

Contrasting effects of nutrient enrichment on below-ground biomass in coastal wetlands

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Summary

1. Anthropogenically enhanced nutrient availability is often cited among the most important drivers of altered ecosystem function and loss of services world-wide. Although the above-ground consequences of nutrient enrichment on plant growth patterns are numerous and well documented, below-ground impacts are less clear but nonetheless critical from a global change perspective. In coastal wetlands, for example, plant–soil–nutrient dynamics directly affect the capacity to sequester carbon as soil organic matter, keep pace with sea level rise and resist storm-induced erosion.

2. Here, we investigate the effects of excess nutrient loading on below-ground plant growth in an oligohaline marsh fertilized for 7 years with a factorial combination of nitrogen (N) and phosphorus (P). We used two common assessment procedures, the ingrowth and standing crop methods, to simultaneously quantify distinct aspects of below-ground plant growth, which are (i) below-ground biomass accumulation into unexploited open resource space and (ii) *in situ*, or maintenance, below-ground biomass of plants in equilibrium with their environment, respectively. Our objective was to determine if plant growth responses to nutrient enrichment differed depending on process and/or biomass component measured.

3. We show that excess N concurrently increased live root biomass accumulation in ingrowth cores and reduced *in situ* live root standing crop. Similar, albeit non-significant, response trajectories were apparent for other below-ground biomass pools using both methods, excepting dead biomass and total standing crop. A review of previously published research supports our results and suggests that nutrient enrichment consistently has contrasting effects on below-ground plant growth depending on whether biomass accumulation or standing crop is measured, and that living biomass components are most responsive to enhanced nutrient availability.

4. Synthesis. We conclude that eutrophic conditions can be both beneficial and detrimental to ecosystem function by either stimulating below-ground biomass accumulation in unexploited soil or reducing the below-ground standing crop required to sustain the nutritional needs of established plants in mature communities. Thus, nutrient enrichment may, in the short-term, contribute to soil organic matter (i.e. carbon) accumulation by increasing below-ground growth as plants exploit new resource space. Over the long-term, however, nutrient enrichment has the potential to negatively impact soil organic matter content as plants equilibrate to excess nutrient availability by down-regulating below-ground standing crop.

Key-words: below-ground biomass, ecosystem function, fertilization, ingrowth method, nitrogen, nutrient enrichment, oligohaline marsh, phosphorus, plant–soil (below-ground) interactions, standing crop method

Introduction

Plant growth responses to anthropogenically enhanced global nutrient availability can elicit diverse effects on ecosystem

structure and function as illustrated by the cascading effects of above-ground biomass stimulation on plant nutrient cycling, competitive hierarchies, community composition and biodiversity to name a few (Bedford, Walbridge & Aldous 1999; Smith, Tilman & Nekola 1999; Suding *et al.* 2005; Elser *et al.* 2007; Bobbink *et al.* 2010). However, similarly definitive responses within the soil environment are, in contrast, currently lacking, despite the important impacts nutrient-induced

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changes in below-ground plant growth patterns may have on the largest global pool of terrestrial carbon, soil organic matter (Post *et al.* 1982; Rasse, Rumpel & Dignac 2005). Understanding the below-ground effects of nutrient enrichment is especially important in coastal wetlands where (i) soil organic matter (i.e. carbon) accumulates at rates comparable to terrestrial forests, despite representing a small fraction of the global surface area (Mcleod *et al.* 2011), (ii) below-ground plant biomass is an essential determinant of ecosystem stability that helps counter-balance the effects of sea level rise by modulating surface elevation change through contributions to soil volume (Nyman *et al.* 2006; McKee, Cahoon & Feller 2007) and maintain soil matrix integrity during high-energy meteorological events, such as hurricanes (Howes *et al.* 2010) and (iii) low elevational positions along coastal margins and hydrological forcings from both the land and sea serve as direct vectors for nutrient input. Consequently, recent research has focused considerable efforts on identifying nutrient enrichment effects on soil processes in coastal wetlands, though it is currently unclear why such conditions can either increase, decrease, or have no effect on below-ground biomass (e.g. Tyler, Lambriños & Grosholz 2007; Darby & Turner 2008a,b; Hunter *et al.* 2008; Langley *et al.* 2009; Ket, Schubauer-Berigan & Craft 2011; Anisfeld & Hill 2012; Deegan *et al.* 2012; Nelson & Zavaleta 2012).

Surprisingly, no attention has been given to the possibility of dual below-ground responses to enhanced nutrient availability depending on the process measured: new below-ground biomass accumulation as plants exploit open resource space vs. maintenance biomass required to sustain the nutritional needs of established plants in mature communities. Currently, multiple approaches are employed to assess the effects of nutrient enrichment on below-ground biomass, including most commonly the ingrowth and standing crop methods. However, the use of these different assessment procedures necessitates interpretations that distinguish the distinct aspects of plant growth measured; the ingrowth method measures new below-ground growth into soil, or similar substrate, after intact roots and rhizomes are severed and removed (i.e. biomass accumulation in open resource space), whereas the standing crop method measures *in situ*, or maintenance, below-ground biomass of plants in equilibrium with their environment. Yet, to date, these methodological differences that may be affecting overall conclusions concerning the below-ground effects of elevated nutrient conditions have not been critically examined or considered as a potential source of variation among results. To our knowledge, Valiela, Teal & Persson (1976) report the only study to simultaneously use both methods to investigate the direct effects of coastal wetland nutrient enrichment on the various below-ground biomass components individually (i.e. live roots, live rhizomes and dead biomass). Interestingly, their results suggested that living below-ground biomass components, but not dead, responded differently depending on whether below-ground biomass accumulation or standing crop was measured, although no explanation was offered as to why this occurred. Thus, discerning nutrient-induced below-ground responses

may require a more nuanced evaluation that explicitly considers the ecological context of the measurement method, as well as biomass pool measured.

