Rhizosphere Processes in Wetlands

Dissertation

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Summary

Wetlands are diverse and important ecosystems in global nutrient and carbon cycles but are declining due to anthropogenic and climatic threats to their areal extent and quality. Wetlands are profoundly shaped by their hydrology that gives rise to unique soil characteristics and well-adapted plant communities. Water level fluctuations are a common feature in many wetlands such as estuarine tidal marshes and freshwater lakes, where increased variability in abiotic factors can have a strong influence on plant productivity and the distribution of plant traits. Vegetation is a key element in carbon sequestration because of carbon fixation and deposition into the soil as various forms of organic matter, including belowground biomass and root exudates. Plant inputs are subsequently transformed by soil microbiota that drive organic matter degradation and contribute to the formation of soil organic matter (i.e. long-term soil carbon storage). The imbalance between input and output of carbon is remarkably large in many wetland types, making them significant carbon sinks. Most information we have on wetland plants and their role in nutrient and carbon cycling stems from investigations of certain aboveground plant traits (e.g. aboveground biomass, plant height, specific leaf area). These aboveground plant traits have been related to nutrient acquisition and depicted along gradients that reflect a variety of plant functional strategies (i.e. plant economic spectrum). Comparatively, belowground plant traits are more challenging to access and study, revealing a research gap in how these traits also relate to overall plant function. Functional plant traits at the individual or species level have been shown to correlate with observations of plants at the community- and ecosystem scales. Therefore, examining root traits, particularly those that influence microbial degradation of organic matter, has great potential to make predictions about ecosystems functioning with respect to carbon sequestration and storage. As root-microbiota interactions are most intense in the rhizosphere, i.e. the immediate soil around the roots, a trait-based approach was used to evaluate of physiological root traits (O_2 release, CO_2 uptake, exudation) on biogeochemical properties of soil or sediments and microbial communities in wetlands.

Plant effects on soil redox conditions were quantified along a hydrological gradient in a salt marsh at the Wadden Sea and in a mesocosm experiment (Chapter 2) to determine the impact of plants on soil reduction using IRIS (Indicator of Reduction in Soils) sticks. The study revealed the ability of wetland plants to act as both net oxidizers and reducers of tidally-influenced soil in a manner inversely related to background redox conditions. Plants generally reduced soil with relatively high redox potential and oxidized the soil when redox potentials were low. Intraspecific variation in radial oxygen loss was more deeply investigated using planar optodes; these observations gave further support for plastic root responses of O_2 release along a gradient of oxygen demand in the rhizosphere. These findings identify substrate characteristics as an important abiotic filter of plant-mediated shifts of soil redox conditions.

Physiological root traits related to gas exchange between the sediment and plant roots (O_2 release, CO_2 uptake) were measured in two life forms of the amphibious plant *Littorella uniflora* (Chapter 3) to evaluate trait performance in each life form under two hydrological treatments and alternating light cycles. The findings of this study indicate that these physiological traits may

not be maintained between the aquatic and terrestrial life forms as the terrestrial form demonstrated limited abilities of both sediment CO_2 uptake and O_2 release compared to the aquatic counterpart, especially upon reintroduction to aquatic conditions (i.e. under submergence). Limitations in either root trait could mean significant consequences for individuals to meet metabolic demands for photosynthesis or respiration. This study underscores the role of phenotypic plasticity as a possible mechanism for adaptation in highly variable environments. Furthermore, the implications of this study could be significant for occurrence of these isoetids and their ecological role in maintaining the oligotrophic status of lakes.

Plants provide electron donors to the soil or sediment via root exudation and thereby indirectly influence the degradation of organic matter by stimulating of soil decomposers. Transcriptional and community composition analysis of prokaryotic soil microbiota (i.e. bacteria and archaea) was conducted on bulk and rhizosphere soil of a common salt marsh plant, *Spartina anglica* (Chapter 4). By performing pulse-labeling of the aboveground tissues of *Spartina anglica* with the carbon isotope ^{13}C, photosynthetically-fixed carbon by the plant could be traced into the soil and used to detect the presence of root exudates. In this way, samples could be distinguished by their ^{13}C signature following enrichment analysis and operationally defined as either rhizosphere samples (^{13}C-enriched) or bulk soil samples (^{13}C signatures that did not diverge from control samples). Key gene analysis of both rhizosphere and bulk soils revealed the prominence of two main energy-conserving metabolic pathways, Wood-Ljungdahl and dissimilatory sulfate reduction. Transcriptional activity of the prokaryotes in the rhizosphere showed upregulation of genes involved in motility, stress response and infection, suggesting that roots stimulate bacterial mobility and organismal interactions. Analysis of community composition demonstrated enhanced relative abundances of certain prokaryotic groups in rhizosphere soil compared to the bulk soil, implying that roots may create favorable conditions for certain taxa who might gain a competitive advantage over other constituents of the community.

Altogether investigating root trait variability at the individual or species level provided mechanistic insight into how plants influence soil biogeochemistry (e.g. soil redox potential) and soil microbiota through physiological root traits. Root traits such as radial oxygen loss and root exudation are both influenced by abiotic factors such as sediment properties and biological drivers of intraspecific variability (e.g. phenotypic plasticity). These insights further the concept of root trait economics by addressing the role of previously neglected functional traits. Future of more physiological root trait measurements will likely enrich the growing knowledge of functional root traits and help to identify further common strategies in the root economic space. As this framework exists to help us predict plant functional responses to a range of environmental gradients, measurements in variable ecosystems like wetlands are particularly needed to make these predictions for wetland-specific plant adaptations. Considering the importance of wetlands not only for carbon sequestration but carbon storage, future research might use a more robust trait-based approach to elucidate the role of functional root traits in carbon stabilization processes as well.

1 General Introduction

Wetland classification and ecosystem services

Wetlands are vastly diverse ecosystems distinguished by periodic or sustained saturation of the substrate which leads to the formation of hydric soils and the establishment of hydrophytic vegetation (Cowardin et al., 1979; Hook, 1993; Council et al., 1995; Cowardin & Golet, 1995). These three attributes (hydrology, substrate, biota) are considered core diagnostic features shared by all wetlands, although classification methods differ in how they consider the relative importance of each (e.g., Cowardin et al., 1979; Brinson, 1993). Wetlands have a global distribution and are conventionally separated in broad categories composed of natural (coastal and inland) and artificial (human-made) wetlands (Scott & Jones, 1995) – for the purposes of this thesis, only natural wetlands (coastal and inland) will be considered with specific examples given for estuarine tidal wetlands and freshwater lakes, respectively. Recent reports approximate that natural wetlands cover an area of at least 13.2-14.2 million km^2 – 1.42 for coastal wetlands (Davidson & Finlayson, 2019) and 11.79-12.79 million km^2 for inland wetlands (Davidson & Finlayson, 2018). Considering their expansive distribution, wetlands exert a large influence on global cycles of nutrients (Martínez-Espinosa et al., 2021) and gases such as carbon dioxide and methane (Chmura et al., 2003; Mitsch et al., 2013).

Wetlands are highly valued for the numerous ecosystem services they provide, including flood protection, shoreline stabilization, carbon sequestration and storage, nutrient cycling, fish and wildlife protection, food and timber production, and cultural services such as ecotourism (Mitsch et al., 2015; Kingsford et al., 2016). Economic valuations of measurable ecosystem services estimate a potential worth of Int$ 47.4 trillion $year^{-1}$ based on the benchmark year 2011 (Davidson et al., 2019). However, wetlands are undergoing global decline in both areal extent (around 35% between 1970-2015; Darrah et al., 2019) and quality (Holland et al., 1995; Assessment, 2005). Wetland loss and degradation results from several anthropogenic and climatic changes. Land use conversion (e.g. urbanization or transformation into arable land) directly reduces the areal extent of wetlands (Mitsch & Hernandez, 2013), encourages habitat fragmentation, and increases vulnerability to eutrophication (Faulkner, 2004). Detrimental changes to one or more wetland functions can cause cascade effects for other ecosystem services, such as habitat provision for migratory birds (Donnelly et al., 2022). Furthermore, the resilience or response of wetlands to climate change such as sea level rise and elevated temperatures are still unclear due to complex ecosystem-climate feedback processes (Schuerch et al., 2018; Hu et al., 2024). Because of its potential in climate change mitigation, carbon sequestration and storage in wetlands (particularly coastal wetlands; Mcleod et al., 2011) has been given much attention (Duarte et al., 2013). Carbon sink potential in wetlands is a result of high productivity (carbon accumulation) and slow processes of microbial decomposition of organic matter (OM) (carbon storage) (Moomaw et al., 2018).

Wetland hydrology

Wetland hydrology is considered the principal diagnostic feature of wetlands (Gosselink & Turner, 1978) because it creates the conditions under which the characteristic substrate (hydric soils) and biota (hydrophytic vegetation) emerge and persist in the environment. In fact, some classification systems (Brinson, 1993; Semeniuk & Semeniuk, 1995) prioritize hydrological and geomorphic features in their categorization of wetland types and assessment of function. Hydric soils are anaerobic soils that form under frequent or constant inundation or waterlogging resulting in saturated soil pores and decreased redox potential (Faulkner et al., 1989; Pezeshki & DeLaune, 2012; Bhaduri et al., 2017). Soil redox potential indicates soil reduction (Pezeshki & DeLaune, 2012) and relates to the amount of dissolved oxygen in the soil (Boyd & Boyd, 2000). The low diffusivity of O_2 (10^4 times lower) in water (Armstrong et al., 1994) combined with oxygen consumption by wetland roots, microorganisms, and accumulated reductants rapidly depletes oxygen levels and shifts biological activity towards anaerobic metabolism. Because the mineralization of carbon is most favorable under oxic conditions, which yields the most Gibbs free energy gain (Canfield et al., 2005), hydric soils are a deterrent for OM decomposition.

The hydrological regimes of various wetland types differ in the frequency, permanence, and degree of inundation or saturation (Cowardin et al., 1979) and therefore soil properties as well (Trettin et al., 2020). Coastal wetlands like mangroves, seagrass beds, estuarine tidal marshes, and tidal flats (Davidson et al., 2019) share a hydrology with some degree of marine influence, i.e. local vertical gradient of tidal inundation and a horizontal salinity gradient with decreasing salt concentrations landward. Because seagrass beds are subtidal and therefore constantly submerged, they are not influenced by tidal cycles, but rather fluctuating salinity (Touchette, 2007). On the other hand, intertidal coastal wetlands like mangrove forests and estuarine tidal marshes experience regular flooding at their lowest elevations that decreases in frequency with increasing surface elevation. Inland wetlands, including rivers, swamps, bogs, fens, and lakes, feature a freshwater hydrology that is solely fed through precipitation and groundwater (Trettin et al., 2020) or even extreme weather events (Skrzypek et al., 2013). While the peatlands tend to have high water retention, stable water table levels in lower peat layers and relatively high resilience to seasonal change (Holden, 2005), lakes (especially shallow ones) are more susceptible to seasonally driven water level fluctuations (Gasith & Gafny, 1990; Leira & Cantonati, 2008).

Impact of water level fluctuations on vegetation

Water level fluctuations (WLFs) are a major component of wetland hydrology that is commonplace for many wetland types (e.g., estuarine tidal marshes and freshwater lakes). WLFs add to wetland hydrodynamics by creating alternating wetting-drying cycles, wave action, and rapid changes in the availability of light, nutrients, CO_2 and O_2 (LaRiviere et al., 2004). These additional processes exert a strong influence over wetland vegetation, including their productivity (Odum et al., 1995) and the distribution and diversity of plant traits (Wooten, 1986; Van Kleunen et al., 2007; Li et al., 2022).

In estuarine tidal marshes, the hydrological regime (tidal-flooding, salinity) interacts with biotic controls (i.e. competition) to create distinct plant zonation along an elevational gradient (Pennings & Callaway, 1992; Bockelmann et al., 2002; Engels & Jensen, 2009). For example, at the meso- to polyhaline zone of the Elbe Estuary, the most frequently inundated areas at lower elevations are occupied by emergent salt-tolerant species (*Spartina anglica, Salicornia europaea*) and are replaced at mid-level elevations by a wider diversity of plant species (e.g. *Atriplex portulacoides, Aster tripolium*). At the highest elevations, dominant plant species have a higher competitive ability but lower salt tolerance (*Elymus athericus, Festuca rubra*) (Engels & Jensen, 2010). At freshwater and oligohaline zones, plant species composition is largely similar between brackish and freshwater marshes with the lowest elevations dominated by rushes (e.g. *Bolboschoenus maritimus*) and mid- and higher elevations dominated largely by *Phragmites australis* (Engels & Jensen, 2009; Struyf et al., 2009).

