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CHANGES IN LEAF MORPHOLOGY, PHOTOSYNTHESIS AND NITROGEN CONTENT IN TWO COASTAL SHRUBS

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Abstract

CHANGES IN LEAF MORPHOLOGY, PHOTOSYNTHESIS AND NITROGEN CONTENT IN TWO COASTAL SHRUBS

By, Elizabeth J. F. Kost B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Major Director: Director: Donald R. Young,
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It is important to understand mechanisms that facilitate expansion of two common shrubs, *Morella cerifera* and *Baccharis halimifolia* in coastal environments. The purpose of my study was to investigate the physiological and structural changes that occur as leaves age. Photosynthesis, incident light, chlorophyll, and leaf C:N ratios were quantified for young, intermediate, and old leaves (distal, central and proximal leaves, respectively). Leaf structural differences were also compared. Leaves did not change morphologically with age. Light decreased with leaf age and during winter months. Photosynthesis showed no seasonal or age related patterns. Chlorophyll increased initially and then declined with age due to self shading. Nitrogen content was highest during spring. Seasonality and leaf age had unique effects on the two study species. Understanding senescence adaptations of these two shrubs can help explain their abundance in coastal ecosystems.

Introduction

Morella cerifera is a native shrub common in the interior of barrier island environments. It is the primary woody species on most barrier islands in Virginia as well as most of the southeastern USA, despite sensitivity to salinity (Young et al., 2007). It is also a symbiotic nitrogen fixer, which facilitates survival in the nitrogen poor soils found on barrier islands. *Morella cerifera* has a dense growth habit in which new leaves quickly self-shade the older ones. Leaves have a lifespan of about 5 to 13 months (Brantley and Young, 2007); as new leaves grow near the distal end of the stem the oldest proximal leaves are shed. This may contribute to decreased photosynthesis of older leaves. *Morella cerifera* has an evergreen leaf habit which helps conserve nitrogen (Monk, 1966), an important adaptation for survival in coastal environments where soil nitrogen is limiting. Photosynthesis decreases with age of *M. cerifera* leaves as it does in many plants (Reich et al., 1991, Kitajima et al., 2002, Vos and Oyarzun, 1986). Perhaps there is a threshold at which cost of maintaining a leaf as it is shaded by newer leaves outweighs usefulness to the plant and it is shed. Decreased nitrogen content as *M. cerifera* leaves age may also contribute to lower photosynthesis. In the low nitrogen environment typical of coastal soils, as the photosynthetic rate decreases the plant may re-allocate nitrogen to more productive leaves; therefore, limiting loss of nitrogen when a leaf is finally shed.

Another shrub common on barrier islands and tidal marshes along the Atlantic and Gulf coasts of Northern America is *Baccharis halimifolia* (Asteraceae) (Krischi, and Denno, 1990). *Baccharis halimifolia* is an ecological generalist adapted to pioneer stages in succession (Westman et al., 1975). *Morella cerifera* and *B. halimifolia* are both

tolerant of a wide range of soil nutrient levels and, have similar growth forms and leaf sizes and shapes. But unlike *M. cerifera, B. halimifolia* is deciduous and does not fix nitrogen. *Baccharis halimifolia* often co-occurs with *M. cerifera* in coastal environments; therefore, it is a model species for comparison to the evergreen, *M. cerifera*.

If these shrubs are conserving nitrogen before leaf senescence, this would impact the total nitrogen budget for the shrub. Further differences in nitrogen concentrations on shed leaves would have consequences for the microbial communities found in the leaf litter and soil beneath the shrubs. Though *M. cerifera* is already a substantial contributor to the soil nitrogen pool by way of leaf litter, from 53- 127 kg ha^{-1} yr^{-1} nitrogen content 70% of which is from fixed nitrogen (Brantley et al., 2007), the constant input of soil nitrogen from *M. cerifera* differs from deciduous systems because evergreen inputs are continuous, as opposed to a single pulse from deciduous species in the autumn (Brantley et al., 2007).

Quantifying changes in photosynthesis, photosynthetic pigments and leaf nitrogen content as leaves age was the primary focus of my research. My objective was to quantify changes for *Morella cerifera* and *B. halimifolia* throughout the growing season. I hypothesized there would be no changes in leaf thickness, trichome density, stomatal density; although as the leaves expanded I expected to see increased area and length. I also examined whether there was a photosynthetic threshold at which *M. cerifera and B. halimifolia* would shed leaves. I identified the threshold by quantifying both photosynthesis directly and by quantifying photosynthetic pigments as estimates of photosynthetic potential. I hypothesized that photosynthesis decreases as leaves age. As leaves continue to develop, older leaves reduce photosynthesis and eventually are shed. I

predicted that the levels of chlorophyll a and b would change as the leaves age. Chlorophyll a should be higher in fully developed young leaves and then decrease while chlorophyll b and carotenoids should increase as leaves age and become significantly more shaded. Chlorophyll b and accessory pigments, similar to carotenoids, allow plants to take advantage of a wider wavelength range in the light spectrum and, therefore, are more advantageous in shaded leaves. I also examined whether *M. cerifera* and *B. halimifolia* reallocate nitrogen content before senescence. I hypothesized nitrogen will remain constant and then decrease just before leaves are shed in *B. halimifolia*, and that this decrease would be less drastic in *M. cerifera* due to symbiotic nitrogen fixation, thereby implying that nitrogen is being reallocated.

Methods

A combination of both field and laboratory measurements over the course of the year were used to gain a perspective of temporal change of photosynthesis, photosynthetic pigments, morphology and nitrogen levels in response to senescence. Field data were collected from the northern end of Hog Island, which is part of the Virginia Coast Reserve Long Term Ecological Research Site (LTER). Most of the island is densely covered in *Morella cerifera* (a nitrogen fixing evergreen) shrub thickets. *Baccharis halimifolia* (deciduous) grows adjacent to *M. cerifera* at these sites on Hog Island.

Morella cerifera (Myricaceae) also known as wax myrtle or southern bayberry is an evergreen species found in the southeastern United States (Radford, 1968), especially in coastal ecosystems (Duncan, 1987). On Hog Island *M. cerifera* is 5.3 ± 0.6 m tall,

with a crown diameter of 4.2 ± 0.5 m (Young, 1994). Seeds are bird dispersed and the shrub forms a symbiotic association with the nitrogen fixing bacterium *Frankia*. It is the most abundant woody species on Hog Island.

Baccharis halimifolia (Asteraceae) is a deciduous shrub, approximately 3.1 ± 0.3 m tall, with a crown diameter of 2.9 ± 0.2 m on Hog Island (Young et al., 1994). It is also found primarily in the southeastern United States (Radford et al., 1968), especially in coastal ecosystems (Duncan and Duncan, 1987). Seeds are wind dispersed and shrubs do not form a symbiosis for nitrogen fixation. It is most common at the edge of *M. cerifera* thickets.

Field Measurements:

A field site was chosen on the ocean side of the island on the leading edge of the *M. cerifera* shrub thicket. The most distal fully expanded leaf, the most central leaf and the most proximal leaf on selected branches of both *M. cerifera* and *B. halimifolia* shrubs were measured. This simplified measurements relative to leaf age and kept constancy due to variation in number of leaves per branch (Young and Yavitt, 1987). Measurement dates were ~ 30 days apart over an 18 month period.

Shoot length was measured for the months of July, August and December. The last five leaves of twenty branches were marked during each visit to the field and their leaf drop was measured during July, August, November and December. Photosynthesis was quantified using a LI-COR model 6200 infrared gas analyzer (IRGA) on a representative leaf of each age group throughout the year. Light levels were measured using a quantum light sensor at the apex of the leaf prior to each photosynthesis

measurement. All measurements were taken within two hours of solar noon. Leaves from each age group were collected for further analysis in the laboratory.

Laboratory Measurements:

Collected leaves were analyzed for chlorophyll a and b and carotenoids, which were used as a relative measure of photosynthetic potential. Samples were first ground and then chlorophyll was extracted in acetone and concentrations were determined using a spectrophotometer. Carbon to nitrogen ratios (C:N) were quantified commercially at Colorado Plateau Stable Isotope facility (NAU) using an elemental analyzer isotopic ratio mass spectrometer. Stomatal and trichome densities were quantified by making thin leaf peels using clear nail polish and then doing counts under a compound microscope. Thickness was measured from crosswise cuts of leaves and measured with an ocular micrometer attached to a compound microscope. Leaf area was determined by leaf tracings on a paper of known density. Leaf length was also quantified.

Statistical Analysis:

I used an ANOVA with Tukey HSD post hoc test to evaluate differences in trichome density and stomatal density due to leaf age. I used MANOVA and Wilks' Lambda with contrast analysis to evaluate leaf area, length and, thickness. Three way ANOVAs with contrast analysis were used to evaluate differences in months, leaf age and species for photosynthesis, light, leaf pigments, nitrogen. I used two way ANVOA with contrast analysis to find statistical differences in branch length and leaf drop for each species throughout the growing season. An ANOVA with Tukey HSD post hoc test

was used to look at differences in tissue chlorides among the leaf age groups of the two species. For all statistical analyses I used a significance level of 0.05. Tukey HSD was used because it is midrange between the very conservative Scheffe's test and the less conservative Fisher LSD. Statistical analyses follow the recommendations of Zar (2009).

