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Short Term CO_2 Enrichment Increases Carbon Sequestration of Air-Exposed Intertidal Communities of a Coastal Lagoon

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In situ production responses of air-exposed intertidal communities under CO_2 enrichment are reported here for the first time. We assessed the short-term effects of CO_2 on the light responses of the net community production (NCP) and community respiration (CR) of intertidal *Z. noltei* and unvegetated sediment communities of Ria Formosa lagoon, when exposed to air. NCP and CR were measured *in situ* in summer and winter, under present and CO_2 enriched conditions using benthic chambers. Within chamber CO_2 evolution measurements were carried out by a series of short-term incubations (30 min) using an infra-red gas analyser. Liner regression models fitted to the NCP-irradiance responses were used to estimate the seasonal budgets of air-exposed, intertidal production as determined by the daily and seasonal variation of incident photosynthetic active radiation. High CO_2 resulted in higher CO_2 sequestration by both communities in both summer and winter seasons. Lower respiration rates of both communities under high CO_2 further contributed to a potential negative climate feedback, except in winter when the CR of sediment community was higher. The light compensation points (LCP) (light intensity where production equals respiration) of *Z. noltei* and sediment communities also decreased under CO_2 enriched conditions in both seasons. The seasonal community production of *Z. noltei* was 115.54 ± 7.58 g C m^{-2} $season^{-1}$ in summer and 29.45 ± 4.04 g C m^{-2} $season^{-1}$ in winter and of unvegetated sediment was 91.28 ± 6.32 g C m^{-2} $season^{-1}$ in summer and 25.83 ± 4.01 g C m^{-2} $season^{-1}$ in winter under CO_2 enriched conditions. Future CO_2 conditions may increase air-exposed seagrass production by about 1.5-fold and unvegetated sediments by about 1.2-fold.

Keywords: intertidal communities, *Zostera noltei*, CO_2 effects, community metabolism, incubation chambers

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INTRODUCTION

The current atmospheric carbon dioxide (CO_2) level is predicted to be doubled by the end of the twenty-first century (IPCC, 2007). Rising atmospheric CO_2 will increase the input of CO_2 levels in the oceans, as 30% of the anthropogenic CO_2 emissions are absorbed in the surface oceans (IPCC, 2014). This raises increased concern on the various impacts, elevated CO_2 will have on the marine ecosystem (Gattuso et al., 1998; Guinotte and Fabry, 2008; Hall-Spencer et al., 2008; Portner, 2008; Porzio et al., 2011). The increase of CO_2 in the world oceans is changing the carbonate chemistry of the seawater, lowering the pH and thus resulting in ocean acidification. This pH levels are expected to decrease further by 0.3–0.4 units by the end of this century (Royal Society, 2005; IPCC, 2014).The low pH of seawater will alter the carbonate chemistry of oceans by increasing the levels of dissolved inorganic carbon (DIC) species [CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-})], which will result in a regime shift of the total DIC in the seawater favoring more toward HCO_3^- and $CO_{2\ (aq)}$ than CO_3^{2-} (Riebesell et al., 2007). This shift is expected to favor those species that can use HCO_3^- as a source for carbon for photosynthesis along with CO_2 (Beer et al., 2002; Mercado et al., 2003).

Seagrass ecosystems are distributed worldwide and contribute significantly to the carbon cycle of coastal areas (Duarte and Chiscano, 1999; Hemminga and Duarte, 2000). They are important marine carbon sinks and contribute significantly to the total carbon storage of oceans (12%) (Duarte, 1990; McLeod et al., 2011; Fourqurean et al., 2012). Saying that, the response of seagrass ecosystems to the rising CO_2 levels is important to understand, as seagrasses are predicted to benefit from the increasing CO_2 levels by increasing their carbon uptake (Beer and Koch, 1996; Zimmerman et al., 1997; Invers et al., 2001) and overcome their limitation under the current carbon dioxide concentrations.

Most of the studies on the effects of elevated CO_2 levels on seagrasses have been on the responses of productivity and light requirements (Beer and Koch, 1996; Thom, 1996; Zimmerman et al., 1997; Palacios and Zimmerman, 2007), photosynthetic and leaf growth rates (Alexandre et al., 2012) and the uptake rate of ammonium and nitrate (Short and Neckles, 1999; Alexandre et al., 2012; Ow et al., 2016). Results so far have suggested that, seagrass meadows can utilize the increased CO_2 concentration from the water column and enhance their photosynthetic activity and community metabolism (Frankignoulle and Disteche, 1984; Frankignoulle and Bouquegneau, 1990; Invers et al., 2001). However, these experiments were carried out mostly on submerged seagrass meadows and data on intertidal meadows is yet scarce. The intertidal seagrass meadows are subjected to daily shifts in light and temperature, due to a combination of tidal cycles and daily irradiance (Short et al., 2001; Vermaat, 2009; Van der Heide et al., 2010). In these intertidal flats, the intensity of light is generally higher during air exposure than in submerged conditions, when the nutrient sources are reduced to sediment pore water (Ouisse et al., 2011). Intertidal seagrasses are exposed to various concentrations of gaseous CO_2 during exposure to air and to various dissolved carbon forms (CO_2, HCO_3^-, and CO_3^{2-}) during submersion (Ouisse et al., 2011; Bahlmann et al., 2015). The seagrass *Zostera noltei,* that inhabits the low intertidal zone, experiences alternate daily periods of submerged and exposed condition to air for several hours especially during spring tides (Silva and Santos, 2003; Coyer et al., 2004; Ouisse et al., 2011). During low tide when exposed to air, the photosynthetic activity of *Z. noltei* may be higher than when submerged (Silva et al., 2005) or decrease due to desiccation (Leuschner et al., 1998).