The present research examines, for the first time, these methodological considerations that may constrain understanding of nutrient enrichment effects on below-ground plant biomass. Although much of the controversial coastal wetland eutrophication literature to date derives from salt marsh research (e.g. Darby & Turner 2008a,b vs. Anisfeld & Hill 2012), it is low salinity wetlands that often dominate landscapes where rivers deliver freshwater with elevated nutrient loads to coastal environments, such as in the Mississippi River Delta (Sasser *et al.* 2008). Therefore, we quantified live and dead below-ground biomass components separately using the ingrowth and standing crop methods concurrently within an oligohaline marsh fertilized for 7 years with a factorial combination of nitrogen (N) and phosphorus (P). We propose that inconsistent below-ground plant responses to nutrient enrichment stem in part from the measurement of different organic matter pools using different methods that require different interpretations: (i) the ingrowth method serves as a proxy for below-ground growth into unexploited soil or open habitat, (ii) the standing crop method represents the quantity of below-ground biomass required to sustain the nutritional needs of established plants and (iii) regardless of method used, measurement of component biomass pools increases the ability to identify nutrient enrichment effects occurring below-ground. We hypothesized that enhanced nutrient availability stimulates the ability of plants to exploit open resource space, but reduces maintenance growth requirements as established plant communities equilibrate to nutrient excess, and that living biomass components, specifically live roots, are the most responsive to elevated nutrient conditions.

Materials and methods

SITE DESCRIPTION AND EXPERIMENTAL DESIGN

Our study site is a *Sagittaria lancifolia* L. dominated, oligohaline marsh located along the west bank of the Tchefuncte River, approximately 1 km north of Lake Pontchartrain, LA, USA (30° 23.205'N, 90° 09.551' W), a brackish estuary with hydrological connection to the Gulf of Mexico. This study site is a relatively fertile, river-fed coastal wetland with a diverse plant community (Graham & Mendelssohn 2010) and Histosol soil (Kenner Series; Trahan *et al.* 1990). Marsh flooding results from a combination of microtidal influence (10 cm tide range; Swenson & Chuang 1983), wind shifts during frontal passages and rainfall driven fluctuations in Tchefuncte River discharge that inundates the soil surface on average (1999–2006) approximately every other day to a depth of 15 cm with surface water containing on average 1.6 g L⁻¹ salinity (indicating oligohaline estuarine conditions), 0.26 mg inorganic N L⁻¹, 0.79 mg total N L⁻¹ and 0.11 mg total P L⁻¹ (Graham & Mendelssohn 2010).

Our fertilization experiment consisted of 40 1-m² plots arranged in a randomized complete block design replicated at five locations spaced 5–10 m apart, parallel to a small drainage canal. At each location, plots were fertilized with one of four N levels (0, 50, 200 or 1200 kg N ha⁻¹ year⁻¹ applied as Nutralene Methylene Urea 40-0-0) in combination with one of two levels of P (0 or

131 kg P ha⁻¹ year⁻¹ applied as Humaphos 0-5-0), yielding eight treatment combinations ($n = 5$ per treatment). Treatments levels were maintained for 7 years by applying granulated, slow-release fertilizer to the soil surface in April and July of 2002 through 2008.

SAMPLE COLLECTION

We quantified below-ground plant biomass responses to N, P and N × P enrichment using both the ingrowth and standing crop methods concurrently. The ingrowth method was employed to estimate below-ground biomass accumulation in root and rhizome free sediment over a 3-year period, 4–7 years after initiating fertilization treatments (2005–2008), while the standing crop method was simultaneously employed to estimate *in situ* below-ground biomass after 7 years of fertilization in 2008. Although we acknowledge that both the ingrowth and standing crop methods have well-recognized shortcomings that limit an accurate estimation of below-ground production (Valiela, Teal & Persson 1976; Neill 1992; Eissenstat & Yanai 2002; Hendricks *et al.* 2006), our objective here was not to determine absolute rates, but rather identify relative responses to nutrient enrichment. Thus, differences in below-ground biomass estimates using both methods accurately reflect treatment effects.

For the ingrowth method, the native soil within each plot was removed to a depth of 30 cm using a 7.62-cm diameter aluminium core tube and, using the same core tube, immediately replaced with creek bank sediment previously collected from a nearby marsh with a similar vegetative community. Roots and rhizomes, both live and dead, were handpicked from the creek bank sediment prior to installation, but the sediment was not sieved in order to maintain a soil environment that contained a natural mix of particulate soil organic matter similar to the study marsh. Upon installation, the location of each ingrowth core was marked with a 4-cm long by 7.62-cm diameter PVC collar inserted to a depth of approximately 2 cm below the soil surface. The entire installation procedure caused no visible disturbance to plants or soil beyond the immediate vicinity (i.e. 10–15 cm) of ingrowth core locations. Following installation, ingrowth core locations were monitored for 3 years to further verify that no lasting disturbance to the plants or soil occurred.

After deployment for 3 years, ingrowth cores were relocated and extracted with the same aluminium core tube used during the installation process. At this time, below-ground standing crop cores (i.e. intact, undisturbed soil cores) of the same diameter and depth as ingrowth cores were also extracted from a separate location within each plot approximately 30–50 cm from ingrowth core locations. All cores collected using both methods were then sieved over a 2-mm mesh screen and the remaining below-ground biomass separated by component as live roots, live rhizomes and dead (roots + rhizomes), dried to a constant mass at 60 °C and weighed. Biomass was categorized as either live or dead using a combination of characteristics that included colour, turgidity and evidence of decomposition (e.g. epidermal lesions and resistance to breakage). When necessary, a dissecting microscope (3× magnification) was used to examine the below-ground material in more detail.

STATISTICAL ANALYSIS

All statistical analyses were conducted using SAS (Statistical Analysis Systems, version 9.3, SAS Institute, Inc., Cary, NC, USA). We used univariate two-way mixed-model ANOVA to identify the effects of N, P and their interaction (N × P) on below-ground biomass pools (i.e. live roots, live rhizomes, live roots + rhizomes, dead roots

+ rhizomes and total live + dead biomass) quantified using the ingrowth and standing crop methods separately. Prior to analysis, we examined normal probability plots and residual plots to verify that the ANOVA assumptions of normality and homoscedasticity were not violated. To meet these assumptions, live rhizome ingrowth data required square root transformation, while the remaining data, excluding live root ingrowth and dead ingrowth and standing crop, required natural log transformation. Post-ANOVA, differences among treatment-means were tested using the Tukey–Kramer multiple comparison test. For presentation of results, untransformed arithmetic means and standard errors (SE) were used.