Results:

Comparisons of Leaf Morphology:

Statistical differences were found in leaf area and leaf length with age for both *M. cerifera* and *B. halimifolia* due to the expansion of maturing leaves (Table 1). There were no significant differences in leaf thickness, stomatal density, and trichome density between age groups for either of the two species, two exceptions being stomatal density on lower leaf surfaces of *M. cerifera* and an increase in leaf thickness of *B. halimifolia* with age (Table 1). There were several differences between species (Table 1). For instance *B. halimifolia* leaves were generally larger than *M. cerfiera* leaves. Also, *M. cerifera* had stomata on the bottom of leaves while *B. halimifolia* stomata occurred on both leaf surfaces (Table 1). *Baccharis halimifolia* also had a much higher density of trichomes on upper leaf surface compared to *M. cerifera* (Table 1). This may reduce water loss caused by stomata also on the top surface (Table 1).

A MANOVA determined that the effects of leaf age were different between species (Wilks' Lambda p = 0.001). Young leaves were thinner than old leaves and there was no statistical difference between middle leaves and young and old leaves. Though when MANOVAs were done separately for each species we saw no significant difference

in leaf thickness based on leaf age for *M. cerifera* ($p = 0.339$) Leaves become larger and longer as they age.

Variations in Light Intensity and Photosynthesis:

As expected, light decreased from young to old leaves due to self shading (Figure 1). In the colder winter months, there was less light than in the warmer summer months (Figure 1). The older leaves in the winter were actually receiving about 10% of full summer sunlight (Figure 1). Variations in light and other microclimate conditions led to complex effects on photosynthesis rates that were difficult to separate (Figure 2).

There were statistical differences in photosynthesis and light based on a three way ANOVA for leaf age ($p< 0.001$), months ($p< 0.001$) and species ($p< 0.001$). Overall old leaves photosynthesized less than young leaves by about 4.67 to 5.43 µmols of CO_2 m^{-2} s^{-1} second and middle leaves photosynthesized less than young leaves by about 3.79 to 4.55 µmols m^{-2} s^{-1}. *Baccharis halimifolia* photosynthesized less than *M. cerifera* by about 1.61 to 2.4 µmols m^{-2} s^{-1}. Overall old leaves had less light than young leaves by about 462.33 to 462.91 µmols m^{-2} s^{-1} and middle leaves had less light than young leaves by about 313.66 to 314.24 µmols m^{-2} s^{-1}. *Baccharis halimifolia* had 99.34 to 99.87 µmols m^{-2} s^{-1} less light than *M. cerifera*.

Comparisons of Leaf Pigments:

In terms of age effects on total chlorophyll for *M. cerifera*, the middle leaves were consistently higher (Figure 3). Throughout the season, I saw a general increase in the total chlorophyll of young leaves and a slight decrease in the total chlorophyll of old

leaves of *M. cerifera* (Figure 3). I found similar trends in *B. halimifolia* with the young increasing through the season and the old decreasing (Figure 3). Though the middle was still relatively high in *B. halimifolia* throughout the year, it was not the highest during every month, and reached a peak around July through September and then decreased slightly (Figure 3). A similar trend was apparent in both chlorophyll a and chlorophyll b (Figures 4 and 5). There were no discernable trends in age or seasonal fluctuations for chlorophyll a/b ratios for either species (Figure 6). Carotenoids showed a different pattern in both *M. cerifera* and *B. halimifolia* (Figure 7). For *B. halimifolia* both young and middle leaves increased throughout the season while the old leaves decreased (Figure 7). In *M. cerifera* the trend was less obvious (Figure 7). Young and old leaves dipped slightly July through November and then began to increase again.

There were significant differences in total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid pigments based on a three way ANOVA for leaf age ($p < 0.001$), months ($p < 0.001$) and species ($p < 0.001$) for all pigments tested. There were no significant differences in chlorophyll a/b based on months, leaf age or species. Overall old leaves had less total chlorophyll than young leaves by about 50.07 to 50.51 mg m^{-2} and middle leaves had more total chlorophyll than young leaves by about 85.85 to 86.29 mg m^{-2}. Overall *B. halimifolia* had 43.38 to 45.79 mg m^{-2} less total chlorophyll than *M. cerifera*. For chlorophyll a, old leaves had less than young leaves by about 34.96 to 35.4 mg m^{-2} and middle leaves had more chlorophyll a than young leaves by about 43.56 to 44.00 mg m^{-2}. *Baccharis halimifolia* had 24.83 to 24.42 mg m^{-2} less chlorophyll a than *M. cerifera*. Old leaves had less chlorophyll b than young leaves by about 14.89 to 15.33 mg m^{-2} and middle leaves had more chlorophyll b than young leaves by about 42.06 to

42.50 mg m^{-2}. Overall *B. halimifolia* had 20.75 to 21.17 mg m^{-2} less chlorophyll b than *M. cerifera*. In terms of c pigments, old leaves had less than young leaves by about 8.47 to 8.92 mg m^{-2} and middle leaves had more carotenoid pigments than young leaves by about 2.27 to 2.72 mg m^{-2}. Overall *B. halimifolia* had 3.76 to 4.19 mg m^{-2} less carotenoid pigments than *M. cerifera*.

Comparisons of Nitrogen Content:

Nitrogen concentration was highest in the young leaves of *M. cerifera* in May (Figure 8). The largest differences in young, middle, and old leaves were found in May (Figure 8). July and September showed little difference in nitrogen content by month, and leaf age for *M. cerifera* (Figure 8). In *B. halimifolia* all three leaf ages were highest in May (Figure 8). There seems to be no consistent patterns in *B. halimifolia* by month or by leaf age for both July and September (Figure 9). As expected the C:N ratios show similar patterns as those discussed above only inverted (Figure 10).

For a three-way ANOVA, significant differences were found in percent nitrogen by months ($p= 0.020$) but there were not significant based on leaf age ($p= 0.591$), and species ($p= 0.210$). However, there were significant differences in C:N based on leaf age ($p< 0.001$), months ($p< 0.001$) and species ($p< 0.001$) based on a three way ANOVA. Overall old leaves had higher C:N values than young leaves by about 1.7 to 2.6, but middle leaves C:N values were not significantly different than young leaves. Overall *B. halimifolia* had C:N values 2.9 to 3.7 less than *M. cerifera*.

Comparisons of Branch Growth and Leaf Drop:

Branches grew less from month to month during the winter as compared to summer for both *M. cerifera* and *B. halimifolia* (Figure 10). Only three months were sampled so it may be beneficial to investigate this further (Figure 10). Leaf drop of the five most proximal leaves per month increased toward the winter months for *M. cerifera* but seemed to remain more constant throughout the growing season for *B. halimifolia* (Figure 11).

There were significant differences in leaf drop and net branch growth based on a two way ANOVA for leaf age ($p < 0.001$), months ($p < 0.001$) and species ($p < 0.001$). Overall *B. halimifolia* had more leaves dropped throughout the year than *M. cerifera*, but grew 0.3 to 1.1 cm less than *M. cerifera* throughout the year.

Comparisons of Tissue Chlorides:

In terms of tissue chloride levels in the leaves, it was higher for both species in the summer (Table 2). The effect of age on salt content for *B. halimifolia* showed a consistently increased level in older leaves (Table 2). The effect of age on salt content in *M. cerifera* seems more complex, though there may be greater salt concentrations in the young leaves (Table 2).

Discussion

My results provide insight as to the mechanisms that facilitate shrub expansion in coastal environments. My goals were to quantify changes in photosynthetic characteristics and leaf nitrogen content as they relate to leaf position with in determinant shoot growth. I also examined morphological differences in leaves as they aged. I chose

M. cerifera and *B. halimifolia* due to their contrasting features. *Morella cerifera* is the dominant shrub on many Virginia barrier island it is an evergreen and a nitrogen fixer. *Baccharis halimifolia* is deciduous, co-occurs with *M. cerifera,* but is much less abundant.

Other than size, leaves did not change morphologically with age or position. There were, however, morphological differences between the two species (Table 1). Light intensity decreased with leaf age due to self shading and also due to lower sun angle during the winter months (Figure 1). No differences or trends were detected in photosynthesis either seasonally or by age group (Figure 2). Many factors other than leaf age, such as light intensity, water availability, temperature and other microclimate factors, confounded dependent variable physiological characteristics such as photosynthesis. Also limited measurements made it difficult to observe trends based on age. Overall chlorophyll concentrations increased in young leaves, decreased in old leaves and remained the highest in middle leaves throughout the year for both species (Figures 3-5). Seasonal patterns in carotenoid pigments differed between the two species (Figure 7). Nitrogen content in new leaves was highest in the spring, which is the opposite seen in the C:N ratios (Figure 8, 9). Branch growth decreased throughout the year (Figure 10). *Morella cerifera* had a large leaf drop in the month of December where *B. halimifolia* had more of a constant leaf drop throughout the growing season (Figure 11). *Baccharis halimifolia* stored more salt in old leaves, but chloride concentrations based on age in *M. cerifera* were less variable with age (Table 2). This could be due to salt storage, salt secretion or higher salt concentrations inside of leaves and requires more research to establish the cause of tissue chloride differences.

