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Exploring the potential for using seaweed (*Ulva lactuca*) as organic fertiliser

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Abstract

The focus of this project is on P recovery by using seaweed (*Ulva lactuca*) as organic fertiliser. The possibilities for using seaweeds as fertiliser, depend on two components. First, to determine whether seaweed is suitable as organic fertiliser, it is necessary to analyse the nutrients taken up by the seaweed, to gain insight in the amounts of nutrients that could be applied to the soil. Phosphorus content of *Ulva lactuca* grown under different initial P concentrations in the water (Pi) was measured. Phosphorus content in relation to Pi concentration showed Michaelis-Menten kinetics with Pmax = 0.46% DW and Km 1.19 µM. N content was also measured and P and N content were found to be related.

Second, it was analysed how *Ulva lactuca* material decomposed over time in an agricultural sandy soil when incorporated in June (2013). The mesh bag method was used to monitor the dry matter decomposition. The relative decomposition rate of *Ulva lactuca* was found to be 0.0413 d^{-1}.

In addition, the effects of seaweed fertiliser on two crops, lettuce and mustard, was studied. Mustard plant height and lettuce diameter were measured throughout the course of the experiment. After harvest, shoot and root dry weight were determined and P and N content were measured. *Ulva lactuca* application increased N content ($p<0.05$) and P content ($p<0.10$) of mustard plants, either due to *Ulva lactuca* application or the reduced dry matter production. *Ulva lactuca* application had a negative effect on crop appearance in both crops and reduced shoot dry matter in lettuce ($p<0.05$) and mustard ($p<0.10$). A delay in crop growth and development was visible under *Ulva lactuca* treatment, but the exact functioning of *Ulva lactuca* in crop performance remains unknown.

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1 Introduction

1.1 Global food security

In the 20th century, due to the rapidly growing population, artificial fertilisers were used more and more to increase food production (Smil, 2000). The application of these fertilisers supplemented the nutrient deficient soils and has led to very high agricultural outputs, thereby reducing the numbers of undernourished people worldwide, despite population growth (Cordell *et al.*, 2009). The tremendous global agricultural production of today, would not be possible without the use of mineral fertilisers and in particular processed rock phosphate.

Whereas atmospheric N_2 is an inexhaustible source of nitrogen, phosphorus availability is limited (Townsend & Porder, 2012). Phosphorus (P) is a nutrient that is gained from mined rock phosphate since halfway the 19th century. Since the mining of rock phosphate started, the market for mineral fertilisers developed quickly (Cordell *et al.*, 2009). Rock phosphate reserves are mined very intensively, however, and are expected to be exhausted within 50-100 years (Cordell *et al.*, 2009; Sattari *et al.*, 2012).

The threatening phosphate scarcity has not found international recognition yet. In comparison: 70% of world's fresh water reserves are used for agriculture and many concerns exist about fresh water reserves in the future. However, 90% of the global demand for rock phosphate is used for food production; both resources are expected to be depleted in the near future (Cordell *et al.*, 2009).

By 2050, the world population is expected to have increased from 7 to at least 9 billion people. Together with the increase in consumption of animal products, this will cause an increase in food prices. In order to secure future global food availability, agricultural outputs have to be increased (Koning & Van Ittersum, 2009). In order to accomplish this, fertiliser availability and affordability are of vital importance. Consequently, more efficient use and increased recovery of phosphorus are indispensable.

1.2 Nutrient flows

More than a third of the world population lives within 100 kilometres from a coastline. Therefore, it is not surprising that nutrient inputs (mainly nitrate and ammonium, but phosphate as well) from human origin in inland surface waters, rivers and coastal waters have increased during the last decades (Brady & Weil, 2002; Charlier *et al.*, 2008; Rees, 2003; Smil, 2000). Annually, 21.5 and 2 million tonnes of N and P, respectively, are transported in rivers (Verkleij, 1992). Smil (2000) states that the global P cycle largely is a one way flow to the ocean, where P is stored into sediments. However, determination of P in seawater and sediments is unreliable, due to the salt error in the measurements and much quantitative data on oceanic P flows is unknown (Burton & Riley, 1956). Despite a lack of reliable information, oceans are presumably the largest P reservoirs on earth, where an estimated 99% of the earth's P is stored. The P flow from the oceans back to the land is very slow, as it depends on the move of tectonic plates. It is assumed that in so called upwelling zones, certain ocean currents transport P from deep sea sediments to more shallow waters (Brady & Weil, 2002; Smil, 2000).

Eutrophication of rivers and coastal waters is a global problem (Arheimer *et al.*, 2004). In Europe, agriculture is responsible for 20-40% of the P found in waters (EFMA, 2000). Although urban sources contribute 50-75% to the P in waters, these sources are easy to identify and regulate compared to the diffuse sources of P in runoff from agricultural areas (Brady & Weil, 2002; EFMA, 2000). These

increased N and P inputs have had large impacts on marine organisms (Lourenço *et al.*, 2006). The excess nutrient levels cause eutrophication in surface waters, resulting in algae blooms, oxygen depletion, fish mortality, loss of biological diversity and consequently loss of recreational value (Arheimer *et al.*, 2004; Brady & Weil, 2002; Cameron *et al.*, 2013; Charlier *et al.*, 2008).

1.3 Aim of the project

In this project, the focus will be on using seaweeds as organic fertiliser. It was mentioned in the previous section, that the P lost to the oceans is not flowing back to the land. As a result of human settlement and activities, phosphorus (and inorganic nitrogen) concentrations in rivers and coastal areas have increased. Not only single-celled algae grow well under eutrophic circumstances, but macroalgae, commonly known as seaweeds, as well. By farming seaweeds in near shore, eutrophicated areas and applying it to arable land, seaweeds could help to recover P, maintain agricultural outputs and in this way contribute to food security in the future.

The focus of this research will be on phosphate, because of the expected future phosphorus crisis (Section 1.1). In addition, analyses of nitrogen contents will be performed. The seaweed *Ulva lactuca* is used in this study, because of its fast growth rate.

The possibilities for using *Ulva lactuca* as fertiliser, depend on two components. First, to determine whether it is suitable as organic fertiliser, it is necessary to analyse the nutrients taken up by the seaweed, to gain insight in the amounts of nutrients that could be applied to the soil. Second, it needs to be analysed how *Ulva lactuca* is decomposed over time in an agricultural soil. In addition, the effects of seaweed fertiliser on two crops is studied. The research questions that follow from this, are:

1. What is the phosphorus content of *Ulva lactuca* compared to the phosphate concentrations in the seawater?
2. How does *Ulva lactuca* decompose over time when incorporated into a sandy soil?
3. How does the incorporation of *Ulva lactuca* affect lettuce and yellow mustard quality and quantity?

The outcome of this research will help to develop the use of seaweed as organic fertiliser and will help to gain insights in seaweed farming as a means for P recovery.

2 Literature study

In the introduction of this report, the expected phosphorus crisis was put into the larger perspective of future food security and the potential of seaweeds for P recovery was put forward. This chapter will elaborate on the basics of agricultural production and the importance of phosphorus recovery for food security. Furthermore, this chapter will go deeper into the characteristics of *Ulva lactuca*. Finally, in this literature study the possible benefits and disadvantages of seaweeds in general, and *Ulva lactuca* in particular for the use as organic fertiliser will be discussed. The aim of this chapter is to gain insights from literature on the suitability of *Ulva lactuca* as organic fertiliser.

2.1 Agricultural production

2.1.1 Introduction

The basic needs for agricultural production are sunlight, water, CO_2, soil and nutrients. Plants acquire nutrients from organic matter and minerals (Ismail & Almarshadi, 2012). Inorganic minerals, organic matter and microorganisms are the major components of a soil. These components affect the physical, chemical, and biological properties of a soil, which determine soil fertility (Mohammadi *et al.*, 2011). The soil quality has a major influence on water and nutrient availability to the crops and is mostly influenced by the soil organic matter (SOM) content.

Soil organic matter is plant or animal material that has been returned to the soil. These materials are decomposed by a wide range of heterotrophic microbial organisms living in the soil. Organic matter must be decomposed by soil organisms before nutrients become available to the crop (Dinesh & Dubey, 1998). The biological activity of these organisms determines the decomposition rate of SOM and is influenced by the quality of SOM added and environmental conditions (Brady & Weil, 2002). Depending on the soil type, most soils have a SOM content of 2 to 10% (Mohammadi *et al.*, 2011).

The presence of organic matter in a soil is extremely important, even when present in very small amounts. Besides being a source of nutrients for earthworms, microorganisms and indirectly crops, SOM contributes to other quality aspects of a soil: i.e. soil structure (aggregate formation), water penetration and drainage, water holding capacity, nutrient retention (cation binding capacity), aeration and pH and heat buffering (Brady & Weil, 2002; Mohammadi *et al.*, 2011). Because of the improving soil quality effects, SOM is the most important factor in reducing soil erosion and nutrient leaching (Ross *et al.*, 2009).

2.1.2 Phosphorus and nitrogen

Phosphorus (P) is an element which is essential for the existence of any form of life and for most plant species, P content ranges between 0.2-0.4% of the dry weight (Brady & Weil, 2002). Phosphorus is completely absent in cellulose, hemicellulose, lignin and amino acids, but it is a basic component of DNA and RNA, the carriers of genetic information and is thereby indispensable for all growth and development processes (e.g. root growth, photosynthesis, maturing, flowering, etc.). Furthermore, the energy needed for the metabolism of every organism is provided by the conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) by the release of a phosphate molecule (Brady & Weil, 2002; Smil, 2000). Phosphorus is also found in phospholipids, the building blocks of membranes surrounding cell walls. Finally, P is indispensable for reproduction: plants store P in their seeds to provide seedlings with sufficient amounts of P for establishment (EFMA, 2000). The amount of P necessary for plant growth and development is relatively small compared to carbon, water and nitrogen requirements, but without P, no production is possible (Brady & Weil, 2002; Smil, 2000). Phosophorus deficiencies result in stunted growth, thin stems, bluish or purple leaves, delayed maturity and poor seed quality (Brady & Weil, 2002).

Phosphorus is often limiting plant growth: the worldwide agricultural area in which P deficiency limits agricultural outputs is estimated to be between 1 to 2 billion hectares (Brady & Weil, 2002). The problem of P deficient soils has three causes:

1. The total level of P in soils is low
2. Compound bound P in soils are often highly insoluble and thus unavailable to plants
3. When soluble sources of P (e.g. fertilisers) are added to soils, they are partially fixed and form highly insoluble compounds.

In general, the amount of inorganic P available to crops is only 0.01% of the total P present in a soil. The large majority of P can be found in calcium bound inorganic P (alkaline soils), iron/aluminium bound inorganic P (acidic soils) and organic bound P (Figure 1). These three compound-P molecules are highly insoluble and release P only very slowly to the soil solution, from which plants can take up P. Calcium bound P becomes more soluble when the soil pH decreases, iron/aluminium bound P when the soil pH increases. In temperate regions, the mineralisation of organic P releases between 5 and 20 kg P ha^{-1} yr^{-1}, of which most is instantly absorbed by growing plants (Brady & Weil, 2002). The P concentration in the soil solution is relatively low compared to other macronutrients, ranging between 0.001 mg/L (very infertile soils) and 1 mg/L (rich, fertilised soils). Plant roots mainly take up P in inorganic forms (HPO_4^{-2} and $H_2PO_4^-$), but P bound to soluble organic compounds can be taken up as well (Brady & Weil, 2002).

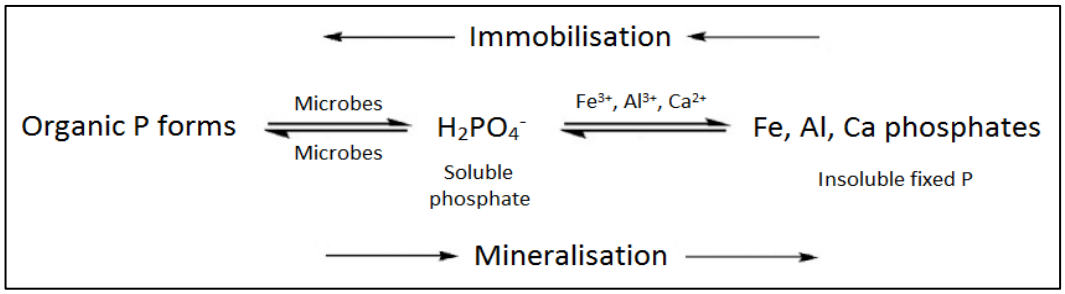

Figure 1: the processes of immobilisation and mineralisation of phosphorus (Brady & Weil, 2002).

Like phosphorus, nitrogen (N) is essential for plant growth. Plants can only take up N in inorganic forms like nitrate (NO_3^-) and ammonia (NH_4^+). Consequently, although 78% of the atmosphere consists of nitrogen (N_2), plants mainly acquire nitrogen from inorganic sources in the soil, like manure, fertilizers and mineralised organic matter (Brady & Weil, 2002; Singh, 2006). However, some plants acquire atmospheric N through symbiosis with N-fixing bacteria (Talgre *et al.*, 2012).

Nitrogen is absent in cellulose and lignin. Amino acids however, all contain an amine group ($-HN_2$) and nitrogen is therefore required for building the variety of proteins occurring in plants (e.g. rubisco). Furthermore, nitrogen is found in DNA and in the pigment chlorophyll (Evans & Poorter, 2001). Consequently, N deficiency is often visible in the yellowing of plant leaves. The loss of chlorophyll causes a reduction in the photosynthetic capacity of the leaves and results in stunted plant growth (Brady & Weil, 2002).

2.1.3 Decomposition and nutrient availability

Besides inorganic nutrients, heterotrophic microorganisms require carbon as a source of energy for living and reproducing. Artificial fertilisers provide nutrients for soil fauna, but do not supply carbon (Mohammadi *et al.*, 2011). Apart from the beneficial effect on soil structure, a low SOM content results in a decrease of soil fauna. Applying organic matter to the soil will cause an increase in soil fauna biomass, which will improve organic matter decomposition and thereby a constant release of nutrients (Brady & Weil, 2002; Holland, 2004). Microorganisms are in particular indispensable for the P cycle, since more than half of the soil P is bound to organic compounds (Smil, 2000).

Soil fauna require 24 times more C than N (based on weight) for energy and maintenance. If the ratio of organic matter exceeds this number, soil microbes will take up N from the soil; if the ratio is below 24, N will be released into the soil (USDA, 2011). The immobilisation of organic bound P occurs when added residues have a C:P ratio higher than 300:1, whereas the mineralisation of soluble phosphate occurs when this ratio is below 200:1 (Brady & Weil, 2002). The C:N and C:P ratios of applied fertilisers will determine the balance between N and P mineralization and immobilization (Brady & Weil, 2002; Holland, 2004). Therefore, a simultaneous application of both organic and inorganic fertilisers provides a crop with the required nutrients and contributes to soil quality and long term nutrient (and in particular P) availability (Mohammadi *et al.*, 2011).

Besides climatic conditions, soil characteristics and C:N and C:P ratios, SOM decomposition rates depend on the quality of the added material (Brady & Weil, 2002). Figure 2 shows the relative decomposition rates of the different components found in plants. Sugars, starches and simple proteins are decomposed very fast, whereas lignins are decomposed very slow by microorganisms. Trials in a Mediterranean climate showed that 40% of oak litter (39% cellulose, 15% lignin) remained in the soil after 3 years (Fioretto *et al.*, 2005). In comparison, 50% of the incorporated mass of white clover (10% hemicellulose, 13% cellulose and 2.3% lignin; Henriksen & Breland, 1999), was decomposed after 12 days (sandy loam soil, temperate climate; McCurdy *et al.,* 2013). The ratio of components in added manure will determine the time needed to be completely decomposed by microorganisms. In addition, lignin physically protects other plant components from decomposition, therefore the breakdown of the different components is not independent (Fioretto *et al.*, 2005). See e.g. Janssen (1984) for the decomposition time of a variety of organic matter sources.

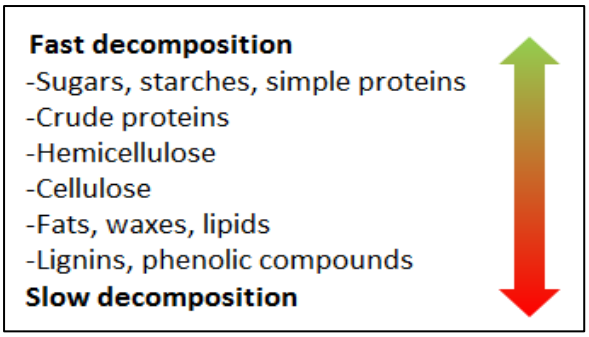

Figure 2: Relative decomposition rates for different plant components. From Brady & Weil (2002)

2.1.4 Organic fertilisers

Mohammadi *et al.* (2011) state that the main advantage of artificial, inorganic fertilisers over organic fertilisers is the convenience in application to the soil. However, artificial fertilisers release nutrients quickly in the soil, possibly with large-scale nutrient leaching and eutrophication as a consequence. The application of organic fertilisers does not cause these problems to this extent, as discussed in Section 2.1.1.

Due to large scale monocultures, abundant use of inorganic fertilisers and pesticides and removal of organic residues after harvests, the SOM content has decreased worldwide (Piotrowska & Wilczewski, 2012; Tejada *et al.*, 2008). As a consequence, large scale soil degradation increasingly occurs in many agricultural areas (Holland, 2004). Nowadays, a paradigm shift is visible: as individual farmers, agricultural organisations and governments increasingly encounter problems like erosion, water pollution and loss of soil fertility, “old-fashioned” practices like the application of organic fertilisers, reduced tillage, etc., aiming to increase the SOM, are more and more seen as the only solution to soil degradation and maintaining agricultural outputs (Block, 2013).

Organic fertilisers like green manures are used to increase the SOM content. Green manures are growing crops that are ploughed under prior to sowing a cash crop. They have the ability to increase crop productivity on the short term and to improve soil fertility on the long term (Dinesh & Dubey, 1998; Tejada *et al.*, 2008). Examples of green manures are clover species, vetch and Lucerne (Talgre *et al.*, 2012). Besides green manures, other organic fertilisers are used in agriculture, like manures, composts and slurries (Piotrowska & Wilczewski, 2012). In addition, post-harvest crop residues are left on the soil to act as mulch or ploughed under. Within the context of soil degradation, seaweed application to soils could contribute to increasing the SOM content of many impoverished soils.

2.2 *Ulva lactuca*

2.2.1 Taxonomy, morphology and reproduction

Seaweeds are multicellular eukaryotic organisms, belonging to the kingdom of the Protista and the phyla Phaeophyta (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae; Raven *et al.,* 2005, p.231). Although all the macroalgae from the different phyla are called seaweeds, they do not share a common multicellular ancestor; they are a paraphyletic group. The seaweed species used in this study is *Ulva lactuca*, a green algae from the family of *Ulva lactucaceae*, genus *Ulva lactuca.*

The common name of *Ulva lactuca* is sea lettuce, because of its morphological resemblance to lettuce. *Ulva lactuca* consists of a large leaf like thallus and a holdfast, which anchors the seaweed to shells, rocks and other materials (Figure 3). The thallus can reach lengths of 1m, but large thalli are more likely to be torn into pieces by the current. As a result, *Ulva lactuca* usually grows to a length of 30cm in nature (Wald, 2010). Especially under eutrophic conditions, mainly free floating thalli of *Ulva lactuca* can be found in natural waters (Malta *et al.*, 1999).

Although *Ulva lactuca* has a strong, plastic like structure, the thallus is only two cell layers thick (Wald, 2010). Normally, thallus colour ranges between light green and dark green, but transparent and olive green spots or edges are observed as well. (Robertson-Andersson *et al.*, 2009) have mapped the colour range of *Ulva lactuca* in relation to the nitrogen content: the more intensely green coloured, the higher the nitrogen content. *Ulva lactuca* is translucent.

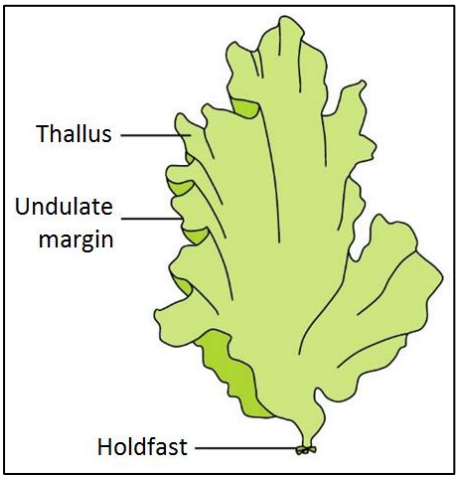

Figure 3: Schematic morphology of *Ulva lactuca.* From Cronodon (2013).

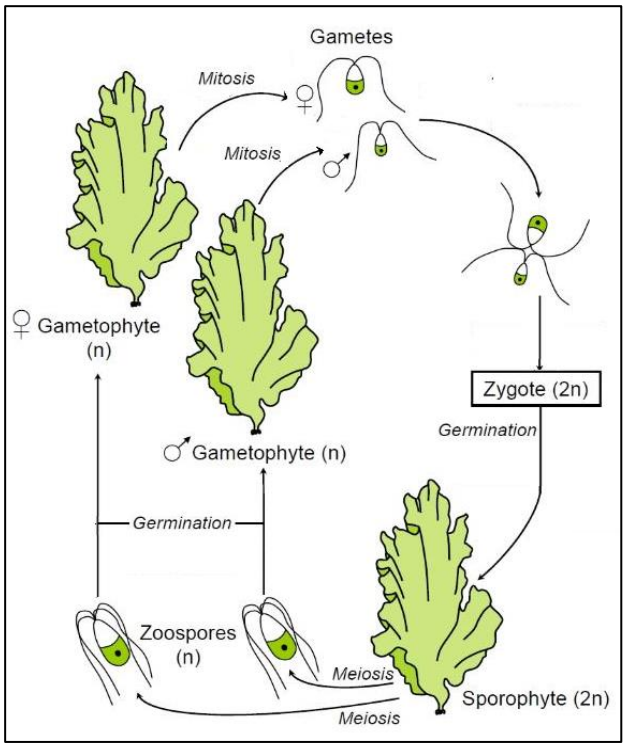

Figure 4: Schematic life cycle of *Ulva lactuca*. From Cronodon (2013).

