frontiers
in Marine Science

The Influence of CO$_2$ Enrichment on Net Photosynthesis of Seagrass *Zostera marina* in a Brackish Water Environment

Liina Pajusalu*, Georg Martin, Arno Põllumäe and Tiina Paalme

Department of Marine Biology, Estonian Marine Institute, University of Tartu, Tallinn, Estonia

Seagrasses are distributed across the globe and their communities may play key roles in the coastal ecosystems. Seagrass meadows are expected to benefit from the increased carbon availability which might be used in photosynthesis in a future high CO$_2$ world. The main aim of this study was to examine the effect of elevated pCO$_2$ on the net photosynthesis of seagrass *Zostera marina* in a brackish water environment. The short-term mesocosm experiments were conducted in Kõiguste Bay (northern part of Gulf of Riga, the Baltic Sea) in June–July 2013 and 2014. As the levels of pCO$_2$ naturally range from ca. 150 µatm to well above 1000 µatm under summer conditions in Kõiguste Bay we chose to operate in mesocosms with the pCO$_2$ levels of ca. 2000, ca. 1000, and ca. 200 µatm. Additionally, in 2014 the photosynthesis of *Z. marina* was measured outside of the mesocosm in the natural conditions. In the shallow coastal Baltic Sea seagrass *Z. marina* lives in a highly variable environment due to seasonality and rapid changes in meteorological conditions. This was demonstrated by the remarkable differences in water temperatures between experimental years of ca. 8°C. Thus, the current study also investigated the effect of elevated pCO$_2$ in combination with short-term natural fluctuations of environmental factors, i.e., temperature and PAR on the photosynthesis of *Z. marina*. Our results show that elevated pCO$_2$ alone did not enhance the photosynthesis of the seagrass. The photosynthetic response of *Z. marina* to CO$_2$ enrichment was affected by changes in water temperature and light availability.

Keywords: carbon dioxide, *Zostera marina*, Baltic Sea, marine acidification, net photosynthesis, seagrass, brackish water

INTRODUCTION

Over the last 20 years, it has been established that the pH of the world's oceans is decreasing which is caused primarily by the uptake of carbon dioxide (CO$_2$) from the atmosphere, also known as ocean acidification (OA). Since the start of the Industrial Revolution in the late eighteenth century, atmospheric carbon dioxide concentration has increased by ∼40% primarily due to the burning of fossil fuels (Raven et al., 2005). The surface-ocean has absorbed ∼30% of anthropogenic CO$_2$ released from the atmosphere, which causes fundamental changes in seawater carbonate chemistry (Sabine et al., 2004). The mean surface ocean pH has already fallen by ∼0.1 units and if global emissions of CO$_2$ continue to rise, the pH may decrease 0.3–0.4 units by the year 2100 (IPCC, 2013).

The projected range of pH changes are not uniform across the globe and there are regional differences. Models project that the surface water pH in the central Baltic Sea may decrease 0.3–0.4 units by 2100 (Omstedt et al., 2012; Schneider et al., 2015). Moreover, the brackish Baltic Sea is sensitive to a future increase in acidity due to low carbonate buffering capacity, which is related to its low salinity, particularly in the northern parts (Hjalmarsson et al., 2008; Omstedt et al., 2015). However, Hjalmarsson et al. (2008) found high alkalinity in the Gulf of Riga caused by extensive inputs of freshwater from the river runoff. In contrast to the open sea, the shallow coastal waters of the Baltic Sea are characterized by high temporal (diurnal and seasonal) and spatial variation of environmental factors (Feistel et al., 2008). Besides light and water temperature, pH and partial pressure of carbon dioxide (pCO_2) show a substantial amplitude of natural variability, especially under summer conditions. These daily pH changes may be of a larger magnitude than the scenario modeling suggests for the surface-water pH decrease in the Baltic Sea by the end of this century (personal measurements, 2013, 2014). However, biological responses to the elevated water CO_2 concentrations in combination with these short-term natural fluctuations of environmental factors have generally received little attention.

Seagrass communities play a key role in the coastal ecosystems. They are important primary producers on soft bottoms (Larkum et al., 2006), contribute to sediment stabilization (Duarte, 2002; Orth et al., 2006), carbon sequestration (Duarte et al., 2005), cycling of nutrients and providing shelter and food for the coastal food webs (Duarte, 2002). Seagrasses are distributed across the globe and there are ∼60 seagrass species (Orth et al., 2006). Unfortunately, seagrass communities are globally in serious decline (Orth et al., 2006; Waycott et al., 2009) mainly due to eutrophication, habitat destruction and climate change (Duarte, 2002).

Seagrass meadows are expected to be influenced by the increase in seawater acidity in the future. However, the response of seagrasses to elevated pCO_2 can vary depending on the seagrass species as well as the habitat (Koch et al., 2013 and references therein). Besides, observed seagrass responses to CO_2 enrichment can be affected by the methods used. Numerous studies have been conducted near natural CO_2 vents, which allow assessment of the long-term effects of ocean acidification (Hall-Spencer et al., 2008; Fabricius et al., 2011; Porzio et al., 2011; Russell et al., 2013; Takahashi et al., 2015), although it is not always clear in these studies whether observed responses are exclusively driven by changes in CO_2 concentration. There are no underwater CO_2 vents in the Baltic Sea, so we used mesocosms to investigate the effects of OA in the field.