Leaves of *M. cerifera* and *B. halimifolia* do not morphologically change with age and seasonality but there was a physiological response in pigments, and nitrogen concentrations. In the spring, the youngest leaves of both species had the highest nitrogen content (Figure 8, 9). It is important to note that because *B. halimifolia* is a deciduous species, in the spring, even the middle and old leaf classes were relatively young. Under optimal natural conditions, leaf N is highly correlated with photosynthetic rate for many species (Reich et al., 1991). But, Reich et al. (1991) found that younger leaves of sugar maples and northern pine oak had higher nitrogen content but not necessarily higher photosynthetic rates as they continued to develop with age. Leaves were either in the process of expanding or had just fully expanded and, therefore, not yet fully functional (Reich et al., 1991). This is consistent with my findings that leaf nitrogen was highest in the youngest leaves of *M. cerifera* and *B. halimifolia* during the spring, even if high nitrogen did not relate to the highest levels of chlorophyll pigments. The relatively higher soil nitrogen found in spring may have contributed to increased nitrogen in leaves.

Morella cerifera and *B. halimifolia* leaves are morphologically different, independent of senescence (Table 1). Stomata occurred only on the abaxial leaf surface for *M. cerifera,* which is the typical position for stomata (Table 1). In comparison, stomata were found on both adaxial and abaxial surfaces of *B. halimifolia* (Table 1). This has interesting consequences for *B. halimifolia* in terms of water use efficiency, especially because barrier islands can go through periods of water stress due to fluctuations in the soil fresh water lens. The congeners, *Baccharis trimera, B. crispa* and *B. articulate,* also have stomata on both leaf surfaces (Cortadi et al., 1999). Trichomes

were present on both leaf surfaces for *B. congenesis* (Cortadi et al., 1999). I also found greater trichome density on the adaxial surface for *B. halimifolia* leaves when compared to *M. cerifera* (Table 1). This may partially offset the water loss caused by stomata on the upper leaf surface. *Baccharis halimifolia* also had slightly larger leaves than *M. cerifera* which may again relate to overall water use efficiency, though Wang and Lincoln (2004) found more trichomes on the abaxial leaf surfaces of *M. cerifera* as compared with my results. These factors could contribute to *M. cerifera* larger success on barrier islands, but most likely not as important as nitrogen fixation and evergreen verse deciduous leaf habit.

Valuable insight towards the physiological performance of leaves can be provided by quantifying leaf pigment concentrations (Sims and Gamon, 2002). The overall trends in photosynthetic pigments for both species seemed to initially increase as leaves developed and then decreased as leaves aged (Table 3-5). This may be due to reduction in light intensity as the leaves were self shaded by emerging new leaves emerge. It also may be caused by a reduction in stomatal conductance with leaf age in response to lower photosynthetic capacity of the mesophyll (Escudero and Mediavilla, 2003). I did measure a decrease in light levels in the winter months (Figure 1).

In agreement with my findings, increased light absorbance in the rain forest tree, *Dryobalanops aromatic*, meant increases in chlorophyll concentrations (Ishida et al., 1999). However I did not find a decrease in chlorophyll a/b ratio which I expected. Seasonal patterns in chlorophyll content vary by species (Lewandowska and Jarvis, 1977). Carotenoid pigments have a tendency to decline less rapidly than chlorophyll pigments during senescence (Sims and Gramon, 2002). Accessory pigments like

carotenoids enable plants to absorb a larger range of wavelengths and therefore increase light use efficiency. This may explain the increase in these pigments in the winter months for both species as light declined.

Most of the research on the interaction of salinity and leaf age is in wetland grasses. Various cereals, *Hordeum vulgare*, *Ricinus communis* and *Atriplex hortensis* revealed increased tissue chlorides with increased age (Klagges et al., 1993). Increased tissue chlorides in older leave have also been found in durum wheat (Sharma, 1996). These findings are consistent with the increase in tissue chloride I found in older leaves of *B. halimifolia*. Salt accumulation in or on old leaves which are then shed may be beneficial in an environment with periodic salt water inundation and sea spray, such as on barrier islands. It is interesting that *M. cerifera* does not show the same trend and in fact seems to have higher tissue chloride concentrations in young leaves (Table 2). These preliminary measurements require more research to understand the primary mechanisms and ecological advantages.

Cost benefit models predict that photosynthetic decline and initial cost of construction are major factors that lead to the timing of leaf senescence (Escudero and Mediavilla, 2003). Photosynthetic nitrogen use efficiency decreases with leaf age. Thus it is beneficial to reallocate nitrogen from old leaves to young leaves; however there is no consistent relationship between leaf life span and tissue nitrogen (Escudero and Mediavilla, 2003). This may be due to the final carbon balance of leaves, initial assimilation rate of carbon, and rate of decline of assimilation with advancing leaf age (Escudero and Mediavilla, 2003). Though I found seasonal variations in nitrogen content I was unable to detect differences among age groups (Figure 8, 9). Most nitrogen re-

absorption happened just before leaves are shed (Escudero and Mediavilla, 2003). This may indicate that my sampling missed the critical period and did not capture the sudden drop in nitrogen in old leaves. My pigment data give more insight into leaf senesce in these two species. In November, just before the large leaf drop for *M. cerifera*, I detected the lowest levels of chlorophyll a and b in old leaves and some of the highest levels of carotenoids (Figure 4, 5, 7, 11). December also showed the lowest light levels overall for *M. cerifera* (Figure 1). This suggests a threshold in photosynthetic pigments or light that occurs before leaves are dropped.

Understanding shrubs adaptations relative to senescence is essential to provide insight towards predicting shrub expansion rates. It is important to determine why *M. cerifera* is successful on Virginia's barrier islands. Shrub expansion is a fascinating yet poorly understood consequence of climate and anthropogenic change, especially the underlying physiological and morphological mechanisms. Virginia barrier islands are excellent for studying this phenomenon, not only because vegetation must contend with other consequences of climate change such as sea level rise and increased storm frequency, but because they have no immediate history of grazing or other anthropogenic influences that might otherwise account for shrub expansion. Therefore the comparison between *M. cerifera* and *B. halimifolia* is especially informative because being able to understand why one shrub is able to thicketize and expand over another shrub can help us make predictions about which species will be more successful in colonizing historic grasslands in the future.

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TABLES AND FIGURES

Table 1: *Morella cerifera* and *Baccharis halimifolia* leaf characteristics for young middle and old leaves collected on Hog Island on 8/27/09 and 6/25/2010 plus or minus the standard deviation. Identical superscripted letters represent no significant differences found with a Tukey HSD post hoc test.

	Young Leaves	Middle Leaves	Old Leaves
Morella cerifera			
Leaf Area (cm^2)	2.7 ± 0.8^{A}	6.9 ± 1.5^{B}	8.4 ± 2.3^{C}
Leaf Length (mm)	37 ± 6.1^{A}	54.2 ± 6.4^{B}	60 ± 8.8^{C}
Leaf Thickness (mm)	0.27 ± 0.08	0.28 ± 0.04	0.26 ± 0.04
Stomatal Density Top (# per mm^2)	0	0	0
Stomatal Density Bottom (# per mm^2)	289 ± 108^{A}	411 ± 89^{B}	331 ± 86^{AB}
Trichome Density (Top) (# per mm^2)	0.6 ± 1.9	1.9 ± 3.1	1.3 ± 2.5
Trichome Density (Bottom) (# per mm^2)	7.5 ± 9.4	2.5 ± 6.3	4.4 ± 1.3
Baccharis halimifolia			
Leaf Area (cm^2)	3.3 ± 0.9^{A}	9.2 ± 2.7^{B}	11.1 ± 2.8^{C}
Leaf Length (mm)	33.6 ± 3.7^{A}	47.2 ± 5.0^{B}	55.1 ± 5.5^{C}
Leaf Thickness (mm)	0.26 ± 0.09^{A}	0.29 ± 0.05^{AB}	0.33 ± 0.08^{B}
Stomatal Density Top (# per mm^2)	72 ± 14	65 ± 16	62 ± 19
Stomatal Density Bottom (# per mm^2)	113 ± 25	94 ± 12	97 ± 25
Trichome Density (Top) (# per mm^2)	10.1 ± 6.3	10.1 ± 6.9	5 ± 5.7
Trichome Density (Bottom) (# per mm^2)	7.5 ± 6.3	8.2 ± 5.7	3.1 ± 4.4

