INVASIVE *SPARTINA DENSIFLORA* BRONGN. REDUCES PRIMARY PRODUCTIVITY IN A NORTHERN CALIFORNIA SALT MARSH

by

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ABSTRACT

Invasive *Spartina densiflora* Brongn. Reduces Primary Productivity in a Northern California Salt Marsh

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Comprehensive primary productivity measurements of the impact of invasive species are important environmental indicators. This study obtained measurements of the impact of invasive *Spartina densiflora* on the primary productivity of a Humboldt Bay (California) salt marsh using above and belowground biomass measurements coupled with paired closed-chamber carbon dioxide flux measurements. Compared to samples dominated by native vegetation, samples dominated by *S. densiflora* displayed higher aboveground net primary productivity, lower belowground net primary productivity, lower net primary productivity, and lower net ecosystem exchange measurements; thus, *S. densiflora* colonization reduced primary productivity. Benthic macroalgal cover was a strong predictor of net ecosystem exchange. In plots dominated by *S. densiflora*, less benthic macroalgae was present and less photosynthetically active radiation reached the substratum. Therefore, increased shading of the sediment surface in plots dominated by *S. densiflora* contributed to lower net ecosystem exchange measurements. These results greatly improve our understanding of the impact of *S. densiflora* on the primary productivity of Humboldt Bay salt marsh ecosystems.

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Words cannot express my thanks for my beautiful wife.

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INTRODUCTION

Invasive Species

Invasive species are non-native species that harm the environment, the economy, or human health (Executive Office of the President of the United States 1999). Few non-native species are classified as invasive, and many native species would be classified as invasive if they were non-native (Lockwood et al. 2007). Invasive species often rapidly evolve as they invade and colonize native ecosystems (Lockwood et al. 2007, Davis 2009). As invasive species colonize native ecosystems they frequently decrease populations of native species; therefore, invasive species are often the subject of ecosystem management decisions made by agencies tasked with the preservation of rare, threatened, endangered, or economically important native species (Dudley and Collins 1995, Bossard et al. 2000, Myers and Bazely 2003, Radosevich et al. 2007). Anthropogenic alterations of native ecosystems have increased the probability of invasion and colonization by invasive species (Chapin et al. 2000, MacDougall and Turkington 2005, Hobbs et al. 2006); this probability will likely continue to increase as a result of the predicted effects of global climate change and predicted increases in trade with emerging markets (Bradley et al. 2012).

When an invasive plant species colonizes an ecosystem, it can increase the primary productivity of that ecosystem by occupying previously unoccupied ecological niches; alternatively, it can decrease the primary productivity of that ecosystem by out-competing native species that were better suited to make use of available resources or by altering herbivory rates (Lockwood et al. 2007, Radosevich et al. 2007, Davis 2009).

Primary productivity is the rate at which autotrophs synthesize new organic compounds via photosynthesis or chemosynthesis (Radosevich et al. 2007).

Salt marshes produce some of the highest rates of primary productivity in the world (Mitsch et al. 2009). Impacts to salt marsh primary productivity from invasive species are important environmental indicators (Westlake et al. 1998, Mitsch et al. 2009). The high levels of primary productivity found in salt marshes represent valuable ecosystem services (e.g., filtering of toxic substances from water, sequestration of large amounts of carbon dioxide from the atmosphere) and also provide energy for extensive food webs that include various endangered vertebrates (Monroe et al. 1973, Mitsch and Gosselink 2007, Mitsch et al. 2009). The overall median annual monetary value, unadjusted for inflation, of ecosystem services generated by wetlands in California has been estimated at 9.96 billion dollars (Allen et al. 1992).

Spartina densiflora in Humboldt Bay

Dense-flowered cordgrass (*Spartina densiflora*) is currently classified as an invasive species throughout California (Cal-IPC 2006). *Spartina densiflora* is native to South America and is rapidly colonizing estuarine environments in Europe, Africa, South America, and North America (Bortolus 2006, Mateos-Naranjo et al. 2007). The colonization of *S. densiflora* in the salt marshes of northern California has decreased populations of native plant species (Daehler and Strong 1996, Kittelson and Boyd 1997).

Sometime during the mid to late 1800s, *S. densiflora* was introduced to Humboldt Bay via lumber trade with Chile and began to invade the salt marshes of Humboldt Bay (Spicher and Josselyn 1985, Bortolus 2006, 2008). *Spartina densiflora* proceeded to displace native vegetation via lateral tiller growth throughout the year, generation of dead biomass that smothered native plants, and prolific seed generation (Kittelson and Boyd 1997). While *S. densiflora* colonized the salt marshes of Humboldt Bay, thousands of hectares of salt marsh land surrounding Humboldt Bay were diked and drained in order to create grazing land, railroads, and a highway (Clifford 2002). By 1980, Humboldt Bay had been reduced from 10,931 ha to 7,920 ha (72 percent of its previous area) (Barnhart et al. 1992). By 1999, the area of salt marsh surrounding Humboldt Bay had been reduced from approximately 3,642 ha to 364 ha (10 percent of its previous area) and *S. densiflora* was present in 94 percent of the remaining 10 percent of the Humboldt Bay salt marsh area, occurring at dense concentrations (>70 percent cover) in 68 percent of the Humboldt Bay salt marsh area (Pickart 2001). By 2010, *S. densiflora* was present in 97 percent of unrestored salt marshes in the Humboldt Bay National Wildlife Refuge (Grazul and Rowland 2010).

The three most common vascular plants currently found in the salt marshes of Humboldt Bay are *S. densiflora*, saltgrass (*Distichlis spicata*), and pickleweed (*Salicornia pacifica*) (Rogers 1981, Eicher 1987). *Spartina densiflora* is a perennial species that only displays partial dormancy in Humboldt Bay (Kittelson 1993) whereas *D. spicata* and *S. pacifica* are dormant during the winter (Rogers 1981). *Spartina densiflora* tends to broadly dominate interspecies competition throughout the ranges of *D.*

spicata and *S. pacifica* in Humboldt Bay (Eicher 1987, Pickart 2001, Grazul and Rowland 2010). Rogers (1981) found that *S. densiflora* displayed higher aboveground primary productivity than *S. pacifica* and *D. spicata* in Humboldt Bay, but did not measure belowground primary productivity or the primary productivity of non-vascular autotrophs.

Study Objectives and Hypotheses

The primary objective of this study was to determine the impact of *S. densiflora* on the primary productivity of a Humboldt Bay salt marsh using comprehensive methods. The secondary objective of this study was to improve upon the methods needed to measure the effect of *S. densiflora* on the primary productivity of Humboldt Bay and adjacent estuaries.

I hypothesized that, compared to a native Humboldt Bay salt marsh, a Humboldt Bay salt marsh dominated by *S. densiflora* would display: (1) higher primary productivity as defined by biomass, (2) lower primary productivity as defined by closed-chamber atmospheric carbon dioxide flux measurements, (3) less photosynthetically active radiation (the spectral range of light used in photosynthesis) at the substratum, and (4) less benthic macroalgae.

MATERIALS AND METHODS

Study Site

The site used for this study was located in both the southernmost portion of the Arcata Marsh and Wildlife Sanctuary and the northernmost portion of the Jacoby Creek Unit of the Humboldt Bay National Wildlife Refuge in Humboldt Bay, California (Figure 1). Vegetation at the site was broadly dominated by *S. densiflora*, especially at relatively low elevations, while in the relatively high-elevation areas *D. spicata*, *S. pacifica*, and *Jaumea carnosa* were variably dominant. Botanical nomenclature follows Baldwin et al. (2012). Tidal creeks were found throughout the site, especially at lower elevations. An abandoned dike that featured several large removed sections lay across the middle of the site. Salt pannes (depressed areas that retain salt water between high tides) were present at lower elevations. These salt pannes were often filled with different types and layers of biofilms and various species of mostly green filamentous algae. Migrating birds were spotted at the site (e.g., Aleutian Cackling Geese) but no nests were observed. No features of this site precluded it from being a reasonably representative example of a Humboldt Bay salt marsh ecosystem.

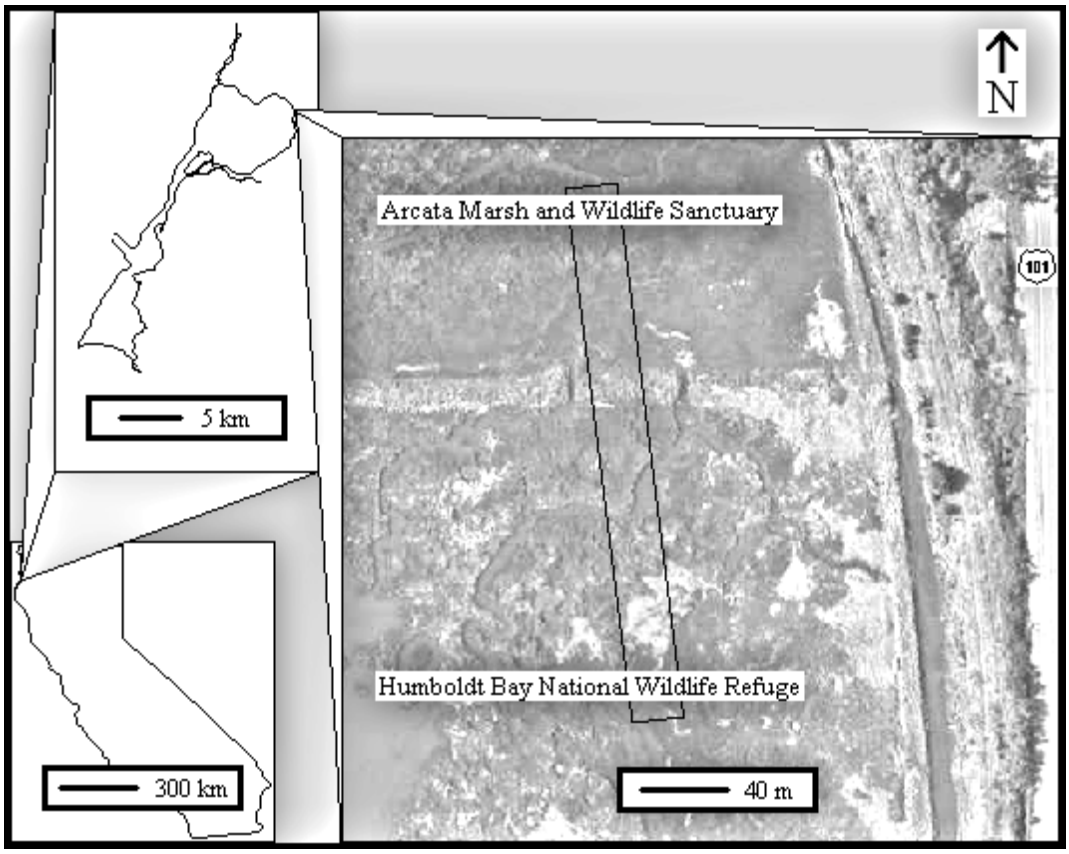

Figure 1. Rectangular study area in both the southernmost portion of the Arcata Marsh and Wildlife Sanctuary and the northernmost portion of the Jacoby Creek Unit of the Humboldt Bay National Wildlife Refuge in Humboldt Bay, California.

