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Biomass Leaching and Dynamics of Nutrients, Microbial Abundance and Activity during Decomposition of Seagrass *Cymodocea rotundata* Necromass

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Abstract

Examining how seagrass decomposition contributes to trophic pathways in marine ecosystems is crucial in understanding seagrass production. Decomposition rates of seagrasses may depend on many factors such as chemical composition and microbial colonization. In this study, microbial colonization and changes in chemical composition of decomposing material (necromass) of Smooth Ribbon Seagrass, *Cymodocea rotundata* of Bogtong Bay, Lahuy Island, Caramoan, Philippines were monitored. Seagrass litter were placed in litterbags and incubated in the seagrass meadow *in situ* for 56 d. Serial dilution, viable plate counts and microbial oxygen consumption analyses were done and gravimetry, Kjeldahl method and acid hydrolysis were used respectively to measure the change in carbohydrate, protein and nitrogen content of the decomposing necromass. Results showed that the decomposition processing rate was 0.27 to 2.51% biomass (g dw) loss per day with a half-life of 2.36 to 2.88 d. Growth of bacteria was greater than fungi throughout the course of experiment. Bacterial abundance (CFU mL^{-1}) fluctuated throughout the experimental period while fungal abundance initially increased but gradually decreased and the initially observed marine fungi ceased to grow in decaying litter until the end of the experiment indicating that heterotrophic bacteria contribute more in the decomposition of seagrass litter. Oxygen consumption as well as protein, lipids and nitrogen content of litter decreased by as over the days of incubation. Therefore, as decomposition proceeds, litter biomass was leached resulting in carbohydrate content loss. But the remaining tissues of decaying *C. rotundata* were eventually colonized by bacteria and fungi. This further contributes to mineralization of the litter and gradual release of nutrients that could be considered as important trophic inputs to the ecosystem.

Keywords: Seagrass decomposition; *Cymodocea rotundata*; Litter bag experiment; Microbial colonization

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Introduction

The high production of seagrass meadows depends on vigorous biogeochemical cycling and coastal trophodynamics resulting to a healthy benthic faunal habitat [1-3]. It is estimated that tropical seagrass meadows have 975 to 3614 g dw m^{-2} a^{-1} annual production making them among the most productive ecosystems in the world [4-8].

Deeper understanding of the process seagrass decomposition is crucial in understanding the fate of seagrass production, especially in under-researched areas like Caramoan Peninsula. Environmental factors such as water temperature, wave action and depth along with the quantity and composition of the decomposing material influence the process of decomposition and deposition of detritus in the seagrass meadow. The overall organic matter reservoir in the meadow and the transfer of nutrients, carbon and energy across trophic levels depend strongly on the rate of decomposition. Information about the condition and resilience of seagrasses are needed to better understand and appreciate the importance of their biodiversity and the need to conserve and use them in a sustainable manner. The coastal management for Caramoan Peninsula should incorporate conservation of good environmental quality of the shoreline in many of its pristine environments, however, this must be based on sound baseline and long-term observational data.

Cellulose, lignin, and soluble matter which are high molecular weight structural compounds constitute vascular plant litter. Bacteria and fungi secrete extracellular enzymes within the plant cells. Enzymatic depolymerization results in the formation of the lower molecular weight compounds, such as carbohydrates, peptides, and amino acids. These compounds are assimilated by decomposers. The decomposers however cannot assimilate the simple phenolic compounds and reducing carbohydrates (i.e., sugar that has a free aldehyde or ketone that can act as a reducing agent) due to their inhibitory effects. But leaching inorganic ions from the litter that end up into as reservoir of dissolved inorganic nutrients accessible for decomposers. Bacteria on the other hand use the parts of vascular plants which have high ratios of C:N and C:P as exogenous source of dissolved inorganic ions [9].

As fragmentation of litters occur due to the herbivores that colonize these materials, the surface area for microbial attach increases. Some of the plant litters are directly consumed by a few invertebrates and vertebrates. Nutrients needed by detritivores are not supplied adequately by the living bacteria and fungi, but microbial exudates increased the food value of detritus and detritivores directly consume the plant remains. Conversely, aging detritus form humus as some components of litter are never decomposed or re-mineralized as they produced aromatic compounds. The humus is enriched with nitrogen, but this does not provide added nutritional value. Lastly, phenolics released from enzymatic digestion of litter and the carbohydrates combine with plant parts rich in nitrogen and exudates of bacteria. Such complexes are composed of labile organic matter and high molecular weight compounds which are resistant to enzymatic attack [9].

Several aspects of litter components have been studied, particularly, decomposition, fragmentation, consumption, changes in chemical composition and nutritional value, and export to other ecosystems [9-12]. Nonetheless, there is also a dearth of research that focused on the estimation of mass material [13] and the use of litter bag experimental technique to examine the seagrass decomposition rates [14-17].

