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Individual and population indicators of *Zostera japonica* respond quickly to experimental addition of sediment-nutrient and organic matter

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ABSTRACT

A manipulative field experiment was designed to investigate the effects of sediment-nutrients and sediment-organic matters on seagrasses, *Zostera japonica*, using individual and population indicators. The results showed that seagrasses quickly responded to sediment-nutrient and organic matter loading. That is, sediment-nutrients positively impacted on seagrasses by increasing N content of leaves and roots, leaf length and belowground biomass. Sediment-organic matter loading lowered N content of seagrass leaves and belowground biomass. Negative effects of organic matter loading were aggravated during nutrient loading, by decreasing N content of leaves, P content of roots, leaf width, shoot number in the middle period of the experiment, increasing C/N ratio of leaves, C/P and N/P ratio of roots and above to belowground biomass ratio of seagrasses. Consequently, *Z. japonica* could be considered as a fast indicator to monitor seagrass ecosystem status in the eutrophic areas and facilitate to interpreting the response of seagrasses to multiple stressors.

1. Introduction

Eutrophication, defined as an increased in the rate of organic matter supply to an ecosystem (Nixon, 1995; Nixon et al., 1996), is typically caused by an excess of nitrogen and phosphorus due to domestic sewage, agricultural fertilizers input, industrial wastes and marine aquaculture (Billen and Garnier, 2007; Bouwman et al., 2013; Gray et al., 2002 and its references). Seagrasses can provide important ecological services for the marine environment (Fourqurean et al., 2012; Hemminga and Duarte, 2000; Orth et al., 2006). In recent years, large-scale losses of seagrasses and risk of extinction for individual seagrass species were reported (Orth et al., 2006; Short et al., 2011). Eutrophication is identified as the main causes for the decline of seagrass meadows worldwide (Delgado et al., 1999; Govers et al., 2014; Orth et al., 2006; Waycott et al., 2009).

Seagrass plant community composition and the spatial distribution of individual species could vary with sediment organic content and nutrient (Barko and Smart, 1983 and its references; Burkholder et al., 2007). Seagrasses responses (e.g. shoot growth, density) to organic matter loading are species specific. Some species (e.g. *Posidonia oceanica, Thalassia hemprichii, T. testudinum*) showed less tolerance to elevated organic matter content in the sediment than other seagrass species (e.g. *Cymodocean nodosa* and *Zostera marina*) (Holmer et al., 2005; Penhale and Wetzel, 1983; Pérez et al., 2007; Ruiz-Halpern et al., 2008; Terrados et al., 1999). Sulphide may accumulate due to the anaerobic decomposition of elevated organic matter, accelerating the mortality of seagrasses (Holmer and Kristensen, 1996; Pérez et al., 2007). Oxygen requirements of seagrass roots and rhizomes fully depend on oxygen transport from the leaves during such anoxic conditions (Smith et al., 1984), which may alter carbon, nitrogen and phosphorus metabolism of seagrass tissues (López et al., 1995; Mateo and Romero, 1997; Pregnall et al., 1984).

Nutrients can differently impact seagrasses depending on their concentration (Burkholder et al., 2007; Huntington and Boyer, 2008; van Katwijk et al., 1997). In oligotrophic areas, nutrient loading could have positive effects on seagrass growth (Burkholder et al., 2007; Lee and Dunton, 1997). In eutrophic areas, enriched nutrients can result in seagrasses mortality mainly due to nitrate and ammonia toxicity (Burkholder et al., 1992; Huntington and Boyer, 2008; van Katwijk et al., 1997). Nutrient enrichment can significantly increase N and P content of seagrass tissues (Bulthuis and Woelkerling, 1981; Invers et al., 2004), also decrease carbon reserves of seagrass tissues because N assimilation requires energy and C skeletons, further affect seagrass survival (Invers et al., 2004). Sediment-nutrient can increase leaf length of *Halodule uninervis*, and cause *H. ovalis* leaves to become longer, wider and thicker (La Nafie et al., 2013).

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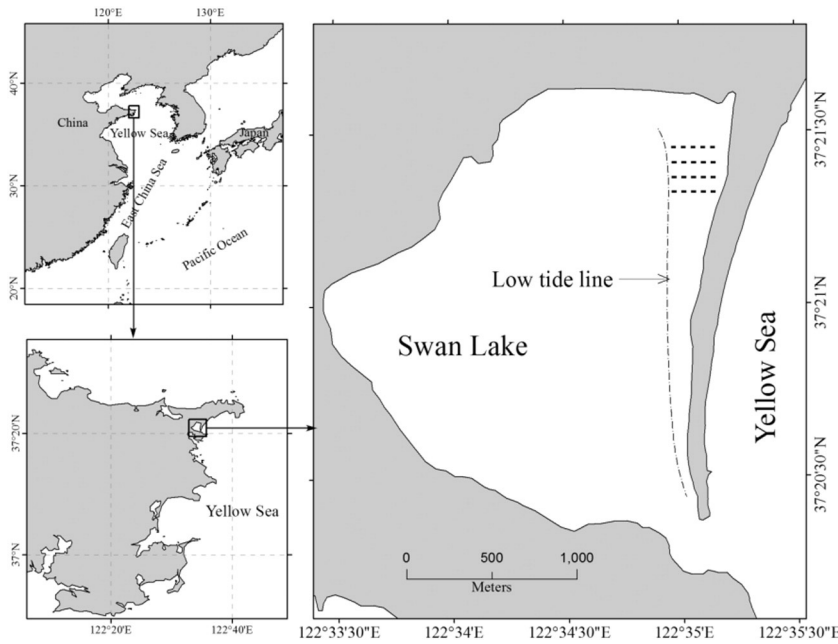*E-mail address:* qyhan@yic.ac.cn (Q. Han).

Fig. 1. Map showing field experimental location.