In freshwater lakes, WLFs are primarily seasonal and are thus often related to excessive rainfall or dry periods (Coops et al., 2003). These fluctuations especially affect vegetation (both submerged and emergent species) in littoral zones and at shallow depths. At higher water levels, emergent plants are effectively drowned, and community composition shifts in favor of submerged species, while at low water levels the opposite trend is observed (Leira & Cantonati, 2008). For some phenotypically plastic species like *Littorella uniflora* that can persist under both conditions, WLFs are not a threat but rather an environmental catalyst for the sexual reproduction. In both wetland types, WLFs act as an environmental driver of observed trait variation either at the community level (tidal marshes) or phenotypic level (*Littorella uniflora*).

Wetland plant adaptations

Vegetation plays a key role in wetland carbon sequestration through the direct fixation of atmospheric carbon and input into the soil as litter, belowground biomass or rhizodeposition (e.g. exudates) (Figure 1.1). Wetlands are considered very productive ecosystems, in part due to positive effects that moderate WLFs have on primary production (allochthonous nutrient input and soil drainage) (Odum et al., 1995; Mendelssohn & Kuhn, 2003). High productivity in these systems is made possible by specific plant adaptations to wetland hydrology and soil.

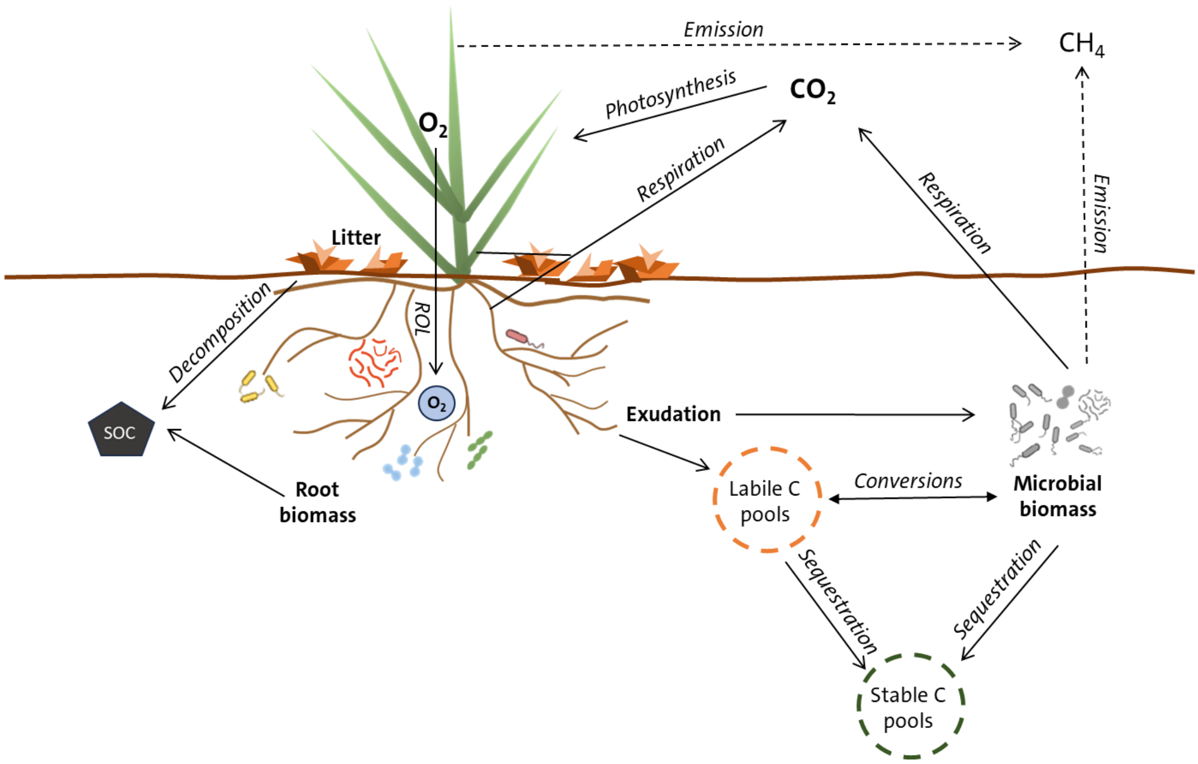

Figure 1.1. Graphical depiction of plant-mediated effects on carbon cycling in terrestrial environments (Jansson et al., 2021). Wetland plants fix atmospheric CO_2 and contribute OM to the system via plant litter, belowground biomass and other plant-derived products, e.g. exudates. These inputs of OM take diverse pathways that ultimately result in re-entry to the atmosphere (respiration, emission) or long-term stabilization into soil carbon pools. Stabilization processes are often mediated by microbially mediated conversion of SOM and are indirectly influenced by other plant traits such as radial oxygen loss.

In response to wave action, coastal wetland plants adapt their stem morphology and canopy height to increase flexibility and reduce turbulence in intertidal zones (Irish et al., 2008; Schoutens et al., 2021). Additionally, many wetland plants have high root, stem and leaf porosities that may be as great as 60% (Armstrong, 1980) to facilitate gas exchange. These high porosities are attributed to the formation of aerenchyma (Figure 1.2), internal plant tissues that contain large gas spaces that provide an internal ventilation system (Laan et al., 1989; Armstrong et al., 1994). Aerenchyma tissues transport oxygen belowground and reduce the volume of respiring tissue (Maricle & Lee, 2002) while simultaneously providing a pathway for outward diffusion of accumulated phytotoxins (Lee, 2003).

Figure 1.2. Example of aerenchyma tissues for gas transport in the rhizome of *Phragmites australis*. Photo: Wilson

Due to the inaccessibility and opacity of the soil, belowground plant adaptations are generally more challenging to elucidate and are commonly referred to as the 'hidden half' (Atkinson et al., 2019). Roots provide anchorage for the plant and are responsible for plant uptake of water and nutrients from the soil, the latter function primarily achieved by absorptive fine roots (< 2mm in diameter) (McCormack et al., 2015).

Functional root traits in the rhizosphere

The rhizosphere is a unique area of belowground compartment where intense interactions between plants, substrate and soil microorganisms occur. Therefore, the rhizosphere is significant in understanding plant controls on OM decomposition. The term 'rhizosphere' was initially defined by Hiltner (1904) as the 'volume of soil influenced by roots' and inhabited by bacteria whose activity and composition of bacteria were distinct from root-free soil, i.e. bulk soil (Hartmann et al., 2008). The modern conceptualization of the rhizosphere has since advanced beyond its earliest description to reflect higher spatial, temporal and functional complexity (York et al., 2016; Kuzyakov & Razavi, 2019). Generally, the rhizosphere can be described as four-dimensional space extending a number of millimeters from the root surface depending on the target gradient (Kuzyakov & Razavi, 2019). Physicochemical gradients in the rhizosphere structure the assembly of rhizosphere microbiota along the vertical root axis (Bonkowski et al., 2021). These gradients include water, pH, nutrients, O_2 and carbon (Marschner & Römheld, 1983; Farrar et al., 2003; Houmani et al., 2015).

Plant roots deposit a number of organic compounds, secondary metabolites, volatiles, and other secretions by decaying or intact root cells, collectively referred to as rhizodeposition, into the surrounding soil matrix (Dennis et al., 2010). Some studies have even reported on the importance of the detritusphere, i.e. the legacy effect of dead roots, in structuring rhizosphere soil (Mueller et al., 2024 and references therein; Witzgall et al., 2024). High molecular weight rhizodeposits, like mucilage, can directly alter physicochemical soil properties such as the soil water potential (Carminati et al., 2010) and aggregate formation and stability (Habib et al., 1990; Xiao et al., 2023), while low molecular weight exudates indirectly stimulate microbially-mediated macroaggregate formation (Baumert et al., 2018) and turnover of microaggregates, i.e. mineral-associated OM (Naveed et al., 2017; Li et al., 2021; Chari et al., 2024). The direction of these rhizodeposition effects on soil aggregation – either stabilizing or destabilizing – is explained by differences in adsorption capacity, recalcitrance, degree of physical protection, and rate of microbial assimilation or transformation of soil organic matter (SOM) (Cotrufo et al., 2013; Mueller et al., 2024).

Many studies have shown that microorganisms in the vicinity of the root are able to utilize the various forms of rhizodeposition released from living and decaying plant roots (Barber & Lynch, 1977; Lynch & Whipps, 1990; Niedeggen et al., 2024). Microorganisms use these products for growth and to fuel their metabolism. Root exudation from the plant can alter the bacterial community or be used to recruit species-specific consortia of microorganisms in the rhizosphere. A recent meta-analysis estimates that root exudates account for approximately 9% of the total global carbon flux (Chari et al., 2024). Roots also form association with other microorganisms in the soil that can be both beneficial or antagonistic and are determined partly by the plant genotype (Zogg et al., 2018). As such, the degradation of SOM in the rhizosphere is a result of the interaction between the assembled microbiota with specific plant factors like plant type (Shann & Boyle, 1994), photosynthetic metabolism (Sivaram et al., 2020), and root morphology and developmental stage (Chaparro et al., 2014; Ajilogba et al., 2022).

Due to barrier formation (e.g. suberization, lignification) to radial oxygen loss (ROL), oxygen release from roots is primarily restricted to the root tip and narrow subapical regions (Armstrong, 1967). The translocated oxygen is then available for root growth and exploration into otherwise anaerobic soil. Interspecific differences have been shown for several wetland plant species in regard to their strategy for soil aeration (Sorrell et al., 2000) and the rate of oxygen release is heavily influenced by the substrate type (Koop-Jakobsen et al., 2017). Plants can directly influence soil redox potential by input of oxygen into the sediment via ROL. As described above, wetland soils are characteristically depleted of oxygen due to a combination of diffusive resistance and high respiratory demand. In the absence of oxygen, alternative electron acceptors are then used for the oxidation of SOM (i.e. electron donors) in the order of their redox potential: nitrate (NO_3^-), manganese (MnO_2), iron (Fe^{3+}), sulfate (SO_4^{2-}) and finally carbon dioxide (CO_2) (Pezeshki & DeLaune, 2012; Marschner, 2021). Studies have shown that root-derived oxygen influx into the soil not only raises the redox potential but stimulates the degradation of SOM in the rhizosphere with benefits of enhanced nutrient uptake for the plant (Wolf et al., 2007; Bernal et al., 2017; Noyce et al., 2023). Like root exudates, oxygen is considered in this thesis as a mechanism by which OM degradation can be stimulated (rhizosphere priming effect; Kuzyakov & Domanski, 2000).

Trait-based ecology

A large body of scholarship exists for the description of aboveground plant traits in wetlands such as vegetation structure, productivity, photosynthetic activity, life forms, germination and flowering ecology (Boutin & Keddy, 1993). Plant traits are defined as morphological, physiological or phenological characteristics of the plant measurable at the individual level (Violle et al., 2007). Plant traits which interact with their environment and influence plant fitness are referred to as functional traits and used to make predictions about ecosystem functions and possible tradeoffs that can be expected with environmental change (Da et al., 2023). For example, aboveground traits such as specific leaf area (SLA) and leaf nitrogen content were used to identify resource-use strategies at the community level, from which a leaf economic spectrum (Wright et al., 2004) emerged. According to this spectrum, fast-growing plants (high SLA, high leaf N) using an acquisitive strategy as opposed to slow-growing plants (low SLA, low leaf N) using a conservative approach. However, a growing body of scholarship is bringing attention to belowground traits and the development of a root economics (multidimensional) space (Prieto et al., 2015; Weemstra et al., 2016; Laliberté, 2017; Yaffar et al., 2022). Root traits do not necessarily correlate to aboveground traits (Bergmann et al., 2017; Carmona et al., 2021), but have been shown to be just as variable (Dawson et al., 2024) and useful at predicting trends in ecosystem functioning, e.g. productivity, decomposition and carbon stocks (Bardgett et al., 2014; La Bella et al., 2024). The lack of coordination between aboveground and belowground traits might suggest different adaptive strategies for distinct plant organs or simply dissimilar function. Therefore, root traits should be measured directly to decipher their relative importance for specific functions (Freschet et al., 2021). Although databases of root traits (e.g. GRooT, FRED) exist, physiological traits (e.g. root exudation) are largely missing (Iversen et al., 2017; Guerrero-Ramírez et al., 2021). Considering that organic carbon is stored primarily in wetland soils and decomposition processes of OM are driven by soil microorganisms, the inclusion of functional root traits directly influencing physicochemical soil properties and soil microbial communities is essential to our understanding of plant influence on carbon cycling.

Project framework

This study is situated within a larger collaborative research training group (RTG 2530) funded by the German Research Foundation (*Deutsche Forschungsgemeinschaft*; DFG). The common research aim of RTG 2530 is to fill existing knowledge gaps related to biota-mediated effects on carbon cycling in estuarine ecosystems. To this aim the associated research features an interdisciplinary approach to unravel biota-mediated processes on various scales and across terrestrial—aquatic boundaries.

