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Leaching of dissolved organic matter from seagrass leaf litter and its biogeochemical implications

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Abstract

Dissolved organic matter (DOM) represents a significant source of nutrients that supports the microbial-based food web in seagrass ecosystems. However, there is little information on how the various fractions of DOM from seagrass leaves contributed to the coastal biogeochemical cycles. To address this gap, we carried out a 30-day laboratory chamber experiment on tropical seagrasses *Thalassia hemprichii* and *Enhalus acoroides*. After 30 days of incubation, on average 22% carbon (C), 70% nitrogen (N) and 38% phosphorus (P) of these two species of seagrass leaf litter was released. The average leached dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) of these two species of seagrass leaf litter accounted for 55%, 95% and 65% of the total C, N and P lost, respectively. In the absence of microbes, about 75% of the total amount of DOC, monosaccharides (MCHO), DON and DOP were quickly released via leaching from both seagrass species in the first 9 days. Subsequently, little DOM was released during the remainder of the experiment. The leaching rates of DOC, DON and DOP were approximately 110, 40 and 0.70 μmol/(g·d). Leaching rates of DOM were attributed to the nonstructural carbohydrates and other labile organic matter within the seagrass leaf. *Thalassia hemprichii* leached more DOC, DOP and MCHO than *E. acoroides*. In contrast, *E. acoroides* leached higher concentrations of DON than *T. hemprichii*, with the overall leachate also having a higher DON: DOP ratio. These results indicate that there is an overall higher amount of DOM leachate from *T. hemprichii* than that of *E. acoroides* that is available to the seagrass ecosystem. According to the logarithmic model for DOM release and the *in situ* leaf litter production (the Xincun Bay, South China Sea), the seagrass leaf litter of these two seagrass species could release approximately 4×10^3 mol/d DOC, 1.4×10^3 mol/d DON and 25 mol/d DOP into the seawater. In addition to providing readily available nutrients for the microbial food web, the remaining particulate organic matter (POM) from the litter would also enter microbial remineralization processes. What is not remineralized from either DOM or POM fractions has potential to contribute to the permanent carbon stocks.

Key words: dissolved organic matter, *Thalassia hemprichii*, *Enhalus acoroides*, leaf litter, leaching

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1 Introduction

Seagrass beds rank among the most productive autotrophic ecosystems on the planet, desipte only covering 0.15% of global sea surface area (Duarte and Chiscano, 1999; Hemminga and Duarte, 2000). A large fraction of seagrass production (up to 50%) is allocated to the growth of aboveground biomass (Duarte et al., 1998), however the relatively high C:N:P ratios and low palatability of seagrass leaves (Vizzini et al., 2002; Duarte et al., 2010) leads to the generally low use of seagrass production by herbivores (Cebrián et al., 1996; Cebrian and Duarte, 2001). Instead, most seagrass leaf production senesces and contributes to the detrital pool (Cebrián et al., 1996; Chiu et al., 2013). Decomposition within the seagrass ecosystems is common for senesced seagrass leaves (Mateo et al., 2006), although it has been recently found that seagrass production can contribute significant biomass to other habitats via export (Duarte and Krause-Jensen, 2017). According to global data summarized, Duarte and Krause-Jensen (2017) estimated that 50% of seagrass biomass produced is decomposed, with export and herbivory accounting for 24% and 19%, respectively.

Leaf litter that enters the decomposition process provides a source of C and nutrient recycling within seagrass meadows and neighboring ecosystems (Ziegler and Benner, 1999; Holmer and Olsen, 2002; Yarbro and Carlson, 2008; Jiménez et al., 2017). The