The individual effects of high nutrient and organic matter enrichment on seagrasses are generally well studied, whereas studies on the combined effects of multiple stressors on seagrasses are relatively rare (but see Govers et al., 2014; Pérez et al., 2007). Pérez et al. (2007) found organic matter and nutrient increased seagrasses mortality and decreased biomass of seagrasses. Govers et al. (2014) studied the effect of organic matter and nutrient in the sediment on the patch of *Zostera noltii*, concluding that patch survival and expansion are constrained at high loads of nutrients or organic matter as a result of porewater ammonium or sulphide toxicity.

Table 1

Results of two-way MANOVA based on categorical data, showing the effects of nutrient (N) and organic matter (OM) on the physiological, morphological and population of seagrasses. Univariate responses are shown as mean per treatment ± SE, with (mean (SE)), to show an indication of the strength of the response, p-values are included in the table.

Variables measured are C, N, P content (% DW), C/N, C/P, N/P ratio of seagrass leaves, roots, rhizomes, leaf length (mm), leaf width (mm), rhizome diameter (mm), rhizome length (mm), dry biomass (g/m^2 dry wt), aboveground biomass (g/m^2 dry wt) and belowground biomass (g/m^2 dry wt), above to belowground biomass ratio, shoot number of seagrasses in the middle and end of experiment (/m^2).

Variables	NN-NO	NN-HO	HN-NO	HN-HO	p-Value		
					N	OM	N & OM
C of leaf	26.9 (0.5)	24.3 (3.5)	26.4 (1.1)	26.0 (1.1)	0.603	0.202	0.366
C of root	23.7 (2.1)	24.7 (5.9)	24.9 (0.4)	25.1 (3.5)	0.711	0.807	0.850
C of rhizome	23.9 (0.3)	27.6 (5.0)	25.5 (2.0)	25.7 (1.4)	0.930	0.253	0.318
N of leaf	2.2 (0.1)	2.0 (0.3)	2.4 (0.1)	2.1 (0.1)	0.070[*]	0.046[**]	0.659
N of root	1.5 (0.1)	1.6 (0.1)	1.7 (0.1)	1.6 (0.1)	0.035[**]	0.887	0.110
N of rhizome	1.2 (0.1)	1.5 (0.3)	1.6 (0.3)	1.4 (0.1)	0.236	0.911	0.068[*]
P of leaf	0.81 (0.17)	1.39 (0.97)	1.34 (0.64)	1.37 (0.29)	0.488	0.412	0.452
P of root	0.28 (0.15)	0.40 (0.08)	0.55 (0.15)	0.33 (0.04)	0.178	0.474	0.030[**]
P of rhizome	0.52 (0.09)	1.06 (0.38)	1.26 (0.74)	0.36 (0.09)	0.929	0.476	0.019[***]
C/N of leaf	12.4 (0.4)	12.1 (0.3)	11.1 (0.4)	12.1 (0.4)	0.511	0.705	0.013[**]
C/N of root	16.2 (0.9)	15.6 (2.5)	14.4 (0.9)	15.5 (2.0)	0.464	0.827	0.410
C/N of rhizome	20.5 (0.3)	18.8 (1.0)	15.8 (2.5)	19.0 (2.4)	0.532	0815	0.049[**]
C/P of leaf	34.3 (7.8)	24.7 (16.1)	24.1 (13.9)	19.5 (3.4)	0.276	0.315	0.715
C/P of root	106.0 (56.5)	63.6 (24.2)	47.6 (13.7)	78.2 (18.4)	0.281	0.765	0.090[*]
C/P of rhizome	47.3 (10.0)	27.4 (5.9)	27.2 (19.6)	75.1 (20.0)	0.153	0.148	0.005[***]
N/P of leaf	2.7 (0.5)	2.0 (1.3)	2.2 (1.4)	1.6 (0.3)	0.418	0.292	0.919
N/P of root	6.4 (3.2)	4.0 (1.1)	3.3 (1.0)	5.0 (0.7)	0.335	0.733	0.083[*]
N/P of rhizome	2.3 (0.5)	1.5 (0.2)	1.9 (1.6)	3.9 (0.6)	0.092[*]	0.306	0.026[**]
Leaf length	71.3 (14.7)	65.6 (10.4)	93.0 (15.8)	91.2 (17.2)	0.053[*]	0.311	0.768
Leaf width	1.5 (0.2)	1.4 (0.2)	1.8 (0.2)	1.5 (0.2)	0.303	0.316	0.248
Rhizome diameter	1.4 (0.1)	1.3 (0.1)	1.5 (0.1)	1.3 (0.2)	0.424	0.539	0.469
Rhizome length	15.3 (2.4)	16.6 (3.4)	13.6 (6.0)	14.2 (12.0)	0.116	0.252	0.905
Dry biomass	16.7 (1.9)	10.2 (5.6)	21.0 (11.8)	19.6 (5.7)	0.218	0.353	0.395
Aboveground biomass	11.6 (1.6)	6.7 (3.4)	15.1 (8.4)	15.0 (4.4)	0.241	0.485	0.313
Belowground biomass	5.1 (1.3)	3.5 (2.2)	5.9 (3.6)	4.5 (2.0)	0.086[*]	0.055[*]	0.907
Above to belowground biomass ratio	2.4 (0.8)	2.2 (0.6)	2.6 (0.4)	3.6 (1.1)	0.418	0.638	0.099[*]
Shoot number in the middle period of experiment	109 (31)	111 (49)	142 (50)	97 (48)	0.763	0.529	0.263
Shoot number in the end of the experiment	977 (233)	586 (307)	1161 (746)	1036 (381)	0.144	0.229	0.526

[*] $p < 0.1$.
[**] $p < 0.05$.
[***] $p < 0.01$.