In most of the intertidal macrophytes, community-level metabolism estimates have been assessed using infrared gas analysis (IRGA) of CO_2 fluxes through gas exchange methods, where flow- through systems or closed chambers have been used for longer periods of time (Streever et al., 1998; Silva et al., 2005). Much of the community-productivity studies utilizing CO_2 flux measurements have focused more on salt marsh than seagrass communities. However, in salt marsh communities, carbon flux measurements have been carried out, by incubation of individual leaves in special leaf chambers with artificial light and controlled temperature (Streever et al., 1998). There is a scarcity in utilization of IRGA for carbon flux measurements in the intertidal seagrasses. Most studies using carbon flux measurements on *Z. noltei* were performed in lab conditions (Leuschner and Rees, 1993; Perez-Loren and Niell, 1994; Leuschner et al., 1998), whereas few measurements have been performed *in situ* (Leuschner et al., 1998; Silva et al., 2005; Ouisse et al., 2010, 2011). In this context, we aimed to measure the short-term effects of high CO_2 concentration (750 ppm) on the net community production (NCP) and community respiration (CR) of air exposed *Z. noltei* community in Ria Formosa Lagoon, Portugal, where the species dominates the intertidal zone. The effects of vegetation on the intertidal ecosystem metabolism were also assessed by comparison with unvegetated areas.

MATERIALS AND METHODS

Study Site

The study site, Ria Formosa lagoon (37°00′ N, 7°58′ W) is in the Southern coast of Portugal. It is a mesotidal system characterized by large intertidal flats (Andrade, 1990). The seagrass *Zostera noltei* dominates the intertidal flats of Ria Formosa lagoon, it's distribution ranges up to 2 m from the mean tide level and plays a significant role in the carbon metabolism of the lagoon (Santos et al., 2004). The *Z. noltei* meadows regularly experience alternate daily periods of submersion and exposure (Site A, **Figure 1**) withstanding extended periods of air exposure of up to 4.5–6 h per tidal cycle. The unvegetated sediment flats are at the same bathymetric zone experiencing similar daily tidal fluctuations. The studied unvegetated flats are located on the north-west direction of seagrass beds approximately 600 m in distance (Site B, **Figure 1**).

Gas Exchange Measurements

Measurements were made *in situ*, through continuous carbon dioxide flux monitoring (IRGA) in short-term incubations using benthic Plexiglas chambers. Incubations were conducted under the range of naturally available irradiance. The community response of *Z. noltei* was compared with that of adjacent un-vegetated sediments. This experiment was carried out in summer (June, July, August) 2014 and winter (November, December, January) 2014–2015 seasons. Gas-tight incubation chambers were made of UV-transparent Plexiglas, providing a closed environment for the incubations. These glass chambers comprised of two separate parts, a basal ring (30 cm in diameter) and a dome shaped lid. Sharpening of the basal ring was carried out, for easy 5–10 cm deep insertion into sediment. To secure both parts of the chamber power clamps were used. To confirm

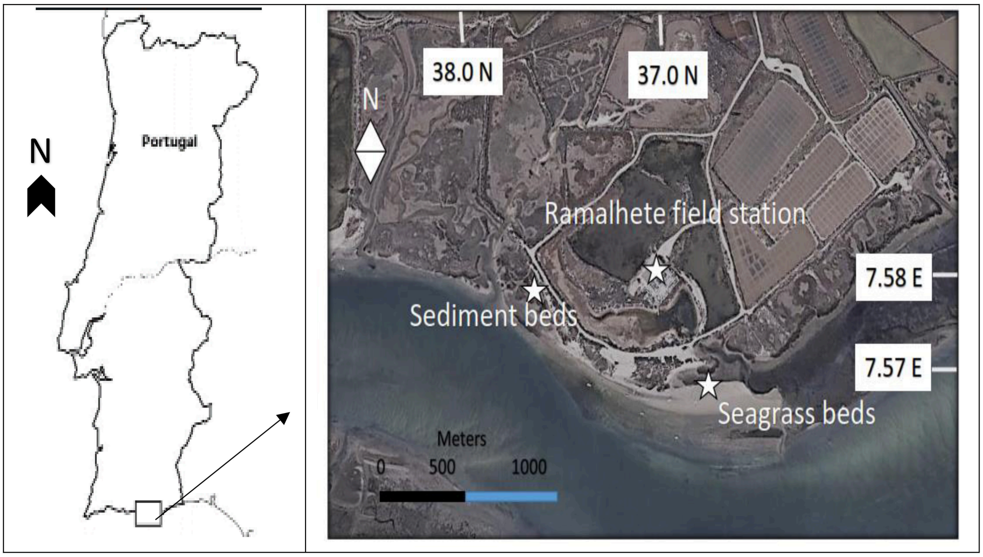

FIGURE 1 | Study area showing *Zostera noltei* beds and unvegetated sediment flats at Ria Formosa Lagoon.

the chamber was sealed a special latex ring was installed in the flange. The chamber has three ports one in the ring (2), one on the top of the dome (1) and the third (3) on the base 5–10 cm above the sediment insertion line where CO_2 gas is injected (**Figure 2**). All ports were fitted with Tygon® ST gas-impermeable tubing. The global operating volume of the chambers ranged between 22 and 26 L, depending on the insertion of ring into the sediment (Silva et al., 2005).