Results

BELOW-GROUND BIOMASS ACCUMULATION (INGROWTH)

After 3 years of deployment that corresponded to fertilization years 4 through 7, live root accumulation in ingrowth cores increased with increasing N enrichment from 121 ± 19 g m⁻² to 249 ± 45 g m⁻². Plots receiving 1200 kg N ha⁻¹ year⁻¹ had significantly greater live root biomass than control plots, while plots enriched with intermediate levels of N (i.e. 50 and 200 kg N ha⁻¹ year⁻¹) accumulated intermediate living root biomass that was not significantly different from either the control or high-N plots (Table 1, Fig. 1a). To a lesser extent, live root accumulation was also influenced by P enrichment when applied in combination with N ($P = 0.08$; Table 1). However, the overall trend was similar to that which occurred with N enrichment alone and N × P treatment-means were not statistically different from each other (see Fig. S1 in Supporting Information). Nutrient enrichment had no significant effect on live rhizome, live or dead (root + rhizome) or total (live + dead) biomass accumulation in ingrowth cores (Table 1), though increasing trends in response to increasing N enrichment were apparent for all component pools, excluding dead biomass accumulation (Fig. 1b–e). On average, plots receiving 1200 kg N ha⁻¹ year⁻¹ had 3-, 2.5- and 2-fold greater live rhizome, live (root + rhizome) and total (live + dead) biomass accumulation, respectively, compared to control plots.

BELOW-GROUND STANDING CROP

After 7 years of fertilization, live root standing crop decreased with increasing N enrichment from 73 ± 12 g m⁻² to 36 ± 12 g m⁻², and a significant reduction compared to the control occurred with 200 kg N ha⁻¹ year⁻¹ (Table 1; Fig. 2a). Further enrichment with 1200 kg N ha⁻¹ year⁻¹ also reduced live root standing crop compared to the control, but this treatment level had no additional effect compared to the 200 kg N ha⁻¹ year⁻¹ treatment. Live rhizome standing crop showed a more variable, but similar non-significant decreasing trend with increasing N enrichment, which resulted in a 2.5-fold biomass reduction in plots receiving 1200 kg N ha⁻¹ year⁻¹ compared to the control (Table 1; Fig. 2b). Nitrogen enrichment tended to decrease combined live (root + rhizome) standing crop as well (Table 1; Fig. 2c).

Table 1. Summary of two-way ANOVAs showing the effects of nitrogen (N), phosphorus (P) and their interaction (N × P) on concurrent measurements of (a) below-ground biomass accumulation using the ingrowth method, and (b) *in situ* below-ground biomass using the standing crop method. Values are *F*-ratios, with associated numerator and denominator degrees of freedom subscripted in parentheses (ndf and ddf, respectively) and *P*-values that are underlined when significant ($P \leq 0.05$)

Model Source	Live Roots		Live Rhizomes		Live (Roots + Rhizomes)		Dead (Roots + Rhizomes)		Total (Live + Dead)	
	<i>F</i> _(ndf, ddf)	<i>P</i>	<i>F</i> _(ndf, ddf)	<i>P</i>	<i>F</i> _(ndf, ddf)	<i>P</i>	<i>F</i> _(ndf, ddf)	<i>P</i>	<i>F</i> _(ndf, ddf)	<i>P</i>
(a) Ingrowth*										
N	3.39 _(3, 27)	<u>0.03</u>	0.94 _(3, 27)	0.43	1.90 _(3, 27)	0.15	0.71 _(3,31)	0.55	1.65 _(3,27)	0.20
P	0.12 _(1, 27)	0.73	0.83 _(1, 27)	0.37	0.05 _(1, 27)	0.82	0.05 _(1,31)	0.82	0.05 _(1,27)	0.82
N × P	2.49 _(3, 27)	0.08	0.87 _(3, 27)	0.47	1.73 _(3, 27)	0.18	1.42 _(3,31)	0.26	2.07 _(3,27)	0.13
(b) Standing Crop†										
N	4.07 _(3, 28)	<u>0.02</u>	1.56 _(3, 28)	0.22	2.56 _(3, 28)	0.08	0.66 _(3, 28)	0.58	0.59 _(3, 28)	0.62
P	2.19 _(1, 28)	0.15	0.44 _(1, 28)	0.51	0.01 _(1, 28)	0.91	0.36 _(1, 28)	0.55	0.04 _(1, 28)	0.85
N × P	0.18 _(3,28)	0.91	1.67 _(3, 28)	0.20	1.31 _(3, 28)	0.29	0.76 _(3, 28)	0.52	0.49 _(3, 28)	0.69

*ANOVA performed on untransformed (Live roots and Dead), square root-transformed (Live Rhizomes) and natural log-transformed (Live and Total) ingrowth data.

†ANOVA performed on untransformed (Dead) and natural log-transformed (all other) standing crop data.

However, nutrient enrichment had no apparent effect on dead (root + rhizome) or total (live + dead) standing crop (Table 1; Fig. 2d,e).

Discussion

We found that below-ground plant biomass, specifically live root biomass, in this oligohaline marsh was significantly affected by N enrichment, but not by P enrichment or its interaction with N enrichment. Previous research at this site also indicates that significant changes below-ground did not occur until nutrient limitation was alleviated and maximum above-ground production was achieved through N enrichment (i.e. ≥ 200 kg N ha⁻¹ year⁻¹; Graham & Mendelsohn 2010). However, N-induced below-ground response trajectories in the present study were dissimilar across the two commonly used below-ground biomass estimation techniques; greater N availability increased live root biomass accumulation in ingrowth cores, but simultaneously reduced live root standing crop. Other biomass components, with the exception of dead biomass and total standing crop, showed similarly distinct, albeit non-significant, N-enrichment trends across methods. These findings that showed dual below-ground responses depending on the process and biomass pool measured are generally consistent with previously published research that investigated the direct effects of nutrient enrichment via fertilization at 34 herbaceous coastal wetland sites in 13 geographical locations across the United States (Table 2).