Higher nitrogen and organic matter contents in sediments contribute to the sediment denitrification (Zhang et al., 2012). Denitrification may be heterotrophic, with organic substrates as energy source, with reduced inorganic compounds such as sulfur or iron used as electron donors (Korom, 1992), which was energy source for anaerobic respiratory processes. Anaerobic environment may promote sediment denitrification (Baker et al., 2000; Zhang et al., 2012 and its references). Nutrient enrichment may further intensify the flux of organic matter to the sediment, thereby enhancing the risk of anoxia and sulphide toxicity (Borum et al., 2005; Holmer and Bondgaard, 2001; Olivé et al., 2009; Pérez et al., 2007). High organic matter content may also enhance the process of nitrate transformed into ammonium (Seitzinger, 1988), consequently increase ammonium toxicity to seagrasses (Govers et al., 2014). The ecological significance of this process in high nutrient and organic matter loading areas is three-folds: *firstly*, organic matter may negatively impact on seagrasses due to hypoxia; *Secondly*, organic matter may decrease the impact of nutrient loading on seagrasses; *Thirdly*, nutrient loading may aggravate the negative effects of elevated organic matter on seagrasses. To our knowledge, there are no studies on the effects of elevated organic matter loading in the sediments during high nutrient enrichment on the individual (physiology, morphology) and population parameters of small seagrasses (e.g. *Zostera japonica*). This is a relevant subject because a better understanding of the eutrophic process is needed to reflect the impacts on seagrasses from a combination of anthropogenic pressures, therefore improve our management capacity.

Consequently, we hypothesize that increased nutrients loading will lead to the negative effects of organic matter to seagrasses by increasing competition for oxygen and resulting in hypoxia within seagrasses, although we expect nutrients may contribute seagrass growth. We therefore carried out a short term (five weeks) manipulative field experiment to test if elevated nutrient enhance the expected adverse effect of organic matter on the seagrass *Zostera japonica*, in the sediments creating gap disturbances, using two level indicators: individual (physiology and morphology) and population. Physiological parameters (C, N and P content of seagrass tissues) were reported to be good indicators of saturating nutrient supply to an environment (e.g. Burkholder et al., 2007; Fourqurean and Zieman, 1992; Fourqurean et al., 1997; Pérez et al., 2008; van Katwijk et al., 2011). Seagrass morphological characteristics (e.g. leaf length, leaf width, rhizome diameter, rhizome length) can be used as indicators in nutrient enrichment (e.g. Burkholder et al., 2007; Lee et al., 2004). Population level indicators (e.g. population biomass, shoot density) could represent characteristic imprints of environmental conditions (e.g. Hauxwell et al., 2001; Hessing-Lewis et al., 2011; Martínez-Crego et al., 2008; Wood and Lavery, 2000). We did a short term manipulative experiment because small seagrasses may rapidly recover by clonal growth after modest sediment disturbance (Han et al., 2012; La Nafie et al., 2013). Moreover, we aim to search good
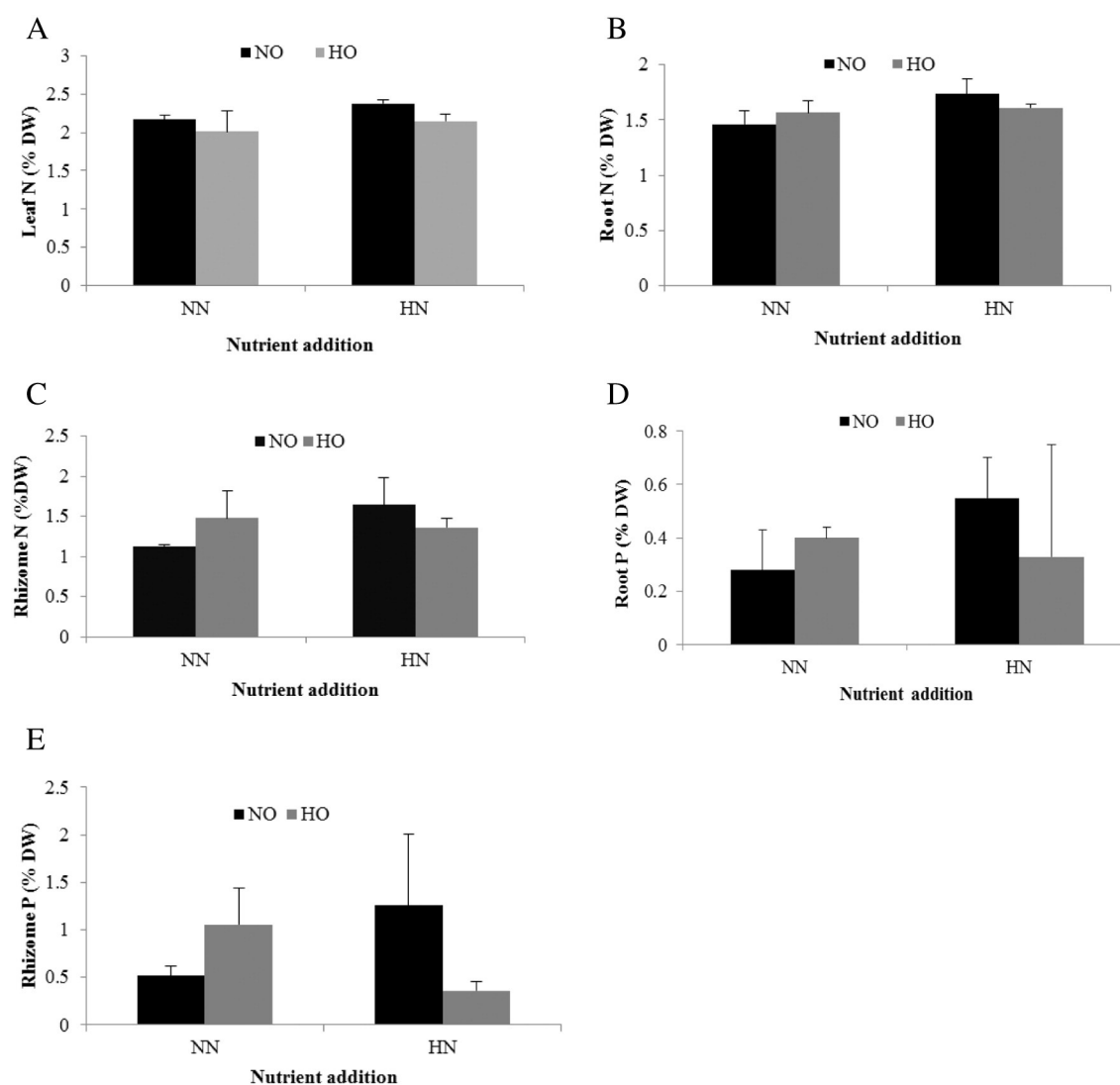

