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Influence of compost, vermicompost and digestate on nutrient status and secondary metabolites, *Lactuca sativa* L. as a model.

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Plant Science – Plant Production Systems

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Andrew James Niday

Abstract

Peat is the most commonly used substrate in greenhouse cultures due to its predictability and performance. Though significant environmental costs and CO_2 emissions are associated with harvesting of peat. It is therefore of great interest to find alternative, environmentally friendly growing media. Compost, vermicompost and digestate are often rich in nutrients and thus represent an interesting alternative to peat. However the availability of nutrients in composts may be limited due to alkaline pH.

Lettuce was chosen as a model for this study due to its sensitivity to factors such as nutrient deficiency, in particular nitrogen. The composts (Mix and Farm), vermicompost (Vermi) and digestate (Biorest) were selected as growing media for this study as they represent typical feedstocks and composting techniques. We wished to examine how the media influenced nutrient status and secondary metabolites in lettuce. Plants were grown with and without fertilizer to see if the media could provide sufficient nutrients. All the alternative media had alkaline pH, and thus lettuce grown in these media were deficient in several nutrients, even when fertilized.

Lettuce grown in both Vermi treatments had sufficient levels of nitrogen (3,5 %), and high levels of potassium (11 %), however growth was inhibited due to other factors, possibly related to high pH. Lettuce grown in both Biorest treatments had high stomatal conductance and chlorophyll levels, but low biomass production. Lettuce grown in the alternative media typically had higher levels of flavonols and anthocyanins than the control, particularly when unfertilized. Plants grown in Farm had the highest levels of anthocyanins and flavonols. Antioxidant levels were twice as high for lettuce grown in Mix and Control when unfertilized, and were generally higher in all unfertilized treatments, with the exception of Vermi. The same trend was seen for total phenol levels, which were higher in unfertilized treatments.

There appears to be an influence of media type on flavonols, anthocyanins, antioxidants and phenols, which can partly be explained by nutrient availability in the media. However adjustments to pH and other parameters would likely have to be made before using compost vermicompost, and digestate as growing media.

Sammendrag

Torv er det mest vanlige mediet i veksthuskulturer på grunn av dets forutsigbarhet og ytelse som dyrkningsmedium. Skjønt uttak av torv bærer med seg betydelige CO^2-utslipp og andre miljøkostnader. Det er derfor av stor interesse å finne alternative, miljøvennlige dyrkningsmedier. Kompost, meitemark-kompost og biorest har ofte høyt innhold av plantenæringsstoffer og representerer et interessant alternativ til torv. Til tross for deres høye innhold av næringsstoffer kan det være en utfordring at disse ikke er tilgjengelige for planten.

Dette studiet valgte salat som en modellplante på grunn av dens sensitivitet til blant annet næringsmangel, særlig nitrogen (N). Materialene som ble valgt som dyrkningsmedium; kompost (Mix og Farm), meitemark-kompost (Vermi) og biorest (Biorest) representerer typiske råstoff og nedbrytningsprosesser. Vi ønsket å undersøke hvordan ulike medier kan påvirke næringsopptak og sekundære metabolitter i salat. Salaten ble dyrket både med og uten gjødsel for å se om mediene kunne forsyne tilstrekkelig med næring. Alle de alternative mediene hadde høy pH, og følgelig var det mangel på flere næringsstoffer i de fleste behandlinger, selv når det var tilført gjødsel.

Salat som var dyrket i meitemark-kompost (Vermi) hadde tilstrekkelig med nitrogen (3,5 %) og høyt nivå av kalium (11%), men vokste dårlig på grunn av andre faktorer, sannsynligvis relatert til høy pH. Salat som var dyrket i Biorest hadde høy konduktans og høye nivåer av klorofyll, men lav biomasseproduksjon. Salat dyrket i de alternative mediene hadde jevnt høyere nivå av antocyaniner og flavonoler enn kontrollen, især i når gjødsel ikke var tilført. Planter som var dyrket i Farm hadde høyest nivå av antocyaniner og flavonoler. Antioksidant-nivå var dobbelt så høyt for salat dyrket i Mix og kontroll når gjødsel ikke var tilført, og var generelt høyere i alle ugjødsla behandlinger, med unntak av Vermi. Den samme trenden var observert for total-fenoler, som var høyere når gjødsel ikke var tilført.

Det ser ut til å være en påvirkning av medietype på flavonoler, antocyaniner, antioksidanter og total-fenoler, som kan delvis forklares av tilgjengelighet av næringsstoffer i mediet. Likevel må pH og sannsynligvis andre parametre justeres før kompost, meitemark-kompost og biorest kan brukes som dyrkningsmedie.

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1. Introduction

In pot-grown greenhouse cultures, peat is the most commonly used substrate, though alternatives such as coconut coir have been adopted by some growers. In Norway, greenhouse lettuce is mostly grown in pots with peat and transferred to hydroponic systems when the plants reach a certain age (Fig. 1), or planted directly in the ground/soil in plastic tunnels. Peat is a non-renewable resource with significant CO2-emissions, and other environmental costs e.g. habitat loss are associated with harvesting peat for potting soil. Peat has many favorable and predictable properties as a growing media; light weight, good water holding capacity and cation exchange capacity. If these properties could be replicated in a compost-based media, growers may have a more sustainable and viable alternative growing media.

Increasingly there is a focus on sustainable practices in agriculture and horticulture, both from consumers, environmentalists and farmers themselves. At the same time there is a significant production of waste from municipal, agricultural and industrial sources. Composting is a tried and tested method of transforming would-be waste in to a useful product, and is a form of controlled decomposition similar to what happens in nature.

The object of this study was to explore the potential of composts as growing media, and to observe how different media affect the growth of lettuce during a growing period of 4-6 weeks. Lettuce was chosen as a model plant, due to its sensitivity and response to factors such as nutritional status, especially to nitrogen (N). To improve our understanding on how lettuce plants respond to different growing media, various physiological parameters were measured. Transpiration, leaf growth, biomass and antioxidant capacity measurements can give an indication of the "contentment" of plants. Furthermore, measurements of nutrient status and antioxidant power can be used in parallel with physiological measurements to improve our understanding of how plants are influenced by growing media. The materials selected for this study; compost (Mix and Farm), vermicompost (Vermi) and digestate (Biorest) represent typical feedstocks and composting techniques.

Figure 1. Pre-cultivation of lettuce in peat (left) before transferring to hydroponic systems at a certain age (right). Foto: Sissel Torre

1.1 Composts

All compost is not created equal; different parent materials will yield compost with differing qualities (Darlington, n.d.), and different techniques of composting yield different end products. Traditional aerobic composts made from e.g animal manure or garden/municipal waste are well known to gardeners and growers/farmers. Darlington (n.d.) broadly defines compost as "the product resulting from the controlled biological decomposition of organic material… with little resemblance to its original form once mature". Lazcano et al (2009) put forth criteria for compost regarding biostabilization; the product should be matured compared to its parent material and should have gone through chemical, microbiological and biochemical changes.

Vermicomposting (with earthworms) is another technique that is gaining interest and is a process that can be used on both raw materials and mature composts. Vermicompost is the result of an interaction between earthworms and microorganisms; a humus-rich, earth-like substrate with a texture somewhat similar to peat (Lazcano et al., 2009). Worm castings are rich in nutrients (Hernandez et al., 2010), but also appear to have growth enhancing properties that go beyond nutrient availability (Ali et al., 2007).

Production of biogas is achieved by anaerobic fermentation, and may thus be deemed a form of composting. In short, gas is extracted from the fermentation vessel and the liquid and solid fractions of digestate are separated. The solid fraction of digestate can be used as growing media or as a component of potting mix. As with compost, the properties of digestate depend on the nature of the feedstock used and the digestion process (Nkoa, 2014) For more details on the process of anaerobic fermentation for biogas production refer to Möller and Müller (2012). Digestate is a relatively new and promising product, but would in most cases need further treatment or processing before use as a growing media. However, due to the high level of nutrients often found in digestate, it shows promise as a soil conditioner or fertilizer (Nkoa, 2014).

When considering the use of compost as potting media, several factors must be taken into account. The quality or horticultural/agricultural suitability of a compost is of the utmost importance. Compost may have high, but unbalanced levels of nutrients and often needs to be mixed with other substrates to achieve a media with desirable characteristics. Perhaps the most significant criteria for the suitability of compost is maturity; a mature compost does not continue to decompose and heat up due to microbial acitivity, and should be free of phytotoxic substances (Clark and Cavigelli, 2005). Composting is a biological process, and thus difficult to replicate exactly from one batch to the next. This inconsistency may be a barrier preventing growers from replacing peat based media with compost. Other factors relevant to the use of compost as potting media are consumer preferences and environmental concerns. Peat is a non-renewable resource, whereas compost mimics nature's own system for decomposing and recycling. Consumers are becoming more conscious of sustainable practices in food production, and growers who adopt better practices may have an advantage with an increased demand for sustainably produced food.

1.2 Some factors influencing plant growth

Plants are strongly influenced by their environment, half of which is in the soil or substrate they grow in. The environment under which plants are grown influences for example morphology, yield and quality. Nutrient uptake is strongly influenced by the pH in the soil or growing media (Marschner, 2012). It is well known that different plants prefer or tolerate different ranges of soil pH, but the availability of nutrients can be limited if the pH is too high or too low (Fig 2). Alkaline media may inhibit the uptake of phosphorus, potassium, calcium, iron, zinc and even nitrogen in strongly alkaline conditions. A simple way to quantify a plants growth rate is to count the number of leaves, but when leaf expansion is limited due to nutrient deficiency, we need to also look at biomass production to gain a better understanding.

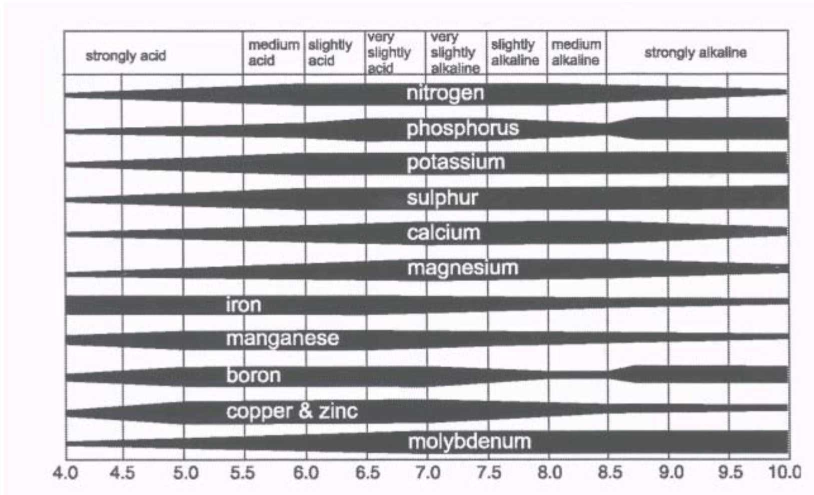

Figure 2 Effect of pH on availability of plant nutrients. Borrowed from A Handbook of Soil Science, Edition: 1st, Chapter: Chapter 3, Publisher: Soil Science Division, Editors: B H Adhikary, pp.12-22

Primary metabolism in plants involves the assimilations of water and mineral nutrients by roots, and carbon-dioxide (CO_2) by leaves, to grow and synthesize carbohydrates, proteins, lipids and nucleic acids. For satisfactory growth plants require sufficient amounts of nutrients, where nitrogen is considered the most limiting factor (Marschner, 2012). Deficiencies in nitrogen can lead to among others stunted growth, chlorosis and necrosis in leaves, and ultimately reduced yields. Nitrogen is an important element for synthesis of chlorophyll; deficiency can lead to reduced photosynthesis, yellowing of older leaves (chlorosis), and a buildup of excess carbohydrates (Taiz & Zeiger, 2010). Chlorophyll is perhaps one of the most important molecules in plants, and contains four atoms of nitrogen with a central magnesium atom. Low levels of chlorophyll may thus indicate nitrogen deficiency. In plants, the rate of transpiration is affected by nitrogen levels, temperature and many other abiotic factors. Stressed and nitrogen-deficient plants tend to have closed stomata and less transpiration. When stomata are closed, less CO_2 is fixed, and consequently plants will produce less biomass. Thus by measuring transpiration we can have an indication of the vitality in plants. Transpiration and photosynthesis are closely linked; stomatal aperture regulates CO_2 uptake and therefore also growth. The opening and closing of stomata is regulated by the stress-hormone Abscisic Acid (ABA), where elevated levels of ABA can lead to closed stomata (Taiz & Zeiger, 2010).