Objectives of the thesis

The objective of this thesis was to investigate the degree to which variation in physiological root traits involved in biogeochemical cycling (O_2 release, CO_2 uptake, exudation) drives interactions between the plant, soil and soil microorganisms. The individual chapters were designed to address multiple aspects of root-mediated ecosystems functioning in wetland systems. Chapter 2 challenges functional generalizations of how wetland vegetation control soil redox conditions.

Box B aims to determine whether the differences in radial oxygen loss are species-specific using individuals of different plant species in a rhizobox experiment. Chapter 3 continues by examining functional trait diversity in different phenotypes of the same species. Finally, Chapter 4 explores the impact of root-mediated soil modifications on biological soil partners in the rhizosphere, their community structure and functional activity. Altogether the findings from these chapters provide additional insight into biota-mediated processes on the ecosystem scale.

2 Root-Driven Soil Reduction in Wadden Sea Salt Marshes

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Abstract

The soil redox potential in wetlands such as peatlands or salt marshes exerts a strong control over microbial decomposition processes and consequently soil carbon cycling. Wetland plants can influence redox by supplying both terminal electron acceptors (i.e. oxygen) and electron donors (i.e. organic matter) to the soil system. However, quantitative insight into the importance of plant effects on wetland soil redox and associated plant traits are scarce. In a combined mesocosm and field study we investigated the impact of plants on soil reduction using IRIS (Indicator of Reduction in Soils) sticks. Vegetated plots were compared to non-vegetated plots along an elevational gradient in a salt marsh of the Wadden Sea and along an artificially created gradient in a tidal tank mesocosm experiment. Our findings from the mesocosm experiment demonstrated that vegetation both enhanced and suppressed soil reduction relative to non-vegetated control pots. The direction of the plant effect (i.e., net oxidizing or net reducing) was inversely correlated with background redox conditions. Insights from high resolution oxygen profiling via planar optode imaging corroborated these findings. In the field study, vegetation consistently reduced the comparatively well-aerated Wadden Sea salt marsh soil. Reduction correlated positively with soil organic matter content and belowground biomass, indicating that greater availability of plant-derived electron donors, in the form of organic matter, increased soil reduction. Challenging the dominant paradigm that wetland plants primarily act as soil oxidizers, our study reveals their potential to exert a net reducing effect. The documented impact of these plant-induced changes in soil redox conditions suggests a previously overlooked role in shaping the stability of soil organic carbon stocks in wetland ecosystems with variable water tables.

Introduction

Wetlands, including coastal blue carbon ecosystems and peatlands are remarkably efficient carbon sinks that store about 20 % of the global ecosystem organic carbon while covering only 1 % of the land surface (Temmink et al., 2022). Extreme rates of carbon sequestration in wetlands are caused by a large imbalance between plant primary production and microbial decomposition (Kirwan & Megonigal, 2013; Temmink et al., 2022). The availability of electron acceptors and donors control microbial decomposition and is reflected in the soil redox potential. As wetland soils characteristically lack oxygen, the most powerful electron acceptor, microbial decomposition of organic matter is inhibited through energetic constraints (Keiluweit et al., 2016) and exo-enzyme suppression driven by the accumulation of phenols (Freeman et al., 2001; Freeman et al., 2004).

Wetland plants act as sources of both electron acceptors (e.g. oxygen) and electron donors (i.e. organic matter, such as root exudates and litter) to the soil, thereby controlling the soil redox potential (Sutton-Grier et al., 2011). Wetland plants increase the availability of electron acceptors via root or radial oxygen loss (ROL), diffusive oxygen release into the rhizosphere. This process supports microbial aerobic respiration of soil organic matter (SOM) in an otherwise anoxic soil environment (Wolf et al., 2007; Mueller et al., 2016). ROL allows plants to maintain nutrient uptake (Bradley & Morris, 1990; Mendelssohn & Morris, 2000; Lai et al., 2012) and protects plant roots from reduced phytotoxins often present in wetland soils (Pezeshki, 2001). In several wetland plants, ROL increases at low redox potentials (Kludze & DeLaune, 1994). The amount of oxygen released by wetland plants can also differ strongly between species. For instance, planar optode measurements showed that in the flooding-adapted salt-marsh grass *Spartina anglica,* only a small amount of oxygen is leaking from a restricted area at the root tip into the soil (Koop-Jakobsen & Wenzhöfer, 2015; Koop-Jakobsen et al., 2018), while the less flooding-adapted salt-marsh grass *Elymus athericus* shows ROL along the entire root-surface under anoxic soil conditions (Koop-Jakobsen et al., 2021).

Organic matter input to soils enhances electron donor availability (Gardiner & James, 2012), a prerequisite for soils to turn anoxic (Jenkinson, 2002). Organic matter input by both root exudation and litter input (Megonigal et al., 2004) fuels microbial respiration and therefore increases soil reduction (Gardiner & James, 2012; Keiluweit et al., 2017). On a conceptual level, the net effect of plants on soil redox should depend on the balance between oxidation through ROL and reduction through organic matter input to the soil system (Mueller et al., 2020). However, quantitative insight into the importance of plant effects on wetland soil redox and associated plant traits are scarce.

This study aims to develop a deeper quantitative and mechanistic understanding of plant effects on wetland soil redox. Our study system was a salt marsh on the European Wadden Sea coast. In recent years, salt marshes and other coastal blue carbon ecosystems have been studied intensively due to their high carbon sink potential (Chmura et al., 2003; Mcleod et al., 2011). In salt marshes, soil redox potentials are predominantly hydrologically driven. Daily inundations lead to periods of water saturation, that are accompanied by a limited oxygen availability, which is reflected in a lower redox potential (Mansfeldt, 2003). These redox dynamics vary along an elevation gradient, representing a flooding-frequency gradient, and in relation to soil depth (Rich et al., 2023; Tang et al., 2023). Wadden Sea salt marshes typically show a distinct vegetation zonation along the elevation gradient. The pioneer zone is flooded twice a day, the low marsh during spring-tides occurring twice per months, and the high marsh only during storm tides, usually less than ten times a year (Butzeck et al., 2015; Jensen et al., 2018). Soil redox can be expected to be lowest either in the pioneer zone, due to frequent flooding, or in the low marsh, due to combined effects of waterlogging and greater organic matter (i.e. electron donor) availability (Mueller et al., 2020). To assess the interaction of plant processes and hydrology on soil redox dynamics, we conducted mesocosm and field experiments including vegetated and non-vegetated treatments along flooding-frequency gradients.

We hypothesized that plant effects on soil redox conditions are controlled by the hydrology-driven background redox conditions (i.e. the redox conditions outside the rhizosphere). With increasing background soil reduction, the net plant effect on the soil redox becomes increasingly positive, i.e., oxidizing. Conversely, with decreasing background reduction, the net plant effect becomes increasingly negative, i.e., reducing. Specifically, we hypothesize that plants act as net reducers in the rarely flooded and well-drained zones and as net oxidizers in frequently flooded and waterlogged zones of the salt marsh.

Material & Methods

Our study combined a full-factorial mesocosm experiment with a field study conducted in a Wadden Sea salt marsh. We used IRIS (Indicator of Reduction in Soils) sticks to quantify soil reduction in both the mesocosm and the field study. To support findings on differences in soil reduction derived from IRIS sticks, we conducted planar optode measurements on an exemplary set of salt-marsh plants.

Full-factorial mesocosm experiment

A mesocosm experiment was set up to analyze plant effects on soil redox under controlled hydrological conditions (Fig. 1). The mesocosm experiment was conducted using a tidal tank facility at the Institute for Plant Science and Microbiology, Universität Hamburg, Germany. Tidal tanks had a volume of 6750 L (300 cm x 150 cm x 150 cm) and were connected to a storage tank (9000 L). An automatic pump was used to move water between storage and tidal tanks. Different flooding frequencies, representing salt marsh-zone-specific inundation regimes, were realized by placing mesocosms at different elevations of a staircase situated inside the tidal tank (Fig. 2.1). The lowest level, representing the pioneer zone was flooded twice a day for two hours. The second level was flooded once a week, and the highest level was flooded twice a month, simulating low- and high-marsh. Salinity was set and held at 20 psu using synthetic sea salt (Aqua Medic GmbH). To create a full-factorial design, each planting treatment (see below) was realized for each flooding treatment in five replicates. During the experiment, mesocosms were moved randomly within each flooding treatment to exclude position effects.

Plant individuals of dominant species from each zone (*Spartina anglica, Atriplex portulacoides, Elymus athericus*) were collected in June 2021. Plants were thoroughly rinsed to remove soil and weighed. The plants were then potted individually into opaque plastic pots (d = 16 cm, h = 21 cm). Experimental soil was a mix part of salt-marsh soil from the Hamburger Hallig site (20 % of the total soil volume), sand (40 %), and standard potting soil (40 %). Unplanted pots containing only mixed soil were also included as a control planting treatment. Five replicates of each planting treatment were established for each flooding treatment for a total of 60 samples (3 zones x 4 planting treatments x 5 replicates).

Field experiment

The field experiment was conducted in a salt marsh on the Wadden Sea coast of Germany, at Hamburger Hallig (54°36'06.2"N, 8°49'00.1"E). Three salt-marsh zones (pioneer zone, low marsh, high marsh) were identified based on the dominant vegetation. Three plots were created within each zone, consisting of a non-vegetated and a vegetated subplot (Fig. 2.1). To create non-vegetated subplots, soil was removed down to a depth of 20 cm in a 20 cm x 20 cm area using a shovel, cleared of roots or rhizomes, and placed back. Plastic pots (diameter = 20 cm, height = 20 cm) were inserted into these non-vegetated areas to function as a root-barrier. Each pot had two holes (d = 9.5 cm) in the lower third, which were covered with a fine mesh, to minimize effects on hydrology. Three tidal flat positions were included as references in the experimental design. Due to the lack of vegetation in tidal flats, these plots were only included for some analysis (i.e. OM and pH).

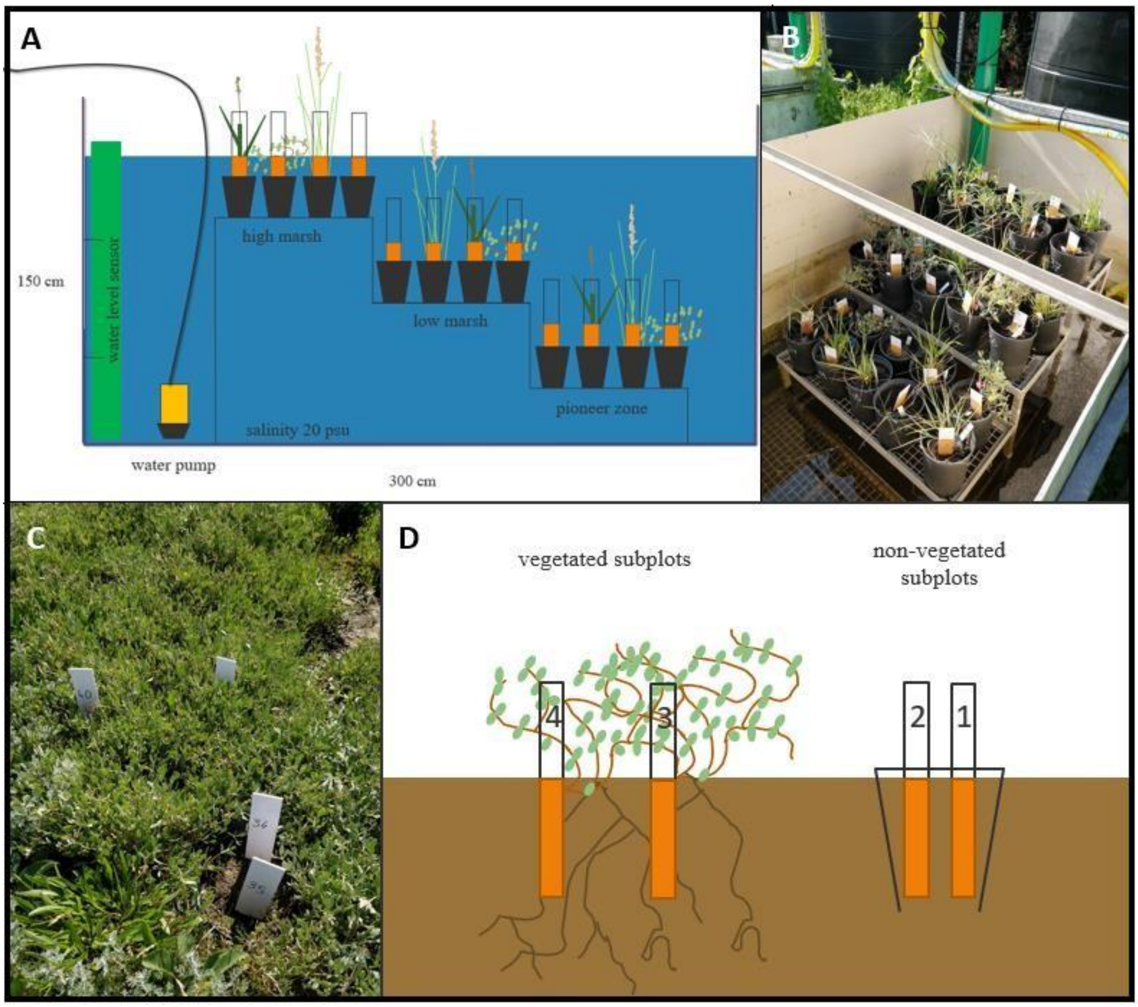

Figure 2.1. Overview of both study set-ups (mesocosm and field experiment). [A, B] The tidal tank experiment. At each of the three flooding levels, four planting treatments were realized: *Elymus athericus* from the high marsh; *Atriplex portulacoides* from the low marsh; *Spartina anglica* from the pioneer zone; non-vegetated control (n = 5 mesocosms per planting treatment). Flooding treatments were set to values that simulate the flooding-frequency of Wadden Sea salt marshes along elevation gradients, i.e., pioneer zone, low marsh, high marsh. [C, D] The complementary field experiment at the Hamburger Hallig (54°36'06.2"N, 8°49'00.1"E). At each of the three elevational zones (pioneer zone, low marsh, high marsh) three non-vegetated subplots were created, allowing for a plant vs. non-vegetated comparison as in the tidal tank experiment.