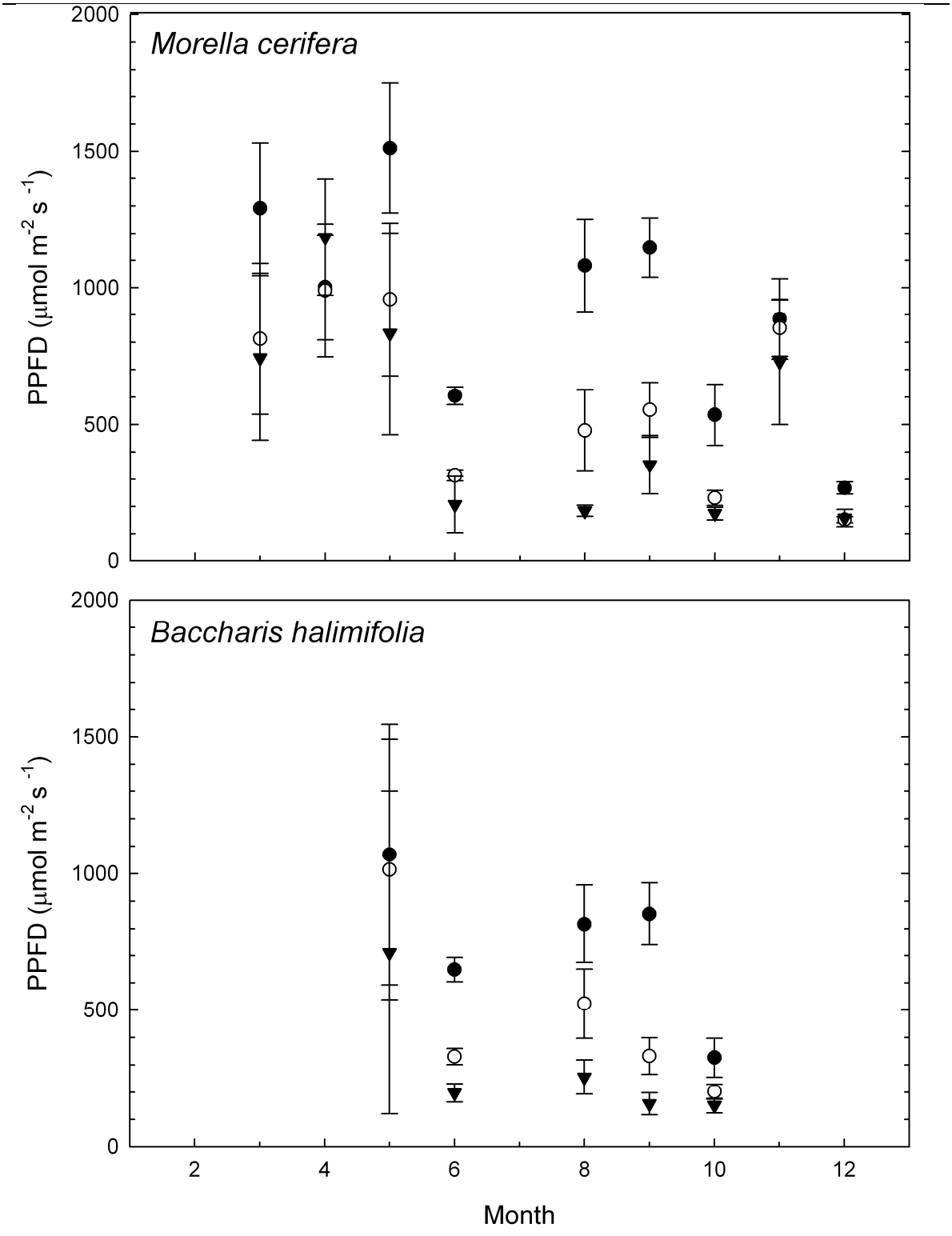

Figure 1: Photosynthetic photo flux density (PPFD) at the apex of young (●), middle (○), and old (▼) leaves of *Morella cerifera* and *Baccharis halimifolia* sampled on the north end of Hog Island. Vertical bars denote ± one standard error

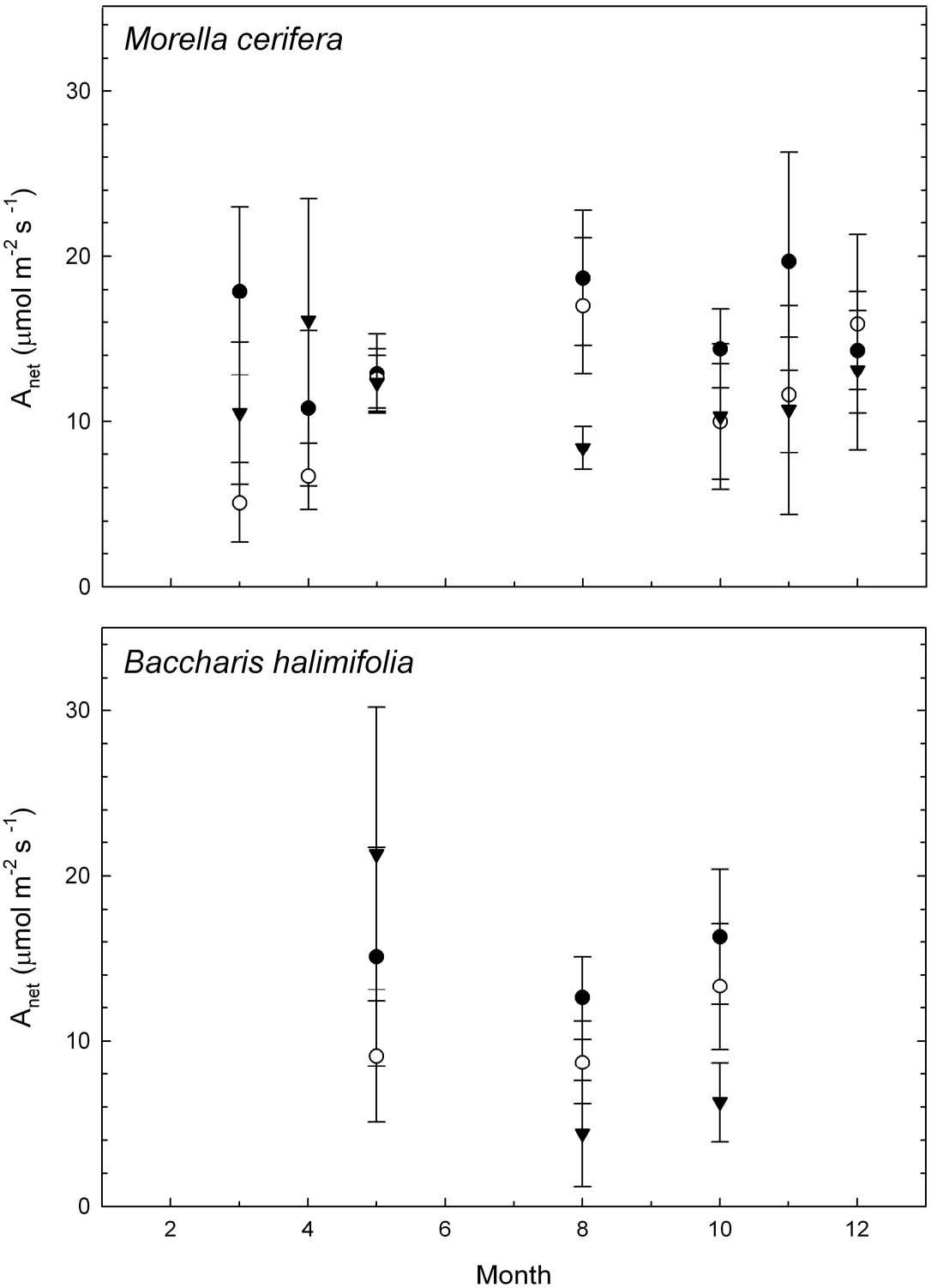

Figure 2: Net photosynthesis (A_{net}) for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves sampled on the north end of Hog Island. Vertical bars denote ± one standard error

