

PHENOTYPIC PLASTICITY OF INVASIVE *SPARTINA DENSIFLORA* (POACEAE) ALONG A BROAD LATITUDINAL GRADIENT ON THE PACIFIC COAST OF NORTH AMERICA¹

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- **Premise of the study:** Phenotypic acclimation of individual plants and genetic differentiation by natural selection within invasive populations are two potential mechanisms that may confer fitness advantages and allow plants to cope with environmental variation. The invasion of *Spartina densiflora* across a wide latitudinal gradient from California (USA) to British Columbia (Canada) provides a natural model system to study the potential mechanisms underlying the response of invasive populations to substantial variation in climate and other environmental variables.
- **Methods:** We examined morphological and physiological leaf traits of *Spartina densiflora* plants in populations from invaded estuarine sites across broad latitudinal and climate gradients along the Pacific west coast of North America and in favorable conditions in a common garden experiment.
- **Key results:** Our results show that key foliar traits varied widely among populations. Most foliar traits measured in the field were lower than would be expected under ideal growing conditions. Photosynthetic pigment concentrations at higher latitudes were lower than those observed at lower latitudes. Greater leaf rolling, reduced leaf lengths, and lower chlorophyll and higher carbon concentrations were observed with anoxic sediments. Lower chlorophyll to carotenoids ratios and reduced nitrogen concentrations were correlated with sediment salinity. Our results suggest that the variations of foliar traits recorded in the field are a plastic phenotypic response that was not sustained under common garden conditions.
- **Conclusions:** *Spartina densiflora* shows wide differences in its foliar traits in response to environmental heterogeneity in salt marshes, which appears to be the result of phenotypic plasticity rather than genetic differentiation.

Key words: anoxia; foliar traits; global climate change; invasive plants; phenotypic plasticity; radiation quality; salinity; salt marshes; sediment texture.

Plant species that successfully invade novel habitats beyond their native range are thought to have broad ecological amplitudes and/or physiological tolerances to environmental change. Phenotypic acclimation of individual plants and genetic differentiation by natural selection within invasive populations are two potential mechanisms that may confer fitness advantages and allow plants to cope with environmental variation (Drenovsky et al., 2012).

Global climate change will affect exotic plant invasions, and it is important to link functional trait-based responses of invaders to environmental changes to improve our understanding of mechanisms that promote invasiveness, predict future invasions,

forecast changes in the distribution of alien species, and manage those at hand (Kirwan et al., 2009; Drenovsky et al., 2012). Phenotypic plasticity may increase the niche breadth of invaders, and plasticity of important functional traits is expected to benefit invasive plants during the invasion process and after establishment due to climate change (Richards et al., 2006; Nicotra et al., 2010). A better understanding of the role of phenotypic plasticity of invasive plants in response to environmental variation will also improve our ability to manage weed invasions and improve conservation efforts in natural landscapes. Thus, studies that integrate biological invasions, climate change, and phenotypic plasticity are needed (Engel et al., 2011).

After a population of aquatic plants is introduced and established in a new range, hydrologic connectivity and the hydrochorous dispersal of propagules can result in their rapid spread at large spatial scales (Shimamura et al., 2007; Okada et al., 2009). When halophytes grow in salt marshes along a wide latitudinal cline, they face environmental conditions such as air temperature, growing season length, photoperiod, light intensity and quality, and salinity that vary among locations (Pennings et al., 2003; Isacch et al., 2006). Cordgrasses (*Spartina* spp., Poaceae) are one of the most geographically widespread of all native and invasive halophyte genera (Adam, 1990). Propagules from nonnative *Spartina* species in estuarine salt marshes along the Pacific coast of North America can be carried

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long distances by ocean currents to new areas (Morgan and Sytsma, 2013). Predictions of the spread and colonization of invasive plants under changing environmental conditions are necessary for effective weed management planning (Daehler and Strong, 1996). However, support for robust predictions is limited since many invasive species successfully colonize habitats with climates and other environmental conditions quite different from those in the native range of the species (e.g., Pysek et al., 2012). Integrated approaches that include the study of natural populations along latitudinal gradients paired with methods such as common garden experiments are a way to increase our understanding of responses of plant species to environmental change (De Frenne et al., 2013). In this sense, Eelsey-Quirk et al. (2011) obtained plant cultures of *Spartina alterniflora* Loisel. from three native populations collected in the Atlantic and Gulf Coast of the United States and compared their growth in a common garden experiment in a Delaware salt marsh. Their results suggest that some of the recorded differences in plant traits were due to adaptation to local conditions while others were due to phenotypic plasticity. Integrated biogeographical approaches are also essential to understand exotic plant invasions, yet the spatial scale of most studies has been limited (Hierro et al., 2005). The invasion of alien *Spartina densiflora* Brongn. across a wide latitudinal gradient from California (USA) to British Columbia (Canada) provides a natural model system for an integrated study of the potential mechanisms underlying the response of invasive populations to substantial variation in climate and other environmental variables.

Spartina densiflora is a halophyte native to southeastern South America (Brazil, Argentina), where it occupies a wide variety of habitats from 23°20'S to 51°33'S latitude (Bortolus, 2006). It has invaded salt marshes in Chile (ca. 33°30'S–42°46'S), Morocco (ca. 34°50'N), the southwest Iberian Peninsula (ca. 36°02'N–37°21'N), and the Pacific coast of North America from San Francisco Bay, California (ca. 37°56'N), north to Vancouver Island, British Columbia (ca. 49°20'N) (Bortolus, 2006; Saarela, 2012). In the Iberian Peninsula, it has invaded very different estuarine habitats (Nieva et al., 2001b), and a variety of phenotypes are recognized (Castillo et al., 2003, 2008; Nieva et al., 2005). Ecotypic differentiation of *S. densiflora* has also been described for populations in its native range. These differences relate to environmental changes driven by latitude (Álvarez et al., 2009), yet the native populations have low detectable genetic variation (Ayres et al., 2008).

Consequently, we hypothesized that populations of *Spartina densiflora* from a geographic gradient along the Pacific coast of North America would respond differently to the environment based mainly on phenotypic plasticity, rather than on adaptive differentiation. Specifically, we expected to see phenotypically plastic responses of leaf traits across geographic latitudes, since the leaf (the organ of photosynthesis and transpiration) is highly sensitive to environmental conditions (Stephenson et al., 2006). To probe this hypothesis, we compared morphological, physiological, and chemical leaf traits in *S. densiflora* growing in populations from invaded sites across a large spatial scale. In the field, we collected data from populations along the Pacific Coast of North America to evaluate the variation of plant responses to changing environmental conditions; these sites varied greatly with respect to sedimentary, tidal, and atmospheric climate factors. Common garden experiments are useful to test whether interpopulation differences recorded in the field are due to genetic differentiation or phenotypic plasticity (Castillo et al., 2005a; Shaw and Etterson, 2012). Therefore, we also collected

plants of *S. densiflora* from each of the studied field sites and grew them in a greenhouse with a generally favorable common environment.