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decomposition of seagrass leaves begins with a rapid initial leaching or autolytic production of dissolved organic matter (DOM), which typically represents most of the labile organic matter content of seagrass leaf (Peduzzi and Herndl, 1991; Lavery et al., 2013). Subsequently, microbial breakdown of more recalcitrant organic matter (e.g., lignin and cellulose) could last for months to years (Godshalk and Wetzel, 1978; Peduzzi and Herndl, 1991). Most of DOM released from seagrass leaf litter occurs during the first few weeks (Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014), but can continue for many months as progressively more cell walls are penetrated by microbes (Harrison, 1989). For example, Lavery et al. (2013) found the *Posidonia sinuosa* released approximately 50% DOC in the first 14 days and estimated it would take about 3 years to release the next 50% DOC. The DOM that is released from seagrass leaf litter provides an important ecosystems service as it supports microbial production and thus microbial-based food webs (Robertson et al., 1982; Vähätalo and Søndergaard, 2002; Lavery et al., 2013). Therefore, seagrass leaf senescence, abscission and subsequent decomposition together represent an ample and constant source of DOM to the ecosystems (Kirkman and Reid, 1979; Mateo and Romero, 1996; Ziegler and Benner, 1999; Apostolaki et al., 2009).

The leaching process can be easily predicted and quantified using a single-component exponential decay model (Maie et al., 2006; Lavery et al., 2013). However, most studies describing these DOM leaching dynamics focus on DOC and chromophoric DOM (CDOM) (Vähätalo and Søndergaard, 2002; Vichkovitten and Holmer, 2004; Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014). As a result, there is little information on the contributions of seagrass dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) to seagrass meadow nutrient cycling. In order to fill in these research gaps, we used a laboratory incubation technique to reveal the release dynamics of DOM from the two tropical seagrass species: *Thalassia hemprichii* and *Enhalus acoroides*. Our aim was to investigate the species-specific leaching patterns of the different DOM fractions: DOC, monosaccharide (MCHO), DON and DOP. The results will help to illuminate the DOM availability of leachate from seagrass leaf litter in a tropical meadow to strengthen our understanding of the effects of seagrass leaf detritus on the biogeochemistry in seagrass ecosystems.

2 Materials and methods

2.1 *Study site and sample collection*

A mixed tropical seagrass meadow lies in the southern shallow waters of the Xincun Bay (18°24′34″–18°24′42″N, 109°57′42″–109°57′58″E), South China Sea, dominated by *Thalassia hemprichii* (Ehrenb. ex Solms) Asch. and *Enhalus acoroides* (L.f.) Royle (Huang et al., 2006). The *T. hemprichii* and *E. acoroides* meadows occupy about 50% and 40% of southern shallow water region in the Xincun Bay (unpublished data), respectively, and are an important source for the Xincun Bay DOM (Liu et al., 2016b). A vast amount of leaf litter of these two tropical seagrass species accumulate in the southern shallow waters of the Xincun Bay. The oldest leaf blades still attached to the shoots were collected from seagrass meadows during low tide. After collection, the seagrass leaves were transported to the laboratory, scraped free of epiphytes using a razor blade, washed with ambient seawater, then cut into pieces for the leaching experiment.

2.2 *Experimental setup*

Before the experiment, a separate subset of seagrass leaves was dried to a constant weight in order to calculate the wet weight: dry weight conversion of the two seagrass species. All glass bottles used in the sample collection and experimental process were acid-cleaned (7 days in 10% HCl), rinsed with Milli-Q water and then pre-combusted (500°C, 5 h) for removing organic carbon. For the leaching experiment, 20 g wet weight (approximately 2 g dry weight) of senescent seagrass leaves were placed into acid-washed 1 000-mL glass serum bottles (n=3) (Maie et al., 2006). Next, 500 mL of sterile, artificial seawater (using the *in situ* salinity of 31) and 2.0 mL saturated $HgCl_2$ solution was added to each bottle to ensure no microbes were active for the experiment, i.e., a "microbe-independent" leaching experiment (Wang et al., 2014). All bottles were left open to the atmosphere, covered slackly with clean aluminum foil and incubated at room temperature (25°C) in the dark for 30 days. At selected times (Days 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30), the incubating seawater was collected for DOC and nutrient measurements. The fresh artificial seawater was analyzed for the initial background contents of DOC and nutrients. In between sampling times, water level was monitored during the incubation, and analyzed concentrations were adjusted for water evaporation losses (Maie et al., 2006). After the 30-day incubation, the seagrass leaves were dried to constant weight and the dry mass measured.