Fig. 2. Seagass tissues nitrogen (N) and phosphate (P) content responses to sediment nutrient and organic matter influence. Significances see Tables 1 and 2.

indicators to show the effects of higher organic matter loading and nutrient enrichment in the short term disturbances, which will provide useful insights for seagrass management, protection and restoration in the situation of nutrient and organic matter enriched areas such as typical areas along the coast of China.

2. Materials and methods

2.1. Field experimental location and design

Swan lake (N36°43′–37°27′, E122°09′–122°42′), a shallow coastal lagoon, is located in the eastern part of Shandong peninsula, northern Yellow Sea of China (Fig. 1). There are Zostera japonica and Z. marina seagrass meadows throughout the lake (Dong et al., 2007). Although Z. japonica seagrass population showed increasing trend worldwide (Short et al., 2011), it is declining in northern China coast (e.g. Swan Lake, Yellow River Delta) in recent years possibly due to human activities (Han et al., 2016). Shellfish aquaculture, sewage discharge and swans feces can enhance high nutrient and organic matter loads into sediments within seagrass habitats, which may contribute to the decline of seagrasses, Z. japonica in Swan Lake. The dam construction (Zhang et al., 2014) and manual R. philippinarum collection during the low tide may disturb the sediment in seagrass meadow at a small scale, which could affect seagrass patch development.

An in situ manipulative experiment was conducted in Swan Lake to test the combined effects of sediment nutrient ("HN" = high nutrient addition, "NN" = no nutrient addition) and organic matter ("HO" = high organic matter addition, "NO" = no organic matter addition) (Fig. 1). The in situ experiment consisted of 3 treatments: i) sediment-nutrient enrichment (HN-NO), ii) sediment-organic matter enrichment (NN-HO) and iii) sediment-nutrient & sediment-organic matter enrichment (HN-HO) plus a control (NN-NO) (i.e., no sediment-nutrient enrichment and no sediment-organic matter enrichment). 5 plots per treatment plus 5 plots for control (20 plots in total) were set up. For preventing nutrient and organic matter cross-contamination, a minimum distance of 5 m between plots in the same treatment and a minimum distance of 35 m between different treatments were implemented. In Swan Lake, hydrodynamics are weak (Han et al., 2016). Our results showing nutrient and organic matter effects also supported sufficient independence between the treatments.

Experimental and control plots were selected in areas with the average Zostera japonica shoot density. At the beginning of the experiment, the cylinder (diameter 50 cm, height 5 cm) was placed within the meadows, and all seagrass materials (including above- and belowground part) were collected to estimate total fresh biomass. Fresh biomasses were not differences among treatments ($F = 2.098$, $p = 0.141$). In the newly created bare areas, fertilization was done using a slow-release commercial fertilizer (g/g ratio N:P:K; 26:11:11; Osmocote). In each fertilized plot, 150 g Osmocote were added into the sediment of the plot for nutrient-enrichment, which was equal to 1500 kg per ha every year. In China coast, nutrient pollution could be more severe than before due to aquaculture and fertilizer used in agriculture (ART, 2006; Miao and Jiang, 2007), so we used higher nutrient loading in our manipulative experiment than other studies (e.g. Govers et al., 2014). Organic carbon content in the sediment is $0.66 \pm 0.19\%$ naturally in seagrass meadows. 400 g organic matters (humic acid) were added into the sediment of the plot for organic matter-enrichment, showing about 0.7% more organic matter into sediment. Organic matter consisted of 4.43% moisture, 11.23% ash, 11.42% volatiles, 70.40% fixed carbon, 2.21% hydrogen and 0.31% sulfur. Nutrients and organic matters were mixed with sediments in the plot for sediment-nutrient & sediment-organic matter enrichment.

2.2. Sampling and measurement

The experiment started in June 2012 and lasted for five weeks. In the middle and end of the experiment, shoot number in each plot was counted. All seagrass materials within the experimental plots were harvested in the end. Seagrass (leaves with rhizomes and roots) samples were collected using a spade (deep into 20 cm sediment) and then cleaned. All samples were kept in a refrigeration box and transported to the laboratory for measurement. The morphological characteristics of seagrasses (leaf length, leaf width, rhizome diameter, rhizome length) were measured within three days following the harvest, and then the seagrass samples were frozen and dried using the freezing dryer (Christ ALPHA 1–4 LD plus). Dry biomass of seagrasses was weighted in the laboratory. The above to belowground biomass ratio of seagrasses was also calculated. Lastly, the C, N and P contents (as % of dry weight) of seagrass leaves, roots and rhizomes were analyzed. The C and N contents of seagrass tissues were analyzed using elemental analyser (Vario Macro cube, Elmentar Company, Germany). The total P content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean and Zieman, 1992). C/N, C/P and N/P ratio of seagrass tissues was also calculated.