Soil properties and reduction index

Indicator of Reduction in Soil (IRIS) has emerged as a practicable approach to get an insight into the redox dynamics of hydric soils (Jenkinson, 2002; Castenson & Rabenhorst, 2006). The IRIS method, following (Rabenhorst & Burch, 2006; Rabenhorst, 2013) utilizes a Fe oxide paint which is applied to PVC tubes. To prepare the Fe oxide paint, anhydrous $FeCl_3$ is dissolved in distilled water, and 1 M KOH is added in a second step. The reddish-brownish precipitate is centrifuged with distilled water, to remove the ions from the Fe oxide cake. The PVC-sticks were sanded using an orbital grinder with fine sanding paper (grid 180). Afterwards the Fe oxide paint was applied to the PVC-sticks (300 mm x 50 mm x 3 mm) in circular movements using a sponge (For more details see Supplement 2.1). The capacity of the Fe oxide paint to be reduced from insoluble Fe (III) to soluble Fe (II) under anoxic conditions and in presence of microbial Fe-reducers allows for the determination of soil reduction (Rabenhorst, 2013). The area of the removed paint functions as a proxy for soil-redox conditions (reduction index), integrated over a 4-week period. Higher amounts of paint removal indicate higher soil reduction. It has been shown that IRIS correlates well with soil redox potentials (Mueller et al., 2020). Compared to redox potential measurements, IRIS sticks are less susceptible to the high variability of soil redox (Bansal et al., 2023). In this study, the method was modified according to Mueller et al. (2020) by using flat PVC sticks instead of tubes to simplify the scanning process. Upon removal, sticks were cleaned carefully with cold tab water to remove soil particles. Each stick was scanned to create digital images for further processing. Using a snipping tool (ShareX), which allows pixel-wise cutting, the individual IRIS images were cut to a standardized size of 115 x 700 pixels. Image analysis was conducted applying a supervised classification on randomly chosen sticks from different field campaigns. Classification was done using the software ArcGIS Pro. In total 4300 points were classified as either reduced, not reduced or errors (background, scanning effects). RGB colour values (0-255) of classified point were retrieved using the *Extract Multi values to Points* function. The classification was included in a Random Forest model (confusion matrix 1.5 %) using the software R. This model allowed for a pixel-wise classification of the scanned IRIS sticks (Fig. 2.2). Sticks were analysed in increments of 5 cm, covering a depth gradient from 0 to 30 cm. Reduction index was calculated as an unitless value ranging from 0 to 1 based on the share of reduced pixel from the total pixels.

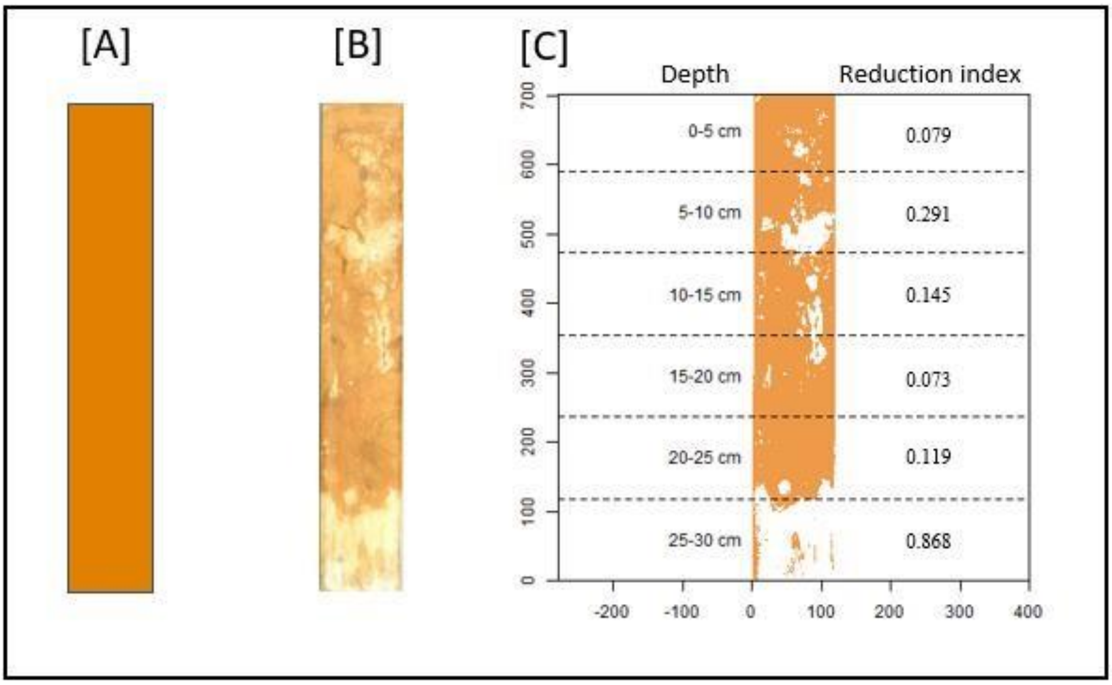

Figure 2.2. Images of an IRIS stick [A] schematic prior to installation with Fe (III) coating. [B] Post installation in the field after 4 weeks. [C] Predicted images after analyzation. Predicted images show not reduced areas (orange), reduced areas (white), errors during scanning are either green or black. Classes were defined based on RGB color values of 4300 randomly set points on test sticks. The reduction index is calculated per depth increment.

In the tidal tank experiment, there was one stick in each mesocosm (N = 60). Sticks were removed after six weeks and replaced with new sticks for a total of two consecutive IRIS campaigns. The first campaign spanned from beginning of August until mid-September 2021, the second campaign spanned from mid-September until end of October 2021.

In the field study, IRIS sticks were collected and replaced at the field site every four weeks beginning in June 2021. Before inserting the sticks into the soil, a pilot hole of 30 cm depth was created using a machete. This step prevents the sticks from scratches and paint removal not attributable to reduction processes. Each plot (N = 18) had two IRIS sticks in vegetated and non-vegetated subplots, resulting in 36 IRIS sticks per 4-week cycle. A total of four campaigns were conducted from June until October 2021.

The organic matter (OM) content of soils, as important electron donor source, was determined via loss on ignition following standard protocols (Dean, 1974; Heiri et al., 2001). Soil cores (volume = 100 cm^3) from every field plot (including the tidal-flat) were taken in October 2023. Samples were dried at 105 °C in ceramic crucibles. After the samples cooled to room temperature inside a desiccator, they were weighed on a fine scale. Samples and crucibles were then placed in a muffle furnace operating at 550 °C. Samples were removed after 2.5 h and placed in a desiccator for cooling. Samples were weighed again to calculate OM contents based on the weight loss following ignition.

Soil pH, as integral part of the soil redox chemistry, was determined for soil samples from both mesocosm experiment pots (N = 60) and field study plots (N = 9). Soil samples were taken at the end of the experiments, in October 2021 and November 2021, respectively. 5 g of air-dried soil was incubated in a 0.01 M solution of $CaCl_2$ for one hour, and pH was measured using a pH-meter (Multi 9310 IDS).

Plant biomass

Plant biomass was harvested in the field experiment in early October 2021. Aboveground and belowground biomass was quantified as potentially important plant traits associated with plant-mediated changes in soil redox. Aboveground biomass was collected in a 20 cm x 20 cm square. Squares were determined around the IRIS stick in each plot. Belowground biomass was collected to a soil depth of 10 cm using a PVC ring (A = 314.15 cm^2). Aboveground biomass was cleaned from remaining soil using tap water and dried for 48 hours at 50 °C in a drying oven. Belowground biomass was cleaned from remaining soil using tap water and a fine sieve (2 mm) and dried at 50 °C for 48 hours. Aboveground biomass and belowground biomass were weighed using a fine scale (FH-2000 Gram).

Planar optodes

We conducted planar optode measurements to analyze plant effects on soil redox at the single-root scale. Planar optode measurements were conducted on an exemplary set of roots (2-3) from individuals of *Spartina anglica* (pioneer zone) and *Atriplex portulacoides* (low marsh). The set-up for planar optode investigations followed standard protocols (Wang et al., 2010; Koop-Jakobsen et al., 2018). The planar optode set-up consisted of a VisiSens TD imaging system (combined camera and excitation light source) facing a rhizobox containing soils and the sample roots. The rhizobox was situated in an aquarium filled with non-sterile water covering the sediment and continuously illuminated by a LED growth lamp. A custom Arduino-controlled, relay switch circuit system ensured regular dark intervals during photography. Roots and root-free soil compartments (i.e. bulk soil), were covered with a luminescent, 10 x 10 cm O_2-sensitive foil. The

VisiSens™ ScientifiCalsoftware detects and evaluates oxygen signals on the sensor foils captured for each frame over a given time series.

Roots were carefully arranged on a freshly re-wetted sand substrate inoculated with soil from the field site. Oxygen concentration of the bulk soil decreased over time (0-24 h) from fully oxic to anoxic driven by increasing microbial respiration upon re-wetting and reduced oxygen diffusion under waterlogged conditions of the rhizobox. Changes in oxygen concentration over time were compared between root vicinity and bulk soil and plotted over 24 hours upon re-wetting.

Statistical analyses

A mixed-model ANOVA was conducted to test for differences in reduction index between planting treatments (non-vegetated, *Spartina anglica*, *Atriplex portulacoides*, *Elymus athericus*), flooding treatments (pioneer zone, low marsh, high marsh) and their possible interaction effect within the tidal tank mesocosm experiment. There were no differences between treatments in the upper 15 cm of the sticks (Fig. S2.1). Therefore, only the bottom 5 cm section of each stick was considered in the statistical analysis (Fig. 2.3). A similar ANOVA was used to test for effects of vegetation presence, marsh zone, and soil depth on the reduction index in the field experiment. A random factor (stick ID) was included to account for statistical dependency of depth segments. Equal sample size was assured and allowed for robust parametric testing using factorial ANOVAs despite moderate deviations from homogeneity of variance (McGuinness, 2002). Normality of residuals was checked visually for each ANOVA. Tukey´s HSD tests were used for pairwise comparison following significant ANOVA tests. Linear regressions were used to test for relationships between reduction index and different environmental parameters measured in the field study (i.e. above- and belowground biomass, organic matter content, soil pH). All statistical analyses were conducted with the software R Studio version 4.4.1 (R Core Posit Team, 2024). Data were modified using psych (Revelle, 2024), dplyr (Wickham et al., 2023) and rstatix (Kassambara, 2023b) R packages. All plots were created using ggplot2 (Wickham & Wickham, 2016), ggpubr (Kassambara, 2023a), ggpmisc (Aphalo et al., 2024) and gridExtra (Auguie & Antonov, 2017) R packages. Mixed model ANOVA was conducted using R package afex (Singmann et al., 2024). Tukey´s HSD post hoc test were conducted using the emmeans (Lenth et al., 2024) R package.

Results

Mesocosm experiment

Soil reduction differed significantly by flooding treatment ($F = 6.45$, $p < 0.01$). The high marsh flooding treatment led to significantly less reduction than the pioneer zone and low marsh flooding treatments (Fig. 2.3a). In planted treatments, reduction was greatest in the pioneer zone. By contrast, background reduction in non-vegetated controls was greatest in the low marsh treatment (Fig. 2.3b). While there was no significant main effect of the planting treatment ($F = 0.75$, $p = 0.53$), reduction was significantly affected by the interaction of flooding and planting treatment ($F = 2.33$, $p < 0.05$). Planting treatment had an effect on soil reduction, but not across all flooding treatments. Magnitude and direction of plant effects differed between flooding treatments (Fig. 3c): the change in reduction index in relation to the non-vegetated control was positive in pioneer zone, negative in low marsh and both positive and negative in the high marsh flooding treatment depending on the plant species (Fig. 3c). pH was 7.42 ± 0.23 and did not differ across planting and flooding treatment combinations (Fig. S2).