The reproduction cycle of *Ulva lactuca* is given in Figure 4. Under natural conditions, the diploid sporophyte sporulates in winter or early spring. This can be observed by a change of colour and breakdown of the edges of the thallus. The spores grow into haploid gametophytes, which are morphologically similar to the sporophyte. A male and female gamete produced by gametophytes together form a 2n zygote which grows into a sporophyte after germination. Germination is stimulated under low temperature combined with high light intensities (Wald, 2010).

2.2.2 Growth and nutrient uptake characteristics

U. lactuca is found along coastlines worldwide (Wald, 2010). The growth season in the Netherlands starts in April and ends in November. *Ulva lactuca* grows well in a temperature range between 10-25°C and a salinity of around 30‰. *U. lactuca* is the only macroalgae which can cause dense blooms (Malta *et al.*, 1999; Wald, 2010). One of the reasons of these blooms, which are seen as detrimental for the recreational value of coast lines, is the high growth rate of *Ulva lactuca* under eutrophicated, warm conditions. As the green colour suggests, *Ulva lactuca* contains the pigments chlorophyll a and b. *U. lactuca* can grow until depths of 15m, but are usually found at depths of 1m, because of light colour and light intensity requirements (Wald, 2010). The chlorophyll contents and therefore growth rates are depending on amount of light, N limitation and sporulation (Geertz-Hansen & Sand-Jensen, 1992). The presence of epiphytes and grazers can restrain growth as well (Kamermans *et al.*, 2002).

In greenhouse experiments, a daily 50% increase in *U. lactuca* biomass was observed (Brandenburg, pers. comm. 2013) and laboratory experiments of Pedersen & Borum (1997) confirmed this high relative growth rate (0.513 d^{-1}). Growth rates in nature are considerably lower due to light, temperature and nutrient limitations. In a variety of articles, *Ulva lactuca* growth rates are mentioned. Frost-Christensen & Sand-Jensen (1990) found specific growth rates of *Ulva lactuca* between 0.20 and 0.35 d^{-1} (Denmark). The studies of Pedersen *et al.* (2010) and Pedersen & Borum (1996) showed maximum relative growth rates for *U. lactuca* of 0.25 d^{-1} (Norway) and 0.40 d^{-1} (Denmark), respectively. These rates are considerably higher than other fast growing algae (Pedersen & Borum, 1996; Pedersen *et al.*, 2010).

Because the thallus of *Ulva lactuca* is only two cell layers thick, the surface area per unit of volume is very large, enabling this seaweed to take up large amounts of nutrients through the cell wall, especially under high growth rates. Under favourable circumstances, *U. lactuca* can take up 4-6 times more nutrients than other seaweed species (Pedersen & Borum, 1997). Especially in highly eutrophicated waters, *Ulva lactuca* outcompetes slow growing macrophytes, like seagrasses and kelps (Pedersen & Borum, 1996). For this reason, *U. lactuca* is often used to remove nutrients from sewage effluents (e.g. Tsagkamilis *et al.,* 2010) or fish farms (e.g. Neori *et al.,* 1991). *U. lactuca* responds to high nutrient levels by increasing nutrient uptake, fast growth and storing nutrients intracellular for future growth (Lourenço et al., 2006).

Maximum observed P uptake rate is 4.15 µmol P g^{-1} DW h^{-1} (Pedersen *et al.*, 2010), maximum observed N uptake rate is 200 µmol N g^{-1} DW h^{-1}, so N requirements of *Ulva lactuca* are much higher than P requirements (Pedersen & Borum, 1996). Growth of *Ulva lactuca* is often limited by N availability during the entire season, whereas P limitation effects on growth are much smaller (Teichberg *et al.*, 2008). However, N and P uptake are suggested to be related and lower P uptake rates were observed when *Ulva lactuca* growth was nitrogen limited (Pedersen & Borum, 1996). The high nutrient uptake rates are suspected to be related with the cell structure of *Ulva lactuca*. Besides the fact that each cell is in contact with seawater, *U. lactuca* is lacking any kind of internal transport system. Therefore, all cells must be able to acquire nutrients, photosynthesize, grow and sporulate and as a consequence, each cell contains high levels of N and P, bound to the various proteins required for these processes (Pedersen & Borum, 1996). Because of this cell structure, *U. lactuca* needs to be able to react quickly to osmotic changes; therefore this seaweed is seen as a stress tolerant species (Raffaelli *et al.,* 1998 in Wald, 2010).

2.2.3 Chemical characteristics

Terrestrial plants are known for their high cellulose (45% DW), hemicellulose (18% DW) and lignin (20% DW) content in cell walls (Brady & Weil, 2002). Cellulose, hemicellulose and lignin are indispensable for the construction of vessels, fortify the entire plant structure and prevent collapse of the plant in air (Martone *et al.*, 2009; Raven *et al.*, 2005). In contrast to land plants, seaweeds do not need support, because they are growing in an aquatic environment. Although compounds comparable to lignin have been found in primitive algae, most seaweeds lack or contain very low levels of lignin (Martone *et al.*, 2009; Yanagisawa *et al.*, 2011).

Cellulose is the most abundant organic compound on earth and is present in both sea and land plants (Raven *et al.*, 2005). However, cellulose from algae species form a more porous network, which differs significantly from higher plant cellulose (Siddhanta *et al.*, 2009). Cellulose contents show a wide variation among different kinds of seaweed: crude cellulose contents of 11%, but also as little as 0.85% of the dry weight were found (Siddhanta *et al.*, 2010). Also, cellulose concentrations vary in different parts of the seaweed (i.e. frond or stipe; Yanagisawa *et al.,* 2011).

Table 1 shows the chemical composition of *Ulva lactuca* collected in Tunisia and the typical composition of green land plants. The insoluble fibres, (hemicellulose, cellulose and lignin) constitute about a third of the seaweed dry matter content, which is much lower than in land plants. Lignin content of *Ulva lactuca* is only 1.6%, which is extremely low compared to the 17-24% found in grasses and leguminous plant families (Vahdat *et al.*, 2011). Cellulose content of *Ulva lactuca* is also much lower than in terrestrial plants. Hemicellulose contents of *Ulva lactuca* and green plants are found to be similar.

Table 1: Chemical composition of *U. lactuca* (Yaich *et al.*, 2011) and representative green plants (Brady & Weil, 2002) based on % of DW.

Component	*Ulva lactuca* (% DW)	Green plants (% DW)
Ash	20	8
Protein	8.5	8
Lipid	8	2
Soluble sugars	0.6	5
Uronic acid	10	ND
Soluble fibres	20.5	ND
Insoluble fibres	31	83
-Hemicellulose	21	18
-Cellulose	9	45
-Lignin	1.6	20

ND: no data available

The ash content of *Ulva lactuca* is about 20% of the dry weight (Bruhn *et al.,* 2011 reported ash contents between 14-35% DW) and contains many different minerals. In a variety of articles, the mineral content of *U. lactuca* is described. The mineral contents of *Ulva lactuca* vary when grown under different mineral concentrations and depend on other growth aspects. Therefore, the different collection sites influence the mineral content of *Ulva lactuca*, for some waters are more eutrophicated than others. In comparison to green manure species, *U. lactuca* contains similar amounts of N and K, has higher amounts of Ca, Mg and Fe, but contains very low amounts of P (Table 2). In addition to the elements described in Table 2, many micronutrients like Cu, Mn, Zn, B, Al, Ni, Cr, Cd and Pb are found in *Ulva lactuca* (Villares *et al.*, 2007).

Yaich *et al.* (2011) found the protein content of *Ulva lactuca* to be 8.5% DW, but Bruhn *et al.* (2011) states that protein content can be as high as 40% DW under high external N concentrations. About

30% of *Ulva lactuca* DW consists of soluble sugars, uronic acid and soluble fibres (e.g. starches). These are sugars or sugar polymers and are decomposed relatively fast (Figure 1).

Table 2: Mineral contents of *Ulva lactuca* and green manure species. *Ulva lactuca* is collected at Hong Kong (Ho), Norway (Pedersen *et al.*), Mexico (Hernández-Herrera *et al.*; Pérez-Mayorga *et al.*), Spain (Villares *et al.*) and Tunisia (Yaich *et al.*). Data on alfalfa and clover: Morón & Cozzolino (2002), vetch: Caballero *et al.* (1996).

Element	Amount (mg/100g DW)	Paper	Green manure species and amount (mg/100g DW)
Nitrogen (N)	2700	Ho (1981)	Alfalfa: 4380
	4950	Pedersen *et al.* (2010)	White clover: 3790
	5800	Pedersen & Borum (1996)	Vetch: 2800
	2000	Pérez-Mayorga *et al.* (2011)	
	2000	Villares *et al.* (2007)	
Phosphorus (P)	100	Hernández-Herrera *et al.* (2013)	Alfalfa: 3660
	145	Ho (1981)	White clover: 2970
	140	Pedersen *et al.* (2010)	Vetch: 310
	150	Villares *et al.* (2007)	
	93	Yaich *et al.* (2011)	
Calcium (Ca)	1700	Ho (1981)	Alfalfa: 1920
	2600	Villares *et al.* (2007)	White clover: 1210
	2700	Yaich *et al.* (2011)	Vetch: 680
Magnesium (Mg)	940	Villares *et al.* (2007)	Alfalfa: 290
	3900	Yaich *et al.* (2011)	White clover: 280
			Vetch: 290
Potassium (K)	1900	Ho (1981)	Alfalfa: 2450
	1970	Villares *et al.* (2007)	White clover: 2100
	630	Yaich *et al.* (2011)	Vetch: 1100
Sodium (Na)	4570	Villares *et al.* (2007)	
	552	Yaich *et al.* (2011)	
Iron (Fe)	820	Ho (1981)	Vetch : 21
	250	Villares *et al.* (2007)	

Pedersen *et al.* (2010) found *Ulva lactuca* C:N and C:P ratios of 6.7 and 238 respectively, but these values depend on nutrient availability and factors such as irradiance (Bruhn *et al.*, 2011). *Ulva lactuca* dry matter content (DMC) varies throughout the season (Brandenburg *et al.*, 2012; Bruhn *et al.*, 2011; Mann, 1972). Bruhn *et al.* (2011) reported *Ulva lactuca* DMC varying between 9.6 and 20.4%; during pilot studies of this research, *Ulva lactuca* dry matter contents of 16% were found.

2.2.4 Applications

Seaweeds are a multipurpose food commodity. Seaweeds can have a high nutritional value: they are low in fat (compared to animal foods) and calories, but high in vitamins and minerals (Smith *et al.*, 2010). Also, *Ulva lactuca* has a high protein content and contains 17 different (including all the essential) amino acids, making seaweeds an excellent source of high quality proteins for human consumption (Yaich *et al.*, 2011). A second important application of seaweeds, is the conversion into biofuels. *Ulva lactuca* is seen as one of the best sources of biomass for producing biofuels, because of its high potential biomass yield of 24 tonnes DW ha^{-1} y^{-1}, which is similar to the potential production of sugar beets and which is three times higher than brown algae (Bruhn *et al.*, 2011; Smit & Willegen, 2011). The polysaccharides in *Ulva lactuca* are easy hydrolysable due to the low lignin content; this results in high bioethanol concentrations per unit weight (Yanagisawa *et al.*, 2011).

Also, in nature, seaweeds are a food source for many aquatic invertebrates (Williams & Smith, 2007). Not only aquatic animals can feed on macroalgae, but terrestrial animals as well. Due to the high

polysaccharide and protein content, seaweeds are suitable as fodder. In coastal regions in Iceland, Norway, Great Britain, Ireland and France, animals are regularly fed with seaweeds, either as fresh material or processed seaweed meals (Verkleij, 1992). Furthermore, (extracts of) seaweeds can be used for their medicinal effects or food supplements (Craigie, 2011; Wald, 2010). European immigrants in New Zealand made puddings with carrageen originating from red seaweeds found on the beaches of New Zealand. Also, during the second world war, dried seaweeds were sent from New Zealand to troops located in the Middle East, probably for their laxative effects. On desert marches, troops chewed on seaweeds, because they quenched thirst more than chewing gum (Smith *et al.*, 2010). Also, the worldwide used culture medium agar, is a product from a red seaweed (Li *et al.*, 2008). Finally, seaweeds have been described as a means to decrease eutrophication, to control red tides and to control biological diseases (Yu & Yang, 2008). Also, seaweed production has been suggested to be integrated with fish and shrimp farming, to diminish the release of dissolved nutrients and convert this into a useful product (Troell *et al.*, 1999).

2.3 Seaweeds as fertilisers

2.3.1 History

In coastal areas throughout the entire world, seaweeds have been used as fertiliser since the beginning of times and are still being used in some areas (Booth, 1963; Caiozzi *et al.*, 1968; Francki, 1960a; Haslam & Hopkins, 1996; Verkleij, 1992). In many Asian countries, the use of seaweeds for multiple purposes has been part of the culture since thousands of years. In Western and in particular European countries, the interest in seaweeds has grown only recently, although in coastal areas the use of seaweeds as manure has not been unusual in the past (Verkleij, 1992). Nowadays, there is a renewed interest in seaweeds because of the industrial components that can be extracted from them (Craigie, 2011). In addition, organic farmers are interested in new sources of organic fertilisers and crop protection agents, since the use of chemical fertilisers and pesticides is prohibited (Verkleij, 1992). Hence they are interested in seaweed products, because of their natural origin.

In areas with easy access to seaweeds, seaweeds are often collected manually and sometimes a selection of certain species is made, indicating that farmers perceive some species to be more beneficial for their crops than others (Villares *et al.*, 2007). Many differences exist in the application of seaweeds as organic fertiliser. Some farmers compost the seaweeds with other plant materials, whereas in other regions, the seaweeds are left to rot over winter (Booth, 1963). In Spain, most of the farmers that use seaweeds as manure, apply the seaweeds directly to the crop, but others leave the seaweeds to rain-wash to remove salts. Some farmers dry the seaweeds before application or let it compost in heaps (Villares *et al.*, 2007). Usually brown algae are selected for the use as manure, such as *Laminaria*, *Fucus* and *Ascophyllum nodosum* (Booth, 1963; Khan *et al.*, 2009).

Since the use of artificial fertilisers became economically more attractive than the use of seaweeds, seaweeds have been used less and less for fertilizing arable land. Now prices of artificial fertilisers are rising and areas are increasingly affected by soil degradation processes, there is an increased interest for natural fertilisers such as seaweeds (Crouch & Van Staden, 1993; Villares *et al.*, 2007). Although in history the use of seaweeds as manure was mainly limited to coastal areas, nowadays seaweed extracts are used as fertiliser in many countries and areas. Large companies in China, France, Ireland and South Africa have developed commercial seaweed extracts (Craigie, 2011). The aqueous extracts are usually made from *A. nodosum*, *Laminaria* spp., *Ecklonia maxima* and *Sargassum* spp., which are all brown seaweeds. Colour, odour, viscosity, solids and particulate matter content vary considerably between the extracts (Craigie, 2011).

The next sections will go deeper into the benefits and disadvantages of seaweed extracts on plant growth, as few literature is available on the effects of crude seaweed material.

2.3.2 Beneficial effects

Since the literature on the manurial use of green algae in general and *Ulva lactuca* in particular is scarce, a separate section is dedicated to the effects of *U. lactuca* on plant growth. In this section, literature on the effects of brown and red seaweeds on crop growth is reviewed. It is assumed that the application of *U. lactuca* to crops can induce the same effects as brown and red seaweed species.

Seaweeds have been used to fertilise a wide range of crop species (e.g. potatoes, cereals, vegetables and horticultural crops; Verkleij, 1992; Villares *et al.,* 2007) on a wide range of soils (e.g. clay, sand, volcanic, organic, acid and calcareous soils; Booth, 1963, Caiozzi *et al.,* 1968; Verkleij, 1992) in a wide range of climates (Booth, 1963; Caiozzi *et al.*, 1968). In literature, many beneficial effects of seaweed (extracts) to crops have been reported. In Table 3, these effects are summarized.

In addition to the beneficial effects mentioned, the following effects are documented:
-Increased resistance to low temperatures (Booth, 1963; Kavipriya *et al.*, 2011; Khan *et al.*, 2009);
-Increased resistance against drought and salinity (Khan *et al.*, 2009);
-Reduced transplant shock (Crouch & Van Staden, 1992; Khan *et al.*, 2009);
-Increased leaf area in lettuce (Crouch *et al.*, 1990) and wheat (Beckett & Van Staden, 1989);
-Increased sugar content in tobacco, melons (Booth, 1963) and sugar beets (Blunden & Wildgoose, 1977).

Some authors reported improved plant growth when seaweed extracts and chemical fertilisers were both applied, in contrast to adding either one of the two. Highest yields for potatoes, leek, cauliflower (Booth, 1963), lettuce (Crouch *et al.*, 1990), olive (Chouliaras *et al.*, 2009) and wheat (Beckett & Van Staden, 1990) were found when sufficient amounts of inorganic fertiliser were applied besides seaweed extracts.

A number of the beneficial changes in plant growth could perhaps be ascribed to a few basic effects of seaweeds on plant growth. For example, enhanced nutrient uptake might be caused by a more extended root system. An overall increase in plant growth might result from a higher photosynthetic capacity, which might affect yield and yield quality as well. Although it is clear that crops benefit in many ways from the application of seaweeds, it is hard to indicate whether seaweed compounds intervene in multiple plant processes directly or alter just a few, which affect many other plant processes indirectly.

It is striking that in a large number of cases, the beneficial effects of seaweeds on crop growth and yield are dose dependent, whereas the optimum usually is found at lower or mediate concentrations applied. This is the case in a variety of crops, e.g. wheat (Beckett & Van Staden, 1990), potato (Blunden & Wildgoose, 1977), mung bean (Kavipriya *et al.*, 2011), grapes (Kok *et al.*, 2010) and even in *Ulva lactuca* production (Robertson-Andersson *et al.*, 2006). These dose dependencies have been documented for the application of seaweed extracts and for raw seaweed material. For extracts, the relatively concentrated seaweed compounds may be the cause of the dose dependence.

At first, the growth enhancing effects of seaweeds were attributed to the extra nutrients added to the soil (Craigie, 2011). In some cases however, crop nutrient uptake was higher than the amount of nutrients supplied by the seaweeds (e.g. Francki, 1960a), thus seaweeds were suspected to promote nutrient uptake. Craigie (2011), Crouch *et al.* (1990) and Khan *et al.* (2009) mention that organic compounds in seaweeds can chelate some nutrients. A chelate is an organic molecule that binds to metals and increases plant availability of these metals if the compounds are soluble (Brady & Weil, 2002). In addition, seaweeds can correct marginal deficiencies of some minerals (Crouch & Van Staden, 1993). Indeed, most seaweeds are known to contain small amounts of indispensable trace elements, like Cu, Co, Zn, Mn, Mg, Fe, Ni, Mo and B (Beckett & Van Staden, 1990; Booth, 1963; Craigie, 2011).

Table 3: Reported beneficial effects of seaweed fertiliser on crops or soils. Percentages are in- or decreases compared to control treatment. In all studies seaweed extracts are used, unless shown in bold. All seaweed species mentioned are brown, unless specified differently.