Selection of Methods

Primary productivity measurements are commonly derived from measurements of primary productivity covariates that change over time (e.g., biomass, atmospheric carbon dioxide) and are commonly expressed in units of grams of carbon per square meter per year (Fahey and Knapp 2007). Different primary productivity measurements are used to evaluate different aspects of primary productivity (Table 1) (Fahey and Knapp 2007).

Several methods of assessing different aspects of primary productivity are currently used, but each method has limitations. Remote sensing using the eddy covariance technique estimates primary productivity effectively over large areas at coarse resolution by evaluating atmospheric gas flux (rate of flow) measurements from vertically spaced atmospheric monitors (Loescher et al. 2006, Burba and Anderson 2010). Remote sensing using low earth orbit satellites can detect variations in the reflectance of small bands of light over very large areas at spatial resolutions as fine as 0.5 m^2, and these data can be used to develop primary productivity estimation models (Hua et al. 2011). Fluorometry techniques measure light emitted by chlorophyll that has been excited (usually with ultraviolet light) in order to determine primary productivity over small areas (generally smaller than a few square centimeters) (Morris 2005, Mateos-Naranjo et al. 2010). Remote sensing techniques are appropriate for measuring primary productivity over large areas at relatively low resolution, and fluorometry techniques are appropriate for measuring primary productivity over small areas (such as a single leaf) at relatively high resolution (Geider and Osborne 1992, Fahey and Knapp 2007).

Table 1. Common primary productivity measurements and covariates used to obtain them.

Measurement	Definition	Covariate(s)
Gross primary productivity	Rate of photosynthesis or chemosynthesis	Atmospheric gas concentration, light reflectance
Net primary productivity	Gross primary productivity minus autotroph respiration rate	Biomass carbon content
Aboveground net primary productivity	Net primary productivity of aboveground portion of autotrophs	Biomass carbon content
Belowground net primary productivity	Net primary productivity of belowground portion of autotrophs	Biomass carbon content
Net ecosystem exchange	Gross primary productivity minus ecosystem respiration rate	Atmospheric gas concentration
Net ecosystem productivity	Net ecosystem exchange plus or minus lateral carbon flow	Atmospheric and soil carbon content

Traditional methods for the assessment of the primary productivity of vascular plants are based on the carbon content of collected biomass samples because these measurements are relatively easy to obtain and contain a large amount of temporal information (Fahey and Knapp 2007). However, biomass collection methods are prone to large inaccuracies (Linthurst and Reimold 1978, Shew et al. 1981, Kaswadji et al. 1990, Fahey and Knapp 2007). For instance, independent net primary productivity estimates measured in Louisiana within two years of each other resulted in one estimate of 1,473-2,895 g C/m^2/year for *Spartina alterniflora* and 1,162-1,291 g C/m^2/year for *Distichlis spicata* (White et al. 1978) and another estimate of 1,381 g C/m^2/year for *S. alterniflora* and 1,967 g C/m^2/year for *D. spicata* (Hopkinson et al. 1980). Additionally, primary productivity measurements that rely only on destructive biomass sampling can produce inaccurate results due to the inability to sample previously sampled plots throughout the year (Fahey and Knapp 2007). Primary productivity measurements that are calculated exclusively from biomass samples do not measure several important variables: herbivory (the rate at which autotrophs are consumed by higher organisms), plant parasitism rates, volatile organic compound production rates (e.g., production of aromatic compounds), root exudate production rates, or rates of organic compound production by autotrophs for symbiotic organisms (e.g., organic acids for rhizobia) (Fahey and Knapp 2007). Additionally, biomass measurements are not sensitive enough to measure the primary productivity of microalgae and cyanobacteria (Geider and Osborne 1992).

A method to compare the net ecosystem exchange of sampled ecosystems has been developed that measures atmospheric carbon dioxide changes *in-situ* in

closed chambers (Streever et al. 1998, Migne et al. 2002, Fahey and Knapp 2007). By directly measuring changes in the primary source of carbon for autotrophs (including algae and cyanobacteria) this method collectively accounts for many of the information gaps encountered with biomass sampling, such as volatile organic compound production rates, root exudate production rates, and rates of organic compound production by autotrophs for symbiotic organisms. By intermittently covering a clear chamber, thereby blocking all photosynthetically active radiation, this method provides measurements of net ecosystem exchange, provides measurements of ecosystem respiration, and allows for a calculation of gross primary productivity (since gross primary productivity = net ecosystem exchange + ecosystem respiration). Ecosystem respiration values collected in this manner contribute to a better understanding of invertebrate herbivory rates and the growth rates of parasitic organisms (Westlake et al. 1998). Gross primary productivity values collected in this manner contribute to a better understanding of carbon cycling within the measured ecosystem (Westlake et al. 1998). Combined with statistical sampling, a comparison of the relative net ecosystem exchange measured in sampled plots can be used to indicate the impact of selected plants on the net ecosystem exchange measured within each sampled plot (Streever et al. 1998, Fahey and Knapp 2007).

For this study I chose to use biomass sampling (aboveground and belowground) because of the temporal information this method provides, and closed-chamber atmospheric carbon dioxide flux sampling because of the inclusive information this method provides. I used both of these methods during the same sampling periods to produce comprehensive primary productivity measurements at the appropriate resolution.

Biomass Measurement

In order to measure net primary productivity values, I obtained a total of 40 biomass samples during each of the months of May 2011, August 2011, November 2011, and February 2012 for a total of 160 biomass samples. These biomass samples were collected with the assistance of crew members of the California Conservation Corps and Humboldt State University student volunteers. We collected these biomass samples at random locations within a 200 m by 20 m belt transect adjacent to and at approximately equal elevation to the permanent paired plots used to measure carbon dioxide flux data. During each sampling period, we collected 20 "native" samples (samples displaying absolute *S. densiflora* cover less than or equal to 10 percent using ocular estimation), and 20 "invaded" samples (samples displaying absolute *S. densiflora* cover greater than 75 percent using ocular estimation). Each sample consisted of unbroken belowground material carefully dug out in one piece using a sharpshooter shovel and then carefully shaped with a hand saw into a rectangular cuboid shape measuring 10 cm by 10 cm horizontal (parallel to soil surface plane) and 40 cm vertical (perpendicular to soil surface plane). These samples were large enough to efficiently minimize spatial variability (Singh et al. 1984). During sample collection, we carefully preserved the aboveground material attached to each belowground sample.

Immediately following biomass sample collection, we cut the aboveground plant material from the belowground material precisely at the soil surface. We then cleaned the aboveground plant material and separated it by species. For each species present in each sample, we counted the number of stems (or culms), visually estimated the mean vascular

plant height using a meter stick, and measured the volume of the aboveground plant material using water displacement in a graduated cylinder. We then separated the aboveground plant parts from each sample into living and dead biomass based on visual examination (Smalley 1958).

Following separation of aboveground and belowground sample components, we cut the belowground portion of each sample into small pieces using a hand saw, rinsed the soil from these small pieces using a high pressure hose, and collected the remaining material in a 1.4 mm mesh sieve (Valiela et al. 1976). The rinsed belowground material consisted of roots and peat. I dried the aboveground live, aboveground dead, and belowground biomass components of each sample in a drying oven for approximately two weeks at 80 degrees Celsius until sample weights had not changed for 24 hours.

Rogers (1981) found a strong correlation between dried aboveground biomass and carbon content, but did not sample belowground biomass. In order to measure the correlation between dry weight and carbon content of the collected belowground biomass, I conducted loss-on-ignition tests of the belowground material collected during the August 2011 sampling period. Two Humboldt State University student assistants and I combusted 24 random samples from the dried belowground biomass collected during the August 2011 sampling period in a muffle furnace at 600 degrees Celsius for 12 hours.

Atmospheric Carbon Dioxide Measurement

I used several measuring instruments to sample atmospheric carbon dioxide flux within *in-situ* closed chambers. I constructed two identical rectangular cuboid chambers

from 0.635 cm clear cast acrylic sheets. Each chamber measured approximately one m by 0.25 m by 0.25 m. Inside each chamber, I affixed an AQ100 circuit board (manufactured by Sundae, Inc.) containing a non-dispersive infrared carbon dioxide gas probe (precision tested at ± 0.02 percent at 390 ppm) as well as an SHT11 temperature (accuracy ± 0.4 degrees Celsius) and relative humidity (accuracy ± 3.0 percent) probe. I connected both circuit boards via universal serial bus connections to a laptop computer outside of the chambers. I also affixed a photosynthetically active radiation probe inside each chamber. The photosynthetically active radiation probes were LI-193 spherical sensors (manufactured by LiCor Biosciences) that measured light received from almost every angle. I connected both LI-193 sensors to a LI-1400 data logger (manufactured by LiCor Biosciences) outside of the chambers and synchronized the data logger to the clock of the laptop computer to which the AQ100 circuit boards were connected before each sampling period. In order to produce sufficient air circulation, I affixed a 12-cm battery-powered fan within each chamber. During half of all sample measurements, I used fitted polyester-film covers to artificially darken the chambers. The AQ100 circuit boards were powered via the USB connections and transmitted measurements every two seconds that the laptop computer logged and graphed in real time. Real-time display of data ensured that problems with probes were dealt with as they occurred.