LIVE VS. DEAD BELOW-GROUND BIOMASS

Irrespective of method used, significant nutrient enrichment effects during the present study were observed only for living below-ground biomass components. However, we did identify a non-significant nutrient enrichment trend for total (live + dead) biomass accumulation estimated using the ingrowth method, which most likely stemmed from enhanced

live biomass accumulation. Previously published results also show corresponding nutrient enrichment effects primarily associated with the living portion of below-ground biomass (Table 2). In fact, Table 2 illustrates that significant responses to nutrient enrichment occurred at 87% of sites in all nine fertilization studies that distinguished live biomass from dead, whereas total (live + dead) biomass and total root biomass responses were observed at only 14% of sites. Furthermore, total ingrowth in tallgrass prairie restoration sites (Camill *et al.* 2004) and along a wet grassland nutrient gradient (Kaplova, Edwards & Kvet 2011), as well as total below-ground standing crop along a salt marsh N-loading gradient (Wigand 2008), a constructed salt marsh fertilized with urea (Boyer, Callaway & Zedler 2000) and a freshwater marsh receiving sewage effluent (Bayley *et al.* 1985) similarly indicate that total below-ground biomass is generally unresponsive to enhanced nutrient availability. As such, the timeframe of our investigation, and most others, may not have been sufficient to detect a response to N enrichment using bulk measurements of total biomass because live roots generally represent a small fraction of total biomass (e.g. 2.3%; Fig. 2a,e) in coastal wetlands.

BELOW-GROUND BIOMASS ACCUMULATION (INGROWTH)

Although the number of fertilization studies using the ingrowth method to directly assess the effects of nutrient enrichment in coastal marshes is somewhat limited in scope, interestingly, not a single one of these studies measured a reduction in below-ground biomass accumulation following nutrient addition relative to unfertilized control plots, while the majority (60%) observed a positive response (Table 2a). Furthermore, nutrient-enhanced root biomass accumulation in unexploited ingrowth soil has also been consistently found in a number of other habitat types including mangroves (McKee, Cahoon & Feller 2007; Castañeda-Moya *et al.* 2011), coastal

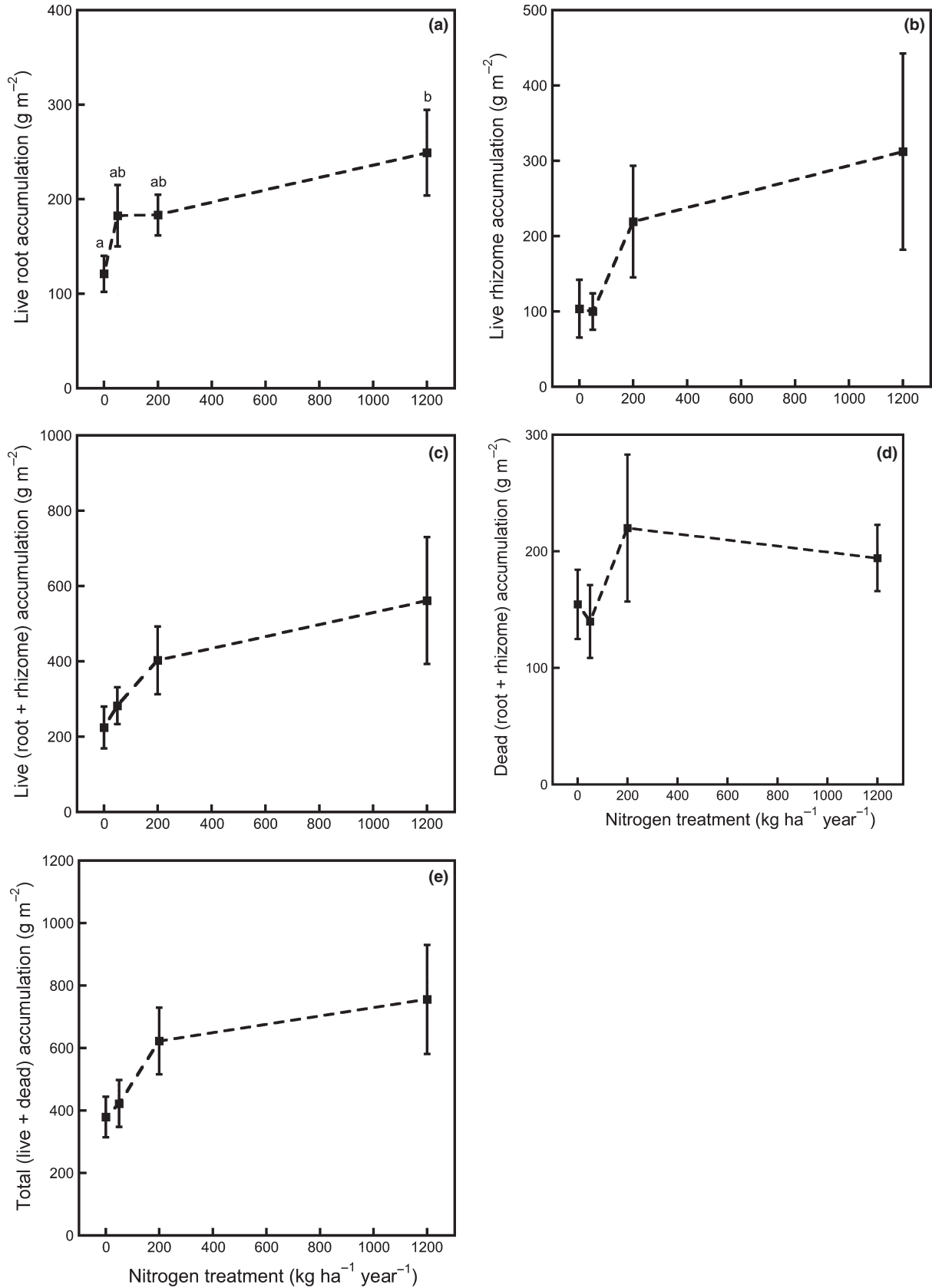


Fig. 1. Below-ground biomass accumulation in ingrowth cores (mean \pm SE; $n = 10$) with respect to nitrogen enrichment after 3 years of deployment that corresponded with fertilization years 4 through 7: (a) live root, (b) live rhizome, (c) live (root + rhizome), (d) dead (root + rhizome) and (e) total (live + dead) biomass. Different letters identify differences among means ($P \leq 0.05$; Tukey–Kramer multiple comparison test).