This study focuses on the seagrass *Zostera marina*, which is a widely distributed seagrass of the temperate coastal waters in the northern hemisphere (den Hartog, 1970; Moore and Short, 2006). Seagrass *Z. marina* is one of the most common macrophytes on the species-poor sandy bottoms and is regarded as a key species of this habitat found throughout the Baltic Sea. These plants provide a secondary substrate for a diverse epiflora and fauna (Boström et al., 2014 and references therein). The majority of investigations on the distribution of *Z. marina* have shown a significant decline of their meadows mainly due to eutrophication in Danish, Swedish, and Polish coastal ecosystems (Boström et al., 2014 and references therein). However, in the Estonian coastal waters (NE Baltic Sea), the effect of eutrophication on their communities is less obvious compared to the other parts of the Baltic Sea (Möller and Martin, 2007).

While the effects of eutrophication on seagrass meadows in the Baltic Sea are well documented, the impacts of the increasing water CO_2 concentrations on *Z. marina* in the Baltic Sea are still mainly unknown. Previous research has shown substantial effect of warming and small positive effect of elevated CO_2 on the growth of *Z. marina* from the Kattegat region of the Baltic (Eklöf et al., 2012). Outside of the Baltic Sea, there are several studies describing the effects of elevated pCO_2 on the temperate seagrass species *Z. marina*. These studies have shown increased growth rate, enhanced photosynthesis, increased reproduction and below-ground biomass of *Z. marina* under elevated pCO_2 (Thom, 1996; Zimmerman et al., 1997; Palacios and Zimmerman, 2007). Brodie et al. (2014) pointed out that *Z. marina* is likely to thrive at high latitudes although the habitat will be altered considerably due to carbonate undersaturation.

The main goal of this study was to examine the effect of elevated pCO_2 on the net photosynthesis of seagrass *Z. marina* in the brackish water conditions. The second goal of this study was to determine whether elevated pCO_2 in combination with short-term natural fluctuations of environmental factors exerts interactive effects on the photosynthetic rate of *Z. marina*. Our hypotheses were that the photosynthetic rate of *Z. marina* increases with the elevated pCO_2 levels and that the response would vary depending on the surrounding meteorological conditions. We tested these hypotheses *in situ* in short-term mesocosm experiments during two different experimental years under summer conditions in the north-eastern Baltic Sea.

MATERIALS AND METHODS

Collection of Seagrass *Zostera marina*

The specimens of *Zostera marina* were collected by SCUBA diving at a depth of 3.0 m from Küdema Bay (58.533°N, 22.238°E) in June 2013 and in June 2014. Whole shoots were carefully removed from the sandy sediment to keep shoots with connected bundles and horizontal rhizomes intact. All specimens were placed in coolers containing water collected at the site and transported to the experimental site immediately.

Experimental Design

The mesocosm experiments were conducted in the shallow semi-enclosed Kõiguste Bay, Gulf of Riga, northern Baltic Sea (58.371°N, 22.980°E). The study area is affected by nutrient inputs from the moderately eutrophic brackish Gulf of Riga (Astok et al., 1999; Kotta et al., 2008).

The experimental design used in the current study has been previously used in a number of studies targeting different macrophyte species (e.g., Pajusalu et al., 2013, 2015, 2016). The mesocosm experiments were conducted during two experimental periods in 2013 and 2014 (18 July 2013–27 July 2013 and 16 July 2014–26 July 2014). The experimental design was identical

during both years of incubation. Approximately 20 *Z. marina* specimens were transplanted to each plastic pot containing the mixture of fine gravel and sand (roughly 20:80). In the both experimental years, the plant material was acclimatized at the experimental site for 3 weeks prior the start of the net photosynthesis measurements. Four plastic pots with ca. 80 individual *Z. marina* specimens were incubated in each mesocosm with manipulated pCO_2 levels. In 2014 also four plastic pots were placed outside of the mesocosm in the sea. Open plastic bags (double wall of LDPE foil, 175 μm each) externally supported by metal frames were used as mesocosms. The bags were floating in the sea, fixed to the bottom by anchors at a depth of 0.8 m. The bags were open on the top, so there was free gas exchange with the atmosphere, but not with the surrounding water. Three plastic bag mesocosms, each with dimensions of 1.2 × 1.0 × 1.5 m, and a volume of 400 l were set up: two mesocosms with elevated pCO_2 levels ca. 1000 and 2000 μatm and one with the untreated level of ca. 200 μatm (control treatment). The levels of pCO_2 in the control treatment was ca. 200 μatm during the photosynthesis measurements. The pCO_2 level of 2000 μatm is much higher than the recommended maximum pCO_2 level of 1000 μatm predicted by 2100 for seawater (Barry et al., 2010). In our experiment, the high target pCO_2 level ca. 2000 μatm was chosen because natural values of pCO_2 in Kõiguste Bay were measured prior to the start of the mesocosm experiments, which turned out to be well above the concentration of 1000 μatm in the summer mornings. Water from the sea area adjacent to the mesocosms incubation site was sieved using a 0.25 mm mesh and used for mesocosms. Food grade pure carbon dioxide from CO_2 tanks was slowly bubbled into the water in the mesocosms. A custom-made controller was used to maintain elevated pCO_2 levels in the treatments. The controller switched on or off the CO_2 supply and treatment according to the CO_2 level measured by underwater (sensor) automatic CO_2 data logger (CONTROS[TM] DETECT 2.0, Germany). However, due to the response lag of the used CO_2 sensor (15–20 min), the actual CO_2 level oscillated around the lower level present by the controller. The pH_{NBS} (National Bureau of Standards scale) values of each treatment were measured during experiments. The measurements of photosynthesis were carried out between 10 a.m. and 4 p.m.