Short-term field incubations using these chambers were conducted in *Z. noltei* meadows and bare sediment beds during several low tides at different times of the day along the season, so that the diel variation of light was captured. Each incubation lasted for a maximum of 30 min and under ambient atmospheric CO_2 concentration (397–399 ppm) followed by CO_2 enrichment to 750 ppm. During the incubation air entered the chamber through the ring port (2) and was out through the port (1), which allowed the air inside the chamber to be homogenized. An external non-dispersive infrared gas analyser (IRGA) (EGM-4, PP Systems, UK) was connected to the chamber, through a low volume (about 25 ml) closed air circuit. Measures of the evolution of CO_2 concentrations within the chambers were done with accurate precision of 1 μatm. The IRGA has a pump installed in it, which gathered the air from the chamber, passed through a desiccation column (Anhydrous calcium sulfate, Drierite) to remove humidity from the air to avoid interferences in CO_2 measurements when passed through the infrared cell. Then this air was send back to the chamber to keep continuous air flow between the chamber and gas analyser. The change in partial pressure of CO_2 inside the chamber was continuously monitored (1 min steps) during the incubation period usually allowing a drop in the CO_2 partial pressure for a 30–40 μatm. During this time the port (3) was clamped, so that no air passes through the tubes. After completion of the air incubation, the clamp was removed and CO_2 gas was injected slowly into the chamber through the port (3), from a premixed air-CO_2 tank until the CO_2 concentration inside the chamber reached 750 ppm. The clamp was closed again. The CO_2 partial pressure was measured, as described previously. Following each incubation, the seagrass was collected, washed and dried for 24 h at 60°C and the dry weight was recorded. Photosynthetic active radiation (PAR) inside the chambers was measured with a Li-Cor system (Li-Cor, 250 A meter, USA) at both the start and end of each incubation. Instantaneous PAR measurements were continuously done at the Ramalhete field station (**Figure 1**) every 30 min using the same sensor to record daily and seasonal light variation. There was approximately 10% of light attenuation inside the glass chambers. Temperature was recorded using a thermometer at the start and end of each air and CO_2 incubation. Similar incubations were also performed for unvegetated sediment beds.

NCP and CR Measurements

Light and dark incubations were conducted for both NCP and CR respectively. The net flux (F) of CO_2 (μmol C m^{-2} h^{-1}) for both air and CO_2 enriched incubations was computed as described in Silva et al. (2005):

$$F = s \times mv \times V/A \times 60/1{,}000$$

where s (ppm CO_2 min^{-1} = μmol CO_2 mol air^{-1} min^{-1}) represents the slope of the change in CO_2 concentration during the incubation period,
V (L) = chamber volume,
A (m^2) = benthic area,
mv (mol L^{-1}) = molar volume or number of gas mole per volume unit, derived from the ideal gas law [$mv = P/(R \times T)$],
where P (atm)= gas pressure,

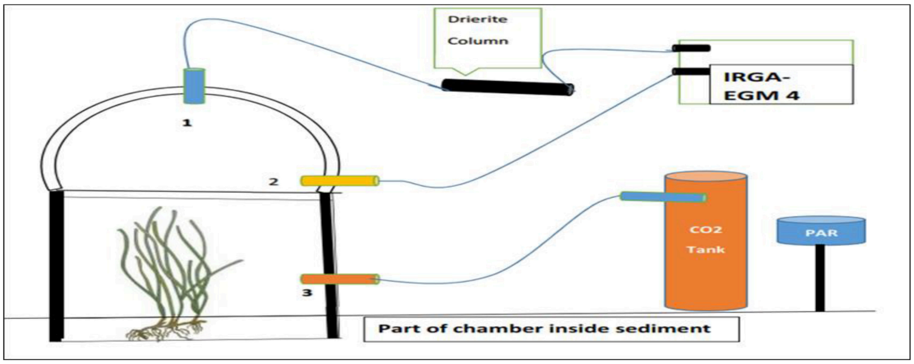

FIGURE 2 | A diagrammatic representation of the incubation work. (1) Port one on top of dome. (2) Port two on ring. (3) Port three for CO_2 injection.

T (K) = temperature, and R (0.082 atm LK^{-1} mol^{-1}) = universal gas constant.

Thus, the net CO_2 flux represents directly the NCP for the given light and temperature conditions. Similarly, the net flux of CO_2 in dark represents the community respiration, CR.

A linear regression model was used to fit the NCP vs. PAR data,

$$Y = -bx + a,$$

Where Y is NCP, −b represent the rate of CO_2 uptake by the community, X is the PAR value and "a" intercept, representing CR. The daily PAR variation reaching the *Z. noltei* community when exposed to the air, was used to estimate the daily NCP variation using the above model. Tidal variations (exposed period) for the study site (*Z. noltei* meadows and sediment beds) for each season (summer and winter) was estimated using a tidal model (Carrasco et al., submitted). The exposed period derived from the model for each day along the month and seasons for seagrass meadows and sediment beds was used to calculate the daily NCP, GCP and CR for each season.

Linear regression was used to model the effects of PAR on NCP. These models were then used to calculate NCP from PAR, which was measured continuously along the year. The daily NCP and CR during exposed period (g C m^{-2} day^{-1}) was estimated by adding every 30 min NCP and CR values along the day when the seagrass beds were exposed to the air. GCP was obtained by adding the NCP and CR during light hours. Seasonal budgets of NCP, CR and GCP were obtained by considering the variability of exposure times related to the alternation of spring and neap tidal cycles and the daily cycle of irradiance.

Statistics

Results are expressed as mean ± Standard error (SE). Significant differences of NCP responses to light were tested using the difference between slopes (Cohen et al., 2003; Soper, 2017). Significant differences between daily and seasonal CR was detected using *t*-tests. Data was tested for normality and constant variance before testing for significant difference. Significant levels were considered at $p < 0.05$.