2.3. Statistical analysis

To test the effect of nutrient (N) and organic matter (OM), and their interaction (N × OM) × OM) on individual indicators including physiological (C, N and P content, C/N, C/P, N/P ratio in seagrass leaves, rhizomes and roots), morphological properties (leaf length, leaf width, rhizome diameter, rhizome length) and population indicators (shoot number in the middle and end of the experiment, dry biomass, aboveground biomass, belowground biomass and above to belowground ratio of seagrasses), we used a two-way ANOVA. The p-value of the

Table 2
Results of student's t-test between HN-NO and HN-HO treatments based on categorical data, showing the effects of organic matter on the physiological, morphological and population parameters of seagrasses mentioned in Table 1 during high nutrient loading. p-Values are included in the table.

Variables	t	p
C of leaf	0.523	0.629
C of root	−0.058	0.956
C of rhizome	−0.191	0.858
N of leaf	4.138	0.014**
N of root	1.840	0.140
N of rhizome	1.341	0.251
P of leaf	−0.066	0.950
P of root	2.586	0.061*
P of rhizome	2.085	0.105
C/N of leaf	−3.074	0.037**
C/N of root	−0.897	0.421
C/N of rhizome	−1.575	0.190
C/P of leaf	0.556	0.608
C/P of root	−2.315	0.082*
C/P of rhizome	−0.693	0.527
N/P of leaf	0.741	0.500
N/P of root	−2.315	0.082*
N/P of rhizome	−2.008	0.115
Leaf length	0.166	0.872
Leaf width	2.126	0.066*
Rhizome diameter	1.630	0.142
Rhizome length	−0.089	0.931
Dry biomass	0.249	0.809
Aboveground biomass	0.027	0.980
Belowground biomass	0.747	0.476
Above to belowground biomass ratio	−1.913	0.092*
Shoot number in the middle period of experiment	2.189	0.060*
Shoot number in the end of the experiment	0.332	0.748

* $p < 0.1$.
** $p < 0.05$.

univariate responses was used to indicate which of these separate variables gave the most significant response. Those data that deviated from normality (Kolmogorov-Smirnov's test) or homoscedasticity (Levene's test) were transformed prior to analyze to meet two-way ANOVA assumptions. The differences of seagrass parameters above-mentioned between HN-NO and HN-HO treatments were tested by a two-sample Student's t-test for independent samples to analyze organic matter effects during high nutrient loading. We set p values as highly significant, significant, and marginally significant when $p = 0.01$, 0.05, and 0.1, respectively (La Nafie et al., 2012; Soissons et al., 2014).

3. Results

3.1. Physiological properties of seagrasses

Nutrient marginally significantly and significantly increased N content of seagrass leaves and roots, respectively (Table 1; Fig. 2A, B). Organic matter significantly decreased N content of seagrass leaves (Table 1; Fig. 2A). Organic matter significantly decreased N content of

leaves in the conditions of nutrient loading (Table 2; Fig. 2A) ($t = 4.138$, $p = 0.014$). N content value of seagrass rhizomes was marginally significantly affected by the combination of organic matter and nutrient addition (Table 1; Fig. 2C). The lowest N content value ((2.0 ± 0.3) %) of seagrass leaves was observed in the NN-HO treatments. The lowest N content value of seagrass roots ((1.5 ± 0.1) %) and rhizomes ((1.2 ± 0.1) %) was observed in the control treatments (Table 1). The highest N content value of seagrass leaves ((2.4 ± 0.1) %), roots ((1.7 ± 0.1) %) and rhizomes ((1.6 ± 0.3) %) was found in the HN-NO treatments (Table 1).

Organic matter marginally significantly decreased P content of roots in the conditions of nutrient loading ($t = 2.586$, $p = 0.061$) (Table 2; Fig. 2D). P content of seagrass roots and rhizomes was significantly affected by the combination of organic matter and nutrient addition (Table 1; Fig. 2D, E). The highest P content value of seagrass roots ((0.55 ± 0.15) %) and rhizomes ((1.26 ± 0.74) %) was found in the HN-NO treatments (Table 1). The lowest P content value of seagrass roots ((0.28 ± 0.15) %) and rhizomes ((0.36 ± 0.09) %) was separately recorded in the control and HN-HO treatments (Table 1).
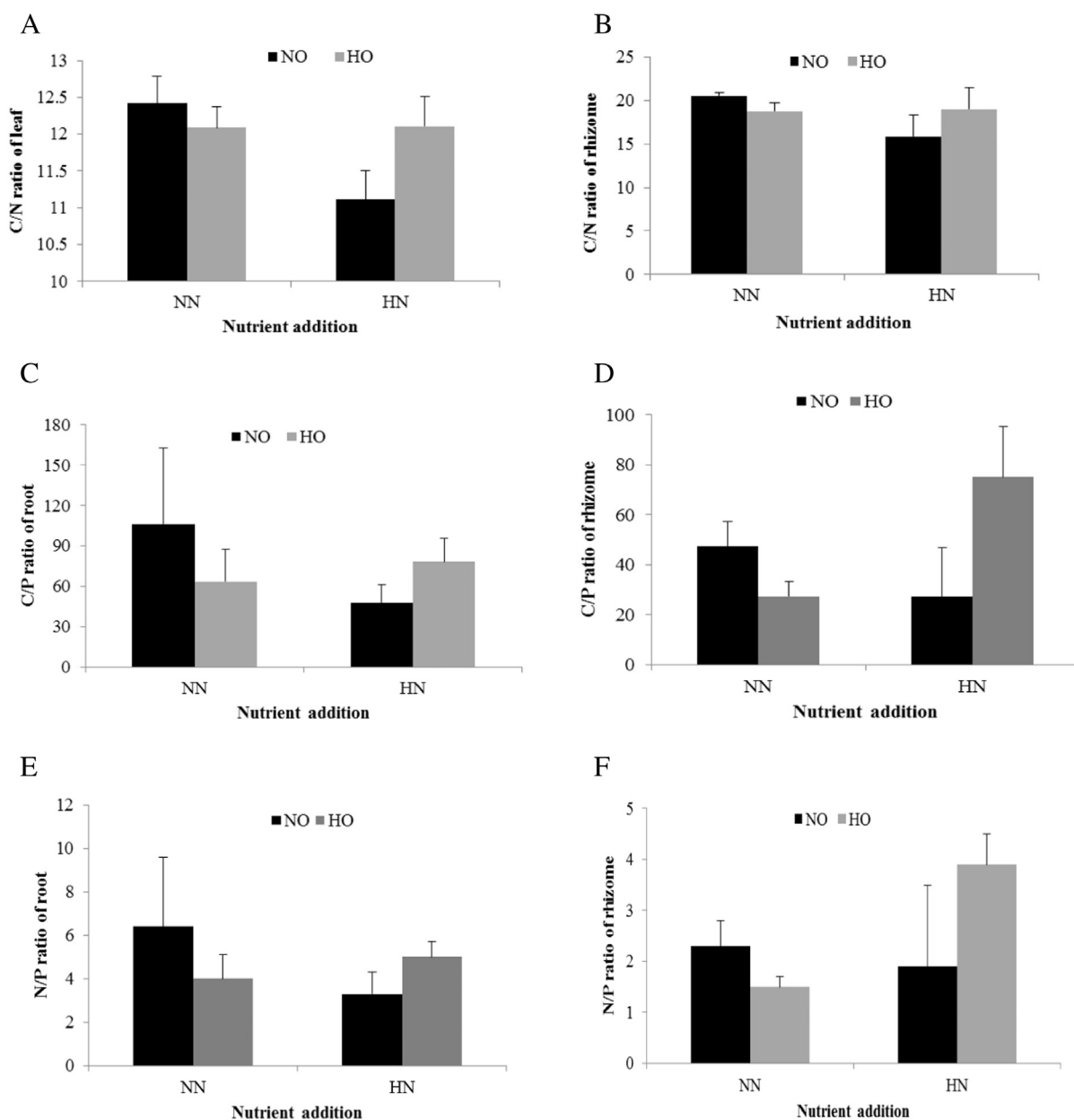

