The effect of temperature and oxygen availability on decay rate and change in food quality of *Laminaria hyperborea* detritus

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Resume

Laminaria hyperborea hører til den ikke taksonomiske gruppe kelp. Kelp er en fællesbetegnelse, for en gruppe arter af store brunalger. Denne gruppe indeholder blandt andet arter fra slægterne *Laminaria, Saccharina* og *Macrocystis. Laminaria* og *Saccharina* er de to mest almindelige slægter i Europa (Araújo et al. 2016). Kelp består af tre morfologisk og funktionelt forskellige dele, en holdfast, en stipes og en bladdel. Holdfasten har en rodlignende struktur, men hvis eneste funktion er at forankre planten til det hårde underlag. Stipes varierer meget i udseende mellem arter, men stipes' generelle formål er at forbinde holdfast og blad eller blade. Bladdelen kan bestå af et eller flere blade, bladdelen for *Laminaria* er et stort blad for enden af stipes (Svendsen & Kain 1971). Kelp som gruppe, er velkendt for at kunne opnå en høj tæthed af individer og biomasse. Kelp kan danne strukturer, som trækker paralleller til de terrestriske skove, her af navnet kelpskov.

L. hyperborea er en art hovedsageligt fundet langs Norges nordvest og nordlige kystlinje, helt op til den Russiske grænse. Her findes den hovedsageligt i den ydre del af fjordene under tidevandszonen, hvor den danner tætte skove af planter op til 3,5 m (Sjøtun et al. 1993; Araújo et al. 2016). *L. hyperborea* er blevet fundet til at trives bedre, i meget eksponeret områder, altså områder med stærk strøm eller bølgepåvirkning. Under disse forhold er den fundet, til at opnå højere produktion og samlet biomasse. Produktionen under ideelle forhold, har været rapporteret til at kunne nå 2000 g C m^{-2} $år^{-1}$, dog er en produktion på under 1000 g C m^{-2} $år^{-1}$ mere realistisk (Mann 1973; Pedersen et al. 2012). Under turbulente forhold har man fundet op til 50 individer m^{-2}, dog er en individtæthed på 7-12 m^{-2} mere normalt rapporteret (Sjøtun et al. 1993; Pedersen et al. 2012). Den høje produktion og individtæthed leder også til en høj densitet af biomasse. Kelpskove kan opnå en densitet på over 18 kg Frisk Vægt (FW) m^{-2} hvor bladet udgøre cirka halvdelen af vægten (Pedersen et al. 2012; Sjøtun et al. 1993). Kun meget lidt af denne biomasse bliver græsset af græssere, kun omkring 18% af kelp biomassen bliver græsset direkte. Dette betyder altså at cirka 82% af biomassen bliver omdannet til detritus (Krumhansl & Scheibling 2012).

Forholdet mellem Carbon (C), Nitrogen (N) og Fosfor (P) er en generel indikator for kvaliteten af føde. Et højere indhold af N og P er generelt forbundet med bedre fødekvalitet. Vækst- og overlevelsesrate er fundet at være korreleret med C:N-ratioen for sekundære producenter, som C:N-ratioen faldt, steg vækst- og overlevelsesraten (Norderhaug et al. 2006; Norderhaug et al. 2003; Duggins & Eckman 1997). Ydermere er der også blevet fundet en sammen hæng mellem N og P indholdet og hvor hurtigt og til hvilken grad materiale nedbrydes. I takt med at N og P indholdet

stiger, stiger nedbrydningshastigheden og hvor stor en procent del som nedbrydes (Cebrian 1999). Den optimale C:N:P-ratio af substrat for mikroorganismers vækst er 106:12:1, ved værdier højere end dette falder produktion og vækst drastisk (Goldman et al. 1987).

Dette speciale er en del af forskningsprojektet KELPEX. Forskningsprojektet har til formål at undersøge produktion af biomasse fra kelpskove, samt produktion af detritus og hvor dette bliver transporteret til. Den akkumulerede viden skal bruges til at lave en økologisk model. Denne model skal bruges til at estimere, den understøttende effekt kelp økosystemer har på de omkringværende økosystemer. Feltarbejdet med estimering af produktion af biomasse og detritus, foregik i den ydre del af Malangen fjord i Norge (69,6˚ N 17,8˚ Ø). Formålet med dette speciale er at undersøge, hvilken kvalitet detritten har, afhængig af hvilket miljø det bliver transporteret til. Nedbrydningen undersøges ved forhold, som har til hensigt at simulere det lave kystnære vand og de dybere dele af fjorden eller dybhavet.

Forskel i raten af nedbrydning og ændringen i biokemiske forhold af kelp detrit blev undersøgt ved to forskellige temperaturer, 4˚C og 10˚C. Som derudover havde enten iltrige eller iltfattige forhold, dette blev sikret ved gennembobling med henholdsvis atmosfærisk luft eller N_2. De to temperaturer blev valgt for at imitere sommer og vinter temperaturerne, samt det koldere dybe vand. De iltrige og iltfattige forhold skulle simulere henholdsvis de lave kystnære områder og de dybere dele af fjorden eller dybhavet. Materiale fra blad og stipes blev nedbrud i henholdsvis 280 og 308 dage. Materiale blev udtaget 10 gange for at måle tilbageværende biomasse samt koncentrationen af C, N, P og fenol. Det forventes at nedbrydningen af biomasse sker hurtigere ved 10˚C temperaturer og i et iltrigt miljø og langsomst ved 4˚C og iltfattige forhold. Dette forventes da aerobe mikroorganismer er i stand til at udfører alle dele i nedbrydningen (Middelburg et al. 1993). Hvor anaerobe mikroorganismer er afhængige af et konsortium hvor de hver er specialiseret til at varetage et led af nedbrydningsprocessen. Derudover er den aerobe nedbrydning mere favorable i forhold til energiudbyttet (Middelburg et al. 1993). Det forventes at fødekvaliteten i form af C:N:P-ratioen falder, og derved bliver bedre. Dette vil ske grundet kolonisering af mikroorganismer med lav C:N:P-ratio (Goldman et al. 1987), vil berige materialet med N og P . Dog forventes det at der vil ske en ophobning af phenoler da disse er svært nedbrydelige, hvilket vil resultere i en forværring af fødekvalitet.

Resultaterne fra dette eksperiment understøtter til dels litteraturen. Betydningen af biomasse i tørvægt (DW) kunne bedst beskrives via en et-fases nedbrydningsmodel. Denne beskrev den hurtige

nedbrydning i starten som aftager langsomt for til sidst at ende ud i et plateau. Plateauet beskriver residual puljen (Westrich & Berner 1984). Denne model beskrev ikke nedbrydningen efter de første 21 til 49 dage, der blev derfor lavet en lineær regression af nedbrydningen herefter. Denne lineære regression viser at nedbrydningen af det ikke let nedbrydelige materiale i en højere grad er påvirket af temperatur og tilstedeværelsen af ilt.

C indholdet steg kraftigt under den tidlige nedbrydning, hvor efter det langsomt faldt igen, hvorimod P indholdet startede med et kraftigt fald hvor efter det steg til en koncentration som var ikke statistisk signifikant lavere end start værdien. C og P indholdet i stipes fulgte en modsat tendens. Her startede C med et kraftigt fald hvor det stabiliserede, ved en koncentration mellem 5 og 10 procentpoint lavere end start værdien. P indholdet steg kraftigt indenfor de første syv dage, men efter 21 dage var indholdet faldet en værdi lig med start værdien. N det eneste element som ændrede sig kontinuerligt under nedbrydningen. Koncentrationen af N næsten tredoblede for både blad og stipes. Indholdet af fenoler faldt drastisk, i modsætning til forventning, især for blad materiale hvor indholdet var omkring detektionsgrænsen efter blot 21 dage, indholdet i stipes faldt støt og nåede samme niveau på 140 dage. Da C:N:P-ratioen er et produkt af indholdet af de selv samme næringsstoffer, var ændringen i denne også varierende. C:N-faldt i alle tilfælde til værdier under 15 og i visse tilfælde under 10 for stipes. C:P forholdet for blad startede med at stige kraftigt, op til 5 gange start værdien, hvor efter den faldt, og udlignende sig på en værdi cirka 50% højere end start værdien. C:P-ratioerne for stipes startede med et fald, hvor efter den steg of stabiliserede på cirka 2/3 af start værdien. N:P-ratioerne steg i alle tilfælde fra en startværdi på 11 for blad og 21 for stipes til værdier mellem 43 og 54. De endelig C:N:P-ratioer på tværs af alle behandlinger resulterede i ratioer som var overraskende ens mellem blad og stipes, henholdsvis 632:45:1 og 538:38:1.

Den primære effekt af temperatur og ilttilgængelighed synes at påvirke nedbrydningshastigeden i en større grad end ændringen i C, N og P. Her spiller især tilstedeværelsen af ilt en rolle, det synes at lede til en hurtigere og mere fuldstændig nedbrydning. Temperaturen påvirker nedbrydnings-hastigheden og i mindre grad den tilbageværende biomasse pulje (residual material). N koncentrationen bliver forskelligt påvirket afhængig af materialetype. Derudover er udsvinget i C, N og P også forskellig mellem de to materiale typer, hvor efter de dog følger mere ens tendenser. Responset til ilttilgængeligheden og temperatur hvad angår C, N og P samt fenol koncentration, syntes derfor at være mindre tydelig.

Abstract

The high quantity of detritus produced by *Laminaria hyperborea* has an important subsidizing effect for the neighbouring ecosystems (Krumhansl & Scheibling 2012; Fredriksen 2003). The kelp detritus have been proven to be of great importance to subsidize ecosystems as far as 9 km from the kelp ecosystems (Harrold et al. 1998; Krumhansl & Scheibling 2012; Duggins et al. 1989). The general assumption has been that the erosion of blade has been the primary source of detritus and kelp is seen as a 'conveyer belt' of food (Mann 1973; Krumhansl & Scheibling 2012). This study implies a change in view of the detrital production by *L. hyperborea* as a pulse occurring simultaneously with the production of new blade between March and May. Destructive grazing fronts of *Strongylocentrotus droebachiensis* have been estimated to be able to graze 30 g DW m^{-2} day^{-1} (Sumi & Scheibling 2005). This is well below the 1000 g DW m^{-2} detritus which are being produced during the blade shredding. This results in either accumulation or transport of detritus. The importance of where the detritus is transported was investigated by decomposition of blade and stipe tissue under 4˚C and 10˚C and under oxic and anoxic conditions. The blade and stipe tissue were decomposed for 280 and 308 days respectively with 10 sampling occasions, the first being after 7 days. The difference in effect of the environmental factors was particularly seen after 140 days, as the difference in decay rate became apparent. Besides that, only few significant differences were found without any discernible trends. Overall the decomposition the decomposition of both stipe and blade tissue led to surprisingly similar C:N:P-ratios. The average across all treatments resulted in ratios of 632:45:1 for blade and 538:38:1 for stipe. Aerobic conditions did however seems to lead to a higher N content for stipe tissue which led to C:N-ratios below 10. Hence the temperature and oxygen availability of the recipient ecosystem does not seem to be of great importance in altering the quality of the detritus.

Introduction

Kelp is a non-taxonomic term for large marine seaweed species of brown algae (*Phaeophyceae)* (Bolton 2016). Kelp is not a strict term and might refer to several genera within *Phaeophyceae*. Some of the most common genera is: *Laminaria, Macrocystis, Nereocystis, Ecklonia, and Saccharina* (Bolton 2016; Mann 2000). Kelp as a group is present globally with *Laminaria* and *Saccharina* being the dominating genera of the northern hemisphere, *Macrocystis* is the main genus along the west coast of the American and south American continents, and *Ecklonia* being the dominating genus in Australia (Dayton 1985). Kelp most commonly inhabits the subtidal zone of highly exposed and turbid rocky shores (Dayton 1985; Mann 2000). Under these turbid conditions, kelp communities can flourish from the intertidal zone to depths of 30 meters (Mann 1973; Mann 2000).

The kelp thallus consists of three morphologically and functionally distinct features: the holdfast, the stipe and the blade. The holdfast as the name suggest is the part of the thallus which anchors the individual to the rocky substrate. The stipe may take different forms but is used for elongation. The blade, which may be found at the end of the stipe as with *Laminaria*, or branching off from the stipe as with *Macrocystis*. Species, that has blade at the end of the stipe usually do not grow larger than 2-3 meters in length, while species branching blades may reach lengths of 60 m (Mann 2000). Kelp communities are highly productive, a production of 1000 g C m^{-2} y^{-1} is not uncommonly reported. Under ideal conditions the production might be as much as approximately 2000 g C m^{-2} y^{-1} (Dayton 1985; Mann 2000). Kelp communities are often referred to as either kelp forest or beds as they reach great densities >50 adult plants m^{-2}. Though densities are more commonly reported to be around 7-12 individuals m^{-2}, which amounts to 18,000 g fresh weight (FW) m^{-2} (Dayton 1985; Pedersen et al. 2012). The morphology of plants and the high density of individuals can visually draw parallels to the terrestrial forests; hence, kelp communities are often referred to as kelp forests or kelp beds depending on the size of the individuals. The high production and densities establishes kelp communities as important components of the ecosystems in which they are situated but also to the surrounding ecosystems. Kelp forests are in many ways commercially important, either harvested for human consumption and as a potential biofuel (Vea & Ask 2011; Moen, Horn, et al. 1997). If left in the water it can act as nurseries and sanctuaries, or as food source for secondary producers which in inhabits the kelp forests and the neighbouring ecosystems (Krumhansl & Scheibling 2012; Araújo et al. 2016; Norderhaug et al. 2007).

In Norway, the three most commonly reported kelp species are *Saccharina latissima, Laminaria digitata,* and *Laminaria hyperborea*. *L. hyperborea* being the most common along the Norwegian coast, as seen in figure 1 (Araújo et al. 2016).

The

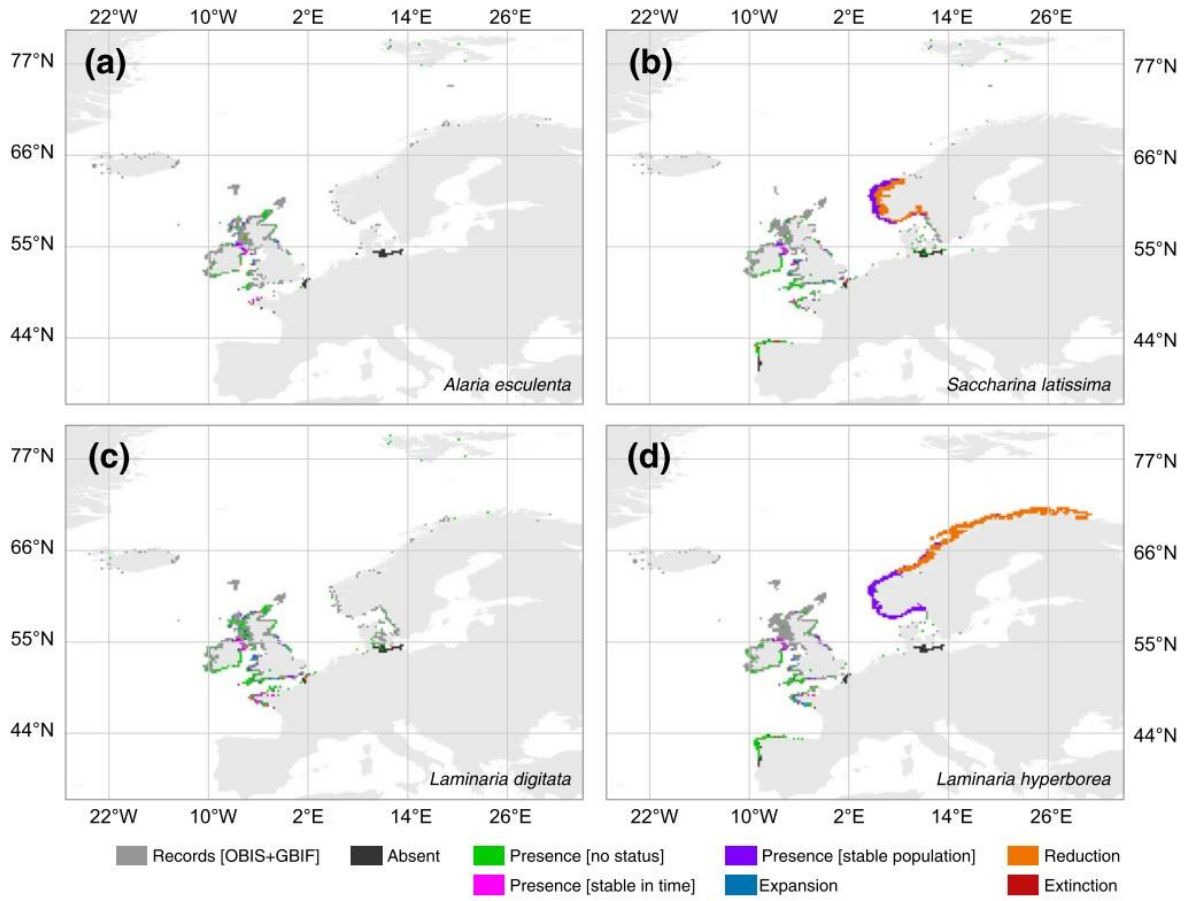

Figure 1: Map over the distribution and estimation of the future extent of kelp beds along the European coasts. (Araújo et al. 2016).