2.3 *Sample analysis*

Water samples were filtered through pre-combusted (450°C for 4 h) GF/F filters. For DOC analysis, samples were stored in acid-washed brown apragaz bottles at -20°C before analysis on a TOC analyzer (TOC-V_{CPH}, Shimadzu, Japan). MCHO was determined by the TPTZ (2, 4, 6-tripyridyl-s-triazine) method (Myklestad et al., 1997). Briefly, samples were measured by oxidizing the free reduced sugar with Fe^{3+} in alkaline conditions, followed by spectrophotometric analysis (UV-2600, Shimadzu, Japan) of a colored product of reduced Fe^{2+} and TPTZ. DON was calculated as the difference between total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN=NO_3-N+NO_2-N+NH_4^+-N). DOP was determined as the difference between total dissolved phosphorus (TDP) and dissolved inorganic phosphorus (DIP=PO_4^{3-}) (Bronk et al., 2000; Barrón and Duarte, 2009). TDN and TDP were measured with the persulfate oxidation method (Valderrama, 1981; Bronk et al., 2000; Pierzynski, 2000). DIN and DIP were measured spectrophotometrically according to standard colorimetric techniques following the methods developed by Grasshoff et al. (2009) using a spectrophotometer (UV-2600, Shimadzu, Japan).

At the beginning of the experiment, the leaf nonstructural carbohydrate content was measured. After drying of the leaf litter, the leaf samples were ground to a size of 0.18 mm (#80-mesh sieve). Approximately, 0.1 g was extracted twice in hot 80% ethanol. The soluble sugar content was determined by the anthrone-sulfuric acid method (Yemm and Willis, 1954). Subsequently, the starch content of the remaining materials was quantified by gelatinization at 100°C for 15 min and solubilization in 70% perchloric acid (Allen, 1989). Nonstructural carbohydrate content was estimated by multiplying soluble sugar and starch concentrations (nonstructural carbohydrate=soluble sugar+starch) (Orth and Moore, 1986; Burke et al., 1996). In addition, the dry weight (DW) and elemental contents of the leaf litter before and after the incubation were measured as well. Leaf samples were oven-dried at 60°C to a constant weight for determining the leaf DW. Elemental C and N using the dried seagrass were measured by Elemental CHNS analyzer model Vario EL cube (Vario EL, Elemental Analyser systeme GmbH, Germany). The elemental P

content was analyzed by a colorimetric method (Fourqurean et al., 1992). Each sample of above parameters was measured two or three times with a coefficient of variation of ⩽2%.

2.4 *Data analysis*

Leaf characteristics, releasing rates, cumulative releasing concentrations of DOC, MCHO, DON and DOP and their stoichiometry ratios during the 30-day incubation were compared between the two seagrass species using student's *t*-test. Data were log transformed if the assumption of homogeneity of variance was violated. The seagrass species effect with α<0.05 was considered statistically significant. Meanwhile, the effect sizes of releasing rates, cumulative releasing concentrations of DOC, MCHO, DON and DOP, and their stoichiometry ratios between the two seagrass species were calculated by using Cohen's *d* test. Napierian logarithm functions were carried out for fitting the accumulation concentrations of DOM from seagrass leaf litter (Maie et al., 2006; Lavery et al., 2013). All above mentioned statistical analysis was performed with Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA), SigmaPlot 12.0 (Systat Software Inc., Chicago, IL) and IBM SPSS Statistics 19.0 software (IBM SPSS Statistics 19, IBM Corporation, Somers, NY).

3 Results

3.1 *Characteristics of seagrass leaves*

Characteristics of the seagrass leaves in the Xincun Bay used for the experiments were summarized in Table 1. There were no significant differences in leaf soluble sugar, starch, C and N content between the two seagrass species. However, the soluble sugar in the leaves of *T. hemprichii* was slightly lower than that of *E. acoroides*, while the opposite was shown for species differences in starch and nonstructural carbohydrates. Elemental contents were similar between *T. hemprichii* and *E. acoroides*. Additionally, after the 30-day incubation, the remaining weights of both seagrass species were ~70% of the initial DW. There was no significant difference in the elemental content between the two seagrass species after 30 days of decomposition. In addition, there was approximately 22% C, 70% N and 38% P released from the seagrass during the 30 days leaching period. In other words, the C, N and P released from the seagrass leaf litter were about 6 000, 1 200 and 30 μmol/g (DW), respectively.