MATERIALS AND METHODS

Study sites—Our field study in the summer of 2010 at five coastal marshes invaded by *Spartina densiflora* along the Pacific Coast of North America spanned the entire known latitudinal range of *S. densiflora* on this continent (Fig. 1). The southernmost *S. densiflora* population evaluated was at Corte Madera Creek (37°56'33"N, 122°30'55"W) in San Francisco Bay Estuary, California (Fig. 1). This population was located in a middle-elevation salt marsh dominated by *S. densiflora*. Plants of *S. densiflora* from Humboldt Bay were introduced to Corte Madera Creek in the late 1970s during a salt marsh restoration project (Faber, 2000). To the north, we studied a population at Vance Marsh (40°49'53"N, 124°10'17"W, Fig. 1) in the Humboldt Bay Estuary (California, USA) where the species had been unintentionally introduced by ships from Chile during the late 19th century (Spicher and Josselyn, 1985) and has now infested more than 90% of the estuary's salt marshes (Pickart, 2001). This population occupies a middle-elevation salt marsh dominated by *Distichlis spicata* (L.) Greene and *Sarcocornia pacifica* (Standl.) A. J. Scott. The third population was near the mouth of the Mad River Estuary that drains to the

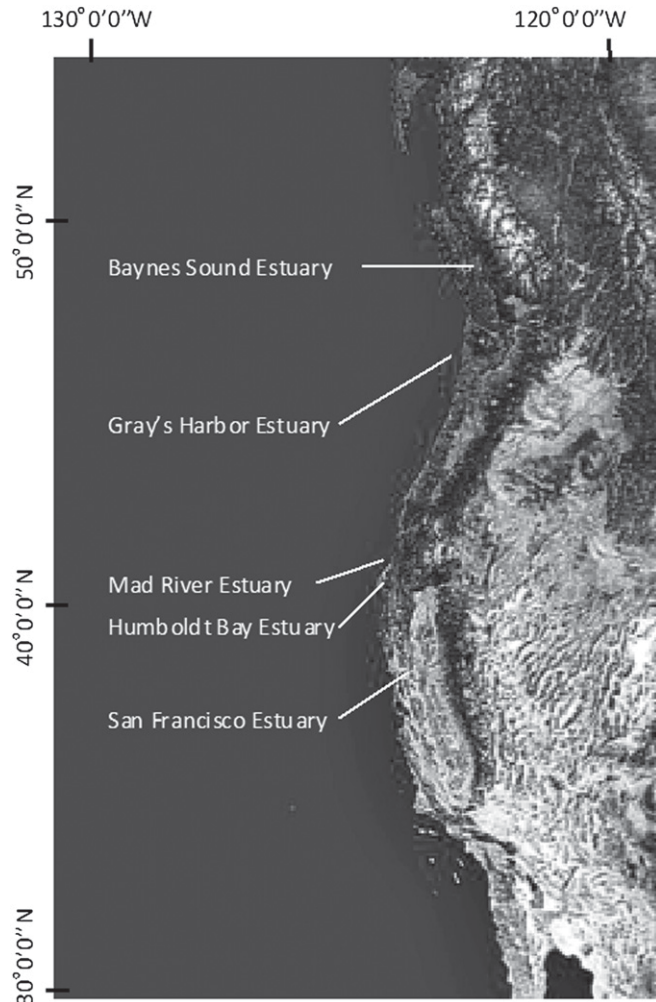


Fig. 1. Satellite orthographic image of the central Pacific Coast of North America showing the sampling locations for *Spartina densiflora* (modified from a public domain image of North America provided by the National Aeronautics and Space Administration, USA).

Pacific Ocean just north of Humboldt Bay (40°56'10"N, 124°7'48"W; Fig. 1). This population of *S. densiflora* has invaded the narrow, fringing intertidal zone at a river bank colonized by isolated clumps of *D. spicata*, *Salix hookeriana* Barratt ex Hook and *Polygonum* sp. Our fourth population was at Grays Harbor Estuary (Fig. 1; Ocean Shores, Washington, USA; 46°57'N, 124°8'08"W). The presence of *S. densiflora* in Grays Harbor was first recorded in 2001 (Pfauth et al., 2003). At our study site, *S. densiflora* occupies a middle elevation marsh where *D. spicata* and *S. pacifica* are codominant. The northernmost *S. densiflora* population in our study was in a fringing salt marsh along the shoreline of Baynes Sound, which is a narrow channel between the east coast of Vancouver Island and Denman Island in the Strait of Georgia (southwestern British Columbia, Canada; Fig. 1: 49°33'N, 124°52'09"W). *Spartina densiflora* was first discovered on Vancouver Island in 2005 (Morgan and Sytsma, 2010). The population was growing on an intertidal cobble plain dominated by *S. pacifica*.

The five sampled localities have mixed tides with climate varying from a Mediterranean climate with cool, wet winters and hot, dry summers with some fog for the San Francisco Bay site, to a marine west coast climate with cooler, foggy summers for the other United States locations, and a wider temperature range with lower low temperatures but less summer fog at Baynes Sound. According to the climatic series (1971–2000), mean daily temperature varied between 9.7° and 17.7°C for San Francisco Bay, 8.8° and 14.8°C for Humboldt Bay, 5.6° and 16.1°C for Grays Harbor and 2.7° and 18.0°C for Vancouver Island. Mean minimum daily temperature among sites was lowest at Vancouver Island in January (−0.8°C), whereas it was similar for every location during June and July (ca. 11°C) (Environment Canada, 2002; NCDC, 2004).

Characterization of habitats of *Spartina densiflora*—Latitude was recorded for every location using handheld GPS technology. Meteorological conditions were characterized using mean daily temperature (°C) for the last month (a period similar to the age of sampled flag leaves) and for the last week before sampling reported in local climatological stations (NCDC, 2013). In addition, we examined mean daily global horizontal insolation (W·h·m^{−2}) for the sampling month (NREL, 1992; NRC, 2009), day length (h) for the sampling day (Lammi, 2008), and the number of annual growing degree days for all sites (Environment Canada, 2002; NCDC, 2004). The total growing degree days, the number of days that daily temperature exceeds a threshold temperature necessary for significant plant growth, reflects both the temperature and the duration of the growing season. We used 10°C as a threshold for *S. densiflora* growth, as applied previously for *Spartina alterniflora* (Kirwan et al., 2009). Mean tidal level and range (m) for each location were obtained from NOAA (2010) and Fisheries and Ocean Canada (2010).

Spartina densiflora populations were visited during low tides, and sediment cores ($n = 10$ per site, 5 cm diameter, 0–10 cm depth) were obtained from randomly selected points within the population. Redox potential (Eh) of the sediment at 10 random sampling points was determined in the field with a portable meter and electrode system (Orion pH/mV 290A, Baton Rouge, Louisiana, USA). Then, sediments were stored in sealed containers and transported to the laboratory. A subsample of the sediment was mixed in a 1:1 ratio with distilled water to determine electrical conductivity (model 9033; Hanna Instruments, Woonsocket, Rhode Island, USA) and pH (pH/mV 290A; Orion, Houston, Texas, USA) ($n = 10$). Loss on ignition (LOI) was used to estimate sediment organic matter concentration ($n = 10$). Gravel percentage was recorded gravimetrically in relation to the other sediment fractions. Sediment texture (as percentage of sand, silt, and clay) was recorded using a hydrometer method in November 2007 ($n = 3$) (Gee and Bauder, 1986). Nitrogen and carbon concentration of sediments were measured using a Perkin Elmer (Waltham, Massachusetts, USA) 2400 CHN/O elemental analyzer, after they had been dried at 70°C for 48 h, weighed for mass loss, and ground in a 40-mesh Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) ($n = 10$).