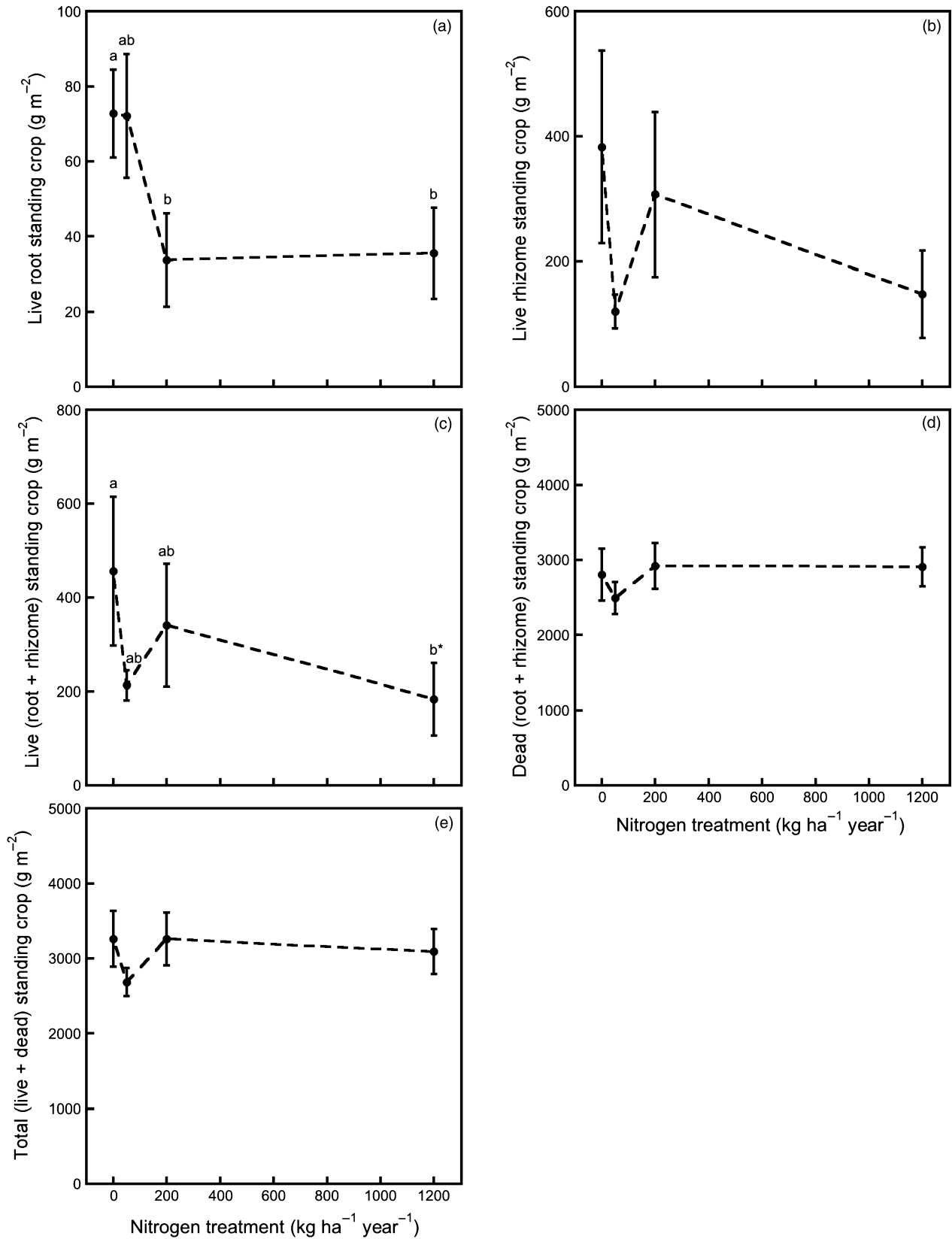


Fig. 2. *In situ* below-ground standing crop biomass in soil cores (mean \pm SE; $n = 10$) with respect to nitrogen enrichment after 7 years of fertilization: (a) live root, (b) live rhizome, (c) live (root + rhizome), (d) dead (root + rhizome) and (e) total (live + dead) biomass. Different letters identify differences among means ($P \leq 0.05$, $0.05 < P^* \leq 0.10$; Tukey–Kramer multiple comparison test).

Table 2. Coastal wetland below-ground biomass response to nutrient enrichment as determined by the (a) ingrowth and (b) standing crop methods