Fig. 3. Seagass tissues C/N, C/P, N/P ratio responses to sediment nutrient and organic matter influence. Significances see Tables 1 and 2.
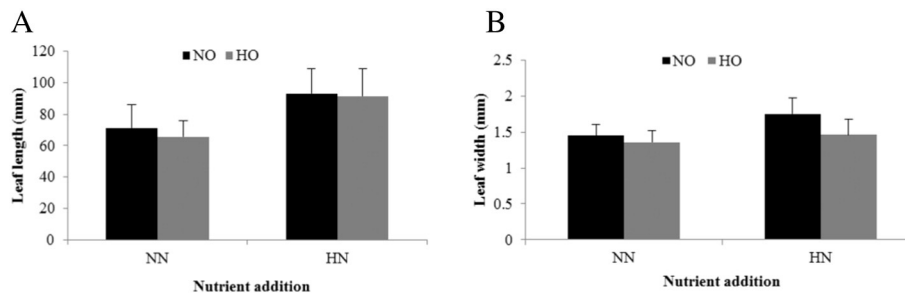
Fig. 4. Morphological characteristics of the seagrass plants responses to sediment nutrient and organic matter loading. Significances see Tables 1 and 2.

Organic matter significantly increased C/N ratio of leaves in the conditions of nutrient loading (Table 2; Fig. 3A) ($t = -3.074, p = 0.037$). C/N ratio of leaves and rhizomes was significantly affected by the combination of organic matter and nutrient addition (Table 1; Fig. 3A, B). The lowest C/N ratio value of seagrass leaves ((11.1 ± 0.4) %) and rhizomes ((15.8 ± 2.5) %) was observed in the HN-NO treatments (Table 1). The highest C/N ratio value of seagrass leaves ((12.4 ± 0.4) %) and rhizomes ((20.5 ± 0.3) %) was recorded in the control treatments (Table 1).

Organic matter marginally significantly increased C/P ratio of roots in the conditions of nutrient loading (Table 2, Fig. 3C) ($t = -2.315$, $p = 0.082$). C/P ratio of roots and rhizomes was respectively marginally and highly significantly affected by the combination of organic matter and nutrient addition (Table 1; Fig. 3C, D). The lowest C/P ratio value ((47.6 ± 13.7) %) of seagrass roots was observed in the HN-NO treatments, while the highest value ((106.0 ± 56.5) %) in the control treatments (Table 1). The lowest ((27.2 ± 19.6) %) and highest ((75.1 ± 20.0) %) C/P ratio values of seagrass rhizomes were separately found in the HN-NO and HN-HO treatments (Table 1).

Organic matter marginally significantly increased N/P ratio of roots in the conditions of nutrient loading (Table 2; Fig. 3E) ($t = -2.315$, $p = 0.082$). N/P ratio of roots and rhizomes was marginally and significantly affected by the combination of organic matter and nutrient addition, respectively (Table 1; Fig. 3E, F). The lowest N/P ratio value ((3.3 ± 1.0) %) of seagrass roots was found in the HN-NO treatments, while the

highest ((6.4 ± 3.2) %) in the control treatments (Table 1). The lowest ((1.5 ± 0.2) %) and highest ((3.9 ± 0.6) %) N/P ratio values of seagrass rhizomes were separately found in the NN-HO and HN-HO treatments (Table 1).

3.2. Morphological properties of seagrasses

Nutrient loading marginally significantly increased leaf length with the highest value ((93.0 ± 15.8) mm) in the HN-NO treatments (Table 1; Fig. 4A). Organic matter marginally significantly decreased leaf width in the conditions of nutrient loading ($t = 2.126, p = 0.066$) (Table 2), with the highest value ((1.8 ± 0.2) mm) in the HN-NO treatments, while the lowest value ((1.4 ± 0.2) mm) in the NN-HO treatments (Table 1; Fig. 4B).

