in protected environments as greenhouses but is more severe in open environment like field experiments.

Conducting an experiment with plants a sufficient sample size is crucial to overcome the variability of the experimental units of different measurements e.g. plant growth and nutrient uptake (Lúcio & Sari, 2017). Weightman et al. (2006) showed in a study that by increasing the sample size from 10 to 40 plants, that it could be expected to decrease the standard error of measurements of tissue nitrate concentration from 16 to 12 % of the mean. In this experimental setup each mini-plot or experimental unit has 8 plants which is also the sample size. The sufficient number of plants to overcome the variability of the growth in a plot has been tested, but results are not clear, one study found 5 plants to be sufficient (Lúcio et al., 2016), and another study found 33 plants as sample size to be sufficient (Santos et al., 2010).

This aspect was not tested for our setup, but it could be an interesting thing to considered for the different observations and analysis conducted in this experiment to evaluate on how large differences of the different treatments has to be to stand out in the setup. For example, with the root observations the sample size is one window per experimental unit, which might not be sufficient to see differences on the root growth. In practices the setup restricts the possibilities of having additional sampling, e.g. the mini-plots could only contain 8 plants, the laboratory capacity did not make it possible to have additional samples for analysis, however the consideration could be valuable.

Another issue was the death of plants, only 17 mini-plots had all 8 plants at harvest. When less plants grew in some tubs additional nutrients and less competitions were present.

*Testing soil of different origin*

In the setup in this project a possible weakness in comparing the two different soils is the fact that the soil was collected at one site in each field. By taking the soil from one site, any site-specific properties of soil in the field would be strongly represented in the experiment.

This is a challenge also found in pot experiments. Wang et al. (2015) conducted a pot experiment taking soil from a long-term field experiment, mixing soil from the 4 replicates of each treatment collected evenly from the plots in the field with a soil core sampler of 5 cm in diameter, then using these composite samples as a treatment in the pot experiment, a similar procedure was used by Röing et al. (2005).

A possible solution could be to collect the soil in a similar manner as the pot experiments described above, by collecting the soil at several sites in the field, and then mixing them together and then using them as a soil treatment.

*Use of farmer field soil*

In the experiment soil was taken from fields with different compost history, but otherwise with similar history and management practice. The two field were of the same farmer and in the same area, however 1.000 m apart which might make up local difference and this can also have influence on the soil.

The two fields were generally comparable, however field history differed, which might influence the content of SOM and soil life interactions with it. For cropping history it is especially the three year grass seed crop in the SnC field 2012-2014 (Table 3.1) that make up a major difference, which possibly has influence on SOM as being a strong method for increasing SOM content. Continuous grass is seen as significantly effective when it comes to storing carbon in the soil. It is found to have an effect of 0.95 $Mg\ C\ ha^{-1}yr^{-1}$ addition to the 0-25 cm soil layer (Taghizadeh-Toosi et al., 2014). In another study a six year experiment with 3-4 cuts of grass and application of mineral fertilizer, 1.1 $Mg\ C\ ha^{-1}year^{-1}$ was stored on average over the period (Christensen et al., 2009).

This means that the two soils that were investigated in the experiment, was two soils with different history, rather than specifically the compost effect. However, it should be emphasized that one of the major differences of the two fields is still long-term compost application. This influence the conclusions that can be made from the experiment.

For a repetition of the experiment the use of soil should be considered. For a new setup soil from long term experiment could be considered, which would possibly give a lot more field information and make it possible to collect the soil in the repetitions of the different treatments used.

*Available soil nutrients*

Another weakness of the setup was an uncertainty of the amount of soil added to each tub, and thereby different availabilities of soil nutrients. In general, the procedure was to fill the tubs to an even bulk density, and in that way add the same amount of soil to each tub. However, when digging the holes for inserting the tubs, the holes had slightly different dimensions. The tubs used were not solid but flexible which meant that the width of each tub deviated after they were filled. Measurements of the tub dimensions after harvest showed that the width of 20 tubs was 42 cm ±0.5, however the last 12 deviated between 40 to 44 cm having influence on the volume. Also, the way the soil settled after harvest showed possible differences in the amount added, and possible different bulk densities when soil was added.