Environmental Variables

In the mesocosm experiments water temperature, oxygen saturation, pH_{NBS}, and salinity were measured continuously using a YSI 6600V2 environmental multiprobe (pH electrode YSI 6589FR). Measurements were performed during a full 24-h cycle with a frequency of 30 s. The irradiance at the incubation depths was measured as photosynthetically active radiation (PAR) using a spherical light sensor (Alec Electronics Co Ltd.). Additionally, in parallel with the photosynthesis measurements, the diurnal fluctuations of water pH, pCO_2, and oxygen saturation were measured outside the mesocosms at a depth of 0.8 m in the natural shallow water macroalgal habitat. Water samples were taken from the experimental site daily and frozen immediately until further laboratory analyses. Nutrient concentrations: total

nitrogen (TN), total phosphorus (TP), phosphates (P-PO$_4$), and nitrites + nitrates (N-NOx) were measured in a laboratory with a continuous flow automated wet chemistry analyzer Skalar SAN[plus] (Skalar Analytic B.V., De Breda, The Netherlands) using the standard methods EN ISO 11905-1[1], EN ISO 15681-2[2], and EN ISO 13395[3].

Measurements of Net Photosynthesis

The photosynthetic rate of *Z. marina* leaves at different pCO_2 levels (mesocosms) was measured once a day. For this procedure about 0.1 g (dry weight, dw) of plant material (one leaf) was incubated in 600 ml glass bottles. The glass bottles were filled with water from the inside of the mesocosm and placed horizontally on the transparent trays hanging at a depth of 0.5 m. All incubations with *Z. marina* were performed in six replicates per treatment. Bottles without plant material (in triplicate per treatment) served as controls. The dw of the plant material was determined after drying at 60°C for 48 h. Net primary productivity (NPP) (given as mg O$_2$ gdw^{-1} h^{-1}) was calculated from the differences in oxygen concentrations in incubation bottles with and without plant material, measured over the incubation period (ca. 1 h) (Paalme, 2005). The dissolved oxygen concentrations were measured with a Marvet Junior dissolved oxygen meter (MJ2000, Elke Sensor, Estonia) using the standard method EN ISO 5814[4].

Statistical Analyses

A one-factor permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations was used to statistically test single and interactive treatment effects on the net photosynthetic rates of *Z. marina*: pCO_2 was used as the fixed factor with 3 levels in 2013 and 4 levels in 2014; photosynthetic radiation (PAR) and water temperature were treated as covariates. Significant effects were explored when necessary with pairwise *post-hoc* tests (with 9999 permutations). Statistical analyses were performed using PERMANOVA (PRIMER, PRIMER-E Ltd., Plymouth, UK). For all statistical tests, a probability of 0.05 was used to determine statistical significance.

RESULTS

Environmental Variables

The average values of PAR during the experimental period were 744 ± 248.09 μmol m^{-2}s^{-1} (± standard deviation, SD) in 2013 and 642 ± 179.13 μmol m^{-2}s^{-1} in 2014. Remarkable differences in water temperature between the two experimental periods were found: the average water temperature at the experimental site was 13.5 ± 2.56°C in 2013 and 21.9 ± 1.45°C in 2014 (**Tables 1**, **2**).

TABLE 1 | Environmental variables (salinity and temperature) and carbonate parameters pCO_2 ($n = 10$) and pH_{NBS} ($n = 10$) in different treatments measured during the experimental period in 2013.

Treatment pCO_2	Salinity	T(°C)	pH	pCO_2 (μatm)
200 μatm	5.7 ± 0	13.5 ± 2.56	9.17 ± 0.15	190 ± 34
1000 μatm	5.7 ± 0	13.5 ± 2.56	7.99 ± 0.17	1010 ± 139
2000 μatm	5.7 ± 0	13.5 ± 2.56	7.54 ± 0.16	2059 ± 101

Carbonate parameters of each of the three different pCO_2 treatments sampled every day before net photosynthesis measurements over the experimental period. Data are the mean $\pm$ standard deviation, SD.

TABLE 2 | Environmental variables (salinity and temperature) and carbonate parameters pCO_2 ($n = 11$) and pH_{NBS} ($n = 11$) in different treatments measured during the experimental period in 2014.

Treatment pCO_2	Salinity	T(°C)	pH	pCO_2 (μatm)
200 μatm	5.7 ± 0	21.9 ± 1.45	8.64 ± 0.12	196 ± 41
1000 μatm	5.7 ± 0	21.9 ± 1.45	7.94 ± 0.11	1023 ± 127
2000 μatm	5.7 ± 0	21.9 ± 1.45	7.47 ± 0.09	2048 ± 98
*Natural conditions	5.7 ± 0	21.9 ± 1.45	8.40 ± 0.13	349 ± 118

Carbonate parameters of each of the three different pCO_2 treatments sampled every day before net photosynthesis measurements over the experimental period. Data are the mean $\pm$ standard deviation, SD.

*Seawater carbonate chemistry was measured in natural conditions outside of the mesocosm.

