Distribution and morphology of established dune species

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Introduction

Human populations along coastlines are ever increasing, with 10% of the world's population already residing within 10 meters or less above sea level (McGranahan et al. 2007). Protecting our coastlines are not only important for infrastructure but for delicate ecosystems that provide a plethora of ecosystem services. Coastal ecosystems are known for providing recreation and habitat for wildlife, but they also provide protection from erosion (Barbier et al. 2011). Sand dunes are the first line of defense against coastal erosion from sea level rise, wind and wave action (Emery and Rudgers 2014, Feagin et al. 2015). Dunes are formed through the feedback between vegetation and sediment. Different species of dune grasses form different shaped dunes at different rates and provide varying degrees of erosion control. Understanding these intricate species differences will be important for future restoration efforts.

Aboveground morphology of dune grasses has been highly studied, with the understanding that plant height, stem density, and biomass are good indicators of a species' ability to capture sand (Hacker et al. 2012, Zarnetske et al. 2012, 2013). Further research has found that it is not only the aboveground portion of the plant that is important, but also the belowground portion which helps bind sediment and reduce erosion (Feagin et al. 2015, Brown et al. 2018). Studies are beginning to research the belowground morphology of native dune vegetation and we wanted to incorporate that into our study as well.

With climate change, species distributions are changing rapidly which has cascading effects on the ecosystem. Along with this, we are seeing an increase in invasive vegetation species. Invasive species disrupt the landscape by limiting areas for natives, which reduces habitat and food sources for native wildlife and pollinators. At Back Bay National Wildlife refuge there has been an invasion of *Carex kobomugi*, Asiatic sand sedge, along the dunes of the northern portion of the refuge. This invasion will disrupt the native vegetation and lead to a myriad of unknown consequences for coastal protection.

The objectives of this study were to **(1) Quantify species presence and abundance across the dune at different elevations to identify species distributions, relationships with elevation, and potential species interactions, (2) Compare above and belowground morphological differences between common native species and the invasive sand sedge, and (3) Compare above and belowground morphological differences between mixed and monoculture plots.**

Methods

The native species included in this study were the three most dominant, *Ammophila breviligulata*, *Uniola paniculata*, and *Panicum amarum* (hereafter referred to by genus). American beachgrass, *Ammophila*, has a distribution from Cape Fear, NC and north along the eastern shore of the US as well as along the great lakes (Goldstein et al. 2018). *Ammophila* grows laterally, forming a long, linear dune ridge through a guerilla growth strategy (Lovett Doust 1981; Brantley et al. 2014). Often discussed in comparison to *Ammophila* is *Uniola*. *Uniola* has a northernmost range at the Maryland border of Assateague Island (Goldstein et al. 2018) and it is the most dominant dune grass species throughout the southeastern coast of the US. It builds dunes of different shapes and sizes compared to its northern counterpart (Woodhouse et al. 1977, Stallins 2005, Goldstein et al. 2017). *Uniola* initially forms dune hummocks using the phalanx growth strategy; these hummocks can coalesce into a linear dune ridge over time (Lovett Doust 1981; Goldstein et al. 2017). These two species historically dominate 2 different ranges but are beginning to overlap more with climate warming. This intermixing is being seen more frequently along the NC and VA coasts, with unknown ecological effects of having them co occur. An understudied species along the eastern coast is *Panicum*, bitter panicgrass, is also a generalist species existing along the entire east coast from Connecticut to Florida. It is often left out of the dune building conversation due to its height, being shorter than the other common natives (Hacker et al. 2019). Nonetheless, it is a dominant species and has other forms of trapping sand, most interestingly its been found to have the thickest leaf width (Hacker et al. 2019) which is helpful for sand trapping and stabilization.