Phosphorus (P) is a key element in adenosine-triphosphate (ATP), is involved in energy metabolism and as a component of nucleic acids in RNA and DNA. Phosphorus deficiency may lead to delayed maturity and excess anthocyanins, and will often manifest as stunted growth and leaf necrosis (Taiz & Zeiger 2010).

Potassium (K) is involved in the activation of enzymes and regulation of osmotic potential in plant cells. Potassium also plays an important role in protein synthesis, photosynthesis and stomatal regulation (Marschner, 2012). Deficiencies in potassium can be seen as chlorotic margins and necrotic tips on older leaves and weakened stems (Taiz & Zeiger, 2010).

Calcium (Ca) functions as a second messenger between external stimuli and physiological processes and as regulatory or structural component of macromolecules (Marschner, 2012). Deficiencies in calcium may be seen as deformed young leaves and necrosis on the tips of young leaves and roots (Taiz & Zeiger, 2010).

Iron (Fe) is required for the synthesis of several proteins and is involved in the biosynthesis of chlorophyll. Thus iron-deficient leaves tend to have lower photosynthetic activity and the characteristic "lime chlorosis" (Marschner, 2012).

Zinc (Zn) is required for many enzymatic processes (Taiz & Zeiger, 2010) and is involved in the expression and regulation of genes and plant tolerance to environmental stresses (Marschner, 2012). Defiencies in zinc may be seen as shortened internodes and reduced leaf size, and is often associated with iron deficiency (Marschner, 2012).

Secondary metabolism in plants is not directly related to growth, rather the compounds formed serve a purpose as attractants, repellants and communication between plants and microorganisms. The enzyme phenylalanine ammonia lyase (PAL) is involved in the synthesis of an important group of secondary metabolites known as phenolic compounds. Under conditions of low nutrient levels, PAL activity is increased, and resources are redirected so that more phenolic compounds are produced (Taiz & Zeiger, 2010). One of the major classes of phenolics are flavonoids, which are comprised of, among others, visible light absorbing (pigmented) anthocyanins and ultraviolet light absorbing (invisible to humans) colorless flavonols. Phenolics, flavonols and anthocyanins have antioxidant activity in the plant, and higher levels are often observed in stressed plants. These compounds serve a protective function in scavenging free radicals such as reactive oxygen species (ROS) in the plant, but may also have antioxidant activity in human health (Kim et al., 2016). Chlorophyll and antioxidant levels appear to be influenced by type of growing media and fertilization.

Sardoei and Rahbarian (2014) showed that growing media can influence chlorophyll and carotenoid production in ornamental plants.

1.3 *Lactuca sativa* L. (Lettuce)

Lettuce belongs to the Asteracea family and is produced worldwide, both in fields and greenhouses. The lettuce crop cycle lasts from 6-8 weeks, with an ideal pH range from 6,0 – 6,5 and temperature range from 15-20 ºC (Omdal, 2005). Lettuce is one of the most consumed vegetables worldwide, and though lettuce is low in calories, it may be a significant source of antioxidants and other health beneficial bioactive compounds (Kim et al., 2016). The variety selected for this study, "Lollo rosso", is characterized by curly leaves with reddish pigmentation. "Lollo" lettuce types account for about one sixth of all lettuce produced in Norway (Omdal, 2005).

Table 1 Sufficiency and deficiency levels of selected nutrients in lettuce plants, adopted from Bævre & Gislerød (1990).

Element	Sufficiency level	Deficiency level
N (%)	3,5 - 5,5	< 2,5
P (%)	0,5 - 0,8	< 0,2
K (%)	5,0 - 10,0	< 2,5
Ca (%)	1,0 - 1,8	< 1,0
Fe (µg/g)	130	< 50
Zn (µg/g)	50	< 40

2. Materials and methods

2.1 Growing media

Four different types of compost, vermicompost and digestate were used (described below) with fertilized peat as a control. The composts were stored for a few days - up to a few weeks in the greenhouse before the experiment began. Each material was mixed with perlite (Substrate Gr 2: 0,6 – 3mm, Pull Rhenen B.V., Netherlands) at a ratio of 80% media to 20% perlite by volume. 12 cm pots were filled (20 pots/media) one day prior to transplanting. Analysis of the media was performed by Eurofins lab (see appendix A, B, C, D).

2.1.1 Farm compost (Farm):

This compost was produced on-farm from a feedstock of horse manure, spruce and cabbage waste, pH 8. The consistency of the finished product was quite hard, almost clay-like. No smell, foreign- or undecomposed objects. When potted up it was dense and not particularly well drained.

Produced by Anders Hørthe, Hørthe Gård, Sylling.

2.1.2 Mix compost (Mix):

The feedstock of this compost was 50% household/municipal food waste and 50% park and garden waste. The compost looked like a traditional compost; dark, no foul odors, various sized particles, but overall well matured, pH 8,4. There were however several pieces of plastic and other trash that do not belong in a well-made compost. The media drained well and retained water satisfactorily.

Produced by Lindum AS, Drammen, waste management, R&D in sustainable food production.

2.1.3 Digestate (Biorest):

Digestate is the solid fraction that remains after biogas is produced by anaerobic fermentation. The feedstock for biogas production was municipal waste from Hadeland and Ringerike area in eastern Norway. The digestate had pH 8,4, a sticky texture and was initially very difficult to break up. The smell was quite foul but subsided once material was aerated for a while. The material displayed very limited ability to retain water after mixing with perlite and placing in pots. At this point the texture was pebble-like aggregates.

Produced by Hadeland og Ringerike Avfallsselskap (HRA), municipal waste company.

2.1.4 Vermicompost (Vermi):

Worm compost produced on-farm, pH 9. The feedstock for this compost was digestate from biogas production with cow manure as a feedstock. The digestate was further decomposed using earthworms *(Eisenia foetidia*). The finished vermicompost had a texture that was rich and loamy. It was dark and smelt of damp earth or forest floor (well matured compost smell). This compost was initially very promising, but proved to perform very poorly as a growing media.

Produced by Knut Vasdal, Foss Gård, Skien.

2.1.5 Control (Fertilized peat potting blend):

Standard commercial peat based potting soil (Tjerbo Veksttorv), pH adjusted and fertilized, pH 5,5 – 6,5. This was chosen as a control due to its known predictability and performance.

Produced by Tjerbo Torvfabrikk AS, Rakkestad.

2.2 Plant material and Experimental setup:

Seeds of *Lactuca sativa* L. var. "Lollo rosso" (LOG, Norway) were sown in three 77-cell plug trays, 1 seed per cell. Seeds were sown in S-jord (soil for sowing - Hasselfors Garden pH 6). Plug trays were placed in the greenhouse room (Centre for plant research in controlled environment, SKP, NMBU, ÅBVH0605) under natural light conditions for germination. The lettuce seedlings emerged 3 days after sowing. 15 days after sowing each medium was mixed with perlite at a ratio of 4:1 as described above (80% media, 20% perlite by volume). Pots were filled with respective substrates and labelled.

16 days after sowing 10 lettuce seedlings/treatment were transplanted from plug trays in to their respective media, one seedling per pot. Temperature and relative humidity (RH) in the greenhouse was set to 20°C and 70% RH respectively. However, both parameters fluctuated due to hot weather and strong sun. The greenhouse was equipped with a Priva Climate

Computer to control and monitor climate in the greenhouse room. Data from the climate computer show that the average measured temperature for the growing period was 22,3°C, with a maximum of 32,7°C and minimum of 16,4°C. The average RH was 65%, with a maximum of <90% and a minimum of 25,23%. The average solar irradiation during the period was 49,02 mol/m^2 (Meteorologiske data for Ås, 2016). Only natural light and no shading was used.

For all media + control, N= 10 pots of lettuce plants were placed on two separate tables in the greenhouse for a total of 100 pots, see figure 3 for schematic of the setup. Each pot was placed inside a small plastic tray to collect any runoff from watering. Though composts can provide a significant supply of nutrients, slow release may be of concern. Therefore, to observe to what extent composts were able to deliver an adequate supply of nutrients, plants were divided in to two separate treatments/tables; one table was only given pure tap water, one table was fertilized twice weekly with Electrical Conductivity (EC) 1,5 mS/cm (Cristalon indigo + Calcinit, Yara, Norway). EC was measured with Orion 420A+, basic pH/mV/ORP (Thermo Fisher Scientific Inc, USA).

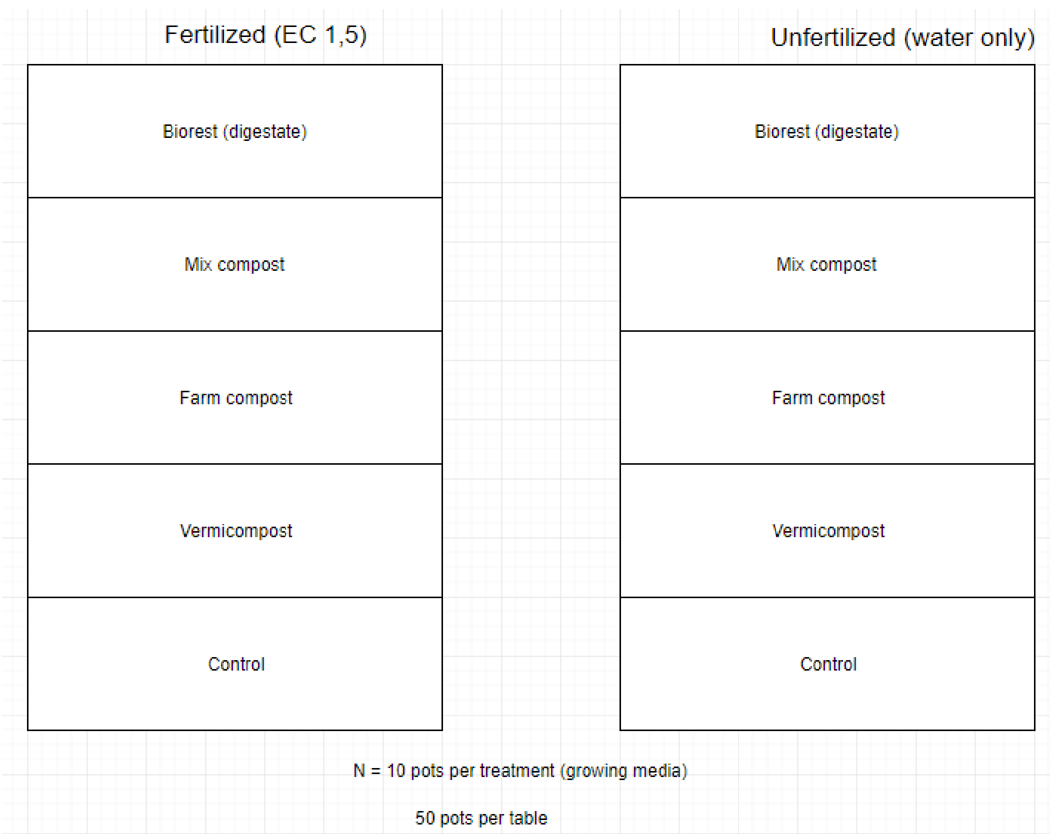

Figure 3 Schematic of table setup fertilized (left) and unfertilized (right), each table has 10 pots per growing media type

All plants were monitored carefully for watering needs throughout the growing period. 4 weeks after transplanting half of the lettuce plants were to be overwatered for the remainder of the period. This was achieved by making sure the pots were waterlogged and the trays they were placed in were filled with water. It was expected that this would lead to stress and possibly growth retardation, however no observable differences in leaf color or growth were seen between the normally watered and the overwatered plants. Therefore most measurements were only performed on the "non-stressed" plants (n=5/treatment).