Leaf traits—Ten adult tussocks of *Spartina densiflora* with between 10 and 80 live shoots and a mean tussock area between 15 and 41 cm² (ca. 2–3 yr old) were individually marked in every studied population. Plants were randomly selected in the population in an effort to sample potentially different genotypes, being separated at least 1 m from each other, and every tussock was identified following Moberley (1956). The following foliar traits were recorded on flag leaves of adult shoots for the marked tussocks in the field ($n = 10$ tussocks; five randomly selected shoots per tussock): (1) leaf length and maximum width; (2) leaf adaxial rolling (calculated as the percentage reduction in leaf width resulting from rolling at the midpoint of the leaf; Premachandra et al., 1993); and (3) apical leaf growth rate. Apical leaf growth rate was measured for all populations

(with the exception of Grays Harbor) by marking five leaves per tussock with permanent sealant at their base and measuring the distance from the sealant to the leaf base 1–2 d later.

Flag leaves were collected in the field from five marked plants per population, placed in sealed plastic bags and immediately frozen on dry ice for analysis of photosynthetic pigments and water content in the laboratory. Photosynthetic pigments of the flag leaf were extracted using 0.05 g of fresh material in 10 mL of 80% aqueous acetone. After the suspension was filtered through filter paper, 1 mL was diluted with a 2 mL of acetone, and concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (C_{x+c}) were measured in a spectrophotometer (Milton Roy Spectronic 401), using three wavelengths (663, 647, and 470 nm). Concentrations of pigments (mg·g^{−1} DW) were calculated as described by Lichtenthaler (1987). Leaf carbon and nitrogen concentration (mg·g^{−1} DW) were measured as described previously for sediments.

Ten entire tussocks similar in size to those marked for further measurement in the field were collected. The dry mass (DW) of five flag leaf drilled circular pieces (0.5 cm diameter) per plant ($n = 10$ tussocks per population) was obtained after drying samples in a forced-air oven at 80°C for 48 h. Specific leaf area (SLA; m²·g^{−1}) was calculated by dividing the leaf area by the dry mass according to Garnier et al. (2001). Leaf water content (LWC) was calculated as LWC (%) = (FW − DW)/FW × 100, where FW is the fresh mass and DW is the dry mass after oven-drying samples at 80°C for 48 h (Castillo et al., 2007).

Common garden experiment—Every marked tussock of *Spartina densiflora* used in the field study was collected, and rhizomes were separated and grown for 27 mo in plastic pots (20 cm diameter × 18 cm height) filled with perlite substrate in the greenhouse facilities of the University of Seville (37°21'42"N, 5°59'15"W). The pot bases were kept permanently flooded to a height of 2 cm, and watered with 20% strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950; Epstein, 1972). Solutions were changed once a week. After 27 mo of growth, every leaf trait recorded in the field was recorded again for the same plants acclimated to common greenhouse conditions to assess whether the differences observed in the field reflect phenotypic plasticity or population differentiation. Common greenhouse conditions included substrate Eh of 234 ± 5 mV, pH 8.3 ± 0.2, electrical conductivity 0.5 ± 0.0 mS·cm^{−1}, mean monthly air temperature 23° ± 2°C and mean monthly air relative humidity 62 ± 2%. Light conditions of mean radiation of 700 μmol photon·m^{−2}·s^{−1} and a daily photoperiod of 16 h (that was extended with incandescent lights, Vialox NAV-T [SON-T; Osram, Munich, Germany] 400 W, giving a spectral continuum) were set up to imitate those recorded at higher latitude sites in relation to photoperiod and light intensity. Fresh water was used to avoid salinity effects on the leaf development. *Spartina densiflora* is a facultative halophyte that can germinate, establish, and develop in freshwater conditions (Nieva et al., 2001a; Castillo et al., 2005b). Thus, experimental settings of the common garden experiment were designed to set up optimum growth conditions for *S. densiflora*.

Statistical analyses—Statistical analyses were carried out using SPSS 12.0 (SPSS, Chicago, Illinois, USA). Deviations were calculated as standard error of the mean (SEM). Data were tested for homogeneity of variance and normality with the Levene test and the Kolmogorov–Smirnov test, respectively ($P < 0.05$). When homogeneity of variance between groups was not found, data were transformed using the following functions: $1/x$ and \sqrt{x} . If homogeneity of variance was not achieved by data transformation, then the means were compared using a Kruskal–Wallis *H* test and Mann–Whitney *U* test. Leaf traits were compared between *S. densiflora* populations by one-way analysis of variance (ANOVA) using the geographical site as grouping factor (*F* test). Tukey's honestly significant difference (HSD) test between two means was calculated only if the *F* test was significant at the 0.05 level of probability. Principal component analysis (PCA) was performed to reduce the number of abiotic and leaf trait variables, analyzing the correlation matrix with 25 maximum iterations for convergence without rotation to extract independent PCA factors with eigenvalues > 1. Multiple linear regression analyses were used to characterize the relationships between PCA factors of the abiotic environment with PCA factors of leaf traits. Simple linear regression analyses were used to characterize the relationships between abiotic variables and leaf traits.

RESULTS

Abiotic environment—Four factors were obtained for the abiotic environmental factors in the field from the PCA,

explaining 87.7% of the variance. The first factor (PC1-A) was positively correlated with latitude, day length, mean tidal level, mean daily temperature during the last week before sampling and with coarse and poorer sediments (with higher sand and gravel percentages, and lower clay percentage, nitrogen and organic matter concentrations), and negatively with the number of growing degree days. The second factor (PC2-A) was positively correlated with mean tidal range and level and negatively with mean daily isolation and more anoxic and acidic sediments (lower Eh and pH). The third (PC3-A) was positively correlated with saltier sediments (higher conductivities) and mean daily temperatures, and the fourth (PC4-A) negatively with the silt percentage (Tables 1 and 2).

Leaf traits—Four factors were obtained for the leaf traits in the field from the PCA, explaining 74.9% of the variance. The first factor (PC1-L) was positively correlated with photosynthetic pigments concentrations (Chl *a*, Chl *b*, Chl *a+b* and C_{x+c}) and negatively with LWC (varying between 46.0–66.9%). The second factor (PC2-L) was positively correlated with leaf length and area (varying between 16.2–22.7 cm, and 3.8–6.8 cm², respectively) and negatively with carbon concentration. The third (PC3-L) was positively correlated with SLA (varying between 0.007–0.011 m²·g⁻¹) and negatively with leaf width (varying between 0.4–0.6 cm), and the fourth (PC4-L) was negatively correlated with nitrogen concentration (Tables 3 and 4).

Each foliar trait showed significant interpopulation differences in the field (ANOVA or Kruskal–Wallis *H*-test, $P < 0.0001$; $P < 0.001$ for photosynthetic pigment concentrations, $P < 0.01$ for LWC and $P < 0.05$ for Chl *a*:Chl *b* ratio). Nearly all of these interpopulation differences disappeared in the common garden experiment. However, leaf area of plants from Grays Harbor was lower than for other populations in both field and greenhouse, and SLA was higher for Grays Harbor samples than for Vancouver Island populations in the field and in the greenhouse (ANOVA or Kruskal–Wallis *H*-test, $P < 0.05$; Table 3).

Relationships between environmental factors and leaf traits—PC1 for leaf traits (PC1-L) correlated negatively with PC1 for the abiotic environment (PC1-A) (Table 5). Specifically, Chl *a* concentration correlated only with latitude and related abiotic factors such as day length and the number of growing degree days. Every chlorophyll pigment concentration (Chl *a*, Chl *b*, and C_{x+c}) decreased with increasing latitude in the field, but this difference among populations disappeared in the common garden experiment (Fig. 2). Moreover, Chl *b* and C_{x+c} concentrations were highest in samples at sites with higher organic matter and nitrogen concentration in sediments. On the other hand, LWC was highest from plants growing in more coarsely textured sediment (with more sand and gravel and less clay) and in sediment with lower organic matter and nitrogen concentrations.

