



Effects of salinity on growth of plant species from terrestrializing fens



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ABSTRACT

Terrestrializing lowland fens may be temporarily exposed to elevated surface water salinity, which may have serious consequences for nature conservation. We investigated the response of five fresh water fen plant species to elevated salinity.

In a controlled greenhouse experiment, these species were exposed to salt concentrations up to 3000 mg Cl[−] l^{−1}.

Total biomass of the five species together was significantly reduced for salinity levels from 200 mg Cl[−] l^{−1}. Four individual species showed leaf death and relative growth rate reduction, with effects at 1000 mg Cl[−] l^{−1} for *Succisa pratensis*, *Thelypteris palustris* and *Viola palustris*, and 3000 mg Cl[−] l^{−1} for *Myosotis scorpioides*. *Comarum palustre* showed no significant (.05 level) sensitivity.

Biomass distribution was investigated as well. Root-shoot ratio of four species was affected by salinity, which in at least two cases seemed to be related to leaf death. Differences in specific leaf area as a result of salinity were only observed for *C. palustre*. Dry matter content increased in four species as a result of salinity.

Salinity tolerance did not correspond to the environmental distributions of the species, nor could species traits be related to tolerance.

Surface water salinity may affect vegetation development in terrestrializing fens at low concentrations. A reduction of plant growth would cause reduced fitness of some species and may lead to reduced root mat growth. Exposure to higher concentrations could eventually lead to a decrease of species richness.

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1. Introduction

Worldwide, delta regions experience increased salinity of groundwater and surface water. Causes include drainage for agricultural and urban purposes, which invokes upwelling of brackish and saline groundwater, and seawater intrusion in rivers (Werner et al., 2013). Climate change may lead to increased evapotranspiration, while fresh water discharge of rivers may reduce. This enhances salinity pressure on wetland ecosystems that are typical for delta regions. The Dutch lowlands are one of those regions at risk of salinity. Water from the river Rhine is used to maintain high water levels in channels and ditches of the polder areas during summers, to suppress land subsidence and saline upward groundwater seepage (De Louw et al., 2010). Salt concentrations in supply

water may increase due to low river discharge (Zwolsman and van Bokhoven, 2007) and high sea water levels.

As a result of this increased salinity pressure, dry summers could lead to increased salt concentrations in freshwater fens in the Dutch lowland region. Terrestrializing lowland fens, found in former turf ponds, are known for their high biodiversity and typical structure of floating root mats, in which gradients of abiotic conditions allow for coexisting successional stages varying from rich fen to poor fen (Verhoeven and Bobbink, 2001). Current standards protect these areas against supplying water with concentrations exceeding 200 mg Cl[−] l^{−1}. It is uncertain whether these standards can be maintained, when supply water salinity may become as high as 1500 mg Cl[−] l^{−1}. Whereas it has been recognized that supply water may cause adverse effects on these ecosystems, due to nutrient loads and internal eutrophication (Smolders et al., 2006), salinity has been identified as a risk (Witte et al., 2012), but its potential effects are mostly unknown.

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Salinity effects on plant communities depend on the response of species. Salinity can affect plant performance in several ways: through osmotic effects, toxicity, and nutrient interactions (Munns and Tester, 2008). Increased electrolyte concentrations decrease the osmotic head in the root zone and decrease water availability for transpiration, resulting in effects similar to drought. Toxicity occurs when salts build up in leaf tissue. Sensitivity to these processes can differ substantially between species. Plants have different mechanisms to deal with salinity, such as closing of stomata to limit transpiration, production of solutes to decrease osmotic pressure in leaves, and the exclusion of salts from sensitive tissue to prevent damage (Parida and Das, 2005). Osmotic stress may cause reduced growth rates and phenotype adjustments, while toxicity will cause chlorosis, necrosis, and death of older leaves (Parida and Das, 2005). Leaf death reduces the photosynthetic surface of a plant and therefore adversely affects its growth rate and competitiveness. Salinity often affects aboveground tissue more strongly than belowground tissue (Munns and Tester, 2008), and may therefore result in increased root-shoot ratio (RSR). Salinity can affect nutrient uptake and partitioning within tissue as well, which may result in additional stress or even nutrient deficiency symptoms for nutrients such as Ca^{2+} , K^{+} , and NO_3^{-} (Grattan and Grieve, 1999).

Salt tolerance has been investigated for crop species (e.g. Shannon and Grieve, 1999) and for species from brackish and saline environments (e.g. Pennings et al., 2005; Macek and Rejmánková, 2007). However, few studies exist about the effects of low salt concentrations on species from freshwater ecosystems, even though these studies show that the effects may be significant (Van den Brink and Van der Velde, 1993).