The dominant invasive species at my study site is *Carex kobomugi*, Asiatic sand sedge. It was first introduced to NJ in the 1920s (Small 1954). Charbonneau et al. (2016) found that the species has an extensive belowground rooting system which could lead to it being a good dune stabilizer. More recently, Charbonneau et al. (2020) found that the spread of *C. kobomugi* in 10 years had expanded 170% along the dune habitat. Much of the expansion was into territory previously occupied by native vegetation and less into open space, indicating that the species is taking over native habitat. The majority of the research on this species has been completed in NJ where it originated, so it has mostly been in comparison to *Ammophila*. One of the goals of my research is to see how this species interacts with other native vegetation in Virginia.

This study was completed at Back Bay National Wildlife Refuge, Virginia along the northernmost mile of dune ridge throughout the summer of 2020. The northern mile of the site is not open to the public and has not been the focus of management at the refuge, with much of the invasive species population still intact. To assess species composition and interactions, percent cover, number of shoots, and maximum height of each species was recorded in 0.25 m^2 plots along transects spaced ~120m apart (n = 15). Plots were positioned ~5m apart and cover the toe, face, crest, and back dune. Locations of mixed species and monocultures of *Ammophila breviligulata*, *Uniola paniculata*, and *Carex kobomugi* were identified for coring (n = 10).

Following modified methods of Charbonneau et al. (2016), root cores were collected to 60cm depth using a slide hammer (AMS, Inc.; American Falls, ID) with a 1.5" diameter, then bagged, and placed in a cooler for further processing. Aboveground biomass within the core was cut at the soil surface and placed in a bag for later processing. Cores were processed by wet-sieving to remove all rooting material and roots were scanned using Epson Perfection V800 picture scanner (Epson America Inc., Long Beach, California, USA) and using WinRHIZO software (Regent Instruments Inc, Québec, Canada). WinRHIZO quantifies the total root length and volume of roots of each diameter class. This allowed for calculations of specific root length (SRL) and root tissue density (RTD). Once all scanning is complete, the total below and aboveground material was dried for biomass at 60℃ for 72 hours. The dried material was then sent to Cornell Isotope Laboratory (COIL) for analysis of percent Carbon and Nitrogen as well as $\delta^{15}N$ and $\delta^{13}C$.

For each species, additional roots were collected from individuals for tensile strength measurements (n = 10). These roots were stored in 15% ethanol until processing. The day of processing they were rehydrated in water for at least 30 minutes (Bischetti et al. 2005, Böhm 1979). Tensile strength measurements were completed using a MTS Insight 30 Universal Testing Machine (UTM) and MTS Advantage Wedge Action Grips (MTS Systems Corporation; Eden Prairie, MN) with a 50N load cell and connected to the software, Testworks 4. This software calculates the peak stress required to break the root, which is what was used as the tensile measurement for the roots. Clamping of roots followed a modified procedure, similar to De Baets et al. 2008.

Statistical analysis for this project was completed in R software (R Core Team). Including species specific differences of all belowground functional traits using one-way ANOVAs, or nonparametric Kruskal Wallis tests when assumptions are not met with basic transformations. Transect data was grouped by dune location (toe, face, crest, back) and then one-way ANOVAs were performed on species cover for each location. Tensile strength analysis included an ANCOVA of species differences in peak stress with root diameter as the covariate. Peak stress was logarithmically transformed to establish normality.

Results

Objective 1: Quantify species presence and abundance across the dune at different elevations to identify species distributions, relationships with elevation, and potential species interactions

The transect data found significant differences for species cover at each location. After completing Kruskal-wallis tests on each location, I found that all locations had significant species differences in species cover (Fig. 1A). The Nemenyi pairwise comparisons found that within the crest *Carex* was significantly different from all species except *Uniola*. Within the face plots

Spartina was significantly different from *Carex* and *Panicum*. The toe and back plots had no significant species differences from running the pairwise test, an indication of the conservativeness of this particular test. Kruskal-wallis tests were also completed on the stem densities per species at each location. The only significance was found at the back dune (p= 0.03) and after running a pairwise comparison the only difference was between *Panicum* and *Carex*, there was no *Ammophila* present in the back dune (Fig. 1B). Height differences between species found expected patterns, *Uniola* the tallest while *Carex* and *Panicum* were the shortest (Fig. 2). Other species occasionally present throughout the transects but not in abundance were: *Gamochaeta purpurea*, *Hydrocotyle bonariensis*, *Krigia virginica*, and *Cakile edentula*.