RESULTS

The mean plant biomass of *Z. noltei* was 293.25 ± 8.08 g DW m^{-2} in summer and 189.66 ± 3.45 g DW m^{-2} in winter. The slopes of the linear responses of the NCP of *Zostera noltei* and unvegetated sediment were higher under CO_2 enriched conditions than ambient conditions in both summer and winter seasons (**Figure 3**). The differences were only apparent at light intensities above the light compensation point for community production (about 200 µmol m^{-2} s^{-1}), i.e., when production is higher than respiration. The NCP of unvegetated sediments was lower than *Z. noltei* within PAR ranges of 800–1,600 µmol m^{-2} s^{-1}, which was most evident in the summer (**Figures 4A,B**). In the summer season under higher light (1,680 µmol m^{-2} s^{-1}) the NCP of sediments was higher than *Z. noltei* under ambient conditions, whereas under CO_2 enriched conditions the NCP of *Z. noltei* was higher than unvegetated sediment community (**Figure 4**).

The NCP of *Z. noltei* and unvegetated sediments responded linearly to the range of PAR observed when the community was exposed to the air, both in summer and winter seasons (**Table 2**). The slope, intercept and R^2-values of the linear regression models are presented in **Table 2**. The high coefficients of determination (R^2) both under ambient and CO_2 enriched conditions (**Table 1**) indicate a good fit of the models to data. The light compensation points (LCP) were always lower under CO_2 enriched conditions for both *Z. noltei* and sediment communities (**Table 2**). The LCP of *Z. noltei* community was higher than the unvegetated sediment community under ambient conditions as opposed to CO_2 enriched conditions (**Table 2**).

The daily NCP of *Z. noltei* was 1.5-fold and 1.3-fold higher in both seasons under CO_2 enriched conditions and CR was lower (**Figures 5A,B**). Consequently, the daily gross community production (GCP) was even higher under CO_2 enriched conditions than ambient conditions (**Figure 5A**). Similarly, under CO_2 enriched conditions the daily sediment NCP was higher in both seasons, whereas the daily CR was higher in

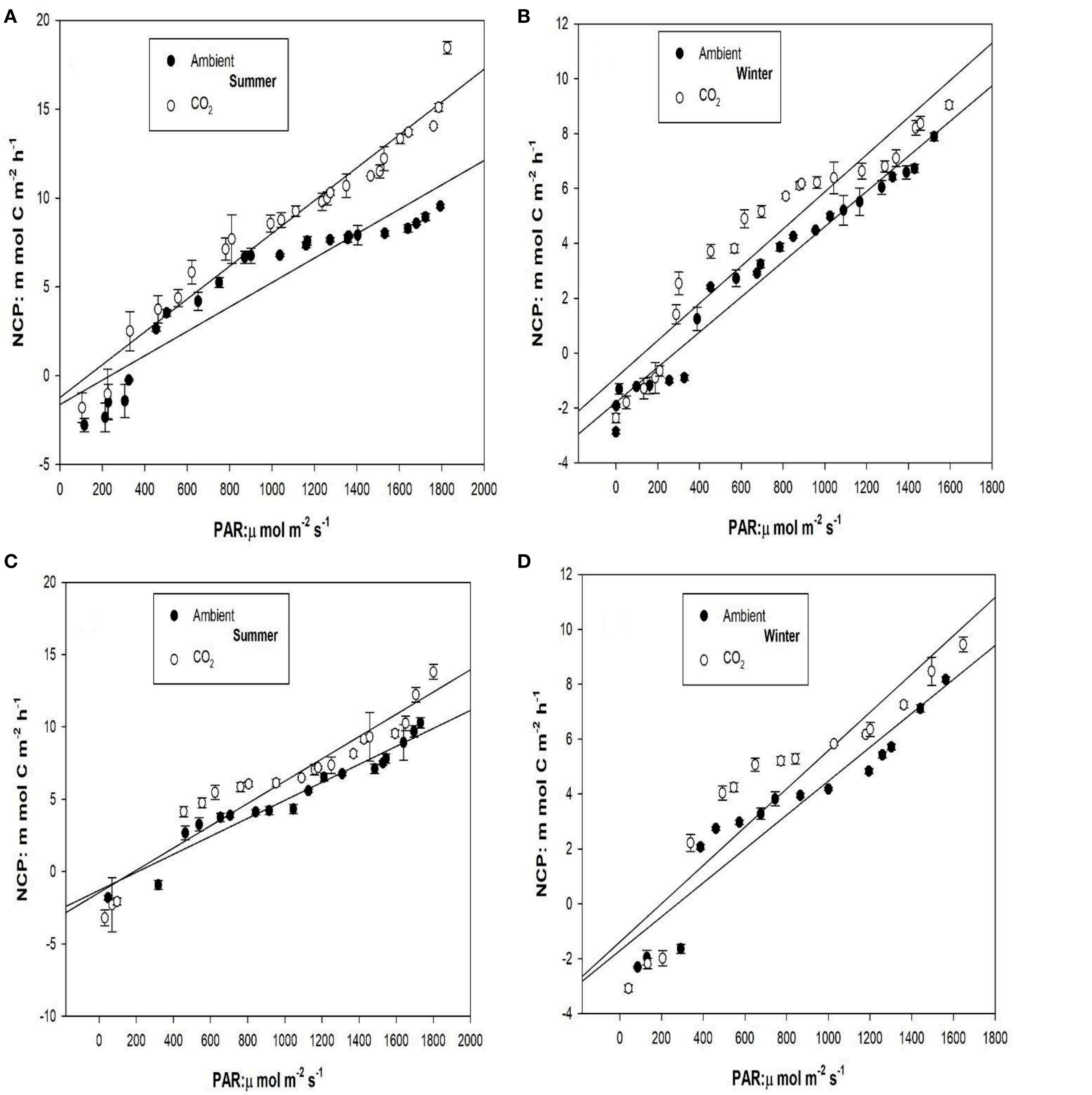

FIGURE 3 | Response of net community production (NCP) to irradiance (PAR) under ambient and CO_2 enriched conditions in summer and winter for *Z. noltei*, **(A,B)** and sediments **(C,D)**. Error bars represent standard errors.