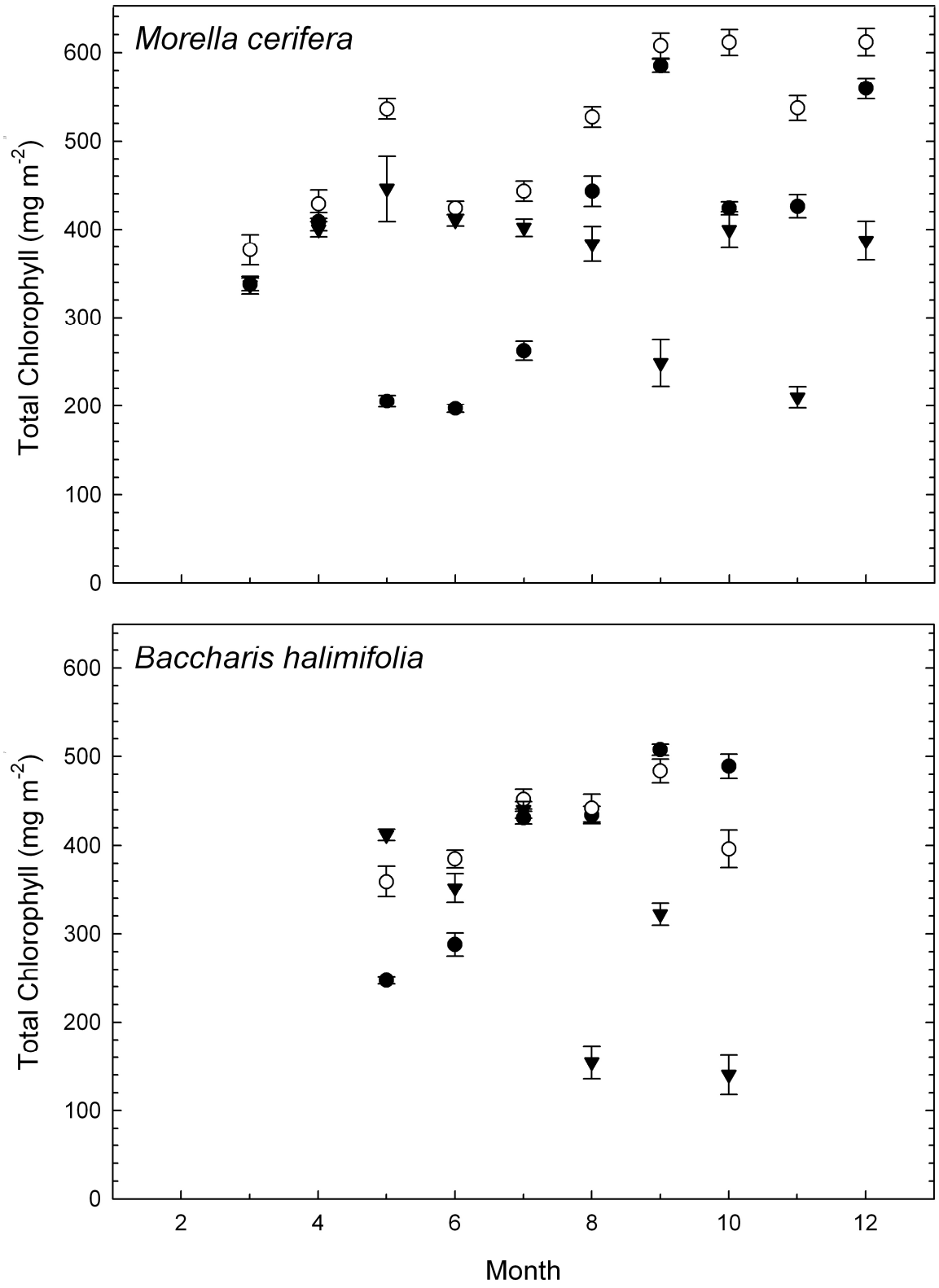

Figure 3: Total chlorophyll content for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island. Vertical bars denote ± one standard error

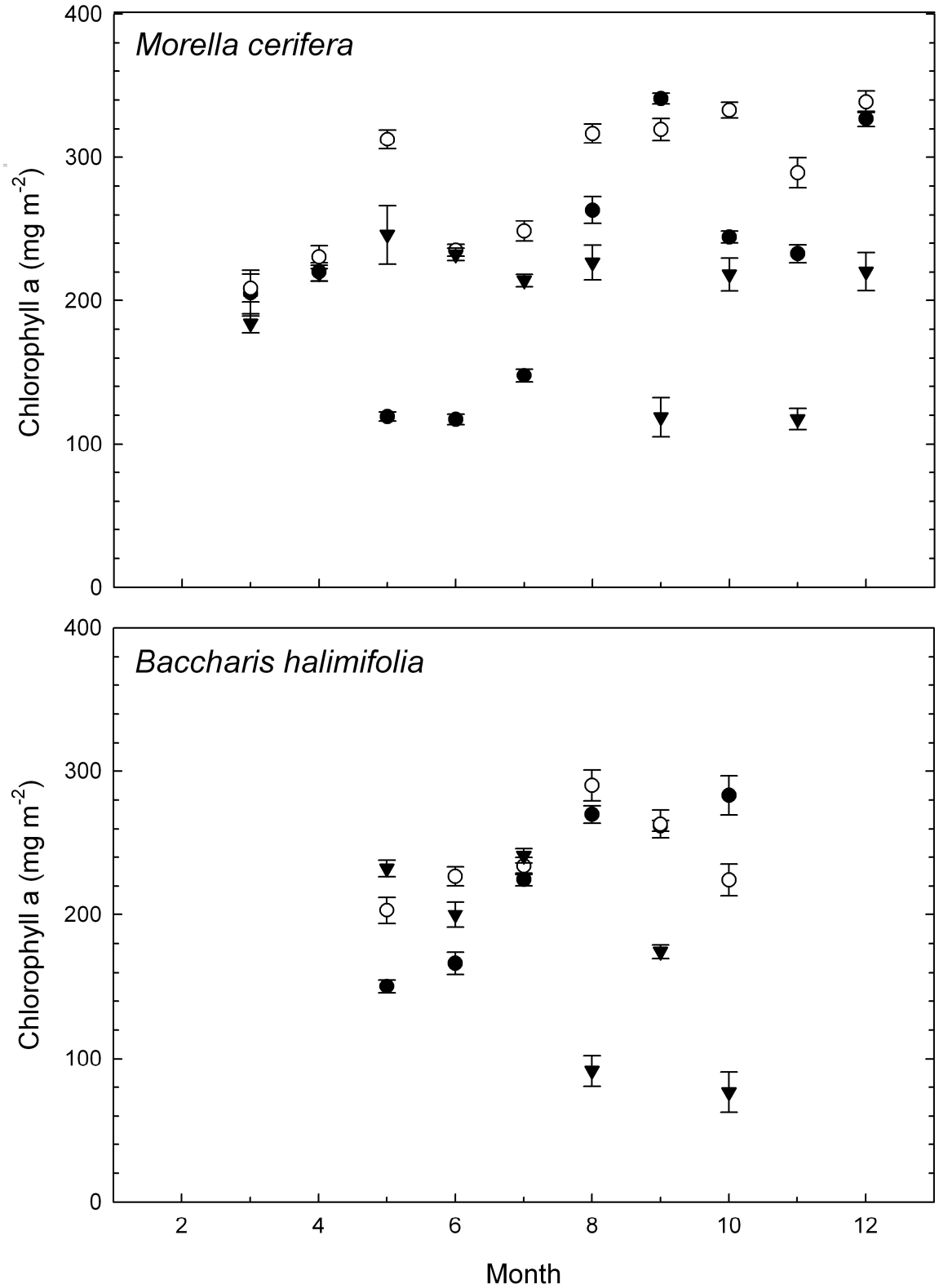

Figure 4: Chlorophyll a content for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island. Vertical bars denote ± one standard error