Seagrass wracks are commonly found on the sandy shorelines of Bogtong Bay, Lahuy Island, Caramoan Peninsula [18]. The accumulation of necromass on the shorelines is influenced by the wrack and litter availability, as well as by height

and speed of waves, and speed and direction of wind. The role that these stranded seagrass litter and detritus on the beach in the deposition of nutrients in the sediment needs further inquiry.

It was shown that spatial and seasonal differences in seagrass wrack, litter and detritus accumulation and the pattern of decomposition based on biomass loss [18]. Among the dominant seagrasses in Bogtong Bay, *C. rotundata* was the least resistant to decay compared to *Halodule uninervis* and *Enhalus acoroides*. The length of incubation could explain only 30% of the variation in biomass loss; about 70% is explained by other factors. It was recommended that additional work must be done to explore seagrass decomposition in Bogtong Bay that should focus on decay rates of seagrass leaves which may be performed under varied conditions, in terms of depth, season (wet and dry) and kind of material (i.e., senescent leaves still attached to the plant versus in leaf litter fragments). The effects of incubation depth and respiratory consumption on biomass loss [19] could be further validated.

Accumulation of seagrass wracks and litters are commonly found on the sandy shorelines of Bogtong Bay, Lahuy Island [18]. These seagrass necromass are reservoirs of detritus that eventually act as a substantial carbon sink [20]. There is dearth of studies in the Philippines in general, and in Bogtong Bay in particular, that investigate the influence of chemical composition, microbial colonization, and physical environment on the decomposition rates of seagrasses. A study that examines how seagrass decomposition contributes to trophic pathways in the marine ecosystem is crucial in understanding the seagrass production [21-22] in Bogtong Bay.

The present study specifically aimed to investigate the microbial colonization and nutrient quality (i.e., changes in the chemical composition) of decomposing material (necromass) of Smooth Ribbon Seagrass, *Cymodocea rotundata* of Bogtong Bay, Lahuy Island, Caramoan, Philippines.

Materials and methods

1) Study site

The seagrass meadow (i.e., estimated meadow size: 0.056 km^2) off the coast of Bogtong Bay, Lahuy Island, Maqueda Channel, northern part of Caramoan Peninsula, Camarines Sur, Philippines (130 53'N, 123^0 56'E) were the site of the study (Figure 1). Bogtong Bay is part of the Lahuy Island, the largest island of the Caramoan Island Group (CIG) on the northern part of the peninsula and famous for its gold mine before the World War II. The bay is an extension of Pocket Bay on the northeastern part of Lahuy Island which is bounded by Guinahoan Island on the left and Cotivas Island on the south. These islands are becoming popular tourist destinations due to the surrounding white beaches and coves. Seagrasses abound these islands and the most abundant seagrass meadows are found in Bogtong Bay.

2) Decay rates (Litter bags - Decay experiment)

Fresh samples of C. rotundata were collected from a seagrass meadow near Bogtong Bay. Leaf blades were excised near the sediment surface. The initial seagrass samples were composed of approximately green leaf blades (60-80%), brown blades (5%), and blades showing a mix color of yellow, brown and green parts (25%). Leaf blades with 0 to 100% epiphytic cover were used. Leaf blades were kept submerged in seawater throughout the harvesting to avoid desiccation of the cuticle that could accelerate decomposition [23].