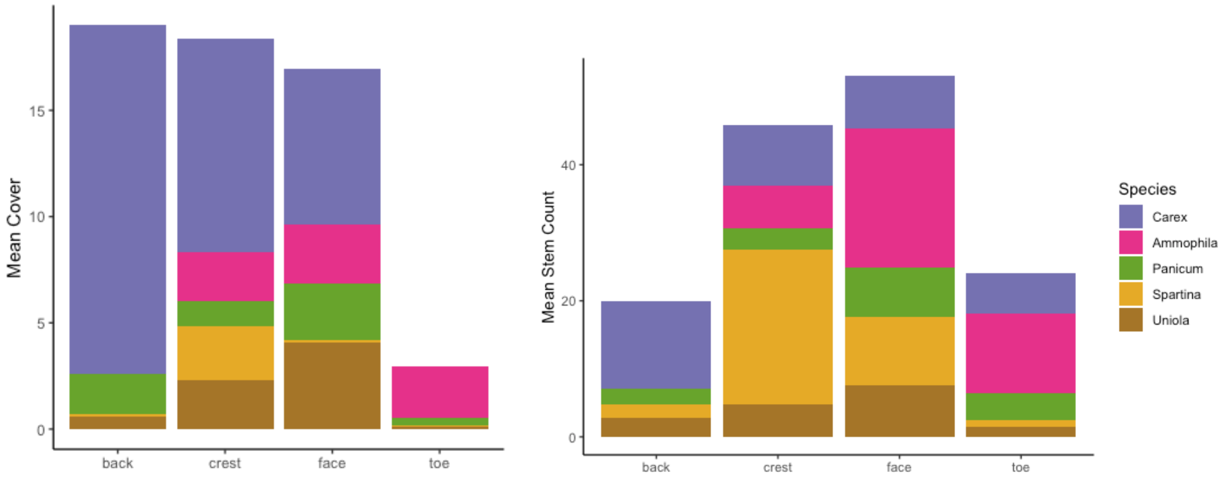

Fig. 1 Average cover (A) and stem counts (B) for species by location, when present (absences were not included in stem counts)

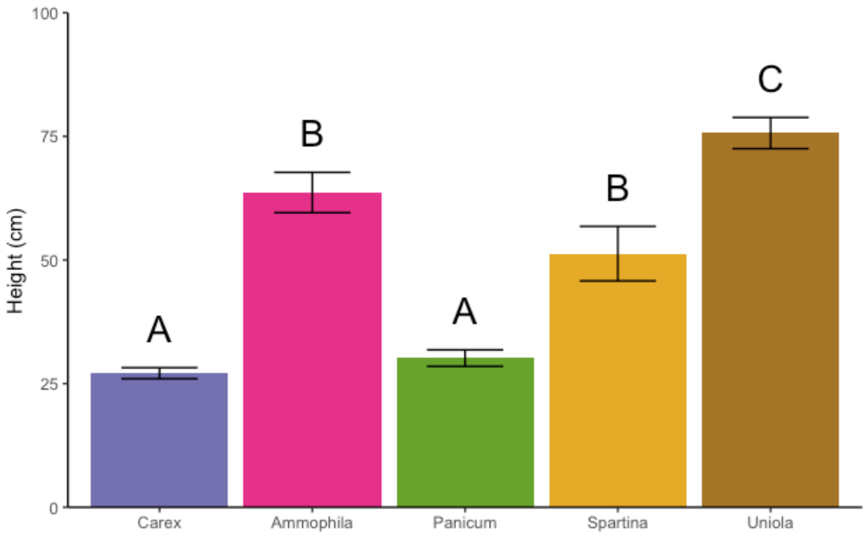

Fig. 2 Height by species results of one-way ANOVA; letter codes represent significant differences from the Tukey multiple comparisons test