PC2-L decreased with PC2-A (Table 5), reflecting that shorter and more rolled leaves with lower ratio of Chl *a+b* to C_{x+c} were associated with more anoxic and acidic sediments (Fig. 3). PC3-L decreased slightly with PC4-A ($P < 0.05$), and PC4-L was correlated positively with PC3-A, reflecting that leaves with lower nitrogen concentration coincided with saltier sediments at higher mean daily air temperatures (Table 5).

TABLE 1. Latitude, sediment organic matter content, carbon and nitrogen concentrations, redox potential, pH, electrical conductivity, texture, mean tidal level and range, growing degree days above 10°C, day length (h) on sampling day, mean daily insolation for the sampling month and mean daily air temperature for five invasive populations of *Spartina densiflora* along the Pacific Coast of North America.

Location, latitude	Sediment					Tides				Atmosphere			
	Organic matter (%)	[Carbon] (mg·g ⁻¹)	[Nitrogen] (mg·g ⁻¹)	Redox potential (mV)	pH	Electrical conductivity (mS·cm ⁻¹)	Texture	Mean tidal level (m)	Mean tidal range (m)	Growing degree days	Day length (h:min)	Mean daily insolation (W·h·m ⁻²)	Mean daily air temperature (°C; mo / wk before sampling)
Vancouver Island 49°33'N	7.3 ± 1.1 ^a	24.7 ± 5.3 ^a	1.8 ± 0.3 ^a	+100 ± 40 ^{ac}	7.2 ± 0.1 ^a	8.8 ± 0.9 ^a	Sandy loam	1.67	1.76	924	15:56	6100	16.2 / 20.3
Grays Harbor 46°57'N	16.6 ± 2.0 ^b	47.0 ± 6.2 ^b	3.9 ± 0.5 ^b	-203 ± 67 ^b	6.3 ± 0.1 ^b	6.9 ± 0.4 ^a	Clay	1.54	2.20	1520	15:52	4964	11.7 / 12.3
Mad River 40°56'N	4.0 ± 0.3 ^c	6.5 ± 0.7 ^c	0.9 ± 0.1 ^c	+131 ± 57 ^{ac}	7.0 ± 0.1 ^a	0.2 ± 0.0 ^b	Loamy sand	1.11	1.47	1600	14:48	5753	12.8 / 13.0
Humboldt Bay 40°49'N	34.1 ± 1.0 ^d	131.1 ± 4.0 ^d	10.8 ± 0.3 ^d	-41 ± 23 ^c	6.4 ± 0.2 ^b	11.9 ± 0.2 ^{ac}	Clay	1.26	1.69	1600	14:48	5753	12.8 / 13.0
San Francisco Bay 37°56'N	13.5 ± 1.5 ^{ab}	35.6 ± 4.5 ^{ab}	3.3 ± 0.3 ^{ab}	+191 ± 21 ^a	6.9 ± 0.1 ^a	13.8 ± 0.7 ^c	Clay	0.96	1.26	3029	14:47	6322	15.0 / 14.1
<i>H</i> -test	$\chi^2 = 40.73$ df = 4 $P < 0.0001$	$\chi^2 = 39.00$ df = 4 $P < 0.0001$	$\chi^2 = 41.21$ df = 4 $P < 0.0001$	$\chi^2 = 22.90$ df = 4 $P < 0.0001$	$\chi^2 = 29.48$ df = 4 $P < 0.0001$	$\chi^2 = 39.53$ df = 4 $P < 0.0001$							

Notes: Data are means ± SEM ($n = 10$). Different letters indicate significant differences between locations for the same abiotic variable (Kruskal–Wallis *H*-test and Mann–Whitney *U*-test, $P < 0.05$).

TABLE 2. Factor loadings (PC-A) of the individual variables obtained by a principal component analysis (PCA) on abiotic environmental variable for five populations of *Spartina densiflora* along the Pacific Coast of North America from San Francisco Bay to Vancouver Island.

	Abiotic variable	PC1-A	PC2-A	PC3-A	PC4-A
Sediment	Latitude	+0.832	+0.465	+0.240	-0.048
	Organic matter content	-0.646	+0.556	+0.288	+0.408
	Carbon concentration	-0.598	+0.541	+0.342	+0.453
	Nitrogen concentration	-0.647	+0.509	+0.312	+0.444
	Redox potential	+0.044	-0.769	+0.140	+0.133
	pH	+0.437	-0.648	+0.160	-0.109
	Conductivity	-0.470	+0.053	+0.739	+0.053
	Sand	+0.650	-0.272	-0.418	+0.516
	Silt	-0.490	+0.110	+0.233	-0.707
	Clay	-0.613	+0.320	+0.450	-0.277
Tides	Gravel	+0.662	-0.316	+0.107	+0.335
	Mean tidal level	+0.718	+0.631	+0.282	-0.030
	Mean tidal range	+0.372	+0.895	-0.120	-0.093
Atmosphere	Growing days	-0.685	-0.521	-0.064	-0.173
	Day length (sampling day)	+0.752	+0.499	+0.184	-0.247
	Mean daily insolation (sampling month)	-0.086	-0.802	+0.558	+0.119
	Mean daily temperature (last month) nth)	+0.355	-0.570	+0.722	-0.015
	Mean daily temperature (last week)	+0.684	-0.221	+0.683	+0.017
	<i>Eigen values</i>	6.782	5.307	2.900	1.673
	<i>Explained variance</i>	35.7	27.9	15.3	8.8

Note: Correlations between the PCA and leaf traits with factor loadings $> \pm 0.600$ are in boldface.

Changes in leaf traits after transplantation and growth in a common garden environment depended on differences in the abiotic environment between the field and the greenhouse (Fig. 4). When Eh of sediment was higher in the common garden than in the field environment of the sampled population, the decrease in leaf rolling and the increase in the ratio of Chl *a+b* to C_{x+c} were more pronounced (Fig. 4).

DISCUSSION

Spartina densiflora is an invasive cordgrass from South America that has invaded habitats with a wide range of environmental conditions along the Pacific Coast of North America. Our results suggest that the plants from these invasive populations are able to adjust key functional leaf traits in response to widely variable climate and other environmental conditions. The plants were especially plastic in their foliar responses to variation in the sedimentary environment (specifically, hypoxia, salinity, and texture) across this latitudinal gradient, and foliar responses were also related to variance in abiotic factors that directly changed relative to latitude such as solar radiation and air temperature.