TABLE 3 | Total nitrogen (TN), total phosphorus (TP), phosphates (P-PO_4), and nitrites + nitrates (N-NO_x) content (mean of the experimental period $\pm$ standard deviation, SD) of the water in mesocosm 2013 ($n = 30$), 2014 ($n = 33$) and water of outside mesocosm 2014 ($n = 11$).

Experimental period	TN (μmol l^{-1})	TP (μmol l^{-1})	P-PO_4 (μmol l^{-1})	N-NO_x (μmol l^{-1})
2013 (water in mesocosms)	27.1 ± 5.50	0.68 ± 0.11	0.29 ± 0.09	0.37 ± 0.55
2014 (water in mesocosms)	34.1 ± 3.36	0.95 ± 0.28	0.26 ± 0.15	0.11 ± 0.11
2014 (water of outside mesocosm)	29.6 ± 3.60	0.98 ± 0.16	0.25 ± 0.07	0.12 ± 0.09

The average salinity measured during the experimental periods was 5.7 ± 0 PSU in 2013 and 5.7 ± 0 PSU in 2014 (**Tables 1, 2**). Carbonate parameters pCO_2 and pH_{NBS} in mesocosms with different pCO_2 levels in 2013 and 2014 are presented in **Tables 1, 2**. Data on nutrient concentrations of the sampling sites for different experimental periods are presented in **Table 3**.

Net Photosynthetic Rate of *Zostera marina* at Different pCO_2 Levels

The PERMANOVA analyses (conducted separately with the data obtained in 2013 and 2014) showed that the photosynthetic rate of the *Z. marina* varied significantly between different pCO_2

levels (**Figure 1**; **Tables 4, 5**; PERMANOVA: $p < 0.05$). In 2013 the variations of NPP rates were also affected by one of the tested environmental variables, namely water temperature, while the effect of the PAR was dependent on the level of pCO_2 and water temperature (**Table 4**; PERMANOVA: $p < 0.05$). The net photosynthetic rates of *Z. marina* increased with increasing water temperature. In 2014 the NPP rates of *Z. marina* were affected by interactions of the PAR with water temperature and with the level of pCO_2 (**Table 5**; PERMANOVA: $p < 0.05$). The results of the NPP measurements in mesocosm experiments are visualized in **Figures 2, 3**.

There were remarkable differences in the NPP rates of *Z. marina* between the two experimental periods: the average NPP rate was 4.08 mg O_2 gdw^{-1} h^{-1} in 2013 and 5.19 O_2 gdw^{-1} h^{-1} in 2014 (**Figure 1**). However, in both experimental periods, the NPP rates showed similar response patterns to CO_2 enrichment in mesocosm experiment. The highest NPP rates for *Z. marina* were measured in the untreated water (control conditions), while at the intermediate pCO_2 level the seagrass had lower rates than at the high pCO_2 levels (**Figure 1**). Based on a PERMANOVA pairwise *post-hoc* test, the differences in the average NPP rates of *Z. marina* at the pCO_2 levels between 1000 and 2000 μatm were significant ($p < 0.01$) in 2013. At the same time, NPP rates of *Z. marina* at the pCO_2 levels between 200 and 2000 μatm were not significant, while at the pCO_2 level of 200 μatm a significantly higher average NPP rate was measured compared to the elevated pCO_2 level of 1000 μatm ($p < 0.01$) in 2013 (**Figure 1**). In 2014 the differences in the NPP rates of *Z. marina* at the pCO_2 levels of 1000 and 2000 μatm were not significant but at the pCO_2 level of 200 μatm a significantly higher average photosynthetic rate compared to elevated pCO_2 levels was measured (PERMANOVA pairwise *post-hoc* test: $p < 0.05$; **Figure 1**). In the natural conditions outside of mesocosm the average photosynthetic rates of *Z. marina* were significantly lower (PERMANOVA pairwise *post-hoc* test: $p = 0.0001$) compared to the NPP values measured in mesocosms in 2014. At the pCO_2 levels of 200 μatm (mesocosm control conditions), the average NPP values of *Z. marina* were 4.38 mg O_2 gdw^{-1} h^{-1} in 2013 and 5.80 mg O_2 gdw^{-1} h^{-1} in 2014. At the high pCO_2 levels of 2000 μatm the average NPP value was 4.08 mg O_2 gdw^{-1} h^{-1} in 2013 and 5.05 mg O_2 gdw^{-1} h^{-1} in 2014, while at the intermediate pCO_2 level the average NPP value was 3.77 mg O_2 gdw^{-1} h^{-1} in 2013 and 4.73 mg O_2 gdw^{-1} h^{-1} in 2014. The lowest average NPP rate for *Z. marina* was measured under natural conditions outside of the mesocosm: 2.94 mg O_2 gdw^{-1} h^{-1} in 2014 (**Figure 1**).