summer and lower in winter (**Figures 5C,D**). The daily GCP of sediment community was higher in summer under CO_2 enriched conditions and there was no significant difference in winter.

The seasonal budgets of carbon sequestered by *Z. noltei* was 1.3-fold and 2.3-fold higher than unvegetated sediment under CO_2 enriched conditions in both seasons (**Table 1**). Under CO_2 enriched conditions, the carbon sequestered by *Z. noltei* was 4.9-fold higher in summer than winter season. The unvegetated sediment community sequestered 10-fold higher carbon in summer than winter season under CO_2 enriched conditions (**Table 1**). Seasonal budget of CR was higher in sediment communities than *Z. noltei* under CO_2 enriched conditions in winter season (**Table 1**).

DISCUSSION

Ambient Conditions

NCP and CR of unvegetated sediment community were lower than *Z. noltei* community in both summer and winter under ambient conditions (**Figure 5**). This suggests *Z. noltei* communities are more diverse and include more autotrophic

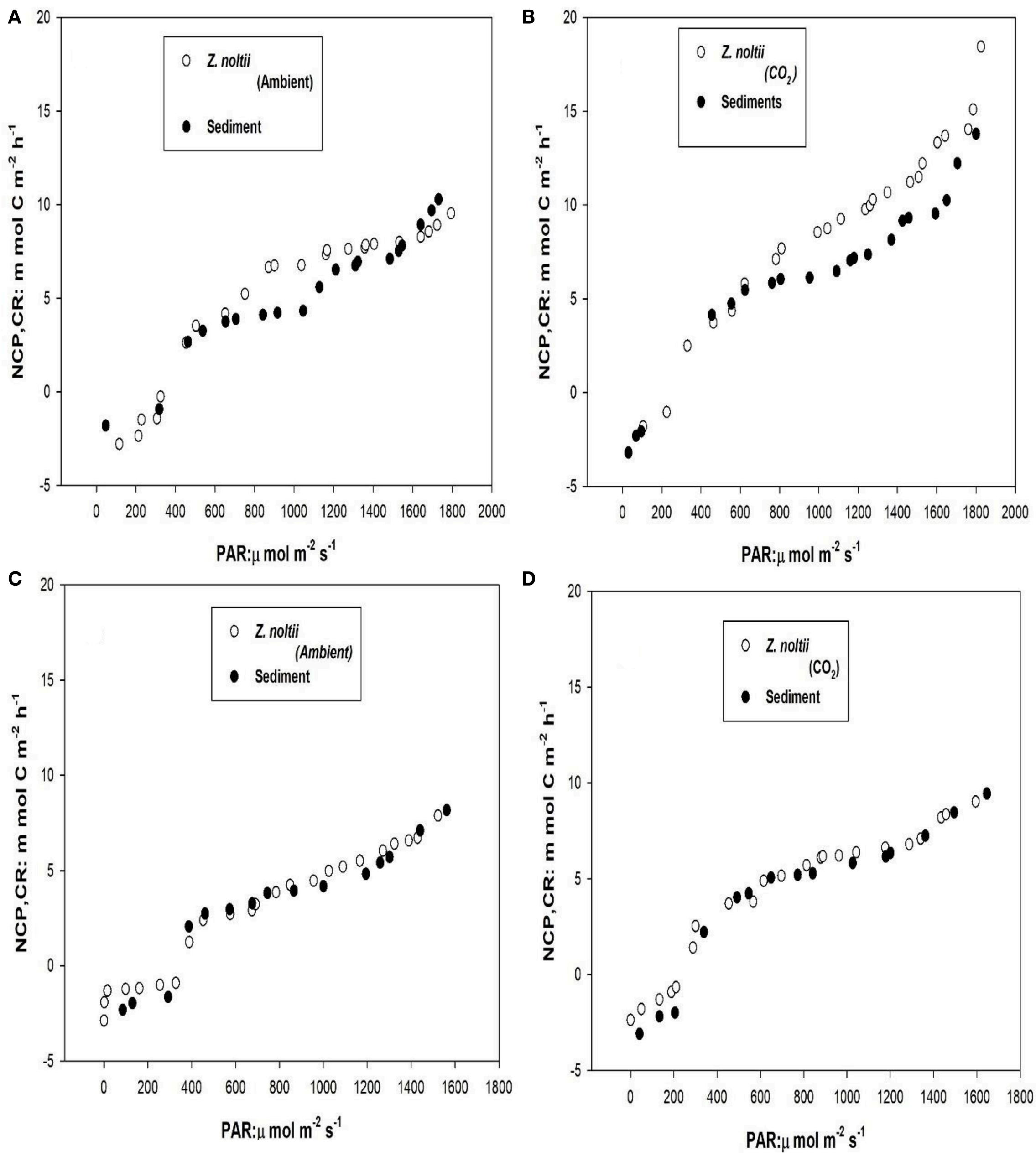

FIGURE 4 | Comparison of net community production (NCP) and respiration (NCP at 0 light level) of *Z. noltei* and unvegetated sediment in summer **(A,B)** and winter seasons **(C,D)** under ambient and CO_2 enriched conditions.

organisms (e.g., epiphytes and benthic microalgae) (Charpy-Roubaud and Sournia, 1990) contributing to the community production than sediment communities. Similar results of higher community production in *Z. noltei* than unvegetated sediment were observed at the Bay of Morlaix, France (Ouisse et al., 2010), and Banco de Mauritania (Clavier et al., 2014).