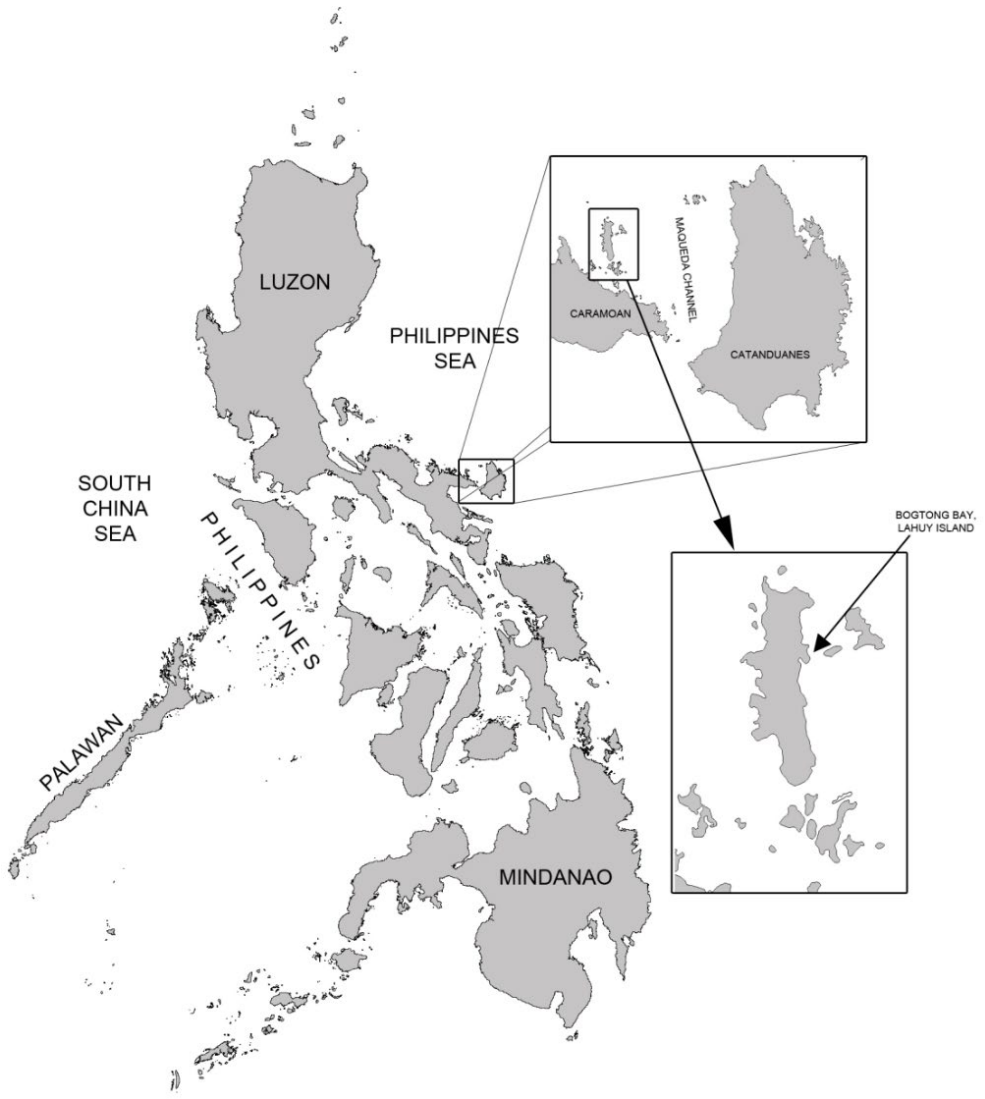

Figure 1 Study site: Bogtong Bay, Lahuy Island, Caramoan Peninsula, Philippines.

Around 30 g wet weight (g ww) of seagrass blades were placed in each of the 80 litterbags. Forty litterbags per sample type were placed in the seagrass meadow 1 m below sea level during lowest ebb tide, while the other 40 litterbags were placed at 3 m below sea level during lowest ebb tide. Litterbags (10 x 20 cm) made up of nylon mesh netting with openings of 3 mm were used. The bags were tied at evenly spaced intervals to a 5-m line which were held to a subtidal mud bottom of the meadow. Three or more bags were harvested at various time intervals and brought to the laboratory in a bucket of seawater for analysis [22].

Change in biomass (ww) were noted. Samples in each treatment/time combination were pooled, then subsamples were analyzed. Five subsamples were dried at 60^{o}C to constant weight. Samples were placed in a muffle furnace (550^{o}C for 3 h) to determine ash free dry weight (AFDW). Weight loss was quantified for the whole sample [22].

Litterbags were collected after 1, 7, 14, 28, and 56 d. During each collection, the samples were prepared at once to assessment of microbial populations and activity, as well as for chemical composition analysis. Other samples were brought to laboratory for drying at 60^{o}C for 3 h to measure the dry weight (dw) and placed in muffle furnace at 550^{o}C for 3 h to measure the ADFW of actual leaf litter [22].

3) Microbial colonization

Samples of decaying seagrass *C. rotundata* collected from the litter bags were subjected to serial dilution and viable place counts. A series of 5-fold dilution was done. Ten grams (10 g) of seagrass litter samples were cut into small pieces and placed in 90 mL filtered seawater. Using vortex, the samples were mixed carefully and from the solution, 1 mL subsample was placed in 9 mL filtered seawater. Three more dilution schemes were done. Then, 1 mL sample from each dilution were transferred into a melted marine agar and another 1 mL into a melted Potato Dextrose agar for detection and enumeration of heterotrophic marine bacteria and marine fungi, respectively. Viable plate counts were observed after incubation. Some plates have many colonies that they are too numerous to count (TNTC). Only plates with 25-250 colonies are used. Counts above 250 are considered to be TNTC because it is impossible to tell whether colonies are separated. No further processing was done for those which showed TNTC but there were recorded and reported. To estimate the concentration of bacteria or fungi in the decaying seagrass litter (bacteria or fungi per mL), colony count was multiplied by the reciprocal of the dilution [24].