With a pot trail it is possible to measure exactly how much soil is added to each pot, whereas in this experiment this is only practically possible to some degree. Instead of weighing the soil, it was tried to have the same bulk density in each tub by filling them to the rim without compacting the soil.

This issue makes it uncertain that all plants has had the same conditions for the same treatments, and this adds variability to the setup negatively impacting the possibility of detecting differences between treatments.

# 6. CONCLUSIONS

## Compost and fertilizer effects

Generally, for the experiment there was found an effect of the soil treatment and less so an effect of the fertilizer treatment.

The intended testing with the soil treatment was of the long and short-term effect of compost application. However, with the soils used it is to a greater extent an effect of two to soils of different origin, and difficult to propagate these findings as a general effect of compost. Further the soil used was only representative for a minor area of the two fields. However, it should be emphasized that a major difference of the two fields was still the use of compost.

The first hypothesis of this project was enhanced growth due compost and fertilizer application, and less affected growth due to suboptimal fertilization.

The effect of fertilizer on plant growth was absent; therefore, no conclusions can be made of the fertilizer implication on growth nor the effect of compost with suboptimal fertilization rates. It was discussed that the termination before full growth and the late application of fertilizer could explain the missing effect of fertilizer.

The soil treatment with compost (SC) showed a lower crop growth than without compost (SnC), which contradicts with the hypothesis. It was discussed that the reduced growth could be due to either a lower N availability of the SC soil because of different starting mineral N levels with soil incubation N levels taken into account. Another explanation could be the effect of compost due to immaturity either due to phytotoxic effects or immobilization of N. These potential explanations cannot be separated due the mixed effect of soil and the recent compost application.

The effect on roots was insignificant. This could either be due to no effect of root growth of the applied treatments or it could be that the method used with one root window per mini-plot is not delicate enough the realize possible differences.

Looking at $NUE_{crop}$ and the risk of leaching, the results of this experiment show that the potential of N leaching was the lowest for the 70N fertilizer treatment without reducing the growth. However, the fact that the growth of the lettuce was terminated before maturity, this fertilization dose could result in limited growth if fully grown.

Findings of the experiment show an elevated microbial activity and microbial biomass in the soil treatment with compost application (SC), which was both found before and after addition of compost to the experiment. This indicates the difference is in the two soils tested. The two assays of enzyme activity were contradicting which makes the conclusion doubtful. Other differences of the two fields could also explain the difference found between the two soil treatments, and it cannot be concluded that it was the effect of compost. However, these findings could be an indication that even that the SnC soil had the highest content of organic C, the rate of the increase of SOM was bigger in the SC soil and that might be explained by a long-term compost effect.

There was an effect of fertilizer on DHA, however the conclusions of this could not be supported by literature and with lack of effect on other parameters this was not seen as a major finding.

### Proposal for a new setup

The approach with the mini-plots gave some problematic issues. With 8 plants per mini-plot and up to 3 death plants per mini-plot, this added a lot of variability to the measurements of crop growth and the estimations of $NUE_{crop}$. The tubs with one window for roots observations per mini-plot gave limited observation of root growth. And then there was a struggle with determining the exact amount of soil filled in each mini-plot due to a relatively large amount of soil (115 kg) added to each tub.

For a new setup it could be considered to use clear PVC tubes with a diameter of about 15 cm and a length of 30 cm with one plant in each, and then the mini-plot would consist of a cluster of these tubes. These could be inserted in the soil in a similar pattern as the tubs. However, this would make each mini-plot uphold the possibility to conduct several root counting's per mini-plot for each tube. For each mini-plot a composite mixed soil sample of all tubes in each mini-plot could be taken for analysis microbial biomass, enzyme activity and soil Nmin, which would cover more variation of each experimental unit in relation to soil properties. The effect of plant death would not disturb the hole mini-plot, but only the tube containing the plant. This might also make it easier to determine the added soil more exactly, due to the lower volume of soil for each tube compared to the tubs.

Another consideration could be to use another model crop, still with a shallow root growth but lower need for space, which would induce the option for more plants per plot which might decrease the variability between plots of the same treatment. This could for example be spinach, leek or radish.

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