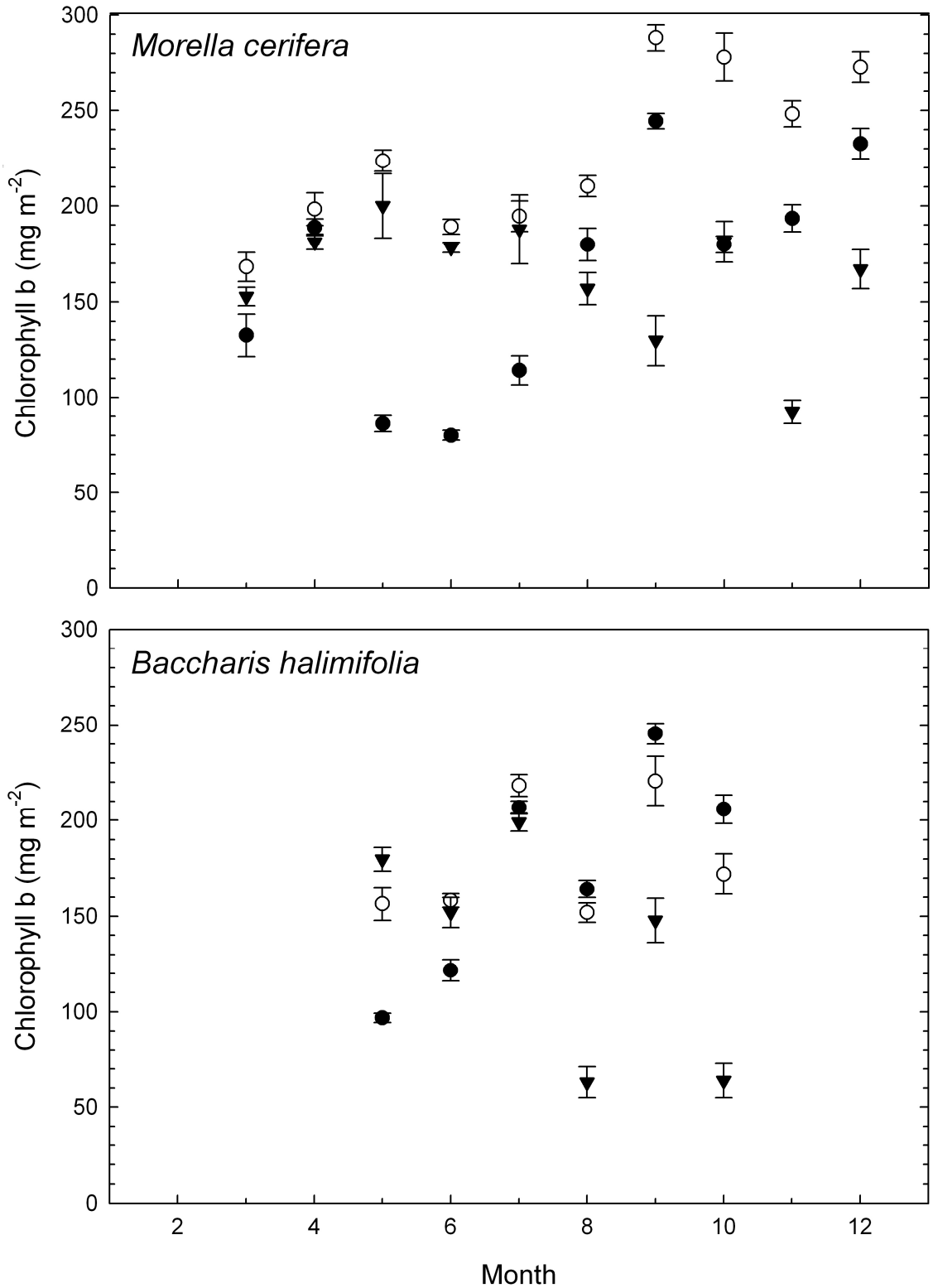

Figure 5: Chlorophyll b content for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island. Vertical bars denote ± one standard error

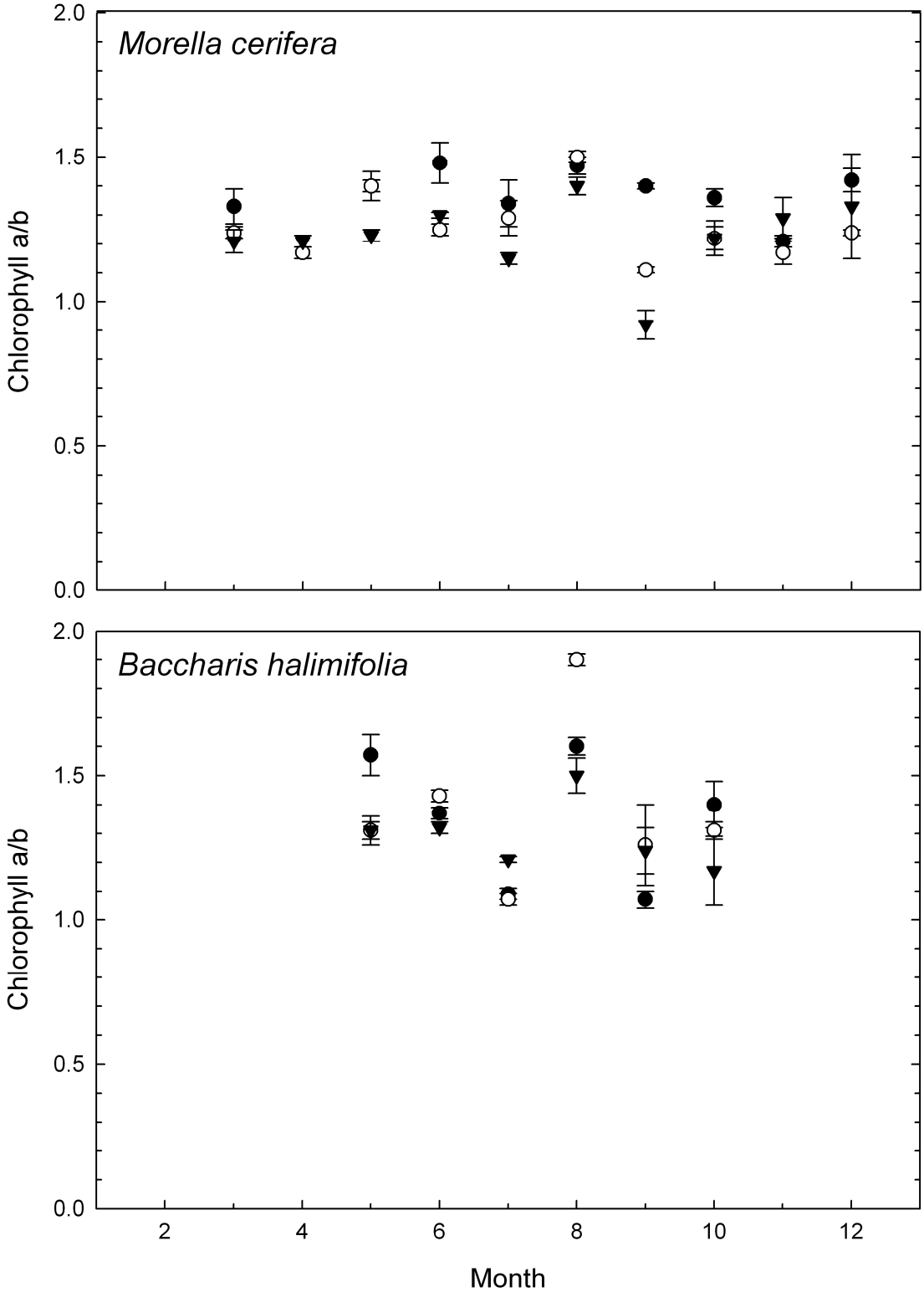

Figure 6: Chlorophyll a to chlorophyll b ratios for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island**.** Vertical bars denote ± one standard error

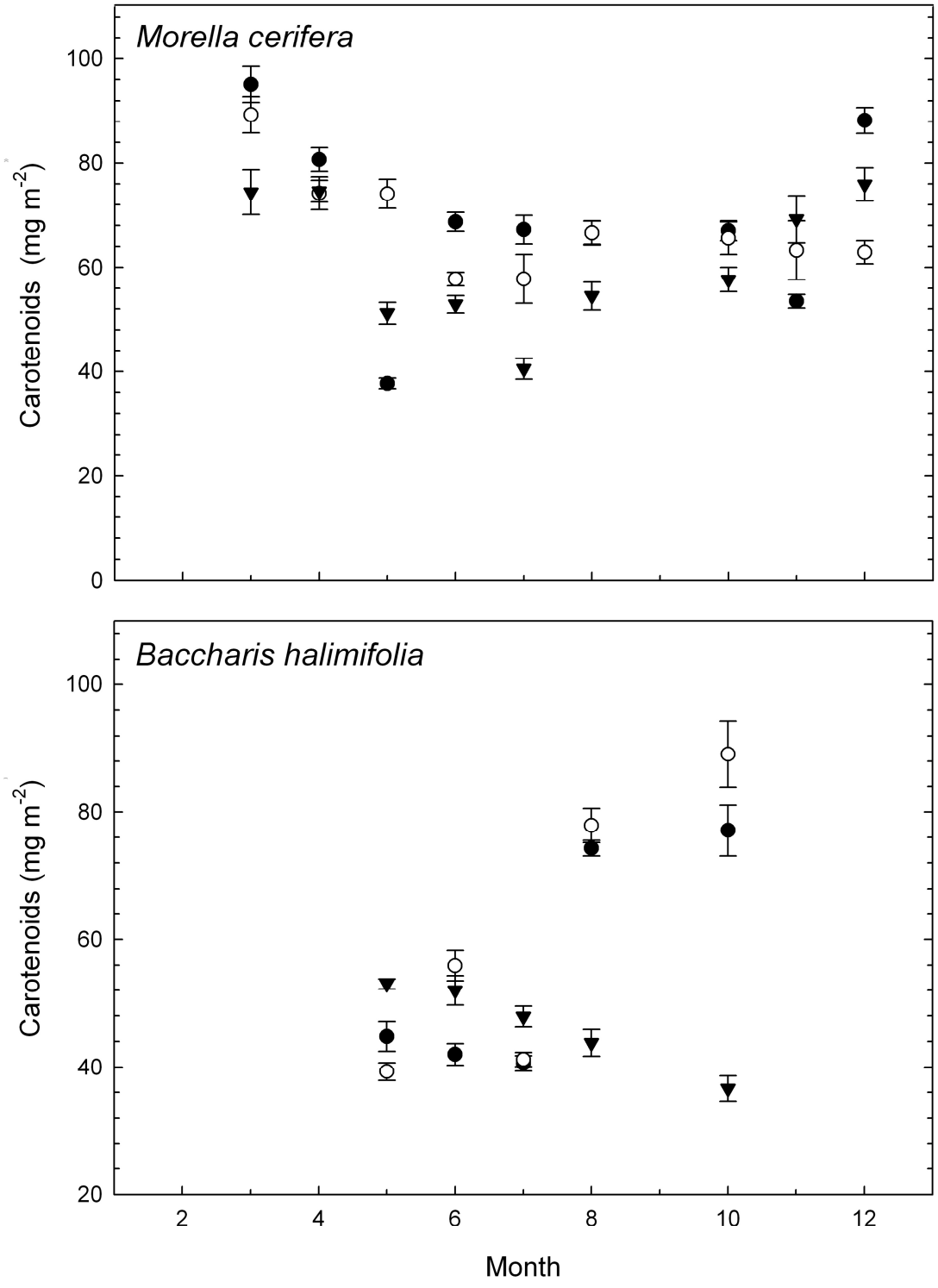

Figure 7: Carotenoid content for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island**.** Vertical bars denote ± one standard error