To measure the microbial oxygen consumption, a detritus subsample (1 g ww) from the litter bags was placed in a 300 mL BOD bottle. From each litter bag, 3 BOD bottles were prepared. Water collected from within the seagrass meadow where incubation was done were filtered and sparged with air for 1 h then placed in the BOD bottles. A set of 3 BOD bottles containing filtered, aerated seawater were used as a control. All the bottles were incubated in the dark for 3 h at *in situ* water temperature. Then the oxygen concentration at the start and end of the incubation time was measured using an oxygen meter [25].

4) Chemical composition

The chemical composition of seagrass litter was compared to the chemical composition of the live seagrass tissue. This allowed an estimation of what remains relative to initial concentration while seagrasses decomposed. All samples for chemical analysis were submitted to SentroTek Laboratory Philippines which used analyses based on the Official Methods of Analysis of Association of Official Agricultural Chemists (A.O.A.C.) [26].

Chemical analyses included determinations of total carbohydrate (g 100 g^{-1}), ash (% w/w), moisture (% w/w), protein (N x 6.25) (% w/w), total lipids (% w/w) and nitrogen (% w/w). The carbohydrate was measured as % carbohydrate (100 – (%moisture + % ash + % protein + % lipids)). Gravimetry was used to measure the ash and moisture while Kjeldahl method was used to determine protein and nitrogen. Total lipid content was measure through acid hydrolysis.

5) Data analysis

Change in necromass litter biomass, nutrient content and microbial abundance was computed by measuring their initial and final value, then subtracting to determine the difference and dividing the change in value by the initial value. To report percent change of the value, the quotient is simply multiplied by 100.

Detritus processing rate is based on the loss of organic mass from litter bags. Decomposition curves was traced according to the exponential decay model [27]: $W_t = W_0 e^{(-Kt)}$ where W_t is the weight of the material left from initial weight W_0 after time t, K is the decay constant (instantaneous decay rate). Decay constant was computed by fitting an exponential regression, which is negative. Litter half-life was computed as $1_{½} = \ln 2/K$ and turnover = 1/K [28].

The mean values of different variables: biomass loss, bacterial and fungal abundance, and nutrient estimates were computed. Then the linear relationships between variables were measured by using Pearson's correlation. Statistical analyses were done using Microsoft® Excel® for Office 365 MSO and IBM SPSS Version 20.

Results

1) *Cymodocea rotundata* litter decay rates

The decomposition of *C. rotundata* seagrass determined done using *in situ* litter bag experiment revealed from the initial 30 g of fresh litter samples (g dw = 4.29), the actual biomass decreased to 4.21 g dw (1.86% loss) in 7 d and in 28 d to 1.28 g dw (or 70% loss), or approximately 0.27 to 2.51% biomass (g dw) loss per day. In terms of ash free dry weight, approximately 2.43% biomass (g AFDW) loss per day (Figure 2).

Half-life (t½), the time needed for a quantity to decrease to half its value determined at the beginning of the time period, showed that the *C. rotundata* has the half-life of 2.36 d based on dry weight and 2.88 d based on ash free dry weight, respectively. The instantaneous decay rates (K) which were all less than 0, confirmed that the calculated time (T) refers to the time it takes to reduce to half or the biomass half-life.

2) Microbial colonization of *Cymodocea rotundata* necromass

2.1) Bacterial and fungal abundance

Bacterial abundance had increased for 96.76% on the initial phase of experiment (Days 1 to 7), decreased for 98.50% (Days 7 to 14), then gradually increased again for 61.95% (Days 14 to 28) before it had increased once more for 97.89% (Days 28 to 56). Compared to bacterial abundance, fungal abundance was lesser throughout the course of the experiment. Fungal abundance had increased for 37.84% initially (Days 1 to 7) and decreased for 30.11% (Days 7 to 14). Marine fungi ceased to grow in the decaying litter that were collected on the 28^{th} d until the end of the experiment (Figure 3).

2.2) Microbial activity

The highest rate of oxygen consumption, 0.200 g ww^{-1} h^{-1}, was associated with the first day and fourteenth of the incubation. From the peak in oxygen consumption on the first day, consumption lowered to 0.133 g ww^{-1} h^{-1} by the 7^{th} d. Consumption of oxygen by the microbes that colonize the decaying *C. rotundata* stabilized at approximately 0.077 to 0.066 g ww^{-1} h^{-1} by the 28 d of decomposition (Figure 4).