Pigment concentrations were higher in plants from low latitude sites compared with higher latitude sites. Furthermore, pigment concentrations from plants in natural field populations were lower at higher latitude sites than concentrations measured in plants from the common garden experiment that experienced similar photoperiod and radiation intensities. However, plants growing in lower latitude field sites (San Francisco Bay, Humboldt Bay, and Mad River) showed similar pigment concentrations in the field and in the greenhouse. These results point to an abiotic limitation of photosynthetic pigments concentrations at higher latitudes, which seemed to be related to changes in the quality of solar radiation with latitude especially for Chl *a*; and also with sediment nutrient concentrations in the case of Chl *b* and C_{x+c} . Sunlight is recognized as the primary determinant of physiological variation in leaves, and Chl formation is mainly

controlled by light. The activation of Chl *a* synthesis by red light, mediated by phytochrome, has been widely found (Beale, 1999). Chl concentrations of *S. densiflora* may decrease with increasing latitude due to the longer twilight periods at the beginning and end of the day at high northern latitudes during the summer. During twilight, light signatures are enriched in far-red wavelengths, which may lead to decreased chlorophyll synthesis (Kasperbauer and Peaslee, 1973). Previous studies have determined that a reduced red to far-red light ratio during twilight hours can control plant growth and the timing of flowering (Linkosalo and Lechowicz, 2006; Lund et al., 2007). This effect could be more significant for species coming from low and middle latitudes such as *S. densiflora*, since responses to red light can change with latitude (Mølmann et al., 2006). Recently, Duarte et al. (2013) recorded a decrease in Chl *a* and Chl *b* concentrations with increasing latitude for *Spartina maritima* during summer in Portuguese salt marshes along a narrow mid-latitude range (ca. 2°). To our knowledge, our results are the first to record a change in the concentration of photosynthetic pigments for a terrestrial plant species along a wide latitudinal cline ($>11^\circ$).

Functional responses of *Spartina densiflora* plants growing in stressful field conditions included greater leaf rolling and less leaf elongation. The water content of leaves was lowest in plants that were grown in clay sediments rich in organic matter, which may be related to lower water availability due to water retention by clay and humus in salt marshes. These clay sediments also tended to be hypoxic (negative Eh), especially when coinciding with high mean tidal level and range. Shorter leaves associated with anoxic sediments had a higher carbon concentration and lower ratio of Chl *a+b* to C_{x+c} than leaves associated with more aerobic sediments. The anoxia-induced stress to the photosynthetic apparatus of *S. densiflora*, in combination with other stressful environmental factors associated with waterlogging, limits its distribution along the tidal inundation gradient (Castillo et al., 2000; Idaszkin et al., 2014). Waterlogging in saline conditions increases Na^+ concentration in roots as a result of low energy status and membrane deterioration, impairing ion

TABLE 3. Foliar carbon and nitrogen concentration, width, length, area, adaxial rolling, growth, specific leaf area (SLA), leaf water content (LWC), chlorophyll *a*, *b* (Chl *a* and Chl *b*, respectively) and carotenoids (C_{x+c}) concentration, and Chl *a* to Chl *b* ratio and Chl to C_{x+c} ratio for five *Spartina densiflora* invasive populations from five locations along the Pacific Coast of North America growing in the field and in a common garden experiment (greenhouse).

Population	Experiment	[Carbon] (mg·g ⁻¹)	[Nitrogen] (mg·g ⁻¹)	Width (cm)	Length (cm)	Area (cm ²)	Rolling (%)	Growth (mm·day ⁻¹)	SLA (m ² ·g ⁻¹)	LWC (%)	Chl <i>a</i> (mg·g ⁻¹ DW)	Chl <i>b</i> (mg·g ⁻¹ DW)	C_{x+c} (mg·g ⁻¹ DW)	Chl <i>a</i> + <i>b</i> (mg·g ⁻¹ DW)	Chl <i>a</i> : Chl <i>b</i>	Chl : C_{x+c}
Vancouver Is.	Field	396.8 ± 4.9 ^a	18.3 ± 0.9 ^a	0.6 ± 0.0 ^b	17.9 ± 0.5 ^a	5.2 ± 0.2 ^a	34 ± 5 ^a	8 ± 1 ^a	0.008 ± 0.000 ^a	66.9 ± 1.9 ^a	0.10 ± 0.02 ^a	0.10 ± 0.02 ^a	0.20 ± 0.02 ^a	0.60 ± 0.05 ^a	5.73 ± 0.97 ^a	3.00 ± 0.13 ^{ab}
	Greenhouse	—	—	0.4 ± 0.0 ^a	59.1 ± 6.1 ^a	12.6 ± 1.6 ^a	26 ± 5 ^a	13 ± 1 ^a	0.009 ± 0.000 ^a	72.4 ± 1.8 ^a	0.98 ± 0.13 ^a	0.29 ± 0.05 ^a	0.33 ± 0.05 ^a	1.27 ± 0.18 ^a	3.46 ± 0.21 ^a	3.91 ± 0.21 ^a
Grays Harbor	Field	433.0 ± 2.7 ^b	15.8 ± 0.8 ^b	0.5 ± 0.0 ^a	16.2 ± 1.1 ^a	4.2 ± 0.4 ^{ab}	21 ± 5 ^b	—	0.013 ± 0.001 ^b	64.3 ± 1.6 ^a	0.58 ± 0.03 ^a	0.17 ± 0.01 ^{ab}	0.30 ± 0.02 ^{ab}	0.76 ± 0.04 ^{ab}	3.39 ± 0.20 ^b	2.57 ± 0.14 ^b
	Greenhouse	—	—	0.4 ± 0.0 ^a	43.7 ± 3.9 ^a	8.1 ± 0.7 ^b	72 ± 6 ^b	11 ± 3 ^a	0.012 ± 0.001 ^b	73.2 ± 2.4 ^a	1.07 ± 0.21 ^a	0.41 ± 0.10 ^a	0.39 ± 0.08 ^a	1.47 ± 0.30 ^a	2.86 ± 0.26 ^a	3.83 ± 0.20 ^a
Mad River	Field	422.9 ± 2.0 ^{bc}	22.7 ± 0.8 ^c	0.6 ± 0.0 ^b	20.6 ± 0.8 ^b	6.8 ± 0.4 ^c	20 ± 2 ^a	2 ± 0 ^b	0.010 ± 0.001 ^b	62.7 ± 2.8 ^a	0.81 ± 0.07 ^{ab}	0.16 ± 0.02 ^{ab}	0.31 ± 0.03 ^{ab}	0.97 ± 0.08 ^{bc}	5.22 ± 0.47 ^{ab}	3.24 ± 0.22 ^{bc}
	Greenhouse	—	—	0.4 ± 0.0 ^a	54.6 ± 3.4 ^a	11.2 ± 1.0 ^{ab}	18 ± 3 ^a	14 ± 1 ^a	0.010 ± 0.000 ^{ab}	71.2 ± 1.8 ^a	1.31 ± 0.06 ^a	0.46 ± 0.04 ^a	0.51 ± 0.02 ^a	1.76 ± 0.07 ^a	2.93 ± 0.22 ^a	3.43 ± 0.08 ^a
Humboldt Bay	Field	419.5 ± 3.3 ^c	17.8 ± 1.2 ^{ab}	0.4 ± 0.0 ^a	16.8 ± 0.7 ^b	3.8 ± 0.3 ^b	83 ± 3 ^b	2 ± 0 ^b	0.007 ± 0.000 ^{ab}	46.0 ± 4.0 ^b	0.93 ± 0.14 ^b	0.24 ± 0.04 ^b	0.44 ± 0.06 ^b	1.17 ± 0.18 ^{bc}	3.87 ± 0.28 ^{bc}	2.68 ± 0.12 ^{ab}
	Greenhouse	—	—	0.4 ± 0.0 ^a	48.5 ± 1.2 ^a	9.0 ± 0.7 ^{ab}	17 ± 4 ^a	16 ± 1 ^a	0.010 ± 0.000 ^{ab}	70.8 ± 2.3 ^a	1.12 ± 0.29 ^a	0.38 ± 0.11 ^a	0.39 ± 0.10 ^a	1.50 ± 0.40 ^a	3.42 ± 0.43 ^a	3.61 ± 0.23 ^{ab}
SF Bay	Field	434.4 ± 1.0 ^b	13.6 ± 0.3 ^b	0.5 ± 0.0 ^a	22.7 ± 0.7 ^b	5.9 ± 0.3 ^{ac}	21 ± 4 ^a	6 ± 1 ^a	0.011 ± 0.001 ^b	62.6 ± 0.7 ^a	1.02 ± 0.08 ^b	0.20 ± 0.02 ^b	0.32 ± 0.02 ^{ab}	1.21 ± 0.10 ^a	5.22 ± 0.38 ^{ab}	3.79 ± 0.08 ^b
	Greenhouse	—	—	0.4 ± 0.0 ^a	55.2 ± 3.1 ^a	11.6 ± 1.0 ^{ab}	21 ± 6 ^a	13 ± 2 ^a	0.011 ± 0.000 ^{ab}	76.6 ± 5.6 ^a	0.95 ± 0.21 ^a	0.26 ± 0.08 ^b	0.33 ± 0.06 ^a	1.21 ± 0.28 ^a	3.98 ± 0.32 ^a	3.55 ± 0.22 ^a
ANOVA or Kruskal-Wallis	Field	$F_{4,45} = 24.26$ $P < 0.0001$	$F_{4,45} = 15.22$ $P < 0.0001$	$F_{4,45} = 12.36$ $P < 0.0001$	$F_{4,45} = 8.74$ $P < 0.0001$	$F_{4,45} = 7.84$ $P < 0.0001$	$\chi^2 = 28.98$ $df = 4$ $P < 0.0001$	$\chi^2 = 24.88$ $df = 3$ $P < 0.0001$	$\chi^2 = 22.18$ $df = 4$ $P < 0.0001$	$\chi^2 = 13.92$ $df = 4$ $P < 0.01$	$F_{4,20} = 6.93$ $P < 0.001$	$F_{4,20} = 5.37$ $P < 0.005$	$F_{4,20} = 5.37$ $P < 0.005$	$F_{4,20} = 6.55$ $P < 0.005$	$F_{4,20} = 3.48$ $P < 0.005$	$F_{4,20} = 4.20$ $P < 0.0001$
H test	Greenhouse	—	—	$F_{4,45} = 1.33$ $P > 0.05$	$F_{4,45} = 2.22$ $P > 0.05$	$F_{4,45} = 2.84$ $P > 0.05$	$F_{4,45} = 0.46$ $P > 0.05$	$F_{4,45} = 1.09$ $P > 0.05$	$F_{4,45} = 3.65$ $P > 0.05$	$F_{4,20} = 0.54$ $df = 4$ $P > 0.05$	$\chi^2 = 2.40$ $df = 4$ $P > 0.05$	$\chi^2 = 4.79$ $df = 4$ $P > 0.05$	$\chi^2 = 6.46$ $df = 4$ $P > 0.05$	$\chi^2 = 2.71$ $df = 4$ $P > 0.05$	$F_{4,20} = 2.31$ $P > 0.05$	$F_{4,20} = 1.04$ $P > 0.05$