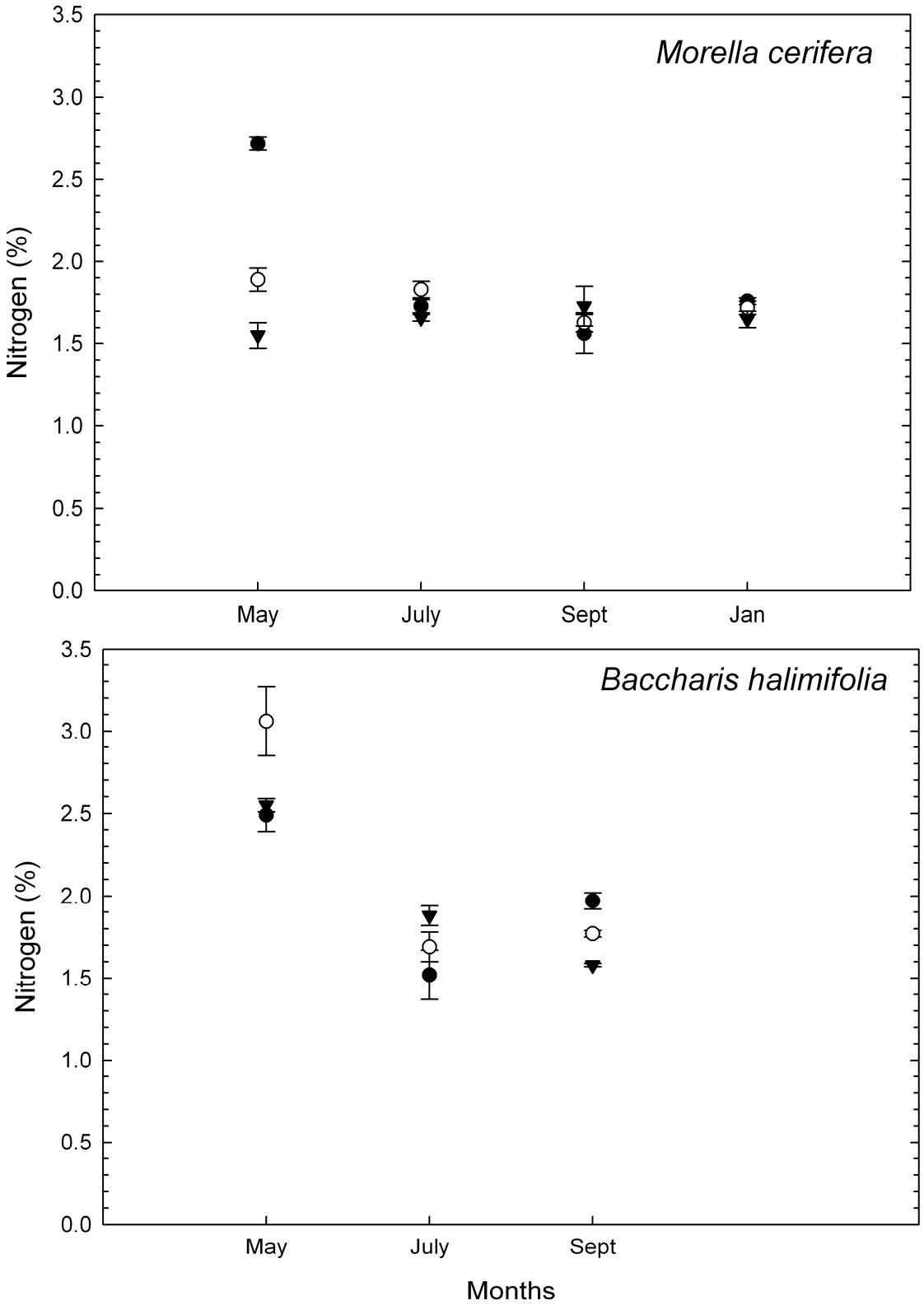

Figure 8: Percent nitrogen content for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island. Vertical bars denote ± one standard error

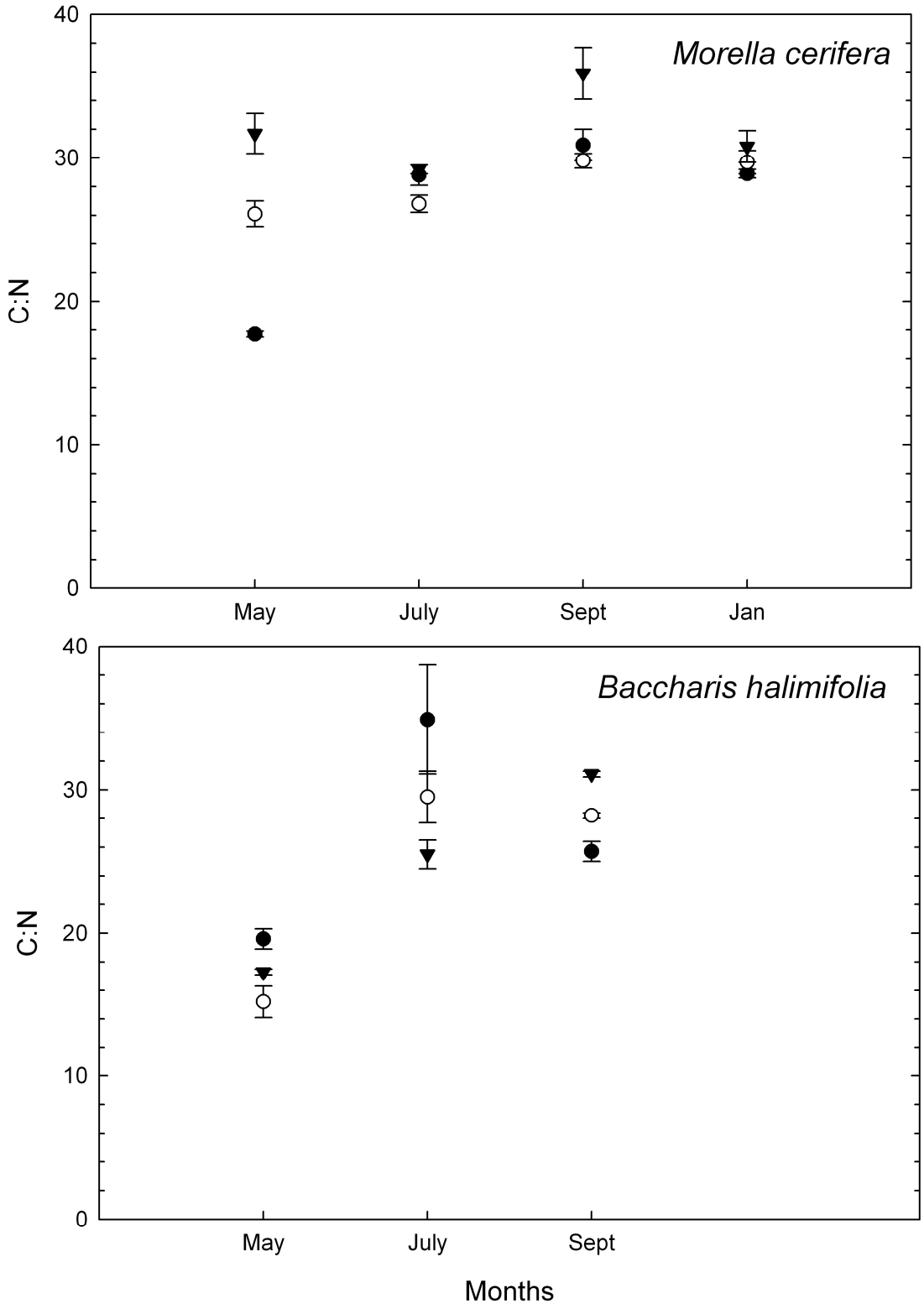

Figure 9: C:N ratios for young (●), middle (○), and old (▼) *Morella cerifera* and *Baccharis halimifolia* leaves collected on the north end of Hog Island. Vertical bars denote ± one standard error