3) Nutritional quality of decomposing Cymodocea rotundata

Litters of *C. rotundata* lost 38.23% of their original carbohydrates in the 7^{th} d and continuously declined by 43.34% in the 14^{th} d. After 28 d of decomposition, *C. rotundata* litter samples lost 48.81% of its carbohydrate content (Figure 5).

Overall, the decaying *C. rotundata* contained more proteins than lipids and nitrogen. Protein, lipids and nitrogen content of litter decreased by 60.10%, 61.50%, and 71.40%, respectively over the first 14 d of incubation. Then, from the day 14 until the termination of the experiment, an increase in nitrogen (50.00%), protein (85.71%) and lipid (28.60%) was observed (Figure 6).

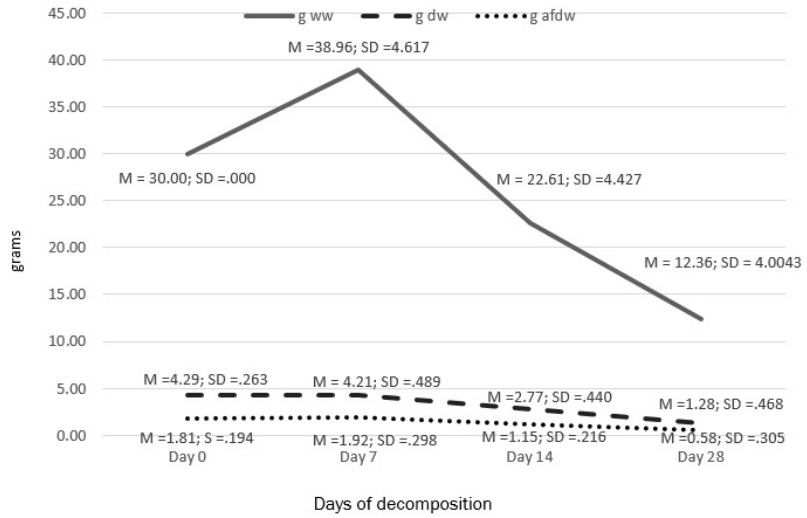

Figure 2 Biomass litter weight loss in terms of wet weight (g ww), dry weight (g dw), and ash free dry weight (g AFDW) of decaying litters of *Cymodocea rotundata.*

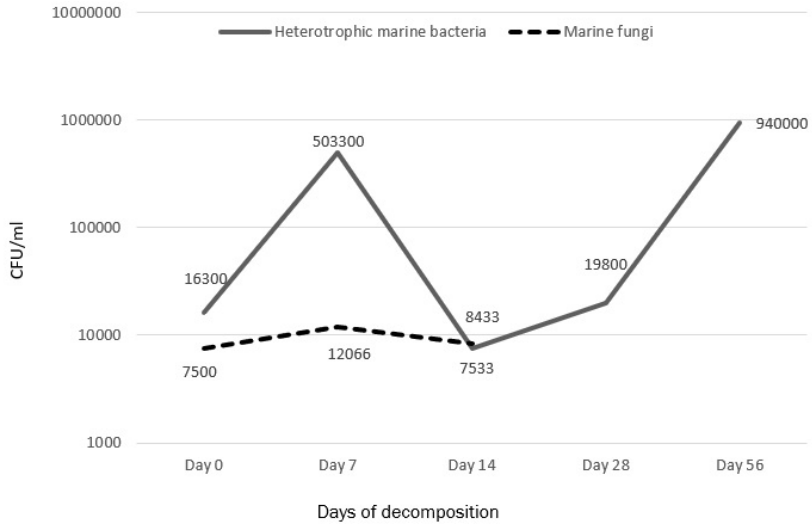

Figure 3 Heterotrophic marine bacterial abundance (CFU mL^{-1}) and marine fungal abundance (CFU mL^{-1}) grown from decaying litters of *Cymodocea rotundata*.

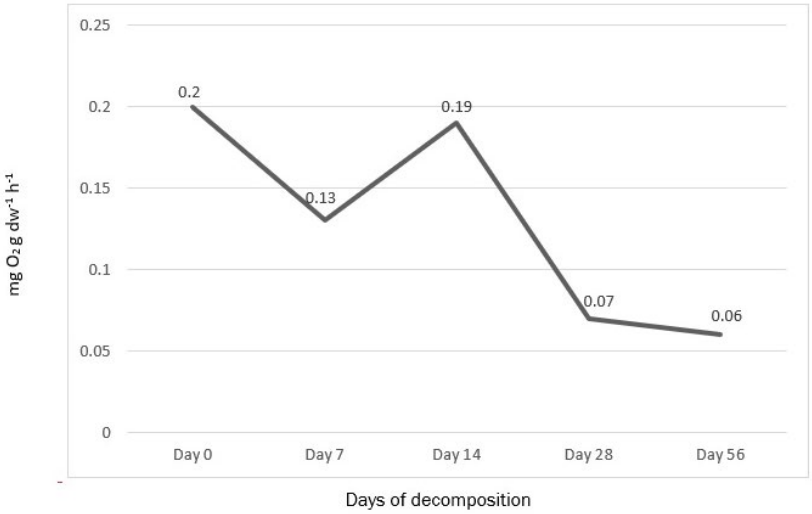

Figure 4 Microbial oxygen consumption g O_2 ww^{-1} h^{-1} by days of decomposition.