In order to build each chamber, I welded the acrylic sheets together using Weld-On 3 (manufactured by IPS Corporation). While building each chamber, I passed the cords connected to each probe through rounded notches at the top of each chamber. I used a thick application of E-6000 clear adhesive (manufactured by Eclectic Products,

Inc.) inside each chamber to create gas-tight seals over the welds and the rounded notches with cords running through them. In order to ensure the integrity of gas-tight seals within the chambers, I darkened the chambers using the fitted polyester-film covers, then placed the chambers in a larger container filled with water to a depth of five cm, then added helium gas to the air inside the chambers in order to alter the concentration of carbon dioxide, and then monitored for changes in carbon dioxide concentration within the chambers for a period of one hour.

In order to establish permanent paired plots, I used a randomly determined coordinate to establish the starting point of a 40 m north-south baseline with a varying elevation approximately 2.1 m above Mean Lower Low Water (MLLW) from start to finish. I measured elevation relative to high tide marks using a clear hose filled with water. This 40 m baseline was located along the eastern edge of a *S. densiflora*-dominated salt marsh area. I then established eight transects at random distances from the southern end of this north-south baseline. Each of these eight transects ran east perpendicular to the baseline and ran across the northern edge of one set of paired plots (Figure 2). While moving away from the baseline along each transect I established one set of paired plots at the first point along that transect where I encountered appropriately distributed plant cover compositions. By starting each transect at approximately the same elevation, I eliminated the potential confounding variables that would be introduced if plots were measured at varying elevations (e.g., soil characteristics). I established both plots in each set of paired plots 170 cm apart from each other. Each set of paired plots consisted of one native plot and one invaded plot. All native plots featured a small

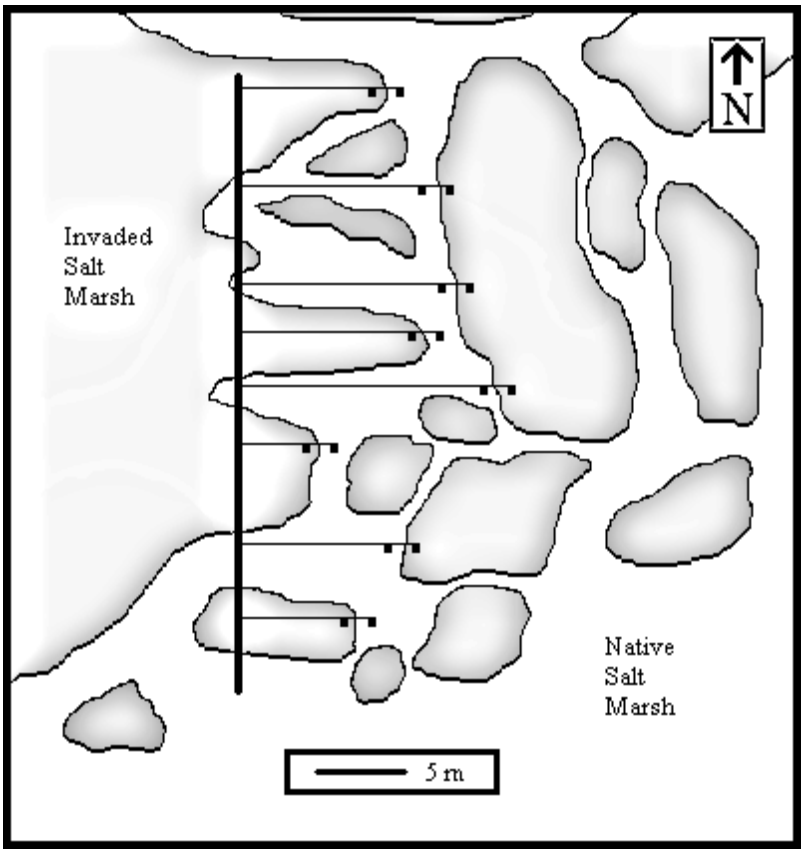

Figure 2. Paired plot locations along transects running east from north-south baseline (shaded areas represent invaded salt marsh and un-shaded areas represent native salt marsh).

amount of *S. densiflora* in order to ensure that soil characteristics in each plot were reasonably similar.

I used the two identical closed-chamber devices to simultaneously measure each set of paired plots during the months of May 2011, August 2011, November 2011, and February 2012 at random times between 8AM and 5PM that featured tides below two m above MLLW. By measuring each set of paired plots at the same time, I eliminated the potential confounding variables that would have been introduced as a result of measuring paired plots at varying photosynthetically active radiation and temperature levels, and I eliminated the potential confounding variables that would have been introduced by diverse photosynthetically active radiation and temperature fluctuation patterns that naturally occurred during measurements. By measuring the exact same set of plots during each sampling period, I eliminated the potential introduction of confounding variables that would have been introduced if different plots were measured during each sampling period (e.g., seasonal bias due to the effects of random plot selection). Measuring the exact same set of plots during each sampling period also allowed me to analyze the collected data using multivariate regression techniques that would have been impossible to use otherwise. By restricting sampling to once every three months, I ensured that disturbance due to trampling of vegetation and soil compaction around (and therefore in) measured plots remained at reasonably acceptable levels. I later summed the measurements obtained at each plot in order to reduce all statistical dependency among samples to only the statistical dependency between plots.

During each sampling period, I sampled each plot once in light conditions and once in dark conditions. I created dark conditions by covering each chamber with a fitted polyester-film cover before placing the chambers over the plots. For each measurement, I placed one chamber over an invaded plot while I placed the other chamber over the paired native plot. During each sampling period, I randomly determined the order of measurement of each set of paired plots, the assignment of each chamber to each plot, and whether the first or second set of measurements at each plot pair would be sampled in dark conditions. By randomizing the order of light and dark conditions, I eliminated the potential introduction of confounding variables that would have resulted from persistent physiological changes in organisms reacting to changes in atmospheric conditions.

During sampling, I maximized measurement accuracy by orienting each chamber so that the probes within the chamber were fastened consistently to the north-facing wall of the chamber. I measured atmospheric carbon dioxide, temperature, and relative humidity 20 cm from the top of each chamber in the path of air flow generated by the fans, and photosynthetically active radiation measurements five cm above the sediment surface in each chamber. I depressed each chamber five cm into the soil and obtained measurements for a period of five minutes. Before placing each chamber over a plot, I placed the chambers on a box so that the chamber openings were held above the soil surface. While the chambers were held open, I monitored the atmospheric carbon dioxide concentration, air temperature, and relative humidity measurements inside both chambers in real time until they stabilized at ambient conditions. Ambient conditions were arrived at via atmospheric mixing aided by the fans inside both chambers. In order to establish a

control and a precision estimate for these chamber measurements, I measured 32 samples using both chambers in a larger container filled with water to a depth of five cm using the above sampling protocol in both light and dark conditions for a total of 64 samples.

Following each chamber measurement, I recorded absolute percent cover classes of vascular plant species, and total benthic macroalgae directly above each plot using 21 cover classes. Cover classes used were: zero percent, one to five percent, six to 10 percent, 11 to 15 percent, and so on up to 96 to 100 percent. I also counted the number of stems of each vascular plant species present in each plot and visually estimated the mean height of each vascular plant species present in each plot using a meter stick.

Biomass Data Analysis

I used various methods to analyze aboveground net primary productivity, belowground net primary productivity, and net primary productivity of native and invaded biomass samples. I used the maximum minus minimum method (Pearsall and Gorham 1956, Waits 1967) and Smalley's (1958) method to calculate aboveground net primary productivity values for native and invaded biomass samples. In order to calculate belowground net primary productivity values, I modified the maximum minus minimum method and Smalley's (1958) method by applying the same calculations that would be used on sampled aboveground biomass measurements to sampled belowground biomass measurements. In order to calculate net primary productivity values, I incorporated belowground biomass measurements into modified versions of the maximum minus minimum and Smalley's method.

Using the maximum minus minimum method and the modified maximum minus minimum method, I calculated above and belowground primary productivity values by subtracting the highest total of dry biomass collected during a sampling period from the lowest total of dry biomass collected during a sampling period. I also modified the maximum minus minimum method to calculate net primary productivity values by adding belowground biomass collected in each sampling period to the aboveground biomass collected during that sampling period and reapplying the above maximum minus minimum calculation.

Using Smalley's (1958) method, I calculated aboveground net primary productivity values in the following manner: (1) if the change in aboveground living biomass since the previous sampling period was positive, then trimonthly aboveground net primary productivity was equal to this change plus any increase in the dead aboveground biomass, and (2) if the change in aboveground living biomass since the previous sampling period was negative, then trimonthly aboveground net primary productivity was calculated as the sum of changes in aboveground living and dead biomass or zero, whichever was larger. The separation of living and non-living peat and root fragments was impractical because careful staining would have been infeasible and I didn't have access to more sophisticated measuring instruments. Instead, based on the results of Valiela et al. (1976), I estimated that 10 percent of sampled belowground biomass was living biomass, and that a decrease in living biomass occurred between the May 2011 and August 2011 sampling periods. I then used the modified Smalley's (1958) method to calculate net primary productivity values by adding belowground biomass

sample weights to the weights of living aboveground biomass in each sampling period and then calculated net primary productivity using Smalley's (1958) calculation method described above. In order to obtain yearly primary productivity values using Smalley's (1958) method and the modified Smalley's method, I used the February 2012 sampling period data to calculate the change in biomass for the May 2011 sampling period.

Atmospheric Carbon Dioxide Data Analysis

Before I could test alternative hypotheses regarding closed-chamber measurements, I first had to convert the ppm carbon dioxide measurements to grams of carbon from atmospheric carbon dioxide. The plant material in each plot occupied a unique volume and changed from season to season; therefore, a simple comparison of carbon dioxide ppm measurements would not have been appropriate since the same change in carbon dioxide ppm values in two different volumes of air would not equal the same change in carbon from atmospheric carbon dioxide.