Beneficial effect on:	Paper	Seaweed	Added to:	Details
Total plant growth	-Booth (1963)	-N.S.	-Orange, grapefruit (seedlings)	-
	-Crouch & Van Staden (1992)	-*Ecklonia maxima*	-Tomato (seedlings)	-Higher shoot and root FW
	-Kavipriya *et al.* (2011)	-Brown	-Seedlings	-
	-Mugnai *et al.* (2008)	-*Fucales, Laminariales*	-Grapes	-Higher leaf, stem and root DW
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster beans	-
	-Robertson-Andersson *et al.* (2006)	-*Ecklonia maxima*	-*Ulva lactuca*	-Extract used in mariculture
Root growth	-Beckett & Van Staden (1989)	-*Ecklonia maxima*	-Wheat	-In K stressed wheat
	-Crouch & Van Staden (1992)	-*Ecklonia maxima*	-Tomato	-
	-Mugnai *et al.* (2008)	-*Fucales, Laminariales*	-Grapes	-
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster beans	-17% increase root weight
Shoot growth	-Crouch & Van Staden (1992)	-*Ecklonia maxima*	-Tomato	-
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster beans	-11% increase in shoot weight
Nutrient uptake	-Booth (1963)	-N.S.	-Melons, Lime	-Ca, Mg, N (melons), Fe, Zn, Mg, B (lime)
	-Caiozzi *et al.* (1968)	-*Macrocystis integrifolia* Bory	-Soil	-P in calcareous soil
	-Chouliaras *et al.* (2009)	-*Ascophyllum nodosum*	-Olive	-K, Fe, Cu (reduced Mn uptake)
	-Crouch *et al.* (1990), A	-N.S.	-N.S.	-N, P, K, Ca, Mn, Mg, Fe, Zn
	-Crouch *et al.* (1990), B	-*Ecklonia maxima*	-Lettuce	-Ca (+52%), K (+46%), Mg (+37%)
	-Mugnai *et al.* (2008)	-*Fucales, Laminariales*	-Grapes	-K, NH_4
Germination	-Crouch & Van Staden (1993)	-Mix of brown species	-Radish, red fescue grass	-
	-Kavipriya *et al.* (2011)	-N.S.	-Table beet, lettuce, faba bean	-
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster bean	-
Yield	-Abetz & Young (1983)	-*Ascophyllum nodosum*	-Lettuce, cauliflower	-Less crop failure (lettuce) Increased weight (cauliflower)
	-Beckett & Van Staden (1989)	-*Ecklonia maxima*	-Wheat	-Only in plants under K stress
	-Beckett & Van Staden (1990)	-*Ecklonia maxima*	-Wheat	-36% increase
	-Blunden & Wildgoose (1977)	-Mix of brown species	-Potatoes	-13% increase
	-Booth (1963), A	-N.S.	-Soy bean, pepper, corn, lime, tomato, tobacco, melons	-No yield increase in cotton
	-Booth (1963), B	-*Hypnea* (Red)	-Okra	-73% increase
	-Booth (1963), C	-**Seaweed mulch**	-Lime trees	-
	-Craigie (2010), A	-*Ascophyllum nodosum*	-Tomatoes, turnips	-

	-Craigie (2010), B	-*Sargassum wightii*	-Cotton	-
	-Chouliaras *et al.* (2009)	-*Ascophyllum nodosum*	-Olive	-Higher productivity, earlier fruit maturation
	-Crouch *et al.* (1990)	-*Ecklonia maxima*	-Lettuce	-Increase of 14%
	-Crouch & Van Staden (1992)	-*Ecklonia maxima*	-Tomato	-Increase of 17%, earlier fruit ripening
	-Kok *et al.* (2010)	-*Ascophyllum nodosum*	-Grapes	-
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster beans	-Increase of 24% in vegetable weight
Yield quality	-Blunden & Wildgoose (1977)	-Mix of brown species	-Potatoes	-Tubers more even, improved storage quality
	-Chouliaras *et al.* (2009)	-*Ascophyllum nodosum*	-Olive	-Higher oil content, better colour and firmness
	-Craigie (2010)	-*Ascophyllum nodosum*	-Strawberries	-Increase of 17% (1yr old plants) and increase of 43% (2yr old plants) of marketable yield
	-Crouch *et al.* (1990)	-*Ecklonia maxima*	-Lettuce	-Higher nutrient content
	-Kok *et al.* (2010)	-*Ascophyllum nodosum*	-Grapes	-Higher tannin content, longer shelf life
Photosynthetic pigments	-Crouch & Van Staden (1993)	-N.S.	-Tomato	-
	-Thirumaran *et al.* (2009)	-*Rosenvingea intricate* (Red)	-Cluster beans	-26% higher chlorophyll content
Resistance to pests and diseases	-Booth (1963), A	-N.S.	-Tomatoes	-Reduced eelworm damage
	-Booth (1963), B	-N.S.	-Melons	-Higher mildew resistance
	-Craigie (2011), A	-*Sargassum wightii*	-Cotton (seedlings)	-74% reduction in blight infection
	-Craigie (2011), B	-*Ascophyllum nodosum*	-Turnips, strawberries (not all varieties)	-Suppressed powdery mildew (turnips) and gray mould (strawberries) infestation
	-Craigie (2011), C	-*Ascophyllum nodosum*	-Broad beans, sugar beets	-Reduced aphid infestation
	-Craigie (2011), D	-*Ascophyllum nodosum*	-Apple, strawberries, chrysanthemum	-Reduced red spider mite infestation
	-Craigie (2011), D	-*Ecklonia maxima*	-Tomatoes, *Arabidopsis*	-Reduced nematode infestation
	-Verkleij (1992), A	-N.S.	-Turnip	-15% powdery mildew infection (control 85%)
	-Verkleij (1992), B	-N.S.	-Strawberries	-5% *Botrytis cinerea* infection (control 23%)
	-Verkleij (1992), C	-N.S.	-Lettuce	-12% of plants diseased (control 82%)
Soil fertility	-Booth (1963)	-N.S.	-Soil	-Large number of N fixing organisms found in heaps of rotting seaweed
	-Haslam & Hopkins (1996)	-***Laminaria digitata***	-Soil	-Increased pore volume, aggregate stability, soil microbial biomass and potential N mineralisation rate
	-Khan *et al.* (2009)	-*Laminaria japonica*	-Citrus trees, papaya, passion fruit	-Improved arbuscular mycorrhizal colonization

N.S. Not specified

Although in some cases yield increases were primarily caused by the nutrients in seaweed extracts (e.g. Beckett & Staden, 1990), other substances originating from seaweeds were found to affect plant functioning as well. Especially the dose dependency and activity at application rates as low as 15 L/ha, indicate that hormones or hormone-like compounds might be present in seaweeds. So far, ABA, auxin, cytokinin and GA are isolated in different brown species of seaweeds, of which most are used in commercial seaweed extracts (Craigie, 2011). Ethylene and strigolactone presence in seaweeds are not reported. Crouch & Van Staden (1993) state that the hormone levels in seaweeds are comparable to levels found in higher plants. A number of effects described in Table 3 can be attributed to the hormones found in seaweeds, e.g. enhanced shoot and root growth, increased germination percentages and increased chlorophyll content.

Besides the plant hormones, many other organic (hormone-like) compounds are found in seaweeds which affect nearly all growth and development processes in terrestrial plants. Craigie (2011) and Khan *et al.* (2009) present a comprehensive review of the variety of compounds present in seaweeds and their functioning in higher plants. A number of these compounds play a role in alleviating abiotic (temperature, drought, salinity) and biotic (insects, nematodes, fungi, bacteria, viruses) stress factors in plants. For example, betaine compounds found in *A. nodosum, Fucus* and *Laminaria,* influence drought, frost and disease resistance in plants. Red and brown seaweeds contain many different polysaccharides which are not found in land plants, but influence a wide range of biological activities, such as the expression of several genes encoding antimicrobial proteins (Khan *et al.*, 2009).

Many remarkable effects of seaweed application on pest and disease control have been observed. Craigie (2011) and Khan *et al.* (2009) present good examples of enhanced plant defence responses after seaweed application. In some cases this response can be attributed to overall increased health of plants, but examples of biological control are given as well. The polysaccharide laminaran in brown and green seaweeds, is found to enhance the activity of soil microbes that digest detrimental fungi. Both Booth (1963) and Khan *et al.* (2009) have reported reduced fungal infestation due to a large increase in the amount of antagonistic bacteria. Seaweeds are also rich in phenols, which are known to have antibacterial and antifungal properties (Khan *et al.*, 2009; Verkleij, 1992). Seaweed extract application to crops is also known to reduce the numbers of detrimental nematodes (soil drench) and insects (foliar spray) in different crops, but the mechanism behind the pesticidal functioning of the extracts is not exactly clear (Booth, 1963; Craigie, 2011; Khan *et al.*, 2009).

Besides the effects on plant functioning and pest and disease control, seaweeds can improve soil fertility by altering soil physical, chemical and biological characteristics. Improved soil structure, water holding capacity, soil aeration and capillary activity have been reported after adding seaweed material to the soil (Haslam & Hopkins, 1996; Khan *et al.*, 2009). This results in better plant growth and improves the microbial activity, which enhances nutrient mineralisation (Section 2.1.3). Also, increased numbers of mycorrhizal fungi and nitrogen fixing and other beneficial bacteria have been reported after adding seaweed material to the soil (Booth, 1963; Haslam & Hopkins, 1996; Khan *et al.*, 2009). In one study soil, pH was found to be slightly increased after adding seaweeds (Caiozzi *et al.*, 1968), which might be a beneficial effect for some acid soils. Also, seaweeds have been reported to remediate soils which are contaminated with heavy metals (Khan *et al.*, 2009). It has to be noted that soil properties are reported to be improved by seaweed material only, not by extracts.

In summary, the application of seaweeds as manure can be beneficial for plant growth, pest and disease control and soil fertility. Besides this, there is one other large benefit of seaweeds for the use as manure, which is linked to the fundamental difference of sea and land plants. Since seaweeds originate from an aquatic, highly saline environment, they completely lack weed seeds and pathogens that could be detrimental to land plants, in contrast to any other source of organic fertiliser (Kavipriya *et al.*, 2011; Villares *et al.*, 2007).

2.3.3 Disadvantages

The amount of literature on detrimental effects of seaweed application to crops is relatively scarce compared to the beneficial effects. One of the earliest notes on side effects of seaweeds as manure dates back to the early sixties (Milton, 1964 in Craigie, 2011). Whereas liquid extracts were thought to be exclusively beneficial for plant growth; whole seaweeds or seaweed meals were reported to inhibit seed germination and plant growth, to reduce N availability on the short term and possibly to release toxic sulfhydryl compounds. These detrimental effects would disappear after 15 weeks.

In the studies of Francki (1960a and 1964) severe manganese toxicity was observed after adding dried meals of *Pachymenia Himantophora* (red) and *Durvillea Antarctica* (brown) seaweeds to tomato plants. The increased Mn uptake was higher than provided by the dried meals, indicating that the seaweeds increased Mn availability in the soil. Soil pH was found to be slightly reduced by adding *Pachymenia* and *Durvillea.* In a subsequent study of Francki (1964), dried *Pachymenia* meals were added to 5 types of soils. Only in the more acid soils, the seaweed meals reduced plant growth, induced waterlogging by disaggregating soil structure and increased Mn uptake by tomato plants. Other results showed that Mn was released in toxic quantities primarily due to waterlogging and not by pH changes in the soil. The negative effect of *Pachymenia* on soil structure is caused by the polysaccharides present in red seaweeds, which behave like weak acids and cause soil disaggregation in acid soils; *Durvillea* (brown) did not change soil physical properties (Francki, 1964). In Chouliaras *et al.* (2009) a reduction in Mn uptake by olive trees was observed after adding *A. nodosum* (brown) extracts.

Francki (1960a) also showed that sodium chloride concentration in the treated soils became much higher after adding the dried seaweed meals. Experiments with radish (salt intolerant), tomato (medium tolerance) and beetroot (salt tolerant) plants however, showed that the increased salinity was not the main cause of the retarded growth of the crops, since the beetroot suffered most from the seaweed meals. Francki (1960a) noted that the detrimental effects of the increased salinity might have been masked by the detrimental effects of the manganese toxicity.

As already mentioned by Milton (1964, in Craigie, 2011), seaweed meals can reduce N availability on the short term. This effect was also observed by Caiozzi *et al.* (1968), Francki (1960b) and Haslam & Hopkins (1996). Beckett & Van Staden (1990) reported reduced N content of wheat grains after adding *E. maxima* extracts, although this reduction was abundantly compensated by the increase in total grain yield. Francki (1960a) showed that leaf N content increased in plants grown with *Pachymenia* (red) meals and decreased with *Durvillea* (brown) meals, attributed to the different C:N ratios of the two seaweed species (Francki, 1964).

Although a large number of commercial seaweed extracts are made from *A. nodosum,* growth inhibitory effects of simple 'homemade' extracts from *A. nodosum, L. saccharina* and other brown seaweeds on mustard are reported by Craigie (2011). Craigie (2011) also noted that *A. nodosum* extracts reduced strawberry fruit weight in one cultivar (not in another) and reduced fruit firmness in both the cultivars.

Another possible side-effect of the use of seaweeds as organic manure, is heavy metal contamination. Smith *et al.* (2010) state that algae can contain high levels of organic arsenic, which could be toxic if mineralised. Large amounts of cadmium were also measured in different kinds of seaweeds (Besada *et al.*, 2009). Verkleij (1992) notes that only in heavily and chronically polluted waters problems are to be expected regarding seaweed quality (for consumption). As long as seaweeds are collected in clean areas, no problems are expected, but monitoring water quality would be necessary when large areas of seaweeds are harvested for fertilisation purposes.

In coastal areas of Spain, some farmers manually collect seaweeds for fertilisation purposes. In interviews, the following disadvantages were mentioned: the costs of labour for collection and transportation of seaweeds is quite high and the drifts irregular, while chemical fertilisers and cattle slurry are abundantly available (Villares *et al.*, 2007). In addition, the salt in the adhering water must be removed from seaweeds before incorporation, otherwise salinization of the soil can occur. This entails different procedures and more work than other organic fertilisers.

Finally, the use of seaweeds or their extracts as manure without any other fertilisers, might not necessarily result in higher financial benefits (Crouch & Van Staden, 1993). In certain specific areas (reducing transplant shock, increase temperature resistance of young plants and such like) the use of seaweeds can be economically feasible (Crouch & Van Staden, 1993). Integrating the use of both inorganic and seaweed fertilisers is necessary to optimise the effects of seaweeds as manure.

The effects of seaweeds on crop growth are dependent on the seaweed species used, the form in which the seaweed is applied (extract, meals), the method of application (foliar spray, soil drench, incorporation into the ground, mulch), duration between application and crop growth, crop and variety used and the soil characteristics.

2.3.4 *Ulva lactuca* as organic manure

In literature, most articles that describe *Ulva lactuca* as manure or biostimulant have used extracts and not crude material. The majority of the studies is of Indian origin and nearly all of the literature found has been published in the last decade. Mostly beneficial effects on crop growth after *Ulva lactuca* extract application have been recorded.

In Table 4, the reported effects of *Ulva lactuca* extract on crop growth parameters are given. Large increases are reported in crop growth, yields, chlorophyll, protein and sugar contents. Except for the studies of El-Sheekh & El-Saied (2000) and Gireesh *et al.* (2011), in all studies different doses of *Ulva lactuca* extract were applied and all studies reported a dose dependent response of crop growth parameters. Usually lower doses enhanced crop growth as well, whereas higher doses sometimes strongly inhibited growth and yield; medium doses were mostly found to be the optimum.

Besides the beneficial effects reported in Table 4, increased number and size of root nodules in cowpea (Bai *et al.*, 2010), increased plumule length in broad bean and tomato (El-Naggar *et al.*, 2005; Hernández-Herrera *et al.*, 2013) and increased nutrient uptake in broad bean (El-Naggar *et al.*, 2005; El-Sheekh & El-Saied, 2000) have been reported. Mugnai *et al.* (2008) observed increased nutrient uptake rates in grapes after application with an extract of different seaweeds from the *Ulva lactucales* order, but the extract did not enhance plant growth in this case. Nabti *et al.* (2009) reported enhanced salt tolerance in wheat inoculated with the bacteria *A. brasilense* NH after *Ulva lactuca* application.

Sridhar & Rengasamy (2010a, 2010b, 2012) reported that growth parameters of marigold, groundnut and chilli peppers were higher under the application of recommended fertiliser than under *Ulva lactuca* extract application, indicating that the nutrient content of the extract cannot fully replace inorganic fertilisers. However, the application of *Ulva lactuca* extracts and 50% of the recommended fertiliser showed better crop growth and yield compared to the 100% of the recommended fertiliser. These results found in different crop types, provide evidence that *Ulva lactuca* (extracts) could decrease the dependency on inorganic fertilisers.

Another study of Sridhar & Rengasamy (2011a) showed that the application of *Ulva lactuca* extract not only increased protein synthesis in roots and shoot, but induced the formation of up to 5 extra proteins in five crop species, including groundnut, chilli peppers and marigolds.

Table 4: Growth parameters of different crops after treatment with *Ulva lactuca* extracts. Values indicate the percentage increase of the growth factor, compared to the control treatment and are copied or calculated from the papers cited.

Increase in	Bai *et al.* (2010) Cowpea	El-Naggar *et al.* (2005) Broad bean	El-Sheekh & El-Saied (2000) Broad bean	Gireesh *et al.* (2011) Cowpea	Hernández-Herrera *et al.* (2013) Tomato	Kavipriya *et al.* (2011) Mung bean	Ramya *et al.* (2010) Cluster bean	Sridhar & Rengasamy (2010a) Marigold	Sridhar & Rengasamy (2010b) Groundnut	Sridhar & Rengasamy (2012) Chilli pepper
Germination %				16	142	28				
Shoot length	19		X	124	X	81	13	55	27	38
Root length	200		X	116	X	19	11	80	110	39
No. of lateral roots	15	67	X			87				
Leaf size	8	50					52		65	
Yield	8	340					30	42	67	158
No. of Pods	100						30		200	
Chlorophyll content		180	377	81			37	40	50	35
Sugar content			36	35			60	37	X	X
Protein content		57	117	115			96	58	X	X

X: Increase was observed, but was not specified or could not be calculated accurately.

In one study, dried *Ulva lactuca* material was used to fertilise broad beans. Total biomass, yield and chlorophyll content were increased (El-Meleigy, 1999, in El-Naggar *et al.* 2005). These results are in accordance with the effects of *Ulva lactuca* extracts on crop growth.

Green seaweeds in general are known to have lower arsenic contents than brown algae (Smith *et al.*, 2010) and contain the polysaccharide laminaran that promotes microbial digestion of detrimental fungi (Khan *et al.*, 2009). Green seaweeds also contain betaine-like compounds, which alleviate salt and drought stress (Hernández-Herrera *et al.*, 2013).

The beneficial effects of *Ulva lactuca* extracts on plant growth are partly dedicated to the hormones present; cytokinins, betaines, auxins and gibberellins have been found in *Ulva lactuca* (El-Naggar *et al.*, 2005). Cytokinin induces shoot growth, regulates changes under abiotic stress (Johri, 2008) and enhances protein synthesis (El-Naggar *et al.*, 2005). Auxin regulates responses to abiotic stress, but also enhances root growth. Gibberellins enhance seed germination (Raven *et al.*, 2005). Abscisic acid is found in other *Ulva lactuca* species, but is not (yet) reported for *Ulva lactuca* (Stirk *et al.*, 2009).

Another reason for these increased plant growth parameters is the enhanced nutrient uptake (e.g. El-Naggar *et al.,* 2005; Nabti *et al.,*2009). For example, in a large share of the articles cited, chlorophyll content was found to be increased significantly. Sridhar & Rengasamy (2011b) and El-Naggar *et al.* (2005) attribute the higher photosynthetic pigment contents to increased uptake of magnesium, an important building block of chlorophyll. Indeed, magnesium uptake was found to be increased with 66% after *Ulva lactuca* extract application (El-Sheekh & El-Saied, 2000).

El-Sheekh & El-Saied (2000), Gireesh *et al.* (2011), Ramya *et al.* (2010) and Sridhar & Rengasamy (2011a) reported the mineral composition of the *Ulva lactuca* extracts they used in their experiments. The variation in mineral content is enormous, for example, El-Sheekh & El-Saied (2000)

reported K content to be as high as 1600 mg/L, whereas Gireesh *et al.* (2011) reported K content to be 0.98 mg/L. Although on average 20 times more N than P is found in *Ulva lactuca* material (Table 2), Gireesh *et al.* (2011) reported higher P (51.35 mg/L) than N (19.05 mg/L) content in the extract. Sridhar & Rengasamy (2011a) reported equal amounts of P (20.2) and N (24.1 mg/L). These differences in nutrient composition might be a result from the different nutrient contents of *Ulva lactuca* and the differences in the preparation of the extracts.

2.3.5 Possibilities and opportunities

There are multiple possibilities and opportunities to use *Ulva lactuca* as green manure. First of all, the seaweed market is expanding and there is growing interest in these relatively new organic products (Craigie, 2011). The value of the world seaweed market is estimated to be around $1.02 billion; the agricultural market for seaweeds (as soil additives, fertilizers, biostimulants and animal feeds) is estimated to be around 1% of the total seaweed market, being worth $10 million (Bixler & Porse, 2010; Craigie, 2011). Currently, mainly *A. nodosum, E. maxima* and *Laminaria* spp. are used for agricultural purposes, but given the beneficial effects of *Ulva lactuca* on crop growth (Section 2.3.4), there are possibilities to introduce *Ulva lactuca* to the expanding market.

Ulva lactuca and other *Ulva* species cause green blooms in many coastal areas. In Brittany for example, annually 100,000m^3 of *Ulva lactuca* drift ashore, causing unwanted ecological changes and economical losses (Charlier *et al.*, 2008). To retain the recreational value of the beaches, *Ulva lactuca* drifts have to be removed. Not only in France, but along coastlines all over the world, *Ulva lactuca* drifts are found. These drifts are great opportunities to obtain *Ulva lactuca* material in a relatively easy way, since it can be collected in great quantities at once without entering the sea. There are also possibilities to cultivate *Ulva lactuca* for the use as organic fertiliser, but this will require the development of technologies and methods, while drifts can be collected easily from the beaches.

3 Materials and Methods

3.1 Nutrient uptake experiment

In this experiment, the P content of *Ulva lactuca* is compared to the phosphate concentration in the surrounding seawater. This experiment was performed in aquaria and lasted 10 days. The results of this experiment will enable us to make an estimation of the P content of *Ulva lactuca* based on the Pi concentration of the water. It is expected that *Ulva lactuca* will have higher P contents when grown under higher Pi concentrations and that the P-uptake relation will show Michaelis-Menten kinetics.

3.1.1 Materials

Test location
The location used for the nutrient experiment is the greenhouse complex Nergena in Wageningen, the Netherlands (51.996 °N, 5.658 °E).

Seaweed species
The seaweed species used in this experiment, is the green alga Sea lettuce (*Ulva lactuca*). From this alga, 3kg material was collected on the 10th of July from Het Veerse Meer (51.561°N, 3.645°E), an eutrophic, brackish lagoon in the Netherlands (Malta *et al.*, 1999). Until the start of this experiment (6th of August), this *Ulva lactuca* was stored in a greenhouse in a 1 cubic meter tank. The tank was filled with Oosterschelde water and was continuously aerated, but not cooled. The *Ulva lactuca* was stored under natural light conditions, but artificial lighting was switched on automatically when the sky was cloudy.

Seawater
The seawater used in this experiment is collected from the Oosterschelde early July (2013), the Netherlands. The Oosterschelde is an estuary which can be closed off from the sea by means of a storm surge barrier. Depending on the tide, fresh or saline water is flowing into the Oosterschelde. The water was collected in Burghsluis, near the surge barrier, which means that the water is rich in nutrients and has a salinity comparable to the North Sea. Concentrations of P and N in Oosterschelde water from the Schelphoek (51.693°N, 3.808°E) in October 2011 were found to be 1µM and 22µM respectively, but these concentrations can vary considerably throughout the year (based on unpublished data by Brandenburg, pers. comm. 2013).