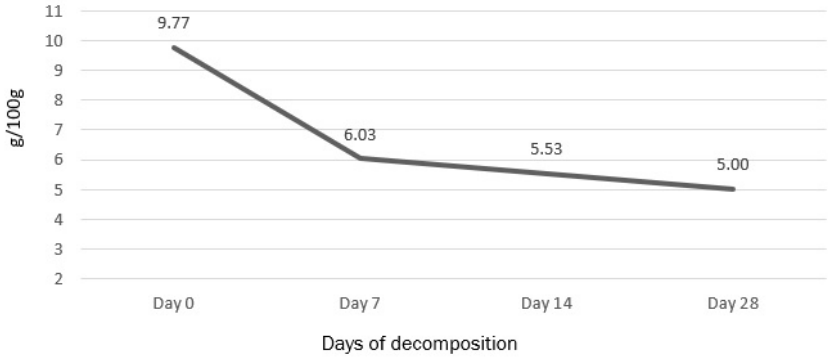

Figure 5 Carbohydrate (g 100 g^{-1}) of decomposing litters of *Cymodocea rotundata*.

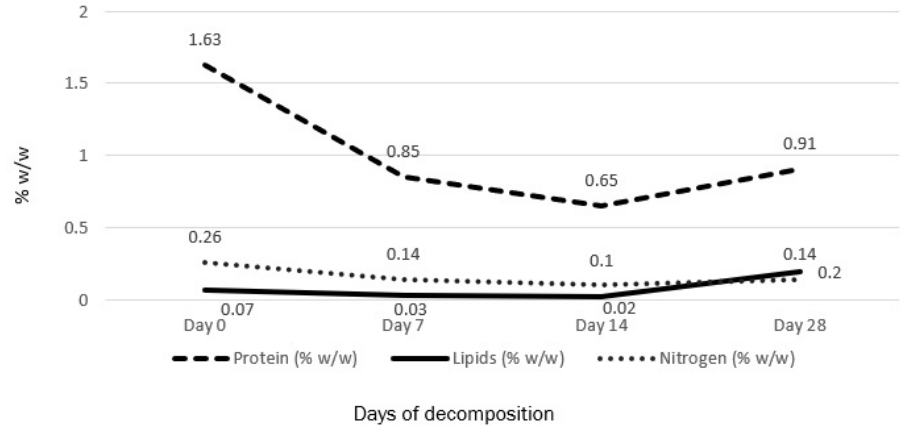

Figure 6 Protein, lipids, and nitrogen of decomposing litters of *Cymodocea rotunda*.

4) Correlations among all variables in the decomposition of *Cymodocea rotundata*

Bivariate correlations among all the variables in the decomposition of *C. rotundata* are shown in Table 1. It was observed that litter bag decomposition days was strongly correlated (all *ps* < 0.01) with abundance of heterotrophic bacteria (r = 0.65), but inversely correlated with litter biomass (r = -0.94), and carbohydrate content (r = -0.62). This imply the intense microbial activity done by heterotrophic bacteria, loss carbohydrate and biomass due to leaching decomposition proceeds.

Lipid and abundance of marine fungi were also significantly inversely correlated (r = -0.59, and -0.62, both *ps* < 0.05) with days of decomposition indicating that the presence of lipid and marine fungi are expected during the early phase of decomposition. Litter biomass was significantly correlated with abundance of marine fungi (r = 0.71, p < 0.01) and carbohydrate (r = .63, p < 0.05) suggesting that as decrease in the biomass of seagrasses during the decomposition may be attributed to the decline of the marine fungi and carbohydrates in the plant litter. In addition, lipid content was inversely correlated with litter biomass, abundance of marine fungi, microbial oxygen consumption and carbohydrate content (-0.65, -0.70, -0.44 and -0.24, all *ps* < 0.05). This confirms that lipid content tends to increase even when litter biomass decreases along with the decrease of marine fungi abundance and carbohydrate content as decomposition proceeds. Microbial oxygen consumption was significantly correlated with carbohydrate content (r = 0.50, p < 0.01) as well as with nitrogen content (r = 0.33, *ps* < 0.05) hinting that microbial activity influence the increase in carbohydrate and nitrogen during the decomposition process.