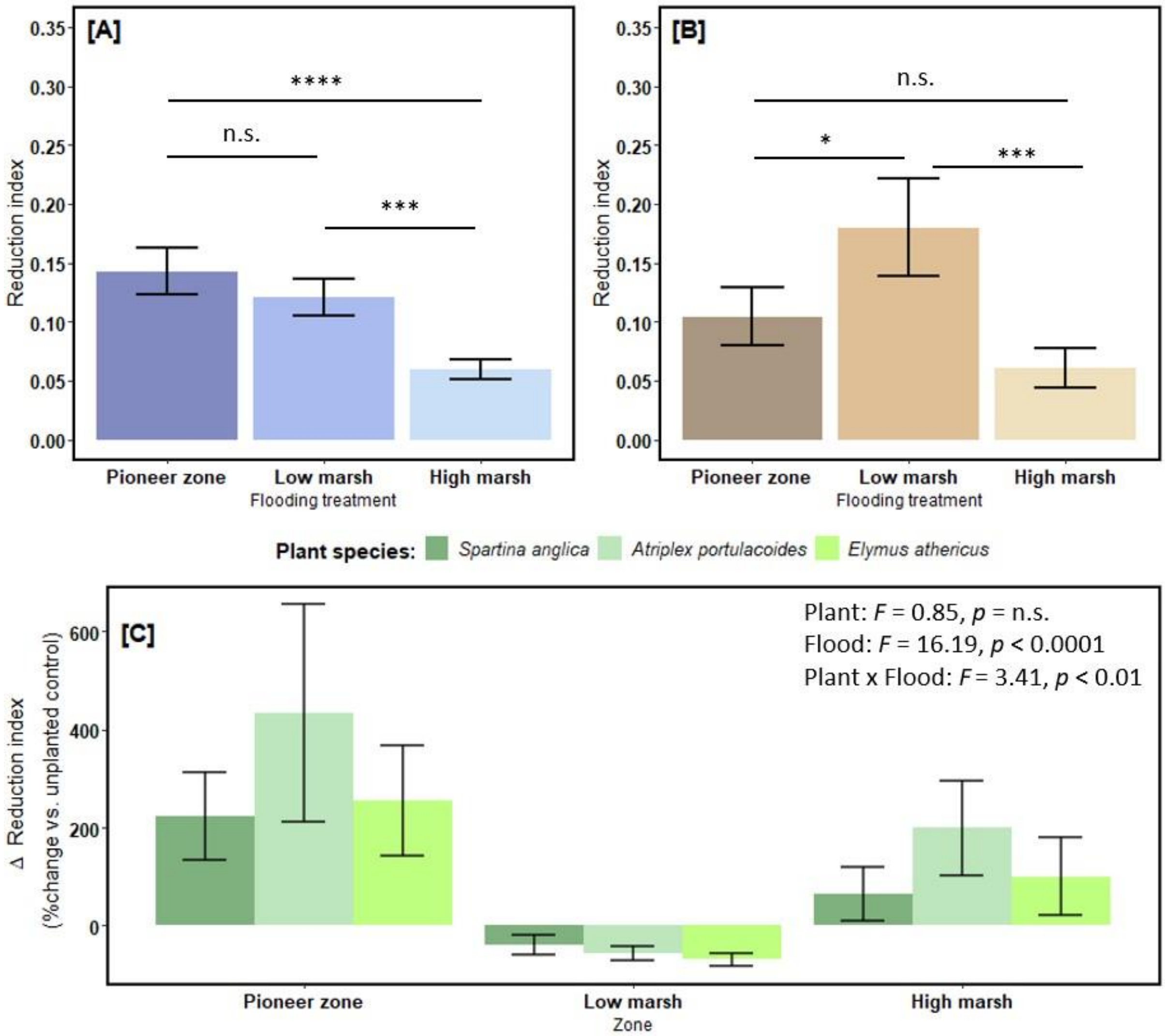

Figure 2.3: Reduction index, i.e. the fraction of reduced Fe paint from IRIS campaigns in the tidal tank experiment across the flooding frequency gradient (pioneer zone, low marsh, high marsh). [A] With all planting treatments across the flooding frequency gradient. [B] Only non-vegetated control planting treatments across the flooding frequency gradient. [C] Δ Reduction index as % change vs. the non-vegetated control, for all plant species (*Spartina anglica, Atriplex portulacoides, Elymus athericus*) across the three flooding treatments (pioneer zone, low marsh, high marsh). Bar plots show mean ± se values. Main effects of mixed model ANOVA are presented in the subpanel [C]. Significant differences based on Tukey pairwise comparisons between all vegetated and all non-vegetated subplot are indicated as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Planar optode results

Both *Spartina anglica* and *Atriplex portulacoides* roots initially showed lower oxygen concentrations compared to the bulk soil (Fig. 4). After 17 hours and 21 hours, respectively, two of the three *Spartina anglica* roots had a higher oxygen concentration compared to the bulk soil (Fig. 2.4a inlet). For *Atriplex portulacoides*, oxygen concentration remained lower in direct root vicinity compared to bulk soil throughout the entire experiment (Fig. 2.4b).

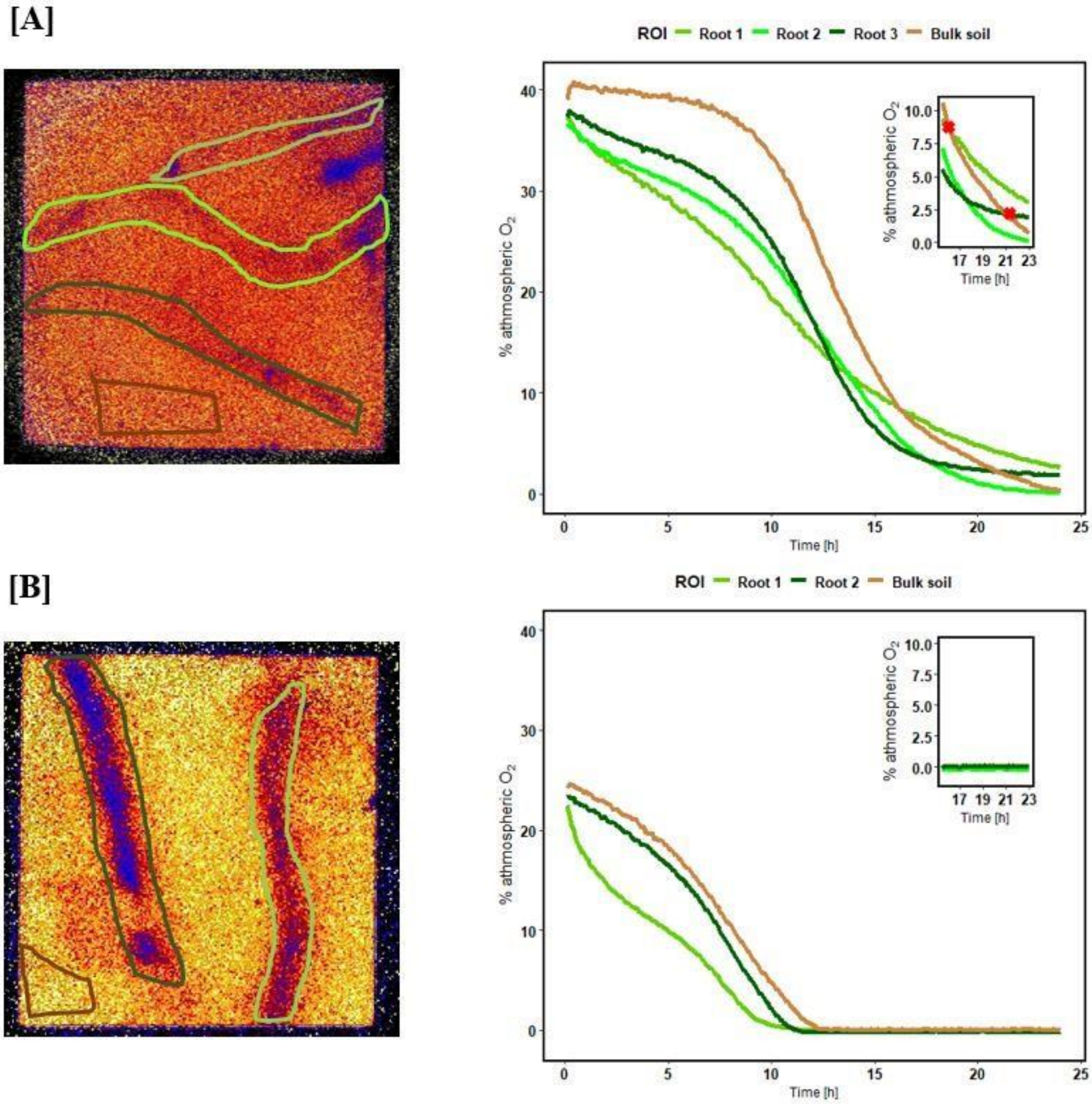

Figure 2.4: Planar optode images of the soil oxygen concentration around roots from [A] *Spartina anglica* and [B] *Atriplex portulacoides*. Different regions of interest (ROI) were selected for roots (green) and a root-free bulk soil control (brown) and tracked over 24 hours. Inlets show timeframe where some roots showed higher oxygen concentrations than bulk soil, crossing points are indicated by red x marks.

Field experiment

Soil reduction differed significantly between zones along the flooding-frequency gradient (F = 73.95, p < 0.001). Reduction across all IRIS sticks was higher in the pioneer zone (0.204 ± 0.011 mean ± se) than in the low marsh (0.046 ± 0.003) and high marsh (0.034 ± 0.004). Zones affected reduction similar for both subplots (vegetated, non-vegetated) and only control subplots (non-vegetated). Reduction increased significantly with soil depth (F = 12.46, p < 0.0001) and was maximized at the deepest section assessed, i.e. 25-30 cm below soil surface. Here, the highest reduction index was 0.342 ± 0.028 in the pioneer zone, 0.088 ± 0.01 in the low marsh and 0.123 ± 0.023 in the high marsh. The soil-depth effect on reduction differed between zones as indicated by the significant zone x depth interaction term of the ANOVA ($F = 3.68$, $p < 0.001$). While soil depth led to a linear increase in reduction in the pioneer zone there were less consistent depth trends in both low marsh and high marsh (Fig. S2.3). Soil reduction was significantly higher in vegetated subplots compared to non-vegetated controls ($F = 4.34$, $p < 0.05$). In both pioneer zone and low marsh, vegetated subplots were significantly more reduced than non-vegetated subplots (Fig. 2.5c). In the pioneer zone, this effect was detected from 0 to 10 cm and in the low marsh from 0 to 25 cm depth (Fig. 2.5c). In the high marsh, this effect was present from 20 to 25 cm depth (Fig. 2.5c).

pH in vegetated subplots was lower compared to non-vegetated subplots across all zones (Fig. S2.2). pH was 7.31 ± 0.15 in vegetated subplots and 7.69 ± 0.48 in the non-vegetated subplots (p = 0.05).