Distribution of *L. hyperborea* extend further east than shown in the figure, as it is found all the way to the Russian border (Sjøtun et al. 1993). *L. hyperborea* has a large coarse hold fast, which anchors it firmly to the hard substrate. The stipe is rod like and rigid with varying lengths depending on the physical conditions, and it has a thick leathery blade, which is situated at the end of the stipe. *L. hyperborea* is most commonly found in turbid waters, if present in a sheltered area the morphology will differ. *L. hyperborea f. cucullata* is a specific morphological form exhibited by organisms growing in low exposure whether it is due to shelter or depths (Svendsen & Kain 1971; Kain & Jones 1971). Both as individuals and as communities kelps exhibit greater production and biomass in areas of high wave exposure and strong currents. *L. hyperborea* has been reported to reach ages of 18 years, and individuals at high exposure sites tends to become older (Sjøtun et al. 1993; Pedersen et al. 2012).

Stipes has been shown to grow longer and at a faster rate with increasing wave intensity, to a maximum of approximately 140 cm in length at high exposure compared to 100 cm at low exposure. Furthermore, the individual biomass have been found to be approximately one-third higher at a highly exposed site compared to a low exposed site 1250 g FW ind^{-1} and 820 g FW ind^{-1}, respectively (Pedersen et al. 2012). An increase in turbidity seems to be correlated with an increased number of individuals and biomass. Pedersen et al. (2012) reported that the biomass at high exposure sites were 2.5 times higher than at low exposure sites, which coincide with their finding of an increased density of 7 ind m^{-2} as opposed to 12 ind. m^{-2} at low and high exposure, respectively. On the contrary Sjøtun et al. (1993) found no clear difference in density nor biomass along an exposure gradient, they found the average biomass of standing stock to be 6-16 kg FW m^{-2}.

The blade is shredded annually and the growth of the new blade is initiated in November by cell division in the meristem (Kain & Jones 1976). The growth rate is highest during the first three months of the year (Kain & Jones 1976). As *L. hyperborea* is a species only found on the northern hemisphere, the growth season is corresponding to the general low productive season of other photosynthetic organisms (Schaffelke & Lüning 1994; Lüning 1986). This strategy results in a markedly change in the biochemical composition during the year (Schiener et al. 2014).

Despite the large production potential of kelp only little to none of kelp biomass is grazed directly, and only few species has been reported to graze kelp do a degree, which can control the biomass (Mann 1982; Schaal et al. 2010; Abraham 2007). Sea urchins are able to establish grazing fronts, which can destructively graze kelp beds and thereby control the abundance of kelp (Mann 1982; Filbee-Dexter & Scheibling 2014). Biomass, which is not grazed, will enter the detritus cycle as particulate organic matter (POM). POM can vary greatly in size as it can be particles which has been eroded off the plant or from dislodgement of whole plants. In this sense POM may vary between the size of a phytoplankton and a 60 m *Macrocystis* (Mann 2000).

Krumhansl & Scheibling (2012) found that an average (across species and spatial extend) of 82% of the kelp primary production enters the detrital pool. The detritus may exist as either small particles or as whole thalli depending on the conditions. The detritus may be transported both horizontally and vertically, and kelp detritus has been documented to subsidize secondary production on a great spatial scale, from neighbouring ecosystems to submarine canyons several kilometres away (Harrold et al. 1998; Duggins et al. 1989; Leclerc et al. 2015). Harrold et al. (1998) found that, at 400 meters of depth, and up to 9 km away from nearest kelp forest, kelp detritus was the largest fraction of the

detritus pool. The low direct grazing has been attributed to poor food quality of fresh kelp (Schaal et al. 2010). As detritus is transported it will undergo both physical and biological decomposition, this may alter the physical and/or chemical nature of the detritus (Duarte & Cebrián 1996; Middelburg et al. 1993).

The food, substrate, or detritus quality is often defined as the relationship between C, N, and P in either molar or percent (Cebrian 1999; Goldman et al. 1987). The C:N:P-ratio varies greatly between taxonomical and functional groups, for example: phytoplankton (106:16:1 (Redfield 1958)), benthic marine macrophytes (550:30:1 (Atkinson & Smith 1983)), and bacteria (45:9:1 (Goldman et al. 1987)). The most commonly used relationship is the C:N-ratio (Duggins & Eckman 1997; Norderhaug et al. 2006; Scheibling & Anthony 2001). Other factors, which are used to estimate food quality is the presence or concentration of certain molecules like lignin or phenolic compounds (Ayres et al. 2014). Cebrian (1999) found a strong positive relationship between primary production and detrital production. Furthermore, an increase in the C:N:P-ratio has been found correlated with a decrease in decomposition rate (Enriquez et al. 1993). Marine bacteria needs a substrate with a C:N:P-ratio of 106:12:1 for optimal growth, at ratios higher than that, the growth and production would decrease drastically (Goldman et al. 1987). Goldman et al 1987 furthermore found that the C:N-ratio had to be below 10:1 before N was remineralized. Norderhaug et al (2003, 2006) found a positive relationship between survival rate and growth as the C:N ratio of aerobically decomposed kelp detritus. Furthermore, a C:N-ratios below 2:1 were found to have a negative effect on growth. Amphipods fed with anaerobically decomposed detritus did not have the same positive response regardless of a lower C:N-ratio. Some molecules, like tannins or phenols, may be attributed deterrent or anti-herbivore properties (Ayres et al. 2014). These properties were not confirmed by Norderhaug et al (2006), as the phenolic content of *L. hyperborea* had no positive nor negative effect on either growth or survival. This was attributed the generally low phenolic content of the tissue.

This project is a part of the KELPEX research project, set out to investigate many aspects of *L. hyperborea* beds in Norway. KELPEX is a multidisciplinary project separated into 4 work packages (WP): WP 1 is set to estimate the biomass production and the detrital production and export. WP 2 tries to estimate the importance of kelp detritus on heterotrophic shore communities. WP 3 investigates the import and importance of kelp detritus in deep-sea communities. The final WP, WP 4 will accumulate the knowledge produced in the other WPs and synthesize this into an ecological model describing the communities in and around areas of kelp beds. WP 1 did investigate the effect of wave and current exposure in relation to the above mentioned factors. This was in practise executed

by performing the same investigations at 10 different sites which were expected to differ in exposure. As an addition to the quantitative production of detritus WP 1 also want to estimate the quality of which the detritus is. The decomposition of *L. hyperborea* is not well studied. Some investigations on the decomposition and food quality of the detritus has been performed, though on detritus which has been reduced to fine particles (Norderhaug et al. 2006; Norderhaug et al. 2003). Others have focused on stipe degradation for gas production in conditions which does not match the *in situ* conditions (Moen, Horn, et al. 1997; Moen, Larsen, et al. 1997).

Figure 2: Map of the location of the sampling sites for the field work. To the right on this map (South) is into the fjord system and to the left (North) is the open ocean, the Norwegian sea.

Microbial decomposition of detritus is ever-present, but the rate may differ greatly depending on the environment and the detritus (Enriquez et al. 1993; Westrich & Berner 1984; Benner et al. 1986; Middelburg et al. 1993). Aerobic microorganisms can individually decompose POM to CO_2. Anaerobic microorganisms are dependent on a consortium of different bacteria to mineralize organic matter into inorganic material. Anaerobic microorganisms are specialized to perform one of three general processes in the microbial food chain (Middelburg et al. 1993). Furthermore, the anaerobic processes are less energetically favourable. All in all this leads to the assumption that anaerobic decomposition is slower than aerobic (Middelburg et al. 1993; Westrich & Berner 1984). Middelburg et *al.* (1993) argues that the difference in decomposition rate is an artefact of the experimental design and that there is no real difference between the two. The bulk of researchers who investigates the

have concluded that aerobic decomposition is in fact faster (up to 3,6 times (Hulthe et al. 1998)) and more complete than anaerobic (Westrich & Berner 1984; Kristensen et al. 1995; Bianchi et al. 2000). The absence of oxygen might inhibit the terminal reaction or at least greatly reduce the rate (Kristensen et al. 1995; Bianchi et al. 2000). Bianchi et al. (2000) found in an investigation of Chlorophyll a and fucoxanthin that decomposition under oxic conditions were fastest, and that the decomposition rate was between 0.04-0.07 nmol d^{-1}, this translate to a maximum rate of 62.5 ng d^{-1}. Kristensen et al. (1995) performed a study on ^{14}C marked diatoms and barley hay, they found that if anaerobic chambers were switched to oxic conditions, an increase in decomposition would occur on the contrary if changed from oxic to anoxic the decomposition would drastically decrease.

Decomposition can be separated into three stages or fractions of material; the initial (G_{01}), which is easily decomposable, the less reactive (G_{02}), which is decomposed slower and is the smallest of the three pools, and lastly the non-reactive pool (G_{NR}), which is not decomposing on a measurable timescale (Westrich & Berner 1984; Kristensen et al. 1995). Organic material, which contain large molecules, often structural or defence molecules are less liable than organic matter consisting of small molecules like sugars. Different molecules will differ in rate of decomposition, depending on size or the strength of the bonds (Middelburg et al. 1993). Middelburg et al. (1993) highlighted and presented several investigations of decomposition rate, they showed that lignin decomposed at rates half of sugars and two-thirds of amino acids. In the study by Kristensen et al. (1995), the carbon mineralization of barley hay occurred at a lower rate compared to that of diatoms, this is due to the higher concentrations of structural components like lignin in hay compared to the more labile matte of the diatoms.

A third often investigated factor, which have been shown to affect the decomposition rate is temperature. Temperature can affect the decomposition rate in more ways, both chemically and biologically. We know from thermodynamics that an increase in temperature can increase the rate and favourability of a process (Middelburg et al. 1993; Pomeroy & Wiebe 2001). Secondly, it may increase the rate as the bacteria reaches their optimum growth temperature, which is often well above their *in situ* temperature (Arnosti et al. 1998; Kristensen et al. 1995). The microbial communities will differ from region to region. Investigations suggest that the efficiency at *in situ* temperatures do not differ greatly, suggesting that cold region communities has adapted to these conditions (Thamdrup & Fleischer 1998; Price & Sowers 2004). Cold water adapted communities even seemed to have a greater potential rate when compared to temperate communities, even though cold water communities generally have a lower optimum temperature (Arnosti et al. 1998; Thamdrup & Fleischer 1998).

Pomeroy and Wiebe (2001) reported that there is a strong relationship between temperature and substrate. They concluded that microorganisms operating close to their lower optimum temperature could be kept active at higher rates if fed higher amount of substrate (Pomeroy & Wiebe 2001).

The major aim of this study is to investigate the rate of decomposition and change in biochemical composition of *L. hyperborea* under different conditions (i.e. two different temperatures and under oxic and anoxic conditions). A secondary aim is to relate the decomposition of detritus to food quality; how food quality changes during the decomposition, and at which rate it changes. It is expected that the total biomass will decrease at a faster rate at higher temperature and under oxic conditions. Furthermore, it is expected that the blade will decompose faster and more completely compared to the stipe. As the stipe serves a structural purpose (i.e. larger structural molecules) whereas the blade is the photosynthetic part of the thallus and therefore contains more labile molecules. They are both relatively nutrient poor detritus (high in carbon) and they are both expected to be enriched with both nitrogen and phosphorous due to colonization of microorganisms. It is also expected that large and less labile molecules will accumulate and up-concentrate as the more labile molecules are decomposed, which may counteract the positive effects of nutrient enrichment

Theory

Decomposition of marine primary producers

Decomposition is a broad term enveloping the numerous processes in which organic matter is broken down until it is reduced to inorganic nutrients. Decomposition concerns both physical deterioration, mechanical and biological breakdown by animals, and the mineralization by microorganisms. Decomposition in a marine ecosystem depends on the biotic and abiotic conditions of the ecosystem and the organic material which is being decomposed. Decomposition is commonly described by a first-order kinetics model, the G-model by Berner in 1964 (Middelburg et al. 1993). The G in G-model is the concentration of metabolizable organic carbon. The G-model was expanded to a multiple-G in which organic carbon is separated into multiple categories of decomposability (Westrich & Berner 1984). Decomposition of particulate organic matter may consist and transition through three stages or types of material G_{01}, G_{02}, and G_{NR} (*sensu* Westrich and Berner 1984). These three stages are characterized by a remarkably difference in decomposition rate G_{01} is the most reactive material which is easily hydrolyzed hence a rapid decomposition (Westrich & Berner 1984; Kristensen et al. 1995). G_{02} is the smallest part of the carbon pool, the decomposition rate of G_{02} is more than a factor ten less than the rate of G_{01} material (Westrich & Berner 1984). G_{NR} is the non-reactive part of the material which is defined as the material not decomposed within the scope of the experiment. G_{NR} material composed of polymeric components and metabolites some from the decomposition of G_{01} and G_{02} (Westrich & Berner 1984). In an experimental study of the multiple-G model Westrich and Berner (1984) found that G_{01}, G_{02}, and G_{NR} accounted for 50%, 16%, and 34% of the carbon, respectively. Thus, the largest compartment in detritus is the easily decomposable organic carbon, the rest is therefore decomposed at a lower rate.

Aerobic vs. anaerobic

The importance of an aerobic environment for microbial decomposition is inconclusive and heavily debated (Middelburg 1989). This inconclusiveness in research is remarkable as oxygen is the strongest oxidizing, from a thermodynamic point of view. Other electron acceptors important for oxidation in the marine environment are nitrate, nitrite, manganese oxides, iron oxides, sulfate, and the oxygen bound in organic matter, in the order of energetic yield (Middelburg 1989). Furthermore, aerobic microorganisms possesses greater potential to completely metabolize large carbon molecules completely to CO_2 and cell biomass compared to anaerobic (Kristensen et al. 1995). The reduced efficiency is assumed to be because anaerobic decomposition is dependent on a mutualistic

consortium of microorganisms. In the consortium, different microorganism is responsible for different steps in the decomposition (Figure 3). The initial steps of the anaerobic decomposition is hydrolyzation and fermentation which are responsible for the cleavage of complex organic structures to smaller low-molecular-weight organic acids (Kristensen et al. 1995). It has generally been found that decomposition in aerobic environments are faster than anaerobic decomposition (Westrich & Berner 1984; Kristensen et al. 1995). Depending on the experimental setup and organic material the aerobic decomposition is found to be 2-3 times faster than anaerobic.

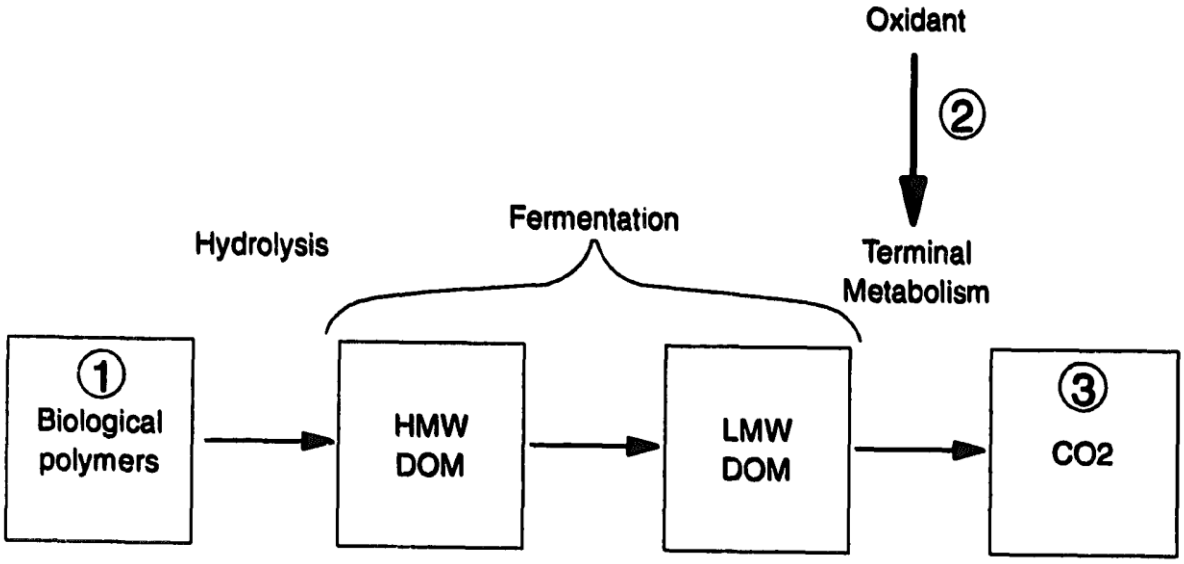

Figure 3: A general illustration of the anaerobic decomposition processes. The first arrow to the left is hydrolysis of particles or biological polymers. The second or middle arrow is fermentation of High Molecular Weight (HMW) Dissolved Organic Matter (DOM) to Low Molecular Weight DOM. The downward arrow indicates an addition of an oxidant which leads to the terminal metabolism, this results in either immobilization of nutrients due to assimilation into microbial biomass or to the mineralization of inorganic nutrients (Middelburg et al. 1993).