Table 1 Correlation matrix between variables in the decomposition of *Cymodocea rotundata*

Variable	1	2	3	4	5	6	7	8	9
1. Days of decomposition	-								
2. Litter biomass	-0.940**	-							
3. Heterotrophic bacteria	0.649**	-0.402	-						
4. Marine fungi	-0.622*	0.712**	-0.035	-					
5. Oxygen consumption	-0.474	0.287	-0.508	0.296	-				
6. Carbohydrate	-0.806**	0.625*	-0.102	0.224	0.499**	-			
7. Nitrogen	-0.586*	0.440	-0.053	0.153	0.332*	0.922	-		
8. Lipids	0.707*	-0.649*	-0.314	-0.697*	-0.436*	-0.240*	0.057	-	
9. Protein	-0.565	0.413	-0.090	0.131	0.347	0.918	0.999	0.079	-

Note: The table shows Pearson correlation coefficients; N= 15 (days of incubation, biomass loss, heterotrophic bacteria, marine fungi and oxygen consumption; N = 12 for carbohydrate, nitrogen, lipids, and protein); **Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

Discussion

Findings of present study indicate that litters of *C. rotundata* underwent leaching leading to biomass loss (~ 2.5% d^{-1}). This value was lesser that those reported in the literature (e.g., Josselyn et al,.1986) wherein seagrass decomposition happened fast, losing over 50% of the initial weight in approximately 3 d (16.67% d^{-1}), but the findings supported a previous report [18].

The rate of decomposition was usually <1% of dw d^{-1} and is affected by chemical composition the seagrass litter when decay begins [9]. As shown by the findings, carbohydrate, proteins, lipids and nitrogen content of *C. rotundata* litters rapidly diminished to approximately 4% d^{-1} from its original nutrient content. Correlation coefficients also showed that as litter biomass diminished the growth of bacteria and lipid content increased, while marine fungi abundance and carbohydrate content decreased. These changes in nitrogen, lipids and carbohydrates in the litter were also generally consistent with literature reports [9, 23, 29]. This rapid loss was presumably related to the rapid decrease of soluble compounds and labile organics. Seemingly, the structural carbohydrates found in decaying seagrass litters are indeed consumed by some animals grazing on these decomposing materials [9].

The fluctuation in the nitrogen content was also observed in the present study. This was consistent with a report that nitrogen content leached from the seagrass litter initially but eventually the absolute nitrogen in seagrass detritus usually increased [29]. Consequently, unless the detritus of seagrasses is consumed by detritivores, these detrituses may serve as nitrogen sink to the sediments [29]. On the other hand, much of the nitrogen were leached and released from the seagrass litter. The lignin and phenolic materials of the remaining seagrass litter could become the source of molecules that constitute resistant complexes with microbial proteins resulting in build-up of nitrogen [9].

Lipid increased on the latter part of the experiment by 28.60% after its rapid loss by 61.50%. Though not characterized in the present study, the lipid components were already isolated in seagrasses [30], namely: neutral lipids, glycolipids, and phospholipids in the leaves and rhizomes of seagrasses.

The observed changes of chemical composition of seagrass litter can be attributed not to leaching during the decomposition process but the colonization by bacteria and fungi and the mineralization of litter that

resulted to the solubilized, gained or lost constituents of seagrass litter [31-32]. This may explain the observed variability in the lipid, protein and nitrogen in the decomposing *C. rotundata*.

Much of the detrital turnover was attributed to the bacterial metabolism [34]. Also, invasive bacteria with marked cellulolytic activity covered the tips of seagrasses, particularly at the epidermal cells showing the signs of decomposition [35]. These observations are aligned with the observed bacterial abundance (163 x 102 CFU mL^{-1}) in the litters of *C. rotundata* in the present study. It was shown also that bacterial abundance increased initially (96.80%) then decreased (98.50%) before it gradually increased for 62.00%. When the experiment ended, a marked increase in bacterial abundance was observed (97.89%). It was explained that to this colonization pattern of microbes in decaying seagrass material [9]. The rapid leaching at the initial phase of experiment left poor substrate for bacteria due to poor amount of inorganic nutrients, presence of inhibitory phenolic compounds and abundance of cellulose and lignin. This was consistent with the finding that bacterial number and growth rates were inversely correlated with detritus supply [36]. The observed bacterial abundance in the phase of decomposition could be accounted for by the fragmentation of seagrass litter as detritivores colonize the litters and increase the surface area available for microbes [9].

It was observed that growth of fungi in the decaying litters of *C. rotundata* was lesser than the growth of bacteria throughout the course of experiment. Normally, the doubling time of bacteria is shorter than that of fungi hence, bacteria must have outnumbered fungi or that incubation conditions may favor the growth of bacteria better than fungi [37]. The fluctuation of heterotrophic marine bacterial growth on day 7 is consistent with the second phase of plant litter decay in which intense microbial activity combines with fragmentation of the substrate [38].