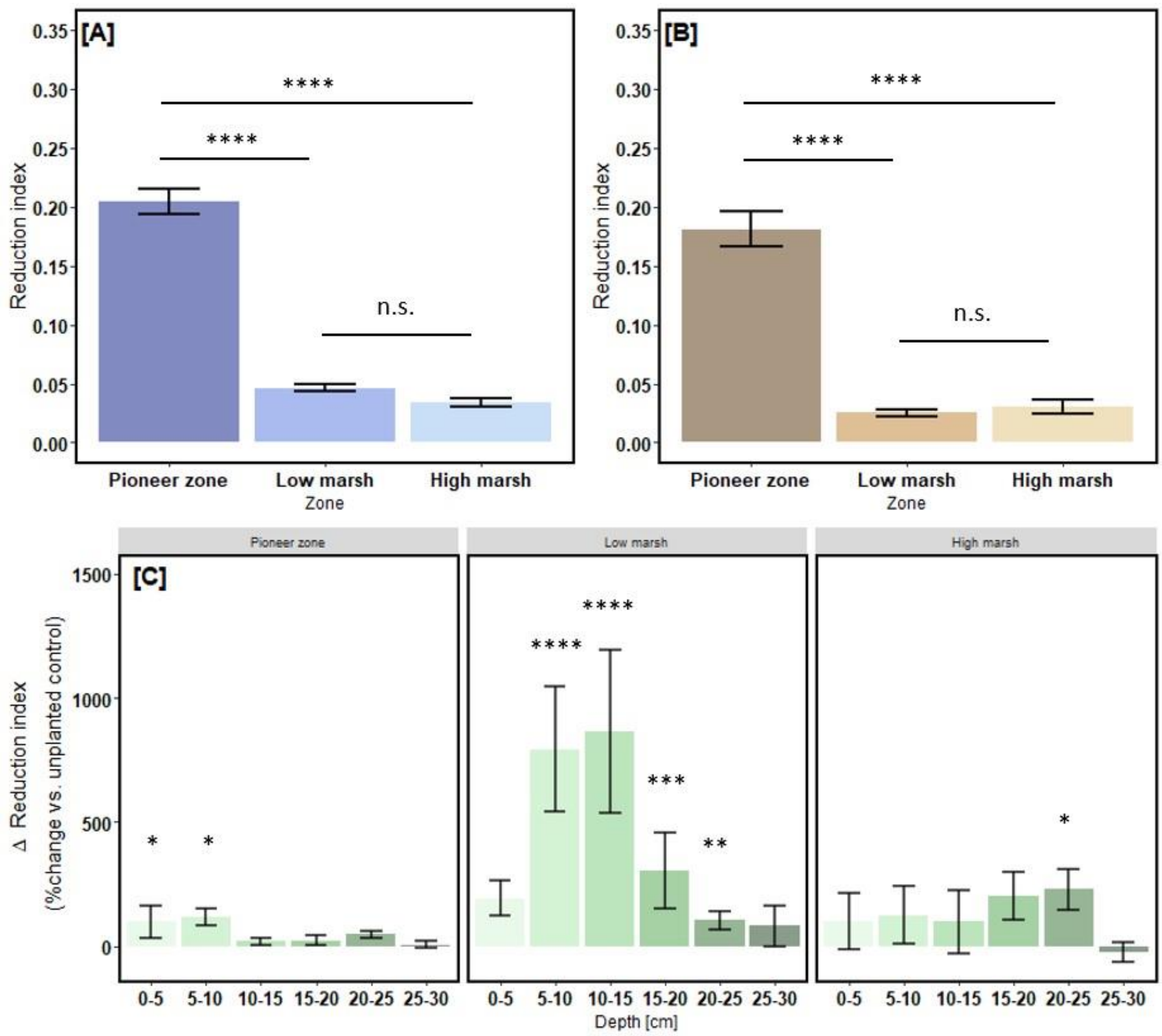

Figure 2.5: Reduction index, i.e. the fraction of reduced Fe paint from IRIS campaigns in the field experiment across the flooding frequency gradient (pioneer zone, low marsh, high marsh). [A] With all planting treatments across the flooding frequency gradient. [B] Only non-vegetated subplots across the flooding frequency gradient. [C] Δ Reduction index as % change vs. the non-vegetated control, for all depths (0-30 cm) across the flooding frequency gradient

(pioneer zone, low marsh, high marsh). Bar plots show mean ± se values. Significant differences based on Tukey pairwise comparisons between vegetated and non-vegetated subplot are indicated as follows * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Field experiment belowground biomass ($p = 0.001$; $R^2 = 0.8$) and soil organic matter content ($p=0.026$; $R^2 = 0.21$) had positive effects on soil reduction (Fig. 2.6). There were no effects by aboveground biomass and soil pH (Fig. 2.6).

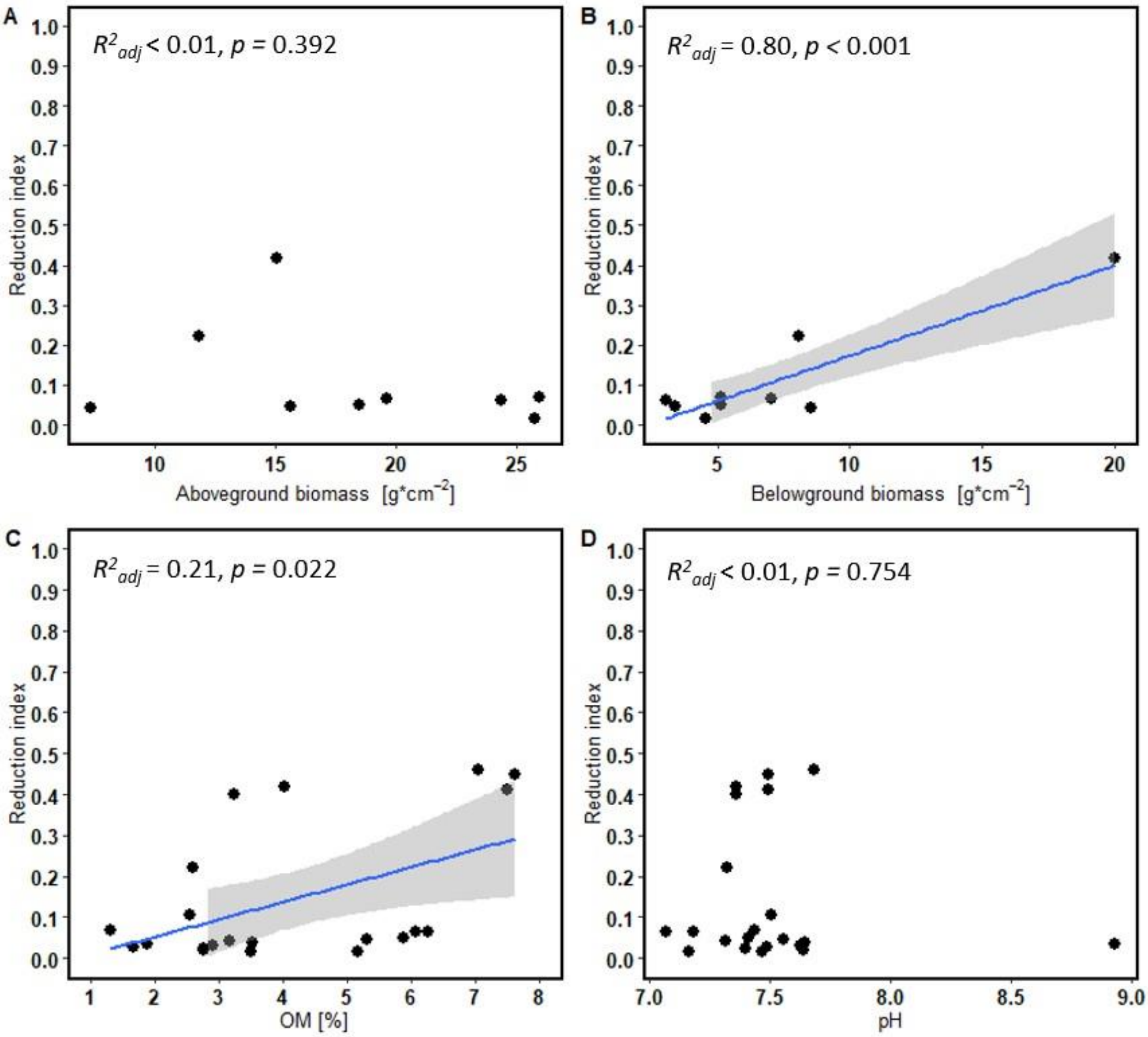

Figure 2.6: Field experiment reduction index, i.e. the fraction of reduced Fe paint, as means across the entire depth (0-30 cm), in relationship to [A] aboveground biomass in g*cm^{-2}, [B] belowground biomass in g*cm^{-2}, [C] organic matter content in % and [D] soil pH values. Plotted lines indicate a significant linear trend ($p < 0.05$). Compare Fig. S2.4 for more details.

Discussion

Mesocosm experiment

Using IRIS sticks as proxy for redox potentials, we found wetland plants to act as both net reducers and net oxidizers of soils. Our data suggest that background levels of soil reduction drove the observed plant effects. Plants acted as net oxidizers in the most reducing flooding treatment, the low marsh treatment, and as net reducers in the less reducing treatments of the pioneer zone and high marsh (Fig. 2.3c). Maximized soil reduction in the low marsh (Fig. 2.3b), despite lower flooding frequency than the pioneer zone, seems counterintuitive but is in line with results from field investigations in hydrologically unaltered Wadden Sea marsh sites (Mueller et al., 2020; Fig. S2.5). It is possible that the observed net oxidizing effect of the planting treatment in the most reducing flooding treatment is the result of root oxygen loss from plants, a process known to increase with decreasing redox potential of the rhizosphere environment (Kludze & DeLaune, 1994).

While no significant differences between plant species could be found, low-marsh derived *Atriplex portulacoides* tended to induce the greatest reduction compared to non-vegetated controls, while high-marsh derived *Elymus athericus* tended to induce the greatest oxidizing effect compared to non-vegetated controls (Fig. 2.3c). The capacity of the grasses *Elymus athericus* and *Spartina anglica* to oxidize soils has previously been shown using planar optodes (Koop-Jakobsen & Wenzhöfer, 2015; Koop-Jakobsen et al., 2021). By contrast, oxygenation capacity of salt marsh shrubs such as *Atriplex portulacoides* have never been investigated. It is also possible that species tend to differ in their potential to oxidize or reduce soils because they differ in their capacity to release electron donors in the form of organic matter. However, our insight into exudation dynamics of wetland plants is scarce.

Planar optode results

Planar optode investigations support findings from IRIS sticks showing that plant roots can act as both net reducers and net oxidizers of soils: roots from *Spartina anglica* acted as both oxygen sinks and oxygen sources to the soil. Planar (Koop-Jakobsen & Wenzhöfer, 2015) and multi-fiber (Koop-Jakobsen et al., 2017; Koop-Jakobsen et al., 2018) optode systems have previously been used to measure in situ sediment oxygenation in *Spartina anglica* rhizospheres and well as other wetland vascular species, e.g., *Elymus athericus* (Koop-Jakobsen et al., 2021), *Juncus spp.* (Blossfeld et al., 2011), and most extensively by Lai et al. (2012). In our study roots were oxygen sinks when background (bulk soil) oxygen concentration was high and (Fig. 2.4a) at lower oxygen concentration of the bulk soil, some of the roots became net sources of oxygen (net oxidizers). These findings are in line with the mesocosms results, showing net oxidizing effects at high background reduction. *Atriplex portulacoides* roots did not show the reducer-oxidizer shift observed for *Spartina anglica*. Instead, roots remained an oxygen sink over the entire 24 hours of the experiment. Koop-Jakobsen et al. (2017) found that different sediment types led to a different extend of oxic root zones, indicating that the background reduction plays an important role in determining the plant effects on soil reduction. Our findings now suggest that soils can have a tipping point of background reduction, which ultimately can lead to net reducing effects of the plants.

Field experiment

Plants consistently acted as net reducers of the soil system throughout all zones (Fig. 2.5c). This finding contrasts from the mesocosm results, indicating more variable plant effects on soil redox. Conditions differed between the two experiments regarding hydrology, plant traits and soil characteristics. Especially differences in alternative terminal electron acceptors as well as electron donors might have led to contrasting findings.

Wadden Sea salt marshes are situated relatively high in the tidal frame, leading to well aerated, oxidizing soil conditions (Erchinger et al., 1996; Mueller et al., 2017). In close agreement with our IRIS data, continuous soil redox records from the nearby low-marsh site at Dieksanderkoog demonstrated frequent oxidizing conditions (Eh > 400 mV) to a soil depth of 15 cm during the growing season (Mueller et al., 2020). We argue that these oxidizing conditions are a precondition for plants to function as strong reducers of the soil system.

Plant-driven reduction in the field experiment was particularly pronounced in the low marsh. We observed highest % change in soil reduction compared to non-vegetated control areas in the low marsh (Fig. 2.5c) at more oxidizing background soil redox compared to pioneer zone (Fig. 2.5b). We attribute the observed net reduction to the dominance of *Atriplex portulacoides*, which showed the greatest net reducing effect in the mesocosm in pioneer zone and high marsh treatments with low background soil redox (Fig. 2.3c). These findings are in line with a study conducted English salt marshes, where *Atriplex portulacoides* stands showed increased carbon stocks, possibly by their comparable high belowground productivity (McMahon et al., 2023).

We show that soil reduction scaled positively with plant belowground biomass and SOM (Fig. 2.6b-c). These findings suggest that greater input of organic matter either via root exudates or belowground root litter input depleted soil oxygen and thus led to greater soil reduction (Gardiner & James, 2012). Studies from other wetland ecosystems (i.e. mangroves) showed that plant roots can reduce the activity of the oxygen-dependent enzyme phenol oxidase (Luo et al., 2018). This finding might be linked to the here described capacity of wetland plants to decrease soil oxygen availability in soils. Further studies are needed to better understand the potential consequences for soil microbial functioning and ultimately soil carbon stability.