Kristensen et al. (1995) measured the CO_2 production of sediment "fed" with ^{14}C marked diatoms. They found the CO_2 production of the aerobic setup to be on average 8.7 times higher than the anaerobic, over a 27-day period. After the 27-day period, they switched the oxygen conditions making the aerobic setup anaerobic and vice versa. Following the switch, the CO_2 production of the pre-switch anaerobic setup increased to the level of pre-switch aerobic. The pre-switch aerobic rate decreased to pre-switch anaerobic level, the new CO_2 production rates were significantly different from the pre-switch rates. A similar experiment were performed with barley hay, in this setup there was no apparent difference in CO_2 production rates of the aerobic and anaerobic setup (Kristensen et al. 1995). In an experiment by Westrich and Berner (1984) they investigated the decomposition rate of aerobic decomposition of phytoplankton. They compared the rates of the aerobic decay rate with rates from anaerobic sediment "fed" with fresh and aged phytoplankton. An acclimatization period

of 5-10 days from addition of material to peak decomposition rate was found. The aerobic decomposition of fresh phytoplankton, measured in particulate organic carbon (POC), was approximately 3 times higher than anaerobic for the G_{01} material 24 ± 4 g POC $\cdot$ yr^{-1} and 8.8 ± 2.2 g POC $\cdot$ yr^{-1}, respectively. The difference in decay rate for the G_{02} was less pronounced and the aerobic decomposition rate were only 1.5 times higher 1.4 ± 0.7 g POC $\cdot$ yr^{-1} and 0.82 ± 0.26 g POC $\cdot$ yr^{-1}, respectively (Westrich & Berner 1984). The availability of oxygen seems to of greater importance during the initial breakdown and decay of easily decomposable G_{01} material, compared to the later stages of decomposition.

Importance of temperature

From thermodynamics, it is known that temperature is an important factor for determining the rate and favorability of a reaction. This can be seen from the Van't Hoff and Arrhenius equations, though it might not be as straight forward to apply the effects of temperature directly to reactions within microbial communities (Thamdrup & Fleischer 1998). The effect of temperature on organic matter degradation by microorganisms both as cultures and natural communities has been aim for several investigations.

Microbial communities will operate at specific minimum, optimal, and maximum temperatures. Presumably the temperature ranges of the communities will be well adapted to those of the region and ecosystem in which they occur. The three most relevant types of heterotroph microorganisms for this project are psychrophilic, psychrotolerant, and mesophilic. Psychrophilic communities are metabolic active at low temperatures often ≤0°C, they will have an optimum temperature ≤15°C and will stop functioning at ≤20°C. Psychrotolerant communities are functioning at higher temperatures. The minimum temperature may still be below 0°C but the optimal and maximum temperatures will be ≤25°C and ≤35°C, respectively. Lastly, mesophilic has an optimum range between 25 and 40°C with a maximum range of 35-45°C (Arnosti et al. 1998).

In situ temperatures are often well below the optimum temperature of the communities (Arnosti et al. 1998; Thamdrup & Fleischer 1998). The metabolic rate of increase linearly with increasing temperatures, until the optimum temperature is reached after which the activity will drastically decrease (Thamdrup & Fleischer 1998; Arnosti et al. 1998; Price & Sowers 2004). In a comparison between arctic and temperate nitrifying bacteria, Thamdrup and Fleischer (1998) found that the potential rates of nitrification were comparable, though the optimum temperature of the temperate community were 20°C higher than that of the arctic. A somewhat similar conclusion was made by

Arnosti et al (1998) who investigated sulfate reduction and hydrolysis. They found that at in situ temperatures hydrolysis were equal despite a significant difference in temperature, -1.7°C as the coldest and 15°C as the median at the warmest site. The rates of the two arctic sites increased approximately two-fold when temperature was increased from in situ temperature to optimum, a leap of 30˚C, whereas the rates of the temperate areas only increased slightly (Arnosti et al. 1998).

In another study, Weston and Joye (2005) investigated the effect of temperature on degradation of organic matter in anaerobic sediment. They correlated the experimental temperatures with seasonal temperatures. Generally, the rates of decomposition increased, until a maximum of 25˚C was reached, after which the rates were greatly reduced. They found that at temperatures between 5 and 25°C the limiting step was the terminal mineralization from DOM to dissolved inorganic carbon and CH_4 above 25°C the initial extracellular hydrolysis from POM to HMW-DOM and the hydrolysis/fermentation of HMW-DOM to LMW-DOM was the rate limiting steps (Weston & Joye 2005).

From the literature reported it seems that there might be little to no difference between the decomposition rates of microbial communities at *in situ* temperatures (Arnosti et al. 1998; Thamdrup & Fleischer 1998). Though an increase in effect and function is evident as the communities are exposed to optimum temperatures often well above in situ temperatures (Weston & Joye 2005; Thamdrup & Fleischer 1998). This suggest that the microbes of colder ecosystems has adapted to these conditions in a number of ways, which may include increased production of extracellular enzymes, increased growth efficiency, or adapting as community rather than on a organismal level (Weston & Joye 2005; Thamdrup & Fleischer 1998).

Methods

Collection of material

The material for the experiment were collected by SCUBA in the period between the 11th and the 15th of October. The material was collected in the outer parts of the Malangen fjord in Troms county, Norway. After collection, the blade and stipe were separated and placed in mesh bags. The mesh bags were then stored, suspended from a jetty to simulate *in situ* conditions. The material was retrieved the 17th of October and packed for transport to Roskilde University on the 18th of October. At RUC, it was stored overnight at 4 °C in dark to slow down degradation. Some material was also gathered to investigate the difference in biochemical content between sites and variation throughout the blade, in both cases the blade material was collected immediately and then stored at -18˚C.

Difference between sites in Malangen

Samples of blade tissue were taken for all 10 sites in October and in March. In March a substantial amount of new blade had grown in the meristematic region, samples of new and old blades were therefore taken. The newly grown blade will be called March (New) and the old blade from the previous growth season will be called March (Old). Five replicates were taken for any site and time. The values of site investigation will be correlated with field measurements of exposure, growth, and erosion, these values were obtained by KELPEX WP1 who allowed me to use the data. The wave and current exposure were measured using HOBO Pendant® G-logger, capable of measuring tilt and acceleration in three planes. The logger was fixed to a submerged buoy approximately 1.5m above the sea bed. The production was measured by punching two holes in the respectively 5 cm and 10 cm from the meristem (*sensu* Mann 1973). The erosion was measured by punching three holes respectively 10, 20 and 30 cm from the distal end. Holes were punched in 20 plants per site, the plants were tagged with cable ties so they could be collected at the following field campaign. After collection, the holes were measured and the change in distance from the meristem or distal is a measure of growth or erosion.

Differences within the blade

Five whole blades were collected from Site 5 to investigate if biochemical content or water content were related to the distance from the meristem. The change through the blade was investigated by cutting 10 cm intervals from the meristem to the tip of the blade. The blades were separated into six 10 cm intervals from Interval 0 to 5. The first interval 0-10 cm from the meristem is called Interval 0, the second from 10-20 cm is Interval 1, and so forth. The 5th interval is defined as any material 50

cm or further away from the meristem. The investigation was done at two separate occasions in October and March.

Experimental setup

The objective of the experimental setup is to investigate, the importance and effect of temperature and oxygen availability, for the decomposition of kelp detritus. The reasoning behind this is to mimic the abiotic condition of the shallow and deeper water areas, in which the kelp detritus might be transported to and degrade. The abiotic conditions of the shallow waters are expected to vary throughout the year, whereas, the conditions of the deep waters are expected to be quite stable. Two temperatures were chosen 4ºC and 10ºC, as these are expected to be representative yearly averages. The temperature of the deep waters is expected to be stable around 4 ºC, whereas the yearly mean temperature of the shallow coastal waters is expected to be around 10 ºC. The experiment was conducted in the dark to exclude photosynthetic activity as a factor of change. Seasonality is not accounted for as the experimental conditions are kept stable and therefore did not factor in the change in air temperature and irradiance of the sun. Furthermore, the treatments were either kept under oxic or anoxic conditions. Again, the deep waters are expected to have a stable low oxygen availability. The shallow waters are expected to have relatively stable aerobic conditions due to the movement of waters. From these different conditions, it is possible to make four combinations.

The lamina and stipe were separated into smaller pieces for the experiment. This was done partly to mimic the *in situ* conditions following a storm surge where pieces of plant material would be teared into smaller pieces. The separation of lamina was also partly done for practical reasons as space was a limiting factor. The bottom part of the lamina, the plate like part above the meristem, were discarded. The reasoning being that this part was expected to have a different chemical composition, due to the proximity to the meristem and the difference in morphology. As for the stipe, the top 25 cm, from the meristem towards the bottom, and the bottom 5cm were discarded to ensure uniformity of the stipe used for the experiment.

The remaining parts of the lamina were haphazardly cut into approximately equal sizes of blade. The pieces were cut in a way to avoid epiphyte covered parts. Each piece was then weighed and assigned a random number between 1 and 160, this was done by drawing numbers out of a bowl to minimize the risk for bias. The lamina part was then put in a mesh bag, and then stored in water with a salinity of 34‰, for the duration of the weighing. The pieces of lamina were stored overnight at 4ºC and

oxygenated water in the dark. 16 laminas were separated into 172 pieces. Very large or small pieces were discarded to ensure a similar weight within a factor of 2.1 from smallest to largest by weight.

The uniformed stipe segments were cut into as many 4 cm pieces as possible per stipe. The stipes were then weighed, age and diameter was not measured. The stipe pieces were then assigned a random number between 161 and 321, same procedure as the lamina. The stipe pieces were then put into a mesh bag, and stored in oxygenated water with a salinity of 34‰ at 4ºC overnight too. 16 stipes yielded a total of 175 pieces of 4 cm. Again, the largest and the smallest were discarded to ensure similarity in size by weight, the weight varied a factor 2.03 from smallest to largest. Stipe parts with an extensive epiphyte cover was discarded too.

Stipe and lamina is separated as these are expected decompose at different rates. Therefore, the full set of treatments can be considered as a matrix and can be seen in table 1. A total of 8 combinations is possible when all factors are taken into account.

Table 1: A table of the combinations of temperatures, oxygen conditions, and tissue types for the experiment. The abbreviated code for the specific combination is given in parentheses.

Stipe: 4 ºC – oxic (AE 4 S)	**Blade: 4 ºC – oxic (AE 4 B)**
Stipe: 4 ºC – anoxic (AN 4 S)	**Blade: 4 ºC – anoxic (AN 4 B)**
Stipe: 10 ºC – oxic (AE 10 S)	**Blade: 10 ºC – oxic (AE 10 B)**
Stipe: 10 ºC – anoxic (AN 10 S)	**Blade: 10 ºC – anoxic (AE 10 B)**

Stipe and blade tissue were individually put into a litterbag and tagged with a number. 10 random litterbags with either blade or stipe tissue were randomly sampled. The 10 litterbags of the same tissue type were placed in the same box for each replicate. Four replicates were made for each treatment. The 10 samples per box will act as the 10 time intervals, which will be used to describe the decomposition rate. The rate of which samples will be taken will vary dependent on the observed rate, and this rate may vary between lamina and stipe. The first sample from each unit was taken one week after initiation. Blade tissue for the seventh sampling time (day 149) of anoxic conditions were lost.

When the samples were removed from their treatment and the content of the litterbags was removed and weighed. This gives the change in fresh weight (FW) per time. After the weighing the material was frozen and stored at -80ºC until further analysis.

Practical setup

The practical setup was made by each replicate consisted of one 23 L plastic box approved for storage of foodstuff, hence there should be no leaching of interfering compounds (Figure 4). In total 32 boxes were used. Each box was filled with sediment collected near Herslev in the Isefjord, Denmark the 6th of October 2016. The sediment was stored in a 10ºC temperature controlled room until used. Each box was filled to approximately one third of capacity with sediment. The plant material was then added to the boxes, the material for the anaerobic conditions was covered with an approximately 5 cm layer of sediment. For the aerobic conditions sediment was scattered loosely onto the material to ensure bacterial colonization as the material was mainly laying on top of the sediment or suspended in the water. Water with a 34‰ salinity was added so each box had an approximately 7 cm water column.

Figure 4: A picture of the setup for the decomposition experiment. The picture is taken of the 4˚C treatment. In the picture, all 16 boxes of the 4˚C treatment can be seen. The abbreviated names for the treatment can be seen written on the box.

Two temperature controlled rooms were used for the experiment one at 4ºC and one at 10ºC. 16 boxes were placed in each room (Figure 4). 8 boxes with stipe material and 8 with laminas material. The aerobic conditions were ensured by bubbling the water column with atmospheric air. The boxes with anaerobic conditions was bubbled with N_2. Oxygen conditions in every box was measured biweekly, measurement was made with a 'Handy Polaris 2' oxygen probe by OxyGuard. Simultaneously with

the oxygen measuring, adjustments to the salinity and water column height was made. Adjustments of salinity were made to a precision of 34 ± 2‰. A temperature logger was placed in each room to monitor the temperatures and to detect possible irregularities.

Analysis procedure

Eight samples of stipe and lamina each were taken from the material also used for the experiment. This was done to determine the average starting values of the detritus. The eight samples underwent the same analytic procedures as the experimental samples.

As for the material sampled from the experiment, a subsample from each sample was taken. The size of the subsample was approximately a quarter of the sample. The remaining of the sample was then returned to the -80ºC freezer. This was done partly for practical reasons as the experimental detritus was relatively large pieces, which would not have been realistic to freeze dry and later crush. Another reason was to keep some material as backup if anything happened with the first or if other analysis were to be conducted at a later point.

Dry weight – fresh weight ratio

The subsamples were transferred into pre-weighed tinfoil pockets. These were then weighed again to get the FW of the subsample. The material was freeze dried for at least 60 hours until stable weight was achieved. Following the freeze drying the subsample and tinfoil pocket was weighed to gain the dry weight (DW). The freeze-dried material was then crushed in an agate mortar until a uniform powder like consistency. This powder was then transferred into capped polyethylene tubes and then stored at -26ºC or lower until further use.

C:H:N analysis

For the determination of Carbon, Nitrogen, and Hydrogen content between 3 mg and 4 mg of material was weighed on a Mettler Toledo XP6 with a 1-5000 mg range. The material was weighed in tin capsules and packed, the exact capsule free weight was noted. The samples were analyzed by a Carlo Erva NA-1500 CHN analyzer, the analysis was performed by specialist staff. The results from the analyzer were given as a percentage of the weighed material.

P - content

The phosphorus content was determined colorimetrically. Between 8-10 mg of crushed dried plant material was weighed into to a glass vial. The glass vial and material was then burned in a muffled oven at 550ºC for 1.5 h. After cooling, 10 ml of 0.2 M HCl was added to the vial and was incubated

in an oven at 100ºC for 1h. A blank of 10 ml 0.2 M HCl without material was started at the same time as the addition of HCL. The incubated material and HCl was left to cool to room temperature before it was transferred into 50 ml nunc centrifuge polypropylene tubes. The total volume of material and HCL was increased to 40 ml by addition of miliQ water. Duplicates of 5 ml supernatant were taken and 0.5 ml activated Reagent-R was added, mixed, and then incubated at room temperature for 15 min. The Reagent-R is made by dissolving 12g $(NH_4)Mo_7O_{24}$, 4 H_2O in 500ml miliQ, followed by addition of 140 ml H_2SO_4, after which 275 mg $K(SbO)C_4H_4O_6$, 0.5 H_2O, lastly demineralized water was added to a total volume of 1000ml. The Reagent-R is activated by dissolving 0.53g ascorbic acid in 50 ml Reagent-R. The absorption was measured at 882nm on a SpectraChrom UV-1601 spectrophotometer equipped with and automatic sipper. A standard curve was made with 1 µM P – 50 µM P from a 2.5 mM KH_2PO_4 solution. The phosphorus content in the supernatant can be calculated by a 1:1 ratio with the KH_2PO_4 concentration.

Phenolic compounds

The phenolic concentration was estimated by Folin-Ciocalteu (F-C) assay modified after the procedure of Anisworth and Gillespie (2007) and Singleton et al. 1999. Approximately 10mg of the freeze dried and crushed material was collected and added to a Pyrex vial. 2 ml 95% (vol/vol) methanol was added to each tube and whirlmixed. This was left to incubate in the dark at room temperature for 24 hours. After the 24 hours of incubation, the samples were whirlmixed again and then transferred to a centrifuge. The samples were centrifuged at 2500 g for 5 minutes to separate the liquid and the solids. After the samples were centrifuge duplicates of 250 µL supernatant was transferred to 15ml reagent tube. A blank was made of 250µL miliQ water. To each vial a 500 µL of F-C reagent was added, this was left to react for at least 3 minutes, but no more than 5 minutes *sensu* Singleton et al. 1999. This was done to ensure an adequate amount of time for the F-C reagent to react completely with all oxidable compounds. The oxidation process was stopped by addition of 2 ml of 700 mM Na_2CO_3. The addition Na_2CO_3 caused the solution to turn alkaline, which started the development of the blue color. The solution was now left for 2 hours in the dark at room temperature to fully develop the color. The samples were then centrifuged at 800g for 1 min, to isolate sediment if produced. The absorbance of the samples was now measured at 765 nm.