Notes: Data are mean ± SEM ($N = 10$). Different letters indicate significant differences between populations in the field or in the greenhouse (ANOVA and Tukey-HSD test or Kruskal-Wallis H-test and Mann-Whitney test, $P < 0.05$).

TABLE 4. Factor loadings (PC-L) of the individual variables obtained by a principal component analysis (PCA) on *Spartina densiflora* leaf traits from five populations along the West Coast of North America from San Francisco Bay to Vancouver Island.

Factor	PC1-L	PC2-L	PC3-L	PC4-L
Carbon concentration	+0.034	-0.629	-0.571	+0.146
Nitrogen concentration	+0.087	+0.495	+0.090	-0.611
Width	-0.193	+0.454	-0.706	+0.110
Length	-0.150	+0.845	-0.124	-0.222
Area	-0.246	+0.796	-0.431	-0.176
Rolling	+0.433	-0.599	+0.124	-0.145
Linear growth	-0.500	-0.066	+0.162	+0.455
Water content	-0.815	+0.100	+0.263	-0.053
Chlorophyll <i>a</i> concentration	+0.789	+0.512	+0.128	+0.270
Chlorophyll <i>b</i> concentration	+0.936	+0.124	+0.020	+0.019
Chlorophyll <i>a</i> + <i>b</i> concentration	+0.849	+0.449	+0.110	+0.227
Carotenoids concentration	+0.952	+0.058	-0.120	+0.052
Chlorophyll : Carotenoids ratio	-0.145	+0.716	+0.372	+0.315
Chlorophyll <i>a</i> : Chlorophyll <i>b</i> ratio	-0.441	+0.352	+0.036	+0.447
Specific leaf area	-0.054	+0.049	+0.683	-0.170
Eigen values	4.575	3.689	1.795	1.173
Explained variance	30.5	24.6	12.0	7.8

Note: Correlations between the PCA and leaf traits with factor loadings ≥ 0.600 are marked in bold.

transport processes in plants (Colmer and Flowers, 2008). Thus, an osmotic alteration in *S. densiflora* tissues under flooding could raise water stress levels, provoking leaf rolling (Kadioglu and Terzi, 2007). As in our study, hypoxia reduces leaf length for cattail (*Typha domingensis* Pers.) and growth rate for several macrophytes, including *S. densiflora* (Castillo et al., 2000; Li et al., 2010; Idaszkin et al., 2014). Leaf rolling and shorter leaves would decrease the potential for photoinhibition by exposing less foliar area to radiation, reduce transpiration by increasing the stability of the leaf boundary layer, and decrease leaf temperature (Heckathorn and DeLucas, 1991). On the other hand, water deficit increases carbon concentration in plant tissues (Muller et al., 2011) and decreases the ratio of Chl *a*+*b* to C_{x+c} as a photoprotection mechanism (Liu et al., 2010).

High salinities decrease nitrogen uptake in different halophytes, such as *Spartina alterniflora* Loisel. (Bradley and Morris, 1991). In this study, higher salinities coincided with higher air temperatures, which are usually associated with higher evapotranspiration rates and, consequently, with salt concentration in

TABLE 5. Correlation matrix (Pearson correlation coefficient *r* and probability level *P*) between factors loadings coming from the principal component (PC) analyses for abiotic environmental variables (PC-A) and for leaf traits (PC-L) for five *Spartina densiflora* invasive populations along the Pacific Coast of North America ($N = 50$).

PC	Statistic	PC1-A	PC2-A	PC3-A	PC4-A
PC1-L	<i>r</i> =	-0.606	+0.244	-0.05	+0.055
	<i>P</i> <	0.0001	0.087	0.975	0.702
PC2-L	<i>r</i> =	-0.164	-0.716	-0.232	-0.092
	<i>P</i> <	0.255	0.0001	0.105	0.523
PC3-L	<i>r</i> =	-0.193	+0.080	-0.114	-0.345
	<i>P</i> <	0.179	0.501	0.431	0.014
PC4-L	<i>r</i> =	+0.232	-0.171	+0.658	-0.006
	<i>P</i> <	0.105	0.235	0.0001	0.966

Note: Correlations with $P < 0.05$ are in boldface.