Nutrients
In order to bring phosphate levels to the desired concentrations, liquid Pokon plant nutrition was used. The Pokon was diluted 25 times with demiwater and P-PO4, N-NH4 and N-NO3 concentrations were measured before using the Pokon solution in this experiment.

Cleaning agents
Before the start of this experiment, the aquaria were cleaned thoroughly with diluted Spirit. Tubes, filters and pumps were soaked overnight in a Spirit solution. After cleaning, all materials were rinsed with affluent amounts of fresh water and left to dry for two days.

Measurements
For monitoring salinity and water temperature, the WTW Cond 315i conductivity meter was used. Total P and N content of *Ulva lactuca* samples were analysed after the end of the experiment.

3.1.2 Methods

Due to time and material limitations, it was not possible to monitor P uptake in the aquaria adequately and accurately. Therefore, it was decided to model P uptake based on the results of a pilot study (Section 3.1.3 and Chapter 1), however, the results were not sufficient to calibrate the model accurately enough. In order to maintain P concentrations at the desired levels, it was decided to refresh the water every other day to prevent large decreases in the phosphate concentrations.

From literature it is known that *Ulva lactuca* can store P to sustain growth under P deficient conditions (Lee, 2000). Pedersen *et al.* (2010) reported that *Ulva lactuca* could potentially store amounts of P needed to sustain growth at maximum growth rates for two weeks. These results were found with *Ulva lactuca* P contents of 3.9 mg P/g DW. In a pilot study, *Ulva lactuca* from the Veerse Meer was found to have a P content of 1.8 mg P/g DW. Based on these results, it was decided to 'starve' *Ulva lactuca* in phosphate depleted Oosterschelde water for 4 days, before the start of the experiment at the 6th of August. This was done to prevent high growth rates and high P content under low P concentrations due to internal storage of P; minimal P content found in literature is 0.93 mg P/g DW (Yaich *et al.*, 2011) .

At the 4th of August, a 1 cubic meter tank was emptied, cleaned thoroughly with fresh water and filled with freshly collected seawater. In this tank, sufficient amounts of *Ulva lactuca* (between 1 and 1.5 kg FW) were grown to deplete the Oosterschelde water from phosphate completely in two days. At the 6th of August, five 150L aquaria were filled with the P depleted seawater and the water in each aquarium was aerated and circulated continuously with an air and water pump. The cubic meter tank was refilled with Oosterschelde water in order to deplete the seawater from P. This water was later used to refresh the water in the 150L tanks.

At the 6th of August, a Pokon solution was added to the five aquaria to obtain phosphate concentrations of 1.0, 2.25, 3.5, 4.75 and 6.0 µM. These concentrations were chosen based on the concentrations found in the Oosterschelde water (1.0 µM), concentrations used by Pedersen (pers. comm. 2013; 2.25 µM) and somewhat higher concentrations, resembling highly eutrophicated waters (3.5, 4.75 and 6.0 µM)

In each of these aquaria, six pieces of starved, good quality *Ulva lactuca* were placed, adding up to a total of around 1g FW per aquarium. This amount was chosen to prevent rapid phosphate depletion in the aquaria during the experiment. The remainder of the starved *Ulva lactuca* was used to analyse P and N content directly after starvation. Every other day (8th, 10th, 12th and 14th of August), the aquaria were emptied, cleaned thoroughly to prevent the bloom of unicellular algae and refilled with P depleted Oosterschelde water. Subsequently, new Pokon was added to obtain the desired P concentrations.

The aquaria were cooled with a cooling plate and the temperature was initially set to 15-16°C; The cubic meter tank was not cooled. After the first change of water at the 8th of August, the *Ulva lactuca* material started to sporulate and/or die. Presumably, the temperature difference between the P depleted water (around 20°C) and the aquaria initiated propagation and decay of *Ulva lactuca*. Therefore, the temperature of the aquaria was increased to 19-20°C to prevent a temperature shock after every change of water. *Ulva lactuca* was grown under ambient light conditions.

The weight of *Ulva lactuca* was measured daily around 10AM during the entire experiment, except for the 11th of August. In addition, salinity and water temperature were monitored. At the end of the experiment, the pieces of *Ulva lactuca* were frozen at -20°C until processing. The material was dried for 24h at 105°C and total P and N content were determined as follows:

Samples were digested with a mixture of H_2SO_4-Se and salicylic acid. The actual digestion was started by H_2O_2 and in this step most of the organic matter was oxidized. After decomposition of the excess H_2O_2 and evaporation of water, the digestion was completed by concentrated H_2SO_4 at elevated temperature (330°C) under the influence of Se as a catalyst. In these digests total N and P were measured spectrophotometrically with a segmented-flow system (Auto-analyzer II, Technicon).

3.1.3 Phosphorus uptake model

During a pilot study, P uptake and growth of *Ulva lactuca* were determined during 11 days. It was assumed that the growth of *Ulva lactuca* is exponential and was not limited during this experiment. The amount of *Ulva lactuca* (g DW) at time *d* can be described by:

Equation 1: $\mathrm{Ulva} = \mathrm{UlvaI} * e^{RGR*t}$

in which UlvaI is the initial amount of *Ulva lactuca* (g DW), *RGR* the relative growth rate in d^{-1} and *t* the time in d. It is assumed that the *RGR* depends on the P concentration and can be described by a Michaelis-Menten equation:

Equation 2: $RGR = \mathrm{RGRmax} * \frac{P\mathrm{conc}}{K\mathrm{m}+P\mathrm{conc}}$

The coefficient PinUlva is the P content of *Ulva lactuca* (mg P/g Ulva DW) which relates P uptake (RPamt; in mg P d^{-1}) to *Ulva lactuca* growth (RUlva; in g d^{-1}):

Equation 3: $\mathrm{RPamt} = -\mathrm{PinUlva} * \mathrm{RUlva}$

Equation 1, Equation 2 and Equation 3 are the main equations of the basic P uptake model. In addition, two model extensions are created for the situation that P is added and *Ulva lactuca* material is removed during the course of the experiment. The extended FST model can be found in Appendix 1.

3.2 Decomposition experiment

In this experiment, the decomposition of seaweed under terrestrial conditions is analysed with the litter bag method (Chikowo, 2004). The goal of this experiment is to analyse the time period needed for *Ulva lactuca* mineralisation in order to estimate the timing of application. Based on the results of a pilot study, a decomposition time of 8 weeks is assumed for 40g (FW) *Ulva lactuca*.

3.2.1 Materials

Seaweed species
The seaweed species used in this experiment, is the green alga Sea lettuce (*Ulva lactuca*). From this alga, 3kg material was collected on the 4th of June from Het Veerse Meer (51.561°N, 3.645°E), a eutrophic, brackish lagoon in the Netherlands (Malta *et al.*, 1999).

Test location
The location used for the decomposition experiment is Unifarm in Wageningen, the Netherlands (51.989°N, 5.662°E). This farm is located in an area with a sandy soil, of which the characteristics are listed in Table 5.

Table 5: Soil characteristics of Unifarm, Wageningen. Soil is analysed in 2007.

Characteristic	Value
pH	5.1
% Organic Matter	1.6
C:N ratio	9

3.2.2 Methods

After collection at June 4th, the fresh seaweed material was stored overnight in a plastic barrel with enough seawater to prevent desiccation. At the 5th of June, the seaweed thalli were washed with a large volume of fresh water and epiphytes and abnormalities were removed. Most of the adhering water was removed by using a salad spinner. The remaining water was removed by leaving *Ulva lactuca* to dry for 10 minutes on a table in a greenhouse (outside temperature 23°C, continuous sunlight). The seaweed was divided into portions of 40g. Each portion was put into a net bag of 20 by 25 cm with a mesh size of 4 by 1.5 mm. During the pilot study, this mesh size was found to be adequate for this experiment; the seaweed did not ooze out. The seaweed was spread evenly in the bags to optimise decomposition of each sample. The samples were put into the ground with an angle of 45° at the test location described in Section 3.2.1. The bottom of the bags are at a depth of 20 cm, whereas the top of the bags are at the soil surface. The average sample depth is 10cm.

Several times a week, three samples were removed from the soil per sampling date. Volumetric water content was determined locally at 10 cm depth, directly after sampling. The seaweed samples were processed within half an hour after sampling. The undecomposed seaweed was removed carefully from the net bag and washed in a bowl to remove sand and soil fauna. The content of the bowl was poured through a sieve with a mesh size of 1.5mm. The remaining seaweed material was frozen in an aluminium tray at -20°C prior to analysis. When around 20 samples were collected, the samples were defrosted and dried for 24h at 105°C afterwards. After drying, the dry weight of each sample was measured. The fresh material was assumed to be uniform and was therefore assumed to have a constant dry matter content.

3.2.3 Climate data

Data of precipitation and soil temperature were collected from weather station Veenkampen (2013) in Wageningen (51.982°N, 5.621°E). This station is located at 2.5 km distance from the test location. In principle, this weather station measures temperature in a bare soil every hour at different depths, i.e. 5cm, 10cm, 20cm and 50cm. However, the sensor at 10cm is broken since June 2012, while the average sample depth in the decomposition experiment is 10cm. To give an accurate indication of the temperature at the decomposition site, a model was used to calculate the temperature at 10cm depth in relation to the temperature at 5cm depth, assuming that the soil at Veenkampen and at Unifarm are comparable.

The temperature fluctuations during a day can be described by a sine:
Equation 4: $T_{x,t} = T_{av} + T_{ampl} * e^{(-x*d)} * \sin(\omega * t - x/d)$

Where:
$T_{x,t}$ = temperature at soil depth x and time point t
x = soil depth
t = hour of the day
T_{av} = equilibrium value of sine
T_{ampl} = amplitude of sine
$\omega = 2\pi/\text{period}$
d = attenuation depth

The values of the parameters are calculated with data from the Veenkampen from June 2011. The model and the calculations can be found in Appendix 3.

The output data from the model was used to calculate average day and night soil temperatures. Official time points of sun rise and sun set were used to calculate day and night length. Day is defined as time from sun rise to sun set, night as the time from sunset to sunrise the next day. Hourly precipitation data was accumulated from sunrise to sunrise the next day. The climate data of the decomposition experiment can be found in Figure 5. Day temperatures at 10cm depth fluctuated between 15 and 33°C, night temperatures between 15 and 29°C. Total rainfall during the experiment was 49.15mm, but was mainly concentrated between day 15 and 27 (43.57mm). During the experiment, the volumetric water content fluctuated between 0.13 and 0.15, but was increased from day 17 until day 31 to values between 0.16 and 0.20 as a result of the rainfall in this period.

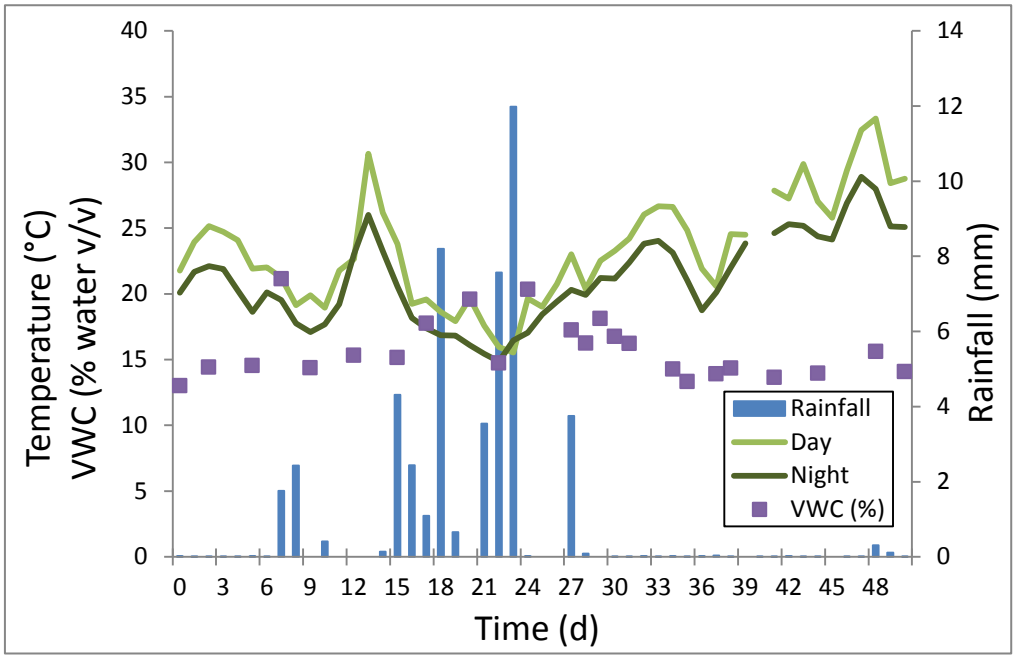

Figure 5: Data of rainfall (daily accumulation), average day and night temperature at 10cm depth in a sandy soil in a temperate climate, from June 5th – July 25th, 2013.

3.3 Crop growth experiment

In this experiment, the effects of incorporated *Ulva lactuca* pieces on crop growth are studied. The goal of this experiment is to find out whether *Ulva lactuca* is suitable as green manure. It is expected that the crops grown with *Ulva lactuca* perform better than the ones grown without.

3.3.1 Materials

Seaweed species
The seaweed species used in this experiment, is the green alga Sea lettuce (*Ulva lactuca*). From this alga, 3kg material was collected on the 10th of July from Het Veerse Meer (51.561°N, 3.645°E), an eutrophic, brackish lagoon in the Netherlands (Malta *et al.*, 1999). Until the start of this experiment (17th of July), this *Ulva lactuca* was stored in a greenhouse in a 1 cubic meter tank. The tank was filled with Oosterschelde water (collected early July) and was continuously aerated. The *Ulva lactuca* was stored under natural light conditions, but occasionally artificial lighting was used when the sky was cloudy. The cubic meter tank was not cooled.

Crop species
The crop species used for this experiment are yellow mustard (*Sinapis alba*) and lettuce (*Lactuca sativa*), variety Twellose gele. Yellow mustard is used as catch crop and has been chosen because of its high growth rate and fast nutrient uptake capacities. Lettuce has been chosen because of its large leaf surface area; differences in nutrient availability, especially nitrogen, could therefore possibly be visible in leaf colour.

Test location
The location used for the decomposition experiment is Unifarm in Wageningen, the Netherlands (51.989°N, 5.662°E). This farm is located in an area with a sandy soil, of which the characteristics are listed in Table 5.

3.3.2 Methods

At the 4th of July, the lettuce was sown in cardboard tubes (diameter 4cm, height 5 cm) filled with sand from the test location. The mustard seeds were sown at the 12th of July in the cardboard tubes. Until transplantation, the seedlings were grown in a room facing north, under room temperature. The sand in the tubes was kept moist continuously by using a plant sprayer.

At the 17th of July, the seaweed thalli were washed with a large volume of fresh water and epiphytes and abnormalities were removed. Most of the adhering water was removed by using a salad spinner. The remaining water was removed by leaving *Ulva lactuca* to dry for 10 minutes on a table in a greenhouse. After surface drying, the seaweed was cut into pieces of approximately 3-5cm.

At the test location, a 20x20x20cm (8L) hole was dug and the sand is collected in a bucket. The soil was mixed by hand and was returned to the hole, either without *Ulva lactuca* for the control treatments, or with *Ulva lactuca* to test its effect on the growth of the crops. No fertiliser was added to the plant grown under the control treatment.

Haslam & Hopkins (1996) applied 8.2 and 16.4g kelp/kg soil and the concentration of 8.2g kelp/kg soil was found to be most beneficial for soil structure. In the decomposition experiment, average soil bulk density was found to be 1.25kg/L, so 8L soil weighs around 10kg. Based on the results of Haslam & Hopkins (1996), it is decided to add 80g of *Ulva lactuca* to each hole (≈8g *Ulva lactuca*/kg soil).

The lettuce and mustard seedlings were placed together with the cardboard tube in the middle of the hole that was dug. The cardboard tube was then torn gently and removed from the soil. During the first two weeks after transplanting to the test location, the seedlings were watered daily with an equal amount of water (around 50mL) to prevent desiccation.

In Figure 6, the experimental setup is given. The plants were grown in blocks. There is sufficient distance between the blocks to prevent shading of the mustard to the lettuce of the adjacent block. The crops grown under the same treatment were placed in two blocks, to prevent variation in the results due to the effect of local circumstances.

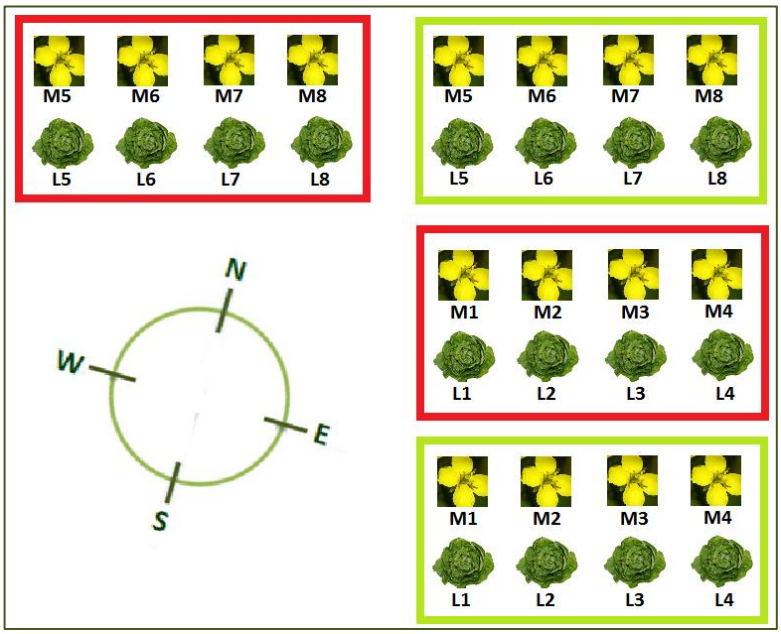

Figure 6: Experimental setup. M: Mustard, L: Lettuce. Green frame: + *Ulva lactuca*, red frame: - *Ulva lactuca*.

At July 31st, August 7th, 15th, 21st and 28th, plant height (mustard) and diameter (lettuce) were measured. Right before harvest (September 2nd), plant height and amount of branches (mustard) and diameter (lettuce) were measured. Directly after the harvest, roots were washed and photos were taken to analyse root and shoot architecture. In addition, shoot and root fresh weights were determined. The samples were dried for 42 hours at 70°C and dry weights were determined. Dried samples of mustard and lettuce shoots were used for total P and N content determination as followed:

Samples were digested with a mixture of H_2SO_4-Se and salicylic acid. The actual digestion was started by H_2O_2 and in this step most of the organic matter was oxidized. After decomposition of the excess H_2O_2 and evaporation of water, the digestion was completed by concentrated H_2SO_4 at elevated temperature (330°C) under the influence of Se as a catalyst. In these digests total N and P were measured spectrophotometrically with a segmented-flow system (Auto-analyzer II, Technicon).

Although the plants are placed in a block-like arrangement, the plants were not distributed randomly and the blocks are chosen manually, instead of randomly. Therefore, it is not allowed to use a Randomized Complete Block Design (RCBD) analysis. The data will therefore be analysed with an independent samples t-test, with a significance level of $p<0.05$.

3.3.3 Climate data

Data of precipitation and soil temperature were collected from weather station Veenkampen (2013) in Wageningen (51.982°N, 5.621°E). This station is located at 2.5 km distance from the test location.

Data of the air temperature are measured every hour. This data was used to calculate average day and night soil temperatures. Official time points of sun rise and sun set are used to calculate day and night length. Night is defined as the time from sunset to sunrise the next day. Hourly precipitation data is accumulated from 0:00 to 24:00. The climate data can be found in Figure 7.

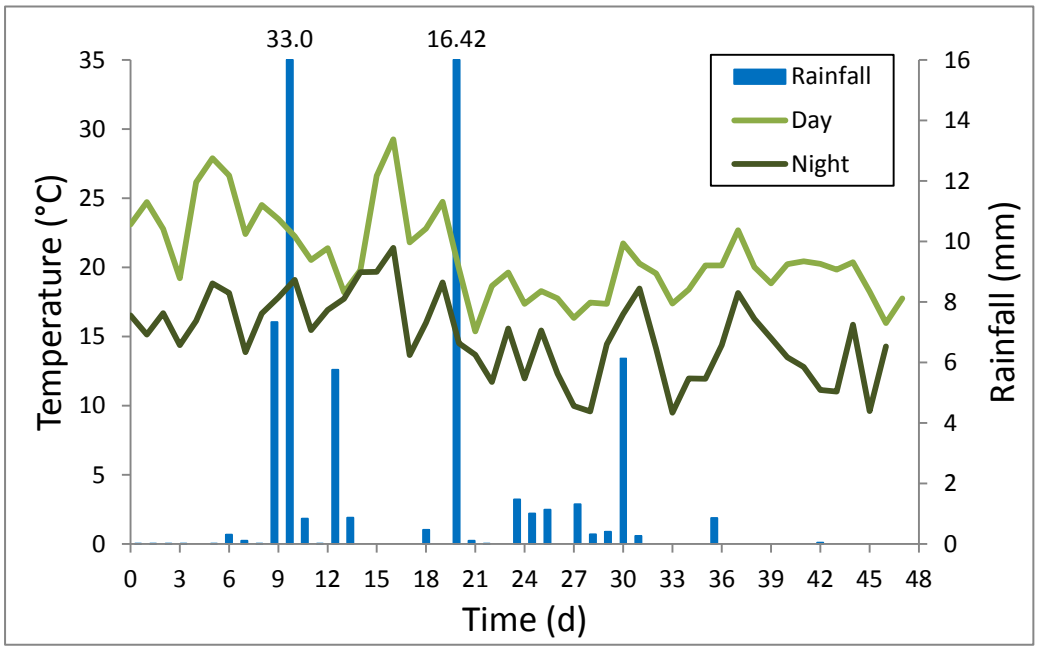

Figure 7: Data of rainfall (daily accumulation) and average day and night air temperature in a temperate climate, from July 17h – September 2nd, 2013.