I obtained values of grams of carbon from atmospheric carbon dioxide using the Ideal Gas Law, partial pressure values converted from ppm measurements, regression analyses of plant volumes, temperature measurements, and stoichiometry. The Ideal Gas Law is commonly expressed using the following equation (Wilson et al. 2007):

$$PV = nRT$$

This equation states that the pressure of a gas (P) multiplied by the volume of that gas (V) is equal to the moles (one mole = 6.022×10^{23}) of molecules of that gas (n) multiplied by the universal gas constant (R) multiplied by the temperature of that gas (T).

According to Dalton's Law of Partial Pressures and the Ideal Gas Law, in a mixture of gases that are not in states of extreme temperature or pressure, the ratio of moles of molecules of a component gas (n_i) to total moles of molecules present (n_{tot}) in the gas mixture is the same as the ratio of partial pressure (P_i) of a component gas to total pressure (P_{tot}) of the gas mixture. This relationship is expressed in the following equation:

$$\frac{n_i}{n_{tot}} = \frac{P_i}{P_{tot}}$$

The above relationship is constant because at the same temperature, all gas molecules have the same mean kinetic energy and therefore generate the same pressure (Wilson et al. 2007). The ppm measurements I obtained can be expressed as ratios of moles of carbon dioxide molecules present in the chamber to moles of air molecules present in the chamber. Using the above equation to convert ppm gas measurements recorded at one atm of total pressure, the molar ratio is simply equal to the partial pressure. Therefore, since the atmospheric pressure at sea level is approximately one atm in a wind controlled environment, the ppm measurements I obtained are approximately equivalent to partial pressure values expressed using the unit atm.

I obtained the volume of air inside each chamber using linear regression analyses of the aboveground measurements of collected biomass samples to generate volume estimation functions for each vascular plant species present in the measured plots. Combinations of some or all of the following five vascular plant species were present during all sampling periods in all paired plots: *S. densiflora*, *D. spicata*, *S. pacifica*, *J.*

carnosa, and *Triglochin maritima*. I used the resulting regression equations to estimate the volume of plant material present in each chamber at the time of each measurement. After the final sampling period, I destructively measured the vascular plant volume in each measured plot and used a *t*-test (assuming unequal variances) to test the alternative hypothesis that a statistically significant difference existed between the difference between measured and estimated vascular plant volumes in native plots and the difference between measured and estimated vascular plant volumes in invaded plots. In order to calculate the volume of air present within each chamber during each measurement, I subtracted the calculated volume estimate of the plant material in the plot from the volume inside the empty chamber when it was depressed five cm into flat soil.

The rest of the required values for the Ideal Gas Law equation were easy to obtain. I used the first and last temperature measurement from each sample. The value of *R* is a constant equal to the product of Avogadro's constant and Boltzmann's constant (Wilson et al. 2007).

Using the pressure, volume, temperature, and constant values described above, I calculated the moles of carbon dioxide present during sample measurement using the Ideal Gas Law equation algebraically rearranged in the following manner:

$$n = \frac{PV}{RT}$$

I then used stoichiometry to calculate the grams of carbon from atmospheric carbon dioxide present inside each chamber. Moles of carbon from atmospheric carbon dioxide equal moles of atmospheric carbon dioxide (since carbon dioxide molecules

contain only one carbon atom), so I multiplied moles of carbon dioxide by the standard atomic weight of carbon to obtain grams of carbon from atmospheric carbon dioxide.

In order to obtain the rate of net ecosystem exchange during sample measurement, I subtracted grams of carbon in atmospheric carbon dioxide present inside the chamber at the end of each five-minute sample measurement in light conditions from grams of carbon in atmospheric carbon dioxide present inside the chamber at the beginning of that measurement. In order to obtain the rate of ecosystem respiration during sample measurement, I subtracted grams of carbon in atmospheric carbon dioxide present inside the chamber at the beginning of each five-minute sample measurement in dark conditions from grams of carbon in atmospheric carbon dioxide present inside the chamber at the end of that measurement. Subtracting measurements in this manner reduced the measurement error associated with each sample measurement (Fahey and Knapp 2007). In order to obtain the rate of gross primary productivity at each plot during each sampling period, I summed the net ecosystem exchange and ecosystem respiration rates measured in the same plot during the same sampling period.

To test for differences between benthic macroalgae and photosynthetically active radiation measurements in native and invaded plots, I used randomization tests to test for statistically significant differences between native and invaded plot measurement data that were not normally distributed. In order to evaluate the relative contribution of all measured cover class categories to net ecosystem exchange values, I used multivariate linear regression to evaluate the relative contributions of measured absolute cover class

categories to summed atmospheric carbon dioxide flux measurements (Streever et al. 1998).

RESULTS

Biomass Results

Each primary productivity biomass analysis calculated higher aboveground net primary productivity in invaded samples, higher belowground net primary productivity in native samples, and higher net primary productivity in native samples (Table 2). Invaded aboveground dry biomass measurements displayed greater changes between sampling periods than native aboveground dry biomass measurements, and native belowground dry biomass measurements displayed greater changes between sampling periods than invaded belowground dry biomass measurements (Figure 3). I used biomass dry weights for all biomass comparisons since Rogers (1981) found a strong correlation between sampled aboveground biomass dry weights and sampled aboveground biomass carbon content, and the loss-on-ignition tests I conducted found a strong correlation between sampled belowground biomass dry weights and sampled belowground biomass carbon content ($R^2 = 0.94$).

Table 2. Aboveground net primary productivity, belowground net primary productivity, and net primary productivity in native and invaded biomass samples calculated using different methods.

Sample Category	Aboveground Net Primary Productivity Using the Maximum Minus Minimum Method (g C/m^2/year)	Aboveground Net Primary Productivity Using Smalley's Method (g C/m^2/year)	Belowground Net Primary Productivity Using the Modified Maximum Minus Minimum Method (g C/m^2/year)	Belowground Net Primary Productivity Using the Modified Smalley's Method (g C/m^2/year)	Net Primary Productivity Using the Modified Maximum Minus Minimum Method (g C/m^2/year)	Net Primary Productivity Using the Modified Smalley's Method (g C/m^2/year)
Native	194	459	5169	4168	5363	4491
Invaded	628	680	1749	1732	2377	1917

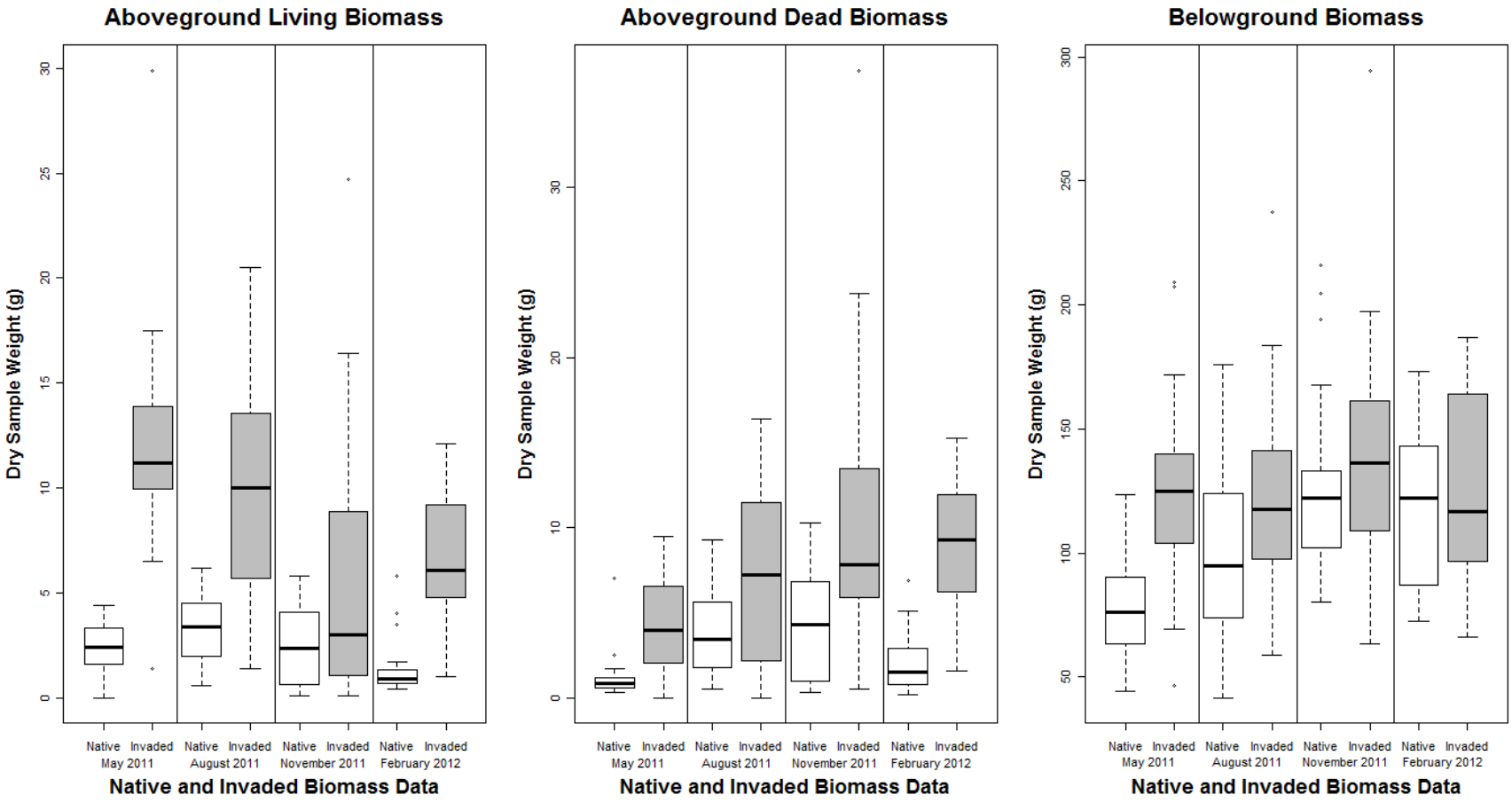

Figure 3. Box plot comparisons of dry biomass measurements obtained during each sampling period (shaded box plots represent invaded sample data and un-shaded box plots represent native sample data).