DISCUSSION

The vast majority of studies have shown that photosynthesis of seagrasses increases with the elevated pCO_2 (e.g., Thom, 1996; Zimmerman et al., 1997; Invers et al., 2001; Ow et al., 2015). However, this effect is highly species specific (e.g., Invers et al., 1997, 2001; Ow et al., 2015). For example, Invers et al. (1997) investigated the effect of pH on photosynthesis in the three species of seagrasses: *Posidonia oceanica, Cymodocea nodosa,* and
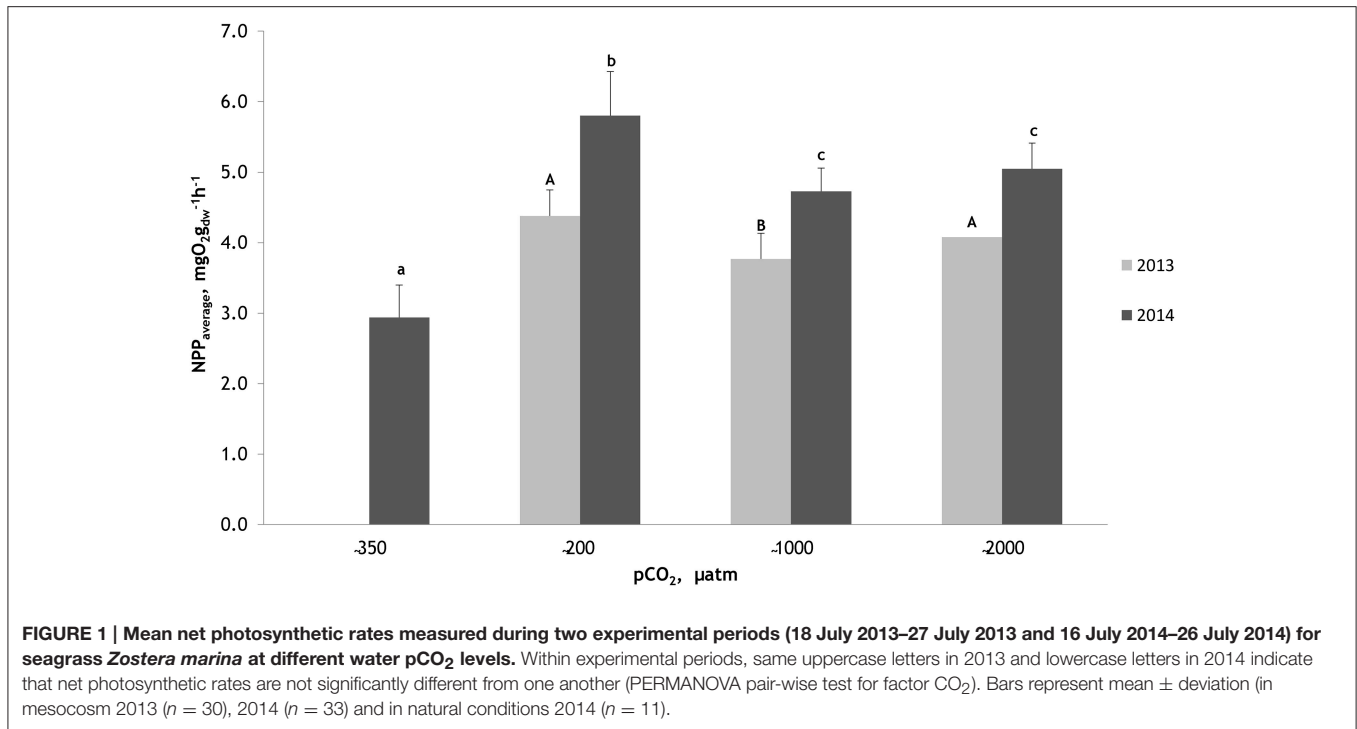
FIGURE 1 | Mean net photosynthetic rates measured during two experimental periods (18 July 2013–27 July 2013 and 16 July 2014–26 July 2014) for seagrass *Zostera marina* at different water pCO_2 levels. Within experimental periods, same uppercase letters in 2013 and lowercase letters in 2014 indicate that net photosynthetic rates are not significantly different from one another (PERMANOVA pair-wise test for factor CO_2). Bars represent mean ± deviation (in mesocosm 2013 ($n = 30$), 2014 ($n = 33$) and in natural conditions 2014 ($n = 11$).

TABLE 4 | Results of PERMANOVA analysis on the separate and interactive effects of pCO_2, PAR, and water temperature on the net primary production rate of *Zostera marina* in 2013.

Source	DF	MS	Pseudo-F	P(perm)	Perms
PAR	1	0.4935	0.3410	0.559	9825
Water temperature	1	59.935	41.42	**<0.001**	9828
pCO_2	2	6.4308	4.4442	**<0.05**	9942
PAR*Water temperature	1	0.0017	0.0020	0.9737	9854
PAR*pCO_2	2	8.909	6.1568	**<0.05**	9956
Water temperature*pCO_2	2	0.8313	0.5745	0.5574	9937
PAR*Water temperature*pCO_2	2	5.7391	3.9661	**<0.05**	9957
Res	168	1.447			
Total	179				

Significant effects ($p < 0.05$ and $p < 0.001$) are indicated in bold.

TABLE 5 | Results of PERMANOVA analysis on the separate and interactive effects of pCO_2, PAR, and water temperature on the net primary production rate of *Zostera marina* in 2014.

Source	DF	MS	Pseudo-F	P(perm)	Perms
PAR	1	5.1443	1.363	0.2382	9830
Water temperature	1	2.398	0.6354	0.4247	9845
pCO_2	3	97.402	25.807	**<0.001**	9946
PAR*Water temperature	1	26.279	6.9627	**<0.05**	9835
PAR*pCO_2	3	39.866	10.563	**<0.001**	9949
Water temperature*pCO_2	3	4.7296	1.2531	0.292	9964
PAR*Water temperature*pCO_2	3	7.2072	1.9095	0.1291	9934
Res	246	3.7743			
Total	261				

Significant effects ($p < 0.05$ and $p < 0.001$) are indicated in bold.