Objectives 2 & 3: Compare above and belowground morphological differences between common native species and the invasive sand sedge, as well as between mixed and monoculture plots

All of the specific species plots were monoculture plots, and each individual species were compared to mixed plots. Biomass throughout the cores was highly variable, with the only consistent significant difference being *Uniola,* having more aboveground biomass than the other species (Fig. 3A). This led to a lower root to shoot ratio for *Uniola* as well (Fig. 4). While root biomass was not significantly different per species, there was some variation in root functional traits. The average root diameter for *Uniola* was thicker than *Ammophila*, with *Carex* at a mid level diameter, not significantly different from either of the natives (Fig. 5). Root tissue density was highest for Ammophila, with all other groups not significantly different (Fig. 6), there were no significant differences found for specific root length. Tensile strength showed significant differences between all 3 species ($p<0.0001$) with highest being *Ammophila*, then *Carex*, and lastly *Uniola*. This also follows the same pattern of highest tensile strength with the smallest diameter of roots (Fig. 7).

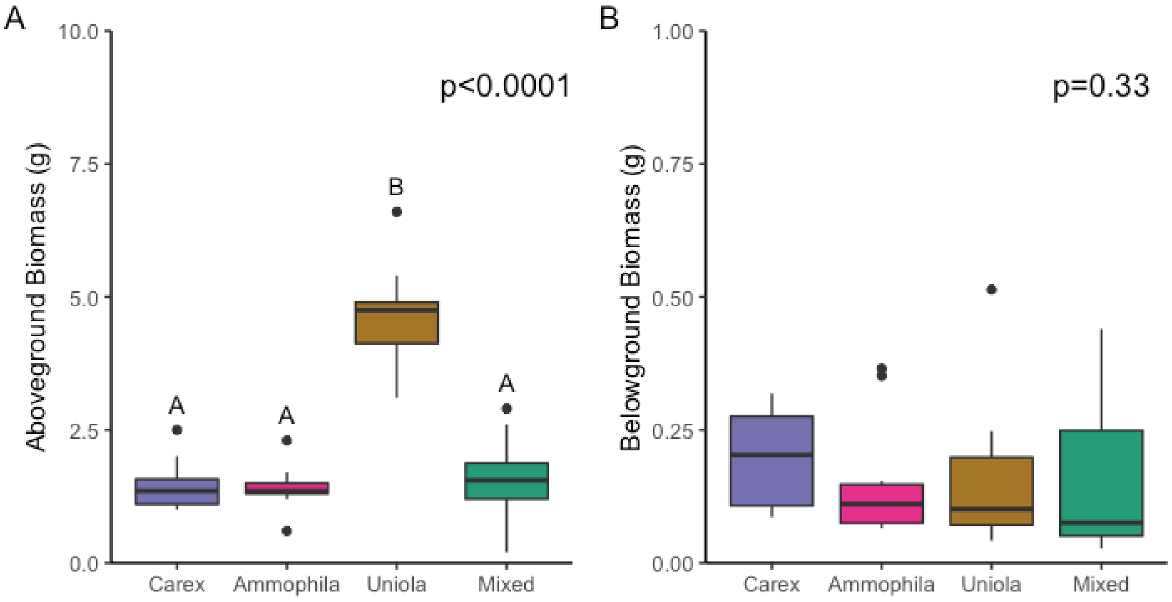

Fig. 3 Biomass of aboveground (A) and belowground (B) material from root cores. P-values displayed are the result of Kruskal-wallis tests. Significant differences are indicated with letter codes.

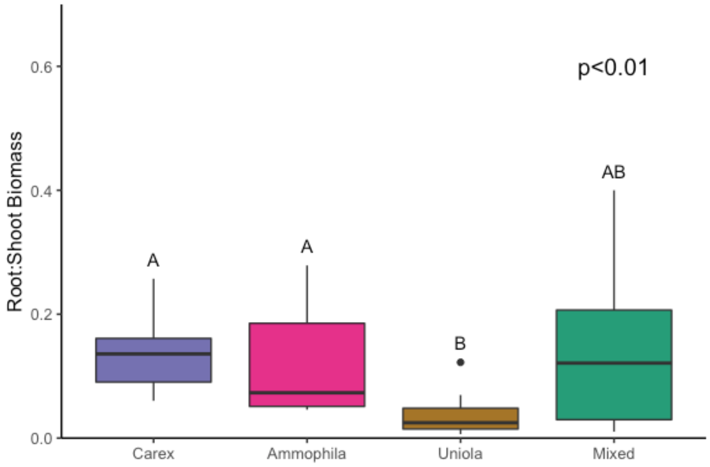

Fig. 4 Root to shoot ratio of biomass material from root cores. P-value is the result of Kruskal- wallis test. Significant differences are indicated with letter codes.

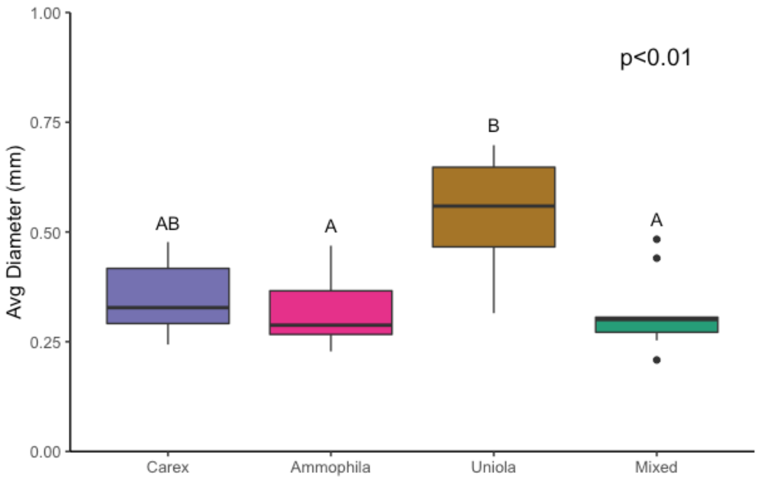

Fig. 5 Average Diameter of roots collected by species and in mixed plots. P-value is the result of Kruskal- wallis test. Significant differences are indicated with letter codes.

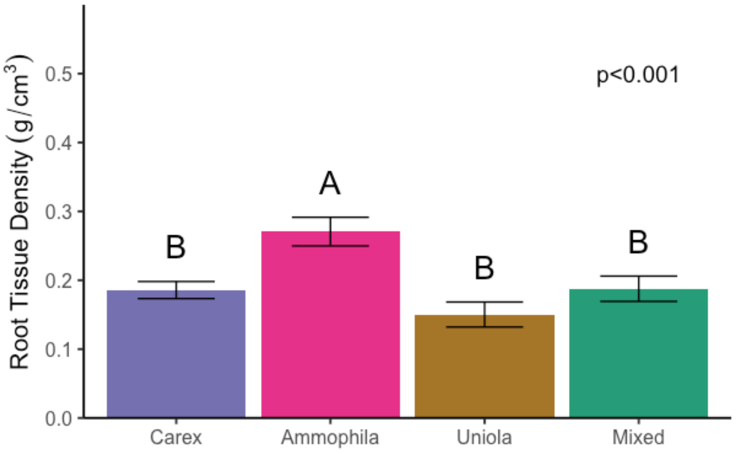

Fig. 6 Root tissue density by species, results of a one-way ANOVA and letter codes indicate significant differences.

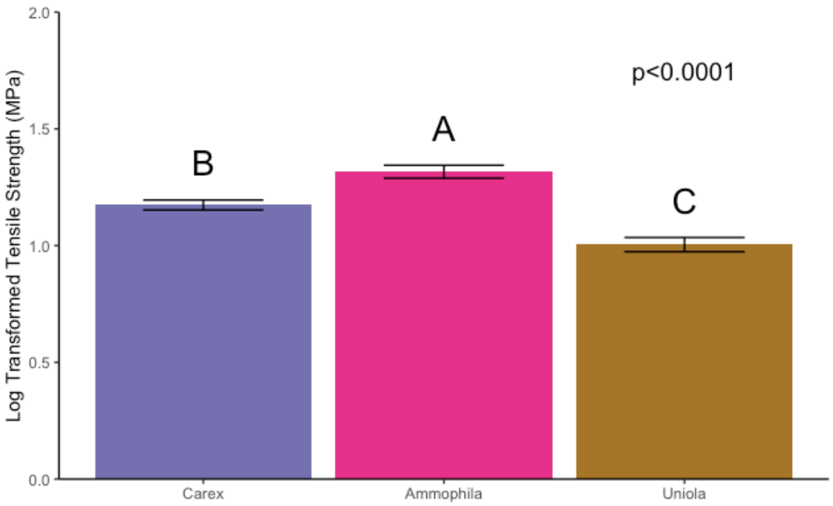

Fig. 7 Log Transformed tensile strength results by species of an ANCOVA. Letter codes indicate significant differences.

Discussion

These results show that *Carex* has the highest cover of any species along the dunes of the northern mile at Back Bay National Wildlife Refuge. Without historic records of species composition along the dunes it is impossible to know if the invasive is taking over open space habitat or pushing natives out of their habitat. However if the spread is anything like they have found in NJ, then we know it is moving into habitat previously occupied by natives. There has been effort at the site to remove the invasive species and plant natives, but this needs to be done in the most efficient way possible to avoid unnecessary costs or damage to the existing dune system. Understanding species interactions and morphological differences will allow for optimal restoration practices.

Previous plantings have consisted primarily of *Ammophila* which is a highly studied dune grass species, but our goal is to make sure this is the correct choice. Through this study we are finding that *Ammophila* has a higher root tissue density and tensile strength than *Carex* or *Uniola*. This is an indicator that *Ammophila* is better for erosion control and can withstand more wind and wave action than the other species. *Uniola* has significantly more aboveground biomass than the other species, which allows for more sediment accretion for dunes. Aboveground growth in *Ammophila* has been found to be stimulated by burial (Brown and ZInnert 2018) which also allows for it to be a good dune builder along with erosion control.

These results show that the natives are preferred for coastal protection over the invasive *Carex* species. Previous studies have shown that *Carex* has significantly more belowground biomass which would make it effective for erosion control (Charbonneau et al. 2017), but that is not what we found here. There was no significant difference of belowground biomass among any of the species tested, but this could be a result of our sampling method. The invasive is also showing no characteristics that will allow it to be a competitive dune builder. *Carex* is also taking over significant portions of the refuge which will need to be removed as to not push out native populations. Restoration of this site will need better understanding of the species specific interactions, which are continuing to be studied. Currently, our understanding of root functional traits is that mixed plots are just more variable than monoculture plots, with no major differences.

Further research on this project is ongoing, including the addition of samples of *Panicum amarum* as well as results for the isotope analysis. *Panicum* samples were collected June of 2021 and are currently being processed for the same analyses as the other species. Isotope samples for the samples collected in summer of 2020 were sent to Cornell Isotope Laboratory in February of 2021 for analysis. The results for which were received in June of 2021 and are currently being statistically analyzed. All results from this project will be published in a manuscript once complete as well as compiled into a chapter of my dissertation.

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Accounting of Expenses

A total of $5950 was spent throughout the last year, with the breakdown of expenses below. Travel included mileage reimbursement to Back Bay National Wildlife Refuge from Richmond, VA. Supplies consisted of various field supplies, including a slide hammer for taking root cores. The majority of the fellowship was spent on the salary for the undergraduate assistant to help with field work and sample processing. The services spent were from COIL (Cornell Isotope Laboratory) to analyze above and belowground samples for carbon and nitrogen isotopes.

	Total Spent
Travel	**$814**
Supplies	**$733**
Undergraduate Assistant	**$3359**
Services	**$1044**