Atmospheric Carbon Dioxide Flux Results

After comparing several regression models, I decided to base my linear regression on the square roots of the volumes of each of the five species present in measured plots (response variable) and the square roots of the products of the number of each of those species and the mean height of each of those species (explanatory variable) (Figure 4). I applied square root transformations to the response and explanatory variables in order to reduce the heteroskedasticity (significant differences in variances) of the plotted values and thereby increase the accuracy of the volume estimations. I set the x and y-intercepts of the regression line to zero because this is a known point. I calculated the estimate of the volume of vascular plant species by squaring the response variable. Although estimated volumes generally underestimated measured volumes, no statistically significant difference existed between the difference between measured and estimated vascular plant volumes in native plots and the difference between measured and estimated vascular plant volumes in invaded plots (p-value = 0.271) (Figure 4). Mean measured vascular plant volumes in native plots equaled 135% of mean estimated vascular plant volumes in native plots. Mean measured vascular plant volumes in invaded plots equaled 128% of mean estimated vascular plant volumes in invaded plots. Cover class variation between sampling periods was greater for native plants than for *S. densiflora* (Figure 5).

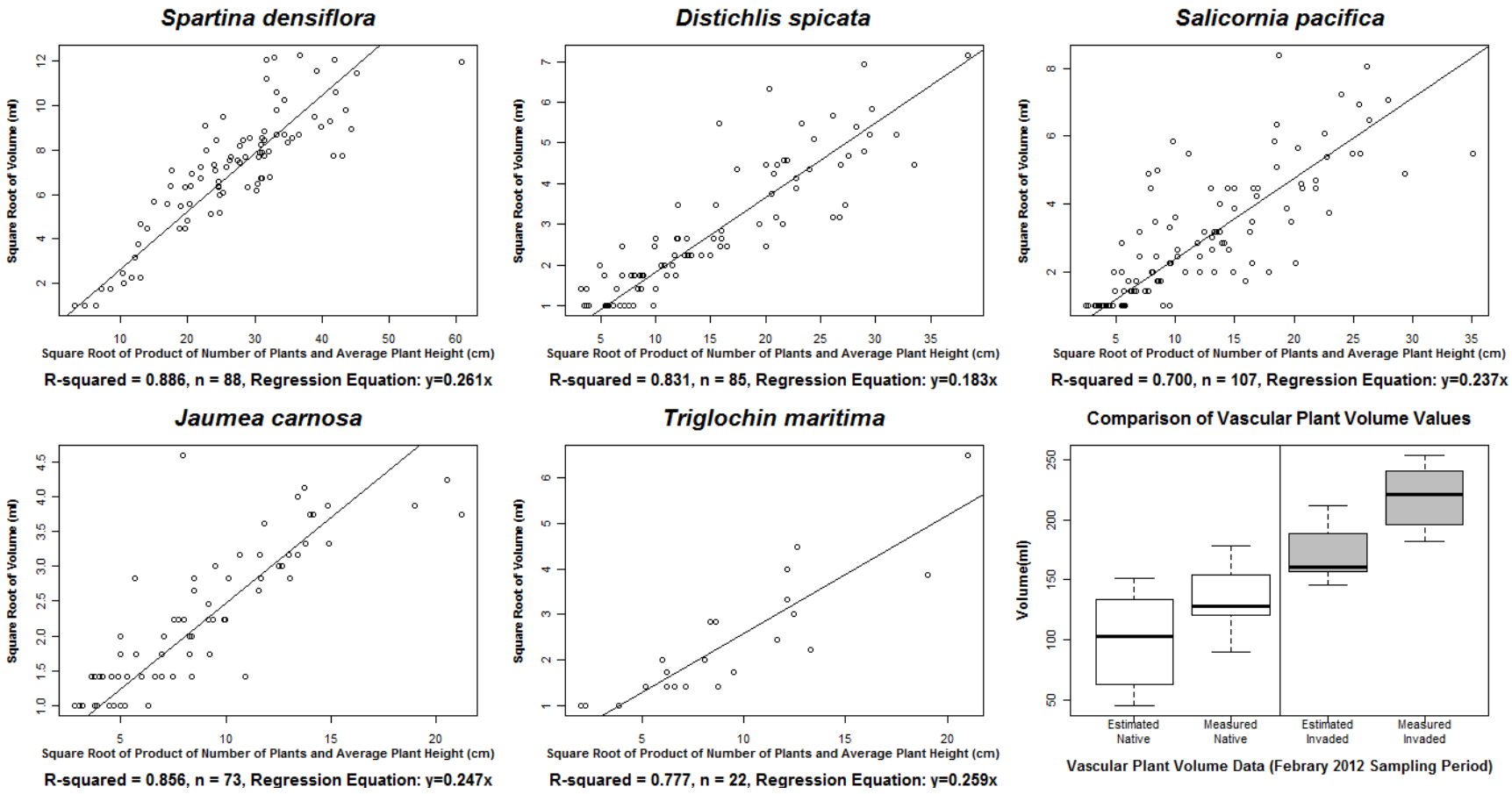

Figure 4. Transformed linear regression vascular plant volume models ($Y = \beta_1 X_1 + \varepsilon$) and box plot comparison of estimated and measured vascular plant volume in native and invaded plots.

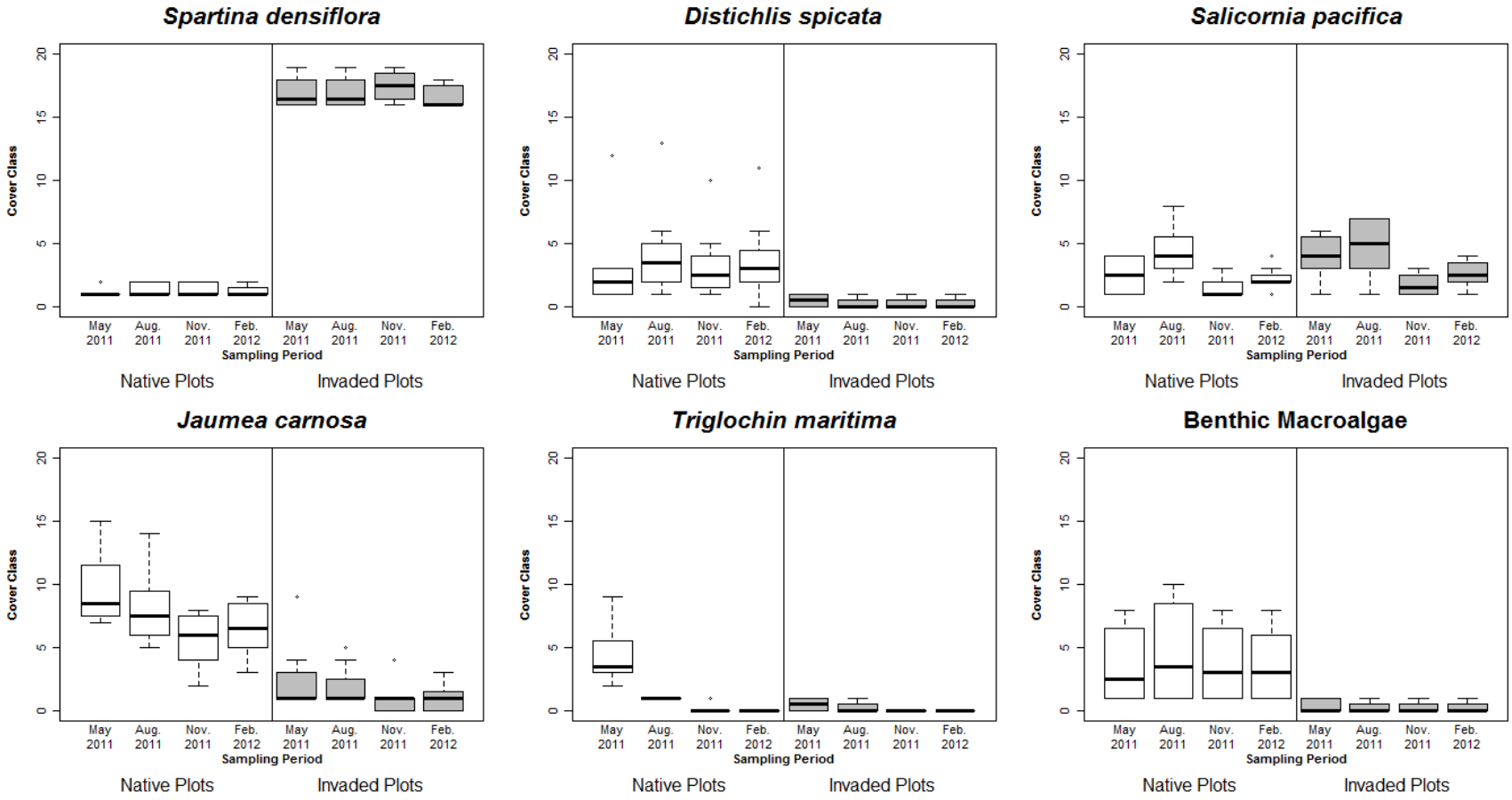

Figure 5. Box plot comparisons of percent cover class measurements of plants in permanent paired plots.

Summing the net ecosystem exchange measurements at each plot caused the distributions of the differences between native and invaded samples to normalize (this was also true for ecosystem respiration and gross primary productivity measurements) (Figure 6). I used the Anderson-Darling normality test to test the alternative hypothesis of non-normal distribution (Anderson and Darling 1954). I then tested the alternative hypothesis that native plots display higher rates of net ecosystem exchange, ecosystem respiration, and gross primary productivity than invaded plots using the summed measurements in paired *t*-tests. Paired *t*-tests revealed that the sampled native plots displayed higher net ecosystem exchange values than the sampled invaded plots (*p*-value = 0.0295); however, there was no statistically significant difference in ecosystem respiration or gross primary productivity between native and invaded plots (*p*-value = 0.241 and *p*-value = 0.0569, respectively) (Table 3). Pronounced differences between atmospheric carbon dioxide flux measurements were detected during the May 2011 sampling period, but differences were less pronounced during all other sampling periods (Figure 7). Low coefficients of determination were found between photosynthetically active radiation and net ecosystem exchange ($R^2 = 0.28$ for un-summed data, $R^2 = 0.0088$ for summed data), relative humidity and net ecosystem exchange ($R^2 = 0.085$ for un-summed data, $R^2 = 0.19$ for summed data), and relative humidity and ecosystem respiration ($R^2 = 0.011$ for un-summed data, $R^2 = 0.0026$ for summed data).