2.3 The measurements

The measurements performed during the course of this experiment can be roughly divided in to non-destructive measurements during growth, and measurements at harvest.

2.3.1 Non-destructive measurements

The number of leaves on each plant was recorded 3 times at weekly intervals, starting 2 weeks after transplanting. An average of the 3 measurements was used for statistical analysis. Stomatal conductance was measured on a middle aged leaf from each pot at 2 and 3 weeks after transplanting, the average of the two measurements was used for statistical analysis. Measurements were performed with the AP4 porometer (Delta-T Devices Ltd, England, UK). Relative chlorophyll content was measured with a handheld chlorophyll meter (Hansatech Instruments Ltd, United Kingdom). Measures were carried out in parallel with measurements of stomatal conductance, and an average of the two measurements was used for statistical analysis.

5 weeks after transplanting anthocyanins and flavonols were measured with the Dualex Scientific sensor (Force-A, France) and were carried out once on three leaves per plant – an old leaf, a middle age leaf and a young leaf. An average of the three measurements was used for statistical analysis.

2.3.2 Measurements at harvest

Fresh weight and dry weight measurements were recorded approximately 6 weeks after transplanting, half of each lettuce head was frozen for FRAP/phenol analysis and half of each head was dried for 3 days and weighed again on the same scale. As only half of each plant was dried, the measured dry weight only accounts for approximately half of the actual dry weight.

For analysis of Ferric Reducing Ability of Plasma (FRAP) and total phenols the KoneLab 30i (Thermo Electron Corp., Vantaa, Finland) was used. Frozen samples were prepared and the measurements were carried out according to the protocols devised by Volden (2007a, 2007b). Material from 5 plants per treatment were blended together with a hand blender (Braun MR400, Karlsruhe, Germany). In order to have sufficient material for the blender to work, material from 2-3 pots per treatment were blended together to give two samples per treatment. Samples were mixed with methanol as solvent and placed on ice in an ultrasound bath (Sonorex RK 100, Bandelin GmbH & Co., Berlin, Germany) before measurements were carried out. The KoneLab 30i measures each sample 3 times to give an average value.

ICP and CN measurements were carried out at NIBIO Ås, according to protocol for the Milestone Ultrawave Single Reaction Chamber Microwave Digestion System (Mileston Srl, Italy). 3 plants from each treatment were ground in to a fine powder and samples were weighed out before preparing for ICP and CN analysis. For more details about the process, see Ogner et al. (2000).

2.4 Statistical analysis

For statistical analysis MiniTab 18.1 (Minitab Inc., USA) was used. One-way ANOVA using the Tukey Pairwise Comparisons was performed, with grouping information using the Tukey Method and a 95% Confidence level. Differences between treatments are considered significant when $p<0,05$. A simple regression analysis was performed with fitted line plots for carbon:nitrogen (C:N) ratio versus anthocyanins and flavonols.

3. Results

3.1 Growing media:

Samples of all media were sent to Eurofins soil lab for analysis. The results are included in the appendix. For the sake of clarity, a few key parameters are included here (Table 2).

Table 2 pH, Electrical Conductivity (EC) and total nitrogen in the growing media, adapted from declaration on peat bag and from Eurofins analysis.

Media	pH	EC (mS/cm)	Total nitrogen (g/100g)
Control	5,5 – 6,5	0,30	0,425
Biorest	8,4	1,60	3,3
Farm	8,0	0,27	0,47
Mix	8,4	0,48	1,4
Vermi	9,0	1,90	2,7

3.2 Growth measurements of *Lactuca sativa* var. "Lollo rosso"

3.2.1 Leaf growth measurements

In general, the fertilized (F) plants produced a higher number of leaves than the unfertilized (U) plants, but not all the treatments were significantly different between F and U. The treatments Farm, Mix and Vermi responded significantly by producing more leaves when fertilized but Control and Biorest did not (Fig. 4). Furthermore, figure 4 shows that lettuce from Control fertilized and unfertilized treatment had the highest number of leaves, though only F was significantly higher than other treatments. Biorest, Farm, Mix and Vermi all had the lowest number of leaves.

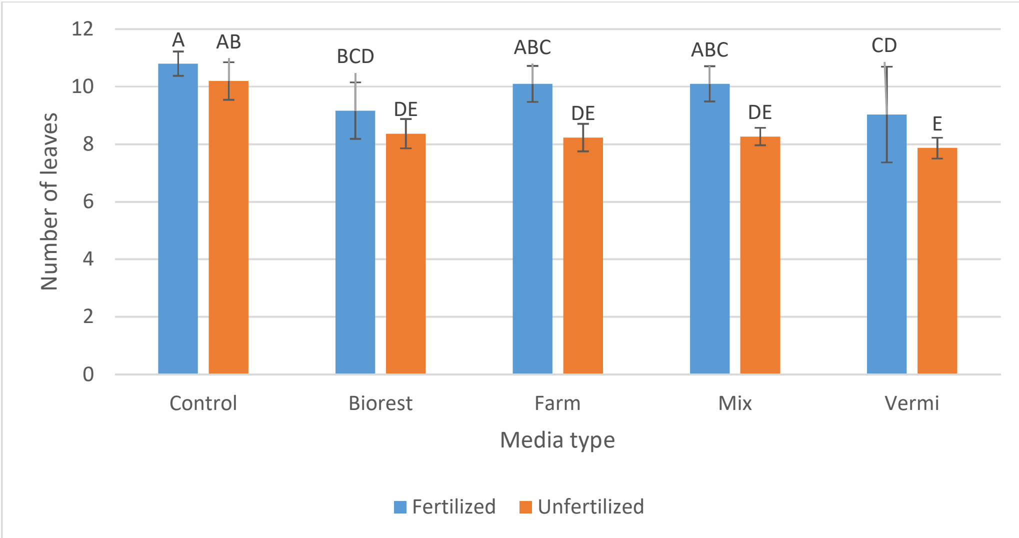

Figure 4 Effect of fertilized and unfertilized growing media on the average number of leaves of lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey's test). n = 10 plants/treatment.

3.2.2 Fresh weight and dry weight

Generally fertilized plants had a higher fresh weight (Table 3). Farm U and Mix U had the lowest fresh weight, and Control U had the highest fresh weight of unfertilized treatments. Control F had a significantly higher total fresh weight than all other treatments, more than twice as much as the lowest; Farm, Mix and Vermi U. Mix F had relatively higher fresh weight than the remaining treatments.

The same trend regarding F and U can be observed for dry weight measurements (Fig 5). Control F had a significantly higher dry weight than all other treatments, and was followed by Mix and Control U. The biggest difference between fertilized and unfertilized treatments was found in the Farm and Mix treatments, where the dry weight was double in fertilized compared to unfertilized plants.

Table 3: Effect of fertilized and unfertilized growing media on average ± standard deviation fresh weight of lettuce (“Lollo rosso”) grown in 5 different media. Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 5 plants/treatment.

Media	**Total fresh weight (g)**	
	Fertilized	**Unfertilized**
Control	93,920 ±14,452 A	44,072 ± 3,012 D
Biorest	39,140 ±4,556 DE	31,941 ± 3,277 EF
Farm	57,082 ±10,150 C	18,609 ± 1,073 G
Mix	69,055 ±9,883 B	24,048 ± 1,506 FG
Vermi	43,272 ±5,219 D	32,246 ± 5,224 EF

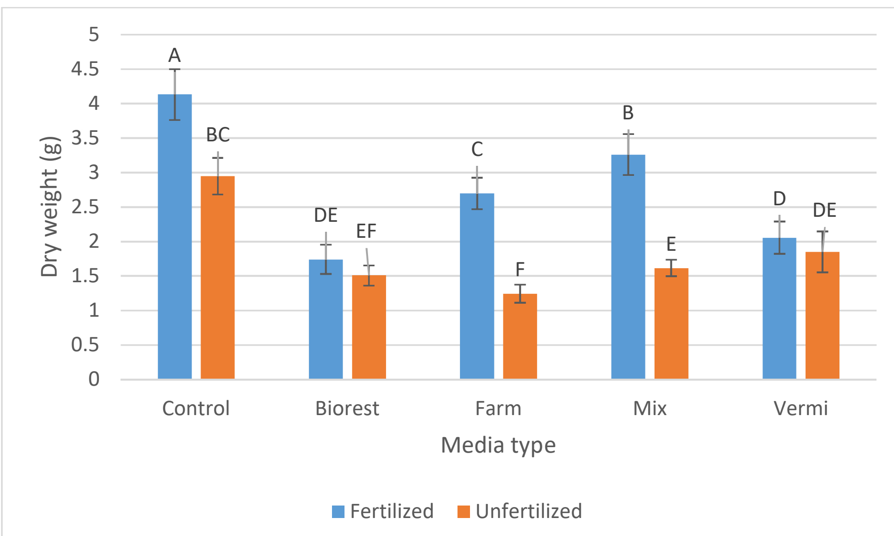

Figure 5 Effect of fertilized and unfertilized growing media on the mean dry weight of the lettuce (“Lollo rosso”) plants. Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 5 plants/treatment.

3.2.3 Stomatal conductance

In general there is a difference in stomatal conductance between media types (Fig 6). There seems to be no clear difference between fertilized and unfertilized treatments of the same media with Control being the exception. F and U are only significantly different in the Control where there is a large difference in stomatal conductance between fertilized and unfertilized plants. Figure 6 shows that lettuce grown in Biorest F had the highest stomatal conductance, but was not significantly higher than Control F, Vermi U and Biorest U. Control U had significantly lower stomatal conductance than all treatments except Farm F + U and Mix F + U.