An explanatory approach to predicting the effects of environmental change is found in the analysis of plant functional traits. These preferably easy to measure parameters can be linked to processes or strategies that affect competitive performance under certain abiotic conditions. Traits, such as leaf mass per area or specific leaf area, have been shown to correlate well with large scale environmental gradients, such as temperature and rainfall (Wright et al., 2004). Eallonardo et al. (2013) found that leaf N content per area correlated with salinity tolerance, and C4 photosynthetic pathway, small leaf size and succulence correlated with salinity gradients in a salt marsh. They suggest that these traits could be related to relatively high water use efficiency. No literature has been found regarding traits that are related to salinity tolerance in low salinity environments. In freshwater fen terrestrialization systems, where water level is constant, there is no necessity for plants to have adaptations to deal with salinity or drought.

In this study, we investigate the effects of elevated salinity on plant species from Dutch terrestrializing fens. Since exposure is likely to occur seasonally, we focus on the direct effects that salt can have on individual species in the growing season, although we are aware that competition processes will play a role as well, especially on longer time scales. We assume that the species that are found in more brackish areas will be scarcely affected, therefore we are interested in the effects on species that are almost exclusively found in fresh water conditions ($<200 \text{ mg Cl}^{-} \text{ l}^{-1}$). We selected five fen species that do not occur in saline environments and exposed them to salinity in a greenhouse experiment to test the following hypotheses:

- 1 Moderate salinity levels (up to $3000 \text{ mg Cl}^{-} \text{ l}^{-1}$) will cause growth reduction and mortality of freshwater plant species.
- 2 Moderate salinity levels will cause changes in biomass distribution of freshwater plant species, as a result of tissue damage.
- 3 Differences of salinity tolerance between freshwater species are related to their species traits.

2. Materials and methods

We exposed the plants to different levels of salinity on a hydroponic setup in a controlled greenhouse experiment. The warm environment of a greenhouse resembles warm summers with little precipitation, when salinity exposure is most likely to occur. Hydroponics allow for good control of exposure to saline solutions. As the selected species grow at edges of root mats that float in surface water (Figs. S1 and S2 in Supporting Information), we considered hydroponic setup a realistic approximation of field conditions.

2.1. Plant species selection

We compiled a list of characteristic plant species from fen terrestrialization habitats, and selected five species that represent the diversity of species that is found in these habitats and that exclusively occur in areas with low salinity (Akkerman et al., 2013): *Comarum palustre* L. (internationally known as *Potentilla palustris* L.), *Myosotis scorpioides* L., *Succisa pratensis* Moench, *Thelypteris palustris* Schott, and *Viola palustris* L. (names after Van der Meijden, 2005). For brevity, we call these by their genus: *Comarum*, *Myosotis*, *Succisa*, *Thelypteris* and *Viola*. All species typically occur in wet, relatively nutrient-poor environments, such as fen meadows, except *Myosotis*, which is frequently found in eutrophic environments.

Locally, *Comarum* and *Thelypteris* are both found at the edges of root mats and function as ecosystem engineers: they form dense root systems, that make up the basis of the root mat, allowing other species to settle. *Myosotis* is often found growing near, or in the surface water, while *Viola* grows on the edge of the root mat. The rosette species *Succisa* does not colonize the surface water and is typically found in slightly more elevated conditions at the edge of the root mats and is considered an indicator of higher species richness (Zelnik and Čarni, 2013). The four higher plants and one fern are all perennial and herbaceous, although *Comarum* has lignifying stolons (Macek and Lepš, 2007). None of the species show succulence and all of them are C3 species (Fitter and Peat, 1994). An overview of plant characteristics is shown in Table S1.

2.2. Plant collection and settling

We collected the plants in the fourth week of May 2013 from Nieuwkoopse Plassen (The Netherlands), a Natura 2000 fen nature reserve with a terrestrialization landscape. Approximately 200 small clones of each species were collected from the edges of different root mats and transported to the greenhouse, where they were temporarily kept in containers with a nutrient solution ($5\times$ the concentration that was used in the experiment) to recover from handling damage. After 3 weeks, all plants were weighed (initial fresh weight) and 112 individuals of each species of similar weight were selected for the experiment and randomly assigned to treatments and containers (plastic, 25 l). Each plant's position on the container was randomly selected to avoid systematic shading effects. Supported by clothespins, the plants were placed in holes in the lid of the container, so the roots could grow freely in the nutrient solution, and the stems were not pressed. An opaque plastic foil cover protected against algal growth and temperature rise. The water was aerated through a tube.

The nutrient solution was made by adding nutrients and salts to tap water (ion concentrations were analysed), which was preferred over rain water due to its constant quality and pH buffering. Total concentrations were in mM: NH_4^{+} , 0.05; NO_3^{-} , 0.1; K^{+} , 0.18; Na^{+} , 1.15; Ca^{2+} , 1.2; Mg^{3+} , 0.3; Cl^{-} , 1.91; SO_4^{2-} 0.36; HCO_3^{-} , 1.3; PO_4^{3-} , 0.05; Si, 0.19; and in μM : Fe (EDDHA), 10; Mn^{2+} , 2; Zn^{2+} , 0.5; BO_3^{3-} , 10; Cu^{2+} , 0.3; Mo^{2+} , 0.1. Nutrient levels were kept in the range as found in moderately rich fens (Bourbonniere, 2009). Nutrient concentrations in the surface water of the study site were

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