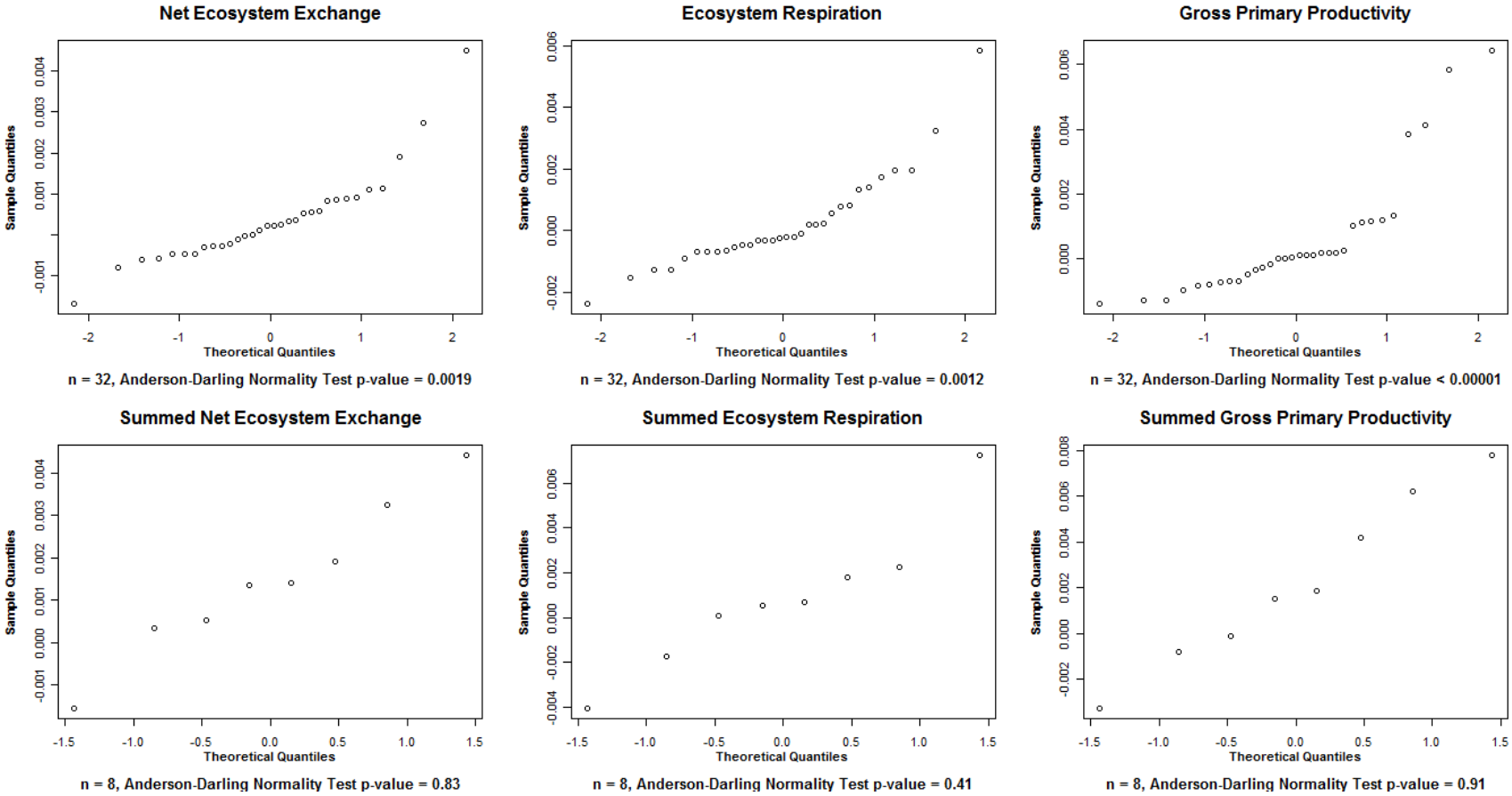

Figure 6. Normal quantile-quantile plots of differences in atmospheric carbon dioxide flux between native and invaded plots before and after summing of each permanent plot.

Table 3. Difference in means of atmospheric carbon dioxide flux hypothesis tests between summed native plots and summed invaded plots.

Measurement	Native Mean (g C/m^2/5 min)	Invaded Mean (g C/m^2/5 min)	*p*-value ($\alpha = 0.05$)
Net Ecosystem Exchange	0.00439	0.00294	0.0295
Ecosystem Respiration	0.0104	0.00953	0.241
Gross Primary Productivity	0.0148	0.0125	0.0569

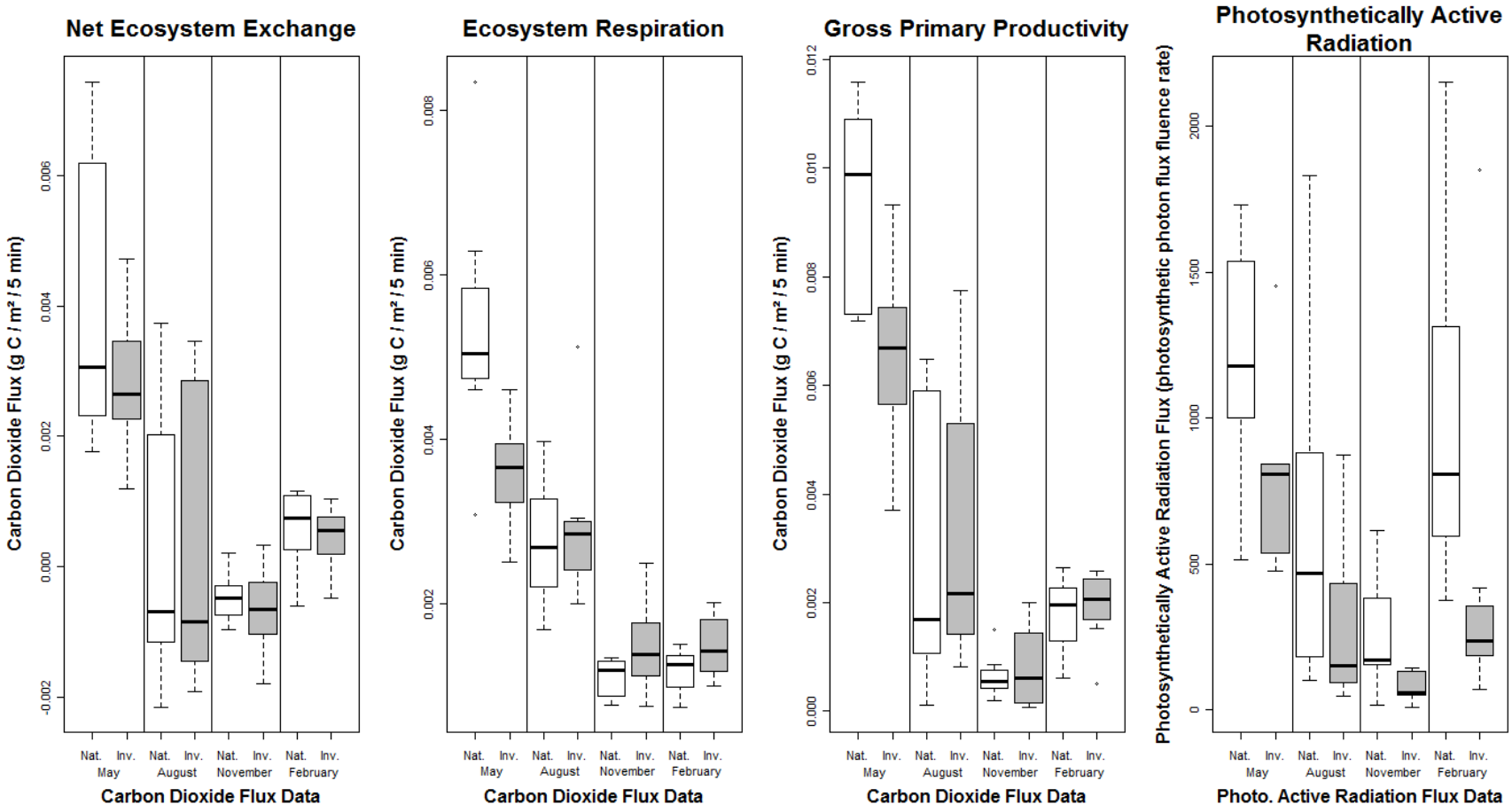

Figure 7. Box plot comparisons of net ecosystem exchange, ecosystem respiration, gross primary productivity, and photosynthetically active radiation in native and invaded plots during each sampling period.

Photosynthetically active radiation values in invaded plots, benthic macroalgae cover class values in native plots, and benthic macroalgae cover class values in invaded plots were not normally distributed (Anderson-Darling normality test p-value = 0.000013, Anderson-Darling normality test p-value < 0.00001, and Anderson-Darling normality test p-value < 0.00001, respectively). Consequently, I tested the alternative hypothesis that more light reaches benthic macroalgae in native plots and the alternative hypothesis that more benthic macroalgae is present in native plots with randomization tests that compared the observed measurements to 1,000,000 random permutations of the obtained measurements (Table 4).

Results of the multivariate linear regression analysis I used to evaluate the relative contributions of measured absolute cover class categories to summed atmospheric carbon dioxide flux measurements show that benthic macroalgae cover class is the strongest predictor of net ecosystem exchange and gross primary productivity (p-value = 0.0197 and p-value = 0.0274, respectively) (Figure 8); no other coefficients shown in Figure 8 are statistically significant at a 0.05 alpha level. Benthic macroalgae in measured plots consisted primarily of *Chaetomorpha aerea*, *Rhizoclonium riparium*, and an unnamed fucoid (Shaughnessy 2012, personal communication).

Table 4. Photosynthetically active radiation and benthic macroalgae presence hypothesis tests between mean native and mean invaded plot measurements.