Reference ¹	Location (USA)	Site vegetation ²	Years fertilized	Nutrients applied ³	Biomass pool ⁴
(a) Ingrowth Method					
<i>Evidence of Reduced Biomass</i>					
None					
<i>Evidence of Increased Biomass</i>					
Valiela, Teal & Persson (1976) ⁵	Massachusetts	SPAL, SPPA	2 or 3	<u>Urea (N)</u> , <u>N + P + K</u>	Roots, Rhizomes
This study	Louisiana	SALA	7	<u>N</u> , P, N + P	Roots
<i>Evidence of No Biomass Response</i>					
Langley <i>et al.</i> (2009)	Maryland	SCAM/SPPA/DISP	2	N	Total fine roots
Anisfeld & Hill (2012)	Connecticut	SPAL	5	N, P, N + P	Total
(b) Standing Crop Method					
<i>Evidence of Reduced Biomass</i>					
Valiela, Teal & Persson (1976) ⁵	Massachusetts	SPAL, SPPA	2 or 3	<u>Urea (N)</u> , <u>N + P + K</u>	Roots
Morris & Bradley (1999)	South Carolina	SPAL	13	<u>N + P</u>	Total
Hines, Megonigal & Denno (2006) ⁶	New Jersey	SPAL	2	<u>N</u>	Roots
Darby & Turner (2008a)	Louisiana	SPAL	1	<u>N</u> , <u>P</u> , <u>Fe</u> , <u>N + P</u> , <u>N + Fe</u> , <u>P + Fe</u> , <u>N + P + Fe</u>	Live
Darby & Turner (2008b) ⁷	Louisiana	SPAL (4)	1	<u>N + P</u>	Live
Darby & Turner (2008b) ⁸	Massachusetts	SPAL	1	<u>N + P</u> , <u>P</u>	Live
Darby & Turner (2008b)	Nova Scotia	SPAL	1	<u>N + P</u>	Live
Darby & Turner (2008b)	Virginia	SPAL (2)	1	<u>N + P</u> , <u>P</u>	Live
Ket, Schubauer-Berigan & Craft (2011)	Georgia	ZIMI	5	<u>N</u> , P, <u>N + P</u>	Rhizomes, Total
Davey <i>et al.</i> (2011)	South Carolina	SPAL	13	<u>N</u> , P, <u>N + P</u>	Total fine roots
Deegan <i>et al.</i> (2012) ⁹	Massachusetts	SPAL	7	<u>N + P</u>	Live
Graham & Mendelssohn (2014)	Louisiana	SALA	13	<u>N + P + K</u>	Live roots
This study	Louisiana	SALA	7	<u>N</u> , P, N x P	Roots
<i>Evidence of Increased Biomass</i>					
Valiela, Teal & Persson (1976) ⁵	Massachusetts	SPPA	2 or 3	<u>Urea (N)</u> , N + P + K	Rhizomes
Tyler, Lambrinos & Grosholz (2007)	Washington	SPAL (3)	1	<u>N</u>	Live
Darby & Turner (2008b) ⁸	Massachusetts	SPAL	1	<u>N + P</u> , <u>P</u>	Live
Hunter <i>et al.</i> (2008)	Alabama	SABI	1	<u>N + P</u>	Total
Nelson & Zavaleta (2012) ¹⁰	California	SAPA	2	<u>N</u>	Total
<i>Evidence of No Biomass Response</i>					
Gallagher (1975) ¹¹	Georgia	SPAL	1	N	Total
Haines (1979) ¹²	Georgia	SPAL	1	N + P + K	Total
Buresh, Delaune & Patrick (1980)	Louisiana	SPAL	1	N, P	Total
Wigand <i>et al.</i> (2004)	Rhode Island	SPPA	3	N, P, N x P	Total
Tyler, Lambrinos & Grosholz (2007) ¹³	California	SPHY (3)	1	N	Live
Hunter <i>et al.</i> (2008)	Alabama	DISP, DISP/SABI	1	N + P	Total
Anisfeld & Hill (2012)	Connecticut	SPAL	5	N, P, N + P	Total

Table 2. (Continued)

¹References listed are peer reviewed journal articles that established a cause and effect relationship by applying fertilizer to attached wetland substrates in which the natural plant community grew under ambient field conditions in tidal freshwater marsh (Ket, Schubauer-Berigan & Craft 2011), oligohaline marsh (Graham & Mendelssohn 2014 and this study), brackish marsh (Langley *et al.* 2009) and salt marsh (15 references) environments. Where applicable, distinct locations and plant communities are identified separately for each reference.

²A comma (,) separates dominant plant species growing in different plant communities, whereas a solidus (/) separates co-dominants growing within the same plant community. A number contained within parentheses indicates that multiple sites within similar plant communities were fertilized. Plant species abbreviations are as follows: DISP = *Distichlis spicata*, SALA = *Sagittaria lancifolia*, SABI = *Salicornia bigelovii*, SAVI = *Salicornia virginica*, SAPA = *Sarcocornia pacifica*, SCAM = *Schoenoplectus americanus*, SPAL = *Spartina alterniflora*, SPFO = *Spartina foliosa*, SPHY = *Spartina alterniflora x foliosa* (hybrid), SPPA = *Spartina patens*, ZIMI = *Zizaniopsis miliacea*.

³Applied nutrients are nitrogen (N), phosphorus (P), potassium (K) and iron (Fe). Nutrient additions that caused a below-ground response are underlined.

⁴Below-ground biomass pools affected by nutrient enrichment are identified. If no effect was observed, the measured pools are identified. Biomass descriptors are as follows: Roots = living root biomass, Rhizomes = living rhizome biomass, Live = living root + living rhizome biomass, Total fine roots = live + dead root biomass < 2 mm (Langley *et al.* 2009) or < 1 mm (Davey *et al.* 2011), Total = live + dead root and rhizome biomass.

⁵Urea (N) was applied for 2 years while treated municipal sludge (N + P + K) was applied for 3 years. Results were not statistically analysed; evidence of nutrient effects on below-ground biomass was based on the authors' written interpretation of the data and by examining Table 1 (ingrowth) and Fig. 3 (standing crop) in Valiela, Teal & Persson (1976). Note: urea had no obvious effect on SPAL root biomass.

⁶ $P = 0.06$.

⁷Data from Darby & Turner (2008a) were excluded to avoid duplicating results.

⁸Data from Valiela, Teal & Persson (1976) were excluded to avoid duplicating results.

⁹ $P = 0.08$.

¹⁰November 2009 measurements ($P = 0.09$); July 2009 measurements ($P = 0.47$).

¹¹No significant effect overall (0–55 cm) or incrementally (0–15 cm, 35–55 cm); $P = 0.10$ at the 15–35 cm depth increment.

¹²Experimental plots were fertilized with dried sewage sludge assumed to contain N, P, K and possibly other micronutrients.