Zostera noltii in the laboratory conditions. Seagrasses *P. oceanica* and *C. nodosa* demonstrated a decrease in photosynthetic rates with an increase of pH and showed a significant reduction in NPP rates at pH 8.8. At the same time, *Z. noltii* showed high photosynthetic rates up to pH 8.8 and significant reduction in NPP rates only at pH 9.0. On the other hand, several studies have found unaltered response in the growth rate of seagrasses under long-term CO_2 enrichment (e.g., Palacios and Zimmerman, 2007; Campbell and Fourqurean, 2013). We hypothesized that in our mesocosm experiments the net photosynthetic rates of *Zostera marina* would increase with the elevated pCO_2 levels. In our mesocosm experiments the highest NPP rate of *Z. marina* was measured in the untreated water (control conditions)

compared to the average values of elevated pCO_2 levels in both experimental periods. Cox et al. (2016) investigated the effect of ocean acidification on the seagrass *P. oceanica* meadows under controlled CO_2/pH conditions using a Free Ocean Carbon Dioxide Enrichment (FOCE) system. They found no effect of elevated pCO_2 on the photosynthesis of Mediterranean seagrass *P. oceanica* from the two enclosures, neither pH manipulated nor control.

However, in 2014 our results showed that the NPP rates of *Z. marina* were significantly lower in the natural conditions outside of the mesocosm as compared to the results from the mesocosm experiment (elevated water pCO_2 levels as well as the untreated water). There might be several explanations for
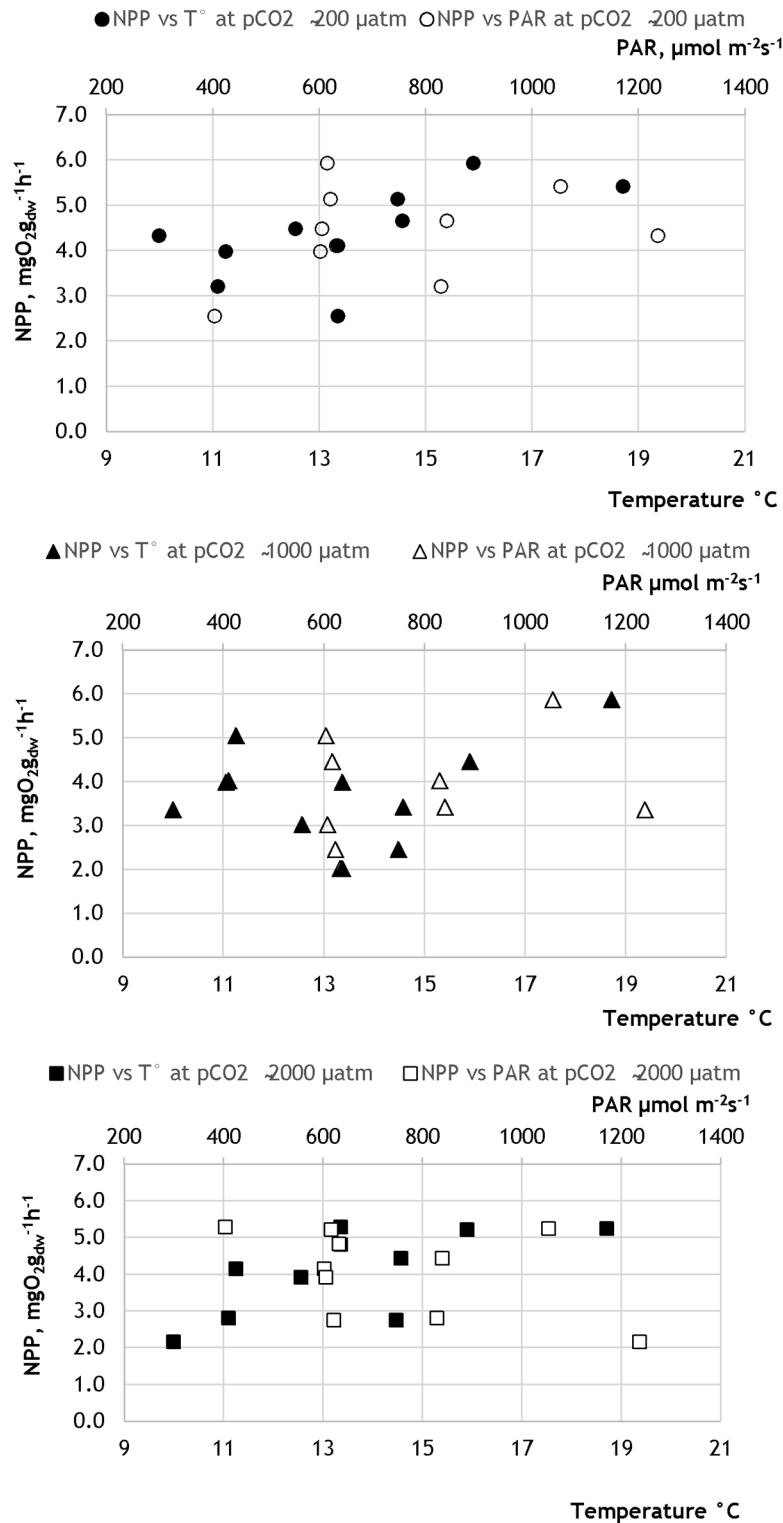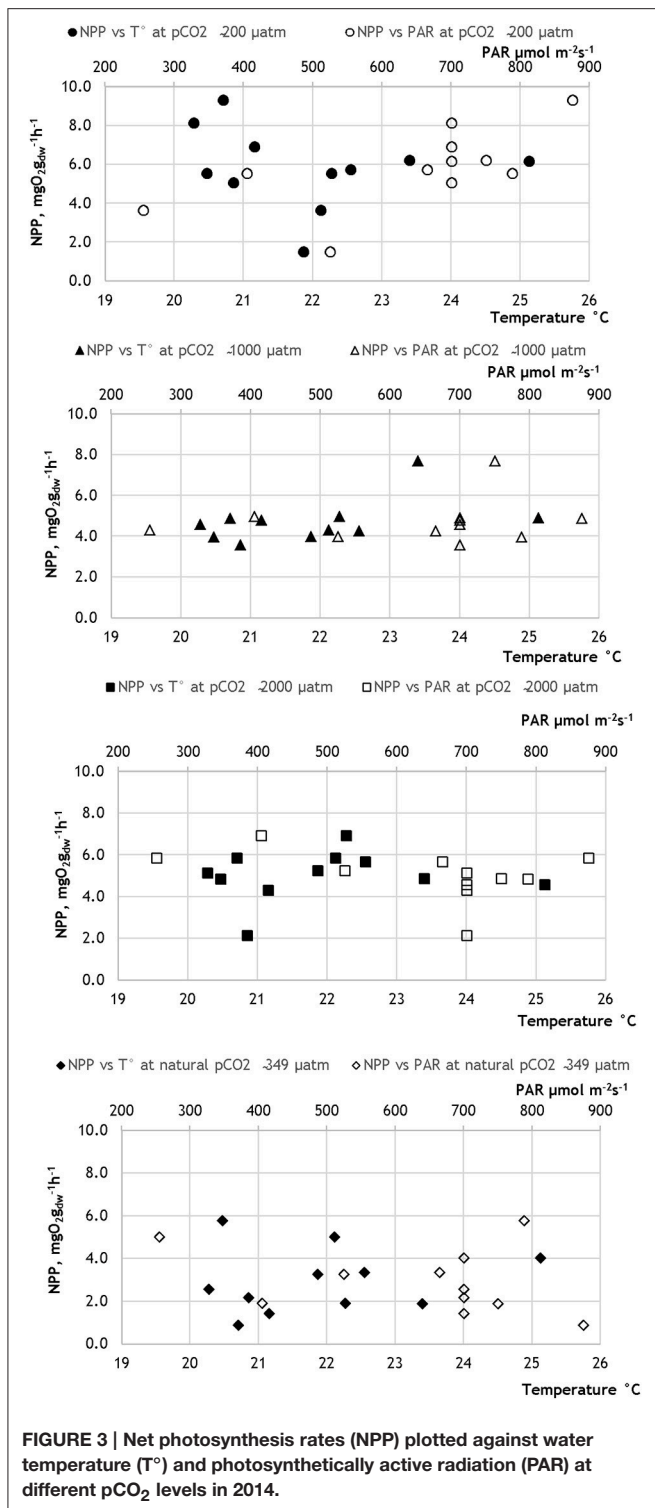
FIGURE 2 | Net photosynthesis rates (NPP) plotted against water temperature (T°) and photosynthetically active radiation (PAR) at different pCO_2 levels in 2013.