Methodological considerations

Soil pH and redox are not independent and need to be studied together in order to understand plant effects on the overall redox chemistry of the soil system. We observed a tendency of lower pH in planted treatments (Fig. S2.2). This effect was pronounced in the field experiment, but negligible in the mesocosms. We attribute this effect to the root release of protons and organic acids (Jones, 1998) as well as increased levels of soil CO_2 through plant and microbial respiration (Koop-Jakobsen et al., 2018). IRIS sticks have proven robust against pH changes at pH > 4.5 (Loffredo et al., 2023). Soil pH was > 7 in both mesocosm and field. We therefore consider the plant effects on iron reduction observed here to be independent of plant mediated pH dynamics. Root exclosures are a useful technique to create *in-situ* control plots without vegetation. However, the installation is destructive and could possibly lead to changes in hydrology. After installation of root exclosures, we allowed the soil to stabilize for four weeks to account for the destructiveness of the installation. To avoid alterations in hydrology, we drilled two holes in each root exclosure, allowing for horizontal water exchange. Remaining artefacts of root exclosures on hydrology cannot be entirely excluded. However, we show that belowground biomass correlated positively with reduction index indicating that differences in reduction between planting treatments were indeed driven by plant effects and not by hydrology artifacts of the installation.

Conclusion

Contrary to the prevailing notion that wetland plants generally act as oxidizers of the soil system, we show that wetland plants can have a net reducing effect on the soil system (Fig. 2.3c, Fig. 2.4c). While the mesocosm experiment revealed plants acting as oxidizers and reducers based on the background soil redox conditions (Fig. 2.3b-c), we found plants to consistently act as net reducers in the field setting. We argue that no net oxidizing effect was observed in the field study due to the comparably well aerated soils of the Wadden Sea salt marshes (Mueller et al., 2020). The here described plant effects on soil redox might play an overseen role in shaping the soil-redox conditions and thus the stability of soil carbon stocks in wetland ecosystems with fluctuating water tables such as peatlands (Evans et al., 2021) or seasonal wetlands (Bansal et al., 2018). Previous studies have indicated shifts in plant communities being closely related to the carbon-sink potential of wetlands (Ward et al., 2013; Robroek et al., 2016). Wetland plants may protect old SOC stocks by providing fresh electron donors, such as low molecular organic compounds, which soil microbial communities preferentially use over old SOC (Robroek et al., 2016). Yet these plant-microbe interactions in wetlands are still poorly understood. Revealing these interactions might require a combination of plant trait and microbial analysis.

Acknowledgments

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Supplement

Supplement 2.1: Fe-oxide paint protocol

Procedure: (For approx. 200ml)

1. For one litre of 1 M KOH, fill a 1 L glass bottle with 56 g KOH and 1 L of distilled water (DW). Let the KOH dissolve.
2. Fill a 2 L glass beaker with 500 ml DW. Place the glass beaker on a magnetic stirrer, install and calibrate a pH-meter. Weigh 16 g of $FeCl_3$ (anhydrous) and let it dissolve in the DW. The pH value should be between 1.2-2.0.
3. Start the magnetic stirrer and slowly add 1M KOH up to 370 mL. In this process the pH-value should rise to 12. Continuously measure the pH-value and stop adding KOH when it reaches pH 12. The suspension is getting thicker as you add KOH. Let the suspension rest for 30 min.
4. After 30 min start the magnetic stirrer and check the pH-value, if it hast dropped below 12, slowly add KOH until it reaches pH 12 again.
5. Then transfer the suspension into two 1000 mL centrifuge glass bottles. Centrifuge at approx. 1000 rpm for 5 min, this should lead to a separation of the Fe-cake on the bottom and the KOH as the supernatant. Remove the KOH supernatant.
6. Add distilled water to the Fe-cake until the volume is doubled. Centrifuge five times at 1000 rpm for 5 min. Remove the supernatant after each centrifugation and refill the glass bottle again. This process helps removing ions from the Fe-cake.
   After the last centrifugation remove the supernatant and carefully fill the whole glass bottles with DW (make sure that the Fe-cake remains on the bottom of the bottle, otherwise centrifuge again). Fill a basin with about 2.5L DW, slowly place the bottles in the basin. Add more DW until the bottles are submerged by DW.
7. The paint should stay in the water basins for 3 days. Using a conductivity meter allows you to check if the Fe-cake is losing Ions. If the value raises above 60 µS the water should be replaced. The Fe-cake should keep its dark red brownish colour.
8. After 3 days the glass bottles can be removed from the basin. Remove the supernatant above the Fe-cake. Add DW and centrifuge at 1000rpm for 5 min and remove the supernatant. The paint is than stored at 4°C for 2 weeks prior to painting.
9. Clean the PVC sticks with acetone than sand both sides of the sticks with a fine sandpaper (grit 180).
10. Fill some paint into a petri dish, put the flat side of the sponge into the paint. Paint the PVC-Sticks with the sponge doing circular movements. The paint should be evenly distributed.

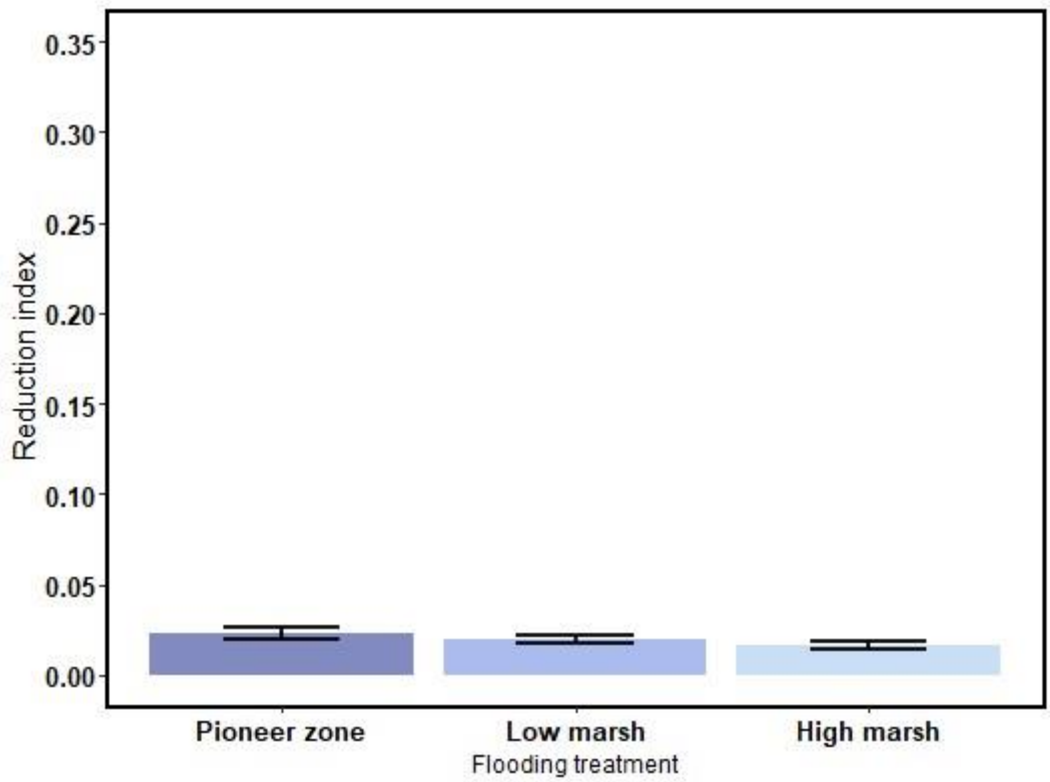


Figure S2.1: Top-soil reduction index, i.e. the fraction of reduced Fe paint across the three flooding treatments in the tidal tank experiment. Shown are values from IRIS stick segments above 15 cm depth. Bar plots show mean ± se values.

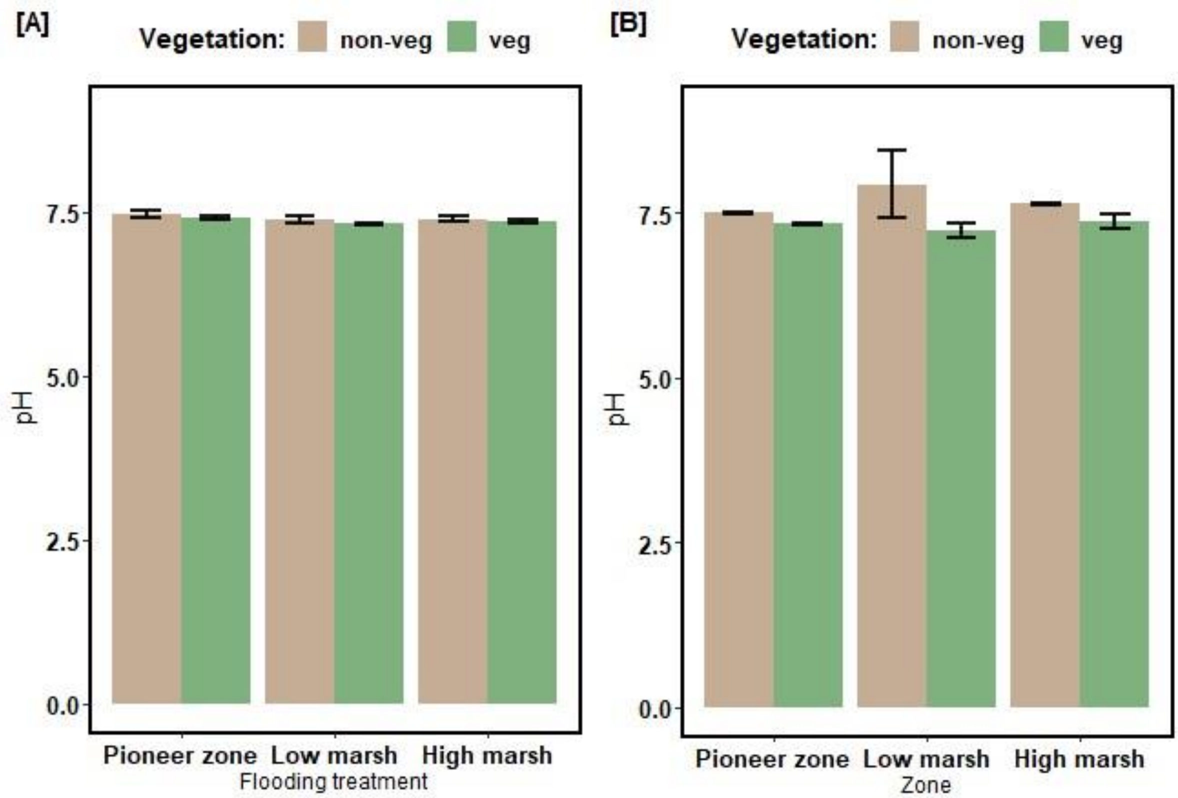


Figure S2.2: pH values of vegetated and non-vegetated subplots across the flooding frequency gradients (pioneer zone, low marsh, high marsh) in both [A] mesocosm experiment in tidal tank and [B] field-study site at the Hamburger Hallig.

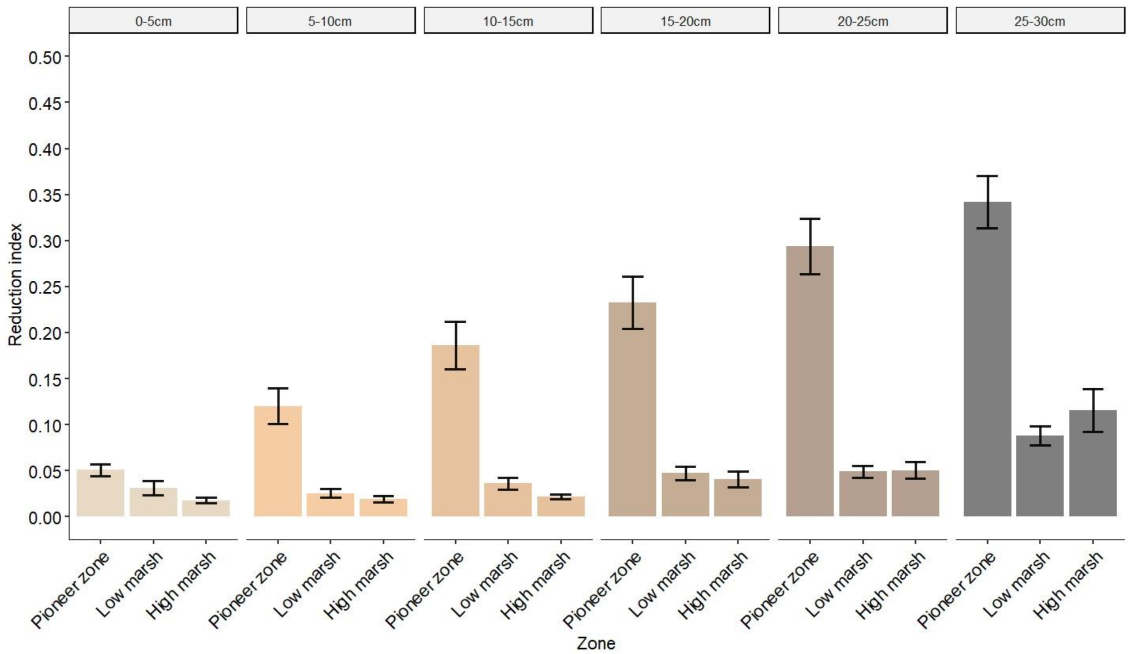


Figure S2.3: Reduction index, i.e. the fraction of reduced Fe paint along the elevational (pioneer zone, low marsh, high marsh) and depth gradient (0-30 cm in 5 cm increments) of the field study. Bar plots show mean ± se values.