¹³The three sites fertilized in California had different sub-dominant vegetation representing different habitat types invaded by SPHY: mudflat, SPFO-dominated marsh and SAVI-dominated marsh.

dunes (Stevenson & Day 1996), tallgrass prairie (Owensby, Auen & Coyne 1994) and a wide variety of terrestrial forests (Cuevas & Medina 1988; Raich, Riley & Vitousek 1994; Helmissaari & Hallbäck 1999; Davis, Allen & Clinton 2004; Gress *et al.* 2007; Gleeson & Good 2010). Indeed, we are aware of only one negative root ingrowth response to fertilization (Cheng & Bledsoe 2002), though this response was isolated to one of several species (*Quercus douglasii*), and total root accumulation from all species combined was unaffected, if not higher on average, in fertilized plots. However, root biomass accumulation does not always respond to fertilization, as shown by ingrowth studies in wet tundra (Nadelhoffer *et al.* 2002), coniferous forest (Ahlström, Persson & Börjesson 1988; Clemensson-Lindell & Persson 1995; Boxman *et al.* 1998; Smith, Coyea & Munson 2005) and pine plantation (Lee & Jose 2003) habitats. Nonetheless, greater biomass accumulation in unexploited soil appears to be a likely response trajectory when nutrient availability is enhanced, considering the weight of evidence provided by studies that used the ingrowth method as a direct assessment of nutrient enrichment in coastal marshes (Table 2a) and other environments (in-text citations). A similar conclusion can also be drawn from numerous greenhouse (i.e. pot) and mesocosm fertilization experiments demonstrating enhanced below-ground biomass accumulation when plants were grown in previously un-vegetated substrate (e.g. Haines & Dunn 1976; Hamilton *et al.* 1998; Svengsouk & Mitsch 2001; Shipley & Meziane 2002; Day, Doyle & Draugelis-Dale 2006; Steinbachova-Vojtiskova *et al.* 2006; Ravit *et al.* 2007; Zhao *et al.* 2009; Langley *et al.* 2013).

BELOW-GROUND STANDING CROP

In contrast to generally positive below-ground biomass accumulation responses found using the ingrowth method, investigations employing the standing crop method observed negative fertilization effects on *in situ* below-ground biomass at 53% of sites compared to positive effects at only 21% (Table 2b). Additional supporting evidence from the literature shows that excess nutrients induced negative root and/or rhizome standing crop responses in mangroves (Castañeda-Moya *et al.* 2011), freshwater marshes and fens (Miao & Sklar 1998; Pauli, Peintinger & Schmid 2002) and numerous upland forests (Gower & Vitousek 1989; Gower, Vogt & Grier 1992; Clemensson-Lindell & Persson 1995; Haynes & Gower 1995; Phillips & Fahey 2007; Cusack *et al.* 2011). However, mixed responses depending on plant species, nutrient supplied and hydrology were observed in freshwater wetlands (Neill 1990a,b), while the duration of fertilization, nutrient supplied and site fertility influenced root standing crop in forests (Alexander & Fairley 1983; Helmissaari & Hallbäck 1999; Ostertag 2001). Although the most common response to nutrient enrichment appears to be a reduction, some forest research found that fertilization increases root standing crop (e.g. Nadelhoffer, Downs & Fry 1999; Ostertag 2001), which may reflect the ability of some tree species to utilize excess nutrients over century-scale growth phases. Similarly, low-level P enrichment ($\leq 400 \text{ mg P m}^{-2} \text{ year}^{-1}$) increased total below-ground standing crop at oligotrophic sites in the Everglades (Daoust & Childers 2004), though porewater P concentrations did not increase after fertilization

indicating that nutrient limited growing conditions likely continued. Therefore, biomass stimulation may be expected when proliferation is essential for nutrient acquisition (Hodge 2004; Cahill & McNickle 2011). Likewise, municipal effluent enhanced total (live + dead) below-ground standing crop in a floating marsh that was developing vertically and horizontally as well as undergoing plant succession (Izdipski *et al.* 2009), suggesting that excess nutrient loading may also accelerate plant-mediated habitat creation. Moreover, greater total root standing crop in fertilized prairie grassland plots corresponded to an N-induced shift from native to naturalized species that accounted for as much as 67% of the above-ground plant biomass (Fornara & Tilman 2012). This response further emphasizes the ability of nutrient enrichment to facilitate plant invasions rather than stimulate the below-ground standing crop of an established plant community in environmental equilibrium.

ECOLOGICAL CONTEXT

Differences among biomass responses observed using the ingrowth and standing crop methods are often interpreted as representing a change in turnover rates (i.e. $\text{ingrowth} \div \text{standing crop} = \text{turnover}$; Eissenstat & Yanai 2002). However, this conclusion assumes, often without direct measurement, that below-ground structures senesce and/or decompose differentially depending on nutrient availability. While results from the present study do not preclude the possibility of diminished below-ground biomass longevity with increased nutrient supply, they do not directly support the necessary assumptions. In particular, dead biomass showed no clear response to nutrient enrichment, regardless of method used (Figs 1d and 2d), though we acknowledge that our measurement timeframe may not have been sufficiently long to detect small changes in turnover that represent a minor fraction of total biomass. Furthermore, numerous coastal wetland fertilization studies (Valiela *et al.* 1985; Jordan, Whigham & Correll 1989; Feller *et al.* 1999; Rybczyk, Day & Conner 2002; McKee, Cahoon & Feller 2007; Anisfeld & Hill 2012), including a concurrent investigation at a nearby site within the same contiguous marsh (Graham & Mendelssohn 2014), have found no effect of nutrient enrichment on organic matter decomposition assessed using the litterbag technique. Rather, the present research in combination with previously published results presented herein offers an alternative context through which the effects of nutrient enrichment on below-ground plant biomass can be viewed: (i) plant establishment and initial below-ground growth into unexploited soil are facilitated by enrichment with the growth-limiting nutrient, (ii) when the plant community becomes established and maximum above-ground growth is achieved through nutrient enrichment, plants equilibrate to nutrient excess by reducing nutrient foraging efforts compared to nutrient limited growing conditions and (iii) these effects are consistently measurable in the living below-ground biomass components, especially living roots as the primary nutrient acquisition structures. Thus, chronic nutrient over-enrichment has the potential to reduce soil

organic matter content over the long-term unless the effects of nutrient enrichment on other processes, such as enhanced above-ground biomass, offset this loss as some research suggests (Morris *et al.* 2002; Anisfeld & Hill 2012; Fox, Valiela & Kinney 2012; Graham & Mendelssohn 2014). Nonetheless, previous research collectively supports our hypothesis that nutrient enrichment has contrasting effects on below-ground biomass in coastal wetlands depending on the process and biomass pool measured. In situations where plants can exploit open resource space, nutrient enrichment generally enhances live below-ground biomass accumulation, as evidenced using the ingrowth method, while in situations where established plant communities sustain nutritional requirements via maintenance growth, nutrient enrichment generally reduces live below-ground biomass, as demonstrated using the standing crop method.