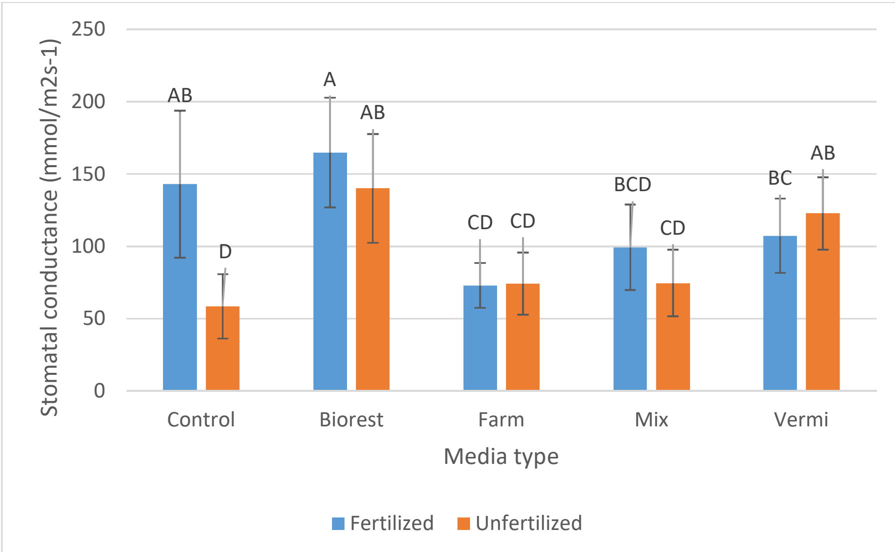

Figure 6 Effect of fertilized and unfertilized growing media on the mean stomatal conductance in lettuce (“Lollo rosso”). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 10 plants/treatment.

3.3 Non-destructive leaf measurements

To gain an insight in to the nutrient status of the plants before they were harvested; chlorophyll, anthocyanins and flavonols were measured.

3.3.1 Chlorophyll

There appears to be an influence of growing media on chlorophyll levels measured in lettuce (Fig 7). Biorest F and Vermi U had the highest levels of chlorophyll, and were significantly higher than Control, Farm and Mix F + U. Farm U and Mix U have the lowest levels of chlorophyll, but these were only significantly lower than both Biorest and Vermi treatments (F + U). Chlorophyll levels were not significantly influenced by fertilizer in any of the media.

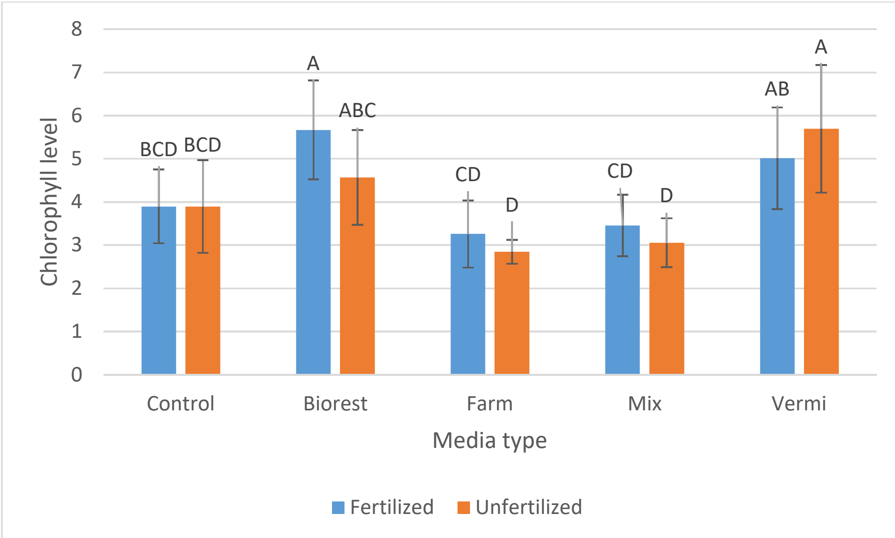

Figure 7 Effect of fertilized and unfertilized growing media on the mean relative chlorophyll content in lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). Clorophyll was measured with Hansatech Chlorophyll-meter. The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at $p< 0,05$ (Tukey's test). n = 5 plants/treatment.

3.3.2 Flavonols and anthocyanins

The dualex uses chlorophyll fluorescence to measure epidermal tissue and gives a value for relative flavonol and anthocyanin levels in this cell layer. This does not necessarily give an accurate depiction of levels in the entire plant, and none of the leaves were uniformly colored. (Fig 8)

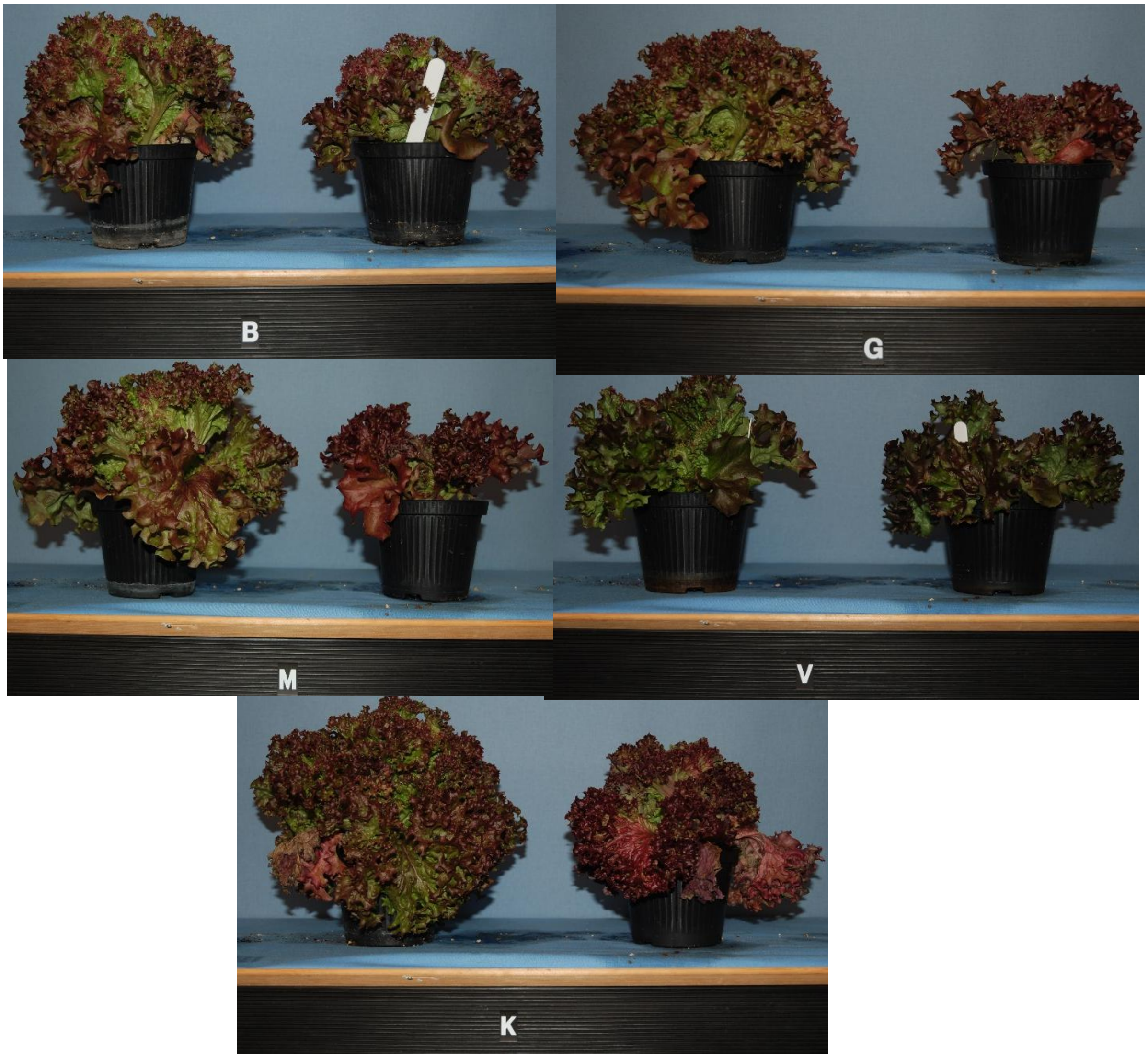

Figure 8 Lettuce plants at harvest from the five growing media: Biorest (B) fertilized (left) and unfertilized (right); Farm (G) unfertilized (left) and fertilized (right); Mix (M) fertilized (left) and unfertilized (right); Vermi (V) fertilized (left) and unfertilized (right) and Control (K) fertilized (left) and unfertilized (right). Photo: Andrew Niday, June 2016.

Figure 9 shows that Farm and Vermi U had the highest levels of flavonols, however they were only significantly higher than Control F and Farm F, the former about half the level measured in Vermi U. The measurement of anthocyanins (Fig 10) follow a similar pattern as the flavonols (Fig 9), however in this case Control U was had the lowest anthocyanin levels, but only significantly lower than Farm and Vermi U. A visual assessment of leaf coloring (Fig 8) can supplement the measured levels of pigmentation.

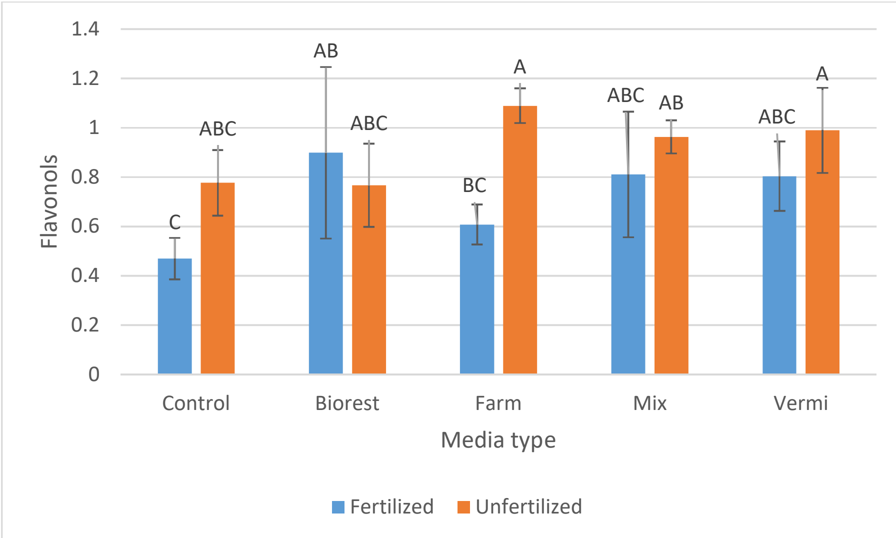

Figure 9 Effect of fertilized and unfertilized growing media on the mean relative flavonol levels in lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey's test). n = 5 plants/treatment.

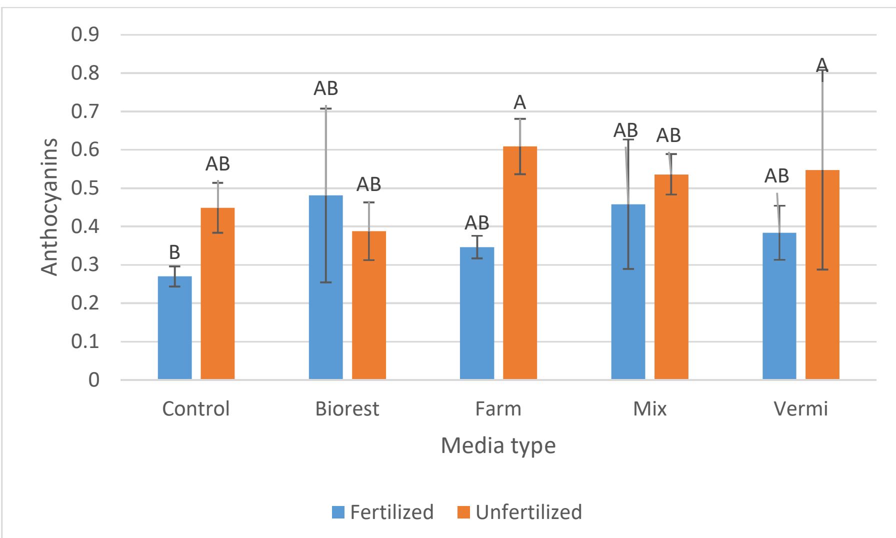

Figure 10 Effect of fertilized and unfertilized growing media on the mean relative anthocyanin levels in lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey's test). n = 5 plants/treatment.