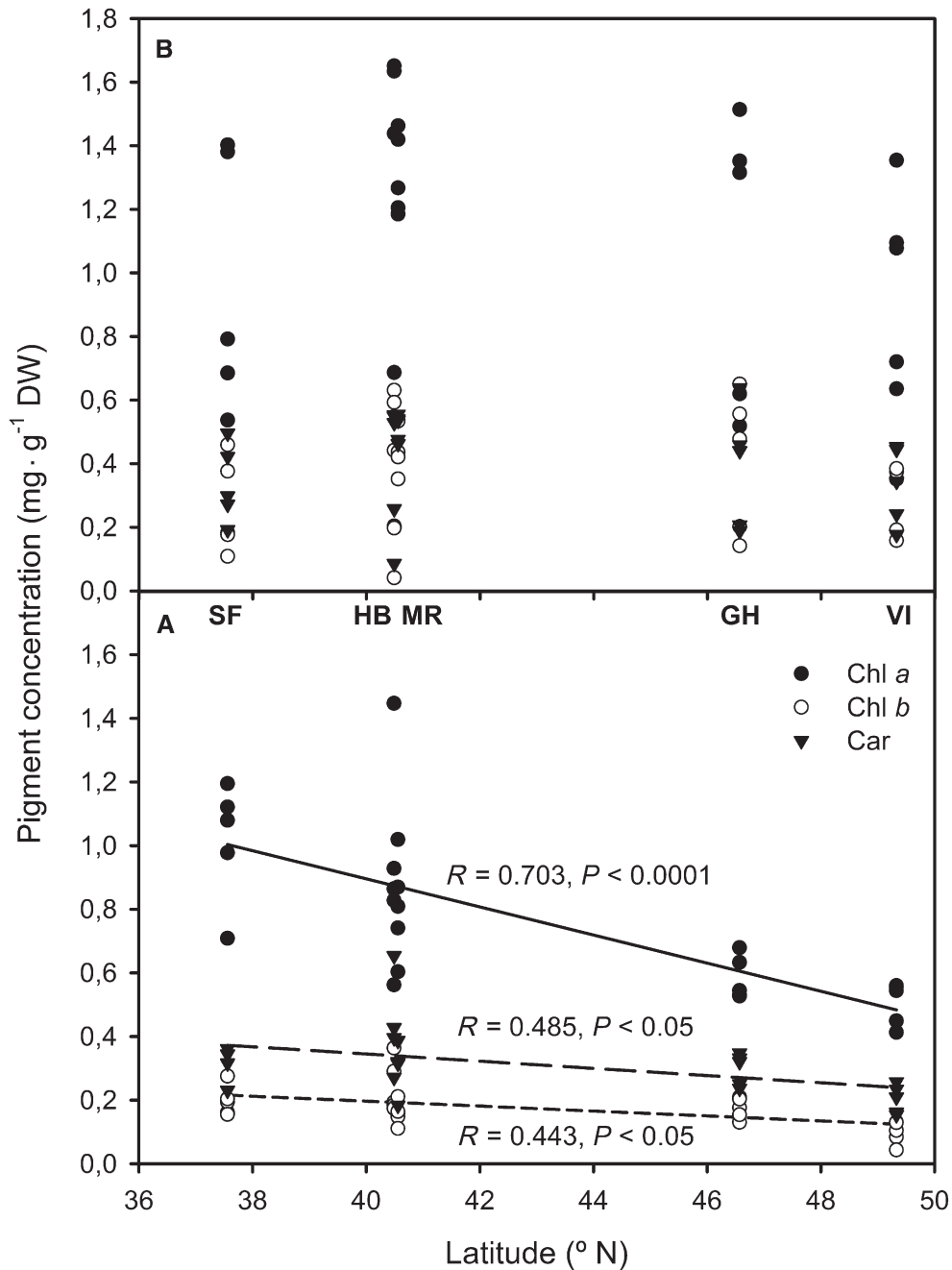


Fig. 2. Relationship between the concentration of chlorophyll *a* and *b* and carotenoids (C_{x+c}) and latitude for tussocks from five invasive populations of *Spartina densiflora* along the Pacific Coast of North America growing (A) in the field and (B) after acclimation to common garden conditions. Populations: SF, San Francisco Bay Estuary; HB, Humboldt Bay Estuary; MR, Mad River Estuary; GH, Grays Harbor Estuary; VI, Baynes Sound, Vancouver Island.

sediment. Invasive *S. densiflora* in the Iberian Peninsula is able to grow in a wide salinity range; however, its stress levels increase markedly with salinity (Nieva et al., 1999, 2003; Castillo et al., 2005b). We found that sediment salinity (recorded as electrical conductivity) varied considerably among *S. densiflora* invaded marshes, and populations from high salinity sites also responded with low concentrations of foliar nitrogen.

Most of the recorded foliar traits we measured at field sites were considerably less robust than would be expected for this species under benign, favorable growing conditions. The field populations that exhibited reduced growth in response to stressful environmental conditions in the salt marshes tended to show the greatest response to the favorable growing conditions we imposed in the common garden experiment in a glasshouse. Thus, as suggested by their foliar responses,

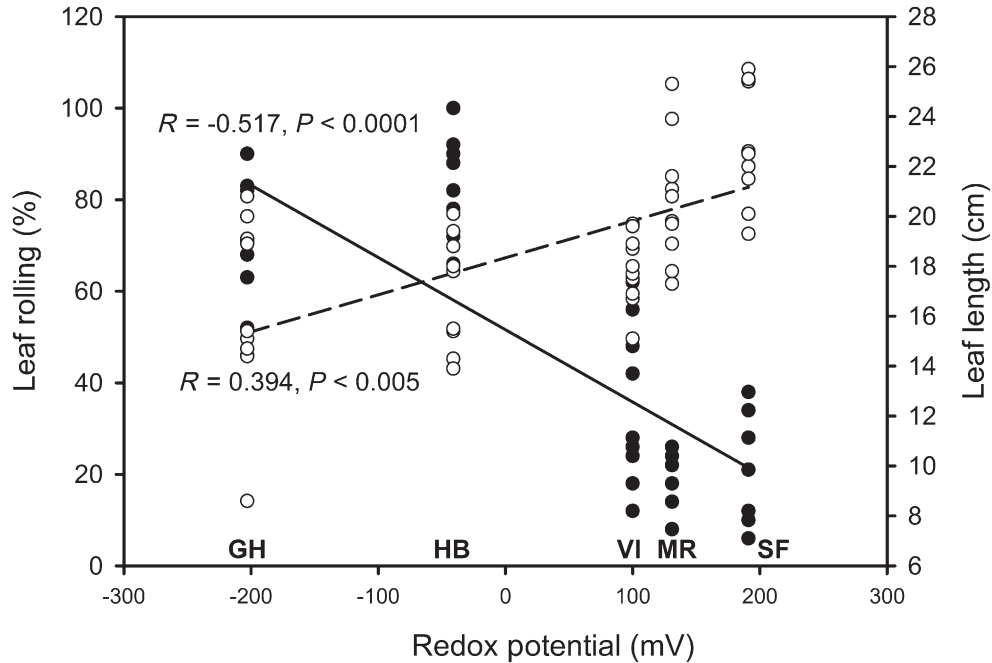


Fig. 3. Relationship between leaf adaxial rolling (%) (full circles) and leaf length (cm; empty circles) with sediment redox potential (mV) for tussocks from five invasive populations of *Spartina densiflora* along the Pacific Coast of North America. Populations: SF, San Francisco Bay Estuary; HB, Humboldt Bay Estuary; MR, Mad River Estuary; GH, Grays Harbor Estuary; VI, Vancouver Island (Baynes Sound).