In the summer, the NCP of *Z. noltei* of Ria Formosa lagoon was 1.5-fold higher than previously reported for *Z. noltei* at Roscoff, France (Ouisse et al., 2011). As well the CR was 2-fold higher than *Z. noltei* community response observed by Ouisse et al. (2011). This resulted in higher GCP of *Z. noltei* of Ria Formosa lagoon than the GCP obtained for *Z. noltei* community at Roscoff (Ouisse et al., 2011).

The GCP and CR of *Z. noltei* observed in winter at Ria Formosa were also higher than previously reported for Z. *noltei* community by Clavier et al. (2011, 2014) and Ouisse et al. (2011). Higher community production (NCP, GCP) is due to higher irradiances (1,522–1,594 μmol m^{-2} s^{-1}) at Ria Formosa than the irradiance (829 μmol m^{-2} s^{-1}) level observed by Ouisse et al. (2011) at Roscoff. The higher respiratory demand of the extended rhizome network of *Z. noltei* community at Ria Formosa is probably due to higher temperature.

The summer NCP of unvegetated sediment in Ria Formosa lagoon (785 mg Cm^{-2} day^{-1}) was higher than previously reported (681.5 mg Cm^{-2} day^{-1}) for the Tagus estuary, Portugal in summer (Uthicke and Klumpp, 1998), whereas NCP (212.52 mg Cm^{-2} day^{-1}) of sediment in winter was lower (255 mg Cm^{-2} day^{-1}). Average NCP and CR observed in our results for sediment community in winter were higher than NCP and CR rates observed in the intertidal sediment community at Banco di Marituania (Clavier et al., 2014).

CO_2 Enrichment

We observed positive responses of *Z. noltei* and sediment communities to CO_2 enrichment. The short-term gas exchange measurements with CO_2 enrichment conditions clearly indicated that *Z. noltei* community production at around the same irradiance level increased 1.5-fold. Higher community production under CO_2 enrichment suggest that community production of *Z. noltei* are carbon limited under the current atmospheric CO_2 concentration, confirming the same conclusions previously obtained for *Z. noltei* (Alexandre et al., 2012) and for *Z. marina* (Beer and Koch, 1996; Zimmerman et al., 1997; Invers et al., 2001). These reports are on the photosynthetic response of individual plants rather than whole community, but it is expected that the increase in photosynthetic rate of individual plants is reflected on the higher organizational level of the community production. On the other hand, Martínez-Crego et al. (2014) did not find a significant effect of CO_2 on the community metabolism of *Z. noltei*, but this experiment was done in submerged conditions. The hypothesis that the future effects of CO_2 on intertidal seagrass will be higher when they are exposed to the air should probably be tested.

The key factor during exposed conditions for higher production under CO_2 enrichment can be higher light availability during exposure periods and abundant CO_2 as substrate from the CO_2 enrichment. Increase in CO_2 as substrate resulting in higher production efficiency was observed in *Z. marina* where elevated CO_2 levels increased plant production (Zimmerman et al., 1997). Secondly sediment microtopography of *Z. noltei* community plays an important role in maintaining hydration for the whole exposed period, due to numerous small depressions in the sediment retaining water (Silva et al., 2005). Thirdly during exposure, a thin layer of water is trapped between the leaves of *Z. noltei* due to higher leaf density (Silva and Santos, 2003), helping the plants remain moist throughout the exposed period.

The desiccations in *Z. noltei* meadows is thus not severe and the *Z. noltei* root system also supplies water to the leaves, which helps in maintaining a thin layer of water at the leaf surface (Leuschner et al., 1998). In these favorable conditions when CO_2 enrichment happens, a rapid diffusion of CO_2 into the thin film of water occurs around the seagrass leaves due to air-water CO_2 gradient, making the CO_2 readily available to the plants as they are consuming it (Leuschner et al., 1998). Therefore, high CO_2 concentrations along with high irradiance levels during air exposure of the *Z. noltei* community, creates the ideal condition for high community production during exposure (Leuschner and Rees, 1993; Silva et al., 2005; Touchette and Burkholder, 2007).

TABLE 1 | Seasonal budget (Mean ± SE, $n = 90$ days) of NCP (g C m^{-2} $season^{-1}$), CR (g C m^{-2} $season^{-1}$) and GCP (g C m^{-2} $season^{-1}$) of *Z. noltei* and unvegetated sediment community under ambient and CO_2 enriched conditions in summer and winter seasons.