FIGURE 3 | Net photosynthesis rates (NPP) plotted against water temperature (T°) and photosynthetically active radiation (PAR) at different pCO_2 levels in 2014.

In the shallow coastal Kõiguste Bay, pH, and pCO_2 show a substantial amplitude of natural variability under summer conditions. In the early morning pCO_2 values were extremely high, being well above the concentration of $1000\,\mu$atm in the natural conditions outside of the mesocosm. But during the daytime, when inorganic carbon was used for photosynthesis, pCO_2 declined to $\sim150\,\mu$atm. In addition, fluctuation of pH between 8 and 9 are common in shallow water macroalgal habitats under summer conditions. There might be remarkable differences in pH and pCO_2 changes between different days. These natural fluctuations of the environmental factors in the shallow coastal waters are driven by direct effects of photosynthesis (increasing pH), respiration (lowering pH) and meteorological conditions. Additionally, in the mesocosm control treatment, during the photosynthesis measurements the average pCO_2 was ca. 200 μatm. At the same time (hour) the average pCO_2 was ca. 350 μatm in the natural conditions outside of the mesocosm. So, considering above-mentioned natural fluctuations of pCO_2, we chose to operate in mesocosms with the pCO_2 levels of ca. 2000, ca. 1000, and control ca. 200 μatm. Similarly, Paul et al. (2015) have used the pCO_2 levels from ambient (240 μatm) up to 1650 μatm in mesocosm experiments carried out in the Gulf of Finland, northern Baltic Sea.