4 Results and discussion

4.1 Nutrient uptake experiment

It was attempted to model the P uptake by *Ulva lactuca*, but the results of the pilot study were not sufficient to calibrate the model accurately enough. More experiments are needed to adjust this model. The basic model and its extensions can be found in Appendix 1.

4.1.1 Growth rate

Figure 8 shows photos of *Ulva lactuca* at day 2, 6 and 10 of the experiment and Figure 9 shows the fresh weight (FW) of *U. lactuca* over time, when grown under different P concentrations. At day 4, *Ulva lactuca* in all aquaria started to sporulate and/or die (photos not shown). At day 6, *U. lactuca* material that sporulated the days before, showed a normal, green colour, however, the FW of *U. lactuca* was decreased (treatment 3.5 and 6.0 µM), remained the same (treatment 1.0 µM) or was only slightly increased (treatment 2.25 and 4.75 µM) compared to day 4. At day 8, the *U. lactuca* material started to sporulate again, but the material did not recover within 2 days, as was the case with the sporulation at day 4. At day 10, most of the material had died, was transparent or olive green (indication of sporulation), therefore the FW was not measured.

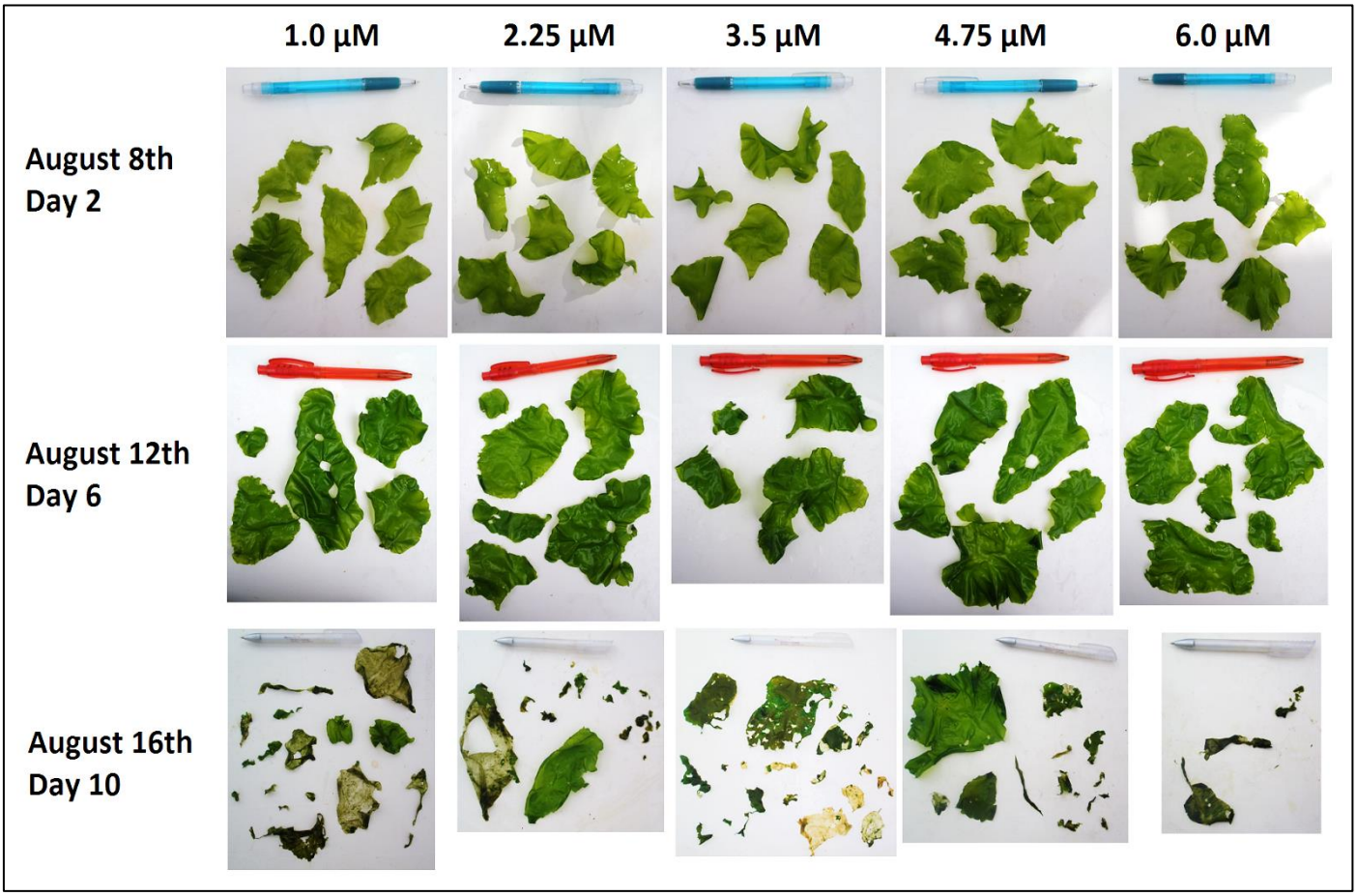

Figure 8: Pictures of *Ulva lactuca* grown under different phosphate concentrations at day 2, 6 and 10 of the experiment. Colours of photos are different from true colours of *Ulva lactuca*, due to light influences and modifications in brightness and contrast.

Presumably, *U. lactuca* started to sporulate due to the large temperature differences between the new seawater and the aquaria (as discussed in Section 3.1.2). As a result of the sporulation and

decay of the material, it is hard to state whether the growth of *U. lactuca* in this experiment was exponential. Also, higher growth rates would be expected under higher P (and N) concentrations, but this was not the case. For example, from day 6 (after the first sporulation) *U. lactuca* grows much faster under 3.5 µM than under 6.0 µM. Because of the sporulation and decay effects on *U. lactuca* weight, it is not possible to say if *U. lactuca* growth in relation to external P concentrations could have an optimal P concentration and the RGR of *Ulva lactuca* could not be determined.

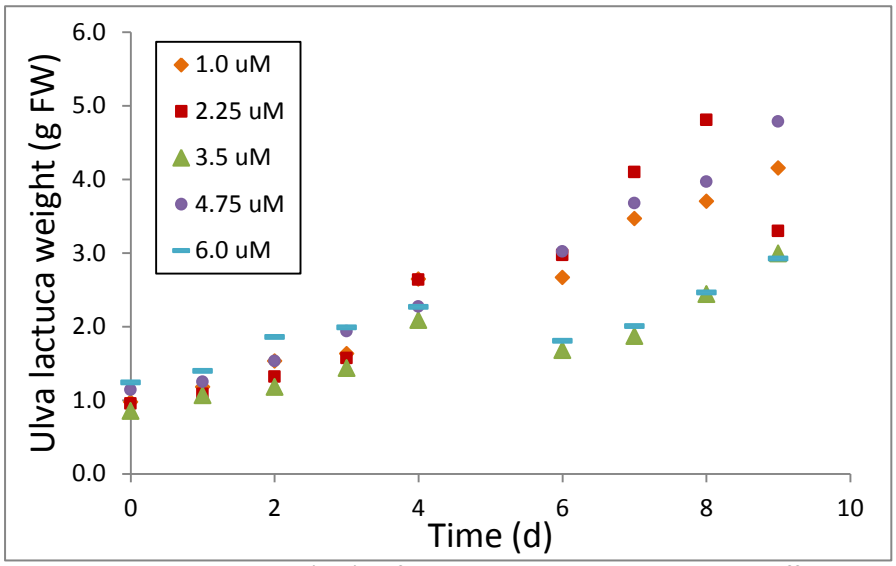

Figure 9: Fresh weight (FW) of *Ulva lactuca* grown under different P concentrations over a time period of 10 days. Day 0 = August 6th, 2013.

Literature shows that the RGR of *Ulva lactuca* mostly is determined by Ni concentrations in the seawater (Pedersen & Borum, 1996; Pedersen *et al.*, 2010). Future studies could find out whether *Ulva lactuca* growth is also dependent on Pi concentrations.

4.1.2 Phosphorus content

Despite the sporulation and decay of *U. lactuca* material, P and N contents were measured. In Figure 10, *U. lactuca* P content in relation to phosphate concentration in the seawater is given. In this figure, average values of the aquaria are used; in Appendix 2.2, data of individual samples are given.

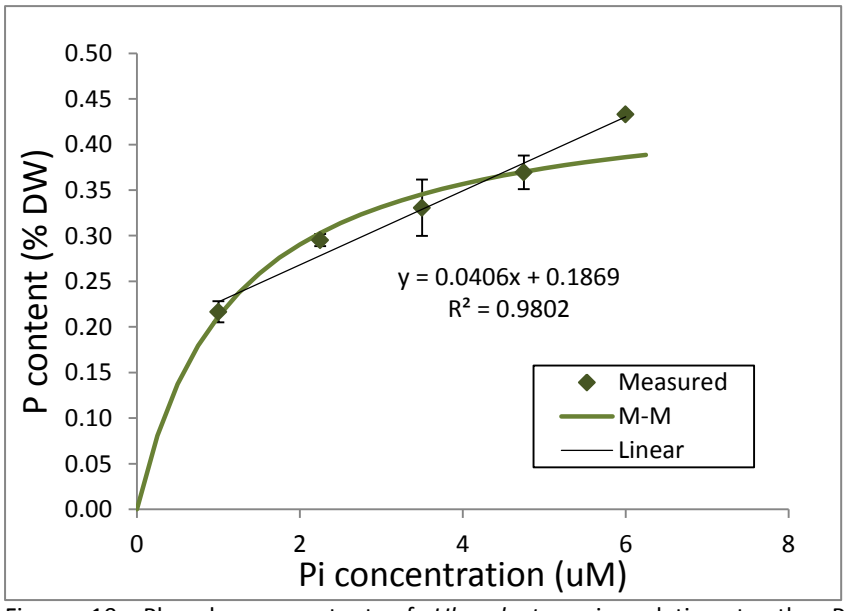

Figure 10: Phosphorus content of *Ulva lactuca* in relation to the P concentration in the seawater. Data are aquarium averages (n=4 for 1.0, 2.25, 3.5 and 4.75 µM; n=1 for 6 µM) ± SE.

A Michaelis-Menten (M-M) curve is fitted to the experimental data. The M-M graph of *Ulva lactuca* P content (P) is given in Figure 10 and can be described by:

Equation 5: $$P = Pmax * \frac{Pi}{(Km + Pi)} = 0.462 * \frac{Pi}{(1.184 + Pi)}$$

in which Pmax is the maximum P content in *Ulva lactuca* (expressed as % of the DW), Pi is the inorganic phosphorus (phosphate) concentration of the seawater and Km is the phosphate concentration at which P content is half Pmax. The values of Pmax and Km are retrieved from the double reciprocal of Pi and P (Lineweaver-Burk) plot. The R^2 value for the Lineweaver-Burk plot is 0.9549 (Appendix 2.3).

Although the R^2 value indicates a good fit of the data to the model, this can be misleading, because only five data points are used. When the data is fit to a linear model, the R^2 value is 0.9802 (Figure 10), which is slightly higher than the R^2 value of the Lineweaver-Burk plot. One of the arguments which support the theory that P content of *Ulva lactuca* in relation to phosphate concentration in the seawater can be described by a Michealis-Menten model, is that P content is most likely to have a maximum (i.e. Pmax), since excessive uptake of nutrients beyond storage capacity are highly inefficient. Another argument is that the data of Pedersen (pers. comm. 2013) in Figure 11 also indicate Michaelis-Menten kinetics.

Pedersen *et al.* (2010) have analysed the relative growth rate (RGR) of *Ulva lactuca* in mesocosms, in relation to the P content of the tissue. They found that RGR was dependent on the P content of the seaweed. This *Ulva lactuca* was grown in 8 land-based, open air mesocosms with 6-12 m^3 of seawater (depending on the tide), which received fresh seawater at a flow through rate of 5 $m^3\ h^{-1}$. Phosphate was continuously added to maintain concentrations 0.06, 0.12, 0.25, 0.5, 1.0 and 2.0 µM above ambient concentrations (two control mesocosms). Pedersen (pers. comm. 2013) provided unpublished data of the precise phosphate concentrations in the mesocosms and the corresponding P contents of *Ulva lactuca* tissues (Figure 11).

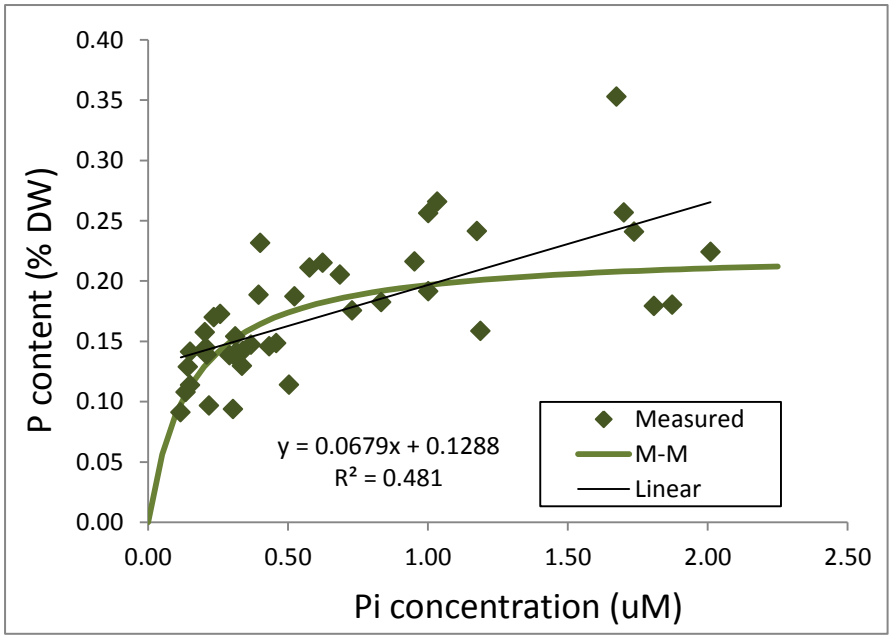

Figure 11: Phosphorus content of *Ulva lactuca* in relation to the P concentration in the seawater. Data from Pedersen (pers. comm. 2013).

A Michaelis-Menten (M-M) curve is fitted to the data of Pedersen (pers. comm. 2013). The M-M graph of *Ulva lactuca* P content (P) is given in Figure 11 and can be described by:

Equation 6: $P = Pmax * \frac{Pi}{(Km + Pi)} = 0.226 * \frac{Pi}{(0.151 + Pi)}$

In which Pmax is the maximum P content in *Ulva lactuca* (expressed as % of the DW), Pi is the phosphate concentration of the seawater and Km is the phosphate concentration at which P content is half Pmax. The values of Pmax (0.226% DW) and Km (0.151) are retrieved from the double reciprocal of Pi and P (Lineweaver-Burk) plot with an R^2 value of 0.5442 (Appendix 2.3). However, the R^2 value of the data fit to a linear model is 0.481 (Figure 11), which is only slightly smaller than the R^2 value of the data fit to a Michaelis-Menten model. From the point of view of the biologically process of P uptake, the data can be described best by the Michaelis-Menten equation.

Pedersen *et al.* (2010) reported the maximum P content of *Ulva lactuca* tissues to be 0.39% DW (125 µmol/g DW), Pedersen & Borum (1996) 0.43% DW and the highest P content in Figure 11 is 0.353%, although this is an outlier. Besides these results, higher *Ulva lactuca* P contents than 0.27% (in Lee, 2005 at phosphate concentrations of 100µM) are not common in literature. It is interesting that the Pmax value found during this study, is 0.487%, which is considerably higher than most of the values found in literature. Since *Ulva lactuca* P contents above 0.27% are mentioned sporadically, it is possible that *Ulva lactuca* growth in the experiments of Pedersen (pers. comm. 2013) and other authors was limited by other factors (e.g. light, N concentration, temperature, etc.) and therefore show lower Pmax than the potential Pmax.

The results of this experiment and the data of Pedersen (pers. comm. 2013), indicate that the P content of *Ulva lactuca* is likely to be higher when grown in seawaters with higher phosphate concentrations. The data does not provide clear evidence whether P content in relation to Pi concentration show Michealis-Menten kinetics or a linear relation, since the R^2 values of both functions are almost equal. Determining P content of *Ulva lactuca* grown under a wider range of Pi concentrations than in this experiment, could indicate if Michaelis-Menten kinetics can indeed be used to describe this relation.

4.1.3 Nitrogen content

The nitrogen content of *U. lactuca* in relation to the Ni concentration (NH_4 + NO_3) is given in Figure 12. Average values of the aquaria are used; in Appendix 2.2, data of the individual samples is given.

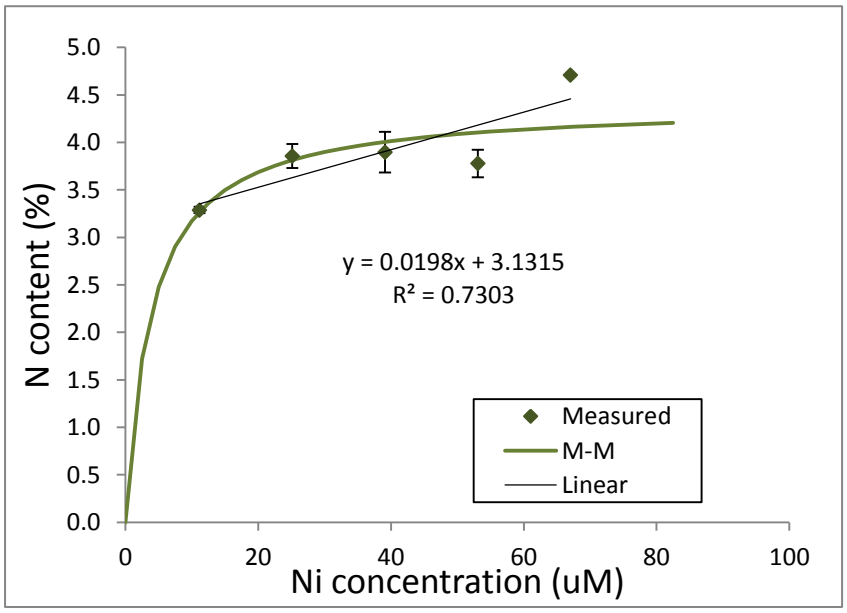

Figure 12: N content of *Ulva lactuca* in relation to the Ni concentration in the seawater. Data are aquarium averages (n=4 for 11.2, 25.1, 39.1 and 53.1 µM; n=1 for 67.0 µM) ± SE.

A Michaelis-Menten (M-M) curve is fitted to the experimental data. The M-M curve of *Ulva lactuca* N content (N) is given in Figure 10 and can be described by:

Equation 7: $$N = Nmax * \frac{Ni}{(Km + Ni)} = 4.404 * \frac{Ni}{(3.889 + Ni)}$$

In which Nmax is the maximum N content in *Ulva lactuca* (expressed as % of the DW), Ni is the inorganic nitrogen (NH_4 + NO_3) concentration of the seawater and Km is the phosphate concentration at which N content is half Nmax. The values of Nmax (4.404% DW) and Km (3.889) are retrieved from the double reciprocal of Ni and N (Lineweaver-Burk) plot with an R^2 value of 0.6789 (Appendix 2.3). However, the R^2 value of the data fit to a linear model is 0.7303 (Figure 12), which is slightly higher than the R^2 value of the data fit to a Michaelis-Menten model.

Pedersen & Borum (1996) reported that N contents of *Ulva lactuca* grown in a brackish estuary ranged between 1 and 6% DW; Pedersen *et al.* (2010) found N contents of 4.94%. The N contents of *Ulva lactuca* in this experiment are comparable with the values found in literature. According to Pedersen & Borum (1996), *Ulva lactuca* has the highest N content of the five macroalgae involved in their study. They also found that the RGR of *Ulva lactuca* increased significantly with Ni enrichment of the medium (not with P enrichment), indicating that *Ulva lactuca* growth usually is limited by Ni availability.

4.1.4 Relation P and N content

In Figure 13 the P content of each individual *Ulva lactuca* sample is plotted against its N content. As Figure 13 shows, P and N content are related and the R^2 value for this relation is 0.5629. Pedersen & Borum (1996) found that the P content of N depleted algae decreased under high Pi availability, presumably because protein synthesis and enzyme activity declines under N shortage. Phosphorus and N content are therefore often related and the fact that this relation is visible in the results of this experiment is in accordance with these findings.

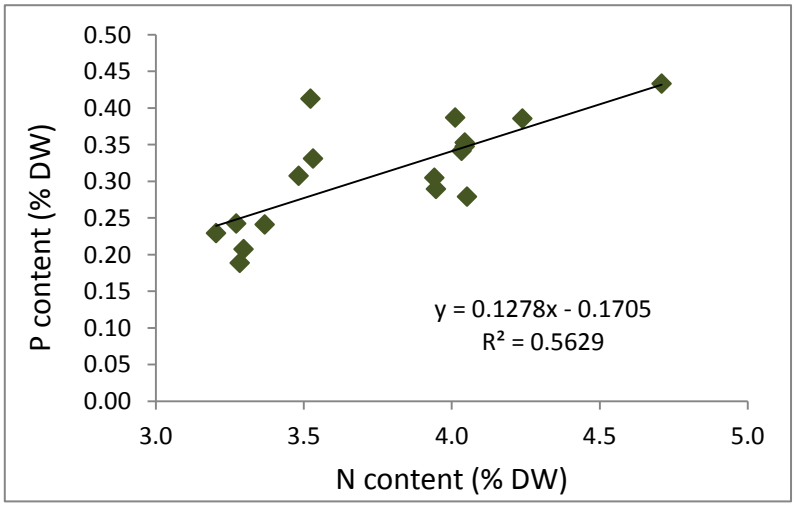

Figure 13: Phosphorus and N content of individual *Ulva lactuca* samples. Contents are expressed as % of the DW.