invasive populations of *S. densiflora* can survive and grow in suboptimal conditions, especially at higher latitudes and in more anoxic and saline marshes. Similarly, Núñez-Olivera et al. (1996) reported that *Cistus ladanifer* L. plants from both latitudinal extremes of its distribution had suboptimum leaf characteristics as compared with plants from intermediate localities. Sultan (2001) studied *Polygonum* sp. with different niche breadths and found successful invaders had better fitness in favorable environments due to phenotypic plasticity while persisting under less than optimal conditions. Along the Atlantic Coast of South America, the highest abundance of *S. densiflora* is observed in the temperate marshes of southern Brazil and south to Buenos Aires Province, Argentina (30–38°S latitude). Suboptimal climatic conditions such as those found in southern Argentina seem to limit *S. densiflora* salt marshes to isolated inlets and coastal islands (Bortolus, 2006), where its C_4 metabolism is constrained (Álvarez et al., 2009). However, new reports suggest that the invasive spread of *S. densiflora* in the Pacific northwest region of North America has accelerated during the last decade (e.g., Pickart, 2001, Smith et al., 2001), and the recent, increased spread farther north into British Columbia may be related to its high phenotypic plasticity that allows it to colonize very extreme environments, despite living in suboptimal conditions. Although environmental conditions appear largely to determine leaf traits in *S. densiflora*, a genetic component influencing these traits due to local adaptation cannot be excluded, since significant interpopulation differences in leaf area and SLA after more than 2 yr in a common environment were still evident. In this sense, Stephenson et al. (2006) described ecotypes of *Ipomea lacunose* L. based on leaf size and form. Furthermore, our common garden experiment may not necessarily have provided conditions for different genotypes to express distinct leaf-traits phenotypes (Thompson, 1991), but our results do

suggest a relatively low level of genetic variation for studied leaf traits in invasive *S. densiflora* populations along the Pacific Coast of North America, in agreement with previous studies that recorded high genetic similarity between *S. densiflora* populations (Ayres et al., 2008).

Our results point to phenotypic plasticity as the main mechanism driving foliar changes of *Spartina densiflora* across a large-scale climate gradient in Pacific coast wetlands. The considerable phenotypic plasticity of *S. densiflora* agrees with that found in *S. anglica* (Thompson et al., 1991), *S. foliosa* (Trnka and Zedler, 2000), and *S. alterniflora* (Elsey-Quirk et al., 2011) and may be related to its ancestral hybrid origin (Fortune et al., 2008). Given the wide latitudinal range of the species in its native South American range, the genotype of invasive *S. densiflora* may have been pre-adapted to environmental fluctuations prior to its unintentional introduction to North America. The five populations of *S. densiflora* we studied ranged widely in their morphological and physiological foliar traits in their naturalized habitats. There was a striking convergence between populations in almost every foliar trait after transplantation to a common environment. In contrast, Qing et al. (2011) recorded differences in plant traits in a common garden experiment comparing native North American populations and invasive Chinese populations of *S. alterniflora*, which may be related with genetic shifts playing a vital role in the invasion success. We conclude that *Spartina densiflora* can readily adjust important functional traits in response to variable environmental conditions encountered in the naturalized range along the Pacific coast of North America. Environmental determination of these functional leaf traits is further supported by the relatively close association of leaf traits with abiotic conditions, both in the natural populations and in their responses to transplantation.

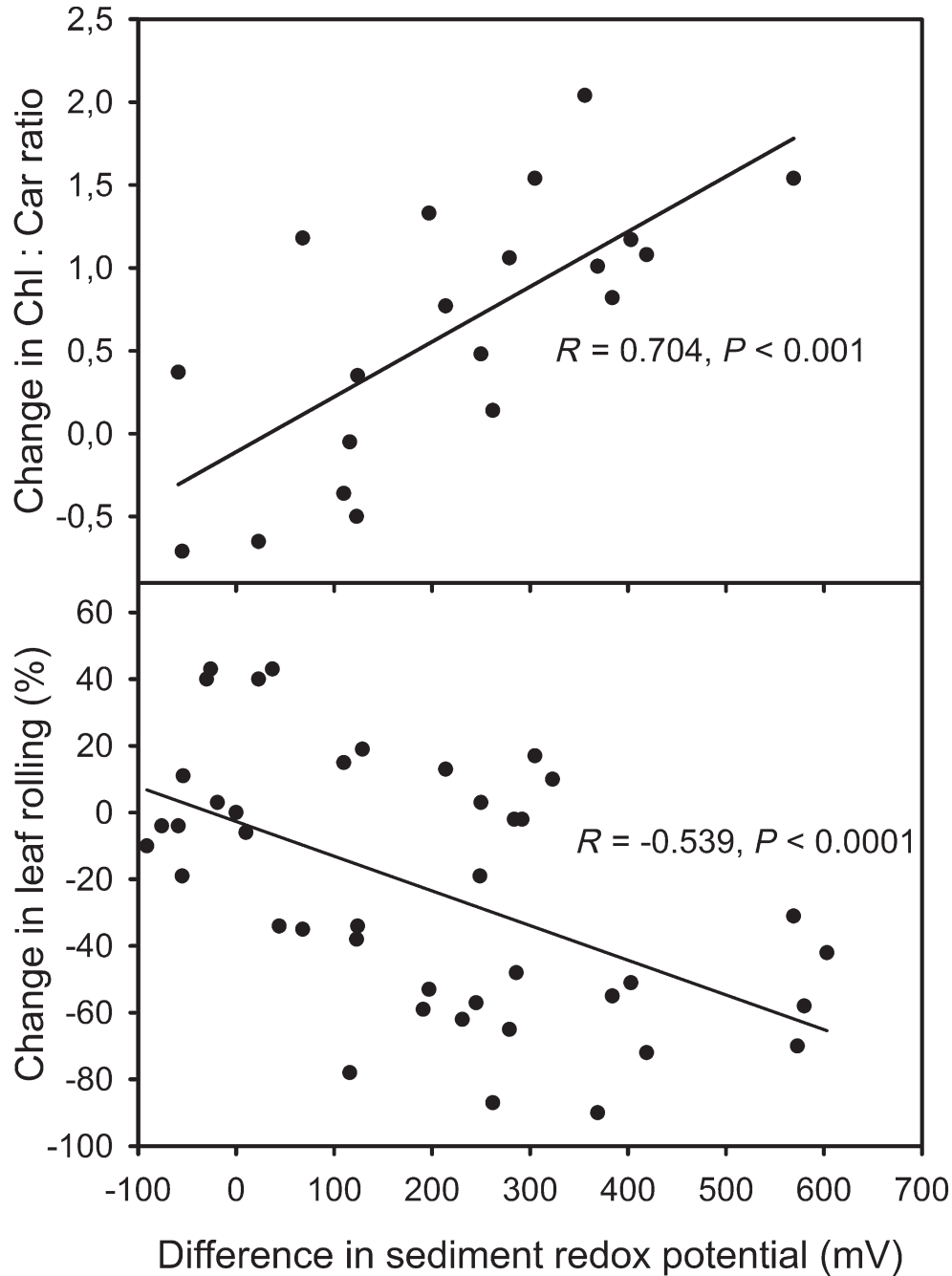


Fig. 4. Relationship between change in leaf length (cm), rolling (%) and the ratio of chlorophylls to carotenoids (C_{x+c}) after growing in a common garden environment during 27 mo and the difference between natural site and common garden site in redox potential (mV) for tussocks from five invasive populations of *Spartina densiflora* along the Pacific Coast of North America.

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