3.3. Population properties of seagrasses

Nutrient loading marginally significantly increased belowground biomass of seagrasses, but which was marginally significantly decreased by organic matter loading (Table 1; Fig. 5A). No significant interaction effects were found. The highest belowground biomass value ((5.9 ± 3.6) g/m^2 dry wt) of seagrasses was recorded in the HN-NO treatments, while the lowest value ((3.5 ± 2.2) g/m^2 dry wt) in the NN-HO treatments (Table 1; Fig. 5A).
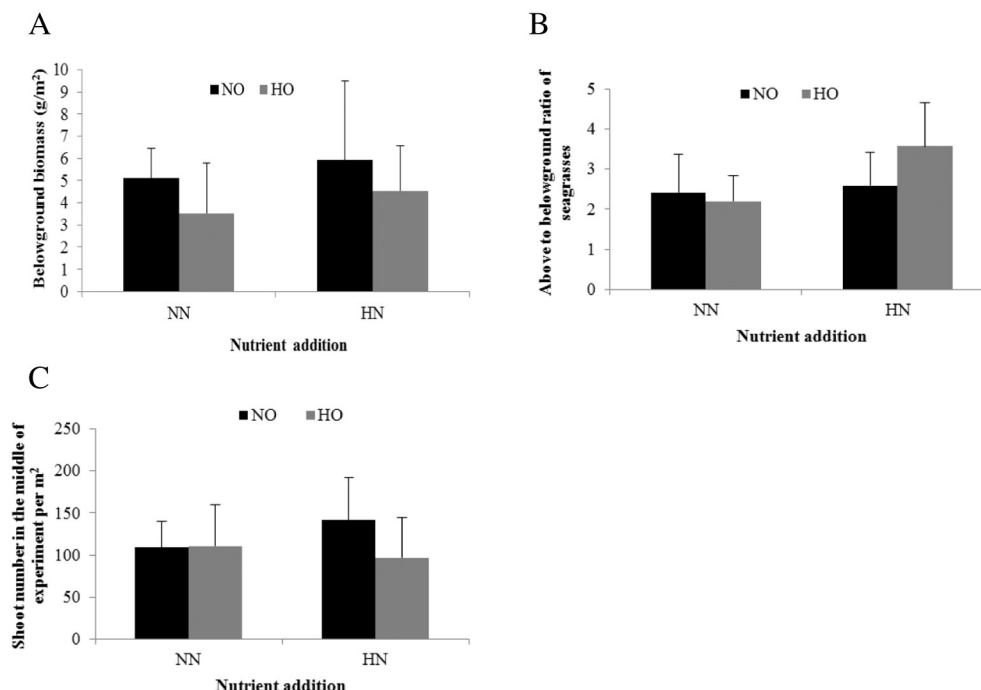

Fig. 5. Population characteristics of the seagrasses responses to sediment nutrient and organic matter loading. Significances see Tables 1 and 2.

Above to belowground biomass ratio was marginally significantly increased by organic matter addition in the conditions of nutrient loading (Table 2; Fig. 5B) ($t = -1.913$, $p = 0.092$), which was marginally significantly affected by the combination of organic matter and nutrient addition with the highest value (3.6 ± 1.1) in the HN-HO treatments, while the lowest value (2.2 ± 0.6) in the NN-HO treatments (Table 1; Fig. 5B).

Organic matter marginally significantly decreased shoot number of seagrasses in the middle period of experiment in the conditions of nutrient loading (Table 2; Fig. 5C) ($t = 2.189$, $p = 0.060$), with the lowest value (97 ± 48) in the HN-HO treatments, while the highest value (142 ± 50) in the HN-NO treatments (Table 1; Fig. 5C).

4. Discussion

Anthropic activity and global climate change can bring nutrients and organic matters into sediments, thereby alter characteristics of seagrasses (Pérez et al., 2007; Rabalais et al., 2009; Short and Wyllie-Echeverria, 1996). Our results showed that seagrasses quickly responded to sediment-nutrient and organic matter loading at individual and population levels. That is, sediment-nutrients positively impacted on seagrasses. Sediment-organic matter loading showed negative effects on seagrasses that was aggravated during high nutrient loading. This has to our knowledge, not previously been reported in seagrasses.

4.1. Positive effects of sediment-nutrient enrichment on individual and population indicators of seagrasses

Plants exposed to high nutrient loads can show physiological change, such as increases in tissue nutrient content (Invers et al., 2004; Peterson et al., 2012; Pérez et al., 2007; Udy and Dennison, 1997a). In our study, N content of seagrass roots and rhizomes was lower than 1.8% of the dry weight (Table 1; Fig. 2B, C), indicating that these seagrasses were probably nutrient-limited at the start of the experiment (Duarte, 1990). In our study at the end of the experiment, nutrient loading significantly increased N content of seagrass leaves and roots (Table 1; Fig. 2A, B), which was in agreement with Peralta et al. (2003) from *Zostera marina* study. Seagrass roots can absorb nutrient from the porewater, and one-third nitrogen fixation activity in sediments was associated with below-ground part of seagrasses (Romero et al., 2006). Our results confirmed that *Z. japonica* roots may supply the N demand of seagrasses by transferring N to aboveground part in nutrient rich sediments, and N content of seagrass tissues could be used as a good indicator for sediment-nutrient loading.

Nutrient availability is reflected within seagrasses via increased growth (Peterson et al., 2012). In our study, sediment nutrient increased leaf length and belowground biomass of seagrasses, which indicated that nutrients are used for seagrasses growth (Short, 1983; Peterson et al., 2012). Previous studies also showed sediment nutrient fertilization increased leaves biomass (e.g. Hemminga and Duarte, 2000; La Nafie et al., 2013; Lee and Dunton, 1999; Peralta et al., 2003; Peterson et al., 2012). Nutrient availability may be incorporated into the carbohydrate reserves of seagrasses (e.g. *Thalassia tesudinum*) (Campbell et al., 2012). Respiration rates of seagrasses may enhance with increasing nutrient enrichment in the sediment (Touchette, 1999), which could help rhizomes and roots of seagrasses to supply the energy and carbon needed to assimilate and reduce nitrate, consequently decrease the toxicity of higher concentration nutrients, facilitate seagrass growth (Touchette, 1999; Touchette and Burkholder, 2000).