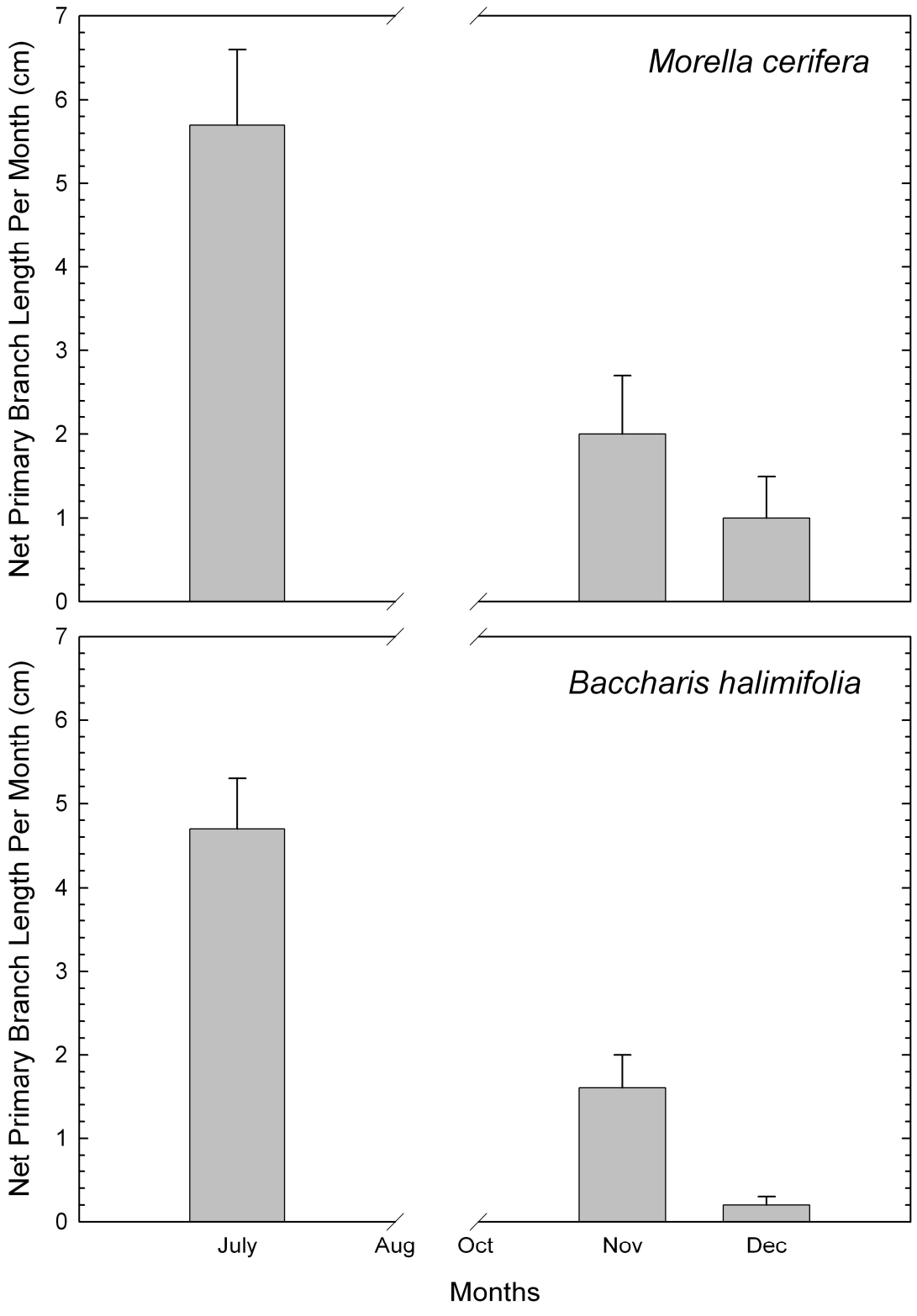

Figure 10: Net shoot growth for *Morella cerifera* and *Baccharis halimifolia* branches collected on Hog Island. Vertical bars denote ± one standard error

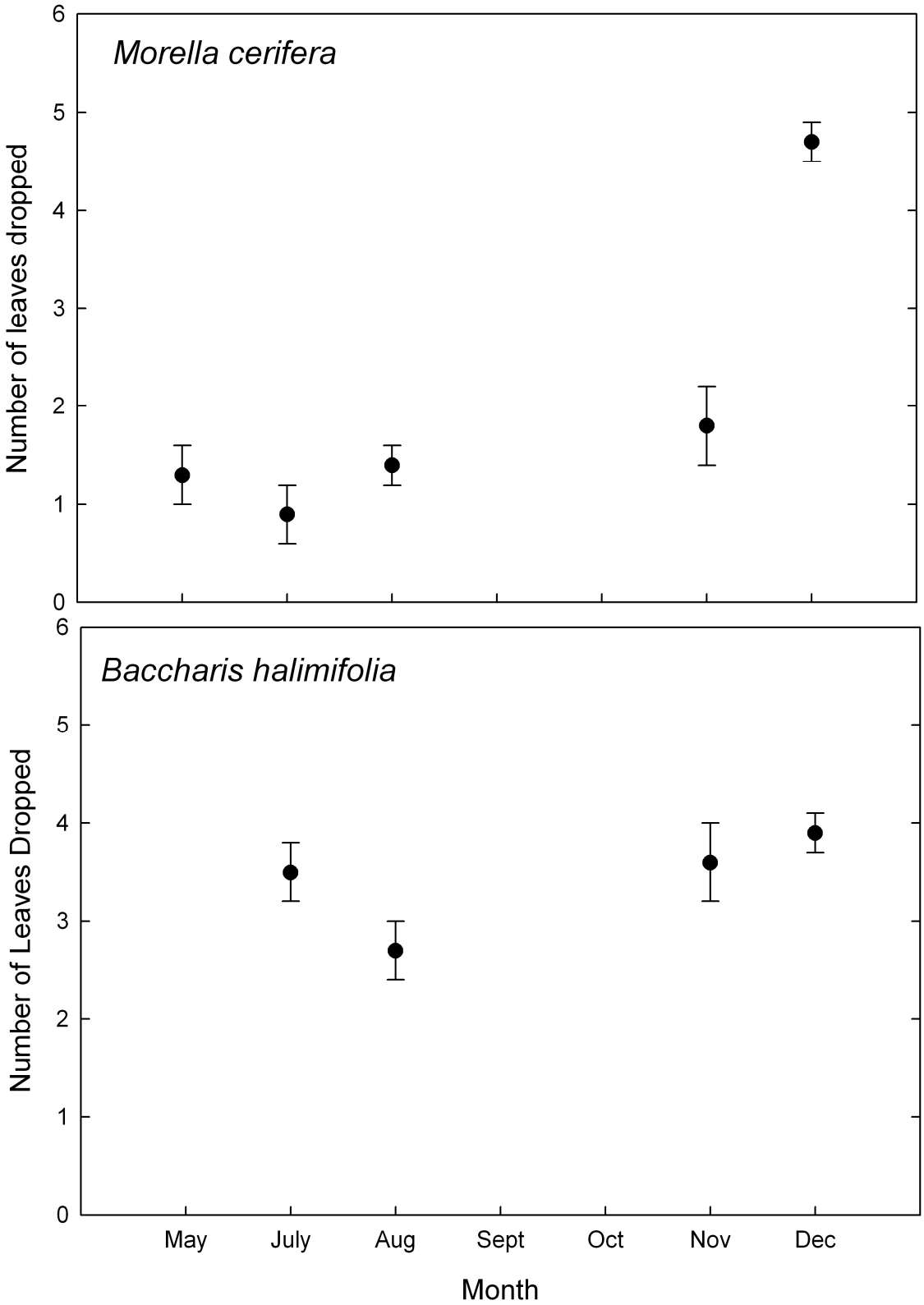

Figure 11: Leaf drop of the last five leaves of *Morella cerifera* and *Baccharis halimifolia* branches collected on the north end of Hog Island. Vertical bars denote ± one standard error

Table 2: Tissue chlorides (mg/g) taken from *Morella cerifera* and *Baccharis halimifolia* for young middle and old leaves collected on Hog Island during the summer and fall of the growing season plus or minus the standard deviation. Identical superscripted letters represent no significant differences found with a Tukey HSD post hoc test

	Young Leaves	Middle Leaves	Old Leaves
Summer			
M. cerifera	8.26 ± 0.89^{A}	6.96 ± 0.53^{B}	7.64 ± 0.87^{AB}
B. halimifolia	8.91 ± 0.58	8.83 ± 0.98	13.97 ± 1.53^{A}
Autumn			
M. cerifera	5.36 ± 0.37^{A}	4.01 ± 0.44	4.03 ± 0.56
B. halimifolia	6.25 ± 0.35^{A}	6.57 ± 1.05^{AB}	7.38 ± 0.72^{B}

VITA

Elizabeth Jennifer Frankel Kost was born on December 7, 1984, in Fairfax County, Virginia. She graduated from Robert E. Lee High School, Virginia in 2003. She received her Bachelor of Science in Biology from Virginia Commonwealth University, Virginia in 2008. She then took a semester of graduate classes before entering the graduate program. She received a Master of Sciences in Biology from Virginia Commonwealth University in 2011