3.2 *Characteristics of leaching dissolved organic matter*

More than 75% of the total DOC, MCHO, DON and DOP was leached from the two species of seagrass leaf litter in the first 9 days (Fig. 1). The accumulated leachate concentrations of DOC, DON and DOP (over the 30 days) were observed not to be significantly different between the two seagrass species (Figs 1a, c, d; p>0.05). However, the comparison (*T. hemprichii* vs. *E. acoroides*) of effect sizes of DOC, DON and DOP using Cohen's *d* were 0.87, –1.87 and 8.48, respectively (small effect=0.2, medium effect=0.5, large effect=0.8). Additionally, the MCHO released from *T. hemprichii* leaf litter was 1.6-fold higher than *E. acoroides* after the 30-day incubation (Fig. 1b, p<0.05, Cohen's d=6.28). There were no significant differences of the leaching rates of DOM between the two seagrass species (Figs 2a, c, d; p>0.05), with the exception of MCHO (Fig. 2b, p<0.05). Meanwhile, the Cohen's *d* test of leaching rates (*T. hemprichii* vs. *E. acoroides*) of DOC, MCHO, DON and DOP were 0.87, 4.45, –0.75 and 3.01, respectively. Furthermore, the released amount of DOC, MCHO, DON and DOP were all described as napierian logarithm functions (Fig. 1, Table 2). However, the DOC, DON and DOP leached from leaf litter were lower than the C, N and P content lost from the leaf litter, respectively. The DOC, DON and DOP accounted for 55%, 95% and 65% of the total C, N and P amounts loss of seagrass leaf litter, respectively.

Over the 30-day incubation, the DOC:DON:DOP ratio of the released DOM from *T. hemprichii* and *E. acoroides* were 161:50:1 and 169:69:1, respectively. In addition, the DON:DOP ratio of the total released DOM from *T. hemprichii* was significantly lower than that of *E. acoroides* (*T. hemprichii* vs. *E. acoroides*, p<0.05, Cohen's d=–0.82). In contrast, the DOC:DON (*T. hemprichii* vs. *E. acoroides*, p>0.05, Cohen's d=0.54) and DOC:DOP (*T. hemprichii* vs. *E. acoroides*, p>0.05, Cohen's d=–0.15) were similar between the two seagrass species (Table 3).

4 Discussion

Nonstructural carbohydrates and amino acids reflect much of the labile organic carbon in seagrass leaves and are typically released during the initial leaching phase of decomposition (Vichkovitten and Holmer, 2004; Lavery et al., 2013). The variation of nonstructural carbohydrate, soluble sugar and starch content of *T. hemprichii* and *E. acoroides* was much higher than previous reports for *Cymodocea* spp., *Halodule* spp., *Halophila spinulosa*, *Syringodium isoetifolium* and *Zostera muelleri*, but lower than *Amphibolis griffithii*, *Syringodium filiforme*, *Zostera marina* and *Zostera noltii* (Table 4). The DOC releasing rates of our study (119.2 μmol/(g·d)) was most similar to that of *Thalassia testudinum* (127.8 μmol/(g·d)) (Maie et al., 2006), but less than other morphologically smaller (sub)tropical taxa, e.g., *S. filiforme* (288 μmol/(g·d)) (Wang et al., 2014).