A standard series was made using known concentrations of Gallic acid in a range between 0.025 mM and 1.0 mM following the same procedure as with the samples. The content of polyphenolic compounds can now be determined by Gallic acid equivalent in a 1:1 ratio.

Statistical analysis

The results of the experiment and investigations were statistically analyzed using GraphPad Prism 7. For the results from the site and interval investigation were tested for significance by one-way ANOVA followed by a Tucky's post-hoc test. For the stipe investigation, the Student's t-test were used as statistical analysis.

The rates from the experiment were tried fitted to several regression models, before the best fit were chosen to represent the general trend. Two different models were found to best describe the data obtained from the experiment. The first is a linear regression:

$$Y_z(t) = S \cdot t + Y0 \quad \text{(Eq. 1)}$$

$Y_Z(t)$ is the value to the time t for the factor Z, S is the slope of the line or the rate of decay, t is the time, and Y0 is the intercept with the Y-axis. The second model is a first-order decay model:

$$Y_Z(t) = Y0 \cdot e^{(-k \cdot t)} + Ressidual \quad \text{(Eq. 2)}$$

Here again the $Y_Z(t)$ is the value of factor Z at the time t, Residual is the steady-state value for Y hence the value at which the Y(t)-Y(t-1) = 0, k is the rate constant, ant t is the time.

Results

Differences between sites

The importance of turbulence on community structure and production of detritus were one of the aims of the WP1 of KELPEX, which were performed in Malangen. 10 different sites (Figure 2) were chosen for their difference in expected wave exposure. The general consensus is that a more turbulent or exposed environment leads to a greater biomass and detritus production (Krumhansl & Scheibling 2012; Sjøtun 1993; Pedersen et al. 2012). Higher exposure is furthermore expected to result in greater density in individual and biomass per area. The variation of biochemical content between seasons is well-documented (Schiener et al. 2014). As the effects of turbulence on biomass and seasonal change in biochemical content is well studied and gave rise to the assumption that turbulence might as well affect the biochemical content of the blade. No apparent studies of the importance of turbulence on the biochemical content has been performed.

There is no clear tendency between erosion nor growth when correlated with the measured turbulence (Figure 5). As detritus production were almost entirely the release of the old blade. The production of detritus was high in the periods between October and March and March and May, which is also the periods of active growth of the new blade. The average standing biomass did not vary statistically between sites neither for blade biomass nor stipe biomass, with an average biomass of 7.15 ± 1.23 kg FW m^{-2} for stipe and 8.04 ± 1.05 kg FW m^{-2} for blade.

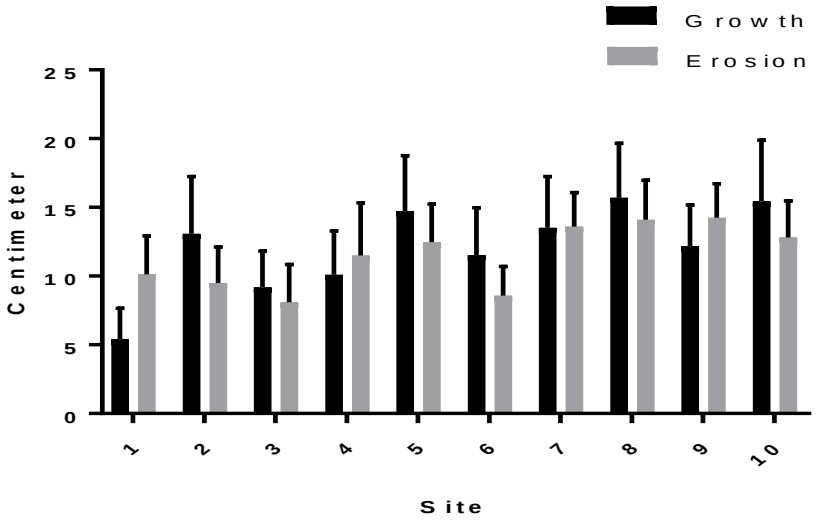

Figure 5: The annual average of growth and erosion. The average was calculated from growth and erosion estimates of four periods: August 2016 to October 2016, October 2016 to March 2017, March 2017 to May 2017, and May 2017 to October 2017. The low growth of site one is a result of lost growth measurement from March 2017 to May 2017, a period with and average growth of 36 ± 2 cm standard error. No significant differences were found by two One-way ANOVA with Tucky's post hoc test, if Site 1 is excluded. All values are the mean with a 95% Confidence Interval.

The tissue used to investigate the difference between sites were separated into three groups, October, March (New), and March (Old). When sites were compared within the same group, only few comparisons were significantly different. Of the 135 comparisons of DW/FW ratio (Figure 6A) only nine were significant. All significant values were within the October group where the tissue of Site 1, 6 and 8 had a significantly higher water content than Site 3, 4, and 10. This confirms the visual difference portrayed in Figure 6A if not accounting for the relatively large 95% confidence interval.

For the Carbon (C) content, only two significant differences were found when comparing sites within the same time. Within the blade tissue from March (Old) Site 6 was found to have a higher C content compared to that of Site 3 and 5 (Figure 6B). Comparisons of the Nitrogen (N) content between sites gave 13 statistically significant results, with seven of them originating from the October samples and six comparisons of the March (New) tissue. Of the October samples six out of seven showed that, Site 1 had a higher concentration of N than Site 4, 5, 6, 7, 9, and 10. The seventh significant difference in the October group was that Site 2 had a higher concentration than Site 10. The tissue from Site 2 had in general a lower N concentration, as it was accountable for all the significant values in March (New) (Figure 6C). The results of the statistical analysis of the Phosphorus (P) concentration yielded 27 significant results, with two originating from October, 15 within the group of March (New), and the last 10 results originated from March (Old) (Figure 6D).

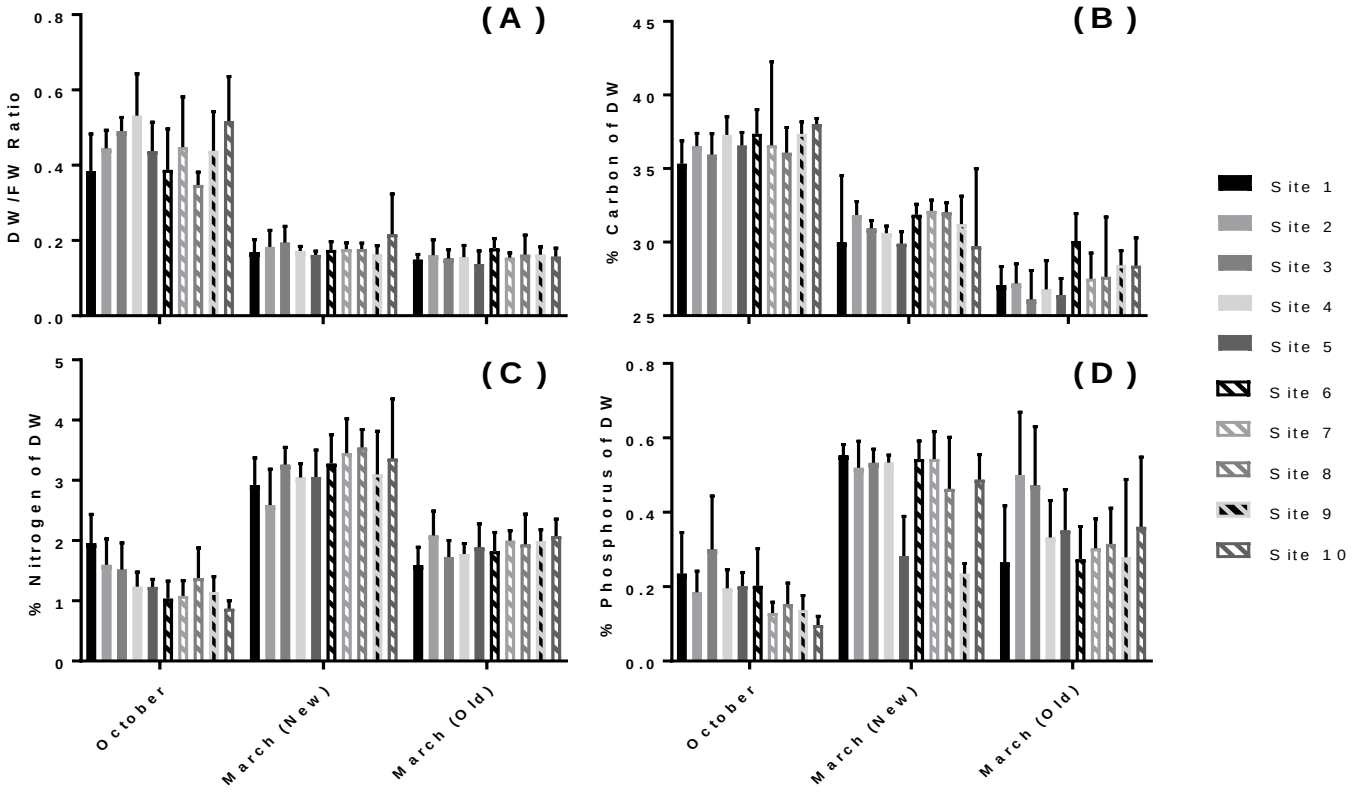

Figure 6: Bar charts of the physical and chemical concentrations of the blade tissue from different sites. In figure (A) the dry/fresh weight (DW/FW) ratio. For the chemical properties, the percentage of the chemical elements Carbon (B), Nitrogen (C), and Phosphorus (D). The March (New) is the results for the newly grown blade and March (Old) is the blade from the previous growth season. The values shown are the mean and the error bars are given as the 95% confidence interval.

There were no discernible patterns when comparing all 10 sites within groups. The blade tissue from Site 5 and 9 in March (New) did in general have a lower P concentration. The P concentrations were for March (Old) was found to be generally higher at Site 2 and 3. There were in general not an evident trend of any site being different to another site in regards to tissue concentration of water or the chemical elements, which were investigated (Figure 6D).

However, when comparing the content of water and chemical elements for each site across groups, almost all were found to be significantly different. The DW/FW ratio for all sites in October were significantly different when compared to both tissue types in March. There was no difference between the groups of March (Old) and March (New), 0.18±0.02 and 0.16±0.01 respectively. The blade tissue of October contained at least twice the amount of dry matter compared to both March tissue groups, with an average DW/FW-ratio of 0.44±0.06. As mentioned earlier, the C content does not differ greatly between sites, but when compared between groups, almost all differ significantly. The tissue from October does always have a higher concentration of C, than any other tissue. The tissue

originating from the new blade in March are also, in general, denser in C compared to the old blade tissue, the only exception is for Site 6 and 10, which is not statistically different.

The tissue samples from October does in general seem to be more depleted in both N and P, when compared to both March groups. The N concentration of the October tissue were in general least rich in N, with an average of 1.31±0.39% N of DW across all sites. The March (Old) tissue were closer related to the October tissue as the N concentration of March (Old) were 1.89±0.28% N of DW. The March (New) tissue were almost twice as rich in N compared to October and March (Old), with an average concentration of 3.32±0.86% N of DW. The New and Old blade tissue from March were in general enriched in P compared to that of October. No real pattern emerges from the comparison between the Old and New tissue from March, though the New tissue does overall contain more P than the Old 0.48±0.12% of DW and 0.34±0.13% of DW respectively. The October tissue were substantially lower than both March groups, with an average of 0.18±0.08% of DW.

These results suggest that the exposure is not relevant for the concentration of water or the chemical elements. The difference between sites is overshadowed by difference in time of sampling and if the tissue is actively growing, that is the new blade, or from and earlier growth season, which is the old blade.

The change in biochemical and water content through the blade

For the decomposition experiment, 16 blades were separated into 160 pieces of relative same sizes. This implies that the pieces used in the experiment originates from different parts of the blade. The age and hence the distance from the meristem may result in a difference in the DW/FW relationship, the composition of the elements C, N, and P, or concentration or presence of certain molecules. It is therefore important to investigate if there is any significant changes within the blade in order to verify the use of haphazardly cutting the blade for the decomposition experiment.

Most importantly for the decomposition experiment is the values for October, as this is the period where the material for the decomposition experiment were sampled. The visual representation of the data in figure 7A through D reveals only small changes throughout the blade. There is no clear trend, nor statistical significant difference within the October samples. Though there might seem to be a trend of declining phosphorus levels, as seen in Figure 7D, moving from the meristem to the distal end of the lamina. No significance was found when Interval 0 was tested against Interval 5 ($p=0.25$), this is highlighted as these were visually most different 0.24±0.08% and 0.13±0.02% respectively.

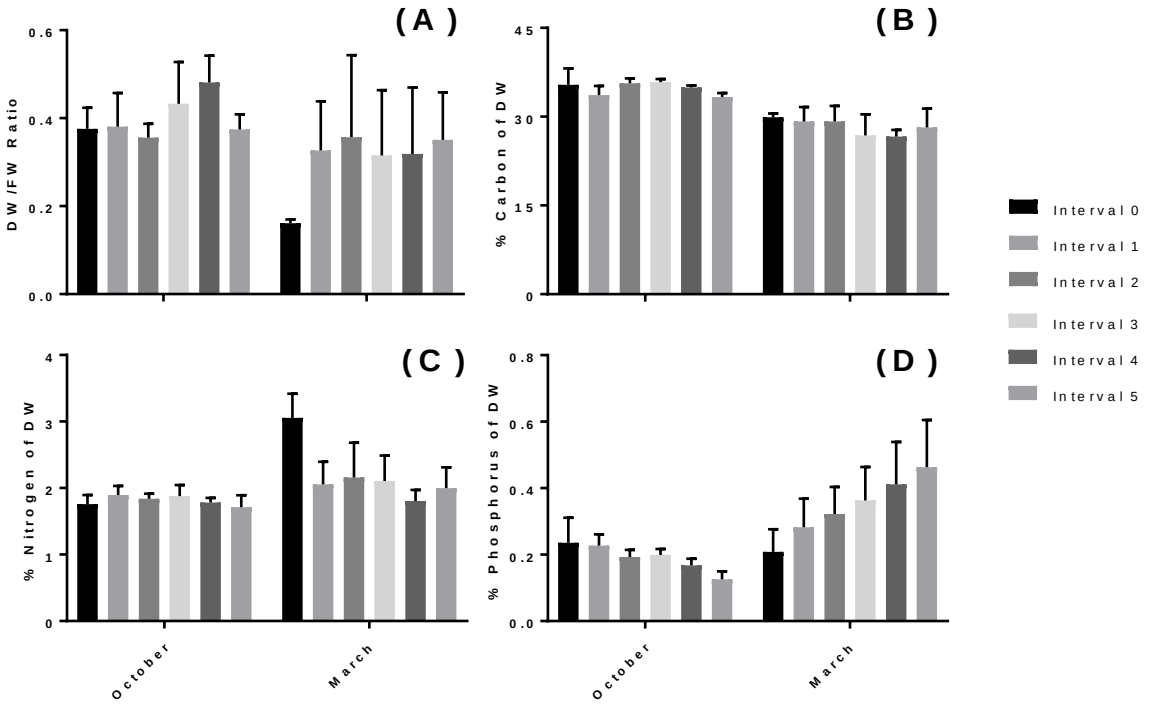

Figure 7: Results from the analysis of the in biochemical change in blade tissue sampled in 10 cm intervals from the meristem. All four figures contain values from blade tissue sampled in October and March. Figure (a) is of the DW/FW ratio, (b) is of the % carbon of DW, (c) is the amount of nitrogen in percent of DW, and (d) is the amount of phosphorus in percent of DW. All values are given as the mean with 95% Confidence Interval error bars.

For the blades cut in March, some values of the Interval vs Interval comparison were significantly different, but no clear pattern emerges from those significant values. The 0th interval, which is the new, and actively growing, part of the blade. The new blade seems to have a higher water content compared to the other intervals, thought there is only significant difference between Interval 0 and Interval 2. This may be a result the large variations within Interval groups, as the dry matter content of Interval 0 is in general half compared to the rest of the blade 16±0.01% and 33±13%, respectively. Carbon concentration does not differ between intervals ($p \geq 0.18$). Only the 0th Interval differed more than once in N and P concentration, when compared to other Intervals. As it can be seen in Figure 7C the 0th Interval has a higher N content than any other Interval, which in all cases is significantly higher ($p \leq 0.0001$). Figure 7D indicates that the P concentration increases with distance to the meristematic region, this is supported by the fact that the 5th Interval is significantly higher than Interval 0 and 1 ($p=0.0002$ and 0.0082). Interval 0 is furthermore significantly less P rich than Interval 4 ($p=0.0045$).

If any interval had been consistently different from the rest, this should have been discarded or only that segment should have been used. Due to the insignificant variations throughout the blade, especially in October, justifies the use of haphazardly cut blade in the experiment. If material were sampled in March, then blade tissue should have been separated into new and old as the biochemical

content varied depending on the origin of the tissue. This observation is supported by the findings in the site investigations.

Difference between peripheral and core tissue from stipes

The peripheral tissue, will further on be referred to as peel, were manually removed from the core of the stipe with a peeler. The peel and core was then freeze-dried and crushed, for analysis. In the Figures 8A through F, it is evident that peel and core differ in their biochemical composition. These differences were statistically tested with a Student's t-test. Which confirmed that peel and core in all cases were significantly different.