3.4 Antioxidant capacity in fresh frozen lettuce; FRAP and total phenols

As seen in figure 11, the highest levels of antioxidant capacity (FRAP) were found in Control, Biorest, Farm and Mix U. These were significantly higher than both Vermi treatments and all other fertilized treaments except Biorest F. Total phenols (Fig 12) shows the same distribution of differences and significance in treatments as in figure 11; Control, Biorest, Farm and Mix U have the highest level of total phenols, the lowest total phenol levels are found in the fertilized treatments and both Vermi treatments.

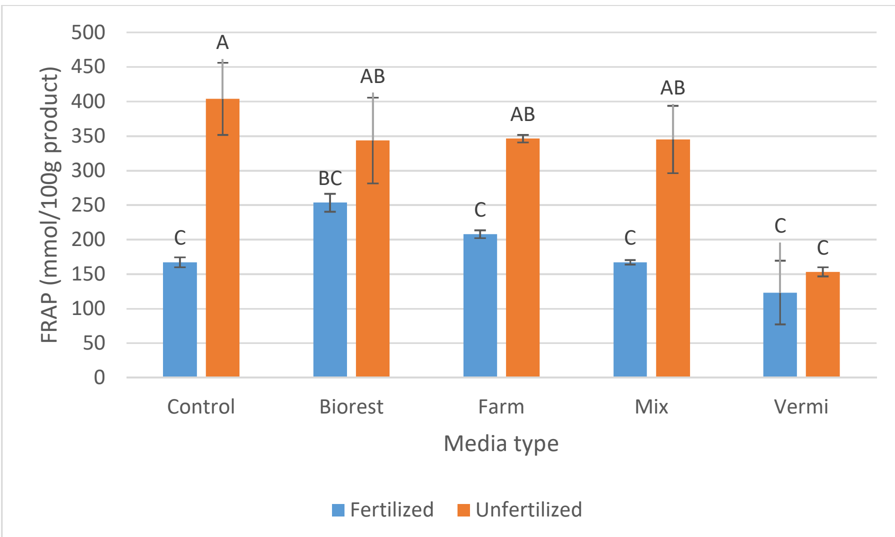

Figure 11 Effect of fertilized and unfertilized growing media on the FRAP levels (mmol/100g fresh product) in lettuce (“Lollo rosso”). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 2 per treatment.

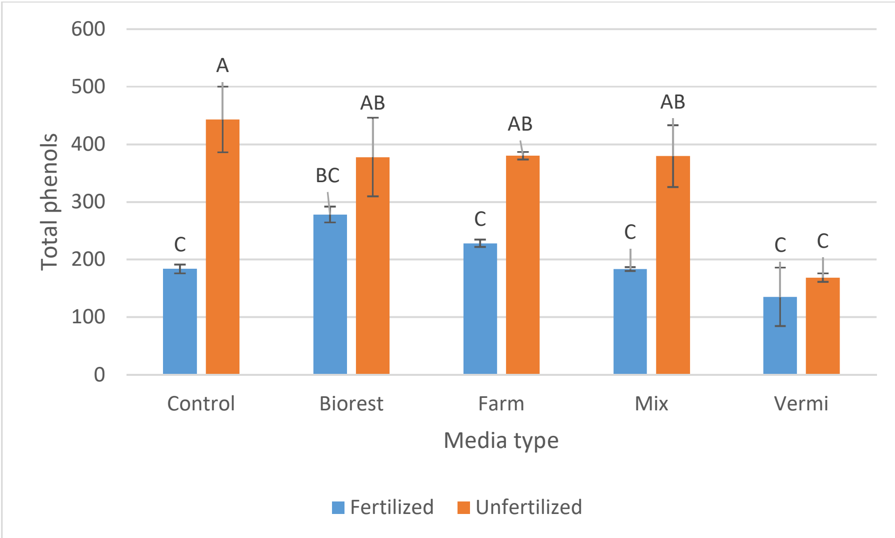

Figure 12 Effect of fertilized and unfertilized growing media on the mean total phenol levels (mg Gallic Acid Equivalents/100g fresh product) in lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey's test). n = 2 per treatment

3.5 Nutrient status of dried lettuce plants (ICP and CN analysis)

3.5.1Nitrogen percentage, phosphorus percentage and carbon:nitrogen ratio

Lettuce grown in Vermi F & U had significantly higher levels of nitrogen (%) than all other treatments (Fig 13). In general fertilized plants had higher nitrogen % than unfertilized plants, no significant difference was found between F and U in Control or Vermi. Lettuce grown in Farm and Mix U had significantly lower nitrogen % than all other treatments. The measurements of phosphorus (P) % (Fig 14) show that Control and Farm F have the highest levels of phosphorus. However, only Farm and Mix U have significantly lower phosphorus levels than the other treatments. Fertilizer only had no significant effect on phosphorus levels when comparing F and U in the same media, except in Farm.

The carbon:nitrogen (C:N) ratio of plants grown in 5 different media (Fig 12) follows a similar, but inverse trend as observed for nitrogen %. The treatments with the highest C:N ratio are the same treatments with the lowest nitrogen % (Fig 15). Farm and Mix U had significantly higher C:N ratio than all other treatments, while both Vermi treatments had significantly lower C:N ratio than all other treatments.

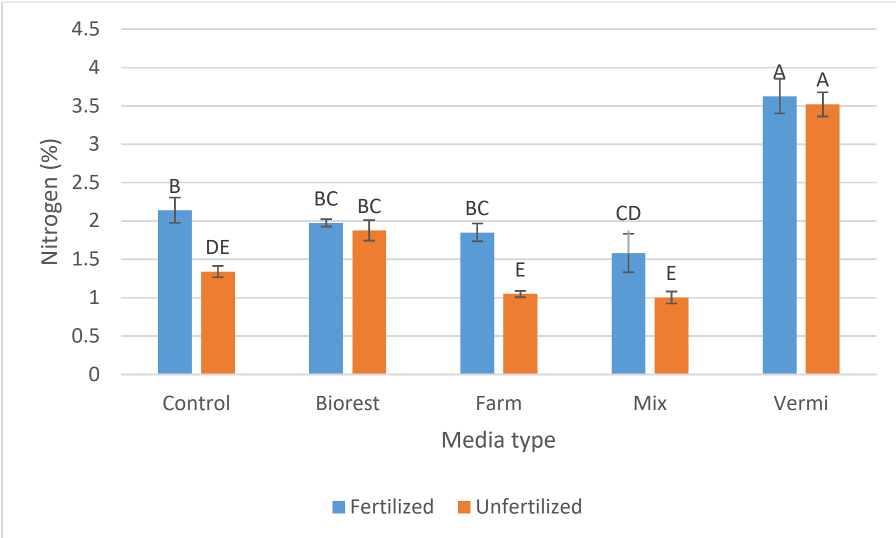

Figure 13 Effect of fertilized and unfertilized growing media on the mean nitrogen % in lettuce (“Lollo rosso”). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 3 plants/treatment.

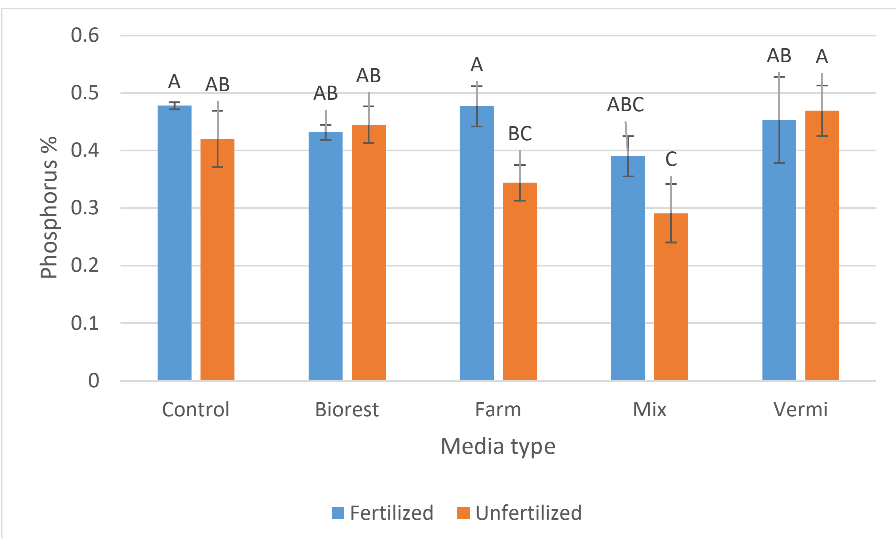

Figure 14 Effect of fertilized and unfertilized growing media on the mean phosphorus % in lettuce (“Lollo rosso”). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey’s test). n = 3 plants/treatment.

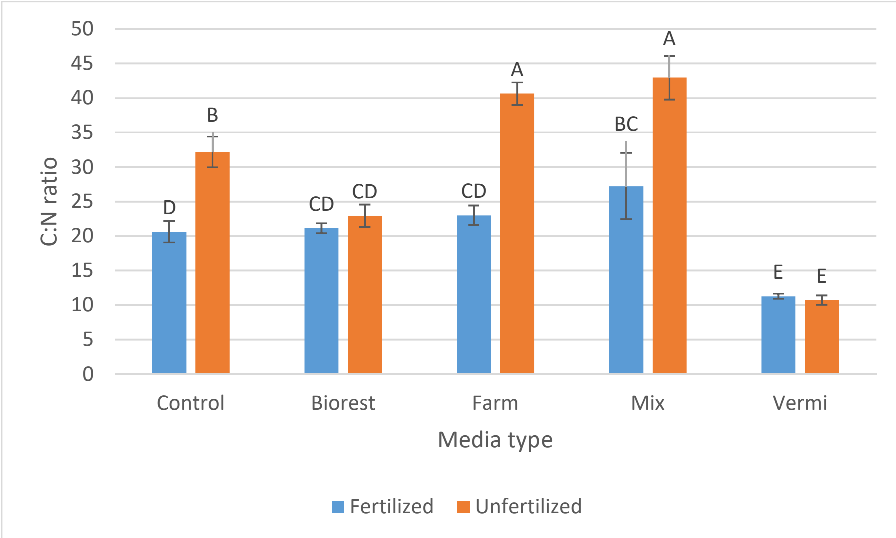

Figure 15 Effect of fertilized and unfertilized growing media on the mean carbon:nitrogen ratio in lettuce ("Lollo rosso"). Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). The bars indicate standard deviation in the 5 different growing media. Means that do not share a letter are significantly different at p< 0,05 (Tukey's test). n = 3 plants/treatment.

3.5.2 Other nutrients (Ca, K, Fe, Zn)

Lettuce grown in Control F + U treatments had the highest calcium (Ca) %, but were only significantly higher than Farm and Vermi F & U and Mix U (Table 4). Vermi U had the lowest calcium levels, but only significantly lower than Control and Biorest F & U and Mix F. The opposite situation was observed for potassium (K) % (Table 4). Both Vermi treatments had significantly higher potassium levels than all other treatments, while both Control treatments and Mix U had significantly lower potassium levels than all other treatments.

Table 4: Effect of fertilized and unfertilized growing media on average ± standard deviation calcium (Ca) % and potassium (K) % in lettuce ("Lollo rosso") grown in 5 different media. Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). Means that do not share a letter within the same element are significantly different at p< 0,05 (Tukey's test). n = 3 plants/treatment.

