Braving the salt marsh – Plant traits over abiotic and biotic gradients

Research Project for MSc Ecology & Evolution

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Abstract

Organisms have two options in dealing with the climatic conditions of their habitat: they can tolerate them as they are, or they can adapt and escape. The ability of a plant, or a plant community, to persist in a certain area, either by tolerating or adapting to the prevailing conditions, depends on certain traits and the plasticity of these traits. This study aims to find out how certain plant traits (e.g. specific leaf area and specific root length) of a salt marsh grassland community are affected by stressors such as salinity, anoxia, light, and stress induced by large herbivores (i.e. trampling and defoliation). All measured plant traits (i.e. specific leaf area, leaf area index and specific root length show the grazed marsh to be much more dynamic than the ungrazed marsh. These results corresponded with our findings on soil oxygen and electric conductivity. We found strong seasonal differences in the underlying abiotic gradient (i.e. salinity and anoxia), that seemed to stabilize as the growing season progressed. Specific leaf area seemed to be closely connected to light stress (i.e. shading), at least for the 2 shading tolerant species, *Elytrichia atherica* and *Festuca rubra,* that experienced lower specific leaf area and abundance outside the patch on the grazed marsh. The grazing tolerant species, *Puccinellia maritima* and *Juncus gerardii*, were much less abundant (outcompeted) inside patches on the grazed, and inside and outside patches on the ungrazed marsh. For the specific root length of *Elytrichia atherica* we found evidence for a 'quantity over quality' strategy; there was a positive relation between SRL and belowground biomass, which was significant on the grazed marsh, but not on the ungrazed marsh, and also belowground biomass was significantly higher outside than inside patches on the grazed marsh. We conclude that there are strong seasonal differences in the abiotic conditions (i.e. anoxia and salinity), but that these abiotic conditions have less effect on plant functional traits (i.e. specific leaf area and specific root length) than originally expected (i.e. between high and low, grazed and ungrazed). Furthermore, the response of plant functional traits to stressors is dependent on their life strategy, and is largely determined by the environment.

Introduction

Organisms have two options in dealing with the climatic conditions of their habitat: they can tolerate them as they are, or they can adapt and escape (Krebs, 2009). The ability of a plant, or a plant community, to persist in a certain area, either by tolerating or adapting to the prevailing conditions, depends on certain traits and the plasticity of these traits (Maestre et al., 2009). Environmental conditions are, however, not static; they are dynamic. So, if conservation and management endeavours are to be successful, we need to determine how well plant communities can adapt (by means of certain functional traits) in response to changing environmental conditions.

Salt marshes present an opportunity to research the effects of abiotic and biotic stress on plant community structure, where different stages of succession are adjacent to each other. (Olff et al., 1997). On a salt marsh, the vegetation is exposed to salinity, anoxia (Schrama et al., 2012) and light, among others. In addition to these stress factors large herbivores (i.e. cattle) also induce physical stress through defoliation (yet at the same time reducing light stress), trampling (i.e. physical damage) and soil compaction (i.e. increasing anoxia), playing a key role in determining the community structure and functioning of grazing ecosystems (Mikola et al., 2009; Milchunas et al., 1988; Olff and Ritchie, 1998; Schrama et al., 2012).

Vegetation heterogeneity, or vegetation mosaics, initially develop from small differences in the vegetation structure that were already present in the landscape due to some local variation in abiotic gradients (WallisDeVries et al., 1999), e.g. salinity, elevation, pH. Grazing by large herbivores can strengthen these mosaic vegetation patterns where short vegetation dominates the relatively heavily grazed patches, a mixture of vegetation dominates in moderately grazed patches and tall vegetation dominates where ungrazed growth discourages grazing (Andresen et al., 1990; Milchunas et al., 1988; Olff and Ritchie, 1998). WallisDeVries et al. (1999) found that when differences between patch types (i.e. short and tall) are easy to detect, the animals will follow a selective foraging pattern within a certain range (over time creating the vegetation mosaic). Large herbivores prefer short, leafy areas of grasslands that contain relatively high concentrations of nutrients (McNaughton, 1984). However, this does not necessarily yield the highest intake rate; they therefore consume abundant low-quality forage (i.e. tall grasses) as well to bulk up and maximize daily intake (WallisDeVries et al., and references therein, 1999; Demment and Van Soest, 1985).

In harsh, limiting environments (e.g. salt marshes), some species are able to ameliorate the environmental conditions (i.e. abiotic stress and grazing pressure) thereby providing more suitable habitats for other species (Flores and Jurado, 2003; Padilla and Pugnaire, 2006; Smit et al., 2007). This is known as the 'nurse plant syndrome' or 'nurse-protégé' interaction (Flores and Jurado, and references therein, 2003). This interaction is considered to be a form of facilitation (Bertness and Callaway, 1994; Flores and Jurado, 2003).

The configuration of species along a stress gradient (e.g. salinity) is determined by plant traits (Maestre et al., 2009). Only plants that are adapted to the high stress end of this gradient can withstand the 'harsh' conditions (Maestre et al., 2009). Stress can affect growth rate (of both shoots and roots) and the quality of leaf and root material (e.g. nitrogen content and photosynthesis rates) (Olff et al., and references therein, 1997). The effect of age (i.e. life stage) on variables is often significant (Olff et al., 1997) and therefore an important factor to take in to account as well. By quantifying the effects of abiotic and biotic stress on plant traits we hope to provide further insight into mechanisms that drive plant interactions, community composition and diversity; we want to find out which plant traits are most important in dealing with salinity, anoxia, light, and stress induced by large herbivores. For this we will determine plant traits of 5 focal species *Elytrichia atherica*, *Festuca rubra, Artemisia maritima, Puccinellia maritima, Juncus gerardii* and traits of the protector species *Juncus maritimus*, under abiotically and biotically driven stress gradients both outside and inside *Juncus maritimus* patches (i.e. tall defended plants), between grazed and ungrazed situations, and along an elevation gradient from low to high marsh.

Earlier (unpublished) work showed that vegetation height on the salt marsh of Schiermonnikoog differed significantly between inside and outside patches of *Juncus maritimus* on the grazed marsh and that these differences were greater at higher elevation. On the ungrazed marsh no differences were found between inside and outside patches of *Juncus maritimus* and elevation was not significant. Therefore we hypothesize for stress-related plant functional traits:

Grazed

- Differences between inside and outside patches of *Juncus maritimus.*
- Differences will be greater at higher elevation.

Ungrazed

- No differences between inside and outside patches of *Juncus maritimus*.
- No differences along the elevation gradient.

Our expectations for plant functional traits are summarized in table 1.

Table 1: *Hypothesized plant functional traits for our two focal species, Elytrichia atherica and Festuca rubra, for the grazed and ungrazed salt marsh both inside and outside Juncus maritimus patches from low to high marsh.*

		Grazed		Ungrazed	
Traits	**Zone**	**Inside**	**Outside**	**Inside**	**Outside**
Vegetation Height	High	++	-	++	++
	Low	+	-	++	++
SLA/SRL	High	++	-	++	++
	Low	+	-	++	++
LAI	High	++	-	++	++
	Low	+	-	++	++

Definition of terms:

- Low marsh is defined as ≤ 150 cm above NAP, high marsh is defined as ≥ 151 cm above NAP.
- Specific Leaf Area (SLA) (mm^2/mg), ↓ SLA = higher quality, typical under stressful conditions, ↑ SLA = lower quality, low stress.
- Specific Root Length (SRL) (cm/g), ↓ SRL = higher quality, typical of fibrous root systems ↑ SRL = lower quality, low stress.
- Leaf Area Index (LAI), measure for the interception of light by the canopy.
- "+", "++" positive effect. The differences between symbols indicate that we expect a significantly larger positive effect, e.g. in ungrazed conditions we expect plants to grow significantly faster and produce more biomass than short grazed vegetation.
- "-", "--" negative effect. The differences between symbols indicate that we expect a significantly larger negative effect.

Materials and Methods

Study System

Field work took place on the salt marsh of the eastern part of the West Frisian Island of Schiermonnikoog (53°29′N 6°10′E), see figure 1. On this island different stages of succession are adjacent to each other, as the island is slowly moving eastwards (Olff et al., 1997). We focused our efforts on the ungrazed marsh (UNG), which has never been grazed by cattle, and the Toekomstig Begraasde Kwelder (TBK), which has been grazed by cattle for 25 years. Each area contains 15 paired plots (i.e. inside patches of *Juncus maritimus* and outside patches) that are similar to each other, apart from grazing. These plots were distributed along an elevation gradient from low to high marsh (i.e. from South to North); low marsh was defined as ≤ 150 cm above NAP and high marsh was defined as ≥ 151 cm above NAP.

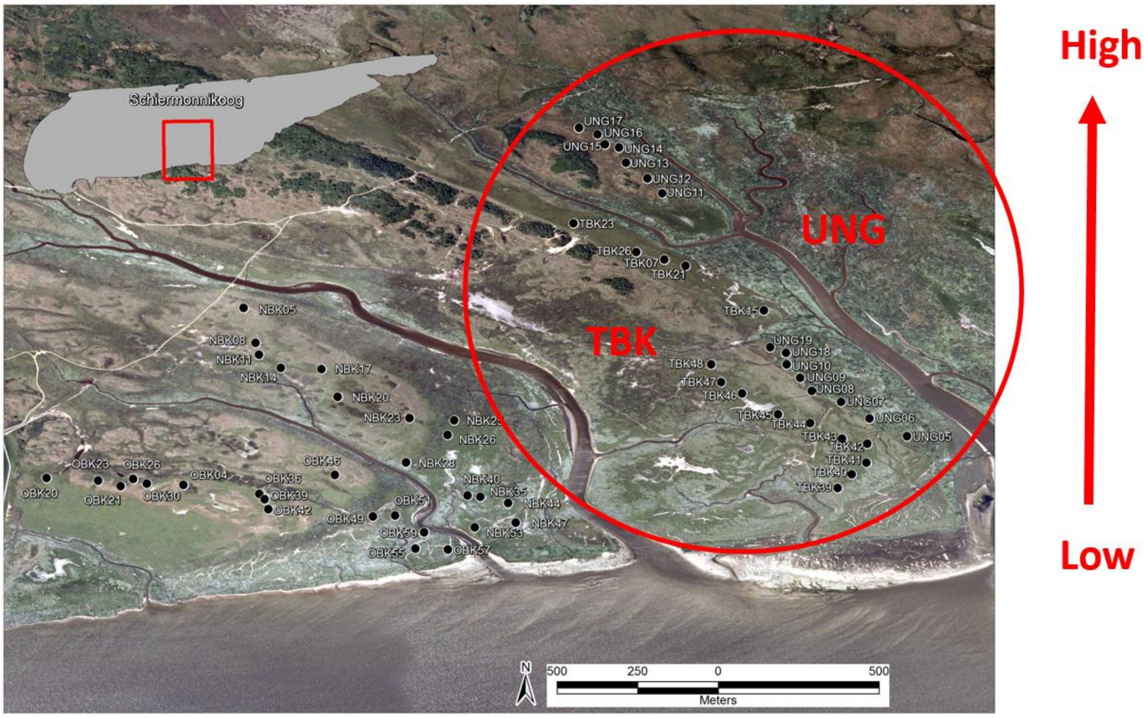

Figure 1: The location of our 30 paired study sites. We used the 25 years grazed marsh (TBK) on the left, and the late successional "ungrazed" marsh (UNG) on the right, with sites ranging from the low marsh (South) to the high marsh (North).

Abiotic variables: soil oxygen and salinity

We used electric conductivity as a proxy for salinity. At each site 2 soil samples were taken with 100 cm^3 sized PF-rings; one inside the patch and one outside the patch. These soil samples were cut in half, using one half to measure the % moisture and bulk density, and the other half for the salinity measurement. The salinity half was dissolved in a fixed volume of demi water (100 mL) and subsequently filtered. The filtered solutions were then measured with an EC meter, type HI98188 (© 2005-2011 Hanna Instruments). Measurements were done in May 2012, March 2013 and June 2013.

Soil redox potential was measured in situ using 5 platinum tipped electrodes and 1 calomel reference electrode (© 2013 Cole-Parmer) connected to a Graphtec GL200 Datalogger (© 2013 Graphtec GB Ltd.), both inside and outside of patches. See Schrama et al. (2012) for a detailed description of use. Measurements were taken at a depth of 5 cm, because this is the main rooting area. Measurements were done in May 2012, March 2013 and June 2013 to capture the possible differences from winter to growing season.

Biotic variables: SLA, LAI and SRL

We sampled plant traits of multiple focal species, *Elytrichia atherica*, *Festuca rubra, Artemisia maritima, Puccinellia maritima and Juncus gerardii,* and traits of the protector species *Juncus maritimus* over 30 paired sites (i.e. TBK and UNG, see fig. 1) and explored that data combined with the data on abiotic and biotic gradients we collected on the salt marsh of Schiermonnikoog in 2012. Additionally, specific leaf area (SLA in mm^2/mg) was measured in the greenhouse to compare optimal growing conditions with the field situation and to give an idea of the genetic (plastic) component of this trait. Seeds of the 6 focal species were collected in fall (2012) and grown in separate pots in the greenhouse for at least 6 months. We can therefore assume that possible elevation, grazing and patch effects were no longer present. For SLA we sampled leaves from both the edge and center of the pots to counter for possible shading effects.

Both SLA (specific leaf area in mm^2/mg) and SRL (specific root length in cm/g) give information about the quality of the plant and investments both above- and belowground (Kembel and Cahill Jr., 2011). For SLA aboveground plant material of 5 individuals of *Juncus maritimus, Elytrichia atherica, Festuca Rubra, Artemisia maritima, Puccinellia maritima and Juncus gerardii* were harvested at sites located along the elevation gradient (i.e. from low to high marsh), inside and outside of *Juncus maritimus* patches and under grazed and ungrazed conditions (fig. 1). This leaf material was dried in the oven at 70 °C for at least 48 hours and subsequently weighed to determine the dry weight. The surface area of the leaves was determined by photographing the leaves and calculating the area with the automated image analysis software program SigmaScan Pro (Copyright © 2013 Systat Software Inc.).

For the SRL we collected soil samples which were carefully washed until only the root material remained. For each site 3 subsamples were taken, separated into absorptive roots and rhizomes for both *Juncus maritimus* and *Elytrichia atherica*, with the remaining unidentifiable absorptive root material categorized as 'unidentified'. Root length was measured using a modified line intersect method from Tennant (1975); root length (R) = 11/14 * number of intercepts (N) * grid unit. The root material was suspended in a water filled Petri dish on top of a piece of paper with 0.5 cm grids. After measuring their length, root samples were dried in the oven at 70 °C for at least 48 hours and subsequently weighed to determine the dry weight.

Plant height (cm) of the 6 focal species was measured to the top of the tallest leaf of an individual by means of a ruler in situ at the study sites (fig. 1). Alongside this we measured the LAI (leaf area index) using an AccuPAR LAI Ceptometer model LP-80 (© 2013 Decagon Devices, Inc.). The LAI is the percentage of ambient light interception by the canopy.

Statistics

All analyses were performed with the R v2.15.2 (R Core Team, 2012) program for statistical computing.
Scatterplot matrices (Zuur et al., 2010) were used to get an overview of the data. This was followed by Principal Component Analysis that was visualized using bi-plots. Elevation data violated the assumption of normality, log-transforming did not change this. A boxplot of elevation over the 3 different elevation zones showed an outlier, site UNG13. A subset was created in which this outlier had been removed and now the elevation data was close to normal, using the Shapiro-Wilk test (Crawley, 2005). The Levene test was used to check the homogeneity of variance. Creating subsets for both the grazed and the ungrazed marsh caused elevation to pass the assumption of normality and homogeneity of variance.

To analyze the effect of grazing on soil oxygen levels (redox) and salinity (electric conductivity), an ANCOVA model was used with redox/salinity as response variable and grazing and patch as explanatory variables, including the interaction between grazing and patch. This was the best fit model after comparing several models' AIC (Aikake information criterion) values. For further analysis robust model selection procedures were used (VIF values for collinearity and backwards stepwise regression for best fit model) until only patch remained as the most important explanatory variable, in order to test for differences between patches on both the grazed and the ungrazed marsh. Barplots (Kabacoff, 2012) were used to show the seasonal trends in redox and electric conductivity (fig. 2a and 3a).

SLA, LAI and SRL were analyzed using ANCOVA models and TukeyHSD. Letters were assigned according to the TukeyHSD results, where means with the same letter were not significantly different (fig. 4, 5, 6 and 7). Greenhouse vs. field comparison of the SLA trait was visualized using barplots (fig. 8) (Kabacoff, 2012). We also performed a detrended correspondence (DCA) analysis, see Oksanen (2013) for details on use. Here we determined for *Elytrichia atherica* and *Juncus maritimus* which environmental variables were most important in explaining the species traits (SLA, LAI, SRL of both absorptive roots and rhizomes, vegetation height and abundance).

Results

Abiotic variables: soil oxygen & salinity

For both the redox and salinity measurements we found evidence of strong seasonal effects over time (fig. 2a and 3a). For each of the measured months, redox values on the ungrazed marsh were significantly higher (ANCOVA (May): $F = 52.4$, $r^2 = 0.56$, $p < 0.001$; ANCOVA (March): $F = 22.2$, $r^2 = 0.31$, $p < 0.001$; ANCOVA (June): $F = 14.1$, $r^2 = 0.30$, $p < 0.001$) than on the grazed marsh. Boxplot data exploration showed the spread in data on the grazed marsh decreasing from March to May to June, so there seems to be a stabilizing effect as the growing season progresses. Figure 2a shows that there are no significant differences in soil oxygen on the ungrazed marsh between inside and outside patches of *Juncus maritimus* in May, March and June. When comparing inside patches to outside patches on the grazed marsh, we find no significant differences (fig. 2a). Splitting the data even further into high and low elevation zones (fig. 2b, 2c and 2d), we find a significant difference in May at high elevation between inside and outside the patch (TukeyHSD: $p < 0.05$, see fig. 2b). For all other groups elevation was not significant.

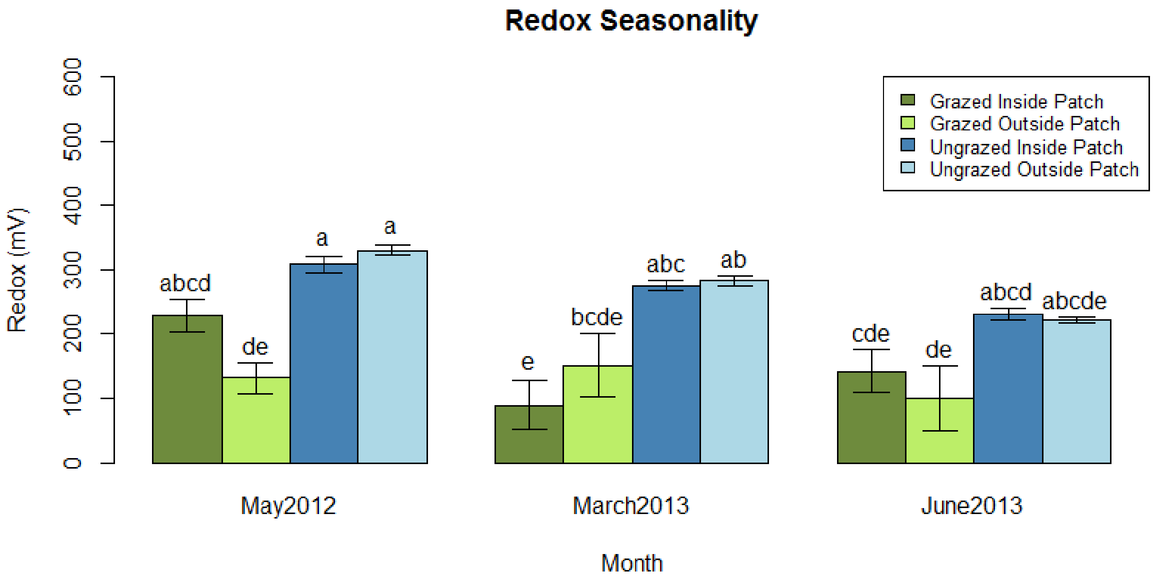

Figure 2a: *Redox (mV) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus. Means with the same letter are not significantly different. Measurements done in May 2012, March 2013 and June 2013.*

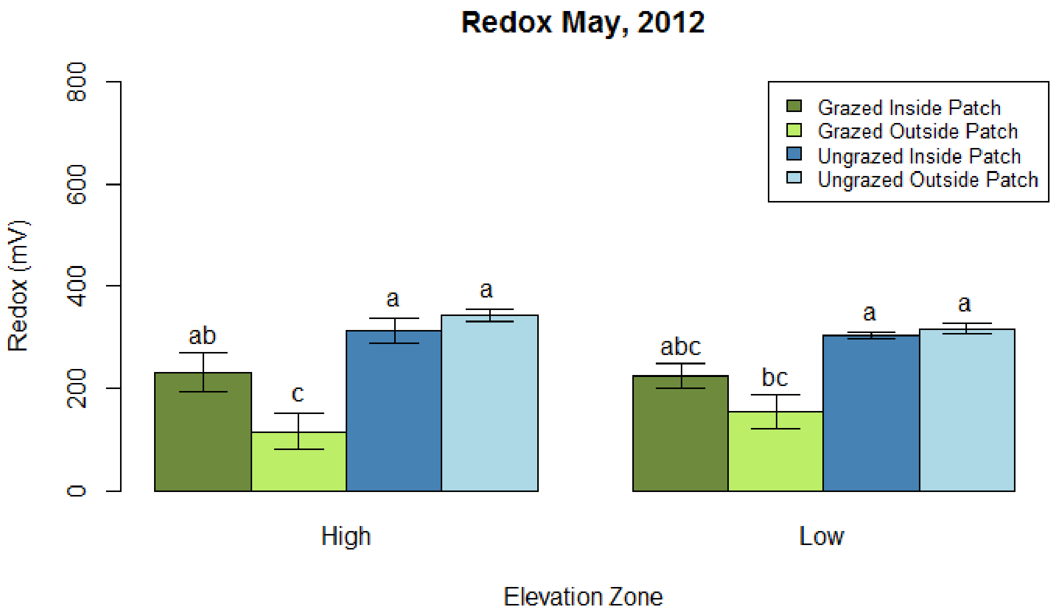

Figure 2b: *Redox (mV) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in May 2012.*

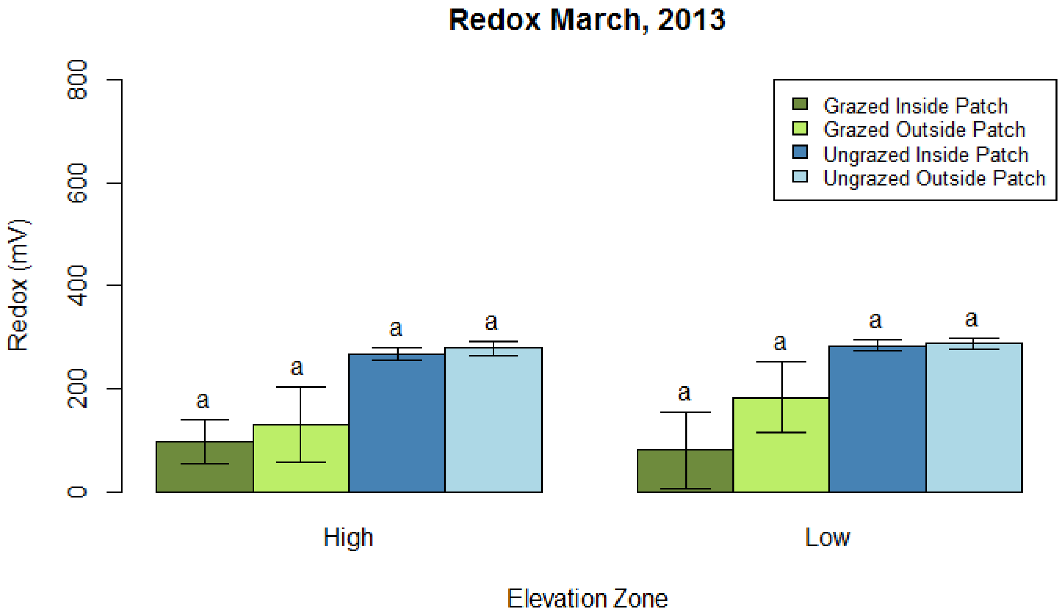

Figure 2c: *Redox (mV) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in March 2013.*

Figure 2d: *Redox (mV) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in June 2013.*

For salinity grazing was significant both in March (ANCOVA: $F = 32.0$, $r^2 = 0.40$, $p < 0.001$) and May (ANCOVA: $F = 7.2$, $r^2 = 0.37$, $p < 0.01$), with electric conductivity always being higher on the ungrazed than on the grazed marsh. In June there was no significant difference in electric conductivity between the grazed and ungrazed marsh. There were, for each of the measured months, no significant differences found between inside and outside patches of *Juncus maritimus* (fig. 3a). Elevation was significant (ANCOVA: $F = 25.4 r^2 = 0.49$, $p < 0.001$), but only in May and only on the grazed marsh. March 2013 showed a unique result after splitting into high and low elevation categories; at high elevation there was a significant difference between the grazed and the ungrazed marsh (TukeyHSD: $p < 0.001$), which was not significant at low elevation (fig. 3b).

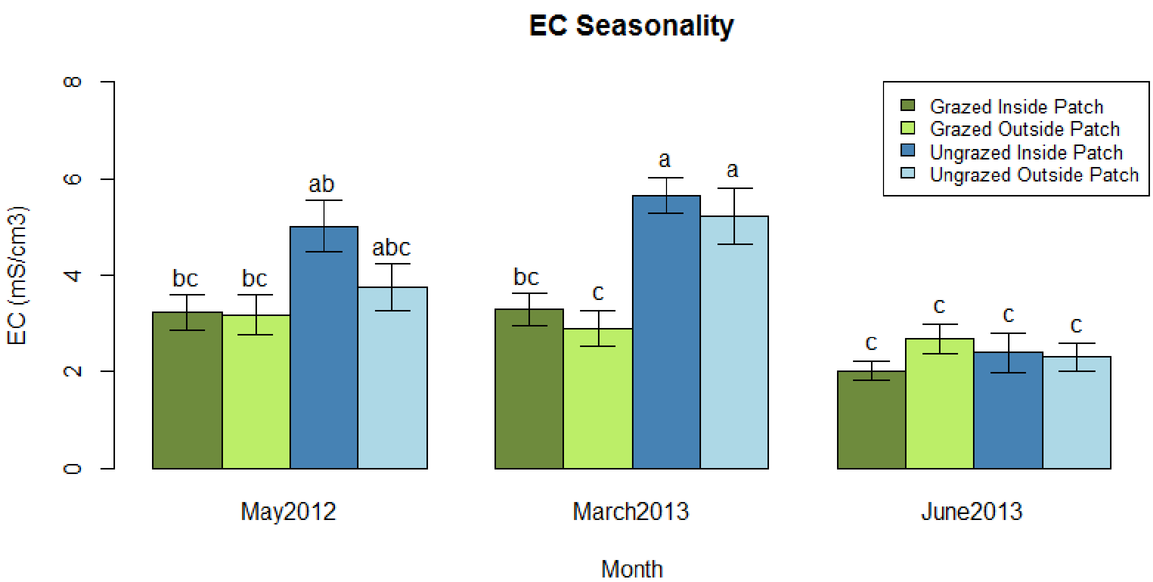

Figure 3a: *EC (mS/cm³) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus. Means with the same letter are not significantly different. Measurements done in May 2012, March 2013 and June 2013.*

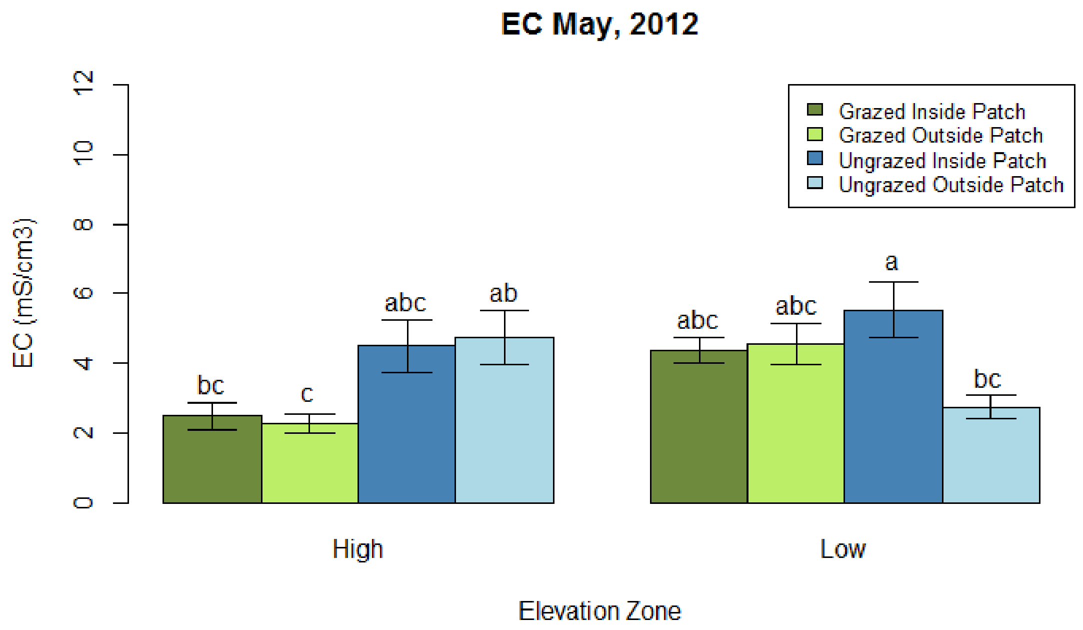

Figure 3b: *EC (mS/cm³) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in May 2012.*

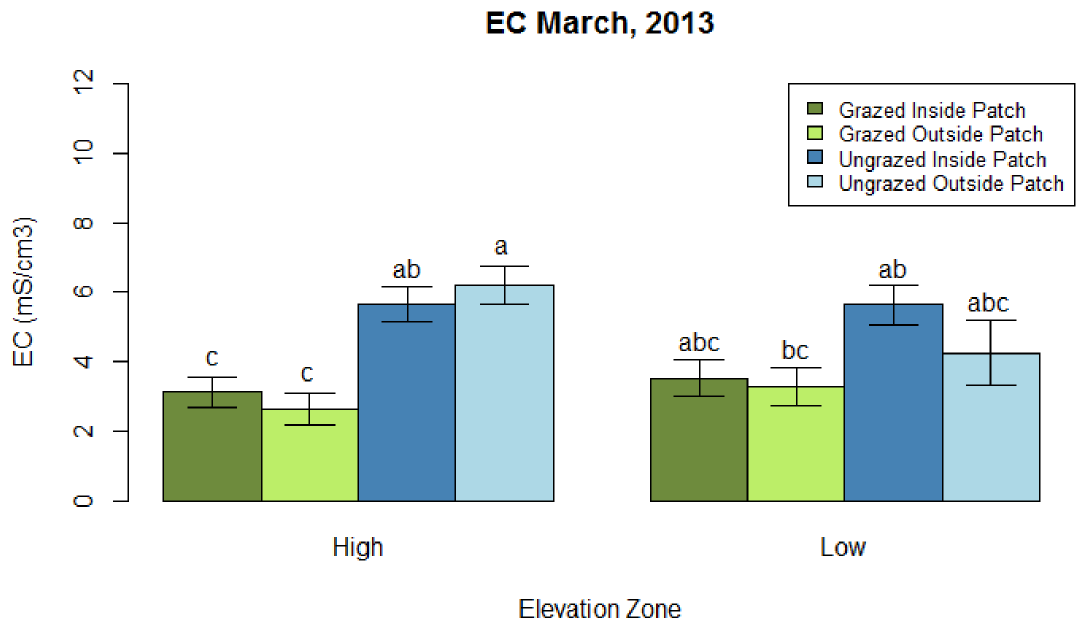

Figure 3c: *EC (mS/cm³) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in March 2013.*

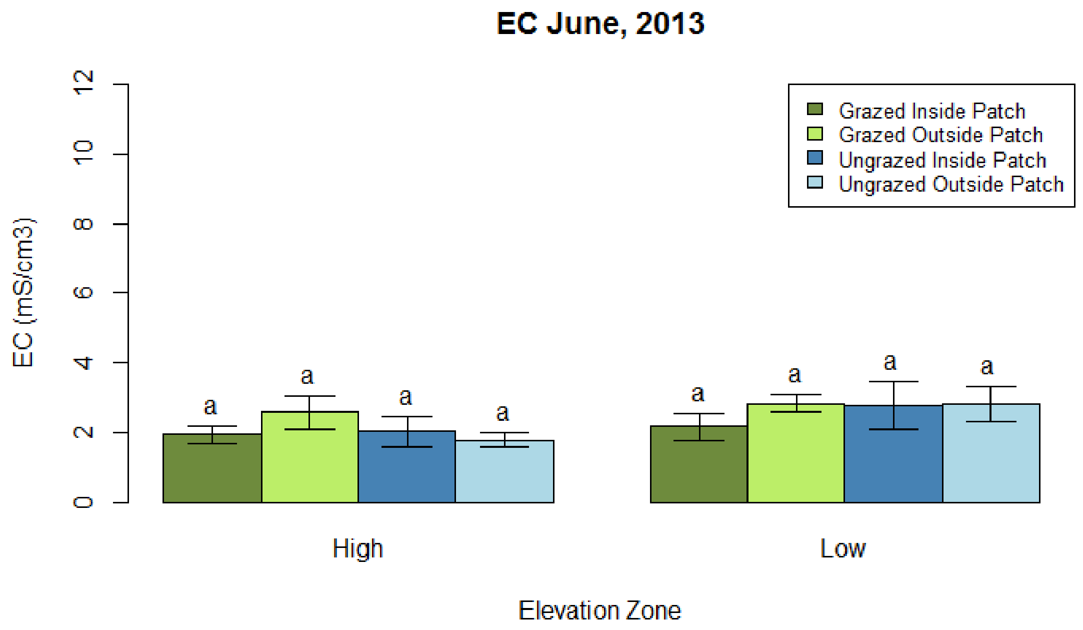

Figure 3d: *EC (mS/cm³) for both the grazed and the ungrazed marsh, both inside and outside patches of Juncus maritimus and at high (≥ 151 cm above NAP) and low (≤ 150 cm above NAP) elevation. Means with the same letter are not significantly different. Measurements done in June 2013.*

Biotic variables: SLA, LAI and SRL

Specific leaf area (SLA) showed no significant differences between the grazed and the ungrazed marsh, between inside and outside patches of *Juncus maritimus* and between low and high marsh (fig. 4a). However, there is a trend with SLA outside patches of *Juncus maritimus* being higher than inside patches of *Juncus maritimus* on both the grazed and ungrazed marsh (fig. 4a).

When using a TukeyHSD on SLA at the community level, we found no significant differences between inside and outside patches and between low and high marsh for all 6 focal species on both the grazed and ungrazed marsh. Figure 4b shows the SLA's for different species, where we see no significant difference between inside and outside patches for *Artemisia maritima, Elytrichia atherica* and *Festuca rubra*. The two grazing tolerant species, *Puccinellia maritima* and *Juncus gerardii*, were absent inside patches and on the ungrazed marsh.

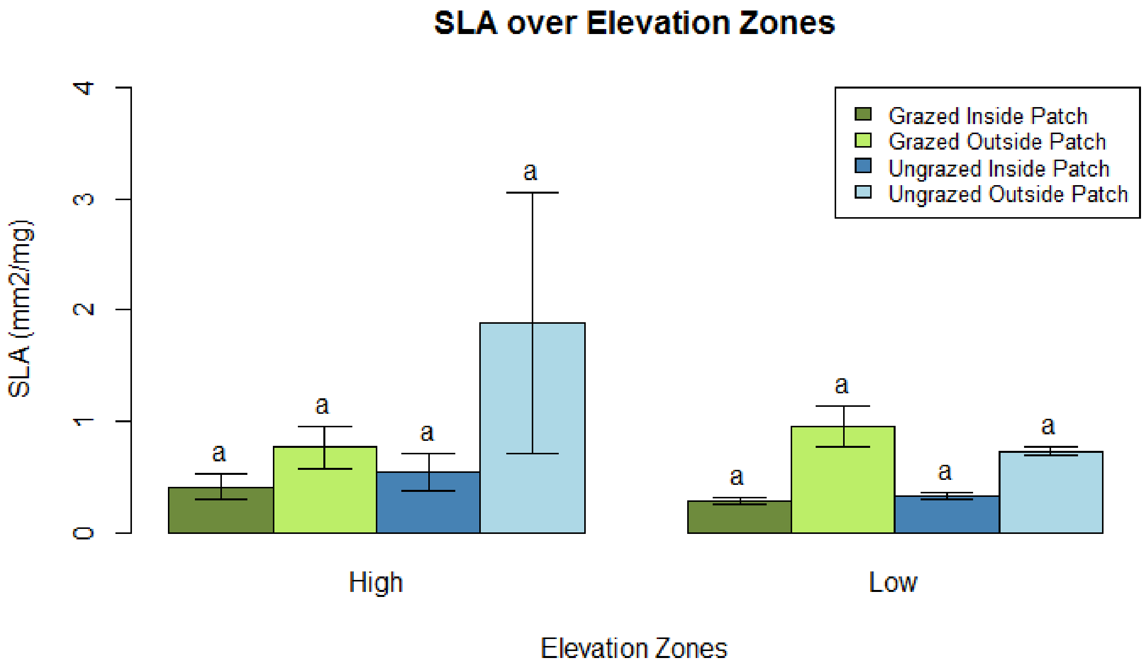

Figure 4a: *SLA (mm²/mg) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus across different elevation zones (i.e. low and high marsh). Means with the same letter are not significantly different.*

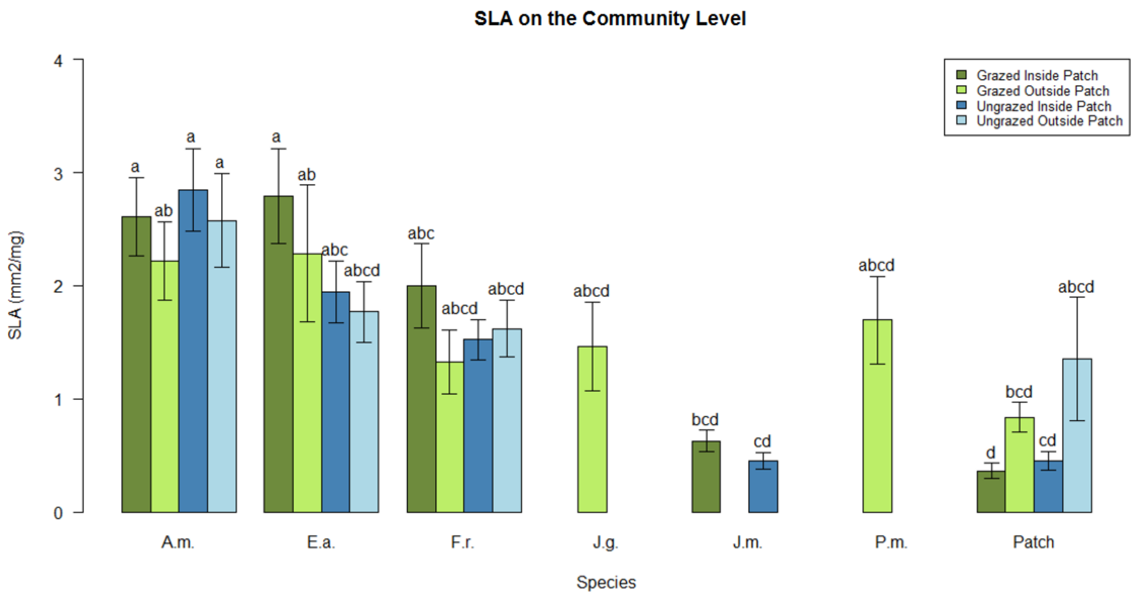

Figure 4b: *SLA (mm²/mg) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus for each of the 6 focal species and a patch average. Means with the same letter are not significantly different.*

The leaf area index (LAI) showed a significant difference between the grazed and ungrazed marsh (ANOVA: $F = 7.7$, $r^2 = 0.36$, $p < 0.01$). There was a very clear significant difference between inside and outside patches on the grazed marsh (ANOVA: $F = 39.4$, $r^2 = 0.58$, $p < 0.001$). Inside and outside patches on the ungrazed marsh were not significantly different (ANOVA: $F = 0.9$, $r^2 = 0.03$, $p > 0.05$). There were no significant differences in LAI between the inside grazed marsh and the inside and outside ungrazed marsh, both at high and low elevation (fig. 5).

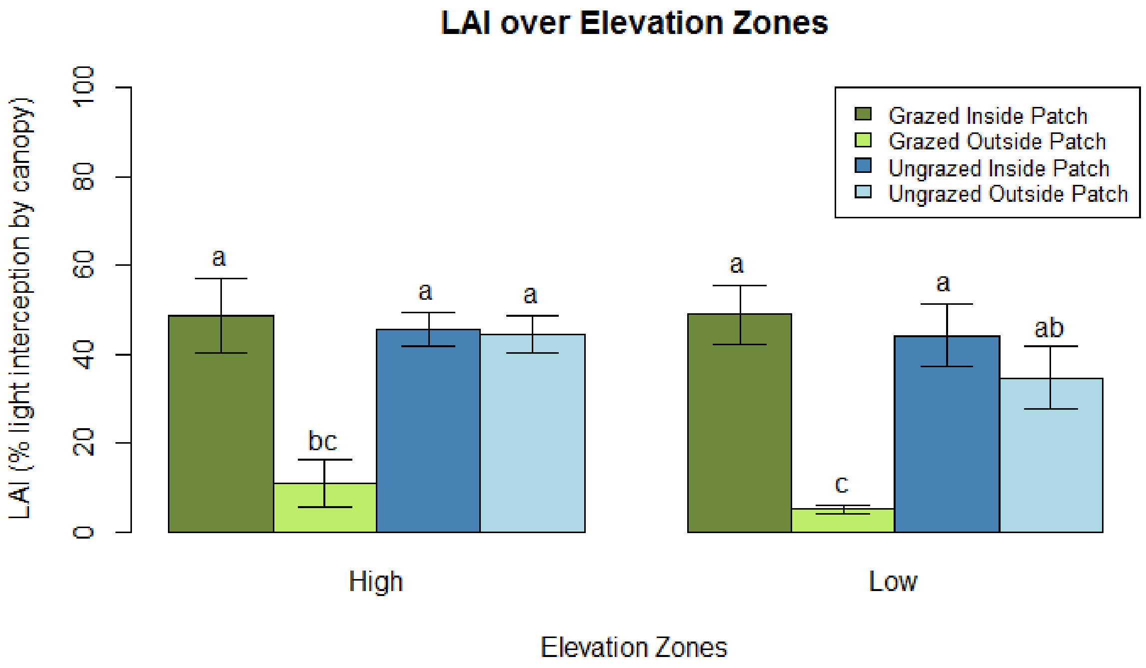

Figure 5: *LAI (% light interception by canopy) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus across different elevation zones (i.e. low and high marsh). Means with the same letter are not significantly different.*

For specific root length (SRL) there was no significant difference between the grazed and the ungrazed marsh. SRL was significantly higher outside patches of *Juncus maritimus* than inside patches of *Juncus maritimus* on the grazed marsh (fig. 6a). The ungrazed marsh showed a similar trend, however, here the difference was not significant (fig. 6a). When separating the data further into high and low marsh categories, SRL outside patches of *Juncus maritimus* is still higher than inside patches of *Juncus maritimus*, both for the grazed and the ungrazed marsh (fig. 6a). The TukeyHSD reported no significant differences between high and low marsh on the patch level. Separating the SRL data even further shows us the trait on a community level with *Juncus maritimus*, *Elytrichia atherica* and the unidentified root material (fig. 6b). At the community level *Juncus maritimus* has the lowest SRL, the unidentified root material has by far the highest SRL and *Elytrichia atherica* has an intermediate SRL (fig. 6b). When looking at root types, rhizomes have a significantly lower SRL than absorptive roots for *Elytrichia atherica*, (fig. 6c). The root types of *Juncus maritimus* show the same trend, but here it's not significant (fig. 6c). Root types showed no significant difference between low and high marsh except for the unidentified root material on the ungrazed marsh, which showed SRL to be higher on the high marsh (TukeyHSD: $p < 0.01$). Figure 6d shows the SRL of *Elytrichia atherica* on the species level separated into different root types (i.e. absorptive roots and rhizomes). Rhizomes of *Elytrichia atherica* had a significantly lower SRL than absorptive roots (ANOVA: $F = 206.6$, $r^2 = 0.64$, $p < 0.001$). Rhizomes showed no significant differences between grazed and ungrazed, and between inside and outside patches (fig. 6d). There was a significant difference in SRL of the absorptive roots between inside and outside patches on the grazed marsh (TukeyHSD: $p < 0.05$), with SRL being higher outside the patch (fig. 6d). The inside patch on the grazed marsh did not differ significantly from the inside and outside patches on the ungrazed marsh (fig. 6d).

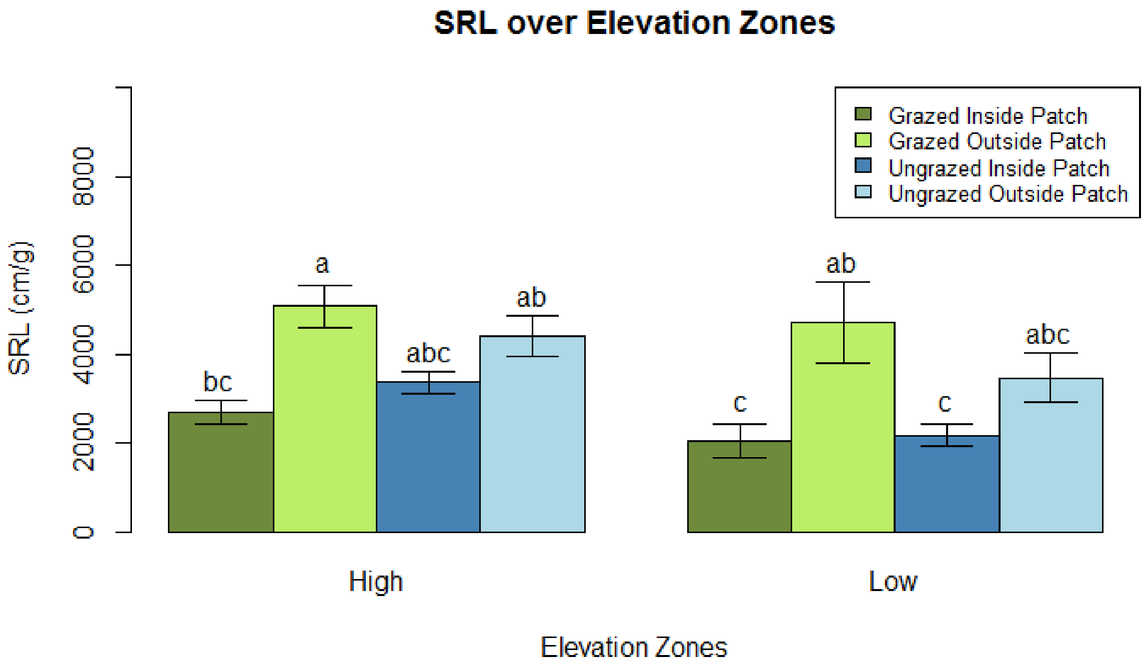

Figure 6a: *SRL (cm/g) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus across different elevation zones (i.e. low and high marsh). Means with the same letter are not significantly different.*

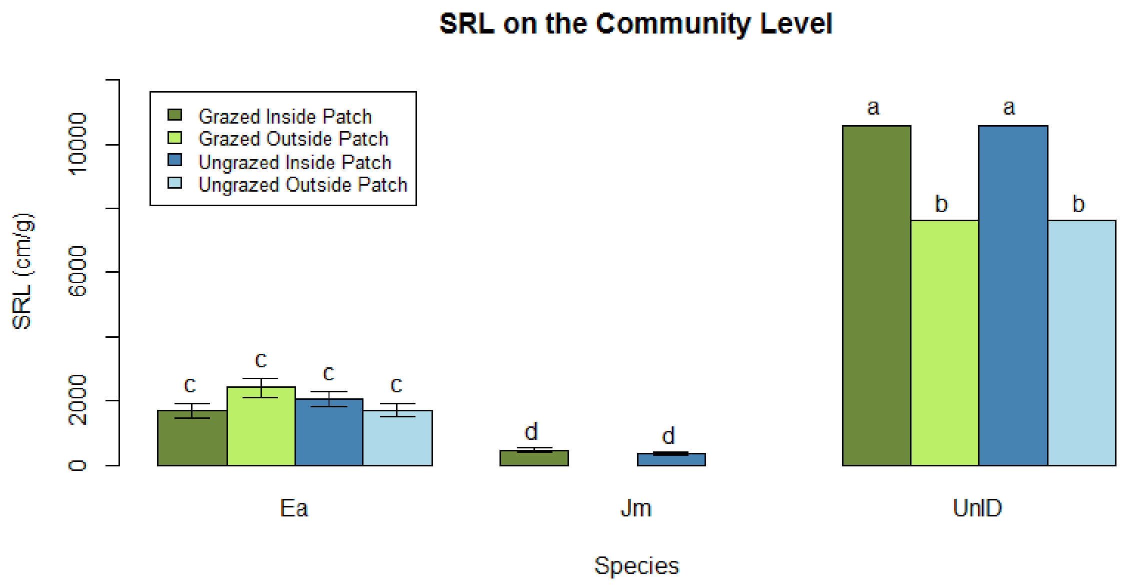

Figure 6b: *SRL (cm/g) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus for 2 focal species (i.e. Juncus maritimus (J.m.) and Elytrichia atherica (E.a.)) and the unidentified root material (UnID)). Means with the same letter are not significantly different.*

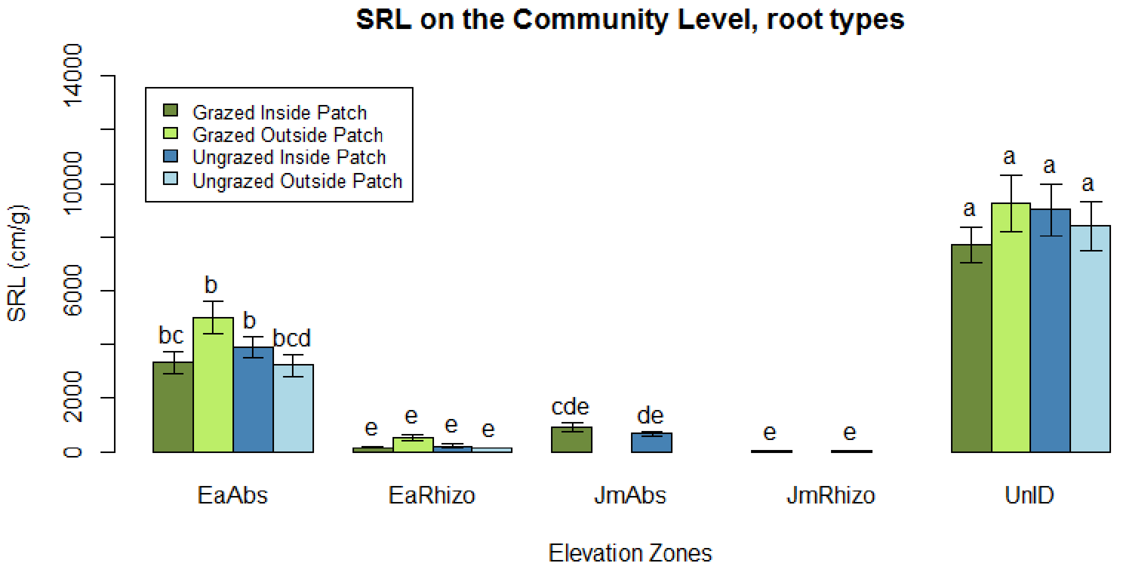

Figure 6c: *SRL (cm/g) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus for 2 focal species (i.e. Juncus maritimus (J.m.) and Elytrichia atherica (E.a.)) and the unidentified root material (UnID) separated into absorptive roots (Abs.) and rhizomes (Rhizo.)). Means with the same letter are not significantly different.*

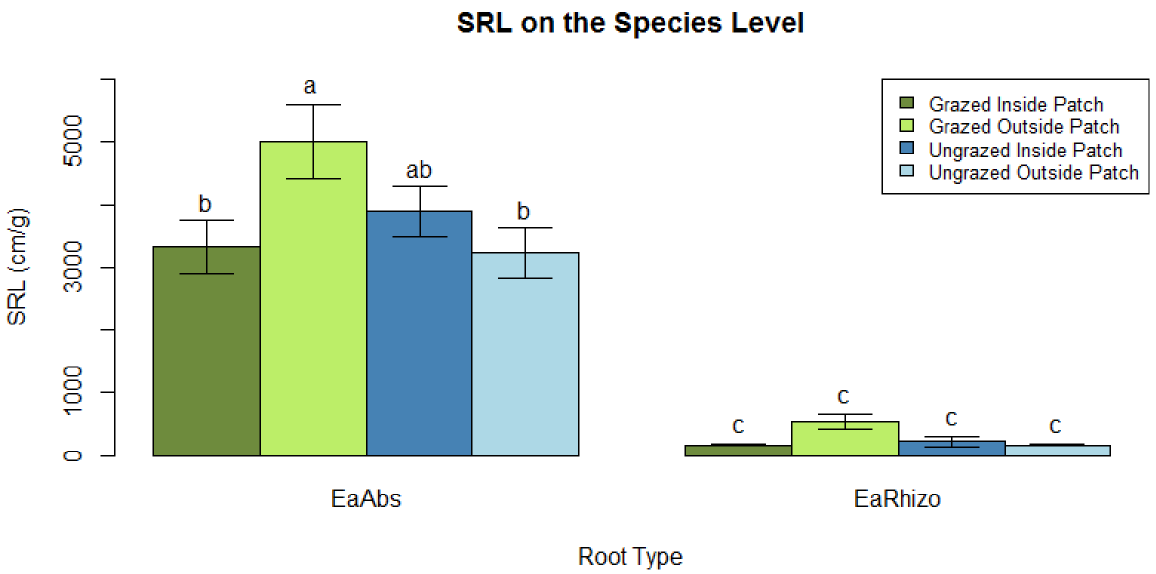

Figure 6d: *SRL (cm/g) of Elytrichia atherica (E.a.) for both the grazed and ungrazed marsh, inside and outside patches of Juncus maritimus separated into root types (i.e. absorptive (abs.) and rhizomes (rhizo.)). Means with the same letter are not significantly different.*

The positive relation between SRL and SLA yielded a significant result, both before (ANOVA: $F = 6.5$, $r^2 = 0.10$, $p < 0.05$) and after (ANOVA: $F = 12.8$, $r^2 = 0.18$, $p < 0.001$) log transforming. SRL proved to be positively related to the belowground biomass, significantly so (ANOVA: $F = 9.1$, $r^2 = 0.40$, $p < 0.01$); further exploration showed this was due to the grazed marsh (ANOVA: $F = 20.8$, $r^2 = 0.48$, $p < 0.001$), as there was no significant relation between SRL and belowground biomass on the ungrazed marsh.

DCA analysis suggested that belowground biomass (BGBM) was important in explaining the quality of the *Elytrichia atherica* rhizomes and absorptive roots, showing a negative relation between BGBM and root quality. For Juncus maritimus root quality was best explained by the percentage of bare soil; root quality seemed to decrease with increasing percentage of bare soil. SLA of both *Elytrichia atherica* and *Juncus maritimus* was not well explained by any of the environmental variables in the DCA analyses.

Greenhouse comparison

Comparing SLA measured in the greenhouse with SLA measured in the field showed for all 6 focal species (i.e. *Juncus maritimus, Elytrichia atherica, Festuca rubra, Artemisia maritima, Puccinellia maritima and Juncus gerardii*) higher SLA values in the greenhouse situation (fig. 7a). Separating the data for different grazing histories does not show a difference in field SLA between the grazed and the ungrazed marsh. No differences between inside and outside patches (see also fig. 4a and 4b). Figure 7a, 7b and 7c contain no error bars and letters, because the greenhouse measurements have no replicas.

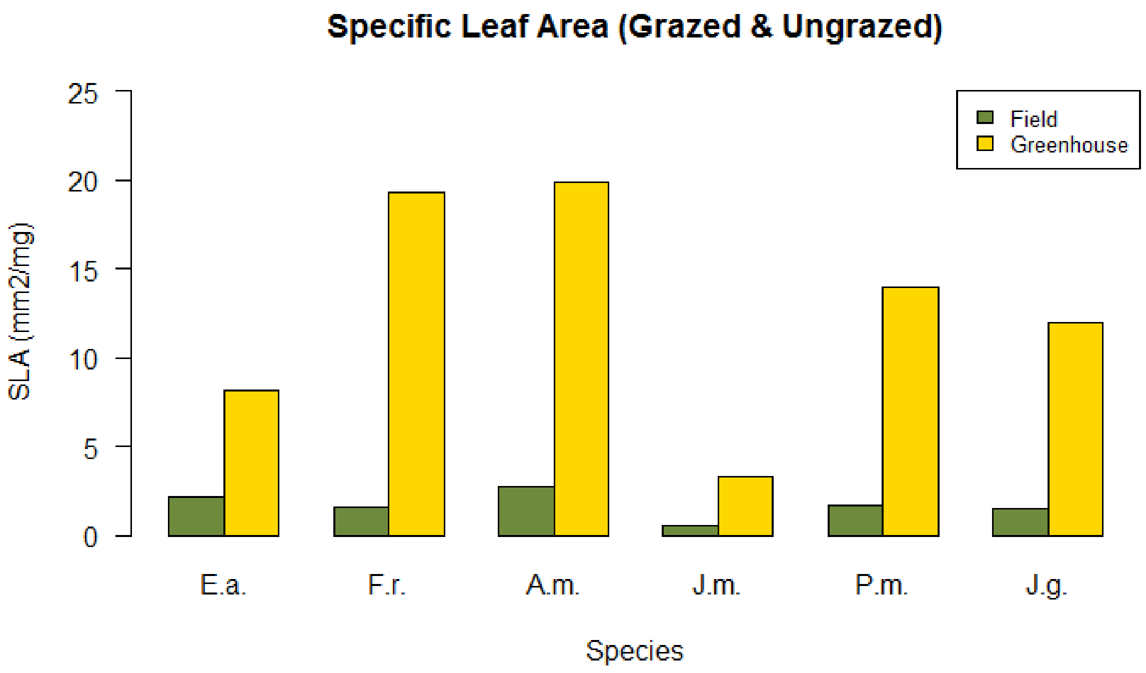

Figure 7a: *SLA (mm²/mg) for all 6 focal species (i.e. Elytrichia atherica (E.a.), Festuca rubra (F.r.), Artemisia maritima (A.m.), Juncus maritimus (J.m.), Puccinellia maritima (P.m.) and Juncus gerardii (J.g.)) measured both in the field (grazed and ungrazed data combined) and in the greenhouse.*

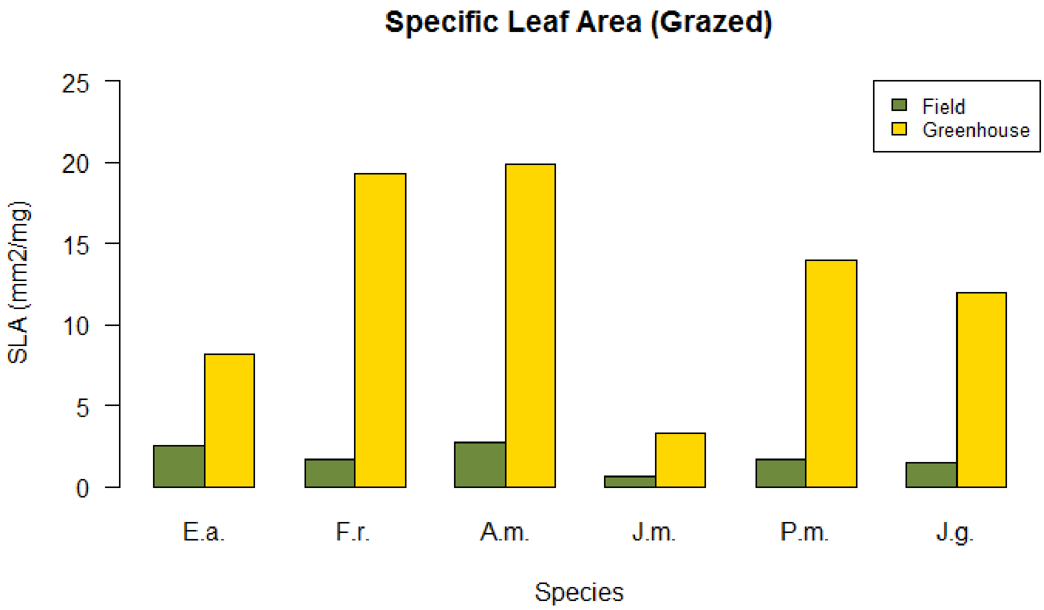

Figure 7b: *SLA (mm^2/mg) for all 6 focal species (i.e. Elytrichia atherica (E.a.), Festuca rubra (F.r.), Artemisia maritima (A.m.), Juncus maritimus (J.m.), Puccinellia maritima (P.m.) and Juncus gerardii (J.g.)) measured both in the field (grazed) and in the greenhouse.*

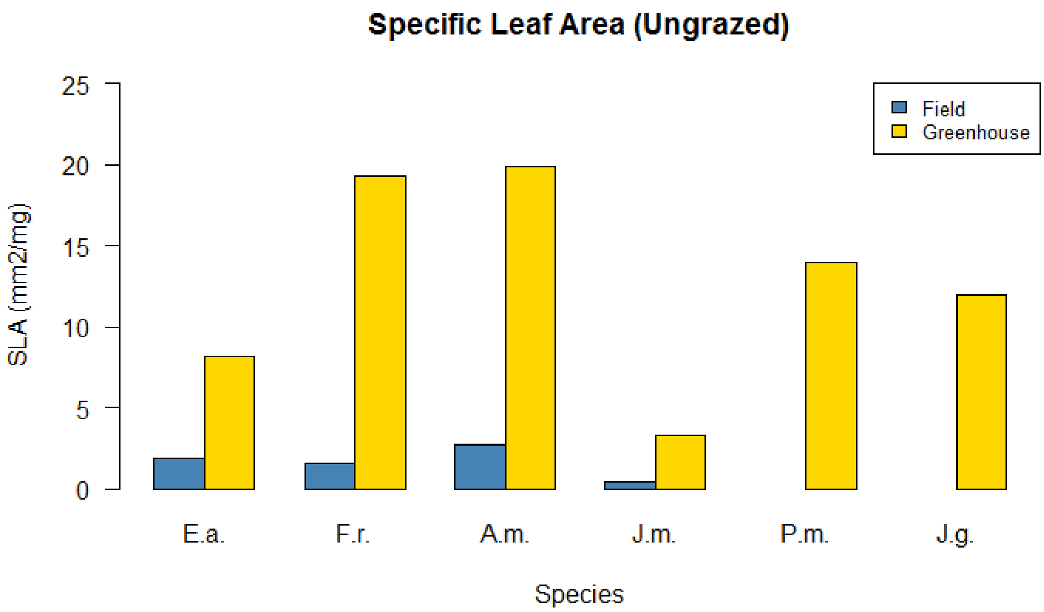

Figure 7c: *SLA (mm^2/mg) for all 6 focal species (i.e. Elytrichia atherica (E.a.), Festuca rubra (F.r.), Artemisia maritima (A.m.), Juncus maritimus (J.m.), Puccinellia maritima (P.m.) and Juncus gerardii (J.g.)) measured both in the field (ungrazed) and in the greenhouse.*

Conclusion & Discussion

Abiotic variables: soil oxygen and salinity

Both the electric conductivity and soil oxygen show seasonal differences (table 2). These changing conditions are more pronounced on the grazed marsh than on the ungrazed marsh (fig. 2a and 3a). From this we can infer that cattle grazing has an effect on the abiotic conditions of the marsh. Also, the effects become more pronounced outside patches of *Juncus maritimus* where the soil is much more compacted (previous unpublished work, 2012). When being inundated compacted soil will hold the water for much longer than less compacted soil leading to low soil oxygen. Inundations happen less frequently as we go from March to June (unpublished work, 2013), so we would expect low soil oxygen to be less of stressor as the growing season progresses, since waterlogged soil is allowed time to drain and the soil becomes more oxic. However, microbial communities could also affect soil oxygen concentrations by consuming oxygen (Mahall and Park, and references therein, 1976; Schrama et al., 2012), but as of yet we don't have any field data from Schiermonnikoog on this. *Elytrichia atherica* produces little aerenchyma (Justin & Armstrong, 1987) and therefore has difficulty to maintain itself in low soil oxygen areas (i.e. outside patches on the grazed marsh).

As we go from March to May to June, we see that, as the growing season starts and the inundations happen les frequently, the difference in electric conductivity between the grazed and the ungrazed marsh becomes smaller (i.e. from being significant to not significant, see fig. 3a). Salinity was found to be significantly higher on the ungrazed marsh in March (fig. 3c). We did not expect this, but a possible explanation might lie in the compaction of the soil; the soil on the ungrazed is very loose due to the burrowing action of *Orchestia gamarellus* (previous unpublished work, 2012), making evaporation of the salty seawater easier, leaving behind the salt on the surface, causing us to find higher electric conductivity values then initially expected. It has been suggested that soil compaction leads to an increase in salinity because of lowered salt leaching and higher evapotranspiration (Chaneton and Lavado, 1996; Olff and Ritchie, 1998). However, because of the significant amount of aboveground biomass on the ungrazed marsh, evapotranspiration could be quite high on the (less compacted) grazed marsh, resulting in a higher salinity. The problem with this explanation is that in June, when we would expect a fair amount of evaporation, we see no differences in salinity between grazed and ungrazed, and between patches.

There is thought to be an inverse relationship between competitive ability and stress tolerance; competitively superior plants occupy the least stressful zones of the marsh and displace competitively inferior plants to the more stressful zones (Pennings et al., and references therein, 2005). If the marsh is irregularly inundated, however, this pattern may not hold, because there may not be a consistent stress gradient (e.g. salinity and anoxia) across the marsh (Pennings et al., 2005).

So in terms of abiotic conditions the grazed marsh is more dynamic than the ungrazed marsh, and outside patches of *Juncus maritimus* are more dynamic than inside patches of *Juncus maritimus* (table 2). We also see a stabilizing effect; differences in both electric conductivity and soil oxygen between the grazed and ungrazed marsh go from being significant in March to not being significant in June (fig. 2a and 3a).

Table 2: *Soil oxygen measured in March (2013), May (2012) and June (2013) across an elevation gradient, now divided into zones (i.e. low and high marsh), for both the grazed and the ungrazed marsh, and inside and outside patches of Juncus maritimus. '+' indicates high values of soil oxygen, '-' indicates low values of soil oxygen and '0' indicates the mean overlapping with both '+' and '-'. Groups with the same symbol are not significantly different. Also included is electric conductivity. '+' indicates high salinity, '-' indicates low salinity and '0' indicates the mean overlapping with both '+' and '-'.*

		Grazed						Ungrazed					
		Inside			Outside			Inside			Outside		
Variable	Zone	March	May	June	March	May	June	March	May	June	March	May	June
Redox	High	-	0	0	-	-	0	+	+	0	+	++	0
	Low	-	0	-	0	0	--	+	+	0	+	+	0
Electric Conductivity	High	-	--	--	--	--	--	+	0	--	++	0	--
	Low	0	0	--	0	0	--	+	+	--	0	-	--

Biotic variables: SLA, LAI and SRL

In correspondence with the abiotic conditions, the leaf and root traits span a wider range on the grazed marsh than on the ungrazed marsh (table 2, 3, 4 and 5).

High LAI values (i.e. increased shading) on the ungrazed marsh and on the grazed marsh inside the patches indicate a strong competition for light (Olff and Ritchie, 1998). This corresponds with the vegetation height where the inside patches on the grazed marsh have generally tall vegetation similar to the ungrazed marsh, and outside patches on the grazed marsh have very short vegetation. Here we can see the role *Juncus maritimus* has as a nurse plant 'protecting' the more palatable plants in its vicinity (Padilla and Pugnaire, 2006). Outside patches on the grazed marsh we see the significant effect of cattle grazing and trampling on the vegetation height, among others, which is absent outside patches on the ungrazed marsh where there has never been grazing by livestock.

The unexpected high values of SRL outside patches of *Juncus maritimus* on the grazed marsh, matched by a very high belowground biomass, seem to correspond with a change in resource management strategy; investing more in quantity and less in quality. This would enable the plant to cope with the physical stress of trampling and occupy more space to take up nutrients and water. Table 5 shows that *Juncus maritimus* has exactly the same strategy on the grazed as on the ungrazed marsh. The unidentified group shows no differences between inside and outside patches of *Juncus maritimus* and no differences between grazed and ungrazed. Of course, separating this group into different species might yield another result, but we don't have that more detailed information. *Elytrichia atherica* is the interesting one showing an increase in SRL outside patches of *Juncus maritimus* on the grazed marsh, most apparent on the low marsh, especially in the absorptive roots. Is this a plastic or a genetic response? We assume it is a plastic response, because of the correlation between SLA (which showed a plastic response) and SRL, but a greenhouse comparison is needed to confirm.

We hypothesized SLA and SRL to show the same patterns, and although the correlation was highly significant, the results turned out to be more complex; both for SLA and SRL the grazed marsh was more dynamic than the ungrazed marsh, but this only became apparent when looking on the community level and separating into different species. For *Juncus maritimus* both SLA and SRL were very low, indicating higher quality leaf and root material and corresponding to a slow growth rate. This result is supported by known literature (Kembel and Cahill Jr., and references therein, 2011). We assumed the grazed marsh to be a stressful environment, especially outside the patch where the soil is more compacted than inside the patch, yet we found SRL to be significantly higher outside the patch. In general low SRL (higher quality root material) corresponds to a stressful environment (Kembel and Cahill Jr., and references therein, 2011), however, we seem to find the opposite. A possible explanation might be ‘quantity over quality’; if roots cannot withstand the stress (no matter the quality), then it is more energetically responsible to make lots of low quality roots (i.e. high SRL) than few high quality roots (i.e. low SRL). We found support for this idea in the positive relation between SRL and belowground biomass, which was significant on the grazed marsh, but not on the ungrazed marsh. The belowground biomass was also significantly higher outside than inside patches on the grazed marsh (unpublished work, 2012). These results correspond with the SRL results of the absorptive roots of *Elytrichia atherica* (fig. 6d), that show the outside of patches on the grazed marsh to be different from the inside patch on the grazed marsh and the inside and outside patch on the ungrazed marsh. An alternative might be to look at the low SRL inside the patches on the grazed marsh and assume those patches to be a stressful environment, with its primary stressor being light (i.e. shading); figure 5 shows the LAI outside patches on the grazed marsh to be significantly lower than inside patches on the grazed and inside and outside patches on the ungrazed marsh, both at high and low elevation. All things considered, it should be noted that root characteristics vary widely among species and appear to be strongly influenced by plant phylogeny (Eissenstat and Yanai, and references therein, 1997). Also, compared to our understanding of aboveground traits, knowledge of root traits (e.g. SRL) and their ecological and evolutionary relationship with leaf traits (e.g. SLA) is limited (Kembel and Cahill Jr., and references therein, 2011). Therefore we should be cautious with drawing far stretching conclusions to our results.

Table 3: *Leaf (SLA and LAI) and root (SRL) traits for Juncus maritimus (J.m.), Elytrichia atherica (E.a.) and unidentified (UnID) measured across an elevation gradient, now divided into zones (i.e. low and high marsh), for both the grazed and the ungrazed marsh, and inside and outside patches of Juncus maritimus. ‘+’ indicates high values of soil oxygen, ‘-‘ indicates low values of soil oxygen and ‘0’ indicates the mean overlapping with both ‘+’ and ‘-‘. Groups with the same symbol are not significantly different.*

		Grazed					Ungrazed				
		Inside			Outside		Inside			Outside	
Traits	**Zone**	J.m.	E.a.	UnID	E.a.	UnID	J.m.	E.a.	UnID	E.a.	UnID
SRL	High	-	+	++++	+	+++	--	+	+++	+	++++
	Low	--	0	++++	++	+++	--	+	+++	+	++++
SLA	High	--	0	0	0	-	--	0	0	0	0
	Low	--	+	++	+	0	--	0	0	0	0
LAI	High	++	++	++	-	-	++	++	++	++	++
	Low	++	+	++	-	--	++	++	++	0	0

Table 4: *Specific root length (SRL) separated into rhizomes (rhizo.) and absorptive roots (abs.) for Juncus maritimus (J.m.), Elytrichia atherica (E.a.) and unidentified root material (UnID) measured across an elevation gradient, now divided into zones (i.e. low and high marsh), for both the grazed and the ungrazed marsh, and inside and outside patches of Juncus maritimus. ‘+’ indicates high values of soil oxygen, ‘-‘ indicates low values of soil oxygen and ‘0’ indicates the mean overlapping with both ‘+’ and ‘-‘. Groups with the same symbol are not significantly different.*

		Grazed								Ungrazed							
		Inside					Outside			Inside					Outside		
Traits	**Zone**	J.m. rhizo.	J.m. abs.	E.a. rhizo	E.a. abs.	UnID	E.a. rhizo	E.a. abs.	UnID	J.m. rhizo.	J.m. abs.	E.a. rhizo	E.a. abs.	UnID	E.a. rhizo	E.a. abs.	UnID
SRL	High	--	-	-	+	++	-	+	++	--	-	-	+	+++	-	+	++
	Low	--	-	-	-	+	-	+	++	--	-	-	0	++	-	-	++

Table 5: *Specific root length (SRL) separated into rhizomes (rhizo.) and absorptive roots (abs.) for Juncus maritimus (J.m.), Elytrichia atherica (E.a.) and unidentified (UnID) measured across an elevation gradient, now divided into zones (i.e. low and high marsh), for both the grazed and the ungrazed marsh, and inside and outside patches of Juncus maritimus. '+' indicates high values of soil oxygen, '-' indicates low values of soil oxygen and '0' indicates the mean overlapping with both '+' and '-'. Groups with the same symbol are not significantly different.*

		Grazed				Ungrazed			
		Inside		Outside		Inside		Outside	
Trait	Zone	rhizo.	abs.	rhizo.	abs.	rhizo.	abs.	rhizo.	abs.
SRL J.m.	High	---	0	na	na	---	0	na	na
	Low	---	0	na	na	---	0	na	na
SRL E.a.	High	--	++	-	++	-	++	-	++
	Low	-	0	0	+++	--	++	-	+
SRL UnID.	High	na	++++	na	++++	na	++++	na	++++
	Low	na	++++	na	++++	na	++++	na	++++

Greenhouse

SLA measured in the greenhouse compared with SLA measured in the field showed for all 6 focal species (i.e. *Juncus maritimus, Elytrichia atherica, Festuca rubra, Artemisia maritima, Puccinellia maritimus and Juncus gerardii*) higher SLA values in the greenhouse situation. If the SLA trait was primarily genetically determined, we would have found SLA in the greenhouse situation to be similar to the field situation; therefore we can infer that the environment plays a significant role in determining this plant trait. When separating into grazed and ungrazed it becomes evident that, for all species, SLA is higher on the grazed marsh than on the ungrazed marsh. This result suggests that our assumption of the grazed marsh being the more stressful environment was wrong. Part of the explanation, as mentioned earlier, might be the light stress (i.e. shading) induced by the tall vegetation on the ungrazed marsh, which could explain the lower SLA values.

It is generally accepted that individuals of a species cannot be good at everything (Krebs, 2009); plants adapted to a certain environment have acquired traits that prevent them from occupying a different environment (Grime, 1979). The shading tolerant species, *Elytrichia atherica* and *Festuca rubra*, showed (although not significant) a trend in their SLA; SLA decreased outside the patch on the grazed marsh (fig. 4b). So a shading tolerant species cannot be a grazing tolerant one at the same time, therefore we see *Elytrichia atherica* and *Festuca rubra* not only having a lower SLA outside the patch on the grazed marsh, but also showing a decrease in abundance (unpublished work, 2013). The grazing tolerant species, *Puccinellia maritima* and *Juncus gerardii*, were much less abundant inside patches on the grazed, and inside and outside patches on the ungrazed marsh then outside patches on the grazed marsh (unpublished work, 2013); apparently they were unable to handle the stress induced by shading.

Further research

The seasonal differences we found in the abiotic conditions show the need for a year-round study, capturing the full range of conditions. For the roots it would be useful if we were able to separate the now 'unidentified' group into species (e.g. with molecular coding), enabling us to correlate fully with SLA on the species level. It would also be helpful to determine how the microbial communities in the soil affect the soil oxygen saturation, as this might shed some more light on the seasonality of this soil parameter (Mahall and Park, and references therein, 1976; Schrama et al., 2012). Another interesting approach would be to determine the phylogenetic relatedness of our 6, and preferably more, focal species as this can affect root-leaf trait correlations (Kembel and Cahill Jr., 2011). Measuring a wider range of plant functional traits, for example leaf nitrogen content and photosynthesis rates, will further add to our understanding of the mechanisms that drive plant interactions, community composition and diversity on the salt marsh of Schiermonnikoog and grasslands in general.

Synthesis

This study aimed to find out how certain plant traits of a salt marsh grassland community were affected by stressors such as salinity, anoxia, light, and stress induced by large herbivores (i.e. trampling and defoliation). All measured plant traits (i.e. SLA, LAI and SRL) show the grazed marsh to be much more dynamic than the ungrazed marsh. These results corresponded with our findings on soil oxygen and electric conductivity. We found strong seasonal differences in the underlying abiotic gradient (i.e. salinity and anoxia), that seemed to stabilize as the growing season progressed. SLA seemed to be closely connected to light stress (i.e. shading), at least for the 2 shading tolerant species, *Elytrichia atherica* and *Festuca rubra,* that experienced lower SLA and abundance outside the patch on the grazed marsh. The grazing tolerant species, *Puccinellia maritima* and *Juncus gerardii*, were much less abundant (outcompeted) inside patches on the grazed, and inside and outside patches on the ungrazed marsh. For the SRL of *Elytrichia atherica* we found evidence for a ‘quantity over quality’ strategy. We found support for this idea in the positive relation between SRL and belowground biomass, which was significant on the grazed marsh, but not on the ungrazed marsh. Furthermore, belowground biomass was significantly higher outside than inside patches on the grazed marsh, again confirming the ‘quantity over quality’ strategy.

We hypothesized there to be no differences in plant functional traits between inside and outside patches of *Juncus maritimus,* and between high and low elevation on the ungrazed marsh. This was false, both the abiotic and biotic conditions showed patch and elevation differences, although this was dependent on time of year and species. We hypothesized there to be differences between inside and outside patches of *Juncus maritimus* and these differences to be greater at high elevation on the grazed marsh. This hypothesis was mostly true; there were clear differences between inside and outside patches of *Juncus maritimus,* for both the biotic and the abiotic conditions, however these differences were not consistently greater at higher elevation. Often elevation was not a significant factor or the differences were greater at lower elevation. Again we see the time and species differences taking effect. The underlying abiotic gradients differ significantly over time, therefore certain stressors (e.g. salinity) can have varying effects on the community. In terms of traits we see the grazed marsh to be much more dynamic than the ungrazed marsh.

We conclude that there are strong seasonal differences in the abiotic conditions (i.e. anoxia and salinity), but that these abiotic conditions have less effect on plant functional traits (i.e. SLA, SRL) than originally expected (i.e. between high and low, grazed and ungrazed). Furthermore, the response of plant functional traits to stressors is dependent on their life strategy, and is largely determined by the environment.

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