Season	Condition	NCP	CR	GCP
		***Z. noltei* community**		
Summer	Ambient	78.21 ± 5.58	16.99 ± 0.85	61.30 ± 5.72
	CO_2	115.54 ± 7.58	12.12 ± 0.67	103.42 ± 7.62
Winter	Ambient	20.13 ± 3.02	26.86 ± 1.52	6.73 ± 3.09
	CO_2	29.45 ± 4.04	12.30 ± 0.74	17.15 ± 4.31
		Sediment community		
Summer	Ambient	72.26 ± 5.08	13.24 ± 0.74	59.02 ± 5.13
	CO_2	91.28 ± 6.32	14.55 ± 0.81	76.73 ± 6.36
Winter	Ambient	19.78 ± 2.98	24.36 ± 1.50	4.57 ± 3.08
	CO_2	25.83 ± 4.01	18.45 ± 1.27	7.37 ± 4.28

TABLE 2 | Slope (b), intercept (a), p, R^2, standard error (S.E.) and light compensation point (LCP, μmol m^{-2} s^{-1}) values derived from linear regression model between PAR and NCP under ambient and CO_2 enriched conditions for *Z. noltei* community and unvegetated sediments community.

Season	Condition	B	S.E.	a	S.E.	PAR vs. NCP (*p*-value)	R^2	LCP
					***Z. noltei* community**			
Summer	Ambient	0.0069	0.00	1.622	0.64	<0.001	0.87	247.50
	CO_2	0.0092	0.00	1.233	0.54	<0.001	0.95	134.02
Winter	Ambient	0.0064	0.00	1.796	0.23	<0.001	0.96	280.65
	CO_2	0.0068	0.00	0.882	0.44	<0.001	0.90	129.70
					Sediment community			
Summer	Ambient	0.0062	0.00	1.281	0.43	<0.001	0.95	206.61
	CO_2	0.0077	0.00	1.436	0.61	<0.001	0.90	186.49
Winter	Ambient	0.0062	0.00	1.707	0.54	<0.001	0.89	275.32
	CO_2	0.0070	0.00	1.391	0.72	<0.001	0.86	198.71

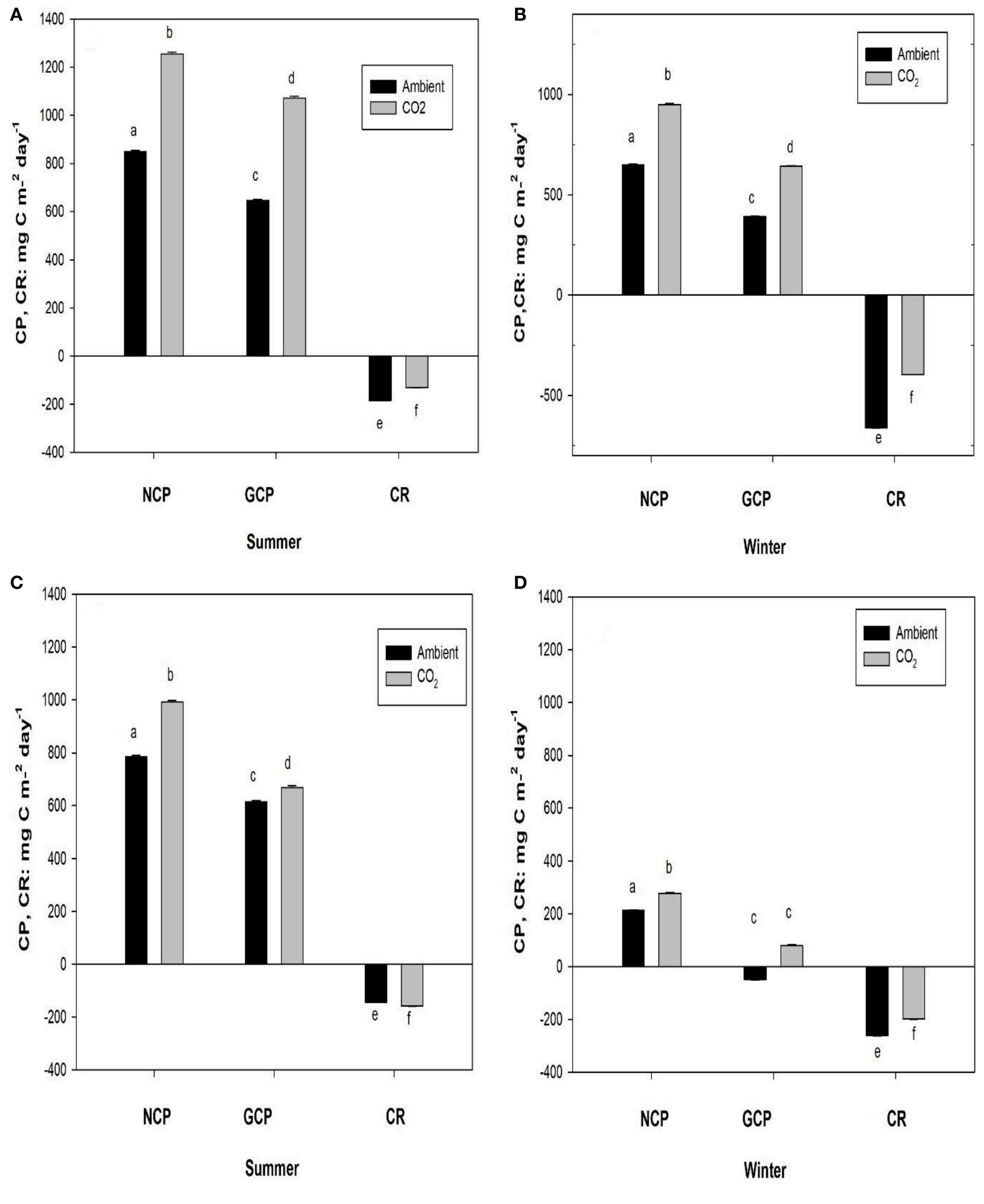

FIGURE 5 | Mean (± SE) daily net community production and respiration (NCP at 0 light level) of *Z. noltei* **(A,B)** and unvegetated sediment **(C,D)** in summer and winter seasons under ambient and CO_2 enriched conditions. Different letters indicate significant difference between ambient (air) and CO_2 enriched conditions. Error bars represent standard errors derived from regression models for NCP and GCP and error bars for CR are derived from field measurements.