Measurement	Mean Native Plot Measurement	Mean Invaded Plot Measurement	*p*-value ($\alpha = 0.05$)
Photosynthetically Active Radiation	771.2 (Photosynthetic Photon Flux Fluence Rate)	397.3 (Photosynthetic Photon Flux Fluence Rate)	0.0023
Benthic Macroalgae Absolute Cover Class	3.9 (Approximately 15% Absolute Cover)	0.28 (Approximately 2% Absolute Cover)	< 0.00001

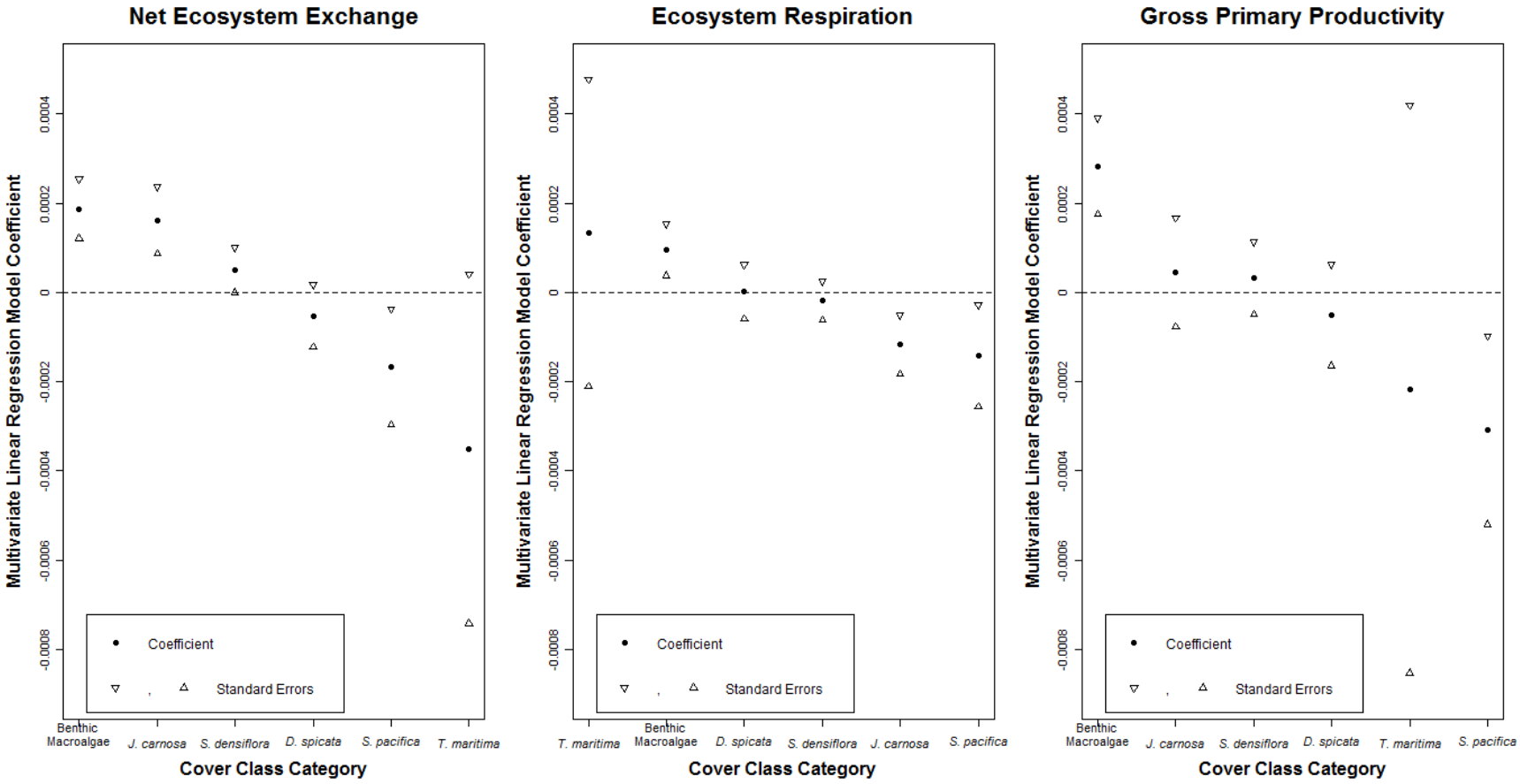

Figure 8. Coefficients of cover class categories from multivariate linear regression used to predict summed atmospheric carbon dioxide flux.

DISCUSSION

Several primary productivity studies have concluded that *Spartina* species displayed greater primary productivity than other salt marsh vascular plant species (Rogers 1981, Shew et al. 1981, Kaswadji et al. 1990, Neves et al. 2010). However, almost all of the conclusions of these studies are based exclusively on aboveground biomass analysis techniques. The aboveground net primary productivity results from this 2011/2012 study are reasonably similar to the results of Rogers' (1981) study (Table 5), but analyses of the differences in belowground net primary productivity and net ecosystem exchange between native and invaded plots were necessary in order to accurately measure the overall impact of *S. densiflora* colonization on primary productivity.

Only an approximate comparison between this study and Rogers' (1981) study can be made for the following reasons: (1) Rogers did not measure mixed native plant community samples, (2) Rogers did not sort *S. pacifica* into living and dead biomass, (3) Rogers sampled at two different study sites, (4) Rogers obtained measurements over 30 years ago, and (5) Rogers measured *S. densiflora*, *D. spicata*, and *S. pacifica* at approximately bimonthly intervals for approximately two years. Smalley's (1958) method has been used effectively to estimate annual primary productivity values from biomass measurements obtained once every three months for a period of one year (Neves et al. 2010).

Table 5. Comparison of aboveground net primary productivity to results from Rogers (1981).

Primary Productivity Assessment	Results (2011/2012) (g C/m^2/year)		Rogers' (1981) Results (g C/m^2/year)	
Maximum Minus Minimum Method	Native Aboveground Net Primary Productivity	194	Mean of *D. spicata* and Succulent *S. pacifica* Aboveground Net Primary Productivity	369
	Invaded Aboveground Net Primary Productivity	628	*S. densiflora* Aboveground Net Primary Productivity	653
Smalley's Method	Native Aboveground Net Primary Productivity	459	*D. spicata* Aboveground Net Primary Productivity	233
	Invaded Aboveground Net Primary Productivity	680	*S. densiflora* Aboveground Net Primary Productivity	1175

Lower herbivory rates in invaded plots could partially explain the greater aboveground net primary productivity displayed in invaded plots. Because invasive plant species are non-native, they frequently fall outside of the diet of native herbivores during invasion and colonization (Lockwood et al. 2007, Radosevich et al. 2007, Davis 2009). If invaded plots experienced less herbivory, they could display greater aboveground biomass as a result, and thereby bias the results of comparative aboveground net primary productivity biomass measurements.

In line with the results of this study, measurements of vascular salt marsh plant species in other salt marshes have found greater belowground primary productivity than aboveground primary productivity (Valiela et al. 1976, Stout 1978, Edwards and Mills 2005, Fahey and Knapp 2007). The greater yearly change in belowground net primary productivity measurements observed in native samples in the study area (Table 2) is understandable in light of the fact that native plots feature more ephemeral vegetation. As the native vascular salt marsh plants enter dormancy, it is possible that they translocate carbon belowground. This belowground carbon could then be rapidly transferred to aboveground portions of native plants during the early growing season. This annual shift of carbon would be consistent with starch storing strategies employed by many temperate zone vascular salt marsh plants (Westlake et al. 1998).

Algae repeatedly accounts for a large portion of the primary productivity of salt marsh ecosystems (Zedler 1980, Geider and Osborne 1992, Westlake et al. 1998, Sullivan and Currin 2000). Figure 8 displays evidence that confirms the hypothesized important contribution of benthic macroalgae to net ecosystem exchange and gross primary

productivity in measured plots. Coupled with the finding that more light reaches the sediment surface in native plots, the finding that benthic macroalgae is a strong predictor of net ecosystem exchange implies that higher net ecosystem exchange in native plots is partially due to more light reaching benthic macroalgae. A study of the effects of shading generated by *S. foliosa* near San Diego (California) demonstrated that the shading generated by *S. foliosa* brings about a microalgal community composition shift (Whitcraft and Levin 2007). *Spartina foliosa* and *S. densiflora* display similar morphology (Rogers 1981, Spicher 1984). Unpublished research conducted by Dr. Mary Kentula found that the shading produced by *S. foliosa* decreases algae primary productivity (Zedler 2010, personal communication). Also near San Diego, primary productivity of benthic algae equaled 76 to 140 percent of the aboveground primary productivity of vascular plants in sampled salt marsh plots (Zedler 1980).

The net ecosystem exchange results (Figure 7) show that more carbon dioxide was transferred from the atmosphere into native plots than into invaded plots. The statistically significant difference in net ecosystem exchange values between native and invaded plots is more likely based on differences in gross primary productivity values than differences in ecosystem respiration values because of the relative difference in *p*-values (*p*-value of 0.0569 is closer to 0.05 than *p*-value of 0.241).

Recently, in an effort to restore coastal ecosystems, government entities have taken steps towards large-scale eradication of invasive *Spartina* species along the Washington-Oregon-California coastline. Action 2.4 of the West Coast Governors' Agreement on Ocean Health (2006) identifies invasive *Spartina* species as a priority for

eradication: "Focus efforts on eradicating non-native cordgrasses (genus *Spartina*), which are transported between the three states on ocean currents, as a pilot coast-wide eradication." The eventual goal of this agreement is to eradicate invasive *Spartina* species from the Washington-Oregon-California coastline by 2018. In line with this goal, the Humboldt Bay National Wildlife Refuge recently received a grant of approximately one million dollars to eradicate *S. densiflora* within its boundaries; this eradication of *S. densiflora* will function as a pilot project for regional eradication of *S. densiflora* from all of the salt marshes of Humboldt Bay and adjacent estuaries (United States Fish and Wildlife Service 2009, 2011).

Since *S. densiflora* displayed greater aboveground net primary productivity than *D. spicata* and *S. pacifica* (Rogers 1981), it has been assumed that if *S. densiflora* were eradicated from Humboldt Bay, the primary productivity of Humboldt Bay salt marshes could be negatively altered (Driscoll 2010). A decrease in the primary productivity of Humboldt Bay would likely reduce the ecosystem services generated by Humboldt Bay and provide less energy to higher organisms within Humboldt Bay. In addition, a decline in the primary productivity of Humboldt Bay could lead to further destructive impacts to salt marsh ecosystems from positive feedback in the form of runaway consumption (Silliman et al. 2005).