The average N:P ratio of the samples is 12.6 and ranges between 8.5 and 17.4. This N:P ratio is much lower than reported by Pedersen *et al.* (2010), who found an N:P ratio (based on % of DW) of 35.3. This is mainly due to the much higher P contents found in this experiment. It is unclear why N:P ratios differ this much.

4.1.5 General discussion

Maximum P content of *Ulva lactuca* was considerably higher than found in literature, but the maxiumum N content was comparable to the values found in literature, although both Pi and Ni concentrations used in this experiment were much higher than in nature. It is unknown why only P content exceeds the values reported in literature. Future studies could further investigate this phenomenon.

At the end of the experiment, morphological different pieces of *Ulva lactuca* were collected in each aquarium. Some of these pieces were normal pieces (green), others were sporulating (olive brown) or had lost all chlorophyll (transparent). During the analysis of the results, no distinction was made between these different pieces, because the P content did not seem to differ between the normal and the sporulating pieces (Figure 14). The P content of one transparent piece was similar to that of the normal pieces grown under the same Pi concentration, but the P content of the second piece was much lower than the sporulating pieces. However, statistically seen, this sample was not an outlier. Because only four samples were collected from each aquarium, it was not possible to see whether P content varied between the morphologically different pieces (i.e. between different development stages). In future studies, analysing P content of morphologically different pieces is recommended, to eliminate potential data bias.

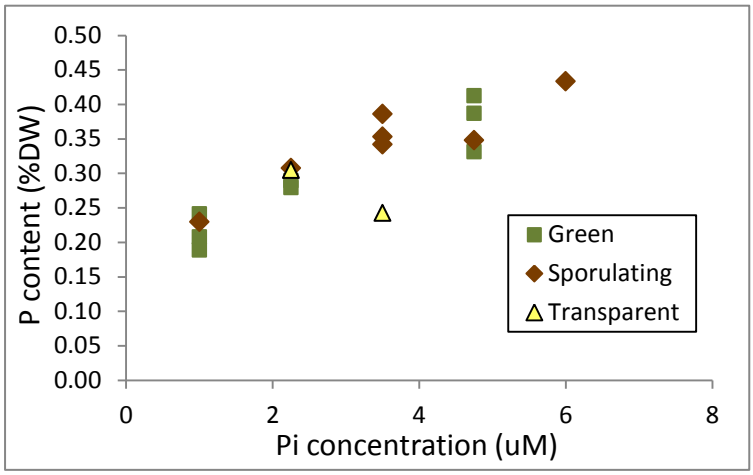

Figure 14: P contents and morphology of *Ulva lactuca* samples for different Pi concentrations.

Knowing that P content of *Ulva lactuca* depends on external phosphate concentrations, different questions rise. For example, are seawater areas with P concentrations of around 6 µM common? Besides the fact that Pi concentrations vary throughout the year, they differ considerably between locations. Seawater Pi concentrations ranged between 2–4 µM in Greece (Tsagkamilis *et al.*, 2010), 0.1–0.4 µM in Norway (Pedersen *et al.*, 2010) and 1.0–9.5 µM in Denmark (Pedersen & Borum, 1996). In the North sea, the Pi concentration is around 0.65 µM, which is seen as the natural concentration. However, Dutch coastal waters have higher Pi concentrations of about 3.2 µM, due to leaching of fertilisers and sewage effluents (Ecomare, 2013). Brandenburg (pers. comm. 2013) reported Pi concentrations of 0.96µM (n=6, SE=0.016) in Oosterschelde water from the Schelphoek (the Netherlands).

Although Pi concentrations in seawaters vary throughout the year, the experimental values of 6 µM are not very common. The P content of *Ulva lactuca* DW, will therefore be much lower than Pmax. Assuming that Dutch seawaters have a Pi of 1 µM, the P content of *Ulva lactuca* will be around 0.197% (63.6 µmol/g DW). This value is based on the Michaelis-Menten model of the data of Pedersen (pers. comm. 2013), since his experiment was executed in situ. When *Ulva lactuca* is grown

under favourable circumstances (as was the case in this experiment), P content when grown under a phosphate concentration of 1 µM will be 0.212% DW.

Pedersen & Borum (1996) stated that P and N content in seaweed species are often related and this relation was also visible in the results of this experiment. To find out what influences *Ulva lactuca* P content, in future studies other factors besides phosphate concentrations could be varied, such as N availability, light quantity and quality, temperature, *Ulva lactuca* variety etc. Determining the factors which influence and to what extent they influence P content of *Ulva lactuca*, will give useful insights for the amounts of P that can be recovered with *Ulva lactuca* farming.

Another question that rises, is whether *Ulva lactuca* is the most suitable seaweed to use for P recovery. Pedersen & Borum (1996) analysed P contents of five macroalgae, including *Ulva lactuca*. The P content of *Ulva lactuca* varied throughout the season and ranged between 0.20 and 0.43% DW. However, compared to the other seaweed species, *Ulva lactuca* had the lowest P content (e.g. the lowest seasonal P content of the red seaweed *Ceramium rubrum* and brown seaweed *Fucus vesiculosis* were 0.40% DW; P content of the green seaweeds *Cladophora sericea* and *Chaetomorpha linum* ranged between 0.20 and 0.70% DW). Smith *et al.* (2010) even reported a P content of 1.30% DW for the brown kelp *Ecklonia radiata*. Despite relatively low P contents, Pedersen & Borum (1996) found that *Ulva lactuca* had a slightly higher N content than four other seaweeds (red, brown and green species).

Although other seaweeds have a higher P content than *Ulva lactuca*, the relative growth rate (RGR) of *Ulva lactuca* is much higher than of other seaweeds. Experiments in land-based mesocosms of Pedersen *et al.* (2010) showed that *Ulva lactuca* RGR (0.196 d^{-1}) was much higher than the RGR of *Ceramium rubrum* (0.136 d^{-1}) and *Fucus vesiculosus* (0.040 d^{-1}). Laboratory experiments of Pedersen & Borum (1997) showed a RGR of *Ulva lactuca* of 0.513 d^{-1}, which was at least twice as high as other seaweeds, including other green macroalgae species. In addition, the RGR of *Ulva lactuca* is also much higher when the P content is higher (Pedersen *et al.*, 2010), but overall, *Ulva lactuca's* RGR is mostly limited by N availability (Pedersen & Borum, 1996). Smit & Willigen (2011) state that growth of *Ulva* species in both near and off shore regions will be limited by N and P concentrations and that fertilisation is needed to obtain high growth rates and therefore a high P recovery rate. When *Ulva lactuca* is used for P recovery, fertilising the seaweed with P and N is contradictory, not to mention the environmental effects due to eutrophication. Further research could find out whether *Ulva lactuca* can be used for P recovery without fertilisation or what fertilisation levels are needed to optimise P recovery.

Due to the high potential growth rate, there are possibilities to use *Ulva lactuca* for P recovery. Farming *Ulva lactuca* in large eutrophicated seawater areas or oceanic upwelling zones could recover relatively large amounts of P per unit of DW of *Ulva lactuca* compared to less eutrophicated areas. Future research, together with the development of P fertiliser costs and the development of suitable *Ulva lactuca* harvesting methods, has to prove whether *Ulva lactuca* for the use of P recovery is economically viable.

4.2 Decomposition experiment

4.2.1 Decomposition rate

In Figure 15, the dry weight of *Ulva lactuca* as measured in the litter bags over time is plotted. The decomposition of *Ulva lactuca* dry matter (DW1) in the soil over time can be described by:

Equation 8: $DW1 = W0 * e^{-RDR*t} = 4.7411e^{-0.0413*t}$

In which W0 is the dry weight at the start of the experiment (g), RDR the relative decomposition rate (d^{-1}) and t time (in days). The values for parameters W0 and RDR are derived from linearizing the experimental values; W0 is found to be 4.7411g and RDR 0.0413 d^{-1}.

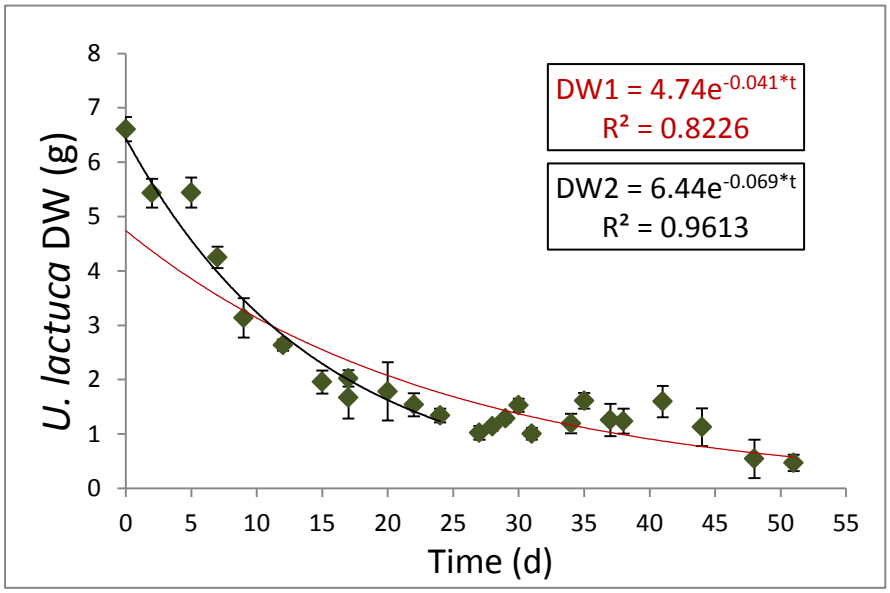

Figure 15: Decomposition of *Ulva lactuca* over time when incorporated into a sandy soil in a temperate climate, from June 5th – July 25th, 2013. Values are averages (n=3) ± SE.

Using Equation 8, it can be calculated that 50% of the *Ulva lactuca* material is decomposed after 16.8 days. In the experiment however, *Ulva lactuca* DW at day 0 is 6.61g instead of the modelled 4.74g. This discrepancy between the measured and modelled data, can be attributed to different decomposition rates during the experiment; the initial decomposition rate from day 0 to 24 is higher than the average rate during the entire experiment and can be described by:

Equation 9: $DW2 = W0 * e^{-RDR*t} = 6.4376e^{-0.0688*t}$

In this new model, it takes 10 days before 50% of the *Ulva lactuca* material is decomposed. In the first 24 days, 80% of the initial *Ulva lactuca* material is decomposed, whereas from day 25 to 51 only 13% of the initial material is mineralised. Therefore it is assumed that the *Ulva lactuca* material indeed has different decomposition rates, probably depending on the stage of decomposition.

The average day temperature from day 0 to 24 is 21.15°C, whereas it is 25.78°C from day 25 to 50 (Section 3.2.3). Therefore, the decomposition rate of *Ulva lactuca* organic matter is expected to be higher during the second half of the experiment. However, this is not the case and therefore other factors must have caused the different decomposition rates.

The decomposition of organic matter has two phases: the decomposition of the labile fraction and the decomposition of the recalcitrant fraction (McCurdy *et al.*, 2013). The labile fraction (sugars, starches, proteins) decomposes relatively fast, whereas the recalcitrant fraction (cellulose, fats, waxes, tannins) decomposes relatively slow. Table 1 shows that *Ulva lactuca* is very low in insoluble fibres such as cellulose (31% of DW) compared to green plants (83% of DW) and has a high content of starches (soluble fibers) and proteins, which are both decomposed relatively fast. Therefore, the initial decomposition rate of the *Ulva lactuca* material might be high, but could be much lower when mostly recalcitrant matter is left.

Decomposition of organic matter depends on many factors (Section 2.1.3) and therefore it is hard to make an accurate comparison between the decomposition of *Ulva lactuca* and other manures. However, some data is published on the decomposition of a variety of organic materials, which gives an indication of the decomposition time of other organic manures. In Table 6, the decomposition of different common green manures is given. These data are calculated from the results of long term trials in Dutch, arable soils. These results are in accordance with findings from Yang (1996), who calculated that 25% of the organic matter of green manures, is left one year after incorporation into the soil (his model is calibrated with experimental data from a wide range of arable soils in China).

Assuming that 25% of the organic matter of common green manures remains after one year, Equation 8 can be used to compare the time needed to decompose 75% of the *Ulva lactuca* material. The time needed (when incorporated in June) is 34 days, which is considerably lower than other green manures (around one year). However, analysis of larger areas with incorporated *Ulva lactuca* are needed to draw reliable conclusions on the decomposition of *Ulva lactuca* in time, since the decomposition rate of organic matter depends on the amount of organic matter in a soil (Section 2.1.2).

Table 6: Decomposition of different green manures (FW) in the Netherlands. From De Haan & Van Geel (2013)

Manure	Application rate	H.C.*	C:N
Yellow mustard	3800 kg/ha	0.23	20
Vetch	2800 kg/ha	0.23	12
White clover	3100 kg/ha	0.27	14
Red clover	4100 kg/ha	0.27	16
Alfalfa (annual)	3000 kg/ha	0.45	13

*Humification Coefficient: the remaining fraction of organic matter, one year after incorporation

Table 7: Hemicellulose, cellulose and lignin contents of *Ulva lactuca* (Yaich *et al.*, 2011) and white clover (Henriksen & Breland, 1999).

Component	*Ulva lactuca*	Clover
Hemicellulose	21% DW	10% DW
Cellulose	9% DW	13% DW
lignin	1.6% DW	2.3% DW

In the study of McCurdy *et al.* (2013), the mesh bag method is used to analyse white clover decomposition in a sandy loam soil in a temperate climate in June. Although McCurdy *et al.* (2013) used only 10g of fresh material per bag, this study provides good results to compare with the results of *Ulva lactuca*. After 12 days, 50% of the white clover organic matter (both the labile and recalcitrant fraction) was found to be decomposed, whereas *Ulva lactuca* lost half of its organic matter to the soil after 16.8 days. It is not surprising that these materials are decomposed in such a short time period, since both white clover and *Ulva lactuca* are low in hemicellulose, cellulose and lignin (Table 7), compared to woody material (Section 2.1.3).

McCurdy *et al.* (2013) incorporated white clover into the soil in March, June and December. The RDR of the labile fraction when clover is incorporated in June is 0.1056 d^{-1} and the RDRs of the labile fraction when incorporated in March and December were respectively 2.9 (0.0367 d^{-1}) and 6.4 (0.0166 d^{-1}) times lower than the decomposition rate in June. It is plausible that the RDR of the labile fraction of *Ulva lactuca* incorporated in March, is 2.9 times lower than the rate when incorporated in

June. This is important when considering the incorporation date in relation to the plant nutrient requirements throughout the growing season of a crop.

The molar C:N ratio of *Ulva lactuca* is found to be 8.7 (Pedersen *et al.*, 2010), which means that N will be mineralised instead of immobilised. The molar C:P ratio of *Ulva lactuca* is found to be 627 (Pedersen *et al.*, 2010), which means P is immobilised (Section 2.1.3). Since *Ulva lactuca* is low in hemicellulose, cellulose and lignin (i.e. recalcitrant organic matter) its application to a soil hardly increases the SOM content on the long term (McCurdy *et al.,* 2013). However, due to the low C:N ratio, application of *Ulva lactuca* leads to a short term high availability of nutrients and could serve as a nutrient boost for crops.

4.2.2 General discussion

In this experiment, fresh seaweed material was used. When seaweeds would be used as organic manure on a large scale, transporting seaweed from coastal to more inland regions could be very costly. Assuming *Ulva lactuca* has an average dry matter content of 15% (Section 2.2.3), this means that mostly water is transported from one location to another, which is very cost inefficient. Future studies could find out whether dried organic matter has the same decomposition rate as fresh material and what drying method is most cost efficient.

The seaweed material that was used to determine the decomposition rate of *Ulva lactuca*, was washed with an affluent amount of fresh water. When seaweeds would be used as organic manure on a large scale, washing the thalli with fresh water is impractical and not sustainable. Further research could point out if adherent seawater influences soil salinity and decomposition rate. Some farmers who use seaweeds as manure reported to use the seaweeds after they were rain-washed (Villares *et al.*, 2007). Additional experiments could indicate if rain-washing seaweed material removes sufficient amounts of salt to prevent the possible salinization of the soil.

In future research, the period of incorporation (e.g. spring or autumn) and effect of soil type on seaweed decomposition can be compared. A downside of the use of *Ulva lactuca* as organic fertiliser, is the low availability at the end of winter/early spring and the high availability during summer. Further research could be done to compare the decomposition of *Ulva lactuca* with the decomposition of other seaweeds, especially brown seaweeds that grow well in autumn and winter and could be applied well before sowing the crops in early spring.

A potential side effect of the use as organic fertiliser, is the odour that is produced by rotting seaweeds (Charlier *et al.*, 2008). In Brittany, a horse rider passed out due to the poisonous sulfhydryl gas produced by heaps of decomposing green algae. The sulfhydryl production can be reduced by aerating heaps of seaweeds (Milton, 1964 in Craigie, 2011). Future studies could find out whether enough oxygen is available to prevent the formation of toxic gases during breakdown when seaweeds are incorporated into a soil on a large scale and if any health risks are present when seaweed is used as organic fertiliser.

4.3 Crop growth experiment

4.3.1 Growth and development

During the experiment, the height of mustard plants and diameter of lettuce plants were measured. The results are graphically presented in Figure 16 and Figure 17. For lettuce, the crops grown with *Ulva lactuca* had a significantly smaller diameter than the crops grown without *Ulva lactuca*, from 21 days after planting until 42 days after planting (5 days before harvest). At harvest date, the difference in diameter has lost its significance, although the p-value is very low (p=0.060). The mustard plants show the same pattern: the plants grown with *Ulva lactuca* are significantly shorter than the ones without *Ulva lactuca*. However, at the harvest date, the difference between the treatments is not significant anymore.

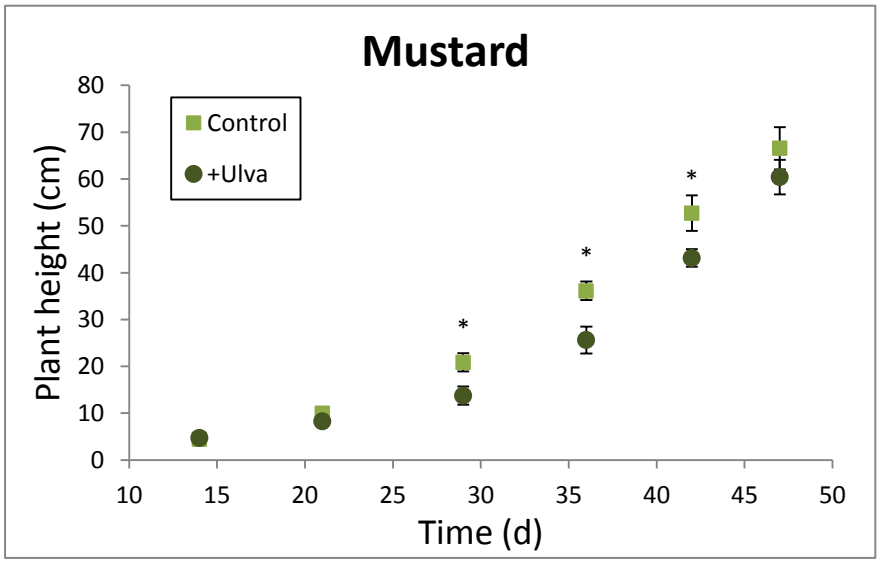

Figure 16: Average plant height of the mustard plants during the experiment. Data are means ± SE, n=7. * indicates a significant difference between the treatments, with P<0.05. Day 0 = July 17th, 2013.

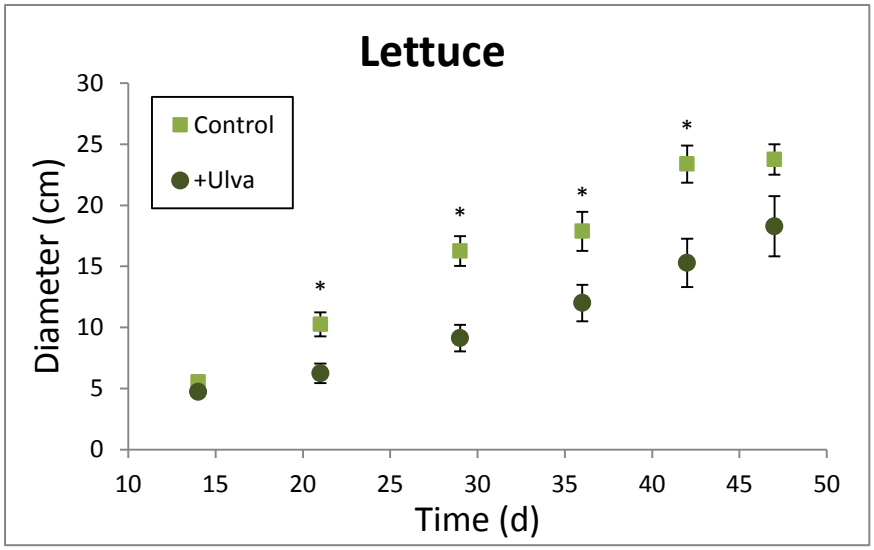

Figure 17: Average diameter of the lettuce plants during the experiment. Data are means ± SE, n=7. *indicates a significant difference between the treatments, with P<0.05. Day 0 = July 17th, 2013.

The number of branches of the mustard plants is not significantly different between the *Ulva lactuca* treatment (14 branches) and the control treatment (16.14 branches), although the p-value is very low (0.055). Of the 7 mustard plants grown without *Ulva lactuca*, 3 plants had 4, 6 and 7 flower clusters. Of the 7 mustard plants grown with *Ulva lactuca*, 2 plants had 1 and 2 flower cluster. The difference between the treatments is not significant however.