On account of the nutrients released from seagrass leaf litter contained not only the DOC, DON and DOP but also the inorgan-

Table 1. The chemical characteristics (soluble sugar, starch, nonstructural carbohydrates, element contents and the total elemental weights for experiment) of seagrass leaves before and after the decomposition incubation experiments (mean±SEM)

Parameters	Before leaching		After leaching	
	Thalassia hemprichii	*Enhalus acoroides*	*Thalassia hemprichii*	*Enhalus acoroides*
Soluble sugar/mg·g^{-1} (DW)	32.0±8.3	35.5±6.5	–	–
Starch/mg·g^{-1} (DW)	47.3±8.7	40.2±11.4	–	–
Nonstructural carbohydrates/mg·g^{-1} (DW)	79.2±17.1	75.7±17.9	–	–
C/% (DW)	33.9±1.2	32.1±1.6	36.8±1.8	36.5±1.0
N/% (DW)	2.30±0.39	2.52±0.15	1.04±0.13	1.02±0.20
P/% (DW)	0.22±0.03	0.28±0.03	0.19±0.04	0.25±0.05
Total C weight/mg (DW)	707±51.8	683±68.5	548±76.9	533±74.5
Total N weight/mg (DW)	48.0±7.8	53.7±6.7	15.5±3.8	14.9±1.1
Total P weight/mg (DW)	4.59±0.16	5.86±0.57	2.83±0.14	3.66±0.23

Note: There were no significant differences for between-species comparisons (p>0.05). DW represents dry weight.

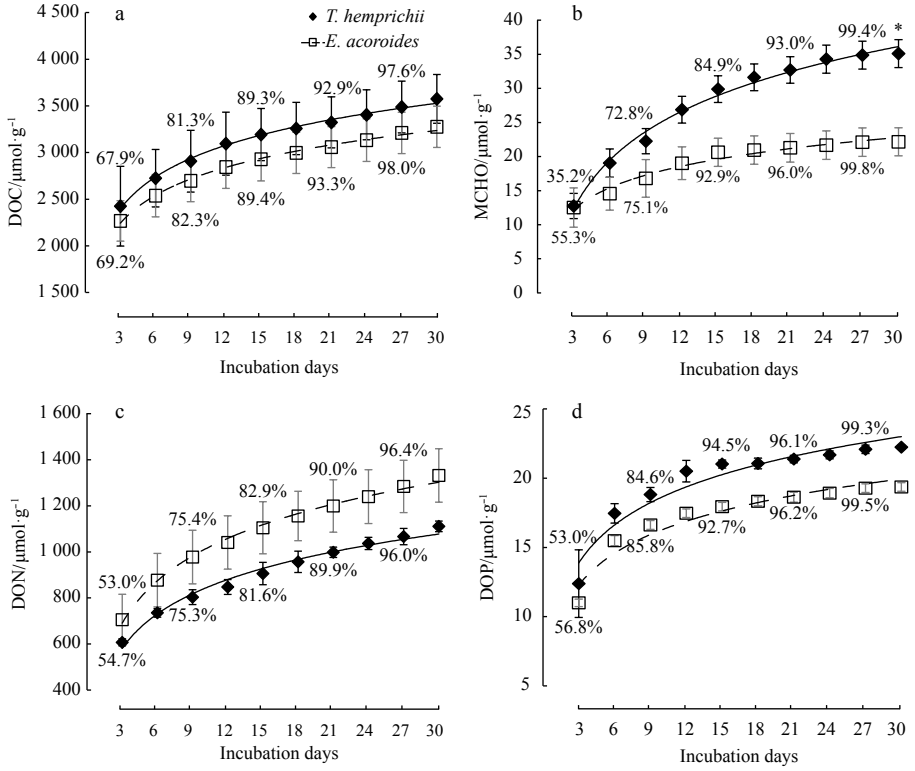

Fig. 1. The cumulative leaching of DOC (a), MCHO (b), DON (c) and DOP (d) from *T. hemprichii* and *E. acoroides* leaf litter during the 30-day incubation in the absence of microbes. Values represent means±SEM. No significant differences between the seagrass species for the DOC, DON and DOP cumulative concentrations were found. Asterisks represents significantly different of MCHO cumulative concentrations between the seagrass species after the 30-day incubation. The molar percentages of the DOM that was leached from *T. hemprichii* and *E. acoroides* (Days 3, 9, 15, 21, and 27) were shown in above or below the error bars.

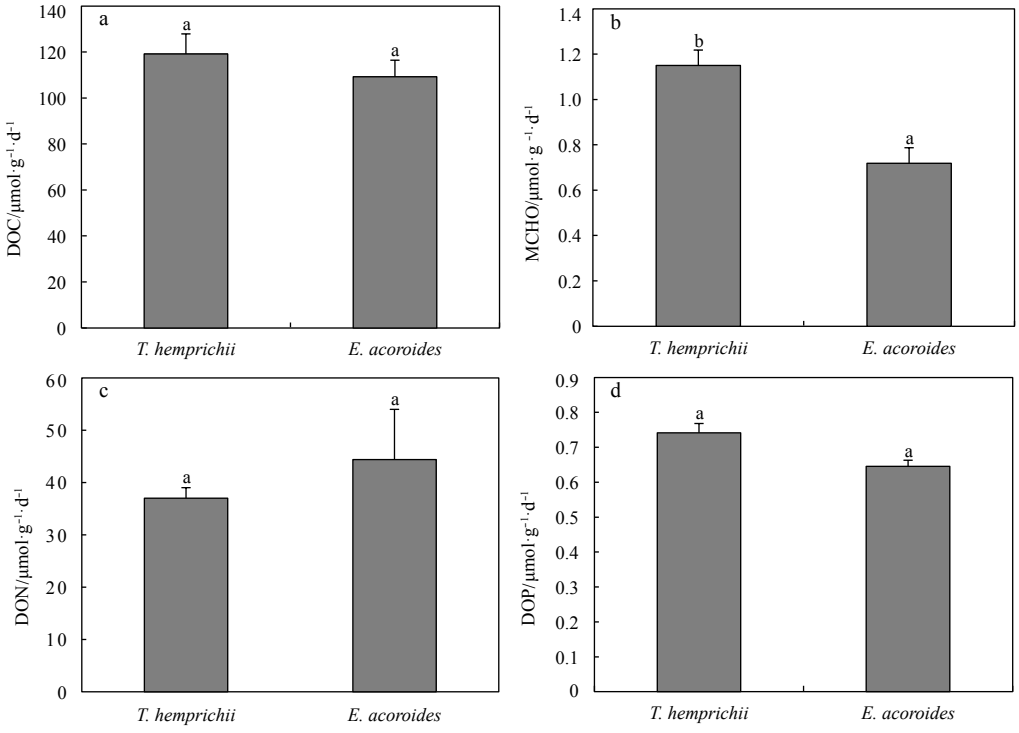

Fig. 2. The leaching rates of DOC (a), MCHO (b), DON (c) and DOP (d) from *T. hemprichii* and *E. acoroides* leaf litter in the absence of microbes after the 30-day incubation. Different superscript letters indicate that the differences in ratios between the two seagrass species were significant (p<0.05). Values are represented as means±SEM.

Table 2. Single logarithm function fitted to the accumulation of DOM fractions over 30 days of leaching

DOM fraction	*Thalassia hemprichii*	*Enhalus acoroides*
DOC	y=1 859.5+490.6ln$x$, R^2=0.995 7	y=1 770.1+431.1ln$x$, R^2=0.995 1
MCHO	y=0.340 0+10.35ln$x$, R^2=0.992 3	y=6.595+4.589ln$x$, R^2=0.972 0
DON	y=344.85+251.4ln$x$, R^2=0.983 1	y=398.44+265.7ln$x$, R^2=0.994 5
DOP	y=9.523+3.964ln$x$, R^2=0.924 6	y=8.613+3.331ln$x$, R^2=0.933 7

Note: x is time in days and y accumulation concentration (μmol/g DW).

Table 3. Ratios of the composition of DOM released from *T. hemprichii* and *E. acoroides* leaf litter

Seagrass species	DOC:DON	DOC:DOP	DON:DOP	DOC:DON:DOP
Thalassia hemprichii	3.22±1.23^a	161±36.8^a	50.0±11.6^a	161:50:1
Enhalus acoroides	2.46±0.67^a	169±40.6^a	68.8±19.8^b	169:69:1

Note: Different superscript letters indicate the differences in ratios between the two seagrass species were significant (p<0.05). Values are represented as means±SEM.

Table 4. Review of literature that reports nonstructural carbohydrate, soluble sugar and starch content for seagrass leaf (nonstructural carbohydrate=soluble sugar+starch)

Species	Nonstructural carbohydrate	Soluble sugar	Starch	References
Amphibolis griffithii	188	38	150	Mackey et al. (2007)
Cymodocea rotundata	9	1	8	Lawler et al. (2006)
Cymodocea serrulata	3	1	2	Lawler et al. (2006)
Halodule uninervis	32	22.1	9.9	Sheppard et al. (2007)
Halodule spinulosa	15	1	14	Lawler et al. (2006)
Halodule minor	9	1	8	Lawler et al. (2006)
Halophila engelmannii		52–124		Dawes et al. (1987)
Halophila ovalis	102	30	72	Longstaff et al. (1999)
Halophila spinulosa	19.2	12.9	6.3	Sheppard et al. (2007)
Posidonia oceanica	40–140			Invers et al. (2004)
Posidonia sinuosa	78	28	50	Collier et al. (2009)
Syringodium filiforme	170–220			Siegal-Willott et al. (2010)
Syringodium isoetifolium	35	19	16	Lawler et al. (2006)
Thalassia testudinum	46–70			Lee and Dunton (1996)
Zostera muelleri	34.3	24.3	10	Sheppard et al. (2007)
Zostera marina	111.13	78.53	32.6	Burke et al. (1996)
Zostera noltii	95.2	90.4	4.8	Olivé et al. (2007)
Thalassia hemprichii	79.2	32.0	47.3	this study
Enhalus acoroides	75.7	35.5	40.2	this study

Note: All data are reported as mg/g (DW).

ic components and other elemental organic matter (Lavery et al., 2013; Jiménez et al., 2017). Thus the DOM content was lower than the total mass lost for each seagrass species. However, we can roughly estimate that the leached DOM accounted for 30% of the lost materials of each seagrass. The average DOC:DON of the two seagrasses in this study was far below the refractory DOM stoichiometry ratios (16:1) for the open ocean (Aminot and Kérouel, 2004), but was close to the *in situ* winter seawater DOC:DON ratio of DOM of the Xincun Bay (6:1) (Liu et al., 2016b). The DOC:DON:DOP ratios from seagrass leachate in this study supports the previous hypothesis that there are high contributions of seawater DOM originating from seagrass sources in winter, while the other contributions to sweater DOM is mainly from river runoff (Liu et al., 2016b). This suggests that the seagrass DOM could be providing substantial nutrients to *in situ* microbial communities (Peduzzi and Herndl, 1991; Vähätalo and Søndergaard, 2002; Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014). For example, Vähätalo and Søndergaard (2002) found 28% of leached DOC was taken up by microorganisms during 30 days. Additionally, the relatively low DOC:DON ratio is likely linked to the high historical nutrient loading that occurs in the Xincun Bay (Liu et al., 2016a), and thus can alter the N content of the leaf standing stock (Duarte, 1990). Nevertheless, we cannot calculate the exact percentage of the seagrass leaf litter contribution to the DOM of *in situ* water column only according to the DOM contents and the ratios. As a matter for future research, we recommend the δ^{13}C analyses of DOC to evaluate the exact percentage of the seagrass leaf litter contribution to the water column DOM.

We would expect that after DOM release that DOP would be preferentially remineralized in the seawater due to P limitations in many tropical seagrass ecosystems, followed by N and C (Ogawa and Tanoue, 2003; Aminot and Kérouel, 2004; Ziegler et al., 2004). The DON:DOP of the DOM leachate from *T. hemprichii* was lower than what was released from *E. acoroides*, suggesting that the DOM from *T. hemprichii* may contribute more dissolved nutrients to the ecosystems than *E. acoroides*. Furthermore, MCHO, one of the highest bioavailability substances among different carbohydrates, can directly and quickly be assimilated by microbes (Peduzzi and Herndl, 1991; Vichkovitten and Holmer, 2004). Previous studies suggest the leached DOM of leaf litter from different seagrass species have distinct bioavailability linked to nonstructural carbohydrate, protein-associated DOM (Maie et al., 2006; Lavery et al., 2013; Wang et al., 2014). Though

the nonstructural carbohydrates were similar between the *T. hemprichii* and *E. acoroides*, the higher releasing rate and amount of MCHO also suggested the higher bioavailability of DOM leaching from *T. hemprichii* than *E. acoroides*.

More than 75% of the total DOM was released during first 9 days, which is similar to those found in previous studies (Maie et al., 2006; Chiu et al., 2013). The releasing rates of DOC, DON and DOP declined rapidly after the first 9 days and remained steady for the remainder of the experiment. The average aboveground biomass of *T. hemprichii* and *E. acoroides* in the Xincun Bay has been reported to be about 43 g/m^2 and 64.24 g/m^2, respectively (Liu et al., 2016a). According to the seagrass distribution area, the total biomass of *T. hemprichii* and *E. acoroides* in the Xincun Bay has been estimated to be 4.33×10^7 g DW and 5.14×10^7 g DW, respectively. Chiu et al. (2013) calculated that about 80% leaf production of *T. hemprichii* can become leaf detritus in southern tip of Taiwan. In consideration of approximate geographical conditions of the Xincun Bay with Chiu et al. (2013) study area, we assume the *T. hemprichii* and *E. acoroides* leaf production is similar. In addition, the *T. hemprichii* and *E. acoroides* leaf turnover time is approximately 45 days and 105 days, respectively (Hemminga et al., 1999). Therefore, the total leaf litter production of *T. hemprichii* and *E. acoroides* is proximately 1.16×10^6 g/d in the Xincun Bay. Assuming the leaching period only last 30 days, and using the DOM leaching rates and the estimated leaf litter production, we estimate that the DOM production in the Xiucun Bay is about 4 035 mol/d DOC, 1 377 mol/d DON and 25 mol/d DOP. Although we only performed a microbe-independent leaching experiment, the microbes do not contribute much to the direct loss of organic matter from detrital leaves of seagrass during initial leaching period (Harrison and Mann, 1975; Vähätalo and Søndergaard, 2002). Rather, the leaf-associated microbial communities play a more active role later on in the decomposition process via enzymatic remineralization of the detritus (Godshalk and Wetzel, 1978). Thus the estimated amount of DOM leached from seagrass was reasonable without the presence of microbes. These sources of DOM provide important pathways for transferring vascular plant production to the microbial food webs and higher trophic levels in seagrass and adjacent coastal waters in the Xincun Bay. In addition, the average DOC:DON:DOP ratios (162:55:1) leached by the two seagrasses was much lower than terrestrial DOC:DON:DOP (1 368:232:1) in the Xincun Bay (Liu et al., 2016b), indicating higher bioavailability of DOM leaching from seagrass than the terrestrial DOM. Furthermore, seagrass leaves contain recalcitrant organic matter and calcium carbonate that likely survives the leaching phase and may contribute to sediment carbon sequestration (Trevathan-Tackett et al., 2017) and sand for the beach or/and dune system (Jiménez et al., 2017), respectively.

5 Conclusions

After 30 days of leaching, about 22%, 70% and 38% of seagrass C, N and P were released, respectively, for near-senescence seagrass leaves. The estimates above indicate that significant quantities of seagrass-sourced DOM is released to the water column independent of microbial processes. Both species contributed similar quantity of DOM, with *T. hemprichii* possibly being of greater bioavailability to the food webs in seagrass meadows. Meadow-wide, the seagrass leaf litter can provide more than 4 000 mol/d DOC, 1 350 mol/d DON and 25 mol/d DOP to the Xincun Bay. However, previous reviews suggest that this rate of DOM release could last several months longer than the timeframe of this study due to further breakdown of the cell walls by microbes (Harrison, 1989; Peduzzi and Herndl, 1991; Mateo and Romero, 1996; Chiu et al., 2013). Therefore, the microbial-dependent release of DOM as well as the remaining POM represent two different pools of C, N and P to the ecosystem. We recommend the use of microbe-centric analyses (e.g., gene expression) in combination of DOM and POM chemical analyses to better understand micro-level exchanges between microbial communities and the nutrients seagrasses provide to create a complete the OM cycle and stoichiometry budget for the Xincun Bay.

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