The belief that *S. densiflora* eradication could decrease the primary productivity of Humboldt Bay is based on two commonly held assumptions. One commonly held assumption is that the results of Rogers' (1981) study are indicative of the net primary productivity of the three plants that he measured; however, the methods employed by

Rogers (1981) only measured aboveground net primary productivity. In addition to being prone to inaccuracies, aboveground net primary productivity values can fluctuate over time in response to environmental changes (Fahey and Knapp 2007). For instance, when Rogers (1981) measured the aboveground net primary productivity of *S. densiflora* in Humboldt Bay he attributed low primary productivity measurements to periods of unusually low rainfall, and when the aboveground net primary productivity of *S. densiflora* was measured in Argentina, low primary productivity measurements were attributed to periods of unusually high rainfall (Trilla et al. 2010). Additionally, as the results of this study show, belowground net primary productivity can significantly contribute to net primary productivity values. A second commonly held assumption underlying the belief that *S. densiflora* eradication could decrease the primary productivity of Humboldt Bay is that the vascular plants Rogers (1981) measured are the dominant contributors to the primary productivity of the salt marshes of Humboldt Bay. However, this second assumption fails to consider the contribution of algae to salt marsh primary productivity observed in this study. Furthermore, according to the results of Harding (1973) and Rogers (1981), phytoplankton would likely display higher primary productivity than *D. spicata* if current measurements were obtained in Humboldt Bay.

The assumption that *S. densiflora* eradication could decrease the primary productivity of Humboldt Bay has also led to an assumption that less energy could be available to higher organisms if native salt marsh plant communities were restored in Humboldt Bay (Driscoll 2010). However, if a plant is not fed on directly, but is instead first fed on by an intermediate organism in a food web, a great deal of energy is lost as

entropy (Batzer and Sharitz 2006). The effects of *S. densiflora* on the food webs that function within Humboldt Bay are largely unknown, but salt marshes in other locations can provide clues. Over the last 35 years, a *Spartina* genus hybrid (*S. alterniflora x S. foliosa*) has spread into several hectares of San Francisco Bay (California) mud flats (Neira et al. 2006). This hybrid has generated a disproportionately large amount of detritus and has had a significant negative impact on the primary productivity of benthic algae (Neira et al. 2006). The shift from an algae-based to a detritus-based food web has led to a substantial shift in the trophic function of San Francisco Bay mud flats and a reduction in the density of lower macrofauna that had previously relied on benthic algae as a primary food source (Levin et al. 2006). These findings from San Francisco Bay are supported by research carried out in Georgia (USA) that examined the isotopic signatures of salt marsh vascular plants and algae in consumer species and discovered that salt marsh algae was consumed directly by higher trophic levels while salt marsh vascular plants displayed little evidence of direct herbivory (Sullivan and Moncreiff 1990, Currin et al. 1995). Therefore, even if *S. densiflora* increased net primary productivity in Humboldt Bay, this increase in primary productivity would probably not result in more energy becoming available to higher organisms because of energy lost as entropy and a likely reduction in benthic macroalgae similar to the difference observed in this study.

Regardless of the effect of *S. densiflora* eradication in Humboldt Bay, a better understanding of the interaction between *S. densiflora* and anthropogenic inputs to Humboldt Bay salt marshes would help to effectively manage the potential effects of invasive species in Humboldt Bay. A study has shown that excess nutrient loading in

Willapa Bay (Washington) and San Francisco Bay increased the susceptibility of intertidal habitats to colonization by invasive *Spartina* species (Tyler et al. 2007). Since *S. densiflora* displays a strong positive correlation with phosphorus levels in Humboldt Bay (Newby 1980, Falenski 2007), and wastewater outflow is likely partially responsible for elevated phosphorus levels in Humboldt Bay (Barnhart et al. 1992), wastewater could be a contributing factor to *S. densiflora* colonization in Humboldt Bay. In addition, since algae growth diminishes excess nutrient loading from wastewater (Mitsch and Gosselink 2007), and excess nutrient loading decreases *Zostera marina* shoot density in Humboldt Bay (Tennant 2006), a reduction in algal growth rates caused by *S. densiflora* colonization in a higher-elevation buffer zone could partially explain decreases in nearby lower-elevation *Z. marina* shoot density. In order to better understand how anthropogenic inputs affect salt marsh plants in Humboldt Bay and adjacent estuaries, I recommend an examination of the effects of experimentally introduced phosphorus treatments on primary productivity and algal growth in randomly located plots.

In order to more completely understand the effects of *S. densiflora* colonization in the salt marshes of Humboldt Bay and adjacent estuaries, future studies could address the following topics: the effect of *S. densiflora* colonization on the net ecosystem productivity of Humboldt Bay, relative rates of herbivory in native and invaded plots, the effect of tides on primary productivity in Humboldt Bay, chemosynthesis rates in Humboldt Bay, and potential energy loss in the form of entropy resulting from *S. densiflora* colonization in Humboldt Bay. At the scale of measurement used in this study, net ecosystem exchange measurements are essentially equal to net ecosystem

productivity since negligible lateral carbon fluxes (e.g., transfer of suspended sediment) occurred during measurements (Randerson et al. 2002). However, if an estimate of net ecosystem productivity occurring throughout Humboldt Bay is needed in order to develop a carbon budget, a full accounting of all lateral carbon fluxes would need to be carried out (Randerson et al. 2002). Rates of herbivory (especially for relatively large herbivores) could be estimated by excluding grazers from *in-situ* plots (Silliman and Bortolus 2003). Primary productivity in salt marshes decreases during high tides (Harding 1973, Streever et al. 1998, Westlake et al. 1998, Migne et al 2002); however, in order to thoroughly compare differences in net ecosystem exchange between native and invaded plots, a future study could measure net ecosystem exchange in native and invaded plots during high tide. During tides above approximately 2.2 m above MLLW, invaded plots displayed more photosynthetic vascular plant material above the surface of the tide water than native plots, but more light was likely reaching phytoplankton in native plots. In salt marsh ecosystems, chemosynthesis accounts for a relatively small portion of primary productivity (Westlake et al. 1998); however, a future study could use closed chambers and soil probes to measure the respiration products of anaerobic bacteria (e.g., methane, hydrogen sulfide) in native and invaded plots in order to estimate relative rates of primary productivity resulting from chemosynthesis. A stable isotope study could be used to test the hypothesis that *S. densiflora* invasion in Humboldt Bay results in less energy available to higher organisms due to energy lost as entropy (Sullivan and Moncreiff 1990, Currin et al. 1995).

CONCLUSION AND RECOMMENDATIONS

Invaded samples displayed lower net primary productivity and net ecosystem exchange measurements compared to native samples; therefore, *S. densiflora* colonization reduced primary productivity in measured samples. Based on multivariate regression analysis, benthic macroalgae cover in measured plots was a strong predictor of net ecosystem exchange. More photosynthetically active radiation reached benthic algae in native plots compared to invaded plots. Ecosystem respiration and gross primary productivity differences between native and invaded plots were not statistically significant.

In order to measure the effect that *S. densiflora* invasion is generating on the primary productivity of Humboldt Bay and adjacent estuaries, I recommend that a future study sample 288 statistically independent pairs of plots (576 plots total). I recommend a stratified spatial sampling design throughout the salt marshes of Humboldt Bay and adjacent estuaries since a completely random sampling design would likely sample a disproportionately high number of invaded samples. Native and invaded areas could be designated using aerial hyperspectral imagery (Judd et al. 2007) or visual estimation (Grazul and Rowland 2010). I also recommend a stratified temporal sampling design in order to eliminate seasonal bias. Samples would be obtained at random times during the daylight hours of each month of a year (48 samples per month). Samples would also need to be obtained during appropriate tide windows. Using paired closed chambers, the following measurements would be obtained at the beginning and end of five-minute sampling periods at each plot: atmospheric carbon dioxide, air temperature, and air

pressure. In order to obtain ecosystem respiration and gross primary productivity measurements, light and dark conditions would be measured at each plot. Plant volumes in measured plots would need to be estimated using available technology.

In order to estimate that 576 samples would be required for the above experimental design, I used the following calculations. Statistically significant ecosystem respiration measurements required the largest sample size of all measured variables in this study; accordingly, I used a retrospective power analysis to estimate that a sample size of 34 summed measurements (instead of 16) would have produced a statistically significant difference ($\alpha = 0.05$, power = 0.80) in summed ecosystem respiration measurements between native and invaded paired plots obtained at the study site. In order to sufficiently capture differences in spatial heterogeneity within a larger sample area, I calculated a sample size increase based on selected coefficients of the *S. densiflora* abundance prediction model developed by Falenski (2007) as well as assumptions that elevation would not vary by more than three meters between all measured plots, reduction potential would not vary by more than 300 millivolts between all measured plots, phosphorus would not vary by more than 45 ppm between all measured plots, and phosphorus site averages would not vary by more than 15 ppm between sites. I multiplied the sample size of 34 by (0.0571[Phosphorus] * 45 + 0.000352[Reduction Potential] * 300 + 0.271[Elevation] * 3 + 0.0493[Phosphorus Site Average] * 15) to get 144. I multiplied 144 by four to conclude that 576 summed measurements would be required. Using summed measurements would be more efficient than using un-summed statistically independent measurements of paired plots (1716 un-summed statistically independent

measurements during a period of one year would be required to obtain a statistically significant ecosystem respiration comparison); however, un-summed statistically independent measurements would allow for a simultaneous analysis of net primary productivity based on destructive biomass analysis of measured native and invaded plots.

Because the study site used for this study is similar to the salt marsh ecosystems found throughout the salt marshes of Humboldt Bay and adjacent estuaries, it is a reasonably good proxy indicator of the relative primary productivity values of salt marsh areas that have become dominated by *S. densiflora* and salt marsh areas that remain dominated by native plants. As such, the results of this study reaffirm the importance of the restoration of native plant communities throughout Humboldt Bay and adjacent estuaries.

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