In the mesocosm experiments some variation between NPP rates within the two experimental periods was found which could be explained by the differences in the weather conditions and other limiting environmental factors. Several studies conducted outside of the Baltic Sea have shown that the capacity of seagrasses to respond to ocean acidification depends on the nutrients content in the water and light levels, and their interactions (e.g., Invers et al., 1997; Palacios and Zimmerman, 2007; Martínez-Crego et al., 2014). Besides seagrasses, Celis-Plá et al. (2015) found that macroalgal responses to elevated pCO_2 depend on nutrients content and light availability. Within the Baltic Sea, the environmental factors vary highly regionally and seasonally (Feistel et al., 2008). In the Estonian coastal waters, the seagrass *Z. marina* grows at its lowest salinity limit (5–7 PSU) and this may cause physiological stress for their communities. Therefore, in the north-eastern Baltic Sea the factors driving the dynamics of *Z. marina* populations and their physiological processes are expected to be different compared to other regions of the Baltic Sea (Boström et al., 2014; Möller et al., 2014). In this study, variation in NPP rates within the two experimental periods could be explained first of all by the differences in the water temperature. In our experiment the higher NPP values of *Z. marina* were measured under the higher water temperatures in 2014 (average water temperature 21.9°C and average rate of NPP 5.19 mg O_2 gdw^{-1} h^{-1}) compared to the values of 2013 (average water temperature 13.5°C and average rate of NPP 4.08 mg O_2 gdw^{-1} h^{-1}). Likewise, the favorable effect of the elevated pCO_2 on the photosynthesis of *Z. marina* was more pronounced at higher water temperature in 2014 than in 2013. Therefore, it could be suggested that a future increase in water temperature under climate change may benefit the net photosynthesis of *Z. marina* and also alter the response of *Z. marina* to CO_2 enrichment. This highlights the fact that in the Baltic Sea, where the temperature is characterized by high seasonal and annual

that since in natural conditions several stress factors might be affecting the condition of the plants which are eliminated in the mesocosm, e.g., water movement or presence of suspended organic particles consuming part of the oxygen production in an incubation bottle during NPP measurement.

variations, the effects of increasing CO$_2$ and water temperature should be observed together. In the Mediterranean, Baggini et al. (2014) found that macroalgal community responses to increased pCO$_2$ are strongly affected by season and this can alter community composition under future ocean acidification.

Light availability controls sea plants carbon fixation and is therefore often the primary limiting factor for seagrass productivity (Hemminga and Duarte, 2000; Zimmerman, 2006). Several studies have shown that the decline of *Z. marina* distribution is caused by deteriorating underwater light climate due to eutrophication in the Baltic Sea (Boström et al., 2014 and references therein). In the both experimental years, our results showed that NPP rates of *Z. marina* were affected by the combined effects of light and pCO$_2$ levels. In the Estonian coastal waters, the main distribution depth of *Z. marina* is between 2 and 4 m (Möller et al., 2014). This low distribution depth of *Z. marina* is probably due to poor underwater light climate. Therefore, seagrass responses to the increasing water CO$_2$ concentrations also depend on the light availability in the north-eastern Baltic Sea.

The response of seagrasses to the elevated pCO$_2$ will depend also on the carbon source they use for photosynthesis as well as on the mechanisms of carbon acquisition. Several studies have found that *Z. marina* is using HCO$_3^-$ as external Ci-source for photosynthesis (Sand-Jensen and Gordon, 1984; Beer and Rehnberg, 1997; Koch et al., 2013). Beer and Rehnberg (1997) investigated two different acquisition systems of inorganic carbon for *Z. marina* and showed that HCO$_3^-$ was used as a main source of inorganic carbon at the normal seawater pH of 8.2. Rather recently Hellblom et al. (2001) detected other HCO$_3^-$ utilization mechanism for *Z. marina* when using the buffer tris (hydroxymethyl) aminomethane (TRIS) for keeping a constant pH. The addition of buffering solution (pH 8.1) to natural seawater reduced net photosynthetic rates of *Z. marina* by some 70% as compared to the values in the non-buffered seawater of the same pH. So, considering above-mentioned results, *Z. marina* can use different mechanisms of carbon acquisition for photosynthesis under different environmental conditions. It could be speculated that in our experiments under high-pH treatment in the enclosed conditions HCO$_3^-$ transport is more efficient for *Z. marina*. However, this statement needs further verification. Similarly, Axelsson et al. (2000) found that brown macroalgae *Laminaria saccharina* appears to be able to utilize the HCO$_3^-$ of seawater even more efficiently at high pH.

Seagrass meadows are amongst the most productive autotrophic ecosystems on the planet (Duarte and Chiscano, 1999). Several studies have found seagrass meadows to be globally significant as carbonate reservoir (e.g., Duarte et al., 2010; Hendriks et al., 2014; Mazarrasa et al., 2015). Seagrass metabolic activity is likely to buffer ocean acidification in the seagrass meadows, but the magnitude of this effect will depend on their metabolic parameters and vary regionally as well seasonally (Hendriks et al., 2014). We can suggest that also in the Baltic Sea conditions seagrass beds could have a buffering role in the case of elevated pCO$_2$ conditions.

In conclusion, our study shows that elevated pCO$_2$ alone did not enhance the photosynthesis of the seagrass *Z. marina*. The photosynthetic response of *Z. marina* to elevated pCO$_2$ levels appears to be the result of interactions with water temperature and light availability in the north-eastern Baltic Sea.

AUTHOR CONTRIBUTIONS

Original idea: LP and GM. Study design: AP, GM and LP. Performing the experiments: LP and AP. Data analyses: LP, AP and TP. Writing a draft of the article LP and TP.

ACKNOWLEDGMENTS

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