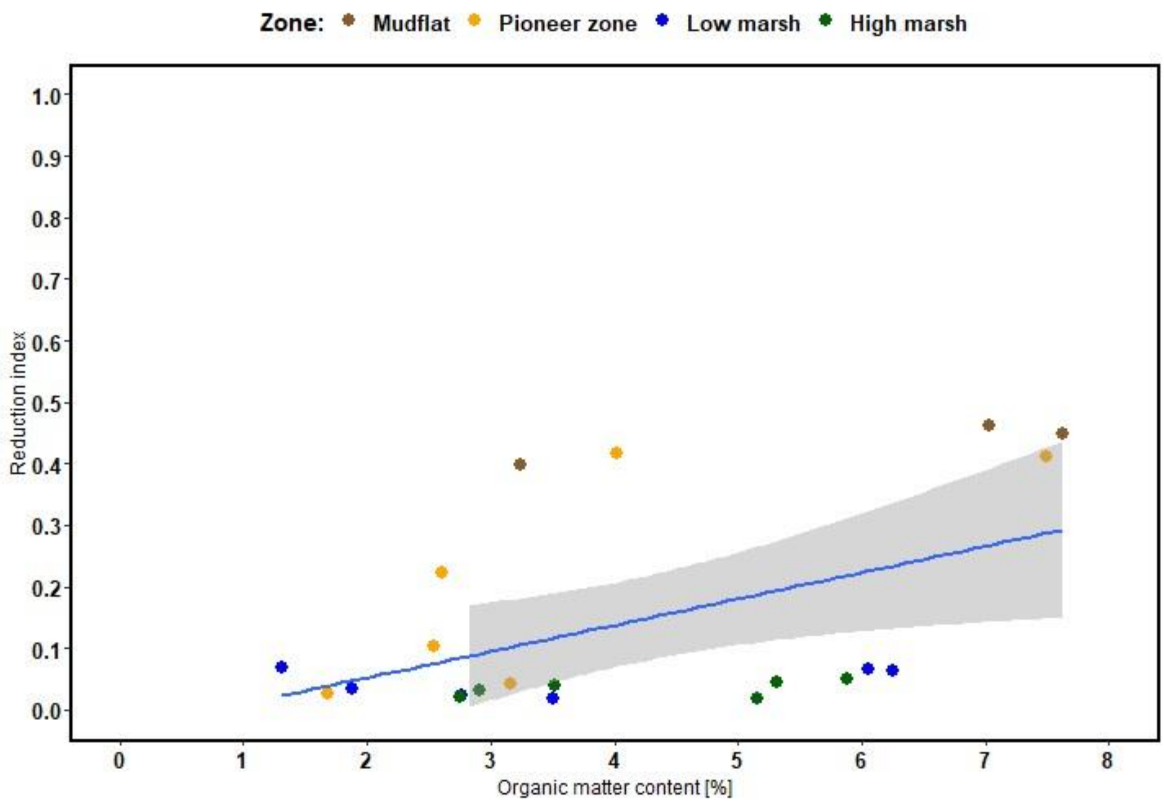


Figure S2.4: Reduction index, i.e. the fraction of reduced Fe paint, in relationship to organic matter content in % across the elevational gradient (tidal flat, pioneer zone, low marsh, high marsh).

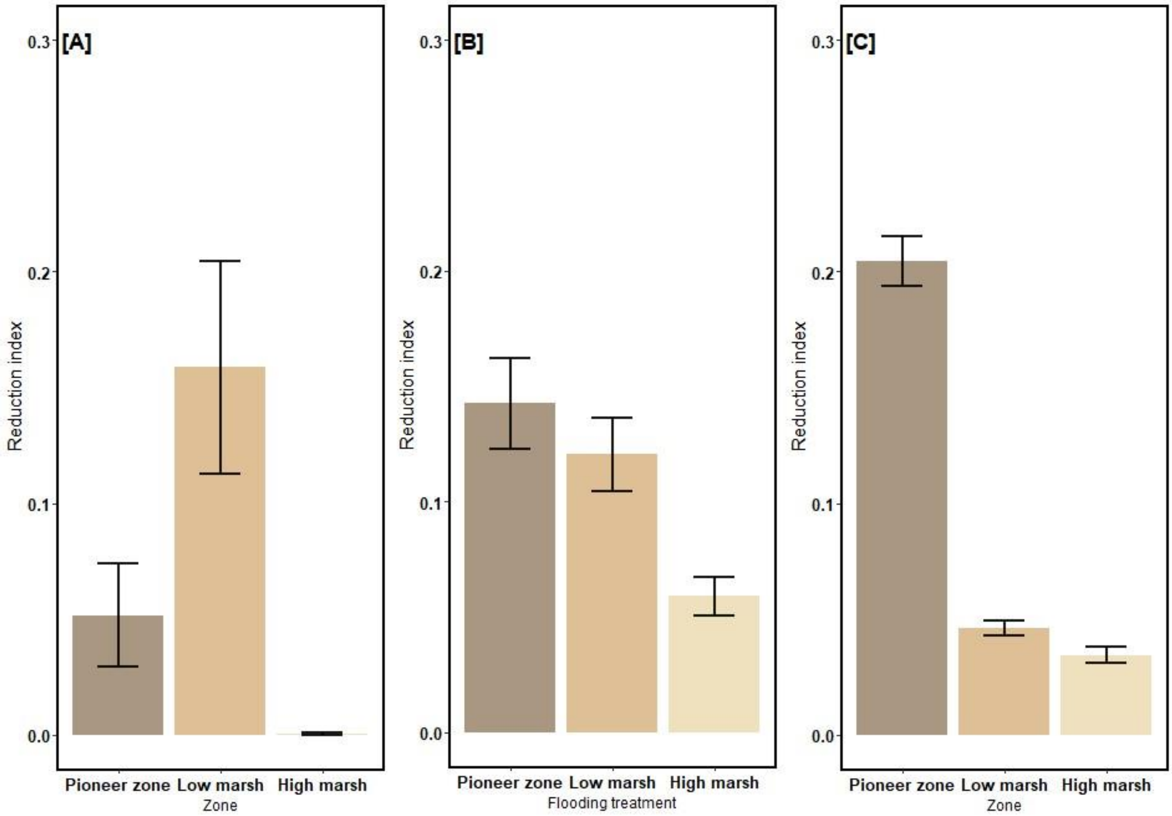


Figure S2.5: Reduction index, i.e. the fraction of reduced Fe paint from IRIS campaigns across the flooding frequency gradient (pioneer zone, low marsh, high marsh) in [A] Natural salt marsh located at Dieksanderkoog (data from Mueller et al. (2020). [B] The tidal tank mesocosm experiment. [C] The field experiment at the Hamburger Hallig.

Box A Observation and quantification of fine roots in a salt marsh using minirhizotrons

Information on the changes in belowground biomass are scarce, despite fine roots (<2 mm diameter) accounting for up to 40% of belowground biomass in some species and having faster turnover rates than coarser roots (Ouyang et al., 2017). Rhizotrons were first introduced in 1873 and refer to large-scale facilities for the non-destructive study of *in situ* root dynamics (Huck & Taylor, 1982). Minirhizotrons, smaller, more convenient alternatives, have also been used for the observation of fine roots employing transparent tubes typically connected to a digital camera system (Taylor et al., 1990). Transparent tubes are usually installed several months prior to imaging to allow soil conditions to readjust and roots to growth over the minirhizotron surface. Additional opaque caps are needed to block light from interfering with root growth.

Installation and theory

Minirhizotrons (n=27) were installed in February 2022 in three vegetated tidal marshes (saline, brackish and freshwater) along the Elbe River in Germany. In each tidal marsh, three minirhizotron tubes were inserted at a 45° angle into each of the three elevation zones (pioneer, low, and high marsh). In this pilot study, repeated measurements were conducted in the pioneer zone of the saltmarsh, Kaiser-Wilhelm-Koog, over four months (June-September) during the growing period (Figure BA.1). The resulting images were used to calculate root productivity, mortality and density (i.e. root number) in the first ten centimeters of topsoil. Data from each time point was analyzed and compared to later measurements from the same soil horizon. Images were corrected for tube distortion and subsequently merged and concatenated using R Studio (v. 4.3.1). Analysis focused on the uppermost ten centimeters of soil as most roots were anticipated at this depth (Steinke et al., 1996).

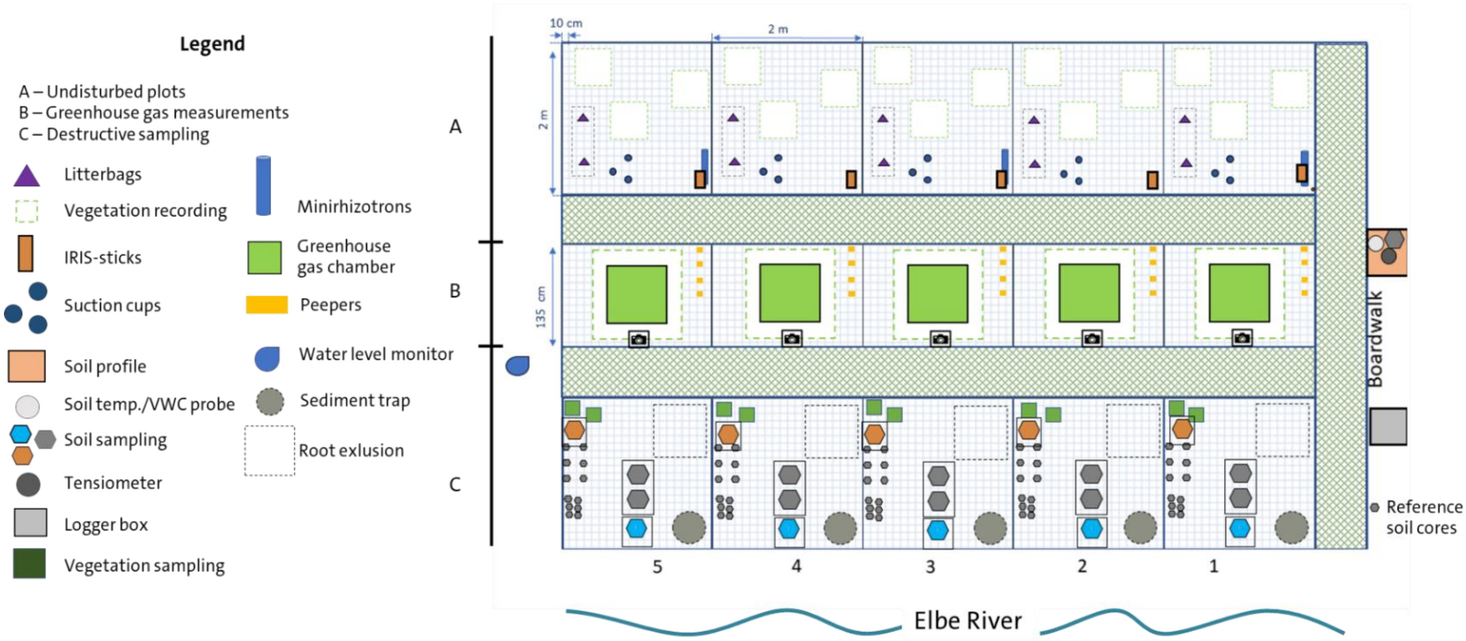


Figure BA.1. Layout of destructive and non-destructive sampling measures at the respective field stations along the Elbe River. Minirhizotron were installed in plots reserved for undisturbed sampling on the side opposite the water source (i.e., closest to the seawall)

Results and implications

- Root density was significantly greater in July than at any other period
- Root productivity was significantly higher in July than in August and September
- Root mortality was significantly higher in August than July and September
- The large variation between replicates can be attributed to fine differences in topology and soil conditions at installations sites and can be improved with a higher replicate number to accommodate spatial heterogeneity
- Root productivity and root mortality generally share an inverse relationship
- Rapidly decreasing root growth from the onset to the end of the growing period may indicate a reallocation of resources from belowground biomass into flowering aboveground foliage, while high mortality at the peak stage of the growing period may indicate suboptimal soil nutrient availability under heightened competition

