Chapter 1

General introduction
An increasing frequency of flooding events

The Intergovernmental Panel on Climate Change (IPCC) states that it is very likely that anthropogenic factors have been responsible for the main part of the observed global warming since the mid-20th century (Ledley et al., 1999; IPCC, 2014a; IPCC, 2014b). The climate changes manifest themselves by increasing temperature and shifting precipitation patterns and intensities. Furthermore, it was already reported that there were intensive changes of land use (Beckers et al., 2013) including deforestation in the world (Kaimowitz, 2004), especially in developing countries, and these changes will increase in the future. Changes of land use and deforestation have potentially large effects on flooding risk, since both of them can cause the decrease of the water-holding capacity of soil.

As consequences of these changes, there was a higher frequency of extreme precipitations in recent decades, and there was an increased occurrence of flooding events in the whole world (Bailey-Serres et al., 2012). Additionally, it is predicted that extreme precipitation events over most temperate regions, such as northern and central Europe, will very likely become more intense and more frequent in the future, particularly in the cold season, but also in the growing season. (Frei et al., 2006; Christensen & Christensen, 2007; Dankers & Feyen, 2009; IPCC, 2014a; IPCC, 2014b). These would result in more severe flood hazards in the future (Dankers & Feyen, 2009).

Effects of flooding on terrestrial plant species

Generally, flooding can result in soil waterlogging or even partial or complete submergence of terrestrial plants. Any kind of submergence negatively affects growth and survival of terrestrial plants, since it severely inhibits gas exchange between the submerged plant parts and the environment due to the 10^4 times lower diffusion rate of oxygen in water than in air (Armstrong, 1979). As a result, the oxygen concentration within plants decreases rapidly upon submergence (Rijnders et al., 2000). Such low oxygen levels will limit aerobic respiration and other essential oxygen dependent processes (Armstrong & Gaynard, 1976; Laan et al., 1990). Anaerobic metabolic pathways, such as fermentation, might compensate the low ATP yield due to the hampered aerobic metabolism (Perata & Alpi, 1993; Gibbs & Greenway, 2003). However, these pathways are far less efficient than aerobic respiration and thus reduce the pool of carbohydrate reserves quickly.
Submergence also hampers root function in flooded soils (Armstrong & Drew, 2002). Dissolved oxygen within sediments is consumed by roots and microorganisms; and slow gas diffusion rate impedes oxygen entry, leading to soil anoxia (Drew & Lynch, 1980). During hypoxic or anoxic conditions the stored carbohydrates in the roots are rapidly consumed. In combination with strongly reduced internal oxygen supply, maintenance of root growth and function becomes difficult or impossible. Mineral uptake by roots is important for growth of the plant but can no longer be accomplished when energy supply, membrane integrity and ion transport in the root are impaired causing nutrient deficiency not only in the root but also in the shoot. In addition, hypoxic conditions also can hamper root hydraulic conductance and result in stomatal closure and wilting in the shoots (Sauter, 2013).

The photosynthesis of submerged plants is strongly limited under water, because both the light quantity and photosynthetic quality are reduced under water compared to the environment above the water. Light is usually attenuated by surface reflection, back-scattering and absorption by water and dissolved particles (Holmes & Klein, 1987; Sand-Jensen, 1989), in which the intensity of red light absorbed by photosystem II also decreases more steeply than other wavelengths in the visible light spectrum (Lambers et al., 2008). Carbon dioxide is an another important limiting factor that may cause the low rate of underwater photosynthesis of submerged plants, due to slower carbon dioxide diffusion rates in the water column (Madsen & Sand-Jensen, 1994) and the thick stagnant boundary layers around the leaves under water, which reduce the gas exchange (Sand-Jensen & Frost-Christensen, 1999). In order to survive, some aquatic species can use the HCO$_3^-$ (bicarbonate) instead of the CO$_2$ in water for the underwater photosynthesis and growth, however, the advantage of HCO$_3^-$ use is very limited for terrestrial species (Pedersen et al., 2013).