NCP and CR of *Z. noltei* are lower during exposed than when submerged under ambient conditions (Ouisse et al., 2010; Clavier et al., 2011), but under CO_2 enrichment conditions the community production is higher and CR lower as observed in our results. Lower CR during exposed condition may increase the overall community production of exposed *Z. noltei* compared to submerged conditions where CR rates are higher. As well, during submersion *Z. noltei* favors the utilization of bicarbonate directly or indirectly to increase its production (Hellblom et al., 2001), but this process is energy intensive (Burnell et al., 2014), whereas the air exposed community during exposure, because of its high affinity and high availability of CO_2 in future conditions, can easily use the CO_2 from the atmosphere for community production (Touchette and Burkholder, 2007). The other factors that can contribute to the higher production of air- exposed *Z. noltei* community under CO_2 enriched conditions can be high light intensity and low turbidity.

Sediment community NCP were also higher with CO_2 enrichment incubations in summer and winter than ambient incubations, which can be related to the presence of microphytobenthos in the sediments during intertidal exposure (Uthicke and Klumpp, 1998; Hubas et al., 2006; Clavier et al., 2014). The intertidal desiccation of the sediments was avoided due to the presence of a thin film of water trapped by the benthos community and water trapped by intertidal burrows (Michael and McIntir, 1983).

PAR Responses

NCP of both *Z. noltei* and unvegetated sediment communities responded linearly to PAR, when the communities are exposed to air both under ambient and CO_2 enriched conditions. This suggests no photoinhibition of the whole community production at highest levels of PAR, which was also observed elsewhere for *Z. noltei* communities under ambient conditions (Ouisse et al., 2010, 2011; Clavier et al., 2011). Under CO_2 enriched conditions both communities increase their production even further without signs of saturation. Even though the photosynthetic production of individual autotrophic species does saturate at high light levels the combination of the responses of individual species that occupy the whole microhabitats of the community results is non-saturating response. In the case of non-vegetated sediments, the vertical migration of microphytobenthos that optimize the whole community production may justify it.

Under CO_2 enrichment, the light use capacity of both *Z. noltei* and unvegetated sediment communities increased as highlighted by the observed lower LCP in relation to ambient conditions. This was also observed in *Z. marina* (Zimmerman et al., 1997 and *Z. noltei* (Alexandre et al., 2012) where increase in CO_2 concentrations increased the light using capacity of individual plants.

The lower LCP obtained from the NCP/PAR models suggest that intertidal communities of coastal ecosystems may become more efficient at low light levels in a future high CO_2 scenario. This may result from a reduction of CR under high CO_2 as observed here for both *Z. noltei* and unvegetated sediment communities and by an increase of the carboxylation rate mediated by RUBISCO as the CO_2/O_2 ratio will increase. The LCP observed in our results were higher than previously reported for *Z. noltei* for both seasons under ambient conditions (Gacia et al., 2005; Ouisse et al., 2010) and lower for CO_2 enriched conditions. The LCPs of sediment community of both seasons under ambient conditions, were also higher than previously observed for sediment communities (Uthicke and Klumpp, 1998; Clavier and Garrigue, 1999). The LCP for CO_2 enriched experiments during exposed conditions are presented for the first time for *Z. noltei* and sediment community.

The lower LCP under CO_2 enriched conditions and higher NCP of *Z. noltei* in summer suggests that the community is more efficient in using low light (Silva and Santos, 2003) and can start using CO_2 for production in low light conditions without community production compensating for CR. In the case of unvegetated sediment community, the LCP was higher than Z. *noltei* under CO_2 enriched conditions, but the NCP was not, which suggests that even with higher CO_2 concentrations the microphytobenthos production is not higher than CR until a certain level of irradiance level is reached.

In situ production responses of air-exposed intertidal communities under CO_2 enrichment were reported here for the first time. The CO_2 enrichment method based on *in situ* short-term incubations of intertidal communities using benthic chambers is a valuable tool for intertidal lagoon metabolic estimates to assess the future trends of carbon cycling in tidal systems. It provides fast and valuable estimation of community carbon metabolism responses during exposed periods. The use of short-term incubations is an important feature in these experiments as the temperature and humidity levels remain constant. More importantly, short-term incubations prevent saturation of photosynthesis due to an increase of O_2 in relation to CO_2, which inhibits the RUBISCO carboxylase activity favoring oxygenase allowing repeatable carbon uptake measurements with minimum disturbance to communities (Olivé et al., 2016).

The good correspondence between carbon metabolism responses to light of the *Z. noltei* community and unvegetated sediment communities across summer and winter provides evidence that community production rates derived from CO_2 enriched incubations are a reflection of photosynthetic activity at ecologically relevant scales for future conditions of carbon dioxide concentrations. Positive production responses, negative respiration responses and lower light requirements under elevated CO_2 levels as observed in our results strongly suggest that the carbon sequestration of intertidal communities, when air-exposed, will increase in the future.

AUTHOR CONTRIBUTIONS

RS, JS, and AM have conceived the idea and the design of the experiments. AM has carried out the field work, data collection and writing of the manuscript. AM and RS carried out the interpretation of results. Both RS and JS have helped in revising and finalizing the final version of the manuscript. RS, JS, and AM approve the final version to be published. RS, JS, and AM agree to be accountable for all aspects of the work to maintain the scientific integrity and the research ideas are appropriately investigated and resolved.

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