Willows in Tidal Wetlands in Times of Climate Change: Ecological Niches in Estuarine Environments



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1. General introduction

1.1 Ecological niches

The niche concept has been central to ecology for decades: more than half a century ago George Evelyn Hutchinson proposed that the ecological niche of a species could be characterised as an abstract mapping of species requirements onto an environmental space, the axes of which are abiotic and biotic factors that determine persistence or extinction (Holt, 2009). Much earlier, in Darwin's (1859) and Wallace's (1876) writings on natural selection and evolution, the term "line of life" was used to describe the role of a species, similar to how species niche is used by biologists today (Chase and Leibold, 2003).

Firstly, the Grinnellian and the Eltonian niche were distinguished (Fig. 1.1): Grinnell (1917) describes the species niche as the response of species to a given set of variables, considered as resources, whereas Elton (1927) focuses on the impact of species on the environment.



Figure 1.1 Distinction of ecological niche definitions: (a) Grinnellian specialization is a species variance in performance across a range of resources (dashed line: mean performance generalist (G), solid line: mean performance specialist (S). (b) Eltonian specialization is the variance in the species' impact on the environment (dashed line: mean impact of a species on a large part of the environment (G) or on a more restricted environment (S). (Devictor et al., 2010)

Gause (1936) experimentally demonstrated the principle of competitive exclusion on interacting protist populations, which influenced the explorations of species niches in the following decades. As introduced at the very beginning, Hutchinson (1957) defined the niche as the "n-dimensional hypervolume" in a more quantitative sense compared to Grinnell and Elton. Here, the niche represents the amount of "n" factors needed for a species to survive.

Moreover, Hutchinson distinguished a species "fundamental niche" from the "realized niche" in which a species is restricted due to biotic interactions.

Secondly, ecological specialization has been developed as a concept to predict the adaptive response of populations to changing environments (Levins, 1968). Ecological specialization was most often used synonymously with limited niche breadth, resulting from evolutionary trade-offs between the ability of species to exploit a range of resources and its capacity to use each one (MacArthur, 1972). However, subsequent authors focused on resource competition and the "niche" was increasingly associated with interspecific competition, which might have downplayed the previously pluralistic view on species niches, including other biotic and abiotic factors (Chase and Leibold, 2003). Moreover, the validity of explorations on patterns of species ´ niches was attacked (e.g. Simberloff, 1978) due to the lack of adequate null hypothesis and called for rigorous experiments.

At the turn of the millennium, the concept on the ecological niche had fallen out of favour and more specific ecological concepts were developed. Moreover, contrary ecological concepts were introduced, such as the neutral theory on biodiversity and species relative abundance by Hubbell (2001), based on the assumption, that species are in essence identical in any ways that might distinguish their population dynamics, and equivalent in all important ecological respects. Thus, ecologists are challenged to rethink the niche concept.

Recently, Chase and Leibold (2003) proposed an integrative approach: connecting modern ecology with classical counterparts is more successful than the ignorance of ecological theories developed in previous decades. Specifically, incorporation of processes that exceed resource competition is required as well as clarification between species requirements and impacts on the environment. In parallel, proper null hypothesis on niche explorations have to be tested using appropriate experimental, statistical and graphical analysis on multiple scales. Then, the niche concept might serve as a nexus of diverging fields in ecology (e. g. evolutionary, population, community and ecosystem ecology). Due to rapid environmental change in some systems, a recent increasing interest in the niche concept might be driven by the urgency of predicting species ' niches as ecological responses (Holt, 2009).

In my thesis, I follow the integral approach proposed by Chase and Leibold (2003). I focus on estuaries as highly dynamic systems with rapid environmental change, corresponding to McLusky and Elliott (2004). They described estuarine environments as mainly being controlled by widely fluctuating physical conditions. Hence, I studied processes - exceeding resource competition - to clarify species requirements on abiotic resources, according to the Grinnellian specialization.

In testing my thesis, I combined experimental approaches with field studies at different scales. With this, I intend to contribute to the ability to predict species' niches in systems with undergoing rapid environmental change, as described herein.

1.2 The estuarine environment and the species that provide its ecosystem services

An estuary is defined as "an inlet of the sea reaching into a river valley as far as the upper limit of tidal rise, usually being divisible into three sections: a) a marine or lower estuary, in free connection with the open sea; b) a middle estuary subject to strong salt and freshwater mixing; and c) an upper or fluvial estuary, characterised by freshwater but subject to strong tidal actions" (Fairbridge, 1980). This definition extends the upstream limit up to the tidal limit and, thus, goes beyond the definition given earlier by Pitchard (1967), which described an estuary as "a semi-enclosed coastal body of water, which has a free connection with the open sea, and within which sea water is measurable diluted with fresh water derived from land drainage." The emphasis of an estuary as a dynamic mosaic characterised by tides and intermediate from land to sea unifies both definitions. Moreover, Fairbridge (1980) emphasised the estuarine gradient of conditions, and recently McLusky and Elliott (2004) characterise the estuarine environment by a constantly changing mixture of salt and freshwater intermediate from sea to land, where species living in estuaries might be distinguished due to their ecological specialization (migrants, oligonaline, true estuarine, euryhaline marine and stenohaline marine organisms) along the estuarine salinity gradient. Furthermore, a dynamic complex of physical factors (e.g. tides, waves and currents), chemical factors (e.g. oxygen and nutrient supply), and sedimentary transport induces a topography of continual change, which requires species adaptive responses to the estuarine environment.

High biological productivity and high variability of abiotic factors are characteristic features of estuaries, forming fluctuating transitions from freshwater rivers and wetlands to marine and oceanic habitats (Bassett et al., 2013). In transitional waters, the species composition in the estuarine fish fauna; including marine estuarine opportunists (e.g. *Clupea harengus*), anadromous (e.g. *Alosa fallax*) and true estuarine species (e.g. *Syngnathus rostellatus*); undergo annual cyclical changes occurring in up- and downstream variations of tidal flooding and salinity (Thiel and Potter, 2001). Moreover, species movement and dispersal parallelled with hydrologic sediment and nutrient transfer link habitats at the estuarine gradient in a physically, chemically and biologically manner both seasonally and during single tidal cycles (Meire et al., 2005).

According to Batzer and Baldwin (2012), tides also dominate many coastal wetlands – contrary to inland wetlands – and thus are hereafter referred to as tidal wetlands.

In European tidal wetlands, field data has showed varying estuarine wetland sedimentation rates depending on (1) tidal wetland age, (2) estuarine variations in mean high water-level rise, and (3) variations in suspended sediment concentrations (Temmerman et al., 2004). In North-American tidal wetlands, corresponding to Batzer and Baldwin (2012), high productivity accompanied by slow decomposition rates due to anaerobic conditions cause an increase in surface elevation relative to sea level rise. Generally, surface elevation and salinity gradients within tidal wetlands lead to horizontal zonation of submergent and emergent plant species.

In marine costal environments, seaweed (green, brown and red algae) constitutes a group of submerged organisms of high significance to ecosystem function. Below the mean low water level, seaweed usually encounters a stable habitat, as the water column above buffers it against strong changes in abiotic parameters, whereas subsequent UV-exposure, tidal currents, salinity changes and waves are stressors on intertidal algae communities (Bischof et al., 2006). Emergent plants consist mainly of vascular plants communities. The estuarine gradient consists of tidal freshwater wetlands, and brackish and salt marshes, exhibiting vertical vascular plant species zonation patterns formed according to the estuarine flooding gradient (Bakker et al., 1993; Engels et al., 2010).

Tidal wetlands of mid-European estuaries exhibit distinct vertical zonation (Engels and Jensen, 2009):

Here, species served as indicators for different elevational zones: in fresh and brackish low marshes, *Bolboschoenus maritimus*; mid marshes, *Phragmites australis*; high marshes, *Phragmites* and tall forbs (e.g. *Angelica archangelica*), and in salt marshes, *Salicornia europaea* (low marsh), *Aster tripolium/Puccinellia maritima* (mid marsh) and *Elymus athericus/Festuca rubra* (high marsh).

Woody species do also play a role in intertidal habitats: in the tropics, mangroves dominate the intertidal zone at the coastline, and tidal inundation as well as salinity contributes to mangrove species distribution (Da Cruz et al., 2013). In temperate climates, willows are the woody counterpart in European tidal freshwater wetlands. According to Struyf et al. (2009), various willow species settle at higher elevated tidal wetlands dominated by *Phragmites* or tall herbs (< 40% flooding frequency) as the first stage of succession to tidal willow wetland forests.

Estuaries are one of the most productive biomes of the world (Costanza et al., 1993). Moreover, estuarine habitats provide important ecosystem services, and it is proposed that habitat response to environmental change (e.g. storm protection and flood control) could be controlled in part by vegetation (Costanza et al., 1997). However, estuaries are major transition zones between land and sea with steep gradients in energy and physicochemical properties and thus estuarine organisms have to cope with hazards in these highly dynamic systems (Jennerjahn and Mitchell, 2013). Hazards to the estuarine environment are grouped by increasing pressures: climate change (e.g. sea level rise), human activities (e.g. changes in river geomorphology) and extreme events (e.g. storm surges) (Fig. 1.2).



Figure 1.2 Hazards to estuarine ecosystems (Jennerjahn and Mitchell, 2013)

During past centuries, tidal wetlands were converted into agricultural, urban and industrial areas (e.g. New York, New Orleans, Shanghai, London, Rotterdam and Hamburg), and today many estuaries have lost most of their natural flood defense capability. Thus, Temmerman et al. (2013) argue that estuarine ecosystem creation and restoration in suitable locations can provide a sustainable flood control alternative to conventional coastal protection. Such protection measures (e.g. breakwater or dams) mitigate threats of flooding and erosion.

Species that provide ecosystem services exhibit the ability to change the physical state of biotic or abiotic materials, thus modulating resource availability to other organisms. Here, Jones et al. (1994) distinguished autogenic engineers (e.g. corals and trees) that change the environment by their physical structure from allogenic engineers (e.g. beavers) that change the environment by transforming material from one physical state to another, and modulating resources exceeding themselves. In subtropical and tropical coastal regions, diverse mangrove species serve as engineers contributing to coastal flood defense (Alongis, 2008; Li et al., 2013).

Furthermore, vegetation in salt marshes is known to decrease the wave impact on elevated floodplains and dikes (Möller et al. 1999), and specifically *Ammophila arenaria* is known as an excellent engineering species, which is used to trap sand and thereby to contribute to dune formation and flood control at sandy coasts.

In temperate climates, tidal wetlands and flood plain willows also contribute to estuarine coastal protection by attenuating waves and stabilising sediments. In the future, they might be planted as ecosystem service species on a larger scale due to their resistance to physical conditions in flood plains (Borsje et al., 2011), and specifically due to their high water flow resistance (Wunder et al., 2013).

Generally, willow species` (genus Salix) engineering capacity exceeds their specific spatial and temporal scale. According to Hastings et al. (2007), species in which the engineering capacity is larger than their specific spatial and temporal scale fit in the ecosystem engineering concept, similarly exemplified by the work of Darwin (1881) on the earthworm as the role of a species in the ecosystem. Corresponding to Odum and Odum (2003), ecological engineering is defined as "the practice of joining the economy of society to the environment symbiotically by fitting technological design with ecological self design." Wetland filtration of heavy metals is mentioned as an example. Willows as fast-growing plants, accumulating above-average concentrations of mobile trace elements (Cd, Zn, B) and sometimes show capacity for phytoremediation (Dickinson et al., 2014). Experimental data on metal uptake showed earlier that willows accumulated levels of metals above normal critical concentrations and thus, heavy-metal contamination (especially Cd) of soils might be decreased by phytoremediation without necessarily losing willows productivity. Punshon and Dickinson (1997) suggested that cumulative treatment enables certain clones of Salix to grow in concentrations which were previously lethal based on willows' phenotypic plasticity and their rapid acclimation to local environments.

Willows' engineering capacity is well studied. However, little is known about willow species requirements on the estuarine environment. Thus, I intend to clarify responses of characteristic willow species to crucial abiotic factors in tidal wetlands. With my thesis, I intend to contribute to a scientific understanding of willows ecological niches in tidal wetlands with regard to possible application of *Salix* species for ecosystem engineering purposes in estuarine environments.

1.3 Willows in tidal wetlands times of climate change

Generally, floodplain vegetation is closely related to a combination of water level fluctuations – differing among floodplain types – and soil moisture, determining the occurrence and absence of individual species, whereas disturbance and dispersal processes additionally contribute to explain pattern of species occurrence in floodplains (Leyer, 2004). Diverse willow species are characteristic in riverine floodplain habitats with high disturbance rates

throughout the northern temperate zone. Large *Salix* plants exhibit mechanical properties (e.g. high bending stability) that enable them to withstand floods and, moreover, uprooted or fragmented plants re-sprout vigorously. Furthermore, *Salix*'s high regeneration capacity is considered an adaptation to regular disturbance. Numerous small and short-lived seeds are produced, are dispersed by wind and water, and germinate and establish on moist exposed riverine sediments after disturbance. Sexual reproduction is limited by the presence of bare-ground sites with suitable hydrologic conditions (Karrenberg et al., 2002).

However, in particular some European floodplain willows (e.g. *Salix fragilis*) propagate by vegetative means. Contrary to the closely related *Salix alba*, *Salix fragilis* showed better rooting ability of vegetative propagules which was correlated with brittleness of twig bases. Thus, disturbed by flooding, twigs easily break off, are spread by water currents and settle at new shoreline locations depending on suitable site conditions (Beismann et al., 2000). Here, vegetatively produced juvenile plants might be in advantage due to their fast rooting and growing capacity (Chmelar and Meusel, 1986), whereas the survival of the small seedlings of *Salix alba* crucially depends on root growth to access water and avoid desiccation (Guilloy et al., 2011). However, in a field survey in a European river flood plain, *Salix alba* regeneration from seeds was high in the first 2–3 years following recruitment, but survival of sexual recruits decreased over time. Here, unseasonal flood disturbances mainly reduced recruitment from seeds.

Moreover, seedlings had a more limited spatial distribution primarily in low elevations with fine sediments, while asexual recruits had a wider spatial distribution at the flood plain (Barsoum, 2002). This confirmed earlier assumptions on *Salix* seedling` vulnerability to soil moisture deficits in North-American (e.g. Stromberg, 1997), and in European (e.g. van Splunder et al., 1995) flood plain willow species distribution.

Willows are distributed worldwide except in Australia, New Zealand and Antarctica. Willow species occur at diverse latitudes: from dwarf willows in the Arctic (e.g. *Salix polaris*), to imposing large trees in the temperate region (e.g. *Salix alba*) up to shrubs and trees in the tropics (e.g. *Salix martiana*). However, the main species distribution of *Salix* is focused in the temperate climate at the Holarctic region. In Europe, the genus *Salix* consists of about 70 species, whereas the total species number is estimated as 300-500 (Chmelar and Meusel, 1986; Newsholme, 1992; Hörandl, 1992).

The wide range in species number is caused by differences in species definition among several authors. According to Neumann (1981), closely related willow species are difficult to determine due to their high variability in growth behaviour and absolute growth features, and in some cases hybridization can be assumed. Zander (2000) exhibited the morphologic diversity exemplarily in the *Salix-repens*-complex, and presented an overview in literature

related to *Salix* systematics and taxonomy. Nonetheless, the percentage of hybrids occurring in nature is estimated as less than 5% (Chmelar and Meusel, 1986).

Moreover, the co-occurrence of closely related species might be limited due to biotic filters on species' ecological similarities within communities. In diverse willows it was found that environmental filtering is important to community assembly along hydrologic gradients. In one study, willows were phenotypically clustered at wet sites, exhibiting ecological specialization to the habitat (Savage and Cavender-Bares, 2012). Karrenberg et al. (2002) assumed that the floodplain environment causes high genetic variability in populations of Salicaceae that predispose hybridization, and proposed a feedback between adaptive life history characteristics and the evolutionary process. They interpreted several life characteristics of Salicaceae (genus *Salix* and *Poplar*) to be adaptations to flood plain environments.

However, flood plain forests might be negatively affected in times of climate change: in tidal wetlands accelerated sea level rise and salt intrusion might lead to declining flood plain forests. Thus, tidal forests with their intolerance to low levels of salinity serve as sentinels of global-warming-driven accelerated sea level rise along the southeastern US coast and elsewhere (Craft, 2012). Moreover, at the North-American coast, predicted sea level by 52–82 cm by 2100 (IPCC) might lead to upriver migration of tidal freshwater and brackish marsh (Craft et al., 2009). Beyond this, the long-term stability of tidal salt marshes is explained by interactions among sea level, land elevation, primary production and sediment accretion that might regulate surface elevation to equilibrium with mean sea level (Morris et al., 2002). Generally, in tidal freshwater wetlands the current distribution indicates that these wetlands might persist against broad gradients of climate and hydrology. However, salt-water intrusion might change the defining features of tidal freshwater wetlands and thus is likely to have the largest long-term impact (Neubauer and Craft, 2009).

In Europe, tidal freshwater wetlands are particularly scarcer compared to other tidal wetlands due to human alterations such as diking, harbor expansion and land use changes for agricultural purposes. Generally, many types of coastal ecosystems, including estuaries, are listed as Ramsar sites and protected as wetlands of international importance (Ramsar, 2014). Moreover, riparian softwood flood plain forests are declining worldwide. Continental alluvial forests comprised of *Salix* species have experienced 99.5% loss of their natural distribution, according to estimations of the UNEP World Conservation Monitoring Centre (2000). In Europe, white willow softwood flood plain forests are vulnerable habitats and thus, are listed in the European Habitats Directive (Annex I European Habitats Directive). A good conservation status of softwood flood plain forests must be fixed or restored.

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Despite substantial loss of habitats so far, tidal freshwater areas with high tidal amplitude still occur today at the Scheldt (Belgium) and the Elbe (Germany). Here, the remaining woody communities in tidal wetlands are dominated by willows (Struyf et al., 2009). The Scheldt estuary is one of the most important estuaries in Europe where future evolution of habitat morphology depends on factors like sea level rise, subsidence, and on human impact. Here, the tidal amplitude near Antwerp has already increased substantially, about twice as much as at the river mouth (Meire et al., 2005). Moreover, tidal wetlands at the Scheldt estuary rise with rising mean high water level, whereby a faster sea level rise will result in a more pronounced elevation difference between land and water level (Temmerman et al., 2004a).

Similarly to the Scheldt estuary, the Elbe estuary, another large estuary in Central Europe, is experiencing a dynamic hydromorphological change. Besides natural processes, human alteration of the river bed via fairway construction and coastal protection influence tidal water levels. Here, the tidal amplitude has increased in conjunction with the shortening of the flood tide and corresponding lengthening of the ebb tide, and tidal velocities (Gönnert et al., 2007).

According to Jensen (2011), tidal wetlands at the Elbe estuary are largely influenced by extreme abiotic factors (salinity and tidal flooding). Recently, climate change might have caused cause variations in precipitation pattern, and is predicted to lead to earlier river discharge maxima parallelled by more pronounced minimum discharge. Furthermore, sea level rise will lead to increasing mean high water (Von Storch and Claussen, 2011) and thus, upstream directed shifts in salinity levels can be expected. Here, tidal freshwater and brackish wetlands are still occurring in approximately 1,050 ha aerial expansion, and the remaining woody communities are dominated by willows (Struyf et al., 2009).

So far it is unclear how these willow communities are distributed along the estuarine gradients. Moreover, these willow communities might be affected by increasing salt water intrusion and tidal flooding in times of climate change. Thus, I aim to answer these open research questions as described below.

1.4 Aim of the thesis and hypothesis

Willows in tidal wetlands serve as valuable ecosystem service species. In parallel, various willow communities contribute to floodplain biodiversity at the landscape scale. Tidal wetland willow communities consist of diverse *Salix* species and contribute to species richness: their local adaptation and phenotypic plasticity additionally promote intraspecific biodiversity.

Generally, observational and experimental studies support the existence of a positive relationship between biodiversity and an ecosystem's function and properties (Bassett et al., 2013). Riverine flood plain *Salix* life characteristics are well studied (see Karrenberg et al., 2002) as well as riverine flood plain willows' habitat distribution and population dynamics in space and time (see Mosner, 2012).

However, less is known about the distribution and ecological specialization of the genus Salix in the estuarine environment. Thus, the overall aim of this thesis is to contribute to understanding the ecological niche of willows in tidal wetlands with regard to tidal flooding and salinity as main factors determining plant species composition in the estuarine environment.

The Elbe estuary, one of the largest estuaries in Central Europe, served as an estuarine model system. I conducted three experimental studies in order to analyse tidal flooding and salinity tolerance of the most characteristic and abundant *Salix* species under defined conditions. Furthermore, I carried out field investigations on *Salix* communities along the estuarine gradient in order to elucidate *Salix* distribution and ecological specialization.

In chapter 2, I present results of a hydroponic greenhouse experiment with salinity treatments on *Salix* vegetative propagules (Salinity is expressed on the practical salinity scale hereinafter, and thus is dimensionsless (McLusky and Elliott, 2004)).

In chapter 3, effects of tidal treatments on *Salix* vegetative propagules which were studied by a mesocosm experiment in order to determine the tolerance to tidal flooding, are presented. In both experiments (chapters 2 and 3); in order to detect possible inter- and intraspecific differences I investigated plant material of two *Salix* species, *Salix alba* and *Salix viminalis*, with two origins: from tidal freshwater wetlands and from oligohaline wetlands.

Furthermore, I investigated sexual regeneration in *Salix alba*, since vegetative regeneration in *S. alba* is less pronounced compared to *S. viminalis*. Thus, in chapter 4 results of a germination experiment in a climate chamber are presented, where effects of different salinities on seed germination and seedlings early growth was documented.

Finally, chapter 5 summarizes results of a field study on *S. alba* and *S. viminalis*: Here, individuals` height and soil water salinity, as well as specimens` morphologic variability, were determined at four sites within the Elbe estuary. With these case studies, I intend to characterize the ecological specialization of *Salix* life stages (vegetative propagules, seeds and seedlings, large shrubs and trees) to tidal flooding and salinity as the most important factors in tidal wetlands in the estuarine environment with regards to protection of willows species in tidal wetlands and their sustainable use for ecosystem service.

Salix species tolerances to salinity were rejected (Ellenberg et al., 1992), and flood plain willows occurrence was determined to be limited to levels above mean high water line (Ellenberg and Leuschner, 2010). However, increasing tidal flooding and salt intrusion in tidal wetlands might be expected due to predicted sea level rise accompanied by changes in river discharge, and human impact on estuarine hydrology.

Contrary to recent literature and based on own field observations, I hypothesise, that characteristic *Salix* species are able to tolerate increasing salinity and tidal flooding in times of climate change. Specifically, I aim to determine which salinity and tidal flooding levels are able to be tolerated by the most abundant and characteristic *Salix* species from varying origins in their different life stages. With my research approach, I intent to contribute to understand the ecological niche of characteristic willows species in tidal wetlands in the estuarine environment in times of climate change.

2. Salt intrusion in tidal wetlands:

European willow species tolerate oligohaline conditions

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2.1 Abstract

Tidal wetlands experience salt intrusion due to the effects of climate change. This study clarifies that the European flood plain willows species *Salix alba* and *Salix viminalis* tolerate oligohaline conditions. *Salix alba* L. and *Salix viminalis* L. are distributed on flood plains up to transitional waters of the oligohaline to the mesohaline estuarine stretch in temperate climates. They experience spatial and temporal variations in flooding and salinity. In the past, willows dominated the vegetation above the mean high water line, attenuated waves and contributed to sedimentation. In recent centuries, human utilization reduced willow stands. Today, the Elbe estuary - a model system for an estuary in temperate zones – exhibits increasing flooding and salinity due to man-induced effects and climatic changes. Willows were described as having no salinity tolerance. In contrast, our soil water salinity measurements at willows in tidal wetlands prove that mature *Salix* individuals tolerate oligohaline conditions. To assess immature plant salinity tolerance, we conducted a hydroponic greenhouse experiment. Vegetative propagules originating from a freshwater and an oligohaline site were treated in four salinities. Related to growth rates and biomass production, we found interspecific similarities and a salinity tolerance up to salinity 2.

Vitality and chlorophyll fluorescence indicated an acclimation of *Salix* viminalis to oligohaline conditions. We conclude, that the survival of *S. alba* and *S. viminalis* and the restoration of willow stands in estuarine flood plains - with regard to wave attenuation and sedimentation - might be possible, despite increasing salinity in times of climate change.

2.2 Introduction

Estuaries form a major transition zone between land and sea with steep gradients in energy and physicochemical properties (Jennerjahn and Mitchell, 2013). Variable and fluctuating salinity is the principal stressor estuarine organisms have to cope with (Odum, 1988; McLusky and Elliott, 2004). Estuarine flood plains are affected by disturbance, such as floods and storms (Mitsch and Gosselink, 2000), and are characterized by two major environmental gradients: the salinity gradient and the flooding gradient. Abiotic stress and disturbance become more severe with increasing salinity and flooding from high to low elevation in estuarine flood plains (Engels and Jensen (2009). Estuarine organisms have to cope with natural disturbances and with effects of increasing pressures due to climate change, human activities and extreme events (Jennerjahn and Mitchell, 2013). In subtropical and tropical climate, mangroves are daily subjected to tidal changes in water and salt exposure and their typical plant species are adapted to live under these harsh conditions (Alongis, 2008). In temperate climate, life characteristics of riparian willows can be interpreted as adaptations to the flood plain environment, which also contribute to high genetic variability and predispose hybridization (Karrenberg et al., 2002). In general, local adaptations can be caused by small scale differences in the environment (Lipowsky et al., 2011). Whether this is also the case in willow species with different origin from flood plains along estuarine salinity gradients has not yet been investigated.

Willow soft wood forests are a characteristic feature of riverine and estuarine flood plains in temperate zones. Willows in these forests are part of the species rich genus Salix, which contains numerous flood plain species (Newsholme, 1992; Karrenberg et al., 2002). Many flood plain willows establish right above the mean water level. Here, both bush-shaped species, such as Salix viminalis L., and tree-shaped species, like Salix alba L., cope with disturbances such as waves and ice scouring by their high regeneration capacity (Ellenberg and Leuschner, 2010). Comparable to the contribution of mangroves to societal goods and services in subtropical and tropical coastal areas (Alongis, 2008; Li et al., 2013), flood plain willows in temperate climate contribute to ecosystem services such as coastal protection by attenuating waves and stabilising sediments (Borsje et al., 2011; Radtke et al., 2012). Furthermore, they might contribute to trap sediments during flooding events similar to the effect which has recently been shown for herbaceous coastal salt marsh vegetation (Callaghan et al., 2010; Suchrow et al., 2012).

Along the coasts, human interventions are mirrored by erosion and subsidence, eutrophication and salinization. These problems are now global and particularly severe at coastal "hot spots" such as river-mouth systems (Newton et al., 2012). However, salinization,

generally regarded as a stressor, also facilitates adapted estuarine organisms, which has recently been shown in the east of the Amazon mouth where an extensive mangrove belt increased in the upper river reaches due to salt water intrusion (Nascimento et al., 2013). In the temperate zone, tidal wetlands historically were locations for the establishment of human settlements and today many large cities (e.g. London, New York, Shanghai) are located here (Baldwin et al., 2009). In Europe especially tidal freshwater wetlands have become scarce due to human alterations such as diking, harbour expansion and land use changes for agricultural purposes. Nonetheless, substantial areas of tidal freshwater areas with high tidal amplitude still occur today at the Scheldt (Belgium) and the Elbe (Germany). Here, the remaining woody communities in tidal wetlands are dominated by willows (Struyf et al., 2009). Thus, willows in tidal freshwater wetlands can be seen as the temperate counterpart of mangroves in subtropical and tropical haline tidal flood plains. Both willows and mangroves are experiencing severe impacts: Beyond human activities, anthropogenic climate change and extreme events are identified as major hazards in estuarine environments (Jennerjahn and Mitchell, 2013). For tidal freshwater wetlands, reduced freshwater discharge and a rising sea level might both contribute to a rapid loss of habitats and biodiversity or to a conversion of vegetation caused by increased salt water intrusion (Baldwin and Mendelssohn, 1998; Neubauer and Craft, 2009). Furthermore, periodic saltwater pulses during storms and periods of low precipitation might contribute to increasing soil water salinity and stress willows in estuarine flood plains.

Salix alba L. and Salix viminalis L. (hereafter referred to as S. alba and S. viminalis) are characteristic willow species in flood plains in temperate zones (Struyf et al., 2009; Ellenberg and Leuschner, 2010). In our investigation, they serve as model species since they are the most widespread willow species in the flood plains of the Elbe estuary (Raabe, 1986). Willows are generally described as glycophytes (Newsholme, 1992), contrary to mangroves, which are found in saline coastal environments around the tropical and subtropical latitudes (Li et al., 2013). In specific, Ellenberg et al. (1992) assigned the above mentioned species as being "not salt tolerant". In contrast, we hypothesised that both species are able to tolerate oligonaline conditions. This hypothesis is of high relevance with regard to increasing salinity in tidal wetlands and due to their possible contribution to ecosystem services in tidal wetlands (see Borsje et al., 2011; Radtke et al., 2012). Against the background of salt intrusion in estuarine ecosystems, we investigated the salt tolerance of European willow species in tidal wetlands. In specific we aimed to answer the question (i) up to which soil water salinity do S. alba L. and S. viminalis L. occur along the salinity gradient of the Elbe estuary. We further asked, (ii) which salinity the species are able to tolerate and whether interspecific differences are existing with regard to salinity tolerance.

Concerning intraspecific differences, we aimed to answer the question (iii) if the salinity tolerance of these species differs between populations with origin from tidal wetlands at the freshwater stretch and the brackish water stretch.

2.3 Material and methods

Study area and study species

The study was conducted on willows in flood plains at the Elbe estuary in Germany. The Elbe estuary exhibits a 142 km long tidal stretch with a salinity gradient from the upstream border at a weir in Geesthacht to the river mouth at the North Sea (Fig. 2.1, A). In the mid-20th century, the tidal freshwater zone (salinity < 0.5) was found between Geesthacht and Glückstadt, the oligohaline zone (salinity 0.5-5) between Glückstadt and Brunsbüttel, and the mesohaline zone (salinity 5-18) between Brunsbüttel and the mouth of the estuary (Caspers, 1959). In the 21st century, variations in precipitation pattern caused by climate change are expected to lead to earlier river discharge maxima and the minimum discharge is predicted to be more pronounced. Furthermore, sea level rise will lead to increasing mean high water (Von Storch and Claussen, 2011) and thus, upstream directed shifts in salinity levels might be expected.

Two sites were selected, which represented close to nature flood plains at the freshwater and at the brackish water stretch, respectively (Fig. 2.1, B). At these sites, the transition zone from reed to woody communities is dominated by shrubs like *Salix viminalis*. At higher elevations, the zonation continues with trees like *Salix alba* (Newsholme, 1992; Ellenberg and Leuschner, 2010).



Figure 2.1 Study area in the Elbe estuary in Europe (A) and northern Germany (B), brackish water site (1) and freshwater site (2).

Soil water salinity

At the freshwater and the brackish water site, Salix stands were marked in aerial images at randomly generated plots. Here, Salix alba and Salix viminalis individuals were chosen as sources for cuttings. Within these plots, we determined S. alba and S. viminalis individuals as sources for cuttings by the presence of stem near-straight shoots. At nine willow stands per site, soil water was sampled seasonally in 2010 and 2011 under those willow individuals which served as donors for cuttings. Salinity was measured using the Practical Salinity Scale by a Multi-Parameter-Analyser (Eijkelkamp ecoTech 18.28).

Hydroponic greenhouse experiment

Experimental design and salinity treatment

We carried out a three factorial greenhouse experiment on cuttings of (i) two species (*Salix alba* and *Salix viminalis*), (ii) from two origins (freshwater and brackish water site), (iii) in four salinities (0.3, 2, 4 and 6) during ten weeks from February to April 2011. We determined the used salinity levels based on the distribution of both species in the flood plains of the Elbe estuary, ranging from the freshwater stretch to the lower limit of the mesohaline tidal stretch. The experiment was conducted in hydroponics in order to measure leaf and root development during the course of the experiment. Non-transparent plastic boxes (18 cm wide, 36 cm long and 20 cm high) were used as experimental mesocosms to avoid algae growth. The mesocosms were filled with well water and supplemented during the experiment. Additive-free sea salt (Meersalz, Alnatura, Bickenbach, Germany) was added to reach the salinity levels 2, 4, 6. Salinity was measured using the Practical Salinity Scale by a Multi-Parameter-Analyser (Eijkelkamp ecoTech 18.28). At the lowest salinity level, well water with salinity 0.3 was used (hereafter referred to as salinity 0). The experiment consisted of five mesocosms per salinity level (N = 5), resulting in 20 mesocosms.

In January 2011, 800 cuttings were harvested from 40 selected *Salix* individuals and stored in plastic bags in a climate chamber at 5 °C. At the start of the experiment, the cuttings were equalized to 20 cm length and weighed. Within the mesocosms, the cuttings were arranged in wooden clips. At least one bud remained above the water and two thirds of the cuttings stayed within the water. Each wooden clip held 10 cuttings of one species from one origin.

Four wooden clips were installed in each of the five mesocosms, resulting in 50 cuttings per species and origin in each of the four salinity levels. The 20 mesocosms were arranged block wise in a greenhouse. Five mesocosms per salinity were placed per block. Due to the sensitive arrangement of the cuttings in the hydroponic, this design had to be retained unchanged during the experiment.

The experiment ran with a mean temperature of 19 °C during 14 h illumination and a mean temperature of 10 °C during dark periods. In addition to sunlight, plants were irradiated with 90 µmol/m²s (LiCor, LI-185B Quantum Sensor).

Plant growth and chlorophyll fluorescence, vitality and biomass

Before the start of the experiment, initial mass of all cuttings was determined. In week 3 and week 7 of the experiment, the number of leaves and roots per cutting were recorded. In week 4 and week 9, chlorophyll fluorescence as maximum quantum yield of photosystem II (Fv/Fm) was determined with a Portable Chlorophyll Fluorometer (PAM-2100, Walz, Germany) based on the principle described by Schreiber et al. (1986). Fv/Fm was calculated as an indicator for the photosynthetic efficiency (Hanelt et al., 1996). Vitality was determined in week 7 according to five predefined vitality classes (5: high, leaves green; 4: medium, leaves up to 50% necrotic; 3: low, leaves more than 50% necrotic; 2: leaves totally necrotic; 1: no leaves developed). At the end of the experiment, shoot and root biomass of the cuttings were harvested, dried separately at 60 °C to constant weight and weighed.

Data analyses

We used Mann-Whitney U tests to analyse differences in soil water salinity between both sites during four seasons since data showed heteroscedasticity of variances.

For the response variables recorded in the hydroponic greenhouse experiment mean and standard deviation were calculated and displayed in graphs to visualize effects of salinity on the measured parameter. Levene's tests on data of plant growth (number of leaves and number of roots), vitality, and dry mass showed heteroscedasticity of variances. Thus, data were square root transformed in order to fulfil the assumptions of ANOVA. As the number of leaves and roots in week three of the experiment approximated zero at salinity 4 and 6, this data was excluded from statistical analyses and ANOVA's were focused on the effects of salinity, species and origin at salinity 0 and 2. For data recorded in week seven, three factorial ANOVA's were applied to test for the effects of salinity, species and origin on square root transformed number of leaves and number of roots and on vitality data at salinity level 0, 2, 4 and 6.

To account for possible differences in cuttings initial weight, the ratio of shoot dry mass: cuttings initial mass and of root dry mass: cuttings initial mass were calculated and multiplied with hundred. Three factorial ANOVA's were applied to test for the effects of salinity, species and origin on square root transformed shoot dry mass: initial dry mass ratio at salinity level 0, 2, 4 and 6. The ratio root dry mass: cuttings initial mass approximated zero at salinity 6 and

thus three-factorial ANOVA's were applied to test for the effects of salinity, species and origin on square root transformed root dry mass: initial dry mass ratio at salinity level 0, 2 and 4. A priori to all statistical analyses, one factorial ANOVA's on the effect of 'block' on plant growth (number of leaves and number of roots), vitality and dry mass were applied. No significant effect was found and thus the effect of 'block' was excluded from further analyses. No statistical test was applied on Chlorophyll fluorescence due to low appropriate sample numbers (leaf necrosis at higher salinities prevented collection of sufficient data). All data analyses were carried out with STATISTICA 9.0 (StatSoft 2009).

2.4 Results

Soil water salinity at willow stands in tidal wetlands along the salinity gradient

Soil water salinity varied between the freshwater and the brackish water site in the Elbe estuary (Fig. 2.2). Large differences in salinity were recorded in summer (Z = -3.5, p < 0.001, N = 18) and spring (Z = 2.9, p < 0.001, N = 13), whereas small differences with lower values occurred in autumn (Z = 3.1, p < 0.05, N = 15) and winter (Z = 2.0, p < 0.05, N = 15). Soil water at the brackish water site exhibited oligohaline conditions during all measurement times.



Figure 2.2 Soil water salinity at four seasons (summer and autumn 2010, winter and spring 2011) at two tidal wetlands dominated by Salix sp. vegetation (freshwater and brackish water site) in the Elbe estuary (*p < 0.05; **p < 0.01; ***p < 0.001).

Salinity tolerance of willows in a hydroponic greenhouse experiment

Plant growth

After three weeks of salinity treatments on cuttings, the number of leaves and the number of roots generally decreased with increasing salinity level (Fig. 2.3). However, the difference in leaf number between salinity 0 and salinity 2 was not significant. Moreover, root number was slightly higher at salinity 2 compared to 0 (F = 3.94, p < 0.05). *S. viminalis* produced significantly more leaves and more roots than *Salix alba* (F = 849.88, p < 0.001 and F = 466.88, p < 0.001, respectively). Cuttings of both species with origin from the freshwater site produced significantly more leaves than cuttings with origin from the brackish site (F = 11.52, p < 0.01). In contrast, no significant effect of cuttings origin on root number in week three was found.



Figure 2.3 Number of leaves in week three of the salinity treatment and number of roots in week three of the salinity treatment (salinity 0, 2, 4, 6) according to species and origin in hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5).

After seven weeks, the number of leaves of both species gradually decreased with increasing salinity (Fig. 2.4). Here, the effect of salinity was highly significant (F = 303.35, p < 0.001). *S. viminalis* produced more leaves compared to *Salix alba* (F = 818.63, p < 0.001). The number of roots increased from salinity 0 to salinity 2 and decreased with further increasing salinity. Within both species, the number of roots at salinity 0 and 2 was higher when cuttings originated from the brackish water site, whereas it was roughly the same within both species at salinity 4 and 6. Overall, neither the number of leaves nor the number of roots of either species showed a significant difference between individuals with freshwater site and brackish water site origin. S. viminalis produced significantly more roots compared to *S. alba*.



Figure 2.4 Number of leaves in week seven of the salinity treatment and number of roots in week seven of the salinity treatment (salinity 0, 2, 4, 6) according to species and origin in an hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5).

Chlorophyll fluorescence

After four weeks of salinity treatments, the maximum quantum yield of photosystem II (Fv/Fm) at salinity 0 and 2 was highest. At salinity 4, variability in Fv/Fm increased, whereas at salinity 6 values generally decreased (Fig. 2.5). After nine weeks, variability of Fv/Fm increased at salinity 2 compared to salinity 0. Here, Fv/Fm of *Salix alba* with brackish water origin was substantially lower compared to freshwater origin. At salinity 4 and 6, Fv/Fm decreased to zero.



Figure 2.5 Fv/Fm in week 4 and in week 9 of salinity treatment (salinity 0, 2, 4, 6) according to species and origin in a hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5, outliers excluded).

Vitality

Vitality significantly decreased with increasing salinity (F = 1015.84, p < 0.001) (Fig. 2.6). After seven weeks of salinity treatment, leaves were totally necrotic at salinity 4, and vitality at salinity 6 was lowest (no leaves developed). *S. viminalis* cuttings performed significantly better compared to *Salix alba* cuttings (F = 67, p < 0.001). Furthermore, a significant interaction between species and salinity on vitality was found (F = 13.73, p < 0.001). *S. viminalis* performed significantly better compared to *S. alba* at salinity 2, 4 and 6, but vitality of both species at salinity 0 was similar.



Figure 2.6 Vitality (5: high, leaves green; 4: medium, leaves up to 50% necrotic; 3: low, leaves more than 50% necrotic; 2: leaves totally necrotic; 1: no leaves developed) after 7 weeks salinity treatment according to species and origin in an hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5). Different letters indicate significant differences in vitality.

Biomass

Shoot dry mass and root dry mass (Fig. 2.7) decreased substantially with increasing salinity. The ratio of shoot dry mass: initial mass decreased significantly with increasing salinity (F = 287.699, p < 0.001). *S. viminalis* performed significantly better than *Salix alba* (F = 17.402, p < 0.001). A significant interaction of species and origin was detected (F = 9.05, p < 0.01): *S. viminalis* cuttings with freshwater origin performed better than *S. alba* cuttings with brackish origin. The ratio of shoot dry mass: initial mass of *S. viminalis* with brackish origin was higher compared to *S. alba* with freshwater and brackish water origin.

The ratio of root dry mass: initial mass decreased with increasing salinity, whereas salinity was excluded from statistical analyses. The decrease was highly significant comparing salinity 0, 2 and 4 (F = 79.59, p < 0.001). The effect of species showed a better performance of *Salix viminalis* compared to *Salix alba* (F = 25.31, p < 0.001). Moreover, a significant interaction of salinity and species was detected (F = 13.79, p < 0.001). The ratio of root dry mass: initial mass in *S. viminalis* was higher compared to *S. alba* at salinity 0 and 2. However, at salinity 4 *S. alba* performed compared to *S. viminalis*. Furthermore, species and origin showed a significant interaction (F = 13.01, p < 0.001): *S. viminalis* cuttings with freshwater origin performed better compared to *S. alba* cuttings from brackish origin. The ratio of root dry mass: initial mass of *S. viminalis* with brackish origin was higher compared to *S. alba* with freshwater and with brackish water origin.



Figure 2.7 Shoot dry mass (A) and root dry mass (B) after 10 weeks of salinity treatment (salinity 0, 2, 4, 6) according to species and origin in an hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5).

Shoot dry mass/initial biomass was higher than root dry mass/initial biomass after 10 weeks of salinity treatment (Table 2.1). The ratio values decreased at salinity 2 compared to salinity 0 since root production relatively increased. Here, the decrease of ratio values was larger in *Salix alba* than in *Salix viminalis*. *S. alba* with brackish water origin produced more roots than with freshwater origin, and S. viminalis with freshwater origin produced relatively more roots than with brackish water origin (Table 2.1).

Table 2.1. Ratio of shoot dry mass/initial biomass to root dry mass/initial biomass after 10 weeks of salinity treatment (salinity 0, 2, 4, 6) according to species and origin in an hydroponic experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/-SD, N = 5).

Salinity	S. alba / freshwater	<i>S. alba /</i> brackish water	<i>S. viminalis /</i> freshwater	S. viminalis / brackish water
0	5.17	6.91	2.94	2.05
2	1.19	1.02	1.03	1.41
4	1.65	1.63	2.71	3.22
6	-	-	-	-

2.5 Discussion

Soil water salinity at willow stands in tidal wetlands

Variable and fluctuating salinity is a major physiological stressor in estuarine transitional waters (Odum, 1988; McLusky and Elliott, 2004). However, *Salix* species mainly occur above mean high water levels in estuarine flood plains (Newsholme, 1992; Ellenberg and Leuschner, 2010) and thus are expected to be less affected by regular flooding and salinity. The soil water salinities at the investigated willow stands reflected the variable and fluctuating salinity in estuarine transitional waters. In the flood plains at the freshwater stretch, soil water salinity beneath *Salix* stands was generally low whereas *Salix* stands at the brackish water stretch exhibited oligohaline conditions with values from 0.4 in winter up to 2.3 during the growing season.

A field survey at flood plains of the river Murray, Australia, confirmed the distribution of closely related *Salix* species even up to a soil water salinity of 7.35 (Kennedy et al., 2003). However, increased salinity was the reason for the replacement of riparian Salix communities by *Tamarix* communities under semiarid Mediterranean climate (Salinas et al., 2000) and *Salix* species native to the lower river Colorado, Mexico, were impaired compared to invasive *Tamarix* species because of their lower salt tolerance (Vandersande et al., 2001). Ellenberg et al. (1992) even consider *Salix alba* and *Salix viminalis* to show no salt tolerance. This general assessment is contrary to our results. We showed that *S. alba* and *S. viminalis* are occurring in flood plains at the transitional waters of the oligohaline to the mesohaline estuarine area and we state a salinity tolerance of mature *S. alba* and *S. viminalis* individuals at least up to salinity 2.

Salinity tolerance of willows in a hydroponic greenhouse experiment

Overall, there were substantial negative effects of increasing salinity on growth parameters and final biomass, vitality and chlorophyll fluorescence of both *Salix* species from both origins in the hydroponic greenhouse experiment. The results clearly showed a decrease in plant growth, in shoot dry mass: initial mass and final root dry mass: initial mass as well as in vitality and chlorophyll fluorescence with increasing salinity. Decreased growth parameters were also found in a desert *Salix* species in response to reduced water supply (Xiao et al., 2005) and in poplar genotypes (Salicaceae) responding to increasing NaCl (Fung et al., 1998). Accordingly, our measured values at salinity 4 and 6 approximated zero and vitality substantially declined as well. This decrease in growth parameters of both species at increasing salinity might be due to a general low salinity tolerance of Salix (Ellenberg et al., 1992; Newsholme, 1992).

In general, plants can either tolerate or avoid temporally stressful environments. In response to drought, tolerators require water throughout the drought to maintain their leaves, while avoiders drop their leaves rapidly and only require water for resprouting (Savage and Cavender-Bares, 2011). In our hydroponic greenhouse experiment with 10 weeks of salinity treatment, both species exhibited growth and biomass, photosynthetic activity and vitality with maintaining their leaves up to salinity 2 what corresponds to the observed distribution of *Salix alba* and *Salix viminalis* in tidal wetlands up to a soil water salinity 2. Thus, regarding the distinction between stress tolerators and avoiders, we reassess *S. alba* and *S. viminalis* as being salt tolerators up to salinity 2.

Interspecific differences in salt tolerance of S. alba and S. viminalis

Interspecific differences in plant growth of Salix alba and Salix viminalis in our mesocosms experiment was rather affected by differences in phenology and ecological behaviour than by interspecific differences in salinity tolerance, since the effect of species on number of leaves and number of roots was significant, whereas no significant interaction of species and salinity was detected. S. viminalis produced a certain number of leaves and roots within three weeks at all salinity levels, whereas leaf and root number of S. alba remained almost zero. Although four weeks later S. alba was also growing, leaf and root number of S. viminalis was still higher. S. viminalis occurs together with other narrow leaved Salix species directly above the reed zone dominated by *Phragmites australis* at the mean water line while S. alba prefers higher elevations (Ellenberg and Leuschner, 2010). Thus, disturbance (e. g. wave action, flotsam and ice scouring) does more severely affect S. viminalis in its habitat. According to Karrenberg et al. (2002), intermediate disturbance in flood plains promotes resprouting of Salicaceae from plant fragments. Thus, the higher resprouting capacity of S. viminalis might be a reason for the faster development of leaves and roots in our experiment, contrary to S. alba, whose twigs do not have this brittleness at their bases and whose rooting ability is low (Beismann et al., 2000). It can be assumed that hydraulic forces restrict the niche of S. alba at low elevations (Mosner et al., 2011). The final dry mass confirmed the differences between both Salix species in leaf and root growth during of the experiment. S. viminalis seemed to have an advantage in growing from cuttings, since growth via vegetative propagules meets the species dispersal characteristics. However, at the end of the experiment, both species had produced substantial dry mass at salinity 0 and 2, representing the general high growth

rates of flood plain willows caused by their life history characteristics (Karrenberg et al., 2002).

Root number of both species and root dry mass of *Salix alba* increased from salinity 0 to salinity 2. However, leaf number and shoot dry mass gradually decreased with increasing salinity. At salinity 0, the calculated shoot:root ratio of *S. alba* was high (5-7), while that of *Salix viminalis* was substantially lower. While there is a tendency for any species to maintain a characteristic shoot:root ratio, this functional equilibrium might be changed by translocation of resources towards the stressed plant organ (Klepper, 1991).

Accordingly, the wetland species *Phragmites australis* responded with lower allocation of biomass to roots and rhizomes at high nutrient availability (Eller and Brix, 2012) and with higher allocation to roots with increasing salinity (Eid et al., 2010). In *Populus* taxa increasing NaCl induced increased biomass allocation to roots (Fung et al., 1998) and native desert *Salix* taxa responded with increased biomass allocation to roots under drought (Xiao et al., 2005). Root growth increased more compared to shoots in willow culitvars used for biomass production in humid climate (Wikberg and Ögren, 2007). This corresponds to our results, since shoot biomass was negatively affected at salinity 2 and might have been balanced by increased translocation towards roots. With increasing salinity, the root:shoot ratio of *S. alba* decreased substantially more compared to *S. viminalis*. Thus, we suppose *S. alba* has a high ability to respond to soil water salinities up to 2 with increasing root growth to optimize water uptake.

Effects of origin on salinity tolerance of S. alba and S. viminalis

We found that effects of origin on salinity tolerance differed between *Salix alba* and *Salix viminalis*. With regard to final shoot and root dry mass at salinity 2, *S. alba* cuttings originating from the freshwater site performed better compared to the brackish origin. This corresponds to the decrease of the maximum quantum yield (Fv/Fm) with increasing salinity which was larger in *S. alba* from brackish water origin than from freshwater origin. Drought responses of willows suggest that their photoprotective strategies vary with habitat affinity (Savage, 2009). Furthermore, the decrease in shoot to root ratio at salinity 2 compared to 0 was larger in *S. alba* with brackish water origin compared to freshwater origin, but lower in *S. viminalis* with brackish water origin compared to freshwater origin. Short-term adaptation of willows to different environments was documented by Wikberg and Ögren (2007) with an higher water use efficiency in willow individuals treated by drought compared to well watered conditions (Wikberg and Ögren, 2007). Willow species originating from

higher growth rates compared to wetland species indicating adaptations to different habitats (Savage and Cavender-Bares, 2011). Accordingly, we suggest *S. viminalis*, but not *S. alba* individuals in tidal wetlands with brackish water origin being acclimated or even locally adapted to salinity 2.

2.6 Conclusions

We provide evidence of willow salinity tolerance in estuarine flood plains. Mature *Salix* viminalis shrubs and *Salix alba* trees are distributed up to the mesohaline estuarine stretch and tolerate oligohaline soil water conditions in estuarine flood plains. Our experiment confirmed that cuttings of both species tolerate salinities up to 2. Thus, we reassess both species as salt tolerators up to moderate oligohaline conditions. Effects of origin were reflected in vitality, final dry mass and chlorophyll fluorescence.

Better performance of S. viminalis with brackish water origin at salinity 2 might indicate acclimation or even local adaptation to increased salinity.

Flood plain willows contribute to wave attenuation and sedimentation and thus are important for ecosystem services like coastal protection (Borsje et al., 2011). Estuaries are "hot spots" at the coastal zone: In these river-mouth systems fluxes of water, dissolved and suspended matter are focused, human populations are concentrated, and problems caused by salinization, erosion and subsidence are particularly severe (Newton et al., 2012). Regarding estuarine flood control, future studies should focus on Salix species salinity tolerance in the natural environment. Further studies on willows in tidal wetlands should separate the role of genetic variation and phenotypic plasticity for salinity tolerance by applying reciprocal transplantation experiments. By doing so, genetically suitable material with possible local adaptations would be determined. This knowledge is important before willows are planted in restoration projects (Mosner et al., 2012).

3. Survivors in drowning wetlands: Willows tolerate tidal flooding

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3.1 Abstract

Tidal wetlands are widely affected due to recent and projected sea level rise. Willows (*Salix alba* and *Salix viminalis*) are characteristic trees in softwood riverine and estuarine flood plain forests in temperate climates. Their tolerance to long-term flooding is well documented. However, effects of increasing tidal flooding on willows are largely unknown. To assess plant tolerance to tidal flooding, we conducted a mesocosm experiment with tidal treatments. Vegetative propagules were harvested from plants at the freshwater and oligohaline stretch of the Elbe estuary - a model system for an estuary in temperate zones – and exposed to increasing tidal flooding.

We detected a general tolerance to a tidal flooding depth of 60 cm in chlorophyll fluorescence, growth rates and biomass production. However, *S. alba* performed better in terms of leaf and shoot growth whereas *S. viminalis* produced more biomass. *S. alba* of brackish origin performed worst, indicating a preweakening due to site conditions.

With this study we clarified that the European flood plain willows species *S. alba* and *S. viminalis* tolerate increasing tidal flooding corresponding to moderate sea level rise scenarios. We conclude that conservation and restoration of willow forests in tidal wetlands might be possible despite sea level rise.

Keywords

Sea level rise, tidal flooding, tolerance, willows

3.2 Introduction

Tidal wetlands are coastal ecosystems that are influenced by tides and that have perceived benefits to society, either direct or indirect, that result from wetland functions. These values include human welfare, environmental quality, and wildlife support. Thus, many types of coastal ecosystems, including estuaries, deltas and tidal flats are listed as Ramsar sites and protected as wetlands of international importance (Ramsar, 2014). However, in recent decades coastal wetlands are suffering immense pressure due to the effects of various natural and human induced factors such as storms, relative sea level rise, coastal engineering, land conversion and construction of dams and dikes. As a result, shoreline location and the changing position of this boundary over time are of great importance to coastal wetland scientists, and managers (Wang et al., 2013). In addition, coastal wetlands that have been modified by anthropogenic activities are often reduced in area. Globally, about 35% of the area of mangrove forests has disappeared since 1980 and the current global rate of loss of tidal marsh is estimated to be between 1 and 2% per year (Pendelton et al., 2012). Specifically, estuaries deserve more attention in science and by the public in times of rapidly expending human uses of coastal zones: estuaries are a major transition zone with steep gradients in energy and physicochemical properties at the interface between land and sea and thus highly affected by pressures that global climate change impose on continents and oceans (Jennerjahn and Mitchell, 2013).

Hydrology, sediment transport, transfer of nutrients, species movement and dispersal link habitats at the estuarine gradient physically, chemically and biologically, both seasonally and during single tidal cycles (Meire et al., 2005). Estuarine flood plains are characterized by two major environmental gradients: the salinity gradient and the flooding gradient. Abiotic stress and disturbance become more severe with increasing salinity and flooding from high to low elevation in estuarine flood plains (Engels and Jensen 2009). However, the abundance and biomass of organisms is usually very high (Meire et al., 2005), and estuaries are cited among the most productive biomes of the world (Costanza et al., 1993). In tropical Amazonian freshwater river floodplain forests more than 1000 tree species grow in an environment subject to extended annual submergence during the flood season. Here, despite a lack of both oxygen and light imposed by submergence for several months, numerous physiological mechanisms and anatomical adaptations may explain the success of species to survive submerged (Parolin, 2009).

In temperate climates, willow species dominate floodplain forests at low elevations. Here, biological characteristics of softwood willow species can be interpreted as adaptations to the floodplain environment since willow softwood forests are a characteristic feature of both

riverine and estuarine floodplains. Willows are part of the species-rich genus *Salix*, which contains numerous floodplain species (Newsholme, 1992; Karrenberg et al., 2002). Wetland willows generally are a disturbance-adapted group of wide ecological tolerance, capable of rapid growth and vigorous reproduction (Timoney and Argus, 2006). Thus, many floodplain willows are able to establish right above the mean water level and cope with disturbances like waves and ice scouring by their high regeneration capacity (Ellenberg and Leuschner, 2010). Flood plain willows in temperate climates contribute to ecosystem services like coastal protection by attenuating waves and stabilising sediments (Borsje et al., 2011; Radtke et al., 2012).

However, climate change strongly affects coastal wetlands ranging from mangrove forests and salt marshes up to tidal freshwater wetlands at the upper estuarine boundary. Mean rate of global averaged sea level rise was 1.7 (1.5 to 1.9) mm per year between 1901 and 2010, 2.0 (1.7 to 2.3) mm per year between 1971 and 2010, and 3.2 (2.8 to 3.6) mm per year between 1993 and 2010. Projected change in global mean sea level rise for the mid- and late 21st century relative to the reference period of 1986-2005 is 24-30 cm until 2046-2065 and 40-63 cm until 2081-2100 (IPCC 2013). Elevation in relation to mean high water largely explains species distribution within studied salt marshes of the mainland North Sea coast (Suchrow and Jensen, 2010). For tidal freshwater wetlands, a rising sea level might contribute to a rapid loss of both habitats and biodiversity or to a conversion of vegetation caused by increased tidal flooding (Baldwin and Mendelssohn, 1998; Neubauer and Craft, 2009). In addition, tidal freshwater wetlands have become scarce due to human alterations like diking, harbor expansion and land use changes for agricultural purposes, especially in Europe. Nonetheless, substantial areas of tidal freshwater areas with high tidal amplitude still occur today at the Scheldt (Belgium) and the Elbe (Germany). Here, the remaining woody communities in tidal wetlands are dominated by willows (Struyf et al., 2009) which might in the future be affected due to increasing sea level.

It is well known that tree physiology, growth, and species distribution are linked to heights and duration of periodical inundations in Amazonian tree species (Marinho et al., 2010). Here, seasonal inundations might be the most important factor influencing the establishment, population dynamics, distribution and growth of floodplain trees (Wittmann et al., 2006). Although it has long been known that floodplain willows are generally flooding tolerant, effects of changing water levels in the intertidal are not well studied yet. In our study *Salix alba* L. and *Salix viminalis* L. (hereafter referred to as *S. alba* and *S. viminalis*) served as model willow species. They are the most widespread willows in the flood plains of the Elbe estuary (Raabe, 1986) and are characteristic tree species in flood plains in temperate zones

(Struyf et al., 2009). Floodplain willows are generally described as tolerant to flooding (Newsholme, 1992). However, Timoney and Argus (2006) found the flooding tolerance of willows to be species-specific in Canadian river flood plains. Moreover, local adaptations in plants can be caused by small scale differences in the environment (Lipowsky et al., 2011). Intraspecific differences in salt tolerance of *S. alba* and *S. viminalis* in plants originating from freshwater and brackish wetlands were recently detected (Markus-Michalczyk et al., 2014). In a hydroponic experiment with salinity treatment, *S. viminalis* cuttings of brackish origin performed better than those of freshwater origin. In contrast, *S. alba* cuttings of freshwater origin performed better than those of brackish origin. Thus, intraspecific differences in tidal flooding tolerance might be possible for *Salix* cuttings originating from tidal wetlands at the Elbe estuary.

We hypothesised that *Salix alba* L. and *Salix viminalis* are able to tolerate tidal flooding projected in moderate climate change scenarios. Specifically, we aimed to answer (i) which tidal flooding the species are able to tolerate and (ii) whether interspecific differences are existing with regard to tidal flooding tolerance. Concerning intraspecific differences we aimed to analyse (iii) if the tidal flooding tolerance of willow species differs between populations that originate from tidal freshwater versus brackish wetlands.

3.3 Materials and methods

A tidal simulation experiment was conducted in mesocosms with defined tidal treatments from May to October 2012 in the Botanical Garden of Hamburg University (N53.561, W9.858) within a temperate climate. Immature plants of (i) two willow species (*S. alba* and *S. viminalis*), (ii) from two origins (freshwater and brackish site) were exposed to (iii) four tidal flooding levels (0 cm, 20 cm, 40 cm, 60 cm).

Plant material

Plant material was collected from two sites at tidal wetlands along the Elbe estuary (Fig. 3.1).



Figure 3.1 Study area in the Elbe estuary in Europe (A) and northern Germany (B), brackish site (1) and freshwater site (2).
The first site, at the lower mesohaline stretch of the estuary (Fig. 3.1, B 1), represents a close to nature brackish water influenced site (hereafter referred to as brackish site). The other site, at the upper limnic stretch of the estuary (Fig. 3.1, B 2), represents a close to nature freshwater influenced site (hereafter referred to as freshwater site). At each site, S. alba and S. viminalis individuals with straight shoots were selected. 12 individuals per species – six of both origins – were used as sources for cuttings. We harvested 24 cuttings on March 28th and 29th, 2012, from each of the selected individuals (576 in total) and stored the plant material in plastic bags in a climate chamber at 5 °C until March 30th. The planting substrate used in the experiment was composed of half river sand and half compost as an organic component. Both components were mixed and sieved, and pots (18 cm diameter, 14 cm high) were filled with the planting substrate. On March 30th the cuttings were equalized to 20 cm length and weighed. In each filled pot, two cuttings of one species and one origin were placed into the planting substrate and watered with spring water. In order to protect them from frost and UV radiation damage, the cuttings were at first stored in a greenhouse for four weeks and then exposed to field conditions in the open. After four weeks, the one of the two cuttings per pot with less developed leaves was removed. The remaining individual per pot served as plant material in the experiment with defined tidal treatments.

Tidal treatments

We established tidal treatments on stairways, which were placed in mesocosms (3 m X 1.5 m; 1.5 m height) and corresponded to the current hydrological conditions of S. alba and S. viminalis habitats ranging from the mean high tide line up to more elevated sites. According to IPCC (2007), average regional water levels rise (hereafter referred to as rwlr) on the German North Sea coast can be expected to be between 20 cm and 80 cm by the end of the 21st century. The amplitude of flooding level in the set-up of the tidal treatments represented the 80 cm sea level rise scenario. Plants on the uppermost step were exposed to hydrological conditions similar to today's lowest natural sites of both Salix species at the mean high tide line, and plants on lower steps of the stairways experienced increasing flooding. The pots height was 14 cm and the difference between steps was 20 cm. The highest flooding level of 80 cm reached the pot's soil surface at the uppermost step and the lowest flooding level of 0 cm reached 6 cm under the pot's bottom at the undermost step of the flooding stairways. Thus, the stairways were flooded by daily tides with different flooding height stepwise: (i) step one: 0 cm - flooded up to the pot's soil surface (corresponds to sites at mean high tide line); (ii) step two: 20 cm - flooded up to 20 cm above the pot's soil surface; (iii) step three: flooded up to 40 cm above the pot's soil surface; (iv) step four: flooded up to 60 cm above the pot's soil surface. Four replicates of two species from two origins (S. alba and S. viminalis from the freshwater and brackish sites) were selected,

resulting in 16 pots on each of the four steps, arranged randomly on the flooding stairways. The tidal treatments were applied from May 11th to October 17th, 2012.

Tidal simulation system

The tidal simulation system consisted of two water storage tanks and four mesocosms. The mesocosms were equipped with timer-controlled electronic pumps (Grundfos Typ Unilift CC7-A1 - model 96280968) and connected to the water storage tanks. Tides were produced by water transfer between the water storage tanks and the mesocoms. During flood tide, well water was pumped from the water storage tanks via tubes into the mesocosms until the water level reached the soil surface of the pots on the uppermost step. During ebb tide, water was pumped from the mesocosms via tubes into the water storage tanks until the water level was below the bottom of the pots on the lowest step. Imitating the conditions at the Elbe estuary, which has a stronger flood tide compared to the ebb tide, the flood tide lasted 5h and the ebb tide duration was 7h. We set up the timer-control of the electronic pump, so that the turning of the tide occurred approximately one hour later each successive day, similar to the daily inequality of semi-diurnal tides in natural systems, in order to align our tidal simulation system with the tide at the Elbe estuary.

Data collection

Before the start of the tidal treatment, the cuttings initial mass was measured and cuttings were allowed to establish from March 30th to May 11th. After establishment, initial number of leaves, number of shoots and shoot length were determined for the cuttings. Then, the established immature plants were placed on the flooding stairways in the mesocosms and the tidal treatments started. During the course of the tidal treatments, chlorophyll fluorescence, number of leaves, number of shoots and shoot length were recorded every two weeks. Chlorophyll fluorescence was recorded as maximum quantum yield with a Portable Chlorophyll Fluorometer (PAM-2100, Walz, Germany) based on the principle described by Schreiber et al. (1986). Fv/Fm was calculated as an indicator of photosynthetic efficiency (Hanelt et al., 1996). To perform the measurements, the pots were removed from the steps during low tide. After carrying out the measurements, the pots were rearranged according to their tidal treatment level randomly on the steps of the stairways.

On August 24th, the experimental set-up in one mesocosm was terminated. Cuttings leaf, shoot and root biomass of plants were harvested, dried separately at 60 °C to constant weight and weighed.

In the other three mesocosms, each cutting's aboveground biomass was removed. In order to analyse plants resprouting capacity under flooding conditions, the tidal treatment continued from August 24th to October 17th. The resprouting rate, the number of resprouted leaves and the resprouted shoot length were recorded every two weeks.

Growth could be limited by light; hence, it is necessary to investigate the photosynthetic energy supply. Photon flux density was recorded during the flooded state of the tidal simulation system in repeated measurements biweekly at high noon (week 0: 26.06., week 2: 09.07., week 4: 24.07., week 6: 07.08.2013) in the tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm). Photon flux density was measured in the water beside the soil surface of the pot and in the air directly above this point above the water column on the pot's soil surface (Li COR Data Logger; LI 192 UW quantum sensor).

Data analysis

We performed ANOVA's to test for the effects of tidal treatments with four flooding levels on two species, *S. alba* and *S. viminalis*, from two origins - freshwater and brackish sites. Repeated measurement ANOVA was applied on chlorophyll fluorescence and pairwise differences were tested with Tukey's post hoc tests. ANCOVAs were applied on aboveground dry mass (leaf mass and shoot mass) and on belowground dry mass (root mass) as well as on total dry mass and on the ratio of above- to belowground dry mass. Here, cuttings initial mass served as covariate. Growth measured as change in leaf number (%), change in shoot number (%), and change in total shoot length compared to initial values was analysed with repeated measurement ANOVAs. Pairwise differences were tested by applying Tukey's post hoc tests.

Mean and standard deviation of photon flux density in water were calculated and displayed in Fig. 8. ANOVA's were applied to test for the effects of tidal flooding depth on relative irradiance (percentage of photon flux density in water in relation to photon flux density in air) and pairwise differences were tested with Tukey's post hoc tests.

Prior to all analysis, Levene's tests were applied to test for homogeneity of variance. Change in total shoot length showed heteroscedasticity of variances and thus were log-transformed in order to meet the assumptions of ANOVA.

All data analysis was carried out with STATISTICA 9.0 (StatSoft 2009).

3.4 Results

Results of the tidal flooding treatment on immature plants of (i) two willow species (*S. alba* and *S. viminalis*), (ii) from two origins (freshwater and brackish site), (iii) in four flooding levels (0 cm, 20 cm, 40 cm, 60 cm) indicate a relative submergence tolerance up to a tidal flooding level of 60 cm.

Chlorophyll fluorescence

Repeated measurement ANOVAs did not show a significant effect of tidal flooding on photosynthetic efficiency (Fv/Fm-values), and inhibition of photosynthesis was not detected. Neither the differences between *S. alba* and *S. viminalis*, nor between origins were significant. Thus, no impact of time, flooding, species and origin on photosynthetic performance was found (Appendix 3.1).

Dry mass



Figure 3.2 Total dry mass after 15 weeks of tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands (mean +/- SD, N = 4).

Overall, total dry mass significantly decreased with increasing flooding level (Fig. 3.2; Appendix 3.2). Tukey's HSD tests confirmed significant differences between 0 cm and 20 cm tidal flooding (p < 0.05), 0 cm and 40 cm (p < 0.01), 0 cm and 60 cm (p < 0.001), 20 cm and 60 cm (p < 0.001) and 40 cm and 60 cm tidal flooding (p < 0.001). *S. viminalis* produced significantly more dry mass than *S. alba* (p < 0.001).



Figure 3.3 Ratio of above- to belowground dry mass after 15 weeks of tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands (mean +/- SD, N = 4).

The ratio of above- to belowground dry mass significantly increased with increasing flooding level (Fig. 3.3; Tab. 3.2). Here, Tukey's post hoc tests confirmed significant differences between 0 cm and 40 cm tidal flooding (p < 0.01) and 60 cm (p < 0.001) whereas ratios remained similar in 20 cm compared to 40 cm tidal flooding. Additionally, *S. alba* of brackish origin developed higher above- to belowground ratios than with freshwater origin (p < 0.05). With regard to plants originating from the brackish site, *S. alba* showed higher ratios of above- to belowground dry mass than *S. viminalis* (p < 0.05).

Growth

The number of leaves increased from May to July (p < 0.001), whereas no significant change in leaf number from the end of July to the end of August was found (Fig. 3.4; Appendix 3.3).



Figure 3.4 Increase in number of leaves (change in leaf number compared to the initial number of leaves) after 15 weeks of tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands (mean +/- SD, N = 4).

Leaf growth at a certain time was similar in each of the four flooding levels and no significant effect of tidal flooding was found. ANCOVA demonstrated, however, a significant interaction between time and species (Appendix 3.3). Leaf number of *S. alba* increased over time more than that of *S. viminalis*. Differences became highly significant at the end of June and remained highly significant until the end of August (p < 0.001).

Growth of total shoot length increased over time (Fig. 3.5; Appendix 3.4) with significant differences between all measurements from May to August (p < 0.001). No significant effect of flooding on changes in total shoot length was found after 15 weeks of tidal treatments.

Substantial differences in changes of total shoot length were found between species. *S. alba* total shoot length was generally higher compared to initial values than *S. viminalis* total shoot length. The interaction of time and species was found to be significant. Here, *S. alba* performed better at each time point except in May (p < 0.001).



Figure 3.5 Growth of total shoot length (change compared to initial total shoot length) (cm) \log^{10} transformed, after 15 weeks of tidal treatment (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands (mean +/- SD, N = 4).

Resprouting rates after shoot removal

After 15 weeks of tidal treatments shoots were removed in order to investigate plant resprouting capacity in tidal treatments. Resprouting rates after shoot removal were 92.63% in *S. alba* and 98.90% in *S. viminalis* at the end of the tidal treatment. The number of resprouted leaves generally increased over time (p < 0.001) and decreased with increasing flooding (p < 0.001) (Fig. 3.6). No significant differences in resprouted number of leaves were found between 20 cm and 40 cm tidal flooding. Furthermore, we detected significant effects of species on number of resprouted leaves over time (p < 0.001). Here, resprouted number of leaves was substantially higher in *S. viminalis* than in *S. alba*.



Figure 3.6 Number of resprouted leaves after 6 weeks of tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/- SD, N = 3).



Figure 3.7 Resprouted total shoot length after 6 weeks of tidal treatment (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/- SD, N = 3).

Repeated measurement ANOVA showed temporal increasing length of resprouted shoots among three dates (p < 0.001) and decreasing total shoot length with increasing flooding (p < 0.001). No significant differences in total shoot length were found between 20 cm and 40 cm tidal flooding at all measurement dates (Fig. 3.7). Temporal shoot length development differed between species (p < 0.001). Resprouted shoots were substantially longer in *S. viminalis* than in *S. alba*.

Photon fluxes

The photon flux densities in the water decreased substantially with increasing flooding depth at each of the four biweekly measuring dates (Fig. 3.8). In week 0, with overall lowest irradiance, values at 20 cm to 60 cm flooding dropped below 200 μ mol/m²s. In week 4, with the second lowest irradiance, values at 60 cm flooding dropped below 200 μ mol/m²s. In week 2 and week 6 with high irradiances, values remained above 200 μ mol/m²s at flooding 0 cm to 60 cm at noon.

Relative irradiance in water generally decreased with increasing flooding depth in week 0 (F = 25.02; p < 0.001), week 2 (F = 196.99; p < 0.0001), week 4 (F = 53.71; p < 0.0001) and week 6 (F = 69.92; p < 0.0001). Here, relative irradiance in water dropped below 50% at 40 cm flooding at each measuring date compared to irradiance impinging on the surface. At week 4 and week 6 values decreased below 25% at 60 cm flooding (Appendix 3.3).



Figure 3.8 Photon flux density in water in biweekly repeated measurements at high noon (week 0, week 2, week 4, week 6) in tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) (mean +/- SD, N= 4).

Relative irradiance in water generally decreased with increasing flooding depth in week 0 (F = 25.02; p < 0.001), week 2 (F = 196.99; p < 0.0001), week 4 (F = 53.71; p < 0.0001) and week 6 (F = 69.92; p < 0.0001). Here, relative irradiance in water dropped below 50% at 40 cm flooding at each measuring date compared to irradiance impinging on the surface. At week 4 and week 6 values decreased below 25% at 60 cm flooding (Appendix 3.4).

3.5 Discussion

Sea level rise due to climate change will cause considerable regional increase in mean high water lines in tidal wetlands (IPCC 2007). Thus, plant species existing right above the mean high water line might be affected due to changes in flooding and air exposure.

Tolerance of willows to tidal flooding in a tidal simulation experiment

Overall, plants survived the tidal flooding during 15 weeks from May to August 2012. Moreover, after shoot removal at the end of 15 weeks more than 90 % of cuttings showed resprouting. The effects of increasing tidal flooding on chlorophyll fluorescence, growth parameters, final biomass and resprouting capacity of *S. alba* and *S. viminalis* of tidal

freshwater and brackish origin in our tidal simulation experiment indicated a general tolerance up to the maximal applied tidal flooding depth of 60 cm. Chlorophyll fluorescence and growth differed marginally, but above- to belowground ratio was higher with increasing flooding depth. Final biomass and resprouting capacity decreased in a similar way at 20 cm and 40 cm tidal flooding, but substantially decreased at 60 cm flooding depth. Thus, a clearly lower tolerance to tidal flooding at 60 cm depth was indicated.

Different flooding types, ranging from soil waterlogging to total submergence of the vegetation, can cause significantly different effects on plants. In flooded soils, excess water filling of soil pores with lower oxygen supply on roots causes physiological stress (Blom and Voesenek, 1996). At complete submergence gas diffusion dramatically decreases and limits the entry of CO_2 for photosynthesis and of O_2 for respiration (Colmer and Pedersen, 2008). However, in our tidal treatment, plants experienced repeated flooding and subsequent exposure to high light conditions. Here, photosynthetic activity of willows, despite twice daily flooding and measurements during exposure to air, was generally optimal during the 15 weeks of tidal treatment (no decrease of Fv/Fm). Plants growing in low light habitats are usually sensitive to UV-radiation and when exposed to a higher UV-irradiance than encountered in their natural habitats, a harmful effect of UV is frequently reported (Hanelt and Roleda, 2009). Intertidal zones are locations with potential for damage by high photosynthetically active radiation (PAR) or UV-radiation (Hanelt et al., 1997). Luo et al. (2009) also hypothesised that post-submergence growth recovery may require efficient photosynthetic acclimation to increased O₂ and irradiance to minimize photo-oxidative damage in two flood tolerant species. However, both studies found that plants were able to maintain the functionality of their photosynthetic apparatus through rapid acclimation to changing O_2 and light conditions. This corresponds to our results of photosynthetic activity (Fv/Fm) of willows which performed optimally after tidal flooding and may have indicated an adaptation to the tidal wetland habitat in which water levels regularly fluctuate.

In contrast, we found a significant decrease in dry mass with increasing flooding depth. This might correspond to the decreased light availability that occurs with an increasing water column (Tab. 4) which, according to Sand-Jensen (1991), might drop below the compensation point so that photosynthesis stagnates and light energy is too low to be converted into biomass. In our experiment irradiance values at noon dropped several times below 200 µmol/m²s so that photosynthesis might have become light limited. Sufficient light supply increases the survival of terrestrial plants under water, indicating that photosynthesis commonly occurs under submerged conditions (Mommer and Visser, 2005) and even submerged plants usually require up to 29% of the incident light measured just below the

water surface (Dennison et al., 1993). Thus, the decrease of the impinging light down to 50% of surface irradiance as observed at a water column of 40 cm, and down to 25% at 60 cm might have caused the significant reduction in dry mass.

However, the above- to belowground ratio increased with increasing flooding. These morphological changes might indicate physiological stress on roots (Blom and Voesenek, 1996). Thus, the dry mass decrease might be mainly due to root necrosis since roots rarely possess the robust anaerobic energy metabolism needed to sustain growth under wet conditions. The decreased belowground compared to aboveground dry mass corresponds to general functional traits of wetland plants exhibiting reduced belowground biomass (Mitsch and Gosselink, 2000) and to findings in experimental flooding treatments on wetland plants (Miller and Zedler, 2003).

Furthermore, our results are in agreement with findings in cutting experiments on a North American willow in flood plains (*Salix nigra*) (Li et al., 2006) and on stress responses in *Salix gracilistyla* cuttings originating from central Japanese river floodplain subjected to repeated alternating flooding and drought (Nakai et al., 2010). Moreover, vegetative reproduction capacities of mid European floodplain willow cuttings showed a more reduced root biomass production in waterlogged compared to moist conditions (Radtke et al., 2012).

Growth in shoot length was not affected by increasing flooding until August, contrasting with the well-known results of a similar study that found that submergence stimulated stem elongation as a distinctive trait of the "escape" strategy of flood tolerance (Bailey-Serres and Voesenek, 2008). Pierik et al. (2010) found in the flood-tolerant species *Rumex palustris* that submergence and shade can induce rapid shoot elongation by elevated ethylene and a reduced red : far-red light ratio (R:FR), respectively. However, we detected no shoot elongation with increasing flooding, which underlines the different effects of stagnant flooding compared to regularly changing tidal water levels in our experiment.

Interspecific differences in tidal flooding tolerance of S. alba and S. viminalis

Interspecific differences in effects of tidal flooding on *S. alba* and *S. viminalis* were detected in final dry mass, growth parameters and resprouting capacity. *S. viminalis* produced more dry mass and showed a higher resprouting capacity, whereas *S. alba* developed more leaves and larger total shoot length compared to initial values.

The higher dry mass and resprouting capacity in *S. viminalis* compared to *S. alba* might be rather due to differences in dispersal and growth characteristics than to interspecific differences in effects of tidal flooding, since the effect of species on dry mass was significant,

whereas no significant interaction of species and tidal flooding was detected. *S. viminalis* is well known for twigs which show brittleness at their bases so that plant fragments commonly serve as vegetative propagules. These propagules have a fast rooting ability and provide rapid establishment and growth (Newsholme, 1992). *S. alba*, however, mainly disperses via seeds, its twigs do not have this brittleness at their bases, rooting ability is low (Beismann et al., 2000), and thus resprouting capacity and biomass development might have been slower in our experiment.

Leaf number of *S. alba* increased substantially more over time than leaf number of *S. viminalis.* This might be due to its well-known ability to cope with long inundation periods (Borsje et al., 2011). However, this feature is described for mature plants mainly, which are only partially inundated. Since we investigated immature plants which - at deep flooding level - were totally submerged, we suggest leaf surface characteristics as a possible explanation for these interspecific differences. *S. alba* leaves are covered with hydrophobic hairs on the leaf upper- and underside (Lautenschlager, 1986; Zander, 2000) whereas *S. viminalis* leaves are hairy only underneath.

Leaf gas films on completely submerged rice facilitate entry of O_2 from floodwaters in darkness and CO_2 entry in light, and thus, have been termed 'plant plastrons', analogous with the plastrons of aquatic insects (Pedersen et al., 2009). Beneficial effects were shown of leaf gas films on internal aeration during tidal submergence contributing to submergence tolerance for *Spartina anglica* and might also occur in other wetland plant species when submerged (Winkel et al., 2011). This might explain the higher leaf growth of *S. alba* in part. However, specific investigations on effects of gas films of *Salix* leaves are needed to confirm this hypothesis, especially since leaves are hypostomatic.

A further explanation of the better leaf performance and higher shoot growth of *S. alba* might be a superior tolerance to long-term flooding (Chmelar and Meusel, 1986; Newsholme, 1992; Lautenschlager, 1994), although Struyf et al. (2009) mentioned both *S. alba* and *S. viminalis* to be part of European tidal freshwater wetlands in the zonation above reed stands. Differences among species in their lowest position on elevation gradients may indicate plant differences in ability to cope with submergence (Blom, 1999). Amlin and Rood (2001) and Timoney and Argus (2006) underlined the species-specific susceptibility: they found that only site-adapted species (e.g. *S. exigua, S. planifolia*) remained after long-term deep flooding. This corresponds to our finding, that *S. alba* could be more tidal flooding tolerant compared to *S. viminalis*.

Effects of origin on tidal flooding tolerance of S. alba and S. viminalis

Generally, the effect of intraspecific differences on tidal flooding tolerance in S. alba and S. viminalis was marginal. However, phenotypic plasticity of distinct clones in response to abiotic stressors is often detected. Eller and Brix (2012) found responses of two contrasting genotypes in the wetland species *Phragmites australis* to temperature and fertilization to be genetically determined and related to the climatic conditions at the site of their origin. Bertolde et al. (2012) elucidated biochemical and physiological characteristics associated with flooding tolerance in two clonal cacao genotypes. In contrast, in our study we did not assume genetic differences between freshwater and brackish origin of plants, but acclimation to environmental conditions instead. However, significant differences related to the plants origin were only found in aboveground- to belowground dry mass and total dry mass of S. alba. A higher ratio and lower total dry mass were detected in S. alba originating from the brackish site compared to freshwater origin and a decrease in performance was pronounced in growth of leaves and shoot length with increasing tidal flooding. This might be explained by assuming that S. alba originating from the brackish site had a generally lower vitality, since these plants might have been negatively affected by the saline soils (Markus-Michalczyk et al., 2014).

3.6 Conclusions

Periodic tidal inundation acts as a stressor by causing submergence and soil anaerobiosis on the one hand, but beyond this, it acts as a subsidy by reestablishing aerobic conditions (Keddy, 2007). Flowing water offers a more oxygenated root zone than if the water is stagnant (Mitsch and Gosselink, 2000), and maintaining the functionality of the photosynthetic apparatus through rapid acclimation to changing CO_2 or O_2 and light conditions may be essential for adaptation to wetland habitats in which water levels fluctuate (Luo et al., 2009). Photosynthetic activity, growth of leaf number and growth of total shoot length showed tolerance to tidal flooding from 0 cm to 60 cm in our tidal experiment on both investigated *Salix* species of freshwater and brackish wetlands origin.

Our study indicates that immature *S. alba* and *S. viminalis* plants are tolerant of tidal flooding up to 60 cm and thus these species might survive the effects of moderate sea level rise with increasing mean high water line in tidal wetlands. However, large or mature willows may be less susceptible to flooding than small or young willows (Parolin, 2004; Timoney and Argus, 2006) and thus their tidal flooding tolerance should be additionally investigated. Moreover, under estuarine field conditions, *Salix* plants' tolerance to tidal flooding might be negatively

affected due to water turbidity, wave energy and current. In addition, biotic interaction and especially competition among flood plain vegetation might change tidal flooding tolerance as well. With regard to conservation of willow softwood forests as priority habitats and protection of tidal wetlands as Ramsar sites (Ramsar, 2014), we propose to undertake studies to elucidate *S. alba* and *S. viminalis* tidal flooding tolerance in the field.

4. Germination and early establishment of white willows in oligohaline conditions

4.1 Abstract

In estuaries, tidal wetland plants experience disturbance and stress across landscapes. Tidal flooding induces sedimentation and erosion processes resulting in modification of flood plain surfaces. Seedlings colonize bare space and influence dynamics of plant communities that are important for managing restoration of tidal wetlands in estuaries.

Salix alba is one of the characteristic species in softwood forests in river flood plains in temperate zones. However, little is known about its germination ecology in estuarine flood plains. Here, saltwater intrusion originating from the sea creates a salinity gradient and tidal flooding might lead to salinity stress during germination and early establishment. Thus, the germination and early growth of *S. alba* originating from tidal wetlands of the Elbe Estuary in Northern Germany was experimentally investigated with salinity treatments (0, 0.5, 1, 1.5, and 2) on seeds originating from an oligohaline wetland. Germination percentage and seedling dry mass remained similar up to salinity 2 whereas chlorophyll fluorescence, root growth and cotyledons performance significantly decreased at salinity 2.

White willow communities as part of tidal wetlands might be restored up to oligohaline conditions via seed sowing on bare space created by disturbance, and managed restoration at appropriate site conditions in times of increasing saltwater intrusion due to predicted sea level rise might be possible.

4.2 Introduction

Riparian softwood flood plain forests are declining worldwide. Continental alluvial forests comprising Salix species and Populus nigra have experienced 99.5 % loss of their natural distribution, according to estimations of the UNEP World Conservation Monitoring Centre (2000). In Germany, only 15-20% of natural flood plains are remaining, and the program has created comprehensive plans for the restoration of these flood plain forests (BMU and BfN, 2009). However, once destroyed, regeneration of flood plain Salicaceae by sexual reproduction requires favorable conditions. In spring and early summer, seeds are produced in abundance and dispersed by wind and water. These seeds germinate immediately on moist surfaces of exposed riverine sediments under favorable conditions. However, seedling establishment might be hampered in such sediments. In general, regeneration of flood plain willow and poplar species appears to be dependent on disturbance by flooding (Karrenberg et al., 2002). According to Grime (2001), disturbance is associated with the partial or total destruction of plant biomass caused by biotic or abiotic events (e.g. trampling and mowing or wind damage and soil-erosion). Despite its destructive character, disturbance is a driving force for vegetation succession and for community pattern and diversity as disturbances continuously alter site conditions on a small-scale (Jentsch, 2004).

In eastern North America and in Europe *Salix, Populus* and *Betula* are important in vegetation succession after disturbance due to their rapid growth and sexual reproduction strategy leading to recolonization and secondary succession at disturbed sites (Grime, 2001). According to Karrenberg et al. (2002), sand and gravel bars in flood plains of rivers created by flood disturbance are colonized by woody pioneer species, in the Northern Hemisphere mainly by *Alnus, Populus* and *Salix* in the Northern Hemisphere. All three genera reproduce sexually by wind dispersed seeds, but also have a high capacity for vegetative reproduction. Because of frequent disturbance by flooding, in addition to rapid growth, anchorage ability of propagules may be essential for plant survival. *Alnus* and *Populus* were found in the study to occupy more stabilized habitats. However, species in the genus *Salix* were detected to be able to colonize highly disturbed river flood plains (Karrenberg and Suter, 2003).

In addition to disturbance in river flood plains, estuarine organisms have to cope with variable and fluctuating salinity as the principal stressor (McLusky & Elliott 2004). Tidal wetland plants experience strong gradients in salinity stress across landscapes declining from salt marshes to oligohaline and tidal freshwater wetlands upriver. In North America, tidal wetland vegetation mirrors this transition: salt marsh plant communities are generally dominated by perennial grasses, but oligohaline marshes are instead dominated by grasses and forbs (Odum, 1988; Crain et al., 2004). Differences in environmental conditions across estuarine salinity gradients induce differences in the ability of colonists to establish and thus also in species interactions during secondary succession. In salt marshes succession is generally facilitated by early colonists, whereas in oligohaline marshes numerous species colonize from seed and are slowly displaced by clonal grasses whose recovery is slowed by competition from seedlings (Crain et al., 2008). Overall, increasing diversity from low to high elevation and from salt to freshwater marshes was confirmed for North American and European estuaries. However, in European estuaries diversity was lower at intermediate elevations in freshwater and brackish marshes. Here landscape evolution and site history might be additional important influences on species richness (Engels and Jensen, 2009). North American studies in low-salinity wetlands have emphasized the importance of seed banks (Baldwin et al., 1996) and competitive interactions between plants as important drivers in seedling establishment on bare space (Crain and Bertness, 2005, Baldwin et al., 2014). Moreover, Crain et al. (2008) found that rates of recovery on bare patches were faster in oligohaline marshes, where the fast recovery was driven by seedling colonization.

Here, river flood plain willows might come into the play: various species of willows and poplars (Salicaceae) are often dominant in flood plain habitats throughout northern temperate zones (Karrenberg et al., 2003). In particular, white willow softwood forests are highly adapted to the hydrological conditions of riparian systems (Ellenberg and Leuschner, 2010). In the tidal stretch of the river Elbe - an estuarine model system in a temperate climate - diking since the 11th century has reduced the active flood plain, and land use conversion has led to fragmentation of white willow softwood forests (Preisinger, 2010). Furthermore, in estuarine transition zones from freshwater to oligohaline conditions, periodic salt-water pulses during storms and periods of low precipitation lead to increased soil water salinity (Mitch and Gosselink, 2000), which might stress willows in tidal wetlands. Salix alba is the characteristic willow species in white willow softwood forests in temperate river flood plains (Ellenberg and Leuschner, 2010), and is widespread in the tidal wetlands of the Elbe estuary (Raabe, 1987). However, white willow softwood forests in Europe are considered to be one of the most threatened natural ecosystems. Thus, they are listed in Annex 1 of the European Habitats Directive and their good conservation status has to be fixed or to be restored.

In former studies, *S. alba* has been generally described as not tolerant to salinity (Ellenberg et al., 1992). In contrast, Markus-Michalczyk et al. (2014) detected a moderate salinity tolerance in *S. alba* up to salinity 2 in a hydroponic greenhouse experiment with salinity treatments on vegetative propagules. Furthermore, they found mature *S. alba* individuals occurring in tidal wetlands with oligohaline soil conditions during the growing season at the

mesohaline stretch of the Elbe estuary. However, the germination capacity of *S. alba* seeds and the early establishment of seedlings under oligohaline conditions has not yet been well studied.

With regard to the sensitivity and vulnerability of white willow softwood forests, I conducted an experiment on germination and early growth of *S. alba* seeds with salinity treatments. Local adaptations in plants can be caused by small scale differences in the environment (Lipowsky et al., 2011), and intraspecific differences in salt tolerance of *S. alba* in plants originating from freshwater compared to tidal wetlands with oligohaline conditions were recently detected (Markus-Michalczyk et al., 2014). Thus, I hypothesise that seeds of *Salix alba* originating from tidal wetlands with oligohaline conditions are able to tolerate a salinity up to oligohaline conditions. Specifically, I aim to answer the questions (i) up to what salinity level are seeds able to germinate and (ii) whether the early growth of seedlings differs between different salinity levels.

4.3 Materials and methods

A germination experiment was conducted in a climate chamber with salinity treatments in 2013. Seeds of *S. alba* were exposed to five salinity levels (0, 0.5, 1, 1.5, 2). Germination and early establishment were recorded during three weeks of salinity treatments.

Plant material

The plant samples originated from *S. alba* populations at the lower mesohaline stretch of the Elbe estuary, Germany. This site represents a close to nature brackish water influenced site (hereafter referred to as brackish site). During June and early July 2013, *S. alba* individuals were frequently observed to determine seed maturity. On July 7th, an individual tree with mature fruits was selected, and open capsules with ripe seeds were collected from all accessible parts of the tree. The plant material was stored in a paper bag at ambient temperature until the next day. On July 8th, the seeds were removed from the capsules and separated from their cottony hairs. To accomplish this, the seeds were carefully rubbed between the fingers to disconnect the hairs.

Experimental design

The experimental design consisted of five salinity levels, each with eight petri dishes (N = 8). Salinity is expressed using Practical Salinity Units (PSU) on the practical salinity scale, and thus is a dimensionless unit. Filter paper discs were put in each of the petri dishes. Salinity solutions were prepared with local spring water and additive-free sea salt (Meersalz, Alnatura, Bickenbach, Germany) to reach the salinity levels (0, 0.5, 1, 1.5 and 2.0). In each of the solutions, 0.01 % Thiram (Dimethyl-Dithiocarbamat) was added in order to avoid fungal attack during germination and early growth. The petri dishes were watered with the solutions until each of the paper discs was saturated. Finally, the *S. alba* seeds were arranged on the paper discs, 50 per petri dish, and lids were placed on the dishes. The closed petri dishes, arranged in rows, were placed in a climate chamber and treated at 25 °C in light (8.73 +/- 0.25 SD μ mol/m²/s) for three weeks.

Data collection

During the course of the salinity treatments, germinated seeds were repeatedly counted but not removed from the petri dishes. Final germination percentage was calculated at the end of the treatments.

Chlorophyll fluorescence was recorded as maximum quantum yield of photosystem II (Fv/Fm) on July 24th after two weeks of salinity treatments by using an Imaging PAM (IMAG M Version, Walz, Germany). Seedlings were left in petri dishes in order to avoid additional disturbance and were dark adapted during a five minute time period. Then, a saturation pulse was applied for 0.8 seconds and data were recorded. Fv/Fm was calculated as an indicator for the photosynthetic efficiency (Hanelt et al., 1996) with ImagingWin Software. Furthermore, images were taken using a digital camera (IMAG K5, Walz, Germany) in order to visually confirm calculated results.

On July 25th, the primary root length was recorded as one of three categories: no primary root, short primary root, and long root (Fig. 4.1). On July 29th, the colour of the cotyledons was determined and classified into three categories: green cotyledons, yellow cotyledons, and brown cotyledons.



Figure 4.1 Categories of primary roots: no primary root (left), short primary root (middle), long primary root (right) in a germination and early growth experiment on *Salix alba* seeds from an oligohaline tidal wetland at the Elbe estuary.

Photon flux density was measured above the surface of each petri dish (Li COR Data Logger; LI 192 UW quantum sensor).

The experiment was finished on July 29th after three weeks of salinity treatment. Then, seedlings in each petri dish were collected in paper bags. Finally, they were stored in a drying furnace at 40° Celsius to constant weight and were weighed (Sartorius Micro M3P, Germany).

Data analysis

In the experiment, effects of salinity on the final germination percentage and final dry mass, on the number of individuals with no roots, short roots and long roots, respectively, and on number of individuals with green cotyledons, yellow cotyledons and brown cotyledons, respectively, were analysed by ANCOVA. Here, irradiance, measured at each petri dish, served as covariate. All ANCOVAs that showed significances were followed by Tukey's post hoc comparisons to detect significant differences between treatment levels.

Prior to all analyses, Levene's tests were applied to test for homogeneity of variance.

Chlorophyll fluorescence (Fv/Fm) was analysed with regard to mean and standard deviation. No statistical test was applied since data from only one petri dish per salinity were recorded. All data analyses were carried out with STATISTICA 9.0 (StatSoft 2009).

4.4 Results

Results of the salinity treatments on germination of *S. alba* seeds and on early seedlings growth in five salinity levels (0, 0.5, 1, 1.5, 2.0) indicate a relative salinity tolerance up to salinity 2.0.



Figure 4.2 Final germination percentage after three weeks of salinity treatments (salinity 0, 0.5, 1.0, 1.5, 2.0) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (mean +/- SD, N = 8).

On the first day after the start of the experiment, more than 85% of the seeds had germinated. The germination percentage was statistically similar at all salinities; 0 (mean 88.5 +/- 3.66 sd), 0.5 (mean 88.5 +/- 4.87 sd), 1.0 (mean 88.5 +/- 3.82 sd), 1.5 (mean 89.25 +/- 2.60 sd) and 2.0 (mean 87 +/- 5.66 sd) (Fig. 4.2). Moreover, since germination percentage increased only slightly during the course of the experiment (approx. 0.2%), germination on the first day after the start of the experiment was assessed as completed and analysed.



Figure 4.3 Dry mass after (mg) three weeks of salinity treatments (salinity 0, 0.5, 1.0, 1.5, 2.0) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (mean +/- SD, N = 8).

Total dry mass of seedlings after three weeks of salinity treatments differed for each of the five salinity levels, ranging between 35.63 mg (+/- 7.96 mg and 42.5 mg (+/- 4.41 mg). Overall, no significant effect of salinity on seedlings total dry mass was found: values were similar at salinity 0 (mean 38.63 + 8.25 g SD), 0.5 (mean 35.88 + 4.61 mg SD), 1.0 (mean 39.63 + 3.34 mg SD), 1.5 (mean 42.5 + 4.41 mg SD) and 2.0 (mean 35.63 + 7.96 mg SD) (Fig. 4.3).



Figure 4.4 Chlorophyll fluorescence of seedlings after two weeks of salinity treatments (salinity 0, 0.5, 1.0, 1.5, 2.0) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (mean +/- SD, N = 50).

The effect of salinity on photosynthetic efficiency (Fv/Fm-values) was substantial. Fv/Fm-values decreased from salinity 0 (mean 0.427 +/- 0.129) to salinity 0.5 (mean 0.341 +/- 138), 1 (mean 0.257 +/- 0.103), 1.5 (mean 0.319 +/- 0.110) and salinity 2 (mean 0.319 +/- 0.094) (Fig. 4.4; Fig. 4.5).



Figure 4.5 Chlorophyll fluorescence **(Fv/Fm, 0-1)** images of seedlings after two weeks of salinity treatments (salinity 0 (left), salinity 2.0 (right)) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (N = 50).



Figure 4.6 Primary root development after three weeks of salinity treatments (salinity 0, 0.5, 1.0, 1.5, 2.0) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (mean +/- SD, N = 8).

The effect of salinity on primary root development was significant (F = 3.25; p < 0.001) (Fig. 4.6). Tukey's post hoc test showed that the number of individuals with long primary roots was significantly higher at salinity 0, 0.5, 1.0 and 1.5 compared to salinity 2.0 (p < 0.01). In parallel, the number of individuals with short primary root was significantly lower at salinity 0, 0.5, 1.0 and 1.5 compared to salinity 2 (p < 0.05). However, no significant differences between salinity levels were detected in the number of individuals in which no primary root was developed.



Figure 4.7 Performance of seedlings cotyledons after three weeks of salinity treatments (salinity 0, 0.5, 1.0, 1.5, 2.0) in an experiment on *S. alba* seeds from a tidal wetland of the Elbe estuary (mean +/- SD, N = 8).

The effect of salinity on the performance of seedling cotyledons was found to be significant after three weeks of salinity treatment (F = 3.16; p < 0.001) (Fig. 4.7). Tukey's post hoc test showed that the number of individuals with green cotyledons was lower at salinity 2.0 compared to 0, 0.5, 1.0 and 1.5 (p < 0.01). The number of individuals with yellow cotyledons was higher at salinity 2.0 compared to 0 (p < 0.01) as well as the number of individuals with brown cotyledons at salinity 2.0 compared to 0 (p < 0.01).

4.5 Discussion

Increasing salinity in tidal wetlands affects flood plain species during differently at different life stages. Germination and early growth stages are more sensitive to salinity and determine species establishment along estuarine salinity gradients (Engels et al., 2010).

In a climate chamber experiment on seeds of *S. alba* originating from a tidal wetland with oligohaline conditions, the percentage of germination and dry mass of seedlings were similar up to a salinity of 2. However, primary root growth and cotyledons performance remained similar up to salinity 1.5, but significantly decreased at salinity 2. Moreover, chlorophyll fluorescence was substantially decreased at salinity 0.5 up to 2 compared to salinity 0. Generally, the salinity range 0.5 to 5 corresponds to oligohaline conditions. Thus, my results are in accordance with Baldwin et al. (1996), who investigated the effect of salinity on the viability and germination of seeds in oligohaline coastal marshes. In that study, salinity 4 reduced seed germination in most of the studied species, and a significant reduction in germination occurred at salinity 2 for some species. Thus, Baldwin et al. (1996) assume that salinity - in the case of relatively slow sea level rise - acts as the most important stressor. They further concluded that salinity might affect species distributions by inhibiting seed germination and thus reducing the abundance of species reliant on seedling recruitment.

Similarly, Engels et al. (2010) - in a glycophyte versus halophyte emergence and establishment mesocosm experiment - found the ability of a species to germinate and emerge under conditions representing a particular position along an environmental gradient being necessary but not solely responsible for determining plant zonation patterns along estuarine marsh gradients. It is clear that emergence of glycophytes is strongly inhibited in mesohaline and polyhaline conditions (Bakker et al., 1985; Baldwin et al., 1996). In contrast to glycophytes, germination of halophytes like *Cakile maritima* is inhibited at concentrations higher than 200 mM NaCl (Devez et al., 2004) or in the case of Atriplex lentiormis at concentrations higher than 250 mM NaCl (Malcolm et al., 2003). However, Engels et al. (2010) showed that competitive exclusion of halophytes by glycophytes is an important driver of marsh plant zonation along estuarine salinity gradients, taking effect during the early seedling establishment phase of the co-occurring plants. Phragmites australis is abundant co-occuring with S. alba in tidal wetlands of the Elbe estuary above the mean high tide line (Preisinger, 2010) and is reported as being salt tolerant and able to germinate in salinity up to 25 (e.g. Mauchamp and Mesleard, 2001). Thus, *Phragmites* might exclude S. alba under saline conditions, whereas it might be outcompeted by S. alba under oligohaline conditions in estuarine flood plains.

Willows are generally described as glycophytes (Newsholme, 1992). However, my results, which showed a consistently high germination percentage up to salinity 2 as well as consistently high primary root growth and cotyledon performance in seedlings up to salinity 1.5 support my rejection of the general assignment of Ellenberg et al. (1992) that *S. alba* is being "not salt tolerant". It is clear that *Salix* communities have disadvantages compared to highly salt tolerant communities, such as *Tamarix* communities, in salinized river flood plains (e.g. Salinas et al., 2000; Vandersande et al., 2001). However, my results give evidence that a distinction must be made between "not salt tolerant" and a certain degree of salt tolerance in *S. alba*.

According to Markus-Michalczyk et al. (2014), who found *S. alba* and *S. viminalis* vegetative propagules to be salt tolerant up to salinity 2 in a hydroponic greenhouse experiment, I also assert *S. alba* seedlings to be salt tolerant to oligohaline conditions based on the results of my seed germination and seedlings growth experiment. Moreover, Van Splunder et al. (1996) detected, in a greenhouse experiment on drought resistance of Salicaceae seedlings, that *S. alba* had 0% drought-induced mortality and *S. viminalis* had 62.5% mortality, which correlated with differences in water-loss characteristics: *S. viminalis* had much higher transpiration rates under well-watered conditions than *S. alba*. Since drought resistance is physiologically related to salinity resistance, this might correspond to my results on *S. alba* seedlings` relative salt tolerance.

Due to the detected relative tolerance up to salinity 2 in *S. alba* seeds and seedlings, with regard to possible increasing salt intrusion in tidal wetlands in times of climate change, and in support of restoration efforts on white willow softwood flood plain forests up to oligohaline conditions, it might be of interest if a *S. alba* seeds salt tolerance in tidal wetlands might be extended. Gul et al. (2013) determinated seed dormancy to be an important means for initiating growth under appropriate conditions in halophytes. Even after imbibition in wet saline environments, seeds of halophytes did not germinate over extended periods of time if the external environment did not favor seedling survival. Moreover, seeds of halophytes are able to recover from a salinity shock and start germination once salinity is reduced (e.g. after rain).

According to Baskin and Baskin (1998), the ability of salt marsh species seeds to recover and germinate after salt intrusion (recovery rate) is frequently observed. Here, seed dormancy was classified into physical, morphological and physiological dormancy types, whereas most salt marsh halophytes belong to families known to have physiological dormancy. However, in my experiment *S. alba* seed germination was already finished one day after sowing. According to Schopmeyer (1974) willow seeds germinate under natural conditions in 12-24 hours on a mixture of moist sand and alluvium. Willow seeds are viable for only a few days and seed dormancy has not been observed in any willow species. In recent literature, Salix species have been found to produce large numbers of wind-dispersed, non-dormant, shortlived seeds (Karrenberg et al., 2002). Because of their limited longevity, Salix seeds were thought to be not tolerant to desiccation. However, recent evidence shows that their longevity is enhanced under dry and cold storage conditions, whereas at 25° C S. alba seeds entirely lose viability within two weeks (Maroder et al., 2000). Because seeds germinate immediately after contact with moist surface, estimates of seed longevity are only relevant for dry conditions (Karrenberg and Suter, 2003). Thus, a further extension of S. alba seed salt tolerance in tidal wetlands might be possible when seeds are stored under dry conditions and germination starts when salinity is reduced, which may happen following rain. Since S. alba flourishes from April to May in Europe (Lautenschlager, 1994; Zander, 2000), dry conditions and different flooding regimes in tidal wetlands along the estuarine salinity gradient might affect germination and early growth. Thus, further studies are therefore recommended on germination and early growth of S. alba in saline conditions under dry versus flooded conditions.

4.6 Conclusions

S. alba is the characteristic species in white willow communities in tidal forests in temperate estuarine flood plains. *S. alba* seeds with origin from a oligohaline site were found to germinate up to salinity 2 in a climate chamber experiment. However, chlorophyll fluorescence, cotyledons performance and primary root growth remained similar up to salinity 1.5, but were reduced at salinity 2. Thus, *S. alba*'s sexual regeneration and restoration via seeds might be possible despite salt water intrusion up to soil water salinity of 1.5 in tidal wetlands. With this study, I intend to support the restoration of white willow forests in tidal wetlands up to oligohaline conditions via seed sowing on bare space created by disturbance.

5. Ecological niches at estuarine gradients:

A field study on Salix alba and Salix viminalis

5.1 Abstract

Rapid environmental change leads to the urgency of predicting ecological responses and determining species ecological niches. Willows (*Salix*) are vulnerable species in European soft wood river flood plain forests, listed in the European Habitats Directive, and generally well known for their flooding tolerance. However, less is known about *Salix* species in tidal wetlands of estuarine environments. In tidal wetlands, projected sea level rise is predicted to lead to increasing salt intrusion and tidal flooding. Thus, two characteristic *Salix* species were investigated at four sites along the estuarine salinity and flooding gradient. The Elbe river mouth is one of the largest estuaries in Europe and thus, served as model system. Here, *Salix alba* and *Salix viminalis* were investigated with regard to individual elevation and soil water salinity at *Salix* stands. Furthermore, specimens' performance with regard to morphological variability was studied in the field.

The elevational optimum was found to be similar in both *Salix* species: 0.77 m (mean +/- 0.87 SD) in *S. alba, and* 0.77m (mean +/- 0.61 m SD) in *S. viminalis.* Recorded minimum was – 0.57 m elevation above mean high water in both *Salix* species. Moreover, soil water salinity increased in tidal wetlands from tidal freshwater conditions to oligohaline conditions (mean 1.31+/- 0.54 SD) at *Salix* stands. However, morphological variability indicated changed performance at elevations under mean high water and in oligohaline conditions.

I conclude that both characteristic *Salix* species might be able to keep pace with moderate sea level rise scenarios, and survive increasing tidal flooding up to 0.6 m in parallel to increasing salt intrusion up to oligohaline soil water conditions in tidal wetlands at estuarine environments.

5.2 Introduction

A recent increasing interest in the ecological niche – basically defined as a hyper-volume in the multidimensional space of ecological variables, within which a species can maintain a viable population (Hutchinson, 1957) – might be driven by the urgency of predicting species' niches as ecological responses to environmental change (Holt, 2009). Estuaries are constantly changing environments intermediate from sea to land, and from salt water to freshwater.

The estuarine environment is characterized by a dynamic complex of physical factors (e.g. tides, waves and currents; sedimentation and erosion), chemical factors (e.g. oxygen and nutrient supply), and a topography of continual change. Species living in estuaries can be distinguished due to their ecological specialization along the estuarine salinity gradient (McLusky and Elliott, 2004). Costanza et al. (1993) described estuaries as the most productive biomes in the world. Moreover, Costanza et al. (1997) cited estuaries as supporters of biogeochemical processes providing important ecosystem services, and furthermore proposed that habitat response to environmental change (e.g. storm protection and flood control) could be mainly controlled by vegetation. In subtropical and tropical climates, mangroves contribute to flood control in coastal areas (Alongis 2008; Li et al. 2013) whereas in temperate climates tidal marshes and flood plain willows contribute to coastal protection by attenuating waves and stabilising sediments (Borsje et al., 2011; Radtke et al., 2012). However, little is known about the ecological niche of willows (genus *Salix*) at the estuarine gradient.

Willows in riverine flood plains are well studied. Diverse willow and poplar species (both *Salix* and *Populus* genera in Salicaceae) are characteristic in riverine flood plain habitats throughout the northern temperate zone. The regenerative capacity of Salicaceae is known as an adaptation to regular disturbance: wind and water dispersed seeds germinate and establish on moist exposed riverine sediments after disturbance. Moreover, larger *Salix* individuals are able to withstand physical disturbances in the flood plain (e.g. waves, currents and ice scouring) due to their high bending stability (Karrenberg et al., 2002). In a flume experiment, the immersion height of willow species and the flow velocity were found to have an impact on the drag coefficient and thus, the flow resistance of flooded plants (Wunder et al., 2013).

Devictor et al. (2010) proposed that species' requirements and species' impacts can both be considered as ecological specialization, at the same time clearly indicating that specialization depends on species-specific characteristics and spatial and temporal environmental conditions. Thus, diverse willow species might be generally assessed as ecologically

specialized to the flood plain environment. With regard to willows value and vulnerability, white willow softwood forests are listed as priority habitats in the EU Habitats Directive. Mosner et al. (2011) considered simple hydrological variables as sufficient to describe occurrence patterns of riparian softwood species by which life stages and interactions have to be considered, thereby permitting the identification of reforestation sites for riparian Salix plantings. However, in tidal freshwater ecosystems in Europe, sedimentation is a further factor determining succession of plant communities that result in willow (Salix spp.) forests as the final stage of succession (Struyf et al., 2009). Moreover, with regard to the whole estuarine stretch, Odum (1988) indicates both salinity and tidal flooding as simultaneous main abiotic factors, forming horizontal (salinity) and vertical (tidal flooding) estuarine gradients. Tidal wetlands along the estuarine gradient are categorized into tidal freshwater wetlands, brackish and salt marshes, exhibiting vertical plant species zonation patterns formed according to the estuarine flooding gradient (Bakker et al., 1993; Engels et al., 2010). Ecological specialization can be measured and defined at various temporal and spatial scales (Devictor et al., 2010) and thus, Salix's specialization along the salinity and flooding gradient might be viewed as its ecological niche in estuarine environments.

With this approach I investigate the ecological niche of characteristic willow species in tidal wetlands along the estuarine salinity and flooding gradient in a field survey. Specifically, I aimed to answer the questions (i) at which elevation and at which salinity do characteristic *Salix* species occur and, (ii) how do *Salix* species vary in morphologic performance along the estuarine gradients. With this investigation, I intend to contribute to identifying the ecological niche of characteristic *Salix* species along estuarine gradients.

5.3 Materials and methods

Study area

This field survey was conducted in tidal wetlands at the Elbe estuary. With a total length of 1,094 km – including a 142km tidal stretch - and a catchment area of 148,268 km², the Elbe is one of the largest river systems in central Europe. The tidal stretch can be determined as a coastal plain estuary, formed by the flooding of a pre-existing valley, with a pronounced tidal inflow compared to the tidal outflow (McLusky and Elliott, 2004).

Human impact has led to loss in the extent of tidal wetland areas at the Elbe estuary due to dike construction for almost 1000 years (Garniel and Mierwald, 1996). Since the 1960s, flood protection measurements have resulted in a loss of about 70% of the tidal wetlands at the estuarine freshwater stretch (Preisinger, 1991). Furthermore, repeated river deepening connected with harbor development has changed the area's estuarine hydrology since the

middle of the nineteenth century, and the mean tidal amplitude increased in Hamburg from 1.8 m in 1840 to 3.6 m in 1996 (Kausch, 1996). Recently, the mean tidal amplitude at the upper head of the estuary was measured as 2 m, increasing towards its maximum at Hamburg harbor, whereas it slightly decreased downstream and finally reached 3 m at the mouth of the estuary (Kappenberg and Grabemann, 2001).

Today, it is expected that climate change will lead to earlier river discharge maximums. Simultaneously, it is predicted that the minimum discharge will be more pronounced due to possible changes in precipitation patterns (Von Storch and Claussen, 2011). Furthermore, in Europe especially tidal freshwater wetlands have been converted for agricultural purposes. Nonetheless, at the Elbe estuary and at the Scheldt estuary (Belgium) substantial areas of tidal freshwater areas with high tidal amplitude still occur today. Here, the remaining woody communities in tidal wetlands are dominated by willows (Struyf et al., 2009). At the flood plain transition zone from reed to woody communities, shrubs like *Salix viminalis* dominate, whereas *S. alba* trees take over at higher elevations (Ellenberg and Leuschner, 2010).

Study sites and Salix study specimens

Four sites were selected along the estuarine stretch, which represented close to nature flood plains. Two sites were selected at the estuarine head, where freshwater enters the estuary and salinity is less than 0.5. Salinity is expressed by using Practical Salinity Units (PSU) on the practical salinity scale, and thus a dimensionless unit. Here, one site (site 1) was situated upstream of the Hamburg harbor (590 Elbe km) and the other (site 2) was situated in close proximity to the harbour (610 Elbe km). The two further sites were located at the estuarine upper reaches, were fresh and saltwater mixes at salinity from 5 to 18, and minimal currents occur especially at high tide, leading to maximum turbidity and high mud deposition. Here, one site (site 3) was situated at 670 Elbe km and the other at 690 Elbe km (site 4), closer to the estuarine mouth.

In order to select *Salix* study specimens, woody patches were marked in aerial images, at the four above described sites. Each of the marked woody patches was assigned to a randomly generated field coordinate. Starting from this field coordinate, 50m x 20m plots were established within the marked woody patches. These plots were examined in the field with regard to the occurrence of *S. alba* and *S. viminalis* specimens. It was intended to determinate 25 *Salix* study specimens at each site. The existence of equal numbers of *Salix* specimens at each site a necessitated the generation of eight plots at site 1, eight plots at site 2, five plots at site 3 and nine plots at site 4. In these plots, it was possible to approximate equal numbers of study specimens. With regard to *S. alba*, it was possible to determine 20 specimens at site 1, 25 at site 2, 22 at site 3, and 29 specimens at site 4. With regard to *S.*

viminalis, it was possible to determine 27 specimens at site 1, 21 at site 2, 26 at site 3 and 17 at site 4, resulting in a total number of 187 specimens.

Sampling

The four study sites are internationally protected via the EU Habitats directive. Moreover, all the study sites except site 4 are protected via regional nature protection legislation, and thus, the sampling time period was strictly restricted. I took measurements and samples from July 2010 up to October 2010 at the *Salix* study specimens.

Each individual *Salix* specimen was leveled in summer 2010 an elevation above sea level was determined. Soil water salinity was measured once in Summer using the Practical Salinity Scale by a Multi-Parameter-Analyser (Eijkelkamp ecoTech 18.28).

For measurements on leaf parameters, 20 leaves per *Salix* specimen were collected from southward orientated branches of each *Salix* specimen in summer. The 20 leaves were prepared as herbarium specimens, arranged on paper and scanned. Then, lamina length and width were measured using WinSEEDLE 2005 (Regent Instruments Inc., Canada), and lamina ratio was calculated (lamina length / lamina width). Petiole length was measured with a slide rule, and petiole ratio was calculated (petiole length / lamina length). The morphological variability of *Salix* was investigated via several parameters. Height, diameter, trunk position and trunk form, and crown area were measured as shown in Table 5.1.

Parameter	Unit	Instrument	Method
Height	centimetre	Altimeter (Blume-Leiss, Germany)	
Diameter	centimetre	Circumference Measuring Tape (Stewe, Germany)	Diameter at 1.3 m height
Trunk position	categories		Gockel 1994; categories: erect (1), inclined (2), prostrate (3)
Trunk form	categories		Gockel 1994: categories: straight (1), arching (2), crooked (3)
Crown area	m²	Measuring Tape	Röhle 1986

Table 5.1 Morphological variability parameters of Salix specimens

Data analysis

The elevation of *S. alba* and *S. viminalis* specimens above sea level and mean high water, and soil water salinity of *S. alba* and *S. viminalis* specimens along the estuarine gradient were visualized in graphs.

We applied two-factorial ANOVAs to analyse the effects of site and species on differences in elevation of *Salix* study specimens. To analyse the effects of site and species on differences in soil water salinity at the plots, two-factorial ANOVAs were used, as well.

For the response variables on morphological variability (height, diameter, crown area, lamina ratio, and petiole length and petiole ratio), mean and standard deviation were calculated and displayed in graphs to visualize the effects of site and species on the measured parameters. Here, measured elevation of *Salix* specimens and soil water salinity per plot were included as covariates and ANCOVAs were applied. *Salix* specimens` trunk position and trunk at the four sites form were measured in categories and thus, median was calculated and Kruskal Wallis test was applied. When significant differences were detected, Tukey's tests were applied post hoc. All data analyses were carried out with STATISTICA 9.0 (StatSoft 2009).

5.4 Results



Elevation of Salix specimens

Salix specimens

Figure 5.1 Elevation (m) of *S. alba* (N = 96) and *S. viminalis* (N = 91) specimens above mean sea level (MSL), (A), and above mean high water (MHW), (B), along the Elbe estuary.

Elevation of *S. alba* and *S. viminalis* specimens above sea level (A) and above mean high water level (B) along the Elbe estuary differed (Fig. 5.1). Mean elevation of *S. alba* specimen above mean sea level was 2.70 m +/- 0.82 SD. The detected minimum was 1.74 m whereas the maximum was 4.65 m above mean sea level. Mean elevation for *S. viminalis* was 2.78 m, and minimum value was 1.57 m whereas maximum value was 4.40 m above mean sea level. *S. alba* mean elevation above mean high water level was 0.77 m (+/- 0.87 SD). Here, the recorded minimum was -0.56 m and maximum was 2.73 m. *S. viminalis* mean elevation above mean high tide level was also 0.77 m (+/- 0.61 m SD). The recorded minimum was 2.26 m elevation above mean high water.



Figure 5.2 Elevation (m) of *S. alba* and *S. viminalis* specimens above sea level (**A**) and above mean high tide level (**B**) in comparison among four tidal wetland sites (site 1/590 km (*S. alba* N = 20; *S. viminalis* N = 27); site 2/610 km (*S. alba* N = 25; *S. viminalis* N = 21); site 3/670 km (*S. alba* N = 22; *S. viminalis* N = 26); site 4/690 km (*S. alba* N = 29; *S. viminalis* N = 17)) along the Elbe estuary (mean; +/- SD).

Elevation above sea level of *Salix* specimens generally decreased from site 1 to site 4 (F = 11.45; p < 0.001; Fig. 5.2). The interaction between site and species was found to be significant (F = 5.43; p < 0.01). At site 1, site 3 and site 4, elevation of *S. alba* compared to *S. viminalis* was similar and no significant differences between species were found. However, Tukey's tests confirmed elevation of *Salix* specimens to be significantly lower in *S. alba* compared to *S. viminalis* at site 2 (p < 0.01). In contrast, *S. viminalis* specimens at site 2 had a significantly higher elevation compared to *S. viminalis* at site 3 (p < 0.01). In contrast, *S. viminalis* specimens at site 2 had a significantly higher elevation compared to *S. viminalis* at site 3 (p < 0.05) and 4 (p < 0.01). Elevation of *Salix* specimen above mean high tide was generally lowest at site 2. Tukey's tests showed that values at site 3 (p < 0.05) and site 4 (p < 0.001) were significantly higher compared to site 2. The site*species interaction was found to be significant (p < 0.01). Here, Tukey's tests showed similar values in both species at sites 1, 3 and 4. However, at site 2 elevation of *S. alba* above mean high tide was lower compared to *S. alba* at site 3 (p < 0.01), and site 4 (p < 0.001). Moreover, values for *S. alba* at site 2 were found to be significantly lower compared to *S. viminalis* at site 2 (p < 0.05) and site 3 (p < 0.01).



Soil water salinity

Figure 5.3 Soil water salinity at four tidal wetland sites (site 1/590 km; site 2/610 km; site 3/670 km; site 4/690 km) along the Elbe estuary according to *S. alba* and *S. viminalis* (mean; +/- SD; N = 8 (site 1 and 2); N = 5 (site 3); N = 9 (site 4)).

Soil water salinity generally increased from site 1 to site 4 (F = 62.55; p < 0.001; Fig. 5.3), but was similar at site 1 compared to site 2. Furthermore, soil water salinity was found to be significantly higher where *S. alba* occurred in comparison to *S. viminalis* (F = 5.9; p < 0.05). At *S. alba*, mean at site 1 was 0.5 (+/- 0.0 SD) and thus, similar to site 2 (mean 0.48 +/- 0.15 SD), but increased at site 3 to a mean of 0.92 (+/- 0.19 SD), and to a mean of 1.76 (+/- 0.72 SD) at site 4. At *S. viminalis* the mean was 0.35 at site 1 (+/- 0.19 SD), similar to the mean of 0.41 (+/- 0.11 SD) at site 2. At site 3 mean values were increased to 0.83 (+/- 0.14 SD) and to 1.31 (+/- 0.54 SD) at site 4.

Morphological variability of Salix specimens

Table 5.2 shows results of ANCOVAs on the effects of site and species on morphological variability y (height, diameter, crown area, lamina ratio, petiole length and petiole index of *Salix alba* and *Salix viminalis* specimens at four tidal wetland sites at the Elbe estuary.

Table 5.2 Two-factorial ANCOVAs on the effects of site and species on morphological variability in a field survey on *Salix* specimens (*S. alba* and *S. viminalis*) at four tidal wetland sites (site 1/590 km; site 2/610 km; site 3/670 km; site 4/690 km) along the Elbe estuary. Elevation of *Salix* specimen and soil water salinity at plots served as covariates (F-values; level of significance indicated as *p < 0.05; **p < 0.01; ***p < 0.001).

Parameter	elevation	soil water salinity	site	species	site*species
Height	0.08	3.01	6.30***	345.85***	6.87***
Diameter	2.38*	0.94	3.06***	13.48***	2.25**
Crown area	4.70*	0.23	1.51	8.92**	2.48
Lamina ratio	0.62	0.12	1.94	218.22***	0.91
Petiole length	0.95	0.01	14.70***	0.83	13.67***
Petiole index	0.55	0.08	13.92***	27.92***	3.40*

Parameters on morphological variability (height, diameter, crown area, lamina ratio, petiole length and petiole index) of *Salix* specimens (*S. alba* and *S. viminalis*) at four tidal wetland sites along the Elbe estuary are shown in Figure 5.4.



Figure 5.4 Parameters on morphological variability (height, diameter, crown area, lamina ratio, petiole length and petiole index) of *Salix* specimens (*S. alba* and *S. viminalis*) at four tidal wetland sites (site 1/590 km (*S. alba* N = 20; *S. viminalis* N = 27); site 2/610 km (*S. alba* N = 25; *S. viminalis* N = 21); site 3/670 km (*S. alba* N = 22; *S. viminalis* N = 26); site 4/690 km (*S. alba* N = 29; *S. viminalis* N = 17)) along the Elbe estuary (mean +/- SD).

Height of *Salix* specimens decreased from site 1 to 4 (p < 0.001). Significant higher values were detected in *S. alba* compared to *S. viminalis* (p < 001). The site*species interaction was found to be significant (p < 0.001). Tukey's tests showed that *S. viminalis* height was similar at all sites. However, height of *S. alba* was significantly higher at site 1, 2 and 3 compared to site 4 (p < 0.001).

Effect of site on diameter of *Salix* specimen was significant (p < 0.001). Generally, values were similar at sites 1, 2 and 4, but substantially lower at site 3. *S. alba* diameter was higher compared to *S. viminalis* (p < 0.001). A significant interaction of site and species was found (p < 0.01).

Table 5.2 Trunk position and trunk form of *Salix* specimens (*S. alba* and *S. viminalis*) at four tidal wetland sites (site 1/590 km (*S. alba* N = 20; *S. viminalis* N = 27); site 2/610 km (*S. alba* N = 25; *S. viminalis* N = 21); site 3/670 km (*S. alba* N = 22; *S. viminalis* N = 26); site 4/690 km (*S. alba* N = 29; *S. viminalis* N = 17)) along the Elbe estuary (median +/- SE). Categories trunk position: erect (1), inclined (2), prostrate (3); categories trunk form: straight (1), arching (2), crooked (3).

Site	trunk position		trunk form	
	S. alba	S. viminalis	S. alba	S. viminalis
1	1.5 (+/- 0.7)	3.0 (+/- 0.7)	2.0 (+/-0.9)	3.0 (+/- 0.7)
2	2.0 (+/- 0.9)	3.0 (+/- 0.7)	1.0 (+/- 0.9)	3.0 (+/- 0.7)
3	2.0 (+/- 0.6)	2.0 (+/- 0.7)	1.0 (+/- 0.7)	2.0 (+/- 0.7)
4	2.0 (+/- 0.7)	2.0 (+/- 0.8)	2.0 (+/- 0.8)	3.0 (+/- 0.5)

Table 5.2 shows measured values on trunk position and trunk form of *Salix alba* and *Salix viminalis* specimens at four tidal wetland sites at the Elbe estuary (median +/- SE).

Differences in trunk position were found to be significant in comparison between species (p < 0.01) but not among sites. Generally, trunk position was more erect in *S. alba* compared to *S. viminalis*. Effects of site (p < 0.01) and species (p < 0.01) on trunk form were found to be significant. Trunk form was generally straighter at site 3 and more arching at site 1 and site 4. Moreover, trunks of *S. alba* were generally straighter (p < 0.01). However, at site 1, trunk form of *S. alba* was highly variable and the median form was arching.

Crown area and lamina ratio were found to be significantly larger in *S. alba* compared to *S. viminalis* (p < 0.001), whereas site was not a significant factor.

Length of petiole differed significantly between sites (p < 0.001). Tukey's tests showed that values were similar between site 1 and site 2, but length of petiole increased gradually from site 2 to site 4 (p < 0.001). The interaction between site and species was found to be significant (p < 0.001). Tukey's tests showed that petiole length of *S. alba* was higher than in *S. viminalis* (p < 0.001) at site 3. In contrast, at site 4 values of *S. viminalis* were higher than in *S. alba* (p < 0.05). Petiole index increased from site 1 to site 4 (p < 0.001), whereas values at site 1 and site 2 were similar. However, Tukey's tests showed that petiole index increased significantly from site 2 to site 3 and site 4 (p < 0.001). Generally, petiole index in *S. alba* was higher compared to *S. viminalis* (p < 0.001). A significant site*species interaction was detected (p < 0.001). Here, petiole index was similar in both species at site 1; *S. alba* petiole index was higher compared to *S. viminalis* at site 2 and at site 3 and became almost equal for both species at site 4.
5.5 Discussion

The approach presented here contributes to the understanding of ecological niches of *Salix alba* and *Salix viminali*s at at the Elbe estuary based on field study. Following Engels and Jensen (2009), I focus on two gradients of environmental stress in tidal wetlands: a horizontal (salinity) gradient on the landscape scale and a vertical (flooding) gradient at each shoreline location and discuss the occurrence and morphological variability of *S*.*alba* and *S*. *viminalis* in tidal wetlands of the Elbe estuary.

Salix ecological niche along the estuarine elevational gradient

It is well known that willow communities settle right above the mean high water line in middle European river flood plains at the longitudinal gradient from the Alps down to the lowlands near the coasts (Ellenberg and Leuschner, 2010). However, at the estuarine river stretch, tidal amplitude determines the mean high tide line, which might restrict the occurrence of willow communities. Generally, mean high water level is higher at upper streams since geomorphology of river beds is narrower, whereas river mouths are wider, and thus water has more space to expand, corresponding to the mean high water level along the Elbe estuary (site 1/2.5 m, site 2/2.3 m, site 3/1.6 m, and site 4/1.5 m above sea level (BSH, 2010)). These differences in mean high water level were reflected in the occurrence of Salix specimens at higher elevations at site 3 and 4. Overall, the recorded elevation of Salix specimens confirmed the general ecological niche of Salix related to the vegetation zonation at middle European river flood plains (Ellenberg and Leuschner, 2010), and specifically at the Elbe estuary according to Preisinger (2010). Moreover, it might correspond to the establishment of willow species in elevated Phragmites australis or tall herb-dominated wetlands where the average flooding frequency is between 0% and 40% at the Scheldt estuary (Struyf et al., 2009).

Firstly, *S. alba* and *S. viminalis* are the characteristic willow species in willow communities in river flood plains (Ellenberg and Leuschner, 2010). Secondly, the studied *Salix* specimens showed site-specific behavior. At sites 1, 3 and 4 both species occurred at similar elevation; *S. alba* elevation was slightly higher compared to *S. viminalis*, which corresponds to the findings of Ellenberg and Leuschner (2010). However, at site 2 *S. alba* was found at substantially lower elevations compared to *S. viminalis*. Based on my findings, I conclude that *Salix* species' niche at the estuarine elevational gradient differs from the general river flood plain elevational gradient: *S. alba* optimum is 0.77 m (mean +/- 0.87 m SD), whereby the ecological amplitude ranges from -0.56 m to 2.73 m elevation above mean high water level. *S. viminalis* optimum is 0.77 m (mean +/- 0.61 m SD), whereby the ecological amplitude ranges from -0.57 m to 2.26 m elevation above mean high tide level.

Soil water salinity at *Salix* plots

The recorded increase in soil water salinity along the longitudinal estuarine stretch, from freshwater conditions at sites 1 and 2 up to oligohaline conditions at sites 3 and 4, corresponds to the general sequence of estuarine salinity zones. From the estuarine head (salinity < 0.5) to the upper zone (salinity 0.5 - 5) salinity varies within small geographic areas mediated by water movement over relatively short time scales (McLusky and Elliott, 2004). My results of increasing salinity from site 1 to site 4 indicate that tidal flooding reached the investigated *Salix* specimen and led to periodic oligohaline soil water conditions at two of the studied tidal wetlands (see also Markus-Michalczyk et al., 2014; chapter 2.1.).

Morphological variability

Generally, height, diameter, crown area and lamina index were larger in *S. alba* than in *S. viminalis*. At each investigated site in tidal wetlands along the estuarine gradient, both species were found growing in a way similar to the description of Zander (2000): *S. alba* with a large crown spread of lanceolate leaves growing up to 20 m in height, and *S. viminalis* as an erect shrub with narrow lanceolate leaves growing up to 7 m in height. Thus, these estuarine sites represent in part the niche of both *Salix* species` requirements on environment.

Nonetheless, certain significant morphological differences between sites and species were found. Height of S. alba was found to be significantly lower at site 4 compared to sites 1, 2 and 3, and diameter was found to be similar at site 1, 2 and 4. Thus, plants age was ruled out as a possible cause of the lower mean height at site 4. However, site 4 is situated close to the river mouth and soil water salinity was found to be highest here compared to the other sites. Thus, oligonaline conditions might negatively affect species performance and thus limit the ecological niche of S. alba, which corresponds to low salt tolerance, indicated by Ellenberg et al. (1992). Negative effects of increasing salinity on the growth parameters of S. alba and S. viminalis vegetative propagules were found in a hydroponic greenhouse experiment (Markus-Michalczyk et al., 2014). Moreover, deviating climatic conditions at site 4, situated close to the North Sea, might have caused reduced height, as well. S. alba is a warm-adapted flood plain willow species, and the northern border of its distribution runs through north-west Germany (Neumann, 1981; Chmelar and Meusel, 1986). According to Holt (2009), controls on species' geographical range limits and how these might shift in our rapidly changing world might be better understood by analyses of species' niches. Thus, projections of climate change in the metropolitan region of Hamburg are of particular interest. Here, a general increase in near-surface air temperature is projected to be more pronounced in winter (Jacob et al., 2013). This might facilitate height growth, and moreover cause a shift in geographical range limits of warm-adapted *S. alba* in the Elbe estuary and other estuaries.

Diameter was found to be significantly lower at site 3 compared to site 2. Specimen elevation and soil water salinity at site 3 was intermediate compared to other sites, and thus another further factor might have caused reduced diameter. Here, more *S. alba* and *S. viminalis* specimen were found per plot and thus higher inter- and intraspecific competition can be assumed. Specifically, light availability per specimen might have been reduced. Flood plain willow species are light demanding pioneer species (Neumann, 1981; Ellenberg and Leuschner, 2010) and thus, increased height might indicate a habitat-specialization in competition for light in dense *Salix* stands. This corresponds to habitat-specialization as a proxy for Grinnellian specialization since habitat is not only the physical place where the species is found, but also encompasses biotic conditions that influence species' performance in various ways (Devictor et al., 2010).

Interestingly, at site 2 S. *alba* trunk position was generally substantially more inclined up to prostrate. According to Preisinger (1991), willow stands are in his study composed of regularly harvested willow shrubs, coppiced willows were confirmed based on early aerial images (taken in 1929), then willow flood plain forest regenerated after land abandonment, and numerous trees were fallen due to increased mean high water level. Increased tidal amplitude, with an increase in mean high tide of 30 cm and decreased mean low tide of 70 cm, have been confirmed since 1962 at Hamburg harbour gauge (IKSE, 2005). The dominance of inclined and prostrate trunks documented in the *Salix* specimens studied at site 2 corresponds to Preisinger (1991), assuming that fallen trees are due to increased high tide level. Moreover, I assume that *S. alba* is more affected by increasing tidal flooding compared to *S. viminalis*, and thus *Salix* species' niche at the estuarine elevational gradient differs from the general river floodplain elevational gradient.

At site 1, trunk form of *S. alba* was highly variable and arching, indicating an effect of stress on the exposed trunk side. The re-orientation of tree stems to maintain verticality against mechanical perturbations (e.g. physical stress) is achieved through the production of reaction wood (Almeres et al., 2001). However, soil instability, storms, wind or other permanent loads might lead to long-term deviation of the stem from vertical orientation (Speck et al., 1990). At site 1, *S. alba* specimens were comparatively more elevated and were exposed at the bank. Thus, wind might have caused modification of trunk form towards arching growth. This effect might become increasingly common due to climate change: in long-term trends calculated for the mid-twenty-first century over northern and central Europe, wind energy potential is projected to increase, particularly in winter and autumn (Hueging et al. 2013).

Furthermore, leaf size and shape are influenced along environmental gradients (e. g. humidity/aridity, solar radiation/shade), and may vary between species as they balance the

need for efficient conductance, net carbon acquisition, and protection against desiccation (Price and Enquist, 2007). In poplar populations (Salicaceae), petiole length showed great divergence when tested for climate-driven adaptation of ecophysiology and phenology (Keller et al., 2011). Here, short petiole length was associated with short growing seasons, and relatively cool and dry summers whereas petioles grew longer in warmer summers and with lower evaporative demand. *Salix*, with respect to its drought response strategy, belongs to the category of 'drought avoiders' versus 'drought tolerators' (Savage, 2009). Thus, my findings on increased petiole length and petiole index in *Salix* specimen at sites 3 and 4 may be due to higher mean annual precipitation rates compared to sites 1 and 2. Here, petiole elongation at sites with high precipitation rate may be viewed as a habitat-specialization of *Salix* along the estuarine gradient.

5.6 Conclusion

Recent interest on species' niches is "driven in part by the urgency of predicting ecological responses to rapid environmental change" (Holt, 2009). Flood plain willows are vulnerable species listed in the European Habitats Directive (Annex I European Habitats Directive) inhabiting rapidly changing tidal wetlands in estuarine environments. Thus, the genus *Salix* served as a model system for understanding species ecological niches along the estuarine gradients in times of global change. Elevation and salinity are crucial factors in determining zonation in flood plain vegetation (Engels and Jensen, 2009). In my study, *Salix* species elevational optimum was found to be 0.77 m above mean high water. Here, the means of *S. alba* and *S. viminalis* were identical. Moreover, minimum and maximum values differed just slightly between both species and specimens of both *Salix* species were detected to survive at approximately -0.6 m elevation above mean high water. Furthermore, I demonstrated that both species occurred up to oligohaline soil water conditions in tidal wetlands.

However, habitat specialization influences a species performance in various ways (Devictor et al., 2010), and, as expected, *Salix* species showed morphological variability and modifications that demonstrated its adaptation to the estuarine environment. The estuarine environment is a dynamic ecosystem with high disturbances rates (McLusky and Elliott, 2004). Increasing mean high water acts as a stressor for flood plain vegetation but might also open windows of opportunity for adapted immigrants, in this case *Salix* specimens, to become established and form reproducing populations at exposed sediments. These populations may respond with greater adaptedness to local natural selection (Levin, 2004). Adaptations to flood plains cause high genetic variability in riverine *Salix* populations (Karrenberg et al., 2002). Beyond my findings on *Salix* elevational and salinity niche, I assert

that the genus *Salix* is an excellent model system, and I highlight the need for further research, including studies of climatic and geomorphological factors, to further characterize the ecological niche of *Salix* species, as well as to better understand niche shifts and evolutionary processes along estuarine gradients.

6. General discussion

Estuaries are highly dynamic systems with tidal flooding and fluctuating salinity as crucial factors. Projected climate change will lead to sea level rise and changes in river discharge which might increase saltwater intrusion and tidal flooding in tidal wetlands. Willows are characteristic woody species in tidal forests in European temperate climates, a vulnerable part of protected softwood flood plains forests and valuable ecosystem service species. In the Elbe estuary which served as the estuarine model system *Salix alba* and *Salix viminalis* are characteristic willow species in tidal wetlands. Both species are described as being not salt tolerant and to occur above mean high water level. Thus, these species were investigated with regard to effects of salinity and tidal flooding at different life stages (large specimens, and vegetative and sexual propagules). In this thesis, experimental approaches were combined with field studies in order to clarify *Salix* ecological niche in tidal wetlands in the estuarine environment in times of climate change.

6.1 Salix salt tolerance in the estuarine environment at different life stages

An increase in global mean surface temperature might result in projected sea level rise from the 1990 level of 0.5 m to a level of 1.4 m in 2100 (Rahmstorf, 2007). Generally, sea level rise and related saltwater intrusion into estuaries might increase soil water salinity and affect organic matter mineralization and greenhouse gas emissions in tidal wetlands (Craft et al., 2009). In some systems, salinization might facilitate adapted estuarine organisms, which has recently been shown in the eastern portion of the Amazon River mouth where an extensive mangrove belt increased in size in the upper river reaches due to salt water intrusion (Nascimento et al., 2013). However, in tidal freshwater wetlands, "because salt water intrusion is going to change the defining feature of tidal freshwater wetlands (i.e. the presence of freshwater), this stressor is likely to have the largest long-term impact," and thus might change species distribution and composition (Neubauer and Craft, 2009). In particular, tree species like bald cypress (Taxodium distichum) in extended North-American tidal freshwater forests, might be negatively affected by long-term salinity increases caused by sea level rise (Krauss et al., 2009), and accelerated sea level rise might lead to decline of tidal forests and expansion of oligonaline and brackish marshes where soil accretion exceeds the current rate of sea level rise (Craft, 2012).

In European tidal freshwater wetlands, woody communities are dominated by willows (Struyf et al., 2009). Thus, willows in tidal freshwater wetlands in temperate climates can be seen as the counterpart to mangroves in subtropical and tropical tidal flood plains, as well as

counterparts of bald cypress tidal forests in North America. Our results of studies on Salix salt tolerance clearly showed, that large specimens of characteristic willow species (Salix alba and Salix viminalis) are distributed up to sites with oligonaline soil water conditions in estuarine environments. Thus, our results are in contrast to expected conversion of North American tidal freshwater forests to oligohaline marshes (Craft, 2012), and large Salix specimens might survive salt water intrusion in tidal wetlands up to oligohaline conditions. However, many floodplain Salix species regenerate and disperse via vegetative propagules (Mosner et al., 2012). Since Salix specimens occurred in tidal wetlands up to the transition from the oligohaline to the mesohaline estuarine stretch, we investigated the salinity tolerance of vegetative dispersal units up to the level of salinity 6 in hydroponic growing conditions. Overall, there were substantial negative effects of increasing salinity in both Salix species from both, freshwater and brackish origins. However, we demonstrated a relative tolerance up to salinity 2, which might ensure regeneration via vegetative dispersal in times of increasing salinity in tidal wetlands. Moreover, immature S. viminalis plants of brackish origin performed significantly better compared to individuals originating from freshwater wetlands, and thus, local acclimation might be indicated.

Our findings are contrary to ecological indicator values of mid-European vascular plants: Ellenberg et al. (1992) considered *Salix alba* and *Salix viminalis* as being not salt tolerant. Hence, we carried out an experiment on *Salix* seed germination and early seedlings establishment, since early life stages of glycophytes of tidal wetlands were found to be extremely reduced under brackish and euhaline conditions (Engels et al., 2010). Moreover, *S. alba* does not exhibit a preformed brittleness at twig bases that enables twigs to serve as vegetative propagules like *Salix fragilis* (Beismann et al., 2000), or like *Salix viminalis*. However, *S. alba* releases small short-lived seeds in spring (Karrenberg et al., 2002), and regeneration via sexual propagation appears to be more crucial. Thus, *S. alba* served as the sole study species.

Germination percentage of *S. alba* seeds was similarly high up to salinity 2 in our climate chamber experiment. However, detected seedling performance decreased at salinity 2 and thus, *Salix* seedlings tolerance up to salinity 1.5 might be suggested. Hence, sexual regeneration of *S. alba* might be possible in tidal wetlands with oligohaline soil water conditions. However, "early seedling establishment is a demographic bottleneck for the sexual regeneration of many riparian tree species" and *S. alba* seedling survival was found to be extremely reduced after an abrupt drop in water table (Guilloy et al., 2011).

Further experimentations on *Salix* sexual regeneration under different soil moisture conditions are recommended. Moreover, in tidal wetlands salt intrusion is related to tidal flooding, and hence, *Salix* tolerance to tidal flooding at different life stages has to be taken into account with regard to survival of drowning wetlands in times of climate change.

6.2 Salix tolerance to tidal flooding in the estuarine environment at different life stages

At the coast, salt marsh species occur in vegetation zonation along an elevational gradient, and elevation is proposed to largely explain species distribution (e.g. Suchrow and Jensen, 2010). In river floodplains, reflection of flooding stress is well documented at the individual plant level in the adaptation and zonation of vegetation (see Blom and Voesenek (1996) for a review). Freshwater flood plains at the Amazon River Basin in South America harbour the most species-rich flood plain forests in the world: more than 1,000 tree species are distributed at the nutrient-rich white water flood plains (Wittmann et al., 2006). However, the number of pioneer species is largely reduced in colonising Amazonian low lying sites: at the lowest sites *Salix humboldtiana* solely forms monospecific stands subjected to long periods of inundation and high sedimentation rates (Parolin et al., 2002). In Central Europe, riverine flood plain forests at low elevated sites right above the mean high water line (Ellenberg and Leuschner, 2010). However, little is known about willow species tolerances to tidal flooding.

We originally hypothesised that *Salix alba* and *Salix viminalis*, the most abundant tree species in tidal wetlands in Germany, are able to tolerate tidal flooding projected in moderate climate change scenarios. Our results on *Salix* specimen elevation relative to mean high water (mean 0.77 m, minimum -0.56/-0.57 m above mean high water) indicate that both species tolerate tidal flooding up to approximately 0.60 m, which corresponds to moderate sea level rise scenarios (IPCC 2007).

According to Colmer and Pedersen (2008) "flooding involves inundation of part or all of the aboveground structures, whereas waterlogging is restricted mainly to inundation of the soil and rhizosphere." Thus, elevation of large *Salix* specimen up to of –0.60 m above mean high water mainly causes waterlogging and inundation of tree trunks at the bases, and no photosynthetically active plant organs of trees would be affected. However, small plants would be affected as a whole and thus, investigations on immature *Salix* plants were carried out.

Our investigation of the effect of tidal treatments on immature plants, where leaves were exposed to simulated tidal inundation twice daily clearly demonstrated that *Salix* plants of both species were able to survive flooding up to 0.60 m inundation after 15 weeks of tidal treatments. However, plants performance was substantially reduced at 0.60 m flooding depth. The specific dry mass was reduced, in parallel with an increased aboveground to belowground ratio. This corresponds to Mitsch and Gosselink (2000) who found that wetland plants generally exhibit reduced belowground biomass, in agreement with other experiments on wetland plants (e.g. Miller and Zedler, 2003). Recently, a multi-species approach on studying tree species in forest flood plain ecosystems at the central European Danube River confirmed the relationships between carbon fine root and carbon aboveground biomass (Rieger et al., 2013). Here, aboveground biomass reduction and higher investment in denser root systems were related to distance to the river, low ground water table, and thus a higher drought sensitivity, which corresponds to our results on flood tolerance.

In our investigation, no difference was found in photosynthetic performance of *Salix* specimens between flooding levels. We measured chlorophyll fluorescence above the water level and demonstrated that the photosynthetic activity was higher after inundation due to tidal flooding twice daily. Thus, we consider that tidal inundation did not cause harmful effects on the photosynthesis of *Salix* plants suddenly exposed to high radiation after tidal inundation, although plants growing in low light habitats are usually sensitive to UV-radiation so that when exposed to a higher UV-irradiance than encountered in their natural habitats, a harmful effect of UV is frequently reported (Hanelt and Roleda, 2009). Moreover, little is known about *Salix*'s ability to perform photosynthesis underwater. It might be hypothesised that *Salix humboldtiana*, a submergence tolerant colonising species of low-lying habitats in Amazonian flood plains with constant leaf number and exposure to flooding up to 230 days (Paralin et al., 2002), is able to be photosynthetically active underwater.

However, so far the ability of *Salix alba* and *Salix viminalis* to perform underwater photosynthesis remains unclear. If *Salix* is generally able to be photosynthetically active underwater, activity might have been negatively affected by decreased radiation with increasing flooding depth and might have yielded a reduced dry mass. Specifically, underwater photosynthesis in the field might be largely negatively affected by high turbidity, especially in the estuarine transition zone from freshwater to brackish conditions, where turbidity is generally highest (McLusky and Elliott, 2004).

Physical soil characteristics, in combination with flooding duration and frequency, are also important in determining plant position in flood plain vegetation (Blom and Voesenek, 1996). Therefore, with regard to estuarine turbidity additional field studies are recommended on *Salix* ecological specialization to tidal flooding in the estuarine environment, including the

study of soil characteristics. Specifically, studies are lacking on soil properties of tidal freshwater flood plain forests although soil accretion in North American tidal forests is described to be less than the recent rate of sea level rise (Craft, 2012). At the Southeastern Atlantic Coast and Gulf Coast, soil properties of tidal forests have a wide range of organic matter content, and pH and N meet conditions in blackwater tidal forests (Anderson and Lockaby, 2007). However, soil accretion of willow softwood flood plain forests is suggested to be higher: in South American Amazonian white water flood plains, 15-25 cm of sediment deposits was measured in a *Salix* stand with a silt fraction of 50% (Wittmann et al., 2001), and a study on White Clay Lake in North America described four willow species as indicators of sediment deposition (Johnston, 2003). Thus, studies are required on accretion in *Salix* stands in tidal flood plain forests in temperate climates specifically regarding rising sea level.

7. Conclusion and perspective

Numerous literature references assign various willow species to diverse ecosystems worldwide. In the North American arctic region, dwarf willow species distribution follows gradients of summer maximum temperatures, whereas the distribution of the tree species in the southern areas follows gradients of fall and winter minimum temperatures. Thus, it is recommended to combine distributional data with environmental tolerance to best determine species niches in order to accurately develop conservation strategies (Stankowski and Parker, 2010). At the South American Paraná River flood plain, species distribution is strongly determined by the flood plain water regime and location at the topographical gradient: here, South American alder (Tessaria integrifolia) and willow (Salix humboldtiana) were found to have similar niches in relation to hydrological fluctuations (Casco et al., 2010). Generally, differences between plants' requirements on resources, response to resources and processes on resources are considered to have a large effect on species composition and ecosystem function (Chapin et al., 2000). Recurrent pattern of plants` ecological specialization were found in four floras and confirmed the existence of evolutionary specialization at the global scale (Diaz et al., 2004). Overall, "taxonomic diversification commonly occurs through adaptive radiation, the rapid evolution of a single lineage into a range of genotypes or species each adapted to a different ecological niche" (Simpson (1953) and Schluter (2000) in Kassen et al., 2004), and ecological gradients have been shown to constrain the size of adaptive radiation, even in the presence of the strong diversifying selection associated with ecological opportunity, by decoupling evolutionary diversification from ecological coexistence (Kassen et al., 2004).

With my thesis, I demonstrated the ecological specialization of *Salix alba* and *Salix viminalis* to salinity and tidal flooding in tidal wetlands. According to Devictor et al. (2010), the variance of species performance across a range of environmental conditions might represent Grinnellian specialization. Thus, I determined *Salix*'s ecological niche by specimens' occurrence and performance related to salinity and tidal flooding in the estuarine environment, according to Grinnell (1917).

We clearly showed both species' ecological coexistence with a mean value of approximately 0.8 m elevation above mean high water and a minimum of approximately -0.6 m elevation above mean high water on the basis of our results in a field study. Here, the recorded mean elevation above mean high tide and the minimum recorded values were similar for both species.

Our results in an experimental approach of the effects of tidal treatments on vegetative propagules indicate plant survival and a relative tolerance to tidal flooding of 0.2 to 0.6 m. However, plant performance was largely reduced at 0.6 m flooding level. Hence, *Salix* long-term survival might be limited by tidal flooding beyond 0.6 m.

Our results contrast with those of Ellenberg and Leuschner (2010), who described *S. viminalis* in river flood plains in Central Europe to occur right above the mean high water line and *S. alba* to follow at higher elevations. I conclude that *Salix* species' niche at the elevational gradient in the estuarine environment differs from its corresponding niche in river flood plains. Both *Salix* species' ecological niche in relation to elevation above mean high tide was similar and thus I conclude that there is ecological coexistence in relation to tidal flooding.

Also, with regard to *Salix* salinity tolerance, our results contrast with Ellenberg and Leuschner (2010). They determined *S. alba* and *S. viminalis* to be not salt tolerant. However, we demonstrated that large specimens of both *Salix* species were distributed up to oligohaline soil water conditions, up to soil water salinity 2, at tidal wetlands in the estuarine environment. Furthermore, in our experimental approach in hydroponics, a relative salt tolerance of vegetative propagules up to salinity 2 was detected, and thus the results of both our field study and experiment are in accordance with each other. Moreover, *S. viminalis* plants of brackish origin performed significantly better compared to individuals originating from freshwater wetlands, and hence, we assume that there was local acclimation to oligohaline conditions. In addition, we investigated *S. alba* sexual propagules and detected similar seed germination up to salinity 2, and similarities in seedling performance up to salinity 1.5. We conclude that the salt tolerance of *S. alba* and *S. viminalis* in the estuarine environment differs from general ecological behaviour and thus both species` ecological specialization to the estuarine environment might be assumed.

Overall, our results support the ecological coexistence of *S. alba* and *S. viminalis* in relation to salinity and tidal flood tolerance in the estuarine environment. Moreover, better performance of *S. viminalis* of brackish origin might indicate the widening of its niche to include more saline conditions. Ackermann and Doebeli (2004) found that in the case of resource competition, niches may be widened, but widening the niche causes additional costs or benefits, reflected in the evolutionary outcome. Thus, if widening the niche is too costly, adaptive diversification is likely to drive species niches. If distinguishing between adaptive diversification and widening the niche via local acclimation is intended, reciprocal transplant experiments might be recommended, and genetic investigations on *Salix* study specimens should be undertaken in parallel. Generally, we recommend the decoupling of our

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stated ecological coexistence of both investigated *Salix* species at the salinity and flooding gradient from evolutionary diversification with regard to diversifying selection associated with ecological opportunity, according to Kassen et al. (2004).

In environments with tidal dynamics (e.g. tidal flats and wetlands at coasts and estuaries), physical forces open various windows of opportunity which might be specifically essential for seedling establishment (Balke et al., 2011). Hydrodynamics (e.g. waves, currents and ice scour) as well as sediment dynamics cause high disturbance rates in estuarine environments (McLusky and Elliott, 2004), and might support bare grounds as suitable sites for *Salix* seedlings establishment (Karrenberg et al., 2002). Arrival at suitable sites for germination and plant establishment is described to be crucial for propagating plants in the floodplain environment (Vogt et al., 2007). Diversifying selection associated with ecological opportunity might be enhanced at tidal wetlands in highly disturbed estuarine environments and further experimental and field investigations on seedlings establishment survival are needed.

While we did focus on salinity and tidal flooding as crucial abiotic factors, biotic interactions were not addressed. Beyond competition in seedling establishment on bare grounds, studies concerning the effect of herbivory on species in European tidal freshwater wetlands are lacking (Engels et al., 2010). Some studies showed *Salix* species interactions with large artiodactyl herbivores through complex changes in plant chemistry and plant morphology which in turn changed feeding strategies (e.g. Stolter, 2008; Stolter et al., 2013). In flood plains, mammalian flood plain inhabitants like beavers are known to interact with *Salix* plants: *Castor canadensis* Kuhl facilitated recovery of riparian vegetation including *Salix* stands after decades of grazing (Demmer and Beschta, 2008) in North America. *Salix* interaction with *Castor fiber* L., the related beaver species native to Europe, might be of interest in European estuarine environments in the future.

Generally, sea level rise and related saltwater intrusion might increase soil water salinity in tidal wetlands (Craft et al., 2009). In tidal freshwater wetlands salt water intrusion is likely to have a large long-term impact, and thus, might change species distribution and composition (Neubauer and Craft, 2009). Since tree species in particular might be negatively affected by long-term salinity increases caused by sea level rise (Krauss et al., 2009), our findings on *Salix* salt tolerance in tidal wetlands are of particular importance for future development of willow soft wood forests in tidal wetlands.

Tidal wetlands perceive benefits to society that result from wetland functions, including human welfare, increased environmental quality, and wildlife support. Thus, many types of coastal ecosystems, including estuaries, deltas and tidal flats are listed as Ramsar sites and protected as wetlands of international importance (Ramsar, 2014).

However, tidal freshwater wetlands in particular decreased in area because of human alteration of estuarine geomorphology (Struyf et al., 2009). In Germany, less than 20% of the recent flood plains are covered by alluvial forests (BMU and BfN, 2009), and remaining softwood forests are fragmented in the Elbe estuary. The value and vulnerability of alluvial forests have been recognized, and white willow softwood flood plain forests are listed in Annex I as a priority habitat (91EO) of the European Habitats Directive (92/43/EEC 1992). A good state of preservation has to be fixed or to be improved. Thus, our findings on the ecological niche of characteristic willow species of white willow softwood flood plain forests might contribute to future measures for aiming to preserve and restore these protected habitats in tidal wetlands in the estuarine environment.

Preservation and restoration of white willow softwood floodplain forests with *S. alba* and *S. viminalis* as characteristic willow species might also contribute to flood defense in estuarine environments. In suitable locations, ecosystem-based coastal defense in times of climate change by ecosystem restoration might provide an economic and ecologic supplement to conventional engineering (Temmerman et al., 2013). It has been proposed to introduce *S. alba* as ecosystem engineering species for coastal protection on a large scale (Borsje et al., 2011).

Overall, we conclude that our results are of high relevance with regard to nature protection and biodiversity preservation, combined with a possible contribution in sustainable flood defense in estuarine environments in times of climate change.

8. Summary

Estuaries are "hot spots" at coasts: these dynamic transition zones from freshwater and terrestrial habitats to marine ecosystems are characterised by continually changing fluctuations of water, dissolved and suspended matter, and concentrated human populations. Today, hazards to the estuarine environment include serious alterations in geomorphology and in land use, and effects of climate change and extreme weather events. These hazards result in increasing salinization and flooding, locally high sedimentation coupled with severe erosion elsewhere, substantial changes in tidal wetlands in general, and impacts on flood protection. In times of climate change, flood defense based on tidal wetland restoration might provide an economic and ecologic supplement to conventional engineering in suitable locations. Willows (species in the genus *Salix*) are proposed as ecosystem engineering species for flood protection measurements in the estuarine environment. Recent interest in species' niches is "driven in part by the urgency of predicting ecological responses to rapid environmental change" (Holt, 2009), and thus willows tolerance to increasing salinization and flooding in times of climate change is of particular importance.

In order to elucidate willows' performance in tidal wetlands and their ecological niche in the estuarine environment, I carried out within this thesis four studies on *Salix's* salt and flood tolerance at different life stages. In the first study, I investigated the soil water salinity at *Salix* stands in tidal wetlands in a European (Elbe) estuary and conducted an experiment with corresponding salinity treatments on vegetative propagules in hydroponic conditions. In the second study, I exposed vegetative willow propagules to tidal flooding in a mesocosm experiment and analysed immature *Salix* flood tolerance. In the third study, I investigated *Salix* seed germination and early establishment in different salinity treatments in a climate chamber experiment. Finally, I carried out a field study, in which I analysed the soil water salinity at *Salix* stands, and *Salix* specimen elevation and performance in tidal wetlands in the estuarine environment.

In the first study, I hypothesised that the characteristic willow species *Salix alba* and *Salix viminalis* from two origins (a tidal freshwater and a tidal brackish wetland) would be able to tolerate oligohaline conditions. Results on soil water salinity at *Salix* stands proved, that both species occur in oligohaline conditions. *Salix* salt tolerance up to salinity 2 was experimentally confirmed via salinity treatments on cuttings. Effects of origin were reflected in measurements of vitality, final dry mass and chlorophyll fluorescence, and better performance of *S. viminalis* of brackish origin at salinity 2 might indicate acclimation or even local adaptation to increased salinity.

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In the second study, I hypothesised that both willow species (*Salix alba* and *Salix viminalis*) from a tidal freshwater and a tidal brackish wetland are able to tolerate tidal flooding up to a level corresponding with moderate sea level rise scenarios. Tidal treatment on vegetative propagules confirmed this hypothesis, whereas origin from freshwater and brackish wetlands showed no effect. Plant performance was found to be reduced in tidal flooding levels of 0.6 m, and thus I assume that there exists a *Salix* tolerance to tidal flooding that is limited to the level of moderate sea level rise scenarios.

Based on the findings of the first study, I aimed to answer the question of whether seeds of *S. alba,* the characteristic species in white willow communities in tidal forests in temperate estuarine flood plains, are able to germinate and establish under oligohaline conditions. *S. alba* seeds originating from an oligohaline site were found to germinate up to salinity 2 and seedlings established up to salinity 1.5 in a third experimental study.

In the fourth study, I aimed to answer the question of up to which elevation and soil water salinity do *Salix* specimens occur and how do they perform in tidal wetlands. In a field study at four sites I found that specimens mean elevation was 0.77 m above mean high water and no interspecific differences were found. Similarly, minimum and maximum values differed just slightly between both species, and *Salix* specimens occurred up to approximately -0.6 m elevation above mean high water.

Overall, I state that *Salix* salt tolerance exists up to oligohaline conditions, and *Salix* tolerance up to a tidal flooding level is predicted in moderate sea level rise scenarios. *Salix* ecological specialization at riverine floodplains is well documented. However, *Salix* was previously assigned to be not salt tolerant and to occur above mean high water level. In contrast, I firstly contribute to redefining *Salix*'s ecological niche in the estuarine environment. Based on the results of three experimental studies combined with one field study on immature and mature specimens, I assessed the *Salix alba* and *Salix viminalis* ecological niche to extend up to salinity 2 and up to 0.6 m tidal flooding. Generally, habitat specialization influences species' performance in various ways. In my studies, I showed that parts of *Salix* are able to perform well under tidal wetland conditions, which might be assessed as being modifications to the estuarine environment.

Beyond my findings on *Salix*'s elevational and salinity niche, I assess the genus *Salix* to be an excellent model system and I highlight the need for further studies, including climatic and geomorphological factors, to proceed in determining *Salix* species' ecological niche, shifts and evolutionary processes along estuarine gradients.

Further studies on willows in tidal wetlands should aim to separate genetic variation and phenotypic plasticity in salinity and tidal flood tolerance. By doing so, genetically suitable material for restoration might be determined, with possible local adaptations.

To conclude: *Salix alba* and *Salix viminalis* are characteristic willow species in softwood flood plain forests, are listed in Annex I of the European Habitats Directive, and their good preservation status has to be fixed or to be restored. In parallel to their vulnerability, *Salix* exhibits high potential as an ecosystem service species in estuarine flood defense. Today, hazards in estuaries lead to increasing salinity and tidal flooding and predicted sea level rise might lead to increasing hazards in estuarine environments.

With this dissertation on the ecological niche of willows in tidal wetlands, I intend to contribute to the preservation and sustainable use of flood plain willows in the estuarine environment in times of climate change.

9. Zusammenfassung

Ästuarien sind "hot spots" an Küsten: Diese dynamischen Übergangszonen von limnischen und terrestrischen Habitaten zu marinen Ökosystemen zeigen kontinuierlich wechselnde Fluktuationen von Wasser, gelösten Stoffen und Schwebstoffen und sind Schwerpunkt menschlicher Ansiedlungen. Weitreichende Veränderungen in der Geomorphologie und der Landnutzung sowie Effekte von Klimawandel und Extremereignissen wirken katastrophal auf die ästuarine Umwelt. Sie resultieren in zunehmende Versalzung und Überflutung, führen zu lokal hoher Sedimentation und Erosion anderenorts sowie zu generell substantiellen Veränderungen in Tideauen und im Hochwasserschutz. In Zeiten des Klimawandels könnte ein auf der Wiederherstellung von Tideauen basierender Überflutungsschutz sowohl eine ökonomische als auch ökologische Ergänzung zu konventionellem Hochwasserschutz an geeigneten Standorten sein. Weiden (Arten der Gattung Salix) werden als Arten für Ökosystemdienstleistungen im Hochwasserschutz in der ästuarinen Umwelt vorgeschlagen. Die Dringlichkeit ökologische Antworten auf schnelle Umweltveränderungen vorauszusagen, befördert ein aktuell zunehmendes Interesse an ökologischen Nischen von Arten (Holt, 2009). Daher ist die Toleranz von Weiden gegenüber Versalzung und Überflutung in Zeiten des Klimawandels von besonderem Interesse.

In der Absicht, die Leistungsfähigkeit von Weiden in Tideauen und ihre ökologische Einnischung in der ästuarinen Umwelt aufzuklären, habe ich vier Studien zur Untersuchung der Salz- und Überflutungstoleranz von Weiden in verschiedenen Lebensstadien innerhalb dieser Dissertation durchgeführt. In der ersten Studie habe ich zunächst die Bodenwasser-Salinität an Weidenbeständen in Tideauen in einem europäischen Ästuar (Elbe) untersucht. Auf Ergebnissen basierend habe ich die Salztoleranz von vegetativen den Ausbreitungseinheiten experimentell untersucht. In einer zweiten Studie habe ich vegetative Ausbreitungseinheiten von Weiden in einem Mesokosmen-Experiment einer Gezeiten-Überflutung ausgesetzt. In der dritten Studie habe ich die Keimung und frühe Keimes-Entwicklung von Salix-Samen in einer Klimakammer unter verschiedenen Salzgehalten untersucht. Darüber hinaus habe ich eine Feldstudie vorgenommen, um die Bodenwasser-Salinität an Salix-Beständen, sowie das Standortniveau und die Leistungsfähigkeit von Weidenindividuen in Tideauen in der ästuarinen Umwelt zu untersuchen.

Für die erste Studie habe ich die Hypothese aufgestellt, dass die charakteristischen Weiden-Arten *Salix alba* and *Salix viminalis* mit Herkunft von Standorten einer Süßwasser-Tideaue und einer Brackwasser-Tideaue in der Lage sind oligohaline Bedingungen zu ertragen. Ergebnisse der Untersuchung zur Bodenwasser-Salinität an Weiden-Beständen bewiesen, dass beiden Arten unter oligohalinen Bedingungen vorkommen. Die Salztoleranz von *Salix* wurde bis zu einer Salinität von 2 durch ein Experiment an vegetativen Ausbreitungseinheiten (Stecklingen) bestätigt. Effekte der Herkunft wurden in der Vitalität, Chlorophyll-Fluoreszenz sowie einer höheren Leistungsfähigkeit von *S. viminalis* mit Herkunft aus der Brackwasser-Tideaue mit der Salinität 2 widergespiegelt. Dies könnte eine Akklimatisation oder sogar lokale Adaptation an erhöhte Salinität anzeigen.

Für die zweite Studie habe ich die Hypothese aufgestellt, dass die Weidenarten Salix alba und Salix viminalis mit Herkunft von zwei Standorten (der Süßwasser-Tideaue und der Brackwasser-Tideaue) in der Lage sind, Gezeiten-Überflutung bis zu einem Niveau eines moderaten Meeresspiegel-Anstiegs zu tolerieren. Eine Studie an vegetativen Ausbreitungseinheiten bestätigte die Hypothese, wobei die Herkunft der vegetativen Ausbreitungseinheiten keinen Effekt zeigte. Die Leistungsfähigkeit der Pflanzen war in einer Gezeiten-Überflutung von 0.6 m reduziert. Daher nehme ich an, dass die Toleranz von Salix gegenüber Gezeiten-Überflutung auf das Niveau eines moderaten Meeresspiegelanstiegs-Szenarios limitiert ist.

Auf den Erkenntnissen der ersten Studie basierend, beabsichtigte ich die Frage zu beantworten, ob Samen von *S. alba*, in der Lage sind, unter oligohalinen Bedingungen zu keimen und sich zu etablieren. *S. alba* ist die charakteristische Art in Silberweiden-Gesellschaften in Tideauwäldern in ästuarinen Überflutungsräumen im gemäßigten Klima. *S. alba*-Samen mit Herkunft vom oligohalinen Standort keimten bis zur Salinität von 2 und Keimlinge etablierten sich bis zur Salinität von 1.5 in einer dritten experimentellen Studie.

Mit der vierten Studie beabsichtigte ich die Frage zu beantworten, bis zu welchem Niveau und bis zu welcher Bodenwasser-Salinität *Salix* Individuen in Tideauen vorkommen können. Mit einer Feldstudie an vier Standorten habe ich festgestellt, dass das mittlere Niveau 0.77 m über dem mittleren Hochwasserniveau beträgt und habe keine interspezifischen Unterschiede gefunden. Die Minimal- und Maximal-Werte zwischen beiden Arten unterschieden sich nur geringfügig und *Salix*- Individuen wurden bis zu einem Niveau von ca. -0.6 m unter dem mittleren Hochwasser vorgefunden.

Daher schließe ich auf eine Salztoleranz von *Salix* bis zu oligohalinen Bedingungen, und eine Toleranz von *Salix* gegenüber einer Überflutung, wie sie in moderaten Meeresspiegel-Szenarien vorhergesagt wird. Die ökologische Spezialisierung von *Salix* in Flussauen ist gut dokumentiert. *Salix* wird als nicht salztolerant und mit einem Vorkommen über dem mittleren Hochwasser-Niveau gekennzeichnet. Demgegenüber trage ich erstmals zu einer erweiterten Definition der ökologischen Nische von *Salix* in der ästuarinen Umwelt bei. Auf Basis dieser experimentellen Studien, kombiniert mit den Ergebnissen der Feldstudie, beschreibe ich die ökologische Nische von *Salix alba* und *Salix viminalis* im ästuarinen Raum wie folgt: Die zwei untersuchten, charakteristischen Weidenarten tolerieren oligohaline Bedingungen bis zu der Salinität 2 und eine Gezeiten-Überflutung von bis zu 0.6 m. Da die Spezialisierung an Habitate generell die Leistungsfähigkeit von Arten in verschiedener Weise beeinflusst, beschreiben meine Studien mögliche Modifikationen von *Salix* an die ästuarinen Umwelt.

Über meine Erkenntnisse zur ökologischen Nische von *Salix* gegenüber Niveau und Salinität hinaus, bewerte ich die Gattung *Salix* als ein exzellentes Modelsystem. Ich betone den Bedarf an weiteren Studien, wobei klimatische und geomorphologische Faktoren einbezogen werden sollten, um in der Determinierung der ökologischen Nische, von Nischen-Veränderungen und evolutionären Prozessen entlang ästuariner Gradienten voranzuschreiten. Weitere Studien an Weiden in Tideauen sollten darauf zielen, genetische Variationen von phänotypischer Plastizität im Hinblick auf die Salz- und Überflutungstoleranz zu separieren. Damit könnte genetisch an die ästuarine Umwelt angepasstes Material bestimmt und für Erhaltungs- und Wiederherstellungsmaßnahmen verwendet werden .

Abschließend stelle ich fest, dass *Salix alba* und *Salix viminalis* charakteristische Weidenarten in Weichholzauen in Überflutungsräumen sind, die in Annex I der Fauna-Flora-Habitat-Richtlinie der EU gelistet sind. Ihr guter Zustand muss erhalten oder wiederhergestellt werden. Parallel zur Schutzwürdigkeit und Schutzbedürftigkeit von Weiden in Tideauen verfügen Vertreter der Gattung *Salix* über ein wichtiges Potential als Arten für Ökosystemdienstleistungen im Hochwasserschutz in ästuarinen Räumen. Massive Einflüsse und Veränderungen in Ästuarien führen zu zunehmender Salinität und Überflutung, und darüber hinaus könnte der projizierte Meeresspiegelanstieg weiter zunehmende katastrophale Einflüsse und Veränderungen in Ästuarien nästuarinen nästuarien bewirken.

Mit dieser Dissertation zur ökologischen Nische von Weiden in Tideauen in Zeiten des Klimawandels beabsichtige ich zum Schutz und zur nachhaltigen Nutzung von Weiden in der ästuarinen Umwelt in Zeiten des Klimawandels beizutragen.

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APPENDIX

Appendix 3.1

Fv/Fm in repeated measurements in tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/- SD, N = 4).

Tidal Flooding	S. alba /	S. alba /	S. viminalis /	S. viminalis /
(cm)	freshwater	brackish water	freshwater	brackish water
0	0.771 +/- 0.025	0.782 +/- 0.026	0.754 +/- 0.025	0.765 +/- 0.021
20	0.761 +/- 0.030	0.747 +/- 0.043	0.761 +/- 0.017	0.773 +/- 0.026
40	0.748 +/- 0.032	0.765 +/- 0.030	0.750 +/- 0.024	0.760 +/- 0.024
60	0.771 +/- 0.036	0.774 +/- 0.030	0.750 +/- 0.039	0.773 +/- 0.022

Appendix 3.2

Three-factorial ANCOVA on aboveground dry mass, belowground dry mass and total dry mass in tidal treatments (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (F; p).

	ratio above-/belowground dry mass		total dry mass	
	F	p	F	p
flooding	8.53	< 0.001	20.54	< 0.001
species	1.64	n.s.	70.72	< 0.001
origin	1.06	n.s.	0.81	n.s.
flooding*species	0.06	n.s.	1.69	n.s.
flooding*origin	0.29	n.s.	0.13	n.s.
species*origin	5.38	< 0.05	2.19	n.s.
flooding*species*origin	0.36	n.s.	0.86	n.s.

Appendix 3.3

Three-factorial ANCOVA on increase in number of leaves and growth in total shoot length in repeated measurements in tidal treatment (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) according to species and origin in a tidal simulation experiment on *Salix* cuttings from tidal wetlands of the Elbe estuary (mean +/- SD, N = 4).

	Increase number of leaves		growth total shoot length	
	F	p	F	p
time	344.12	< 0.001	1220.51	< 0.001
time*step	2.72	< 0.001	4.10	< 0.001
time*species	17.97	< 0.001	179.73	< 0.001
time*origin	4.17	< 0.001	1.16	n.s.
time*step*species	2.41	< 0.001	5.43	< 0.001
time*step*origin	0.92	n.s.	1.45	n.s.
time*species*origin	0.30	n.s.	0.26	n.s.
time*step*species*origin	1.35	n.s.	0.60	n.s.

Appendix 3.4

Relative irradiance in water (% (depth to surface)) in biweekly repeated measurements at high noon (week 0, week 2, week 4, week 6) in tidal treatment (tidal flooding 0 cm, 20 cm, 40 cm, 60 cm) (mean +/- SD, N = 4).

Tidal Flooding (cm)	week 0	week 2	week 4	week 6
0	80.82 +/- 0.11	83.97 +/- 0.03	80.78 +/- 0.11	86.74 +/- 0.07
20	59.75 +/- 0.12	63.47+/- 0.01	54.59 +/- 0.10	68.1 +/- 0.11
40	36.52 +/- 0.07	46.41 +/- 0.03	30.29 +/- 0.04	41.81 +/- 0.03
60	29.99 +/- 0.06	36.74 +/- 0.03	13.45 +/- 0.01	20.81 +/- 0.06

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Monday, 3rd March 2014

Re: 'Willows in Tidal Wetlands in Times of Climate Change: Ecological Niches in Estuarine Environments,' by Heike Markus-Michalczyk

To whom it may concern,

As a native English speaker and manuscript Proofreader, I do hereby confirm that the abovementioned paper is written in precise and correct English (U.K.). I can additionally confirm that the standard to which this paper is written is of the highest order, and is worthy of publication without let or hindrance.

Yours sincerely,

vie & to

Michelle L. Arthur, MSc. English Proofreader

List of papers

This thesis is based on three experimental (chapter 1.-3.) and one field study (chapter 4.).

A publication based on the first experiment was published in 2014 (chapter 1.), and a manuscript on the second experiment was submitted in 2013 (chapter 2.).

- I. **Markus-Michalczyk, H.**, Hanelt, D., Ludewig, K., Müller, D., Schröter, B., Jensen, K., 2014. Salt intrusion in tidal wetlands: European willow species tolerate oligohaline conditions. Estuarine Coastal and Shelf Science 136, 35-42.
- II. **Markus-Michalczyk, H.**, Hanelt, D., Jensen, K., Survivors in drowning wetlands: Willows tolerate tidal flooding. Submitted to Wetlands, 17.12.2013.

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift

" Willows in Tidal Wetlands in Times of Climate Change: Ecological Niches in Estuarine Environments"

selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Heike Markus-Michalczyk Hamburg, 5.3.2014