Consequently, these changes of plants upon submergence will affect the natural ecosystems and probably will result in changes in species distributions, abundances and changes in timing of ecological processes in both terrestrial and aquatic systems (Naiman & Decamps, 1997; IPCC, 2014a; IPCC, 2014b). In addition to the negative effects at the level of individual terrestrial plants,
increased flooding events may also have positive effects on species richness of plant communities, depending on the environmental context and flooding severity. The intermediate disturbance theory states that intermediate disturbance leads to species-rich communities, while too low or high disturbance regime results in species losses (Connell, 1978; Pollock et al., 1998). Some literature has mentioned that relatively mild flooding events at sites with more frequent water level fluctuations during the year may have positive effects on species richness, as such regimes may benefit otherwise less competitive species that are adapted to them (Jansson et al., 2005; Stromberg et al., 2007; 2009).

O2 sources and adaptive traits of submerged plants

Oxygen deficiency is considered to be one of the major factors negatively affecting survival and growth of submerged plants, because, as mentioned above, it leads to energy deficits due to hampered aerobic metabolism. For submerged terrestrial plants, there are three main oxygen sources: (1) Some terrestrial plants have underwater photosynthesis in the presence of light, the photosynthetically produced O2 can diffuse within the plant to other organs, via the aerenchyma, thereby potentially providing an endogenous supply of O2 to the root system. Even though underwater photosynthesis of terrestrial plants usually occurs at a much lower rate than that in air due to physical restrictions of the aquatic environment (Sand-Jensen, 1989), it still can improve the internal oxygen status of submerged tissues. The photosynthetically produced oxygen contributes to radial oxygen loss from the root to sediment, leading to substantially higher oxygen concentrations in the rhizosphere. Such a mechanism has been shown in a number of submerged species (Waters et al., 1989; 1995; Pedersen et al., 1998; 2004).

(2) The dissolved oxygen in the water column is considered as another main O2 source for submerged plants. Generally, the concentration of dissolved oxygen in freshwater at equilibrium with the atmosphere at 20°C is about 284 µmol/L O2 or 20.6 KPa. However, dissolved O2 can rise to well above air saturation in the afternoon when there is a lot of O2 produced by plant and algal photosynthesis (Borum et al., 2005; Sand-Jensen et al., 2005; Pedersen et al., 2006). This dissolved oxygen in the water column affects the internal O2 partial pressure of submerged plants (Pedersen et al., 2006; Rich et al., 2013), because it can be absorbed by acclimated leaves, which have low resistance for gas exchange with
the water column (Mommer et al., 2004); it also can be easily absorbed by leaves with a gas film on the surface (Winkel et al., 2014).

(3) Partially submerged plants have an additional aerial pathway by which a connection of shoots above and below the water surface allows for oxygen exchange with the atmosphere and movement within submerged plants via low-resistance gas pathways (aerenchyma; see below) (Bailey-Serres & Voesenek, 2008; Colmer & Voesenek, 2009). Thus partially submerged plants are considered to have less stress than completely submerged terrestrial plants. The benefits for plant aeration offered by shoot emergence above the water surface has been demonstrated via measurements of gas flows and O2 concentration in many emergent wetland plants. Shoot emergence considerably enhances internal O2 in submerged parts, which is also beneficial to root aeration, particularly in darkness (Pedersen et al., 2009; Pedersen et al., 2010). Therefore, several plant species demonstrate dramatic changes in shoots upon complete submergence. For instance most species elongate their shoots in order to enhance the contact with the atmosphere (Voesenek et al., 2006; Luo et al., 2016).

Aerenchyma formation

Aerenchyma is tissue containing large gas-filled spaces (lacunae) that interconnect longitudinally to provide a low-resistance pathway for long-distance gas transport across plant organs (Voesenek et al., 2006). The presence of aerenchyma is essential for the oxygen transport in the submerged plants, especially in partially submerged plants which can more efficiently transport the oxygen. Depending on the species and environmental conditions, aerenchyma forms in newly emerged adventitious roots (Drew et al., 1979); young seminal roots (Konings, 1982; Thomson et al., 1990), and stems, petioles, and rhizomes (Liang, 1940; Brix et al., 1992). In roots, aerenchyma usually forms in the cortex, whereas in stems (including rhizomes) aerenchyma can occur both in the cortex and in the pith cavity (Armstrong, 1979). The volume of aerenchyma formed depends on genotype (species, as well as cultivar/accession) and environmental conditions (Colmer, 2003). Flooding usually causes oxygen stress for submerged plants; most of them respond by increasing the size or proportion of aerenchyma in tissue in order to improve the internal oxygen status. Some literature demonstrates that in many wetland plants aerenchyma forms constitutively, but is further enhanced in response to flooding; in most terrestrial species, aerenchyma is largely absent until
it is induced by environmental conditions, such as flooding (Armstrong, 1979; Colmer, 2003).