For mustard plant height, the loss of significance between the treatments at the harvest date might be a result of the end of the growth phase. Flowering indicates that the plants are in transition from the vegetative to the generative development stage. The mustard plants grown with *Ulva lactuca* have less flower clusters than the control treatment. Although the number of plants with flower clusters and the amount of flower clusters per plant were not significant between treatments, it might be an indication that the mustard plants grown with *Ulva lactuca* have a delay in development. The delay in development under *Ulva lactuca* treatment was observed for lettuce as well: three lettuce crops grown without *Ulva lactuca* were in the generative stage at harvest date, compared to none of the crops grown with *Ulva lactuca*. This seems to indicate that *Ulva lactuca* application retards crop growth and development.

4.3.2 Crop morphology

The mustard plants of the control treatment were more uniform in size than the ones grown with *Ulva lactuca*; under *Ulva lactuca* treatment, three plants were considerably smaller than four others, whereas under the control treatment, the plants were of similar size (Figure 18).

The roots of mustard plants of the control treatment seem to be a bit more extended, but the variation within each treatment is too large to draw any reliable conclusions.

Figure 18: Morphology of mustard shoots and roots at harvest date, 47 days after planting. Length of ruler is 30cm.

The lettuce crops grown under the control treatment look more healthy and are more uniform than the crops grown under *Ulva lactuca* treatment, since six crops have the same size (three crops are in the generative development stage, three in the vegetative stage at harvest date) and two crops are much smaller. The crops grown under the *Ulva lactuca* treatment have three different sizes and are smaller in diameter compared to the control treatment (Figure 19).

The root systems of lettuce plants grown with *Ulva lactuca* treatment are less extended than grown under the control treatment. This is also visible in the reduced root dry matter production under *Ulva lactuca* treatment (Section 4.3.3), although this difference is not significant (p=0.104). No differences in greenness of lettuce plants are observed.

Figure 19: Variety in morphology of lettuce shoots and root at harvest date, 47 days after planting. Length of ruler is 30cm.

It was found by Abetz & Young (1983) that yield quality of lettuce increased with extracts of the brown seaweed *Ecklonia maxima*, but in this study, lettuce grown with *Ulva lactuca* looked less healthy than for the control treatment (Figure 19). The results might be different from what is found in literature, because another seaweed species is used. It is recommended to do research on the effects of *Ulva lactuca* on crop growth and nutrient content compared to other (brown and red) seaweed species in order to find out whether *Ulva lactuca* is the species most suitable to manure crops with.

4.3.3 Dry matter production

After harvest, dry weight of roots and shoot of both lettuce and mustard were determined. Figure 20 shows the dry matter production of mustard plants; no significant differences were found between the treatments, although shoot dry weight was found to be lower for the *Ulva lactuca* treatment (p=0.080). Dry matter production of mustard roots is also lower under *U. lactuca* treatment, but the difference is not significant (p=0.210). The shoot/root ratio was altered (lower) with *U. lactuca* application compared to the control treatment, but this difference was not significant (p=0.245). Since the difference in mustard length between treatments was not significant, is not surprising that the dry weights do not differ significantly. It is possible that the dry matter production was significantly lower for *U. lactuca* treatment compared to the control during most of the vegetative state, as was the case with plant height.

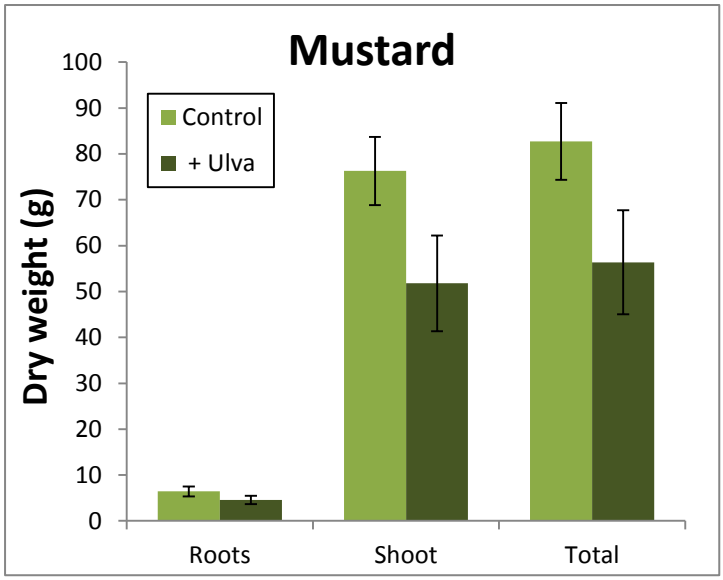

Figure 20: Average dry matter production of mustard plants. Data are means ±SE, n=7.

Figure 21 shows that lettuce shoot dry matter production for *Ulva lactuca* treatment is significantly lower than for the control treatment (p=0.015), which is in accordance with the smaller diameter of the crops. Dry matter production of roots is also lower under *Ulva lactuca* treatment, but the effect of the treatment is not significant (p=0.104). As with the mustard plants, lettuce shoot/root ratio was altered (lower) with *Ulva lactuca* application compared to control treatment, but this difference was not significant, although p-value was very low (p=0.061).

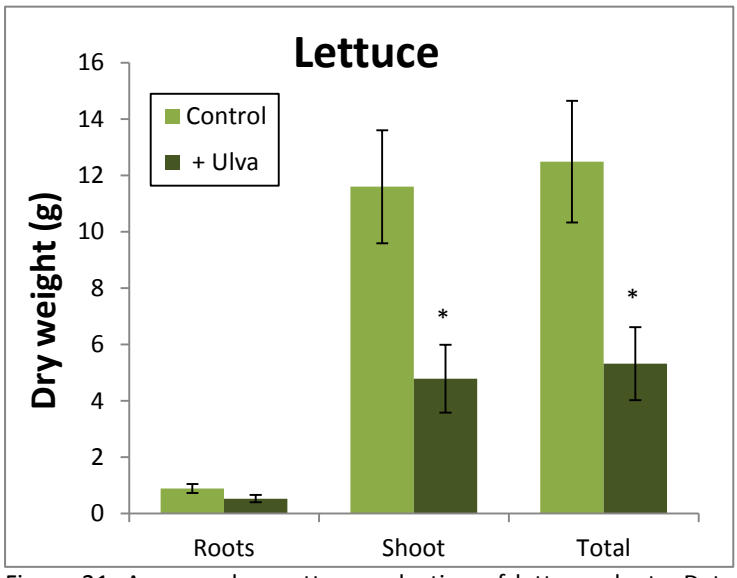

Figure 21: Average dry matter production of lettuce plants. Data are means ±SE, n=7. * indicates a significant difference between the treatments, with p<0.05.

The reduced shoot dry matter production of lettuce (significant) and mustard (not significant, p=0.080) supports the conclusion that *Ulva lactuca* application retards crop growth. In literature it was found that seaweed meals (in contrast to extracts) can have a detrimental effect on crop growth due to different reasons (Section 2.3.3). It is not clear what caused the reduction in crop growth in this experiment.

4.3.4 Phosphorus and nitrogen content

Figure 22 and Figure 23 present data on the P and N contents of mustard and lettuce plants. As Figure 23 shows, the N content of mustard plants with *Ulva lactuca* is higher than the control treatment (p=0.022). Average phosphorus content of mustard was also found to be higher under *Ulva lactuca* treatment, but the difference was not significant, although the p-value was very low (p=0.085). The differences in P and N content of lettuce plants are respectively higher and lower for *Ulva lactuca* treatment compared with the control treatment and the differences between different treatments are not significant (p=0.555 and p=0.531, respectively).

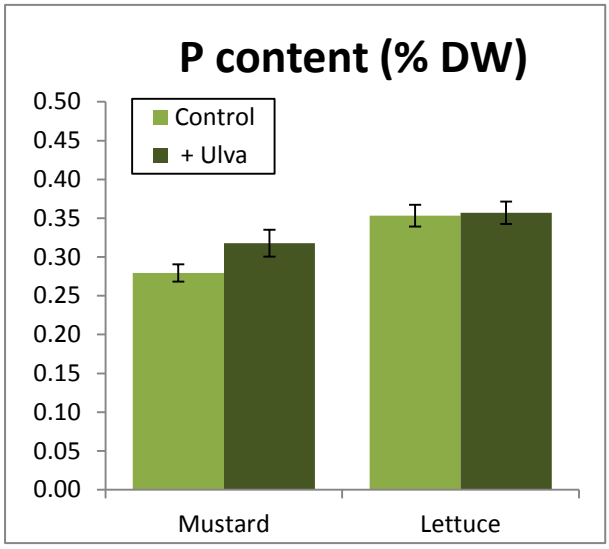

Figure 22: Phosphorus content (% of DW) of mustard and lettuce. Data are means ± SE, n=7.

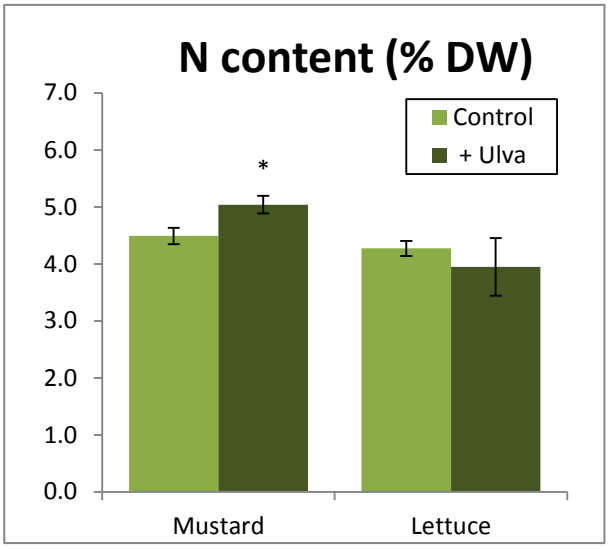

Figure 23: Nitrogen content (% of DW) of mustard and lettuce. Data are means ± SE, n=7. * indicates a significant difference between the treatments, with

These results show that *Ulva lactuca* might increase the nutrient content of crops when incorporated into the soil, as was the case with the mustard plants. In literature it was found that nutrient contents (Ca, K and Mg) were increased after the application of seaweed extracts in lettuce (Table 3), but these results do not provide clear evidence for that. The effect on nutrient content is probably only visible in mustard plants, because this green manure has a fast nutrient uptake. At the harvest date (47 days after planting), 86% of the initial *Ulva lactuca* DW is decomposed (Equation 8) and The C:N ratio of *Ulva lactuca* is found to be 8.7 (Pedersen *et al.*, 2010), which means that some N should be mineralised and should have been available for crop uptake. The C:P ratio of *Ulva lactuca* is found to be 627 (Pedersen *et al.*, 2010), which means P is immobilised (Section 2.1.3) and the possible higher P content cannot be explained by that. Despite the higher P and N content under *Ulva lactuca* treatment, total P uptake from the soil is lower compared to the control, due to the smaller dry matter production. It is not clear whether *Ulva lactuca* only adds extra nutrients to the soil or also enhances crop nutrient uptake from the soil.

Francki (1960a) showed that leaf N content increased in plants grown with *Pachymenia* (red) meals and decreased with *Durvillea* (brown) meals, attributed to the different C:N ratios of the two seaweed species (Francki, 1964). It is recommended to compare the effect on nutrient content between *Ulva lactuca* and other species, since the effects might be seaweed species specific. The effect of *Ulva lactuca* application on nutrient content might also be crop specific, therefore it is recommended to study the effect on a variety of crops.

4.3.5 General discussion

In summary, the following effects of *Ulva lactuca* application to mustard plants were visible: higher N content ($p<0.050$), reduced number of branches, reduced shoot dry matter production and higher P content ($p<0.010$). The following effects were visible in lettuce crops: reduced shoot dry matter production ($p<0.05$), reduced diameter and lower shoot/root ratio ($p<0.010$). These effects are a strong indication that *Ulva lactuca* application delays crop growth. Because nutrient uptake is increased under *Ulva lactuca* application treatment (in mustard), the inhibiting effect on plant growth must be caused by other factors than nutrient availability. This supports the theory that hormonal compounds in *Ulva lactuca* could affect crop growth (Section 2.3.2). The possible delay in crop development is also a strong indication of hormonal functioning of *Ulva lactuca* in mustard and lettuce plants.

Another factor that might have influenced crop growth is salt stress. The lettuce crops seem to be more affected by *Ulva lactuca* application than the mustard crops and lettuce is less salt tolerant than mustard plants. However, as Table 2 shows, the mineral content (Na, K, but also Ca and Mg) of *Ulva lactuca* are in the same range as other green manures, therefore salinization of the soil is not expected when thoroughly washed *Ulva lactuca* is added to crops.

The seaweed material that was used to fertilise mustard and lettuce crops, was washed with an affluent amount of fresh water. When seaweeds would be used as organic manure on a large scale, washing the seaweed manure with fresh water is impractical and not sustainable. Francki (1960a) showed that sodium chloride concentration in the treated soils became much higher after adding the dried seaweed meals, but it was not clear whether the salt influenced crop performance. Further research could point out if adherent seawater influences soil salinity and crop response.

Although mostly beneficial effects of the addition of seaweed *extracts* have been reported, the application of seaweed meals were found to inhibit plant growth by Milton (1964, in Craigie, 2011) and other authors (Section 2.3.3). It is not specified which species where used in the studies described by Milton, and the seaweed species used by Francki (1960a) were red and brown; the detrimental effect of green seaweed species on crop growth is not reported. It is interesting to see that differences between extracts and meals were found in literature. This might be caused by certain compounds that are broken down during the processing of seaweeds into extracts. These compounds might be retarding crop growth and development, despite the increase in nutrient content of the crop. Bai *et al.* (2010) state that seaweed meals take a month to become available as plant nutrients, because the carbohydrate material, absent in seaweed extracts, has to be broken down. Future studies could find out which compound (carbohydrates, hormones, etc.) in *Ulva lactuca* or which mechanism (nutrient immobilisation or mineralisation of toxic nutrients, etc.) is responsible for the delay in crop growth and development. This could give more insights in why seaweed meals and extracts have different effects on crops.

Some seaweed species are known to have a high heavy metal content (Besada *et al.*, 2009). Villares *et al.* (2007) showed that heavy metal contents of *Ulva lactuca* are comparable with heavy metal contents found in other seaweed species. No problems with heavy metal contamination are expected when *Ulva lactuca* is used as organic manure.

In literature, seaweed meals were applied fresh (Haslam & Hopkins, 1996), dried and ground (Francki, 1960a) or composted (Villares *et al.*, 2007). Future studies could find out if certain methods of processing (i.e. drying or composting) benefit crop growth and development more than adding unprocessed material.

5 Conclusions and recommendations

5.1 Nutrient uptake experiment

Phosphorus and nitrogen contents of *Ulva lactuca* increase under higher Pi and Ni concentrations respectively, as was expected. Phosphorus and N contents can be described by both a Michaelis-Menten and linear relation (R^2 values are similar), although the Michaelis-Menten model is the best way, biologically seen, to describe the data. Pmax of the Michaelis-Menten equation in this experiment was found to be 0.462% DW and Km 1.194 µM, Pmax being much higher than other values found in literature. This is probably due to limiting growth circumstances of *Ulva lactuca* with other authors. Nmax of the Michaelis-Menten equation in this experiment was found to be 4.404% DW and Km 3.889 µM, Nmax values being comparable to the values found in literature.

It remains unknown why Pmax is much higher than values found in literature, whereas Nmax is comparable to the findings of other authors; future studies could perhaps clarify this. The results of this experiment show that *Ulva lactuca* has the potential to clean eutrophicated waters from excess P and N. In this study, P and N content in *Ulva lactuca* are found to be positively correlated; P content is higher under higher N contents.

In future research, the different factors that influence P content of *Ulva lactuca* besides Pi concentration, could be determined. It could also be determined whether the relative growth rate is dependent on the P content of *Ulva lactuca* and whether these high growth rates can be obtained without P fertilisation. Finally, estimations of the potential P recovery could be done.

5.2 Decomposition experiment

The relative decomposition rate of 40g *Ulva lactuca* DW is 0.0413 d^{-1}; which means that half of the *Ulva lactuca* material is decomposed after 17 days, which is very fast compared to green manures which are commonly used. This leads to the conclusion that *Ulva lactuca* cannot be used to increase SOM on the long term, since it is decomposed in a short time span. However, since *Ulva lactuca* has a high decomposition rate, it can serve as a nutrient boost for crops. Further research could analyse the effect of the time of incorporation on the decomposition rate. In future studies, decomposition of *Ulva lactuca* could be compared to the decomposition of other seaweeds and effect of seaweed application on soil salinity could be analysed. Finally, the potential hazards of toxic gas production could be assessed.

5.3 Crop growth experiment

Ulva lactuca application increased N content ($p<0.05$) and P content ($p<0.10$) of mustard plants, but not of lettuce. Despite the higher nutrient contents in mustard, *Ulva lactuca* application had a negative effect on crop appearance in mustard and also in lettuce. *Ulva lactuca* application reduced shoot dry matter in lettuce ($p<0.05$) and mustard ($p<0.10$). In literature it was found that seaweed extracts mainly had beneficial effects on crop performance, but the results of this study show that seaweed application does not have to benefit crop growth and development. Future studies could identify why seaweed meals and extracts influence crop growth in a different way. It remains unclear why crop growth and appearance was affected in a negative way by *Ulva lactuca* application, because it was expected that crops grown with *Ulva lactuca* would perform better than crops grown without; Future studies could try to gain more insight in this mechanism. In addition, further research could compare the effects of *Ulva lactuca* application to the effects of other seaweed species.

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Appendix

1 P uptake model

To model P uptake of *Ulva lactuca* in a 150L aquarium over a time period of at least 10 days, an FST model was created. The goal of this model is to gain insight in the growth and P uptake dynamics of *Ulva lactuca*. This model was calibrated with data from literature and results of the pilot study. First the basic model and calibration is given for the situation that no P is added after the start of the experiment. In Section 1.2, model extensions are given for situations that additional P is added after the start of the experiment and *Ulva lactuca* is harvested during the experiment.

1.1 Basic model

Acronym	**Explanation**
Volume	Content of the aquarium
UlvaI	Initial amount of *Ulva lactuca* in the water
PconcI	Initial P concentration in the water
PamtI	Initial amount of P in the water
PinUlva	Stoichiometric coefficient of P uptake in *Ulva lactuca*
Ulva	Amount of *Ulva lactuca*
Pconc	P concentration in the water
Pamt	Amount of P in the water
Mu	Relative growth rate of *Ulva lactuca*
Mu_max	Maximum relative growth rate of *Ulva lactuca*
Km	Constant where mu is half mu_max due to Pconc
RUlva	Rate of change of the amount of *Ulva lactuca*
RPamt	Rate of change of amount of P in water:

Model

```
TITLE  Ulva lactuca growth with phosphorous limitation
Units are in liter, gram, milligram, day

INITIAL
PARAM Volume = 133.0
*     Liter
INCON UlvaI = 0.151
*           g (DW)
PARAM PconcI  = 555.E-3
*     mg/L
PamtI   = PconcI * Volume
*     mg
PARAM PinUlva = 14.15
*     mg P / g Ulva lactuca (DW)
PARAM mu_max  = 0.3095
*     d-1
PARAM Km  = 838.5E-3
*     mg/L
```

```
TIMER STTIME = 0.; FINTIM = 11.; DELT = 0.1; PRDEL = 1.
TRANSLATION_GENERAL DRIVER='RKDRIV'
PRINT Ulva, Pconc, Pamt, mu
SET P_CumAddition = 0.0[1]

DYNAMIC
Pamt  = INTGRL (PamtI, RPamt)
*     mg
Ulva = INTGRL (UlvaI, RUlva)
*     g
Pconc = Pamt/Volume
*     mg/L

RUlva = mu * Ulva
mu    = mu_max *( Pconc /(Km + MAX(0.,Pconc)))
RPamt = - PinUlva * RUlva

END
STOP
ENDJOB
```

It was assumed that *Ulva lactuca* growth was proportional to the amount of *U. lactuca* present and that the relative growth rate of *U. lactuca* (mu), in relation to the phosphate concentration in the water (Pconc), could be described by a Michealis-Menten (M-M) curve. The rate of P uptake (RPamt), was assumed to be equal to the growth (RUlva) times the P content of *U. lactuca* (PinUlva).

Calibration

In a pilot study, five 5cm pieces of *Ulva lactuca* originating from different plants, were cultivated in a 133L aquarium. After P depletion of the seawater, new P was added at day 0 of the experiment. The pilot experiment had two replications and lasted 11 days. Water samples were taken at day 0, 1, 2, 3, 4, 7, 9 and 11. At day 0, *U. lactuca* FW in the aquaria was measured and DW was calculated with the dry matter content of the plants that were used to obtain the 5cm pieces. At the end of the experiment, *U. lactuca* DW was determined and water samples were analysed for P and N. The mean of the two replications were used to calculate the value of parameters UlvaI, PconcI, PinUlva, mu_max and Km.

Initially, the P concentration was set to 0.620 mg/L (20 µM), but the Pokon solution used was lower in P than reported. Therefore, the initial PconcI was 0.555 mg/L. More than 80% of the phosphate was taken up after 11 days, which means 14.15 mg P was taken up per g of *Ulva lactuca* DW (PinUlva). However, the relative growth rate of *U. lactuca* was 0.3095 d^{-1} and this was assumed to be the maximum growth rate (mu_max) since very high P concentrations were used and literature only showed lower maximum growth rates (e.g. Pedersen *et al.,* 2010). Km was changed until the model fitted the observed data. The optimum fit was found at 838.5E-6 mg P/L (see Figure 24).