4.2. Negative effects of elevated organic matter on individual and population indicators of seagrasses during high nutrient loading

Organic matters significantly decreased N content of seagrass leaves (Tables 1, 2; Fig. 2A) and increased C/N ratio of leaves in the conditions of nutrient loading (Tables 1, 2; Fig. 3A). It may be because organic matter can induce sediment redox in the conditions of nutrient loading, which reduces the absorption of seagrass roots to N, especially nitrate available forms by denitrification from bacterial metabolism (Peralta et al., 2003; Udy and Dennison, 1997b; Worm and Reusch, 2000; Worm et al., 2000). In the conditions of higher nutrient loading, denitrification requires a supply of organic matter as energy source (Seitzinger and Nixon, 1985 and its references), resulting in anaerobic respiratory processes (Korom, 1992), which may conversely promote sediment denitrification (Baker et al., 2000; Zhang et al., 2012 and its references). Nutrients absorption of seagrass roots may impact on uptake of seagrass leaves to nitrogen (Thursby and Harlin, 1982, 1984). Also epiphyte overgrowth due to additive sediment-nutrient and organic matter loading may hinder N uptake by seagrass leaves, therefore decrease N uptake of seagrass leaves (Apostolaki et al., 2012; Balata et al., 2010; Cancemi et al., 2003; Delgado et al., 1999), although we did not observe epiphytes in this study.

Phosphate enrichment of the seagrass (e.g. *Heterozostera tasmanica*) sediments can increase P content in the rhizomes (from 0.16–0.18%) (Bulthuis and Woelkerling, 1981). In our study, phosphate loading in the sediment did not significantly impact on P content of *Zostera japonica* tissues, because P content of seagrass tissues was above the threshold (0.2%) for limitation for seagrasses outlined by Duarte (1990), indicating there is not P limited in our study area. Roots can assimilate phosphate from the porewater of sediment (Brix and Lyngby, 1985). Organic matter marginally significantly decreased P content of roots ($t = 2.586$, $p = 0.061$) (Table 2; Fig. 2D) and increased C/P and N/P ratio of roots in the conditions of nutrient loading (Table 2; Fig. 3C, E). That copes with Pérez et al. (2007), who found organic matter reduced P content of *Posidonia oceanica*. Plants require more P for higher growth rates (Ågren, 2004; Sterner and Elser, 2002), because P is required during the metabolic energy transfer process (Duarte, 1990; Stitt, 1997). Organic matter can result in low energy status of seagrasses (Erskine and Koch, 2000; Pérez et al., 2008), therefore negatively affect P uptake of seagrasses by increasing decomposition under low-oxygen conditions (Day et al., 1989; Pérez et al., 2007). Phosphate may play a key role in reducing ammonium toxicity (Brun et al., 2002, 2008). When carbon turnover and ATP consumption increase as a result of ammonium assimilation in the conditions of nutrient loading, an enhancement of phosphate demands would be expected (Brun et al., 2002; Villazán et al., 2013). Phosphate also easily adsorbs to organic particles during higher organic matter loading (Froelich, 1988; Stumm and Morgan, 1996). Consequently, high nutrient and organic matter loading process limiting phosphate availability and acquisition might make seagrasses more vulnerable to ammonium toxicity (Villazán et al., 2013).

Organic matter marginally significantly decreased leaf width, shoot number in the middle period of the experiment during nutrient loading (Table 2; Figs. 4B, 5C), reduced belowground biomass of seagrasses (Table 1; Fig. 5A), but increased above to belowground biomass ratio in the conditions of nutrient loading (Table 2; Fig. 5B). Our findings are in accordance with those of previous studies (e.g. Pérez-Lloréns and Niell, 1993; Terrados et al., 1999). Shoot:root (including rhizome) ratio in *Zostera noltii* was higher in the high organic muddy sediments in Palmones River estuary, Spain (Pérez-Lloréns and Niell, 1993). Terrados et al. (1999) also found some bigger seagrasses were negatively affected by the addition of organic matter to the sediment in terms of decreased *Thalassia hemprichii* shoot density and *Z. marine* leaf growth. Enhanced organic matter sedimentation can cause anoxia and sediment sulphide intrusions into the plants (Holmer and Bondgaard, 2001; Olivé et al., 2009; Pérez et al., 2007). High organic matter content may enhance the process of nitrate transformed into ammonium (Seitzinger, 1988), consequently increase ammonium toxicity to seagrasses (Govers et al., 2014), therefore negatively affect seagrasses growth.

Sediment anoxia can promote seagrass growth inhibition and mortality by increasing the oxygen demand of the sediment with the addition of organic matter (Han and Liu, 2014; Terrados et al., 1999), because carbon translocation from leaves to roots is stopped due to

relevant metabolic adaptation for controlling carbon losses under anaerobic conditions (Zimmerman and Alberte, 1996). Sulphide, one of the main causes of the regressions of the seagrsses, is known to have inhibitory effects on plant growth, although seagrasses demonstrate some tolerance at least to short-term sulphide exposure (Erskine and Koch, 2000; Koch and Erskine, 2001). Nitrogen fixation in seagrass sediments is dependent on photosynthates exuded by seagrass roots (Welsh et al., 1996, 1997). Nutrient enrichment increased seagrasses demand for carbon to support metabolic processes in anaerobic sediments with organic matter addition (Burkholder et al., 1992; Irlandi et al., 2004; Lee and Dunton, 1999), which may explain the negative effects of organic matter loading on seagrass growth during sediment-nutrient enrichment.

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