Media	Ca(%)		K(%)	
	Fertilized	**Unfertilized**	**Fertilized**	**Unfertilized**
Control	1,48 ± 0,41 A	1,51 ± 0,57 A	3,17 ± 0,41 C	2,25 ± 0,18 C
Biorest	1,09 ± 0,12 AB	0,91 ± 0,07 ABC	5,31 ± 0,02 B	5,08 ± 0,25 B
Farm	0,67 ± 0,11 BCD	0,63 ± 0,09 BCD	6,31 ± 0,64 B	5,31 ± 0,40 B
Mix	0,88 ± 0,21 ABC	0,60 ± 0,03 BCD	5,212 ± 0,95 B	3,55 ± 0,28 C
Vermi	0,30 ± 0,01 CD	0,18 ± 0,03 D	11,800 ± 0,58 A	11,23 ± 0,42 A

Iron (Fe) levels in lettuce were highest in both Control treatments, but were only significantly higher than Farm and Mix U, which were significantly lower than all other treatments (Table 5). Zinc (Zn) levels in lettuce (Table 5) were significantly higher in both Vermi treatments (and Control F) than in all other treatments, which were not significantly different from each other.

Table 5: Effect of fertilized and unfertilized growing media on average ± standard deviation iron (Fe) and zinc (Zn) levels (µg/g) in lettuce ("Lollo rosso") grown in 5 different media. Plants were fertilized with full spectrum fertilizer (EC 1,5) twice weekly, or unfertilized (tap water only). Means that do not share a letter within the same element are significantly different at p< 0,05 (Tukey's test). n = 3 plants/treatment

Media	Fe(µg/g)		Zn(µg/g)	
	Fertilized	**Unfertilized**	**Fertilized**	**Unfertilized**
Control	286 ± 186 A	255,80 ± 166,80 AB	98,30 ± 113,30 BC	30,62 ± 4,32 C
Biorest	91,52 ± 17,29 AB	60,86 ± 3,90 AB	37,91 ± 8,95 C	33,12 ± 1,70 C
Farm	111,50 ± 30,7 AB	51,02 ± 5,28 B	31,33 ± 5,98 C	30,54 ± 2,52 C
Mix	76,40 ± 19,8 AB	34,59 ± 1,12 B	39,41 ± 7,94 C	31,43 ± 2,04 C
Vermi	79,21 ± 5,4 AB	55,80 ± 11,55 AB	251,60 ± 21,20 A	197,60 ± 18,20 AB

3.6 Anthocyanins and flavonols vs. C:N ratio

To investigate whether there is a correlation between anthocyanins, flavonols and the nutrient status of the lettuce plants, a simple linear regression was performed. We chose to use C:N ratio rather than simply using nitrogen, because they give approximately the same information. The levels of flavonols (Fig 9) and anthocyanins (Fig 10) appears to correlate well with C:N ratio (fig 15). However a simple regression analysis reveals that the correlation is weak when Vermi is included; R-Sq = 11,7% for flavonols vs. C:N ratio and R-Sq = 21,6% for anthocyanins vs. C:N ratio. When Vermi is removed the correlation is stronger (Figures 16 & 17).

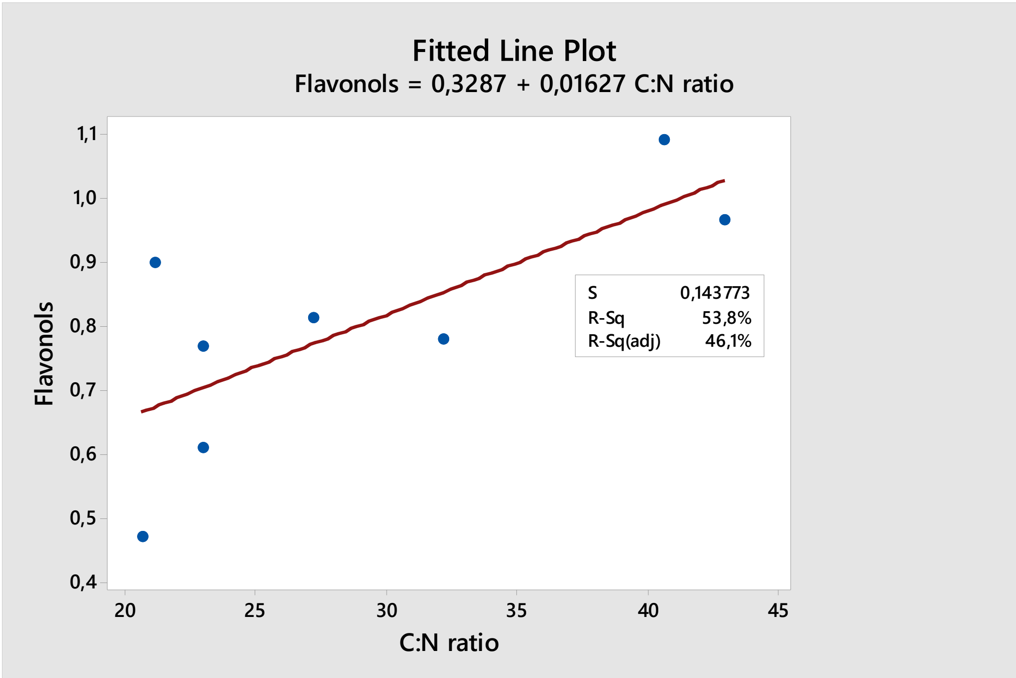

Figure 16 Fitted line plot of flavonols versus C:N ratio. p = 0,038

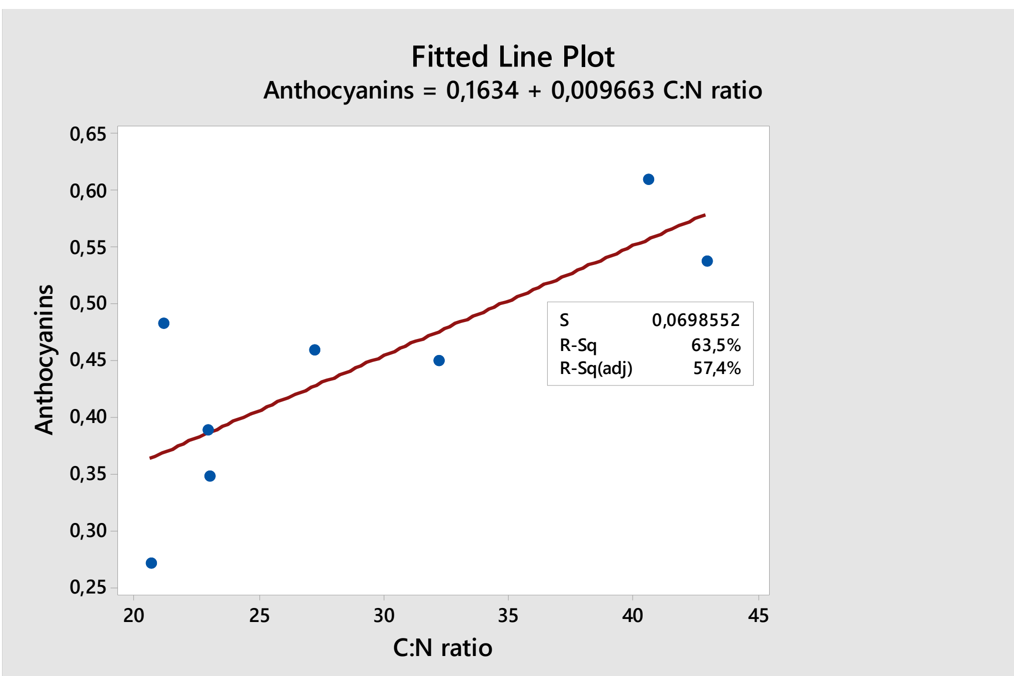

Figure 17 Fitted line plot of anthocyanins versus C:N ratio. p = 0,018

4. Discussion

4.1 Growing media

In general, the composts, vermicompost and digestate performed poorly as growing media, compared to the peat Control. Biomass production was reduced and antioxidant levels in plants were typically higher in unfertilized than fertilized treatments. A favorable pH in the growing media ensures optimal nutrient uptake and growth. All media except the Control had a relatively high pH, ranging from 8 to 9, and this may be the simplest explanation of the poor performance seen. Tavakkoli et al (2016) showed that alkaline stress leads to significantly reduced growth in Gerbera plants. The availability of nutrients such as nitrogen, phosphorus, potassium, iron and zinc may be limited at alkaline pH. Exstensive research on alkaline stress in various plants exists, but little is done on lettuce in particular. However Youssef et al. (2017) showed that application of protein hydrolysate and biostimulants was able to enhance lettuce tolerance to alkalinity. The problems related to high pH, especially in Vermi (pH 9), could have been avoided if a complete analysis of each material had been carried out prior to the experiments instead of in parallel with the experiments. We could have for example blended the compost with a more acidic media such pure sphagnum peat, which has a naturally low pH.

The existing literature related to use of compost, vermicompost and digestate as potting media are mostly from experiments where different levels of compost are blended with conventional potting substrates such as peat, sand, perlite etc. Lazcano et al., (2009) found that tomato biomass was increased when low doses of compost and high doses of vermicompost replaced peat, with peat as a control. Santos et al (2016) performed a similar experiment to this, using 5 treatments/types of compost each mixed with sand. They found that compost amendments lead to increased antioxidant activity in lettuce, compared with peat and sand as a control. Clark and Cavigelli (2005) found that when lettuce was grown in compost with high salinity, nitrogen mineralization and yields were reduced. They also found that when the compost with high salinity and EC was mixed with peat, the problems were ameliorated. Hernandez et al. (2010) found that vermicompost and compost could supply sufficient amounts of several macro- and micro nutrients, but insufficient amounts of nitrogen and phosphorus. Ali et al. (2007) conclude that low level applications of vermicompost in potting mix is the most beneficial for lettuce growth.

Overall, poor growth was seen in treatments that were unfertilized. Farm and Mix compost did not perform as well as Control, but in general plants performed better than in Biorest and Vermi. Farm was closest to Control in several growth parameters, but would need some adjusting for optimal performance. Vermi and Biorest performed poorly as growing media, however research shows that these materials can be used as growing media (Lazcano et al., 2009, Crippa et al., 2013) and reduce the use of synthetic fertilizers (Hernandez et al., 2010) due to high nutrient contents. However, test needs to be done where Vermi and Biorest are mixed with other relevant media.

4.2 Can compost, vermicompost and digestate provide adequate nutrients to lettuce plants?

One of the objectives of this study was to examine whether the different media could provide sufficient levels of nutrients to grow lettuce plants. To evaluate the nutrient status of the plants in this experiment, we have compared the measured levels of nutrients with the suggested levels of sufficiency and deficiency in table 1. According to the suggested levels, all plants except those grown in Vermi (F + U) are deficient in nitrogen, i.e. < 2,5 % (Fig 13). Biorest and Vermi media were high in nitrogen (Table 2), but as plants from Biorest were deficient, the availability of nitrogen must have been limited. As nitrogen is considered the most limiting nutrient (Marschner, 2012), it is difficult to say whether deficiencies in other nutrients have had an effect on growth. Reduced growth as a result of nitrogen limitation, may entail a reduced demand of other nutrients (B. Føreid, personal communication, 2018). Nonetheless it is interesting to look at levels of other nutrients in the lettuce plants, as they give an indication of the media's ability to supply nutrients.

Phosphorus levels are lower than the suggested sufficiency levels, but are higher than the deficiency levels in all treatments (Fig 14). Phosphorus is less available at alkaline pH, so this may explain the low levels in plants grown in compost, but should be sufficiently available in Control. Potassium levels in plants from Vermi treatments are high, and plants from Biorest and Farm F & U and Mix F are at the lower end of the suggested suffiency level. Neither Control treatments were sufficient in potassium, but only Control U has low enough levels to be considered deficient i. e. < 2,5 % (Table 4). Deficiencies in nitrogen, phosphorus or potassium can lead to altered levels of plant hormones such as ABA and Cytokinin (Marschner, 2012). ABA is involved in stomatal regulation and Cytokinins influence shoot growth (Bævre & Gislerød, 1990), and could explain the small leaves observed in Biorest and Vermi.

Multiple nutrients were deficient, likely due to high pH. Farm, Mix and Vermi were not able to supply sufficient levels of calcium, even when fertilized (Table 4), this could be linked to the high pH in these media. All plants except those grown in Mix U have iron levels (Table 5) over the deficiency level (< 50 µg/g). However only Control (F + U) has sufficient Fe levels (130 µg/g), while Farm F is close behind (111,5 µg/g). It is possible that these low levels are related to alkaline pH. However it is surprising that Biorest and Vermi are not deficient in iron, as they have the same pH as Mix, or higher. Zinc (Table 5) appears to be deficient (< 40 µg/g) in all plants except those grown in Control F. This seems reasonable, as the availability of zinc is limited at the pH levels of all media except Control.

4.3 Physiological effects of growing media on lettuce

4.3.1 Influence of growing media on leaf growth and transpiration

The measurements performed on living plants give an impression of the plant's wellbeing. Healthy lettuce plants should have functioning stomata and fully expanded green leaves, with reddish pigmentation in "Lollo rosso" type lettuce. Looking at the number of leaves only gives a clear picture of the growth rate when coupled with biomass production – the measured dry weight. Only small differences in the number of leaves per plant were observed between treatments (Fig 4), however plants grown in unfertilized Farm, Mix, Biorest and Vermi had very small leaves compared to the other treatments (Fig 8). These treatments also had the lowest biomass production (Fig 5). Reduction in leaf area is often a result of stress, and this phenomenon is often seen when plants are exposed to stresses such as nutrient deficiency, drought or too much light (Taiz & Zeiger, 2010).

Lettuce has stomata on both sides of its leaves, but only the bottom side was measured in this study. Plants tend to close their stomata when under stress, a process that involves increased levels of the stress-hormone abscisic acid (ABA). Closed stomata results in less fixed CO_2 and subsequent reduction in photosynthesis and growth (Taiz & Zeiger, 2010). Nutritional status and especially nitrogen has a strong influence on stomatal conductance. Shimshi (1970) showed that stomatal function was affected by nitrogen-status in beans (*Phaseolus vulgaris* L.) and that nitrogen-deficient plants neither opened or closed their stomata as much as nitrogen-sufficient plants. Plants with the lowest stomatal conductance (Fig 6); Farm and Mix

F + U and Control U have closed stomata, and also the lowest nitrogen-levels. One would expect to find a correlation between stomatal conductance, chlorophyll and biomass, however this is not the case for all treatments. Plants from Biorest F + U have the highest stomatal conductance and among the highest levels of chlorophyll (Fig 7), but also the lowest biomass production. While plants from Vermi have open stomata, high levels of chlorophyll and low biomass. Plants from Farm on the other hand have closed stomata, and when unfertilized, the lowest chlorophyll and biomass. The number of stomata was not measured in this experiment, but can also explain differences in conductance between treatments. Further studies are necessary to understand the relationship between growing medium and conductance. On the other hand, both biochemical and physical properties of the medium can influence transpiration. It may have been useful to perform a physical analysis of the media, other than subjective judgement when blending and potting the different media. This could have given an indication of the water holding capacity of each media. The gardener in charge of watering the lettuce plants observed that the Farm compost media retained water very strongly and required slow watering to absorb everything. On the other hand, Biorest was troublesome to water due to poor water retention.

Mahlangu et al. (2016) showed that chlorophyll levels in hydroponically grown lettuce increased linearly with nitrogen fertilization, while total phenols and antioxidant capacity peaked at 100 and 120 mg/L nitrogen. Ali et al. (2007) showed that leaf chlorophyll content was significantly reduced when lettuce was grown in pure vermicompost with green waste as feedstock. In contrast, plants from this study grown in Vermi have high levels of chlorophyll. Clearly it is difficult to say with certainty that a particular type of media has a specific effect; as different feedstocks give end products with different properties. However, it is interesting to note that there was a significant difference in chlorophyll levels between the media, but levels were unaffected by fertilizer within treatments. This phenomenon may warrant further investigation.

4.3.2 Influence of growing media and nutrient status on secondary metabolites

Bævre and Gislerød (1990) state that when all other factors are optimal, the nutrient content in leaves has a strong influence on plant growth; weak growth can be observed at low nutrient concentrations, but small increases in the deficient nutrients will lead to large increases in growth; also known as law of the minimum. It is generally accepted that plants grown under nitrogen limitation experience the deficiency as a stress. Plants with insufficient levels of nitrogen tend to show reduced growth, produce more phenolic compounds, other secondary metabolites and other non-nitrogen compounds (Fritz et al., 2006). Some of these phenolic compounds are related to pigmentation, e.g. anthocyanins and flavonols. Thus one could expect to see more pigmentation in nitrogen-deficient plants. The plants grown in Vermicompost do not fit with this explanation, as they had a surplus of nitrogen and a relatively high degree of pigmentation. Phosphorus deficiency usually induces a reddish color due to increased level of antochyanins. However, the plants grown in in Vermi were not deficient in phosphorus either (Fig 14). One must therefore assume that other factors in the media than nitrogen or phosphorus have caused stress in plants from Vermi. We can not say with certainty which factors contribute to stress, but the high pH may be involved.

A close relationship was found between C:N ratio and anthocyanins (Fig 17). This is in keeping with the principles of measurements from the Dualex instrument; that elevated levels of flavonols and/or anthocyanins are associated with a nitrogen deficiency (Bumgarner et al., 2012). Galieni et al. (2015) found that for lettuce grown under zero nitrogen fertilization, yield was greatly reduced and polyphenol content was high, compared to the control. The plants with the lowest nitrogen % (Fig 13), have high levels of both flavonols and anthocyanins (Figures 9 & 10). This seems to hold true for most treatments except Vermi. Both Vermi treatments have significantly higher nitrogen % than all other treatments, yet also similarly high relative levels of flavonols and anthocyanins as most other treatments. On the other hand, plants from both Vermi treatments have the lowest levels of FRAP (antioxidant capacity) and total phenols (Figures 11 & 12). A possible explanation for this discrepancy could be that FRAP and total phenols are measured in the whole plant, while relative flavonols and anthocyanins are only measured in the leaf epidermis.

It is worth noting that phenolic compounds not only have an important function in plants, but may have beneficial properties for human health as well. Polyphenols derived from whole foods can be metabolized by intestinal bacteria to become more bioavailable, but may also influence the actual composition of bacteria in the gut (Duda-Chodak et al., 2015). Lettuce is a good source of bioactive compounds such as polyphenols (Kim et al., 2016), thus it could be interesting to examine further how these compounds are influenced by growing media.

4.4 Practical implications and concluding remarks

It is interesting to ponder whether it could be possible to grow plants with longer crop cycles in compost with sufficient nutrient levels, given that more nutrients may become available over time. Though likely unbalanced levels of nutrient would still be a problem. Is it possible to find a balance between sufficient nutrients in the media and enhanced antioxidant capacity from the media itself? Low level applications of 5-20% (v/v) of Biorest or Vermi in a potting media would be interesting to examine. Other research has shown that a small addition of composted and fresh spent coffee grounds supplemented in growing media at 2,5-30% (v/v) led to increased photosynthetic capacity and antioxidant activity (Cruz et al., 2014).

Overall, the media appear to be unable to supply sufficient levels of key nutrients, but this is likely due to unfavorable pH. One of the most important take home messages from this experiment is that when using compost as a potting media, it is very important to know the quality of the compost. If these experiments were to be repeated, it would be interesting to see if a blend of different composts or other materials could be mixed to provide an optimal growing media. By adjusting pH, nutrient levels and various physical parameters related to water holding capacity, it should be feasible to produce a growing media from compost. It is also interesting to note that higher levels of antioxidants in lettuce may be induced by the media itself. A possible mechanism of root-to-shoot phenolic uptake is put forward by Santos et al (2016). They found a correlation between simple phenolics present in the composts used as growing media, and phenolics in lettuce grown in the respective media. They propose that specific flavonoid compounds in the compost act as signal factors, leading to enhanced synthesis and accumulation of phenolic compounds in lettuce. With more understanding of the mechanism behind this phenomenon, growers in the future may be able to boost antioxidant levels in their produce simply by making adjustments to growing media.

5. Appendix

Eurofins soil analysis

5 A: Biorest

Prøvenr.:	**439-2016-04200472**	Prøvetakingsdato:	20.04.2016
Prøvetype:	Kompost	Prøvetaker:	Astrid Solvåg Nesse
Prøvemerking:	Biorest fra HRA BIO.HRA	Analysestartdato:	20.04.2016

	Analyse	Resultat	Enhet	LOQ	MU	Metode
b)*	Fosfor (P-AL)	0.57	g/100 g tørrstoff	20	20%	SS 028310 + T1
b)	Kalium (K-AL)	0.58	g/100 g tørrstoff	20	20%	SS 028310 + T1
b)	Kalsium (Ca-AL)	3.6	g/100 g tørrstoff	100	20%	SS 028310 + T1
b)	Magnesium (Mg-AL)	0.33	g/100 g tørrstoff	10	20%	SS 028310 + T1
b)*	Natrium (Na-AL)	0.41	g/100 g tørrstoff	50		SS 028310 + T1
c)	Arsen (As)	4.9	mg/kg TS	0.5	30%	NS EN ISO 17294-2
c)	Bly (Pb)	12	mg/kg TS	0.5	40%	NS EN ISO 17294-2
c)	Kadmium (Cd)	0.40	mg/kg TS	0.01	25%	NS EN ISO 17294-2
c)	Kobber (Cu)	86	mg/kg TS	0.5	30%	NS EN ISO 11885
c)	Krom (Cr)	16	mg/kg TS	0.3	30%	NS EN ISO 11885
c)	Kvikksølv (Hg)	0.051	mg/kg TS	0.001	20%	NS-EN ISO 12846
c)	Nikkel (Ni)	6.0	mg/kg TS	0.5	30%	NS EN ISO 11885
c)	Sink (Zn)	260	mg/kg TS	2	25%	NS EN ISO 11885
	pH målt ved 23 +/- 2°C	8.4		1		NS-EN 12176
*	Konduktivitet/ledningsevne	160	mS/m			NS-EN ISO 7888
	Totalt organisk karbon (TOC)	>40	% TS	0.1		Internal Method 1
c)	Aluminium (Al)	3000	mg/kg TS	10	15%	NS EN ISO 11885
c)	Bor (B)	50	mg/kg TS	5	25%	NS EN ISO 11885
	Fosfor (P)	0.75	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c)	Jern (Fe)	6800	mg/kg TS	30	25%	NS EN ISO 11885
	Kalium (K)	0.48	g/100 g tørrstoff	0.0002		NS EN ISO 11885

Kalsium (Ca)	3.6	g/100 g tørrstoff	0.0001		NS EN ISO 11885
Magnesium (Mg)	0.35	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c) Mangan (Mn)	480	mg/kg TS	0.3	20%	NS EN ISO 11885
Svovel (S)	0.46	g/100 g tørrstoff	0.00015		NS EN ISO 11885
b)* Ammonium-N (2 M KCl)					
b)* Ammonium (NH4-N)	0.4971	g/100 g tørrstoff			Internal Method 5
c) Total tørrstoff glødetap	73.0	% tv	0.1	10%	EN 12879
b)* Nitrat-N (2 M KCl)					
b)* Nitrate nitrogen	0.00146	g/100 g tørrstoff			Internal Method 5
c) Total tørrstoff	24.7	%	0.1	10%	EN 12880
a)* Total nitrogen (mod. Kjeldahl)	3.3	g/100 g tørrstoff	0.01		EN 13654-1

Merknader:
K < K-AL, men innenfor MU.

5 B: Farm

Prøvenr.:	**439-2016-09260287**	Prøvetakingsdato:	21.09.2016
Prøvetype:	Kompost	Prøvetaker:	Bente Føreid
Prøvemerking:	Gårdskompost GÅRD	Analysestartdato:	26.09.2016

Analyse	Resultat	Enhet	LOQ	MU	Metode
b)* Fosfor (P-AL)	0.050	g/100 g tørrstoff	20	20%	SS 028310 + T1
b) Kalium (K-AL)	0.31	g/100 g tørrstoff	20	20%	SS 028310 + T1
b) Kalsium (Ca-AL)	0.40	g/100 g tørrstoff	100	20%	SS 028310 + T1
b) Magnesium (Mg-AL)	0.068	g/100 g tørrstoff	10	20%	SS 028310 + T1
b)* Natrium (Na-AL)	0.013	g/100 g tørrstoff	50		SS 028310 + T1
c) Arsen (As)	4.8	mg/kg TS	0.5	30%	NS EN ISO 17294-2
c) Bly (Pb)	13	mg/kg TS	0.5	40%	NS EN ISO 17294-2
c) Kadmium (Cd)	0.23	mg/kg TS	0.01	25%	NS EN ISO 17294-2
c) Kvikksølv (Hg)	0.018	mg/kg TS	0.001	20%	NS-EN ISO 12846
c) Tørrstoff	54.5	%	0.1	5%	EN 12880
c) Kobber (Cu)	23	mg/kg TS	0.5	30%	NS EN ISO 11885
c) Krom (Cr)	23	mg/kg TS	0.3	30%	NS EN ISO 11885
c) Nikkel (Ni)	23	mg/kg TS	0.5	30%	NS EN ISO 11885

c)	Sink (Zn)	95	mg/kg TS	2	25%	NS EN ISO 11885
	pH målt ved 23 +/- 2°C	8.0		1		NS-EN 12176
*	Konduktivitet/ledningsevne	27	mS/m			NS-EN ISO 7888
	Total tørrstoff glødetap	16	% TS	0.02		NS 4764
	Totalt organisk karbon (TOC)	2.1	% TS	0.1	20%	Internal Method 1
c)	Aluminium (Al)	14000	mg/kg TS	10	15%	NS EN ISO 11885
c)	Bor (B)	9.2	mg/kg TS	5	25%	NS EN ISO 11885
	Fosfor (P)	0.12	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c)	Jern (Fe)	21000	mg/kg TS	30	25%	NS EN ISO 11885
	Kalium (K)	0.42	g/100 g tørrstoff	0.0002		NS EN ISO 11885
	Kalsium (Ca)	0.53	g/100 g tørrstoff	0.0001		NS EN ISO 11885
	Magnesium (Mg)	0.40	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c)	Mangan (Mn)	590	mg/kg TS	0.3	20%	NS EN ISO 11885
c)*	Svovel (S)	1200	mg/kg TS	4	20%	NS EN ISO 11885
b)*	**Ammonium-N (2 M KCl)**					
b)*	Ammonium (NH4-N)	0.000518	g/100 g tørrstoff		20%	Internal Method 5
b)	Kompaktert labdensitet	730	g/dm³		5%	EN 13040
b)*	**Nitrat-N (2 M KCl)**					
b)*	Nitrate nitrogen	0.0181	g/100 g tørrstoff		20%	Internal Method 5
a)*	Total nitrogen (mod. Kjeldahl)	0.47	g/100 g tørrstoff	0.01		EN 13654-1

5 C: Mix

Prøvenr.:	**439-2016-09260286**	Prøvetakingsdato:	21.09.2016
Prøvetype:	Kompost	Prøvetaker:	Bente Føreid
Prøvemerking:	Mix kompost MIX	Analysestartdato:	26.09.2016

Analyse	Resultat	Enhet	LOQ	MU	Metode
b)* Fosfor (P-AL)	0.17	g/100 g	20	20%	SS 028310 + T1 tørrstoff
b) Kalium (K-AL)	0.39	g/100 g	20	20%	SS 028310 + T1 tørrstoff
b) Kalsium (Ca-AL)	4.0	g/100 g	100	20%	SS 028310 + T1 tørrstoff
b) Magnesium (Mg-AL)	0.14	g/100 g	10	20%	SS 028310 + T1 tørrstoff

b)* Natrium (Na-AL)	0.15 g/100 g	50		SS 028310 + T1 tørrstoff
c) Arsen (As)	2.2 mg/kg TS	0.5	30%	NS EN ISO 17294-2
c) Bly (Pb)	14 mg/kg TS	0.5	40%	NS EN ISO 17294-2
c) Kadmium (Cd)	0.48 mg/kg TS	0.01	25%	NS EN ISO 17294-2
c) Kvikksølv (Hg)	0.034 mg/kg TS	0.001	20%	NS-EN ISO 12846
c) Tørrstoff	44.6 %	0.1	5%	EN 12880
c) Kobber (Cu)	22 mg/kg TS	0.5	30%	NS EN ISO 11885
c) Krom (Cr)	5.3 mg/kg TS	0.3	30%	NS EN ISO 11885
c) Nikkel (Ni)	4.7 mg/kg TS	0.5	30%	NS EN ISO 11885
c) Sink (Zn)	130 mg/kg TS	2	25%	NS EN ISO 11885
pH målt ved 23 +/- 2°C	8.4	1		NS-EN 12176
* Konduktivitet/ledningsevne	48 mS/m			NS-EN ISO 7888
Total tørrstoff glødetap	41 % TS	0.02		NS 4764
Totalt organisk karbon (TOC)	16 % TS	0.1	20%	Internal Method 1
c) Aluminium (Al)	2600 mg/kg TS	10	15%	NS EN ISO 11885
c) Bor (B)	11 mg/kg TS	5	25%	NS EN ISO 11885
Fosfor (P)	0.21 g/100 g	0.0001		NS EN ISO 11885 tørrstoff
c) Jern (Fe)	4100 mg/kg TS	30	25%	NS EN ISO 11885
Kalium (K)	0.33 g/100 g	0.0002		NS EN ISO 11885 tørrstoff
Kalsium (Ca)	3.7 g/100 g	0.0001		NS EN ISO 11885 tørrstoff
Magnesium (Mg)	0.20 g/100 g	0.0001		NS EN ISO 11885 tørrstoff
c) Mangan (Mn)	330 mg/kg TS	0.3	20%	NS EN ISO 11885
c)* Svovel (S)	1600 mg/kg TS	4	20%	NS EN ISO 11885
b)* Ammonium-N (2 M KCl)				
b)* Ammonium (NH4-N)	0.000608 g/100 g tørrstoff		20%	Internal Method 5
b) Kompaktert labdensitet	600 g/dm³		5%	EN 13040
b)* Nitrat-N (2 M KCl)				
b)* Nitrate nitrogen	0.013 g/100 g tørrstoff		20%	Internal Method 5
a)* Total nitrogen (mod. Kjeldahl)	1.4 g/100 g	0.01		EN 13654-1 tørrstoff

Merknader:

K < K-AL og Ca < Ca-AL: men innenfor MU.

5 D: Vermi

Prøvenr.:	**439-2016-04200471**	Prøvetakingsdato:	20.04.2016
Prøvetype:	Kompost	Prøvetaker:	Astrid Solvåg Nesse
Prøvemerking:	Markkompostert kumøkk KUKOMP	Analysestartdato:	20.04.2016

	Analyse	Resultat	Enhet	LOQ	MU	Metode
b)*	Fosfor (P-AL)	0.63	g/100 g tørrstoff	20	20%	SS 028310 + T1
b)	Kalium (K-AL)	4.5	g/100 g tørrstoff	20	20%	SS 028310 + T1
b)	Kalsium (Ca-AL)	1.4	g/100 g tørrstoff	100	20%	SS 028310 + T1
b)	Magnesium (Mg-AL)	0.57	g/100 g tørrstoff	10	20%	SS 028310 + T1
b)*	Natrium (Na-AL)	0.48	g/100 g tørrstoff	50		SS 028310 + T1
c)	Arsen (As)	1.2	mg/kg TS	0.5	30%	NS EN ISO 17294-2
c)	Bly (Pb)	2.5	mg/kg TS	0.5	40%	NS EN ISO 17294-2
c)	Kadmium (Cd)	0.14	mg/kg TS	0.01	25%	NS EN ISO 17294-2
c)	Kobber (Cu)	28	mg/kg TS	0.5	30%	NS EN ISO 11885
c)	Krom (Cr)	5.5	mg/kg TS	0.3	30%	NS EN ISO 11885
c)	Kvikksølv (Hg)	0.017	mg/kg TS	0.001	20%	NS-EN ISO 12846
c)	Nikkel (Ni)	7.5	mg/kg TS	0.5	30%	NS EN ISO 11885
c)	Sink (Zn)	240	mg/kg TS	2	25%	NS EN ISO 11885
	pH målt ved 23 +/- 2°C	9.0		1		NS-EN 12176
*	Konduktivitet/ledningsevne	190	mS/m			NS-EN ISO 7888
	Totalt organisk karbon (TOC)	36	% TS	0.1	20%	Internal Method 1
c)	Aluminium (Al)	1000	mg/kg TS	10	15%	NS EN ISO 11885
c)	Bor (B)	38	mg/kg TS	5	25%	NS EN ISO 11885
	Fosfor (P)	0.75	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c)	Jern (Fe)	3000	mg/kg TS	30	25%	NS EN ISO 11885
	Kalium (K)	3.2	g/100 g tørrstoff	0.0002		NS EN ISO 11885
	Kalsium (Ca)	2.0	g/100 g tørrstoff	0.0001		NS EN ISO 11885
	Magnesium (Mg)	0.52	g/100 g tørrstoff	0.0001		NS EN ISO 11885
c)	Mangan (Mn)	280	mg/kg TS	0.3	20%	NS EN ISO 11885

Svovel (S)	0.53	g/100 g tørrstoff	0.00015		NS EN ISO 11885
b)* Ammonium-N (2 M KCl)					
b)* Ammonium (NH4-N)	0.00216	g/100 g tørrstoff			Internal Method 5
c) Total tørrstoff glødetap	69.2	% tv	0.1	10%	EN 12879
b)* Nitrat-N (2 M KCl)					
b)* Nitrate nitrogen	0.3783	g/100 g tørrstoff			Internal Method 5
c) Total tørrstoff	18.1	%	0.1	10%	EN 12880
a)* Total nitrogen (mod. Kjeldahl)	2.7	g/100 g tørrstoff	0.01		EN 13654-1

Merknader:

K < K-AL og Mg < Mg-AL, men innenfor MU.

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