Growth of marine fungi was no longer observed in the decaying litter collected on the 28^{th} d until the end of the experiment. This is consistent with the observation that fungi appeared to be insignificant in seagrass decomposition [9]. Lastly, the pattern of increase and decrease in microbial oxygen consumption was recorded to peak during the 1^{st} and 14^{th} d of litter bag experiment and was lower on the 7^{th} and stabilized on the 28^{th} until the termination of experiment. This pattern was inconsistent with the observed pattern of microbial abundance, although, correlation coefficient showed that as microbial oxygen consumption increased, the nitrogen content also increased. These results support the specific studies [22] that assessment of seagrass decomposition rate using estimates of oxygen consumption of microbes that colonize decaying seagrasses provided very high variances and unpredictable results.

Based on the findings of the present study, decomposition involves the physical and chemical disintegration of dead organisms. Various organisms including fungi and bacteria are involved in the process. Rapid decay of seagrasses *C. rotundata* occurred *in situ* plausibly contributing to the trophic input in the seagrass system. The present study showed that decomposition process is influenced by the net chemical constituents and the colonization of microorganisms.

Figure 7 presents a conceptual model summarizing the findings of the current study. It indicates that as decomposition proceeds, litter biomass is leached resulting in loss of carbohydrate content. But the remaining seagrass tissues are eventually colonized by bacteria and fungi that further contribute to the mineralization of the litter and gradual release of nutrients.

The results of the study warrant that further research must be done to understand completely the fate of seagrass production. The present study only elucidated the rapid decay of seagrass litter that remains within the meadow. Primary production of seagrasses may also decay while accumulating in along the shorelines or exported to other nearby ecosystems such as mangrove forest and coral reefs. The decay rates of available seagrass necromass revealed in this study may be compared with the estimates of production to assess the possible variations in the amount of litter that decomposed *in situ* and amount of exported litter. Grazing, detrivory and decomposition pathways will be understood completely if more studies are done to synthesize knowledge on how trophic energy transfer in seagrass systems.

Acknowledgements

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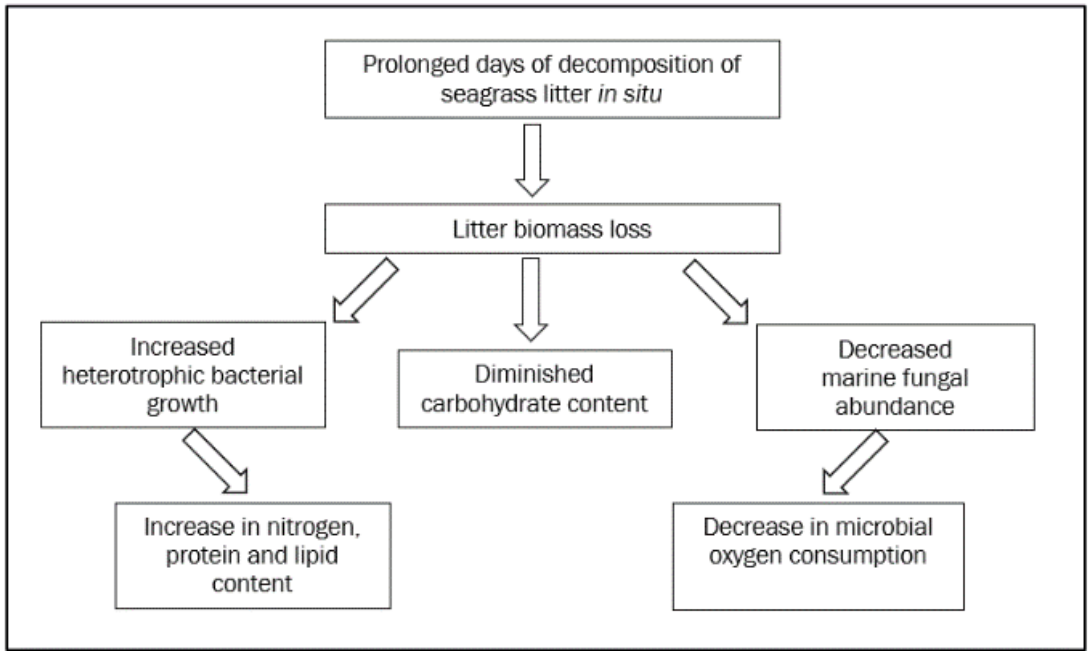

Figure 7 Conceptual model showing the interrelations between the variables contributing to the decomposition of *Cymodocea rotundata*.

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