This dual response is further reinforced when differences among plant species' life histories or environmental settings are taken into account. As such, some of the below-ground responses presented in Table 2b can be interpreted as representing new growth into unexploited soil rather than maintenance growth of plants in environmental equilibrium. For example, fertilization stimulated below-ground standing crop of the annual plant *Salicornia bigelovii* but not the perennial plant *Distichlis spicata* or mixed *S. bigelovii/D. spicata* stands (Hunter *et al.* 2008), suggesting that nutrient enrichment facilitates plant establishment and new below-ground growth when plants propagate from seed. In a second example, nutrient-enhanced root standing crop coincided with high soil mineral matter content (75% by mass) resulting from a continuous sedimentation rate of 2–5 mm year⁻¹ (Nelson & Zavaleta 2012), which again supports our hypothesis that nutrient enrichment stimulates new growth into unexploited substrates. Thirdly, N enrichment increased live (root + rhizome) standing crop of *Spartina alterniflora* invading previously un-vegetated mud flats (i.e. open habitat) in Willapa Bay, WA (Tyler, Lambrinos & Grosholz 2007). Together, these examples illustrate nutrient-enhanced below-ground plant biomass in unexploited resource space, as indicated by ingrowth measurements in the present study and others (Table 2a). Accordingly, when these case studies are interpreted as representative instances of new growth into unexploited substrate rather than the quantity of below-ground biomass required to sustain the nutritional needs of established plants (i.e. more similar to ingrowth than standing crop), along with the additional inference that measures of total biomass are much less responsive to enhanced nutrient availability than living biomass components, the evidence supporting contrasting effects of nutrient enrichment on below-ground biomass at the 34 herbaceous coastal wetland sites identified in Table 2 increases to 89% by site, while opposing evidence decreases to 15%.

Broadly, these distinct below-ground responses to nutrient enrichment can likely be explained in large part by the equilibrium state of the ecosystem. Coastal wetlands in transition or otherwise out of environmental equilibrium (i.e. non-steady state) may benefit from anthropogenically elevated nutrient

loads that enhance the development of functional processes by stimulating below-ground growth as plants exploit open resource space created, for example, by sedimentation, annual plant senescence, disturbance or as plant invasions occur. However, in the absence of a mechanism that continuously creates new resource space, potentially negative impacts on soil organic matter content may occur once the plant community matures to a quasi-steady state and equilibrates to excess nutrient availability by down-regulating below-ground standing crop. Of course, site-specific conditions such as hydrology and plant community composition, among other factors, may modify nutrient enrichment effects under both scenarios. Nonetheless, the ecological consequences of these distinctly different below-ground responses to anthropogenically enhanced nutrient loading will likely be considerable through corresponding feedback effects on soil biogeochemical processes that influence soil microbial community structure and function, infaunal composition and abundance and food-web complexity, to name a few.

Concluding remarks

Below-ground biomass is a primary determinant of ecosystem function, with specific implications for soil carbon accumulation on a global scale. Thus, identifying the effects of global change drivers, such as eutrophication, on functional processes, such as below-ground plant growth, is of critical importance from ecological, economic and social perspectives world-wide. On the basis of the present research, as well as previous studies investigating the effects of nutrient enrichment on below-ground biomass in coastal wetlands and other ecosystems, we conclude that (i) eutrophic conditions can affect ecosystem function beneficially by stimulating below-ground plant growth in previously unexploited soil, or detrimentally by reducing the below-ground standing crop required to sustain the nutritional needs of established plants in mature communities and (ii) measures of living compared to total (live + dead) biomass are the most responsive indicators of these effects. Although differences among plant species growth patterns, environmental settings and specific nutrient inputs may modify outcomes, our results clearly show distinct, simultaneous below-ground plant responses to excess nutrient loading depending on the process measured. We have also provided supporting evidence that suggests nutrient-enhanced below-ground growth can occur with continuous sedimentation or when soil mineral matter content is high, where annual plants dominate or propagation by seed is prevalent, during the process of plant succession and when plants invade or create new habitats. In all of these cases, we predict that enhanced below-ground growth is sustained by greater nutrient availability in under- or unutilized soil that is capable of supporting plant growth, all else being equal. Conversely, reduced below-ground biomass is expected in chronically eutrophic areas that maintain established mature plant communities containing perennial plants that store nutrients in below-ground structures during non-growing seasons. In these instances, we predict that

reduced below-ground growth occurs when nutrients are available in excess because plants can acquire the nutrients necessary to sustain maximum growth with less below-ground standing crop.

Acknowledgements

Funding for this research was provided by the National Oceanic and Atmospheric Administration's (NOAA) Center for Sponsored Coastal Ocean Research (CSCOR) and the Louisiana Sea Grant Program, a part of the National Sea Grant Program maintained by NOAA. We thank Kristen Laursen for initial field site set-up and Joe Baustian, Kate Lingoni, Josh Roberts and Camille Stagg for their assistance in the field and laboratory. Thoughtful comments by Brian Silliman, Amy Austin and three anonymous reviewers improved upon an earlier version of this manuscript.

Data accessibility

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.1hn2b> (Graham & Mendelssohn 2015)

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Received 19 December 2014; accepted 20 October 2015

Handling Editor: Brian Silliman

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The interactive effect of N and P enrichment on live root accumulation in ingrowth cores.