Discussion

In this section, the model and the calibration of the model are discussed. The P uptake model was not found to be reliable in predicting the P uptake of *Ulva lactuca* grown under different P concentrations. The first reason why the model cannot predict P uptake accurately enough, is the large variation in the results of the experiment. For example, the standard error as a percentage of

[1] P_CumAddition is used in the extended model only, but is given in this listing so the extensions can be inserted without changing the basic model.

the average value of the two replications, was 21% (growth of *Ulva lactuca*) 15% (P uptake) and 20% (N uptake). Given the variability of the results, more replications are needed to calibrate the model.

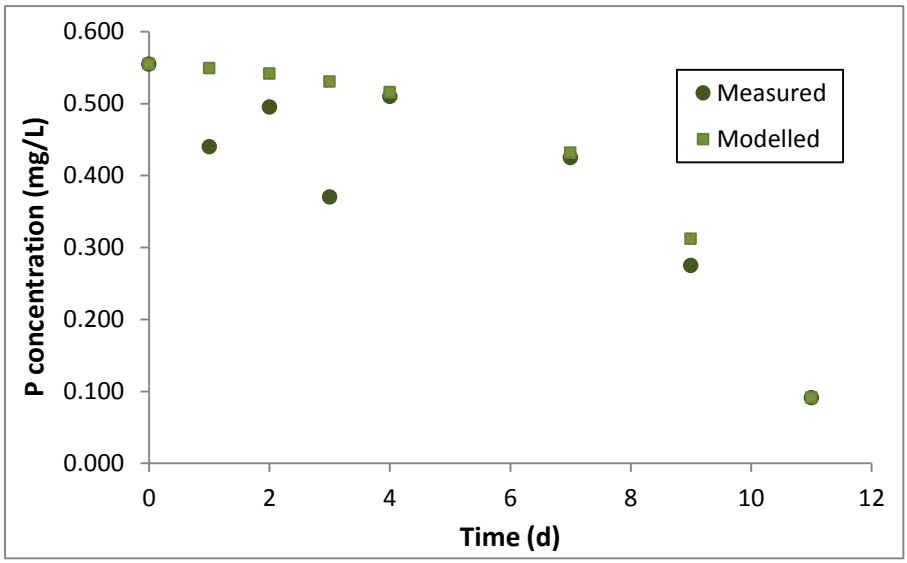

Figure 24: Modelled and measured P concentrations during the pilot experiment.

Second, the experiment was performed with a PconcI of 0.550 mg/L (17.75 µM), which probably resulted in the high P uptake of *Ulva lactuca* (14.15 mg/g DW). In literature, the highest P content of *U. lactuca* was found to be 3.9 mg/g DW, indicating that *U. lactuca* had taken up much more P than was necessary for growth. This means that the value for PinUlva is higher than in reality. Both Pedersen *et al.* (2010) and Lee (2000) noted that *U. lactuca* can store P for future growth. As a result, P uptake by *U. lactuca* over a time period of 10 days might vary considerably between different P concentrations, for P uptake is partly dependent on the P concentration in the seawater (as was already shown by Pedersen *et al.,* 2010).

Furthermore, when P uptake was modelled, Km of the growth curve of *Ulva lactuca* was changed until an optimal fit was found (Figure 24). Seawater samples for the determination of P concentrations were taken at 7 different days. The model was fitted to these values, however, two values were outliers, resulting in a model fit to 5 data points. Although the average difference between the model and the 5 observed data points was 10.4% (compared to the model values), more data points are needed to calibrate the model more accurately.

Finally, other factors like temperature, light, CO_2, pH, density and N depletion are not taken into account in this model, despite the fact that *Ulva lactuca* growth and P uptake are influenced by these factors (see e.g. Msuya & Neori, 2008 and Frost-Christensen & Sand-Jensen, 1990). *Ulva lactuca* growth is often N limited (Section 2.2.2) and reduced P uptake was observed under N limited growth of *Ulva lactuca* (Pedersen & Borum, 1996). In the pilot study, the aquaria were depleted or nearly depleted from NO_3^- and NH_4^+ in 11 days. Growth and therefore P uptake might have been influenced strongly by the fast depletion of inorganic N levels.

1.2 Model extensions

In this section, two model extensions are presented. The first extension concerns the addition of extra inorganic P after the start of the experiment when P concentrations reach a certain level. The second extension includes the harvest of *Ulva lactuca*. Harvesting *Ulva lactuca* during the experiment might be necessary, for example because the *Ulva lactuca* density becomes too high.

Acronyms[2]	**Explanation**
Frac	Fraction of PconcI at which new P is added
Pconc_min	Minimum allowed concentration of P
P_Amount	Amount of P added when Pconc_min is reached
P_CumAddition[3]	Cumulative amount of P added after the start of the experiment
Ulva_max	Maximum allowed amount of *Ulva lactuca*
P_Ulva	Amount of *Ulva lactuca* harvested when Ulva_max is reached

Model

```
PARAM Frac  = 0.8
Pconc_min = Frac * PconcI

EVENT
     ZEROCONDITION Pconc - Pconc_min
           P_Amount   = (PconcI - Pconc) * Volume
     NEWVALUE  Pamt       = Pamt + P_Amount
     NEWVALUE  P_CumAddition = P_CumAddition + P_Amount
ENDEVENT

Ulva_max = 15.
*     g

EVENT
     ZEROCONDITION Ulva_max - Ulva
     P_Ulva  = Ulva_max - 5.
     NEWVALUE  Ulva    = Ulva - P_Ulva
ENDEVENT
```

The minimum concentration of P can be set by changing the fraction (in this case 0.8). When the concentration of P decreased below the fraction of 0.8 of PconcI, new P is added. This results in a new value of Pamt (i.e. PconcI). Ulva_max is set to 15g and when this amount is reached, 10g will be harvested (P_Ulva = 15 - 5). Frac and Ulva_max can be given any desired value.

[2] These additional acronyms are not included in the PRINT section of the basic model

[3] This acronym is already inserted in the basic model

2 Data nutrient uptake experiment

In this appendix, data on temperature and salinity and *U. lactuca* P and N uptake during the nutrient uptake experiment can be found. In addition, the Lineweaver-Burk plots and the calculations of Pmax and Km used in Sections 4.1.2 and 4.1.3 are given.

2.1 Temperature and salinity

Table 8: Temperature (°C) and salinity (‰) during the nutrient uptake experiment. Given concentrations are phosphate concentrations in the aquarium.

		Aquarium 1 1.0 µM		Aquarium 2 2.25 µM		Aquarium 3 3.5 µM		Aquarium 4 4.75 µM		Aquarium 5 6.0 µM	
Date	**Day**	**Temp**	**Sal**	**Temp**	**Sal**	**Temp**	**Sal**	**Temp**	**Sal**	**Temp**	**Sal**
Aug 6th	0	21.1	31.0	20.5	30.9	20.3	31.0	19.9	30.9	20.0	31.0
Aug 7th	1	15.9	31.1	14.7	30.9	14.8	31.0	14.4	30.9	15.5	31.0
Aug 8th	2	20.4	30.7	19.8	30.7	20.1	30.7	20.5	30.7	20.7	30.7
Aug 9th	3	16.2	30.7	15.0	30.7	15.0	30.7	14.8	30.7	15.4	30.8
Aug 10th	4	21.4	31.0	21.2	31.0	21.3	31.0	21.2	31.0	21.3	31.0
Aug 12th	6	20.3	31.1	20.2	31.1	20.2	31.1	20.2	31.1	20.2	31.1
Aug 13th	7	19.3	31.2	19.1	31.1	19.0	31.2	18.9	31.2	18.7	31.2
Aug 14th	8	19.3	31.2	19.1	31.2	19.1	31.2	19.0	31.3	19.2	31.3
Aug 15th	9	19.6	31.3	19.4	31.3	19.3	31.3	19.1	31.4	19.0	31.4
Aug 16th	10	20.5	31.5	20.2	31.3	20.0	31.4	19.9	31.5	20.1	31.5

2.2 Phosphorus and nitrogen content – individual samples

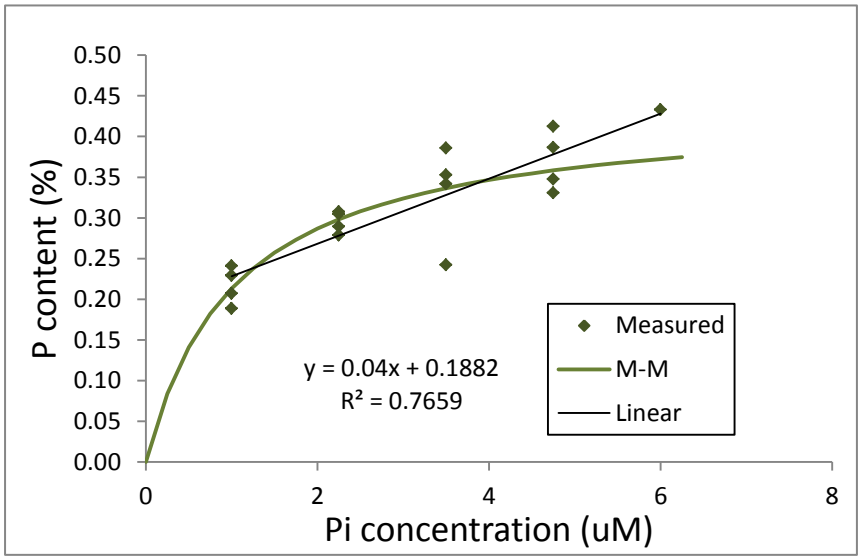

Figure 25: Phosphorus content of individual *Ulva lactuca* samples in relation to the Pi concentration in the seawater. R^2 of Lineweaver-Burk plot is 0.7732.

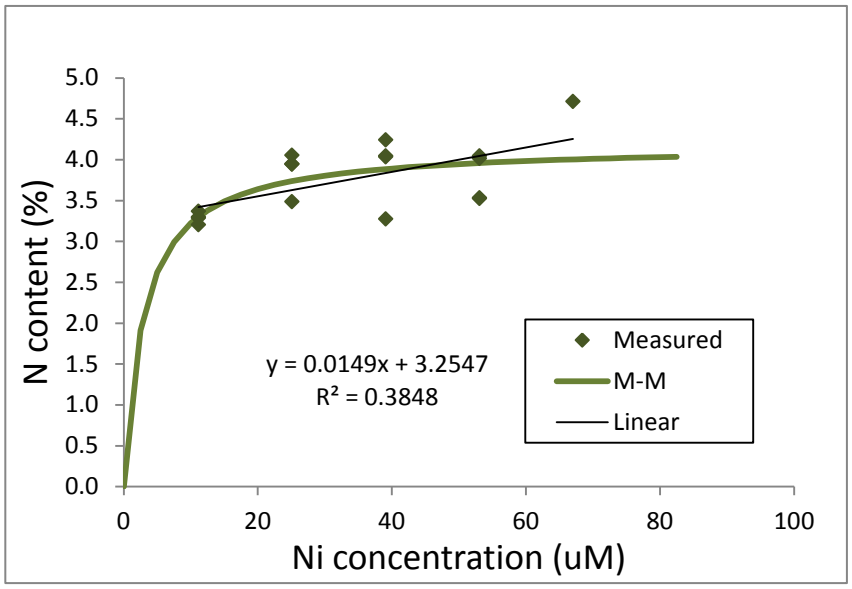

Figure 26: N content of individual *Ulva lactuca* samples in relation to the Ni concentration in the seawater. R^2 of Lineweaver-Burk plot is 0.4615.

2.3 Lineweaver-Burk plots

The values of the slope and intersection with the y-axis of the Lineweaver-Burk plots can be used to retrieve the values of Pmax and Km of the optimal fit of the data to a Michaelis-Menten equation. Figure 27 shows the Lineweaver-Burk plot of the data on Pi concentration of the seawater and P content of *Ulva lactuca*. The value of the intersection with the y-axis of (x=0, y=2.1624) is the inverse of Pmax. Therefore, Pmax = 1/2.1624 = 0.4625% DW. The value of the slope of the Lineweaver-Burk plot (2.5612) is Km/Pmax, therefore Km = 1.1844.

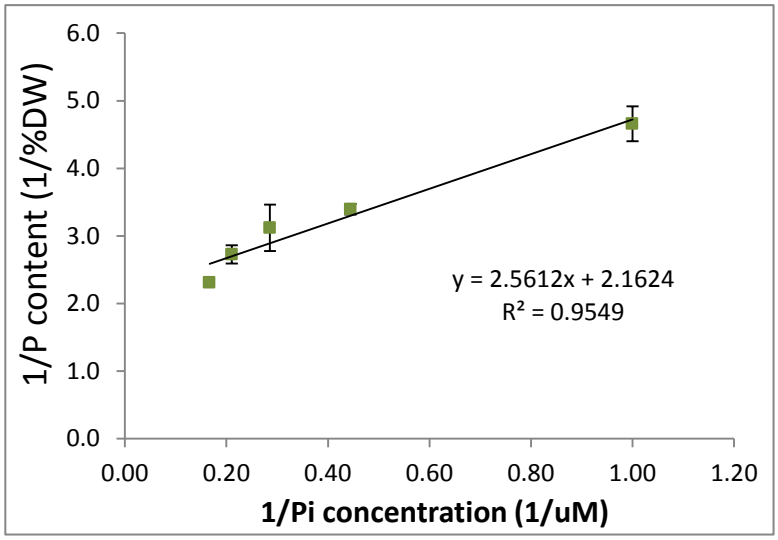

Figure 27: Inverse of Pi concentration plotted against the inverse of P content of *Ulva lactuca*. Data are aquarium averages ± SE.

A Lineweaver-Burk plot is created from the data of Pedersen (pers. comm. 2013) to retrieve the values of Pmax and Km of the Michaelis-Menten equation of this data set (Figure 28). Pmax = 0.226% DW and Km = 0.151.

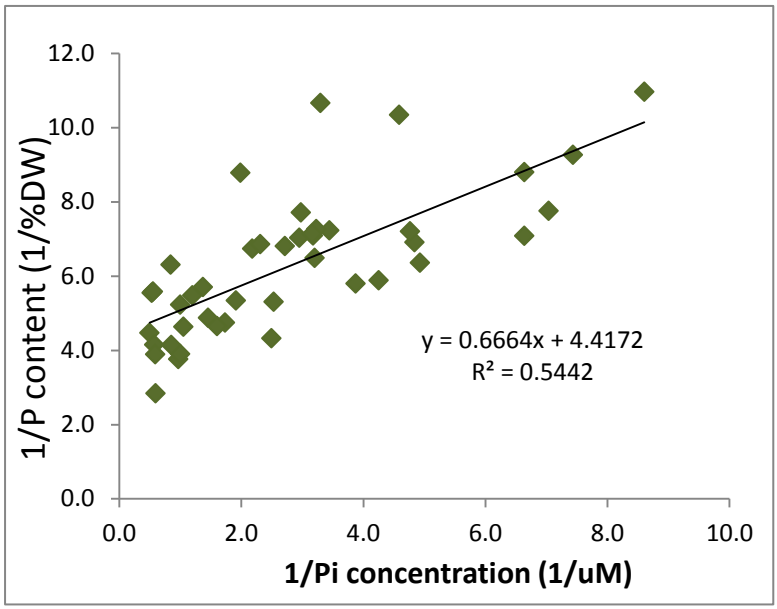

Figure 28: Inverse of Pi concentration plotted against the inverse of P content of *Ulva lactuca*. Raw data from Pedersen (pers. comm., 2013).

The values of Nmax and Km of the Michaelis-Menten equation of the experimental data on Ni concentration and *Ulva lactuca* N content are calculated as mentioned before (Figure 29). Nmax = 4.4042% DW and Km = 3.8893.

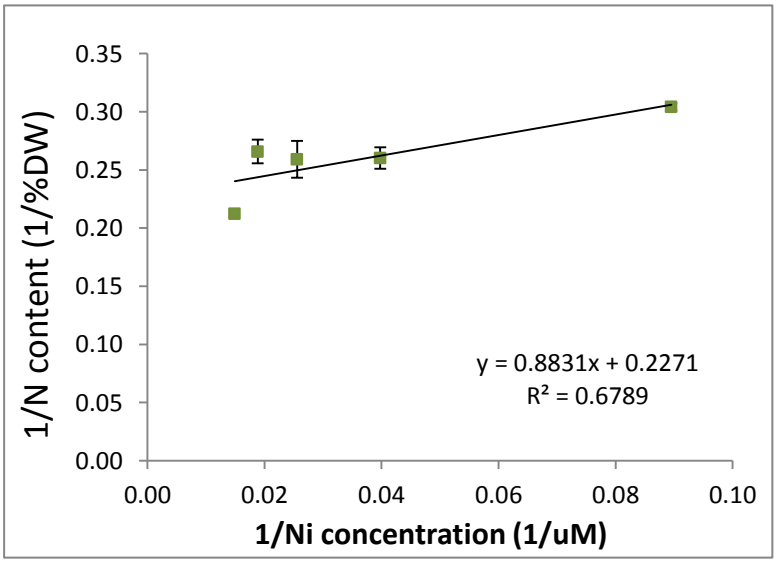

Figure 29: Inverse of Ni concentration plotted against the inverse of N content of *Ulva lactuca*. Data are aquarium averages ± SE.

3 Temperature model

The temperature model as described by Leffelaar *et al.* (2013) is used to calculate the temperature at 10cm depth in relation to the temperature at 5cm depth. Temperature data at 5cm depth were used, because temperature data at soil surface were not found to be a good reference for determining the temperature at 10cm depth. Climate data of soil temperature under a bare soil from June 5st to July 26th (2013) is used from the Veenkampen (2013).

Temperature fluctuations during a day can be described by a sine (Equation 4):
$T_{x,t} = T_{av} + T_{ampl} * e^{(-x*d)} * \sin(\omega * t - x/d)$

in which:
$T_{x,t}$ = temperature at soil depth x and time point t
x = soil depth in m
t = time of the day in s
T_{av} = equilibrium value of sine
T_{ampl} = amplitude of sine
ω = 2π/period
d = attenuation depth in m

The attenuation depth can be described at the depth at which the amplitude is 0.37 times the amplitude at the soil surface (or in this case the amplitude at 5cm depth).

The lowest and highest temperature at 5cm depth are selected manually. Since each day has a different lowest and highest temperature, values for T_{av} and T_{ampl} vary daily. The average of the lowest and highest temperature is used as the equilibrium value (T_{av}). The difference between the peaks and the equilibrium value is used as the amplitude value (T_{ampl}). Soil depth x at 10cm depth is 0.05 (since 5cm depth is the reference point). Naturally, the period of one oscillation is 86400s (24 hours) and ω is therefore 2π/86400.

Attenuation depth (d) is calculated with Equation 10 (from Leffelaar *et al.,* 2013):
$$d = \sqrt{2 * \lambda/(\omega * Ch)}$$

where:
λ = heat conductivity in J/(m s °C)
C_h = heat capacity in J/(m³ °C)

The value of λ (0.40 J/(m s °C)) is obtained from Koorevaar *et al.* (1983) and the value of C_h ($1.412*10^6$ J/(m³ °C)) is calculated below in Table 9. Attenuation depth (d) is calculated with Equation 10, it can be calculated that d = 0.088 m.

Table 9: Calculation of the C_h value of the Unifarm soil.

Soil component	C_h in J/(m³ °C)*	Fraction of component in soil	C_h value in J/(m³ °C)
Quartz	$2.0 * 10^6$	0.362	$0.725 * 10^6$
Organic matter	$2.5 * 10^6$	0.0059	$0.015 * 10^6$
Water	$4.2 * 10^6$	0.16	$0.672 * 10^6$
Air	$0.0013 * 10^6$	0.472	$0.001 * 10^6$
		Total:	**1.412** $* 10^6$

*From Koorevaar *et al.* (1983)

Figure 30 shows the modelled temperature at 10 cm depth, calculated with Equation 4. The output data from the model was used to calculate average day and night soil temperatures. Official time points of sun rise and sun set were used to calculate day and night length. Day is defined as time from sun rise to sun set, night as the time from sunset to sunrise the next day.

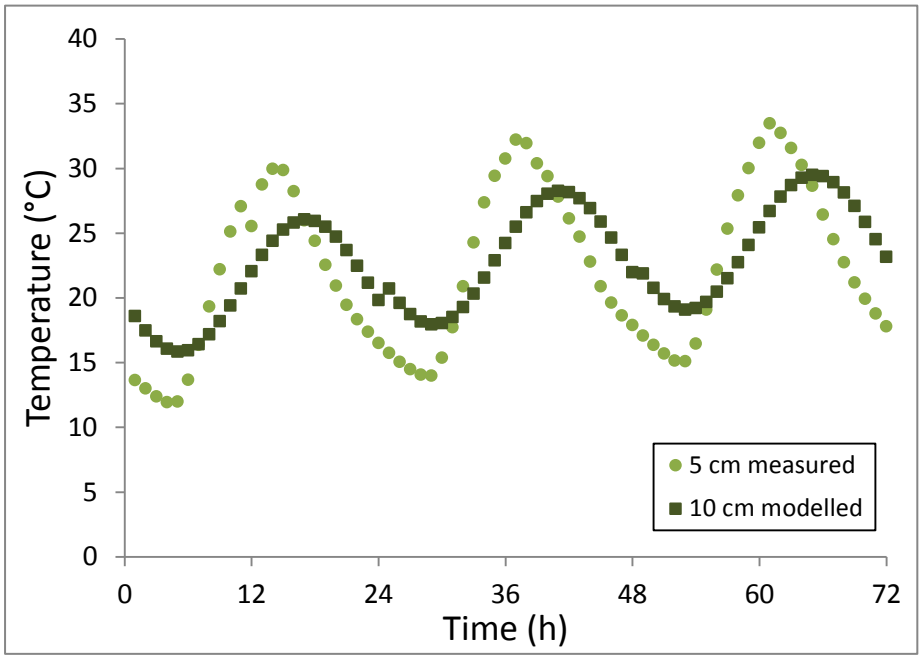

Figure 30: Temperature at 5 cm depth (data from Veenkampen, 2013) and the modelled temperature at 10 cm depth of June 5 – 7, 2013.