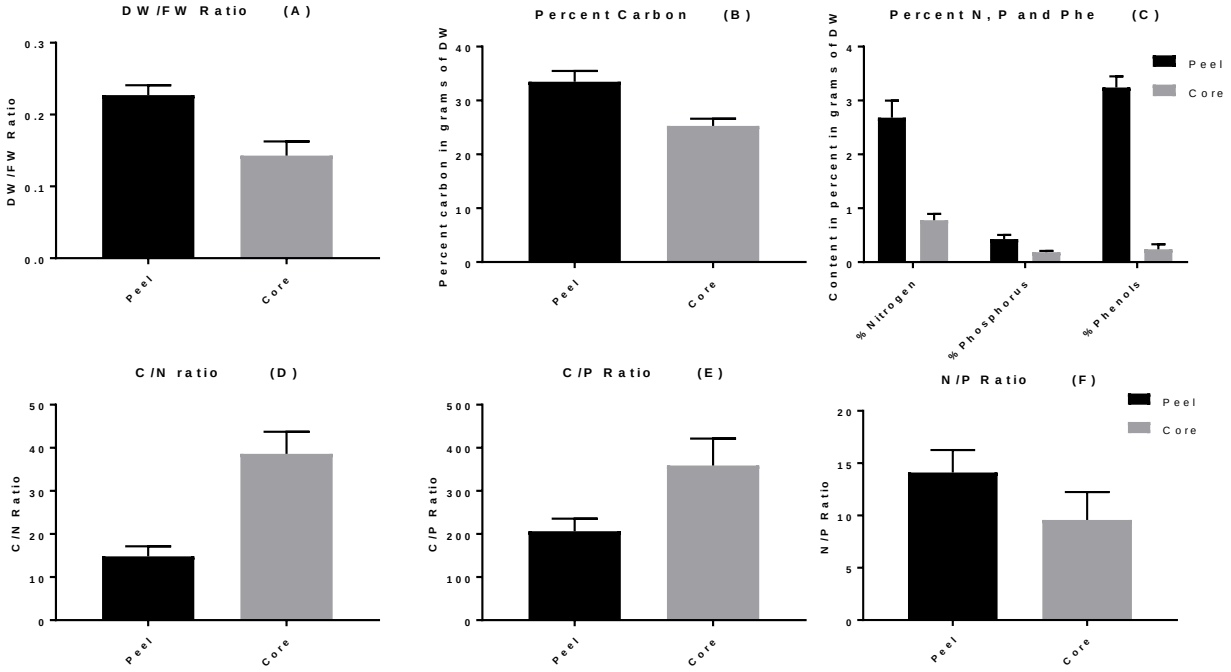

Figure 8: Results from the investigation in difference between the pheriphal tissue (Peel) and the core of the stipe. In Figura A shows the Dry weight (DW) and Fresh weight (FW) ratio. Figure B contains the values of the Carbon (C) content, Figure C contains the concentration of Nitrogen (N), Phosphorus (P), and phenolic compounds, all are given in percent of DW. Figure D, E, and F displays the C/N, C/P, and N/P atomic ratios in that order. All values are the unadjusted mean with an 95% Confidence Interval error bar.

The dry matter content of both the peel and core is 23±1% and 14±2% respectively (Figure 8A). The peel was found to contain significantly more dry matter ($p<0.0001$). The peel was significantly more dense in C, N, and P than the core, the p-value were in all cases below 0.0001 (Figure 8B and C). The C concentration of peel were almost 10 percentage point higher than the core, 33.5 ± 0.8% for peel and 25.3 ± 0.5% for the core. The N concentration were more than thrice as high for peel than for core respectively 2.7 ± 0.1% and 0.8 ± 0.05%. The peel were more than twice as rich in P (0.43 ± 0.03%) than the core (0.19 ± 0.01%). The higher concentration of C, N, and P in the peel led to the C/N and C/P ratios of the peel to be 2.6 and 1.7 times higher compared to the core (Figure 8D and

E). The N/P ratio where higher of the peel is significantly higher than N/P ratio of the core (p=0.007). The N/P ratio of the peel (14.1 ± 0.9) is approximately 50% higher than the value of the core (9.6 ± 1.1) (Figure 8F). The higher N/P ratio of peel is misleading as nitrogen concentration and phosphorus are both significantly higher than the core. In this case the total amount of chemical element may be a better indicator for food quality than the atomic proportion, in which they are found. The concentration of phenolic compounds is a staggering 13.5 times higher in the peripheral tissue compared to the core (Figure 8C). This supports the idea of the peripheral tissue as a protective layer.

Decomposition of *Laminaria hyperborea*

It was hypothesized that the blade would decompose at a faster rate compared to the stipe. The assumption was, that the blade is the photosynthetic part of the thalli and therefor would contain more easily decomposable molecules. The stipe was expected to have a high C:N:P ratio due to the morphology and structural function, hence being a worse food source than blade.

Change in biomass

Decomposition of stipe and blade material has been found described best by a first-order decay model. The initial decomposition rate at 4 ˚C are very similar, with the anaerobic depicted as slightly faster until day 49 (Figure 9A), though the rate constant (k_{DW}) is roughly three times higher for anaerobic decomposition than aerobic (Table 2). The higher decay rate for the anaerobically decaying blade material is due to the large difference in residual material. From the regression, it is estimated that there will be almost twice as much remaining biomass under anoxic conditions compared to oxic (table 2). The residual material is not well estimated as the aerobically decaying biomass as the measured values of Figure 9A is well below 16%. The estimate of residual material for the anaerobically decaying material seems to a better estimate.

Table 2: Table of the best-fit values from the one-phase regressions of remaining biomass in dry matter. Y0 is the intercept with the Y-axis, k_{DW} is the decay rate, Res is the residual or non-reactive biomass, and $t_{0.5}$ is the half time. AE denotes aerobic conditions, AN is anaerobic, 4 and 10 is 4 ℃ and 10 ℃ respectively, B is for blade, and S is for stipe. Values are given with ± 1 Standard error.

	Y0 (%)	k_{DW} (days^{-1})	Residual material (%)	R square
AE 4 B	89.32 ± 5.8	0.0453 ± 0.0103	16 ± 2.58	0.793
AN 4 B	97.62 ± 5.2	0.124 ± 0.0258	27.31 ± 1.97	0.821
AE 10 B	99.48 ± 5.3	0.191 ± 0.0349	11.99 ± 1.80	0.859
AN 10 B	99.21 ± 4.8	0.1423 ± 0.0261	24.73 ± 1.78	0.857
AE 4 S	93.4 ± 3.5	0.0079 ± 0.0018	10.22 ± 7.37	0.876
AN 4 S	92.21 ± 4.9	0.021 ± 0.0063	43.2 ± 3.23	0.671
AE 10 S	93.09 ± 5.8	0.0111 ± 0.0032	14.34 ± 7.15	0.745
AN 10 S	95.49 ± 5.0	0.0307 ± 0.0078	37.54 ± 2.70	0.752

The material decomposed at 10 ˚C are decomposed faster under aerobic conditions than under anaerobic conditions (Figure 9B). The residual material estimated from the regression does again fit badly for the aerobic decay, whereas it seems to be fitting for anaerobic decay. The decay rates were between 0.0453±0,0103 d^{-1} at aerobic 4 ˚C conditions and 0,191±0,0349 d^{-1} at aerobic conditions and 10 ˚C. The decay under aerobic conditions were in general more complete than under anaerobic conditions, which is evident from the amount of residual material (Table 2). The estimated residual material from the regression were between 12% and 27% depending on the treatment (Table 2). The largest amount of residual biomass was found under anaerobic conditions at 4 ˚C, and the lowest was found at aerobic conditions at 10 ˚C. The rate of which the tissue decays differ greatly depending on the origin.

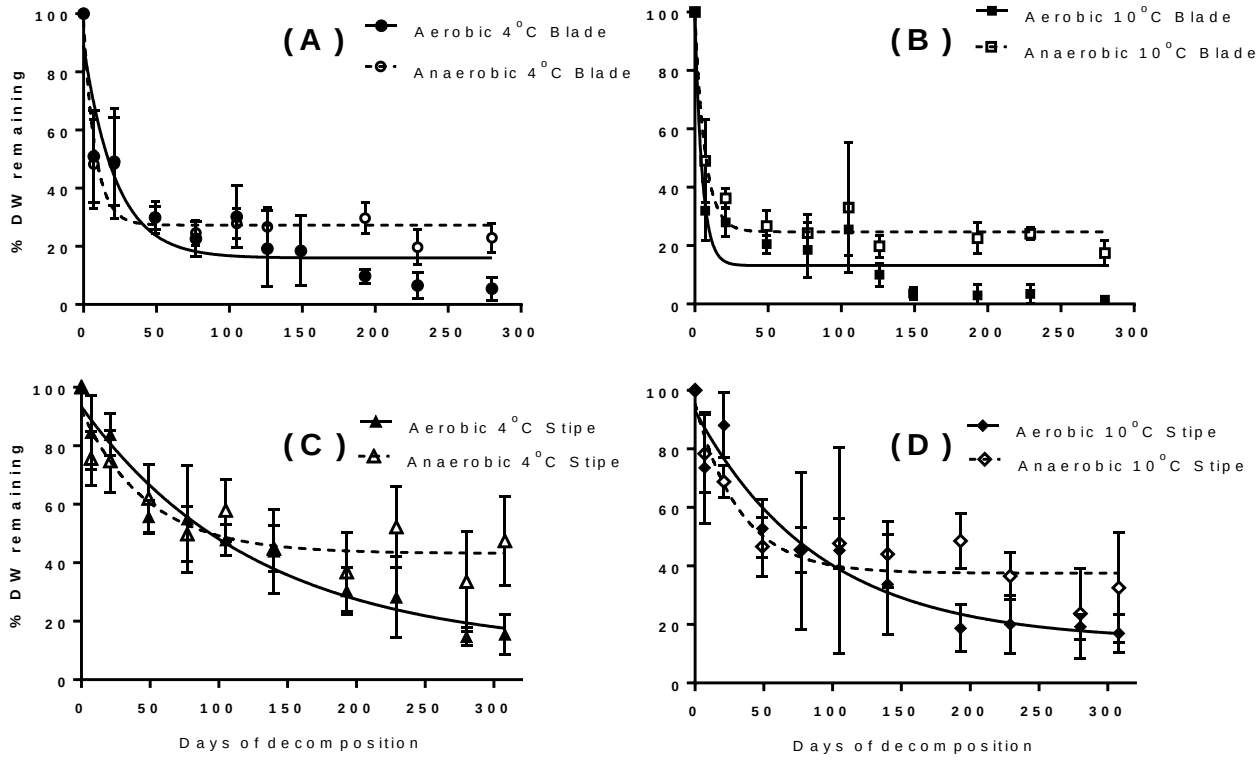

Figure 9: A figure of the results from the decomposition of blade tissue and stipe in percent remaining dry weight (DW) biomass. Figure A displays the results of the aerobic and anaerobic decayed blade at 4˚C and Figure B displays those decomposed at 10˚C under aerobic and anaerobic conditions. Figure C and D shows the results of stipe decay. With Figure C containing values of aerobically and anaerobically decomposed stipe tissue at 4˚C and Figure D showing that of aerobic and anaerobic decay at 10˚C. In all cases the a one-phase decay model were fitted to the data. All values are shown as the mean with an 95% Confidence Interval.

At both 4˚C and 10˚C were the decay rates faster under anoxic conditions, this can also be seen in Figure 9C and D. The residual material were though notably lower for aerobic decay. Temperature seemed to have a positive effect on the rate and amount of decay, though not statistical significant. The regression model seems to fit better on the decay of stipe tissue, as the residual material is visually

similar to the measured results. The blade tissue were decaying faster and more completely than the stipe tissue. The decay rate differed by a factor of five or more. The estimated residual biomass did not vary greatly for aerobic decay, though a visual difference is evident in Figure 9.

The residual biomass values from the regressions of aerobically decomposed blade and stipe tissue are comparable despite the difference in temperature. When comparing the latter two measured biomasses, the blade seems more fully decomposed, and in several cases litterbags were sampled with no biomass left, which were not the case for stipe tissue. A linear regression of the decay after the rapid initial decay were performed using the values from day 49 and forth. Linear regressions of the values following the initial rapid decay are shown in Figure 10.

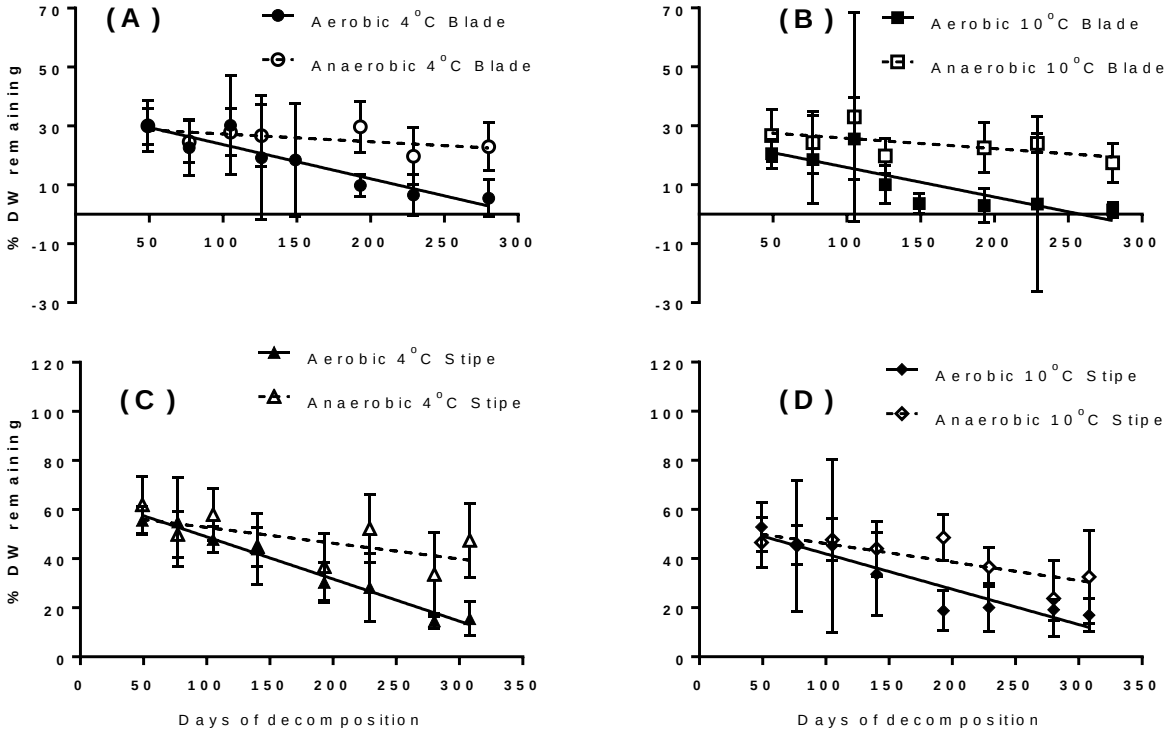

Figure 10: A figure of the decomposition, after day 49, of blade tissue and stipe in percent remaining dry weight (DW) biomass. Figure A displays the results of the aerobic and anaerobic decayed blade at 4˚C and Figure B displays those decomposed at 10˚C under aerobic and anaerobic conditions. Figure C and D shows the results of stipe decay. With Figure C containing values of aerobically and anaerobically decomposed stipe tissue at 4˚C and Figure D showing that of aerobic and anaerobic decay at 10˚C. A linear regression are fitted to the data. All values are shown as the mean with an 95% Confidence Interval.

The "starting" values for blade are roughly the same with 4˚C anaerobic the highest remaining biomass of 30% and 10˚C aerobic the lowest with 20%. The "start" remaining biomass for the stipes are 61% as the highest from anaerobic 4˚C and 46% as the lowest from anaerobic 10˚C treatment. When comparing the slope of the aerobic and anaerobic decay it is evident that they are visually

different in all cases. This is also evident from the results of the regression analysis, as seen in Table 3. The slope of the line (S_{DW}) are on generally notably steeper for aerobic decay compared to anaerobic, the difference is a factor two or more. This is also evident from the x-intercept which is the amount of days required to decompose all material, under the assumption that there is no pool of residual material. The decay rate does also vary between temperatures, though not in a matter which is comparable with oxygen regime.

Table 3: Values of the linear regression of the decay after day 49. S_{DW} is the decay rate in % dry weight biomass per day. x-intercept is the intercept with the x-axis or the amount of days needed for complete decay of biomass. AE denotes aerobic conditions, AN is anaerobic, 4 and 10 is 4 ℃ and 10 ℃ respectively, B is for blade, and S is for stipe. Values are given with ± 1 Standard error.

	S_{DW} ($days^{-1}$)	X-intercept (days)	R^2
AE 4 B	-0,116 ± 0,018	304	0,868
AN 4 B	-0,0264 ± 0,016	1132	0,356
AE 10 B	-0,100 ± 0,027	258	0,691
AN 10 B	-0,0351 ± 0,022	834	0,348
AE 4 S	-0,172 ± 0,01	384	0,980
AN 4 S	-0,0643 ± 0,032	921	0,398
AE 10 S	-0,144 ± 0,022	391	0,881
AN 10 S	-0,075 ± 0,022	713	0,651

Stipe tissue does in all cases have a higher rate of decay when compared with blade decay of the same treatment. The difference is not as evident when comparing the x-intercept as less blade tissue is remaining after the initial decay. The difference in decay rate for stipe seems to be less temperature and oxygen dependent, as difference between treatment are smaller.

The initial rapid loss of biomass may be due to a rapid decomposition of easily decomposable molecules rich in energy and C, N, and P. It may also be due to leaching of molecules to DOM. It is generally assumed that C is decomposed at the same rate as biomass, as it constituent of biomass. If biomass and C degrade at the same rate no change in C concentration should be evident.

Change in C, N, P content during decomposition

Carbon

From the visual representation in figure 11A and B, an initial enrichment of C can be seen between day 0 to day 7, followed by a decline to values not different to the initial values. Only in three of 40 cases values were significantly lower, all cases are found in at 105, 229, and 280 days for the aerobic and 10 ˚C treatment. The C concentration of the tissue fluctuate greatly under aerobic conditions, especially at 10 ˚C. The C content of the blade tissue varies greatly between samples of the same time

and conditions, this made the 95% CI very broad leading to difficulty in noticing any possible pattern. Despite the large variations, a general trend of mineralization is evident, after the initial increase.

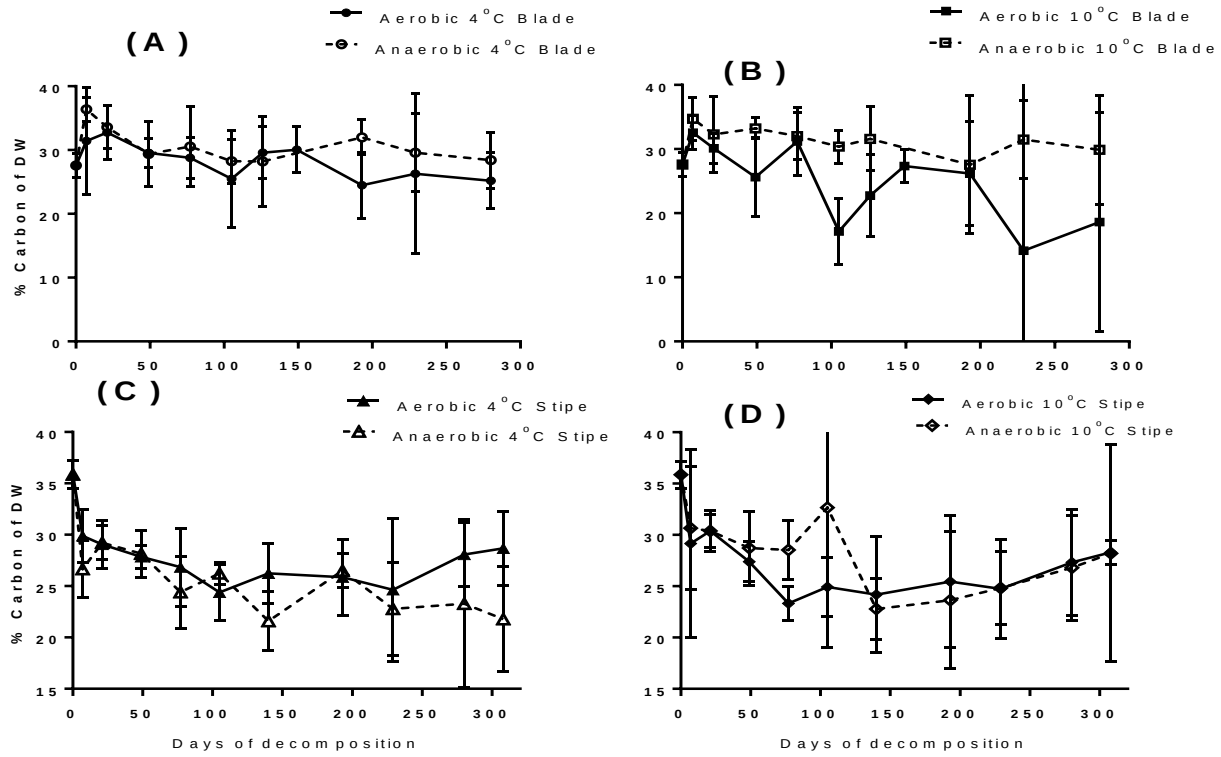

Figure 11: Four figures of the change in Carbon (C) concentration during decomposition of blade and stipe tissue. Figures A and B are the change in C content of the blade both with linear regressions fitted to the values. Figure A is for aerobic and anaerobic decay at 4˚C. Figure B is for anaerobically and anaerobically decayed biomass at 10˚C. Figures C and D show the change for stipe tissue, in all cases the one-phase decay model is used to describe the changes. Figure C contains the values of aerobic and anaerobic decayed stipe tissue at 4˚C. Figure D contains the values for stipe decomposed at 10˚C, both aerobically and anaerobically. All values are given as the mean with 95% Confidence Interval error bars.

Common for both 4˚C and 10˚C is an initial loss of carbon after which they seem to change by a temperature dependent pattern (Figure 11C and D). Stipe material at 4˚C seems to stabilize at concentration similar to those after the initial loss or slightly decrease. Whereas at 10˚C a pattern of carbon mineralization reduces the C concentration until day 150 where the C concentration is roughly 23% after which C is immobilized reaching a C concentration of 28% at day 308. The final C content is between 28.7% and 28.2% for all treatment except anaerobic decay at 4˚C which has a significantly lower C content at 21.8%.

The starting values of stipe and blade tissue differs by roughly 10 percentage points, with 27.6 ± 4.1% and 35.9 ± 2.4 % for blade and stipe respectively. During the initial decay of biomass leads to different changes in C concentrations. Blade tissue is initially enriched after which the C concentration declines, whereas the C of the stipe is initially remineralized. The final C concentration measured for

aerobic 10 ˚C blade was 19 ± 37% confidence interval, if this was excluded then the final values did not differ greatly between tissue types, the ranges were 25%-30% for blade and 22%-29% for stipe.

As biomass and hence C is decomposed, a colonization of microbial decomposers is expected. These microbial decomposers do in general have a higher concentration of N and P compared to primary production, as they have a higher concentration of proteins, amino acids, and other nutrient rich molecules. Hence a colonization of bacteria and protozoans would lead to an increase in N and P.

Phosphorous

Throughout 280 days of decomposition the tissue concentration of P is roughly halved. From figure 12A and B, an initial and rapid decrease in blade tissue P concentration can be seen within the first seven days. After the initial decrease in P the concentration increases slightly, between day seven and 49, to reach a stable or slight decreasing concentration during the remaining biomatter decay. This is confirmed by an ANOVA followed by a Tucky's test which finds that the starting value is significantly ($p<0.05$) higher than the concentrations measured at other times in 37 of 40 comparisons. There were no significant differences when comparing between treatments at the same time.

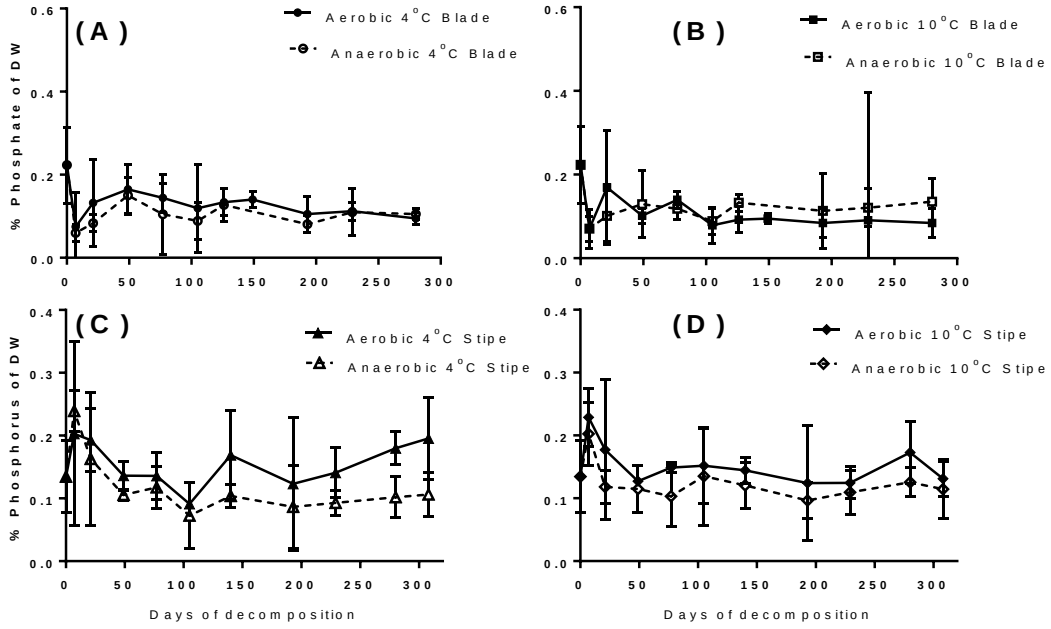

Figure 12: The change in phosphorus concentration over time. Figure A and D depicts the change in P concentration for blade tissue over a 280 days decomposition period described with a linear regression. Figure C and D shows the change in P content for stipe tissue over a 308 days period. All values are given as the mean with 95% Confidence Interval error bars.

Instead of an initial decrease in P concentration as in the blade, the stipe showed an increase in P within the first 49 days (Figure 12C and D). The stipes were enriched until day 49, after which the P content evened out at concentrations not different from the initial values ($p \geq 0.0922$). After 49 days, the aerobically decomposed stipes had P concentrations which on average were significantly higher than the stipes exposed to anaerobic conditions ($p \leq 0.006$). Tissues of equal oxygen regime had tissue P concentrations which did not differ significantly despite difference in temperature, $p=0.91$ and $p=0.14$ for blade and stipe respectively.

The initial loss of P in blade tissue is somewhat equal to the loss of carbon which might indicate that the easily decomposable material is rich in phosphorus. It may indicate that the microorganisms are either C or N limited as P is released and not assimilated into biomass.

Nitrogen

Nitrogen was the only chemical element found to consistently increase throughout the decomposition experiment. In all treatments, the N concentration of the blade tissue increased by more than a factor of two (Figure 11A and B). The pattern of change were very similar between treatments and tissue types except for blade at 10˚C which varied a lot between times. The rest followed a trend of a slow increase during the initial 105 days by which the N concentration had roughly doubled. After day 105 a rapid increase took place, especially notably for 4˚C anaerobically decaying blade, and all stipe treatments. All stipe tissue almost doubled in N content again between day 105 and 140 and stabilized. The blade tissue at 4˚C did also increase drastically, under anoxic conditions it doubled in content before day 193, whereas the aerobically decaying only increased by half. The blade tissue N content for the 4˚C treatments stabilized after 193 days of decomposition. Whereas the N content of stipe tissue were stable between day 140 and 280, but declined between day 280 and 308. The loss of N were especially pronounced for the both 10˚C treatments and the anoxic 4˚C treatment with a loss of at least 25% of the N content, the latter losing 50%. The blade decomposed at under oxic conditions and 10˚C did not change in any discernable pattern except for a slight increase in N content. The N content varied little between tissue at the same time, but varied greatly between times.

The initial N content were approximately 0.1% higher in blade tissue than stipe tissue 1.3% and 1.2% respectively. The average final measured N concentrations across all treatments were $3.5 \pm 0.7\%$ for blade and $2.9 \pm 0.7\%$ for stipe. With the anaerobically decomposed blade tissue being significantly ($p \leq 0.025$) richer than the aerobically decomposed tissue. The opposite was true for stipe where

aerobically decomposed tissue had a significantly ($p \leq 0.002$) higher N content than anaerobically decomposed tissue.

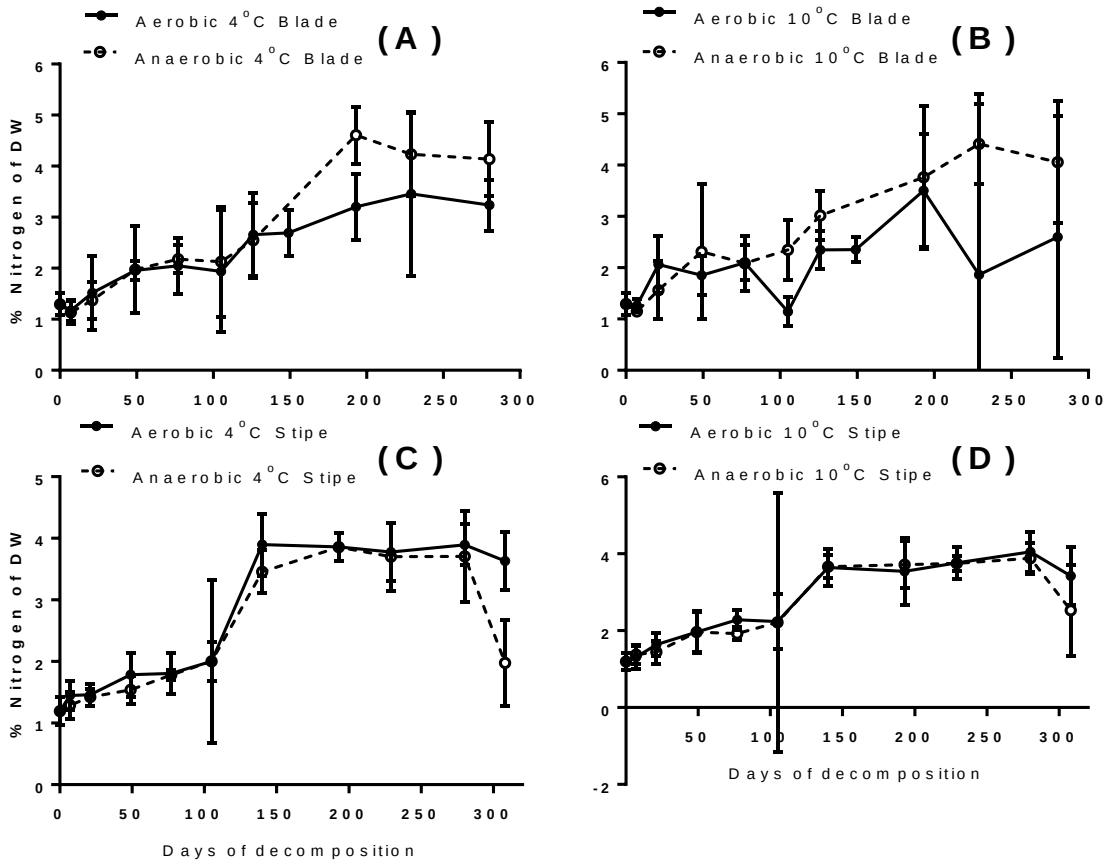

Figure 13: The change in nitrogen concentration over time. Figure A and D depicts the change in P concentration for blade tissue over a 280 days decomposition period described with a linear regression. Figure C and D shows the change in P content for stipe tissue over a 308 days period. All values are given as the mean with 95% Confidence Interval error bars.

This may indicate that a micro biotic colonization of especially the blade under anaerobic conditions were assimilating nutrients from the surroundings. It might also be because large amounts of N were bound in resilient molecules, which could not be decomposed under anaerobic conditions. These molecules would then only be present in blades as stipe tissue decomposed under anaerobic conditions in general had the lowest N content.

Phenol

The loss of phenolic compounds from blade tissue were rapid during the first 21 days (Figure 14A and B). The concentration of phenolic components in the blade tissue exposed to 10 ˚C decay was

reduced from an average starting value of 1.251 ± 0.245% to between 0.038 ± 0.035 for aerobic decay and 0.101 ± 0.056 for anaerobic decay within the first 7 days. The tissue exposed to 4 ˚C had a slower loss of phenolic compounds, as they reached equal results after 21 days, 0.042 ± 0.034% for aerobic and 0.086 ± 0.112% for anaerobic conditions.

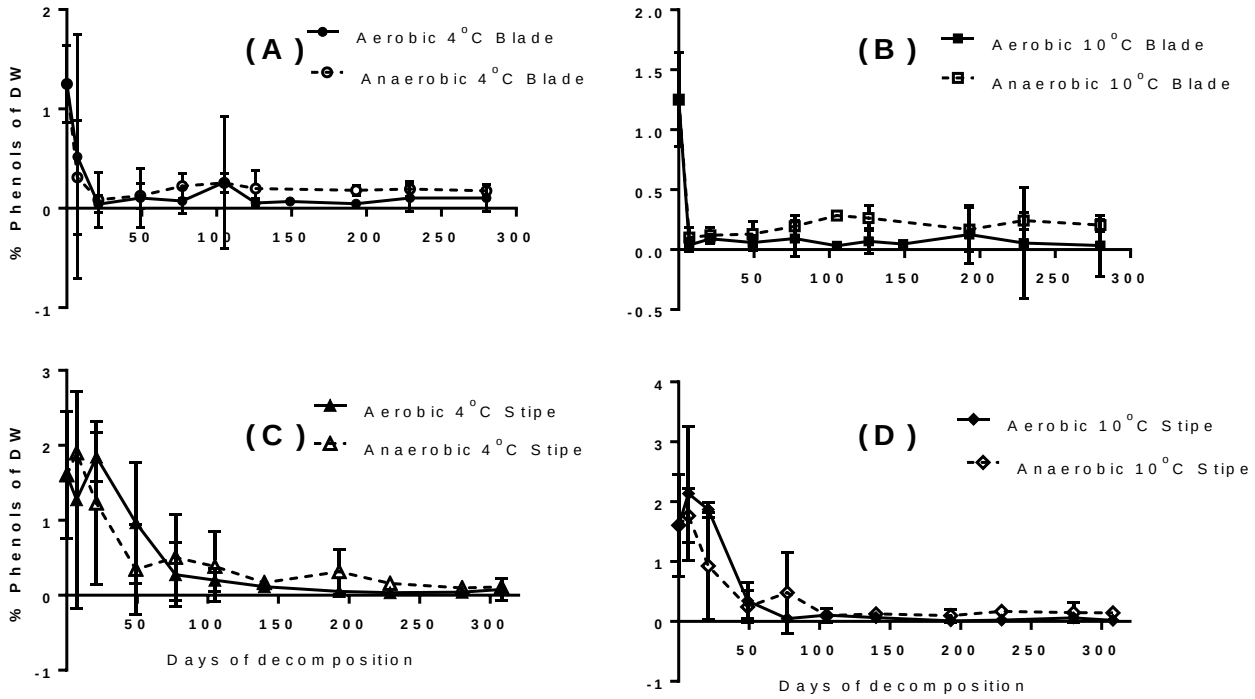

Figure 14: The change in phenolic concentration over time. Figure A and D depicts the change in P concentration for blade tissue over a 280 days decomposition period described with a linear regression. Figure C and D shows the change in P content for stipe tissue over a 308 days period. All values are given as the mean with 95% Confidence Interval error bars.

The initial loss of phenolic compounds was slower for the stipe tissue than for the blade. A slight increase between day 0 and 21 can be seen in figure 14C and D. In all cases the tissue reached a steady concentration of phenolic compounds after 49 days at 10˚C and 105 days at 4˚C. After which the changes in phenolics were not detectable.

The stipe did in general have a higher starting concentration of phenolic components compared to the blade. This tendency was not evident after the 280 and 308 days of decomposition respectively, as the tissue concentration of phenolic compounds were below 0.2%. However, a tendency of a more efficient breakdown of phenolic compounds under aerobic conditions may be emerging. The final concentration of phenolic compounds were generally twice as for anaerobically decomposed tissue

compared to aerobically decomposed tissue. There temperature does not affect the final concentration to the same degree.

The loss of phenolic compounds is almost instantaneous for blade and more gradual for stipes. It was expected that that phenolic compounds would build up during decomposition as they are regarded as non-reactive. The levels found in this experiment may be too low to retard decomposition, hence the phenolic compounds present in the blade does not act as defense against microbial attacks.

Change in atomic ratios

Blade

In Figure 15 the change in atomic ratios for blade tissue during decay calculated as the C:N, C:P and N:P-ratioes. The top three figures, Figure 15A, B and C, are for aerobic and anaerobic decomposition at 4˚C, whereas the bottom three figures, Figures 14D, E, and F, are for decomposition at 10˚C. Figure 14A and D are both displaying the change in C:N ratio during the 280 days of decay. Figure 14B and E contains the C:P ratios for the blade tissue. In Figure 15C and F the calculated N:P ratios are shown. In all figures the mean values and the 95% CI are shown for each treatment and time.

The change in C:N-ratio at both 4˚C and 10˚C seems to follow the same pattern and change at similar rates (Figure 14A and D). The initial blade C:N-ratio was 25.3 ± 1.1, this increased to 33.8 ± 1.8 within the first seven days. After the first seven days the C:N-ratio gradually decreased to an average across treatments at 14.3 ± 0.9 which is significantly lower than the starting value.

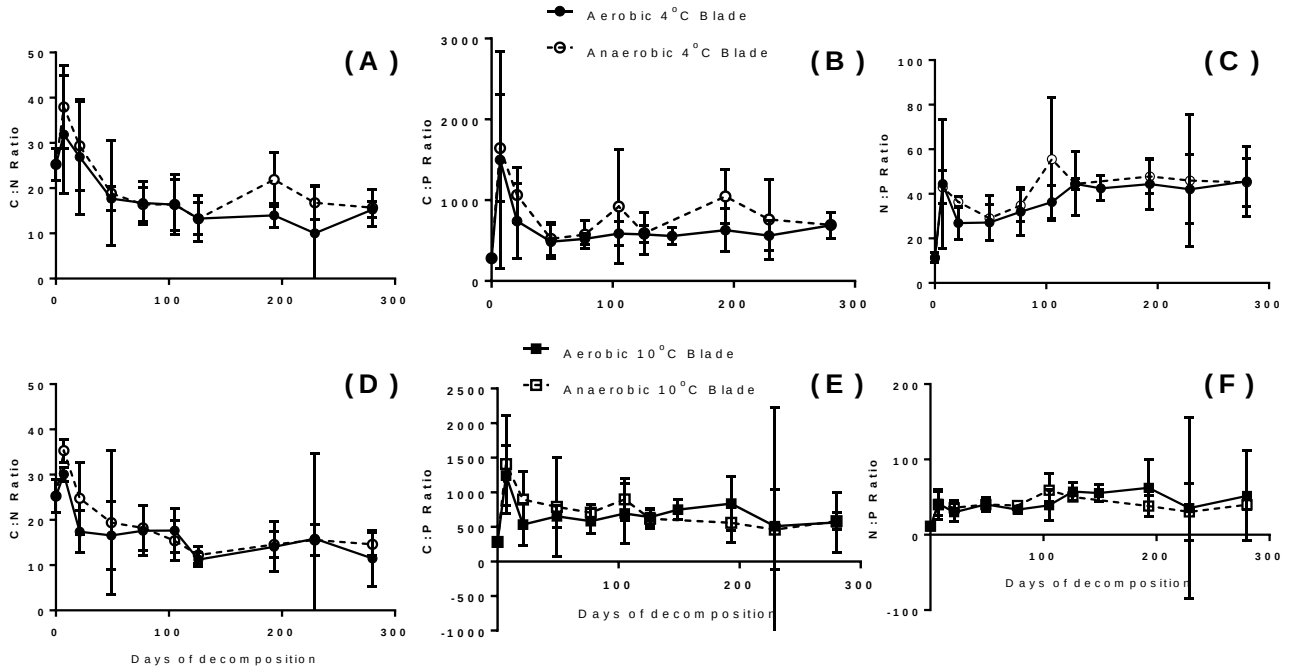

Figure 15: A figure of the change in atomic ratios. Figure A is the C:N-ratios at 4˚C blade. Figure B is displaying the C:P-ratios for 4˚C blade. Figure C is the N:P-ratios for 4˚C blade. Figure D is the C:N-ratios for 10˚C blade. Figure E is the C:P-ratios for 10˚C blade. Figure F is for N:P-ratios for 10˚C blade. All values are shown with 95% confidence interval.

The C:P-ratios of Figure 15B and E, also seems to follow a similar trend. The initial C:P-ratio of 282 ± 9 is the lowest value throughout this investigation, though it is not significantly lower in most cases. The initial increase in C:P-ratio is more pronounced under 4˚C conditions as the C:P-ratios rose to an average of 1571 ± 106 as opposed to an average of 1324 ± 82 under 10˚C. After the increase the C:P-ratios decreased levels approximately half of the peak. Between day 49 and 149 the tissue decaying at 4˚C had a slightly lower C:P-ratio. Between day 149 and 193 the C:P-ratios of tissue decaying at 10˚C had decreased to levels below tissue decaying at 4˚C. The average of the final C:P-ratio measured of the tissue decaying is also lower for 10˚C decaying material than for 4˚C 582 ± 8 and 692 ± 2 respectively.

The change in N:P-ratio does also have an initial increase from 11.3 ± 0.7 to an average of 43.6 ± 0.8 for 4˚C decay and 40.7 ± 0.8 at 10˚C decay. At day 21 the N:P-ratios had decreased with approximately 10 percentage point. With exception of anaerobically decaying tissue at 4˚C, N:P-ratios started to increase after day 21, for anaerobic 4˚C it started after day 49. The tissue at 4˚C steadily increased in N:P-ratio from day 49 to the end of the experiment. Whereas the tissue at 10˚C increased until day 193 after which a slight decrease may be seen. The final measured N:P-ratios were in all cases not different from those of the initial increase.

Stipe

The change in chemical element proportion were also calculated for stipe. The change in the ratios of C:N, C:P, and N:P had for stipe the same issue as for blade. Figure 16A through F displays the change in the given ratios throughout the 308 days of decomposition. The top three figures, Figure 16A, B, and C contains the results from the 4˚C treatments. Whereas the bottom three Figure 16D, E, and F displays the results of the 10˚C decay treatments. Figure 16A and D contains the C:N-ratios for 4˚C and 10˚C treatments respectively.

The change in C:N-ratio drops by approximately 10 within the first seven days (Figure 15A and D). After which the change is a steady decline which is not notably different between treatments. The general trend is that aerobic conditions leads to a slightly lower C:N-ratio as both aerobically treatments led to C:N-ratios below 10. Furthermore, tissue decomposed at 4˚C temperature had a lower C:N-ratio than 10˚C. The effect of oxygen regime is almost 10 times more larger than the effect of temperature.

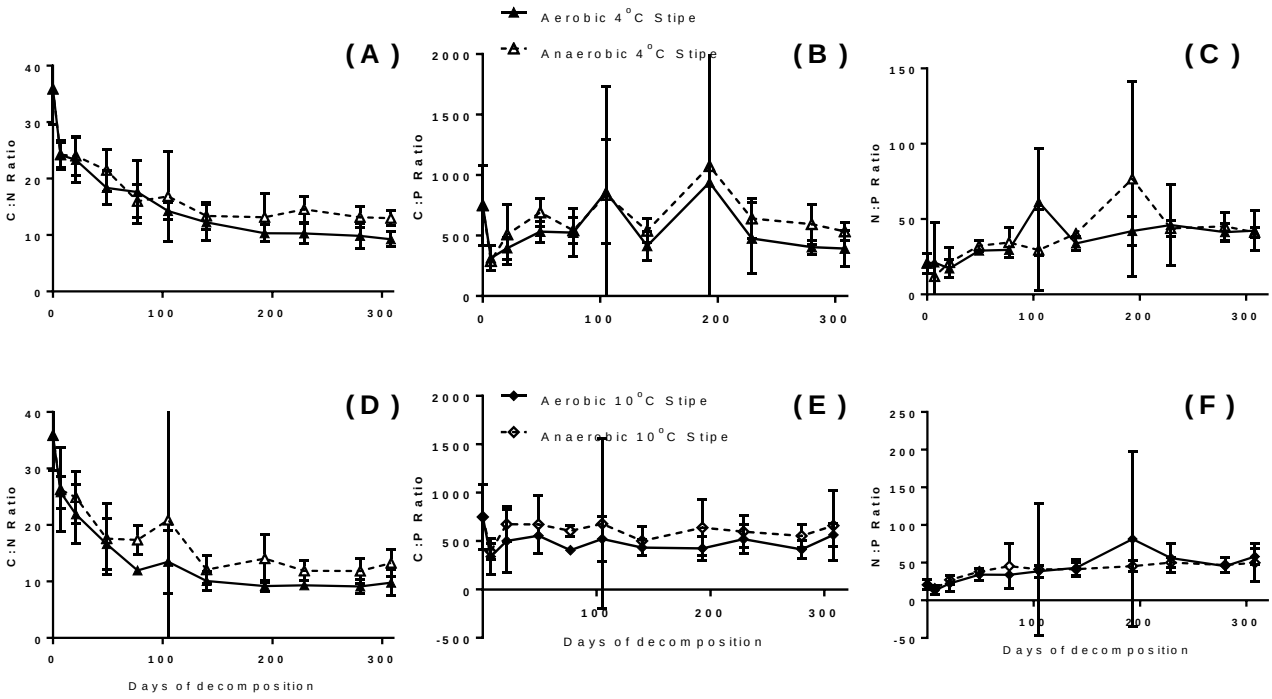

Figure 16: A figure of the change in atomic ratios. Figure A is the C:N-ratios at 4˚C stipe. Figure B is displaying the C:P-ratios for 4˚C stipe. Figure C is the N:P-ratios for 4˚C stipe. Figure D is the C:N-ratios for 10˚C stipe. Figure E is the C:P-ratios for 10˚C stipe. Figure F is for N:P-ratios for 10˚C stipe. All values are shown with 95% confidence interval.

There seems to be no markedly change in C:P-ratios as shown in Figure 15B and E. Initially the C:P-ratios drop from a starting value of 749 ± 104 to 299 ± 12 for tissue decaying at 4˚C and 366 ± 31 at 10˚C. After this increase the values increase to levels which seems relatively stable. The lowest C:P-

ratio were found in tissue decaying under oxic conditions at 4˚C 393 ± 42 . The highest value was found in anaerobically decayed tissue at 10˚C 660 ± 113. A trend seems to be that oxygen availability and lower temperatures leads to a lower C:P-ratio.

The N:P ratios of decomposing stipe does not seem to vary greatly between treatments. As the only significant differences were only found for day 193 which had large variations within treatments. Though the values are not significant a general trend seems to be that the N:P-ratios are lower at 4˚C than at 10˚C. This is evident from the final measured values as the average N:P-ratio at 4˚C is 42 ± 2 and 54 ± 4 at 10˚C. Furthermore, anaerobic decay may lead to a slightly higher N:P-ratio as the final values when compared between oxygen conditions are 45 ± 4 for anoxic conditions and 50 ± 4 at oxic conditions.

Discussion

Detritus production and transport

This thesis seeks out to investigate the rate of decay for *Laminaria hyperborea* detritus fragments at different temperatures and oxygen conditions. Furthermore, the change in the concentration and proportion of chemical elements were investigated, which in turn is used to determine the food quality of the detritus. Lastly, the phenolic concentration of the tissue were also measured as these molecules have anti-microbial and herbivory properties (Ayres et al. 2014; Enriquez et al. 1993).

The results of the fieldwork performed by KELPEX WP1 in the outer part of Malangen may change the view of kelp as a conveyer belt of FPOM detritus. The production of detritus by erosion was found to be independent of wave exposure (Figure 5). The detritus production by erosion seemed to be less important than the detritus produced by dislodgement of the old (Results "Site"). If dislodgement of old blade is confirmed to be the primary cause of detritus production by *L. hyperborea*, the detritus would change status from "conveyer belt" to pulse. The rapid burst of detritus would coinciding with the production of the new blade between March and May (Schaffelke & Lüning 1994; Kain & Jones 1976). A simultaneous release of old fronds would lead to the release of amounts approximately equal to the standing stock biomass found in October, 7 kg FW m^{-2} or approximately 1000 g DW m^{-2}. *Strongylocentrotus droebachiensis*, a species commonly known for its destructible grazing pattern had a maximum feeding rate of 0.6 g DW ind^{-1} day^{-1} (Scheibling & Anthony 2001). This sudden burst in large fraction detritus is presumably exceeding the grazing capacity of secondary producers capable of grazing upon these large fragments. The density of sea urchins within and around the kelp bed is 5-100 m^{-2} , which would lead to a grazing pressure of 3 - 60 g DW m^{-2} (Mann 1982; Scheibling & Anthony 2001). This is well below the estimated input of approximately 1000 g DW m^{-2}, as this is the standing stock blade biomass in October, before the shredding of the old blade. The remaining detritus, which is not grazed directly will be transported away by wave motion or currents. These fragments were found to accumulate in either crevices and trenches or scatter evenly over barren ground (pers. obs.). The large particle detritus may have a smaller dispersal range as they are heavier than small particles, hence more likely to sink to the sea bed (Schiel 2004). The transport of detritus as large fragments is not well understood but preliminary investigations have indicated, that the transport is slow (Unpublished data, KELPEX WP1). Large fragments of *Macrocystis* has been found to be transported to a continental shelf nine kilometers from the nearest kelp forest (Harrold et al. 1998). *Macrocystis* is naturally buoyant, which greatly affect the dispersal efficiency (Harrold et al.

1998), as opposed to the tissue of *Laminaria hyperborea*, which is negatively buoyant (Pers. Obs). The surplus of detritus will accumulate in trenches and depressions as observed *in situ*. These accumulations might act as reserves of POM or DOM during periods of low detritus production.

Degradation

To describe the change in biomass the one-phase G model were used, even though the most commonly used model is the multiple-G model (Kristensen et al. 1995; Westrich & Berner 1984; Middelburg 1989). The one-phase decay model was chosen over a multiple-G model as the estimated values of the regression were not viable, several values could not be determined due to variation being too big. Thus, the one-phase were chosen as the decay rate could be determined via this model though it do not accurately describe the decay of less liable material. From the measured data, a decomposition with multiple compartments of matter can be identified, this is also evident in the visual representation of the data (Figure 9A through D). An initial rapid decomposition or leeching of material can be seen for blade and stipe, which is accurately expressed by the one-phase decay model. This initial loss may be due to rapid decomposition of especially labile molecules (Kristensen 1991; Westrich & Berner 1984). Between 49% and 68% blade biomass is lost within the first seven days. These values are comparable with values reported for loss in *Fucus veciculosus* (Buchsbaum et al. 1991), barley hay (Kristensen et al. 1995), and phytoplankton (Westrich & Berner 1984). The difference in initial release of DOM by blade and stipe tissue coincide well with the fact that material with a higher content of structural molecules release less DOM (Kristensen 1991; Buchsbaum et al. 1991). Several investigators have compared the difference in decomposition of marine macrophytes and terrestrial vascular plants as these differ greatly in structural compounds (Enriquez et al. 1993; Lettice et al. 2011). The loss of phenolic compounds in marine macrophytes have been thoroughly investigated. The loss of phenolic compounds seems to be strongly correlated with the nature of the material being investigated rather than the total amount (Results “phenols”). The loss seems to be somewhat correlated with the amount of structural molecules. This is evident as material from seagrasses has a steady loss of phenolic compounds compared to macroalgae which has a rapid loss and almost total loss of phenolic compounds during the initial month of decay (Buchsbaum et al. 1991). The phenolic concentration reaches levels similar to that of the blade after 140 days, which is approximately 120 days later than the blades. This correlates well with the general tendency of a slower release or decomposition of phenols in the stipe compared to the blade.

Temperature

The relation between decomposition and temperature have often been found to be linear, until a threshold value after which it drastically decreases (Arnosti et al. 1998; Weston & Joye 2005). The results of the regression suggest that the decay of biomass may be up to four times faster at 10˚C as opposed to 4˚C (Table 2). This is mainly due to the fit of the regressions, as the initial decay is overestimated for the aerobic 10˚C treatment. Visually only small differences in remaining biomass can be seen (Figure 9). A difference in decay rate this large has normally not been reported and a doubling in rate is often found to happen every 10˚C - 15˚C (Arnosti et al. 1998; Weston & Joye 2005; Thamdrup & Fleischer 1998). The linear regression of the decay from day 49 and forth, confirms that the loss of biomass is not four times faster at 10˚C compared to 4˚C. The effect of temperature for the linear regression leads to an approximately 20% higher decay rate at 10˚C than 4˚C (Table 3). A one-way ANOVA followed by a Tukey's test supports the fact that the rate of decay does not vary significantly between temperatures. As no significant differences between remaining biomass were found at any time for stipe and after 21 days of decay for blade.

The temperature did have some effect on the initial loss of blade biomass, as the amount of remaining biomass at 10˚C were significantly lower than at 4˚C. This might indicate that with large amount of labile material the temperature affects the chemical processes more than the biological (Weston & Joye 2005; Pomeroy & Wiebe 2001). From the linear regression of the results from day 49 to the final sampling it is evident that temperature has little effect on decay rate. Only stipe decay at anoxic conditions are as the slope of the curve is approximately half for degradation 4˚C compared to 10˚C. This may be explained by the fact that sulfate-reducing bacterial are more sensitive to decreasing temperatures (Weston & Joye 2005). The residual values of the one-phase regression suggest that temperature should not affect the amount of remaining biomass greatly, given enough time to decay. This assumption is based mainly on the results of the aerobically decomposed blades as almost all material was decomposed. It is furthermore strengthened by the fact that the decay rate does not differ greatly between temperatures.

The change in nutrients concentration of the detritus, seemingly depended little on the temperature at which it was decomposing. The change in C, N, and P followed similar trends in all cases independent of temperature. Hence the final concentration did not vary between temperatures. With the exceptions that blade C content under oxic conditions at 10˚C were lower than the that of 4˚C. Furthermore, blade tissue at 4˚C seemed to become more enriched in N compared to tissue decomposed at 10˚C. The concentration of phenolic compounds is also largely independent of temperature. As the phenolic

compounds are depleted to levels around the detection limits within the first third of the experiment. Though the loss of phenolic compounds seems to be slightly faster and more complete at higher temperatures. This was especially pronounced under oxic conditions as the concentration were approximately four times lower at 10˚C than at 4˚C for both blade and stipe. The concentrations under anoxic condition varied by only approximately 25% as tissue at 4˚C had a slightly lower phenolic content than at 10˚C. This lower concentration at 4˚C might be because the higher amount of remaining biomass. If peripheral tissue contains the bulk of the remaining phenolic compounds and only little remains in the core, a larger amount of biomass would result in a lower overall concentration.

The change in C and P seems to happen at the same rate as biomass as the only substantial change in concentration is during the initial phase of leaching and rapid decomposition of labile molecules. The enrichment with N is correlated with loss of biomass and may be due to respiration of organic carbon by colonization of high N microorganism. Another possible reason to the enrichment may be caused by N being bound in large molecules with low liability, such as pigments which has a low decay rate or due to the N binding ability of some phenolic compounds (Bianchi et al. 2000; Ayres et al. 2014). The lack of significant results may be due to the difference in temperatures were too low. If a higher and thus less realistic maximum temperature were chosen a difference might have been found.

Oxygen

The effect of oxygen availability in regards to decomposition rate is a disputed topic (Middelburg et al. 1993; Westrich & Berner 1984; Kristensen et al. 1995). Aerobic decay has often been found to be several times faster depending on the material. The decomposition of *L. hyperborea* detritus seem to be strongly correlated with the oxygen regime. The initial rapid decay does not seem to be affected by the availability of oxygen. This is evident as the stipe tissue at varying times did not differ between oxygen regimes in any recognizable pattern. This was confirmed by an ANOVA followed by a Tuckey's test, as no treatments were consistently different. Though there was significantly less remaining biomass under oxic conditions than under anoxic conditions at day 308.

The proportion of remaining biomass did only become significantly different after 193 days of decomposition. As the remaining biomass of the aerobic decay became smaller than the anaerobic, by continuing a steady loss of biomass while the anaerobic decay seemed to stabilize. The estimated residual biomass was in general more than a factor two higher under anaerobic conditions. Similarly, from the linear regression of the decay from day 49 aerobic rates were in general twice as fast (Table

3). The rates found in this study is not directly comparable with other studies as rates here are given in percent and not weight. Though the difference in rates between aerobic and anaerobic decomposition is very like the literature (Bianchi et al. 2000; Westrich & Berner 1984). These results correlate well with the conclusion by Kristensen et *al.* 1995. They concluded that neither aerobic nor anaerobic decay was obviously faster during decay of the easily decomposable material. Though the decomposition of less liable material which had accumulated during the initial phase were faster and more complete under oxic conditions compared to anoxic. Hence the age and quality of material, which are being decayed has a major influence (Kristensen et al. 1995). This supports the continual loss of biomass under oxic conditions and the stabilizing trend of the anoxic conditions. The previous decay may have resulted in a build-up of large structural and aromatic macromolecules accumulated in the remaining tissue retarding or halting the anaerobic decomposition (Kristensen et al. 1995).

Oxygen availability did not have an obvious impact on the change in C and P tissue concentration. No patterns were evident from the ANOVA's and Tukey's tests. Oxic conditions had opposite effects depending on the tissue type. Blade tissue decomposed under anoxic conditions accumulated significantly more N. Whereas stipe tissue decomposed under oxic conditions achieved significantly higher levels of N. For blade the difference in N content became evident after day 193 for the 4˚C ($p=0.0001$) while at 10˚C differences became significant at day 229 ($p<0.0001$). This finding is not directly applicable with the findings of Kristensen et *al.* 1995 who found no difference in PON concentration at the end of their experiment with diatom fed marine sediment.

The loss of phenolic compounds was not significantly affected by oxygen availability. Though the final values of the present experiment might indicate that aerobic decomposition is slightly faster and more complete. Under oxic conditions the phenolic content reached levels of 0.1% or below for both blade and stipes. The concentration of phenolic compounds under anoxic conditions were lower at 4˚C than at 10˚C. At 4˚C the aerobically decomposed material had 25% lower concentration of phenols than the anaerobically decomposed material. For the 10˚C treatments the difference in phenolic content varied by at least a factor of six, as oxic conditions led to notably lower concentrations than the anoxic conditions. These findings are supported by Norderhaug et al. 2003 who found that both the rate and final concentration were different. They found that aerobically decomposed particles of *L. hyperborea* lost phenolic compounds faster and to a lower level than anaerobically decomposed. They furthermore found that aerobically decomposed detritus had a concentration of phenolic compounds comparable with detritus sampled *in situ* (Norderhaug et al. 2003). The phenolic content found in this experiment is in general well below the values of

Norderhaug et *al.* 2003. This might be due to the time of sampling as *L. hyperborea* generally have the lowest phenolic content during October (Schiener et al. 2014).

Quality of kelp detritus

Secondary producers in proximity to kelp beds has been shown to be greatly dependent of kelp detritus (Duggins et al. 1989; Fredriksen 2003). Many secondary producers are relying on the detritus as fine particles due to their feeding strategies as filter or suspension feeders (Fredriksen 2003; Duggins & Eckman 1997). It is unknown if the detritus produced from erosion is enough to support secondary production. Due to the limited amount of FPOM found by WP1, an additional FPOM production from the dislodged thalli might be important for subsidizing secondary production within and around the ecosystem. The dislodged blade and stipes must undergo chemical or mechanical degradation to become FPOM. The degradation is needed to fragment the large detritus to smaller particles, which can be ingested by secondary producers. After the deterioration of the detritus an upwelling is needed to release the fine particles to the water column, by which it becomes available to the secondary producers (Schiel 2004). As the atomic ratios of C, N, and P is a product of the tissue concentration of the relevant nutrient the change in atomic ratio follows the change in nutrient concentration. As with the total content of C and P the C:P-ratio does not change during decomposition. The C:P-ratio for blade initially increases by a factor of 4-5, after which it decreases and stabilizes at levels approximately twice as high as the initial value (Figure 15A and D). On the contrary the stipe C:P-ratio initially decreases by roughly one-third at which the C:P-ratios stabilizes. The final C:P-ratios are very similar, with the stipe tissue having one-sixth lower ratio than the blade. This is remarkable as structural tissue normally has less P and are in general regarded as having poorer food quality (Cebrian 1999; Duarte & Cebrián 1996). Though the origin of the P in the stipe tissue has not been investigated and could be bound in non-degradable compounds in the peripherical tissue (Cebrian 1999; Enriquez et al. 1993). Hence degraded material will have a better stoichiometry of at least C and P compared to the detritus produced from erosion. As the decomposed detritus would have regained some of the initially lost nutrients, by enrichment of colonizing microorganisms.

As N was the only element to change consistently throughout the experiment, so did the C:N and N:P-ratios. Neither temperature nor oxygen availability had a significant impact on the change of the final proportion of atomic C:N or N:P. Though some differences could be seen between treatments. Aerobic decay of especially stipe tissue seems to lead to a lower C:N-ratio as both aerobic treatments led to C:N-ratios below 10, whereas the anaerobic decayed stipe tissue had a ratio about 13 (Figure

16A and D), for blade no trend were evident (Figure 15A and D). Though earlier investigations have found the degradation of similar material would lead to a higher C:N-ratio at anaerobic conditions (Kristensen et al. 1995; Norderhaug et al. 2003). The *L. hyperborea* blade detritus investigates by Norderhaug et *al* 2003 had a C:N-ratio more than twice as high as the detritus investigated in this study. Their final C:N-ratios after 44 days of decay were also twice as high for aerobic decomposition and three times as high for anaerobic. They furthermore found that *in situ* degraded detritus had a C:N-ratio comparable with that of aerobic decay. The results of the present experiment follows the theory better in terms of change in C:N-ratio as bacteria is expected to respire C as long as the C:N-ratio is above 10-15 (Kristensen 1991).

The N:P-ratio did also change in a non-dependent manner on temperature and oxygen. Though the stipe tissue had an initial N:P-ratio almost twice that of the blade, the final N:P-ratios differed little (Results "Change in atomic ratios"). The N:P-ratios of the blades were in general slightly higher than the stipe ratios, though not significantly. Only the N:P-ratio of the 10˚C aerobically decomposed tissue differed notably from the other treatments. It did in general have a N:P-ratio which were one-fourth higher than the rest. These results indicate that decomposition at higher temperatures and under oxic conditions might lead to slightly poorer food quality. Though no values were found significantly different. These finding differ from the generally reported beneficial properties of aerobic decay (Norderhaug et al. 2003; Enriquez et al. 1993; Goldman et al. 1987; Kristensen et al. 1995).

The detritus experienced a rapid change in C:N:P-ratios during the initial 21 days of the experiment. The general changes throughout the experiment resulted in C:N:P-ratios which did not differ greatly between treatments nor tissue type. The blade detritus did in general become depleted in P whereas the stipe tissue was enriched during decomposition. The final C:N:P-ratios of the detritus are on average 632:45:1 for blade and 538:38:1 for stipe. Both of these ratios are well above the C:N:P-ratio of 106:12:1 for optimal bacterial growth (Goldman et al. 1987). The C:N:P-ratio of microorganisms is relatively invariant at approximately 45:9:1 (Goldman et al. 1987). Hence the colonization by microorganism should enrich the detritus with P. The high C:N:P-ratios of the detritus might indicate that colonization of microorganisms may be of little importance for the P content of the detritus. The quality of *Laminaria* detritus as a food item for secondary producers has centred around the total phenolic content and the C:N-ratios. Common for all investigations is a rapid initial loss of phenolic compounds. Furthermore, they have concluded that growth and survival is not affected by the concentration of phenolic compounds presumably due to the generally low levels of phenolic compounds in *Laminaria* tissue (Duggins & Eckman 1997; Norderhaug et al. 2003; Norderhaug et

al. 2006). The C:N-ratio has been found to be a good estimator of food quality for kelp associated fauna. Survival and growth of detritivores have been found to positively related with a decreasing C:N-ratio of aerobically decomposed detritus (Norderhaug et al. 2003; Norderhaug et al. 2006). Though at C:N-ratios below 4:1 the beneficial effect diminishes and at ratios of 2:1 is correlated with increased mortality and inhibition of growth for amphipod detritivores (Norderhaug et al. 2006). The a C:N-ratio above 30 seems to be negatively correlated with survival and growth for detritivores and a reduction in C:N ratio from 12 to 6 had a positive effect on growth for a suspension feeding worm (Norderhaug et al. 2003; Duggins & Eckman 1997). Detritus in this study rapidly stabilized on C:N-ratios below 30. Norderhaug et *al.* 2003 found that the survival of *Gammarus locusta* were negatively correlated with days of anaerobically decomposition of detritus despite lower C:N-ratios. This might indicate that fouling molecules may accumulate in the anaerobically decomposed. Kristensen 1994 found that even though decomposed material had the same C:N-ratio as the fresh material an accumulation of humic compounds due to condensation had occurred (Kristensen 1991). If fouling or large non-liable molecules accumulate to a greater degree under anoxic condition than oxic conditions the aerobically decomposed material would be of greater quality.

Conclusion

The detritus produced by *L. hyperborea* has been found to be mainly in the form as large fractions of blade tissue or whole blades. This detritus is produced mainly between March and May as the new blade is detached from the newly grown blade. This results in a release of as much as 7 kg FW of detritus during a short period of time. This amount of detritus is arguably a bigger input of detritus than detritivores could consume, leading to an accumulation of detritus in the ecosystem or transport of detritus out of the ecosystem. The main influence of temperature and oxygen availability is the rate of which the biomass is decomposed. The initial phase of decay is not affected by the temperature nor the oxygen availability. Yet as the detritus ages the expected effect of lower temperatures and especially anoxic conditions becomes more pronounced, leading to lower decay rates. It is at least not possible to generalize the effect of oxygen availability as it had opposite effects on blade and stipe in regard to the N concentration. The initial change in C, N, and P were also opposite between the two tissue types. But the decomposition led to the C:N:P-ratios of stipe and blade to be more similar than the starting ratios. Temperature and oxygen availability does not seem to greatly affect the concentration or stochiometric relation between the elements investigated. If the C:N:P-ratio can be used as the primary estimator for food quality then aerobically decomposed tissue, might be a slightly better food source. Though effect of oxic or anoxic conditions on the production of fouling compounds needs to be elaborated, to fully understand the change in quality of the detritus.

Perspective

To further elaborate on the understanding of detrital subsidization and food quality of detritus derived from *L. hyperborea* several factors has to be investigated. Several other analyses of the material from the decomposition could have been performed as the accumulation of fouling compounds might have been measurable. Similar it would have been interesting to investigate in which molecules the C, N, and P were bound throughout the decomposition. Furthermore, it would be interesting to investigate to which degree the detritus had been colonized by bacteria. A way to further clarify the decomposition of detritus would be to monitor the release of gasses from the experiment, though this would be a herculean task.

Furthermore, it could be interesting to investigate the effect of fragment size on transport. To evaluate how long and to what degree the detritus will settle under different environmental conditions. The grazing and shredding large detritivores would also be a valuable subject to investigate. As these may be a vector in the breakdown of large fractions to smaller fractions. Furthermore, grazers may contribute with colonization of bacteria through faecal production or sloppy feeding. This would could lead to an investigation of the effect of particle size on the decomposition rate. It is well known that the volume and surface area does not change at the same rate. Hence smaller particles has a bigger surface to volume ratio. This could result in colonization of more microorganisms per volume, which presumably would lead to a faster decomposition.

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