**Adventitious root formation**

Flooding-adapted plants employ a range of morphological, anatomical and metabolic mechanisms that enhance their probability of survival during flooding, since slow oxygen exchange between the submerged plant parts and the environment results in energy and carbohydrate deficits in plants. Flooding often results in complete or near complete loss of the sediment roots because of toxic effects of the accumulation of reduced elements such as Mn$^{2+}$, Fe$^{2+}$ and S$^{2-}$ in soil (Colmer & Voesenek, 2009). At slow gas diffusion in the water column, the gaseous hormone ethylene accumulates in submerged tissues to physiologically active concentrations within a few hours (Visser & Voesenek, 2005; Bailey-Serres & Voesenek, 2008), while the accumulated ethylene always promotes the formation of adventitious roots (Negi *et al.*, 2010; Vidoz *et al.*, 2010). Flooding-tolerant herbaceous plants and trees produce adventitious roots in response to submergence to absorb water and nutrients thereby replacing the functions of sediment roots (Sauter, 2013). The new adventitious root system is usually produced from the upper portions of the primary root systems or from the shoot base of underground stem portions, and if floodwaters rise above the soil surface, adventitious roots can also form from submerged stem parts (Kozlowski, 1997). The formation of adventitious roots as a response to soil waterlogging or flooding is found across a wide range of species, from dicotyledons to monocotyledons (Rich, 2011). The replacement of the sediment root system by adventitious roots was shown to improve plant growth and survival in the perennial wetland species *Cotula coronopifolia* and *Meionectes brownii* (Sauter, 2013), because the new root system can take up water and nutrient from the surrounding environments, and probably improve the internal oxygen status.

**Challenges and knowledge gaps**

While aerenchyma is usually considered to provide a low-resistance pathway for oxygen transport to the root system, only a limited subset of plant species contain continuous aerenchyma. Many species do not have continuous gas passages but instead have internally separated gas “rooms”. The most common type of separated gas rooms are sequential compartmented pith cavities in stems and
rhizomes, separated by thick nodal diaphragms. These nodes and compartmented stem pith cavities are a paradox in the context of partial submergence, as they may strongly constrain the advantages of aerenchyma to local internode conditions only. We found that quantitative information on oxygen conductance through nodes (especially nodes with thick diaphragms without visible lacunae), and on how this trait affects the growth and survival of submerged plants, is rather limited.

Furthermore, it is generally accepted that the growth of an adventitious root system improves the survival of submerged plants, since it can take up nutrient and water from the water column instead of from the sediment root system. However, there is no knowledge about the theoretical possibility that adventitious roots can also take up oxygen from the water column, which, if true, could improve the internal oxygen status of submerged plants and their survival. Related to this novel hypothesis, it is also unclear how the morphology of adventitious roots is affected by the oxygen and nutrient availability in the surrounding environment.

While flooding in general may become more common in the near future, also the average depth of flooding is likely to increase as a result of both increasing natural water level fluctuations as related to climate warming (see above) and increasing artificial water level fluctuations due to the construction of river dams (Mallik & Richardson, 2009; Su et al., 2013). As a result, deep submergence of terrestrial plants is likely to become increasingly common. The depth range a species occupies is determined by its tolerance to a combination of potentially multiple factors that all vary with water depth, notably light regimes, dissolved oxygen availability and water pressure. There is a knowledge gap about how these factors individually, or interactively, affect plant performance under different water depths, and which factor is the most important for plant survival. To fill these knowledge gaps it will be essential to carry out experiments that vary one factor at a time, or more factors in a standardized, factorial way; and for such experiments a representative species needs to be identified.

**Alternanthera philoxeroides as a model species**

*Alternanthera philoxeroides* (Mart.) Griseb., a perennial evergreen terrestrial plant species of the *Amaranthaceae* family, which is commonly known as alligator
weed, originates in South America, but has spread to many parts of the world and is considered an invasive species in the United States, Australia, New Zealand, Thailand and China. Under normal conditions, *A. philoxeroides* grows to a 50-120 cm height, with a long, single or sparsely branched stem. This species is considered to have a good tolerance to submergence at different water depths. It has compartmented pith cavities in stems, which are separated by solid low-porosity nodal diaphragms; and the volume of aerenchyma in its stems can be promoted by submergence or hypoxic conditions. Another response of this species to submergence is the production of adventitious roots on nodes of submerged stems. The production of adventitious roots on the stems shows a strong dependence on the submergence conditions, which makes it a suitable system for studying the plant response to submergence. The aerenchymatous stem and adventitious roots seem to benefit plant growth and survival upon submergence. *Alternanthera philoxeroides* is, therefore, used in this thesis as a model species.

**Aims and outline of this thesis**

The aims of this thesis are to (1) investigate the traits related to oxygen uptake and transport of terrestrial plants, here represented by the model species *A. philoxeroides*, upon different submergence regimes, especially the traits relating to aerenchyma and adventitious roots, and (2) the consequent influences or benefits of these responses to the growth and survival of the same submerged plants. Figure 1 conceptualizes how the different chapters in this thesis tackle key aspects of the these aims.

**Chapter 2**

In chapter 2, we present a novel aerenchyma blocking method, in combination with *in situ* oxygen measurement, for directly and experimentally investigating the role of compartmented stem pith cavities in a plant’s internal aeration, adventitious root formation and growth in response to partial submergence.

**Chapter 3**

In chapter 3, we applied a new methodology of experimentally pruning adventitious roots of submerged plants and measured its impacts on their hypothesized oxygen uptake and on plant survival. We monitored the oxygen
uptake by adventitious roots during complete submergence in the dark; and measured the oxygen concentration in stem nodes with adventitious roots versus in stem nodes with all adventitious roots removed; we also quantified the survival status of plants with or without adventitious roots (due to pruning treatment) during prolonged complete submergence in the dark.

Chapter 4

In chapter 4, we conducted two separate partial submergence experiments, with proportionally comparable environmental stress gradients of nutrient concentration and dissolved oxygen concentration in the water column. We measured the plants’ responses in terms of growth, adventitious root production, biomass allocation to adventitious root, and morphology of adventitious roots under different nutrient and dissolved oxygen treatments. These measurements and results allow us to get the experimental demonstration of (1) the key role for aerenchyma tube dimensions within adventitious roots for oxygen transport and for the associated plant performance under partial submergence; (2) how different agents of environmental stress related to flooding can induce both similar and fundamentally different plant responses.

Chapter 5

In chapter 5, we set up a unique full factorial experiment with three factors at two levels (2 light regimes × 2 dissolved oxygen concentrations × 2 water pressure levels (i.e. depths)), to quantitatively disentangle the interacting effects of water pressure, light and dissolved oxygen on the growth and survival of terrestrial plants under complete submergence at different depths.

Chapter 6

In the final chapter, the General discussion, the main findings of the different chapters are synthesized with respect to the research questions and aims, and in the context of a conceptual framework focused on feedbacks on oxygen cycling in flooding-prone ecosystems. In addition, future steps to be taken in flooding tolerance study of terrestrial plants are proposed.
Figure 1. Schematic representation of the four research chapters. Chapter 2 focuses on the main function of compartmented pith cavity to transport the oxygen to lower parts of partially submerged plants (in blue rectangle). In chapter 3 the new function of adventitious root is revealed (in green rectangle). In chapter 4, we measure the growth of adventitious root under different dissolved oxygen and different nutrient levels, including the morphology of adventitious roots under different partial submergence (in red rectangle). Chapter 5 focuses on the effects of water pressure, dissolved oxygen and light regime on the growth and survival of deeply submerged plants (in orange rectangle). The different font sizes of “O2” represent the different concentrations of available O2. The red arrows indicate the fluctuation of water depth. Darker blue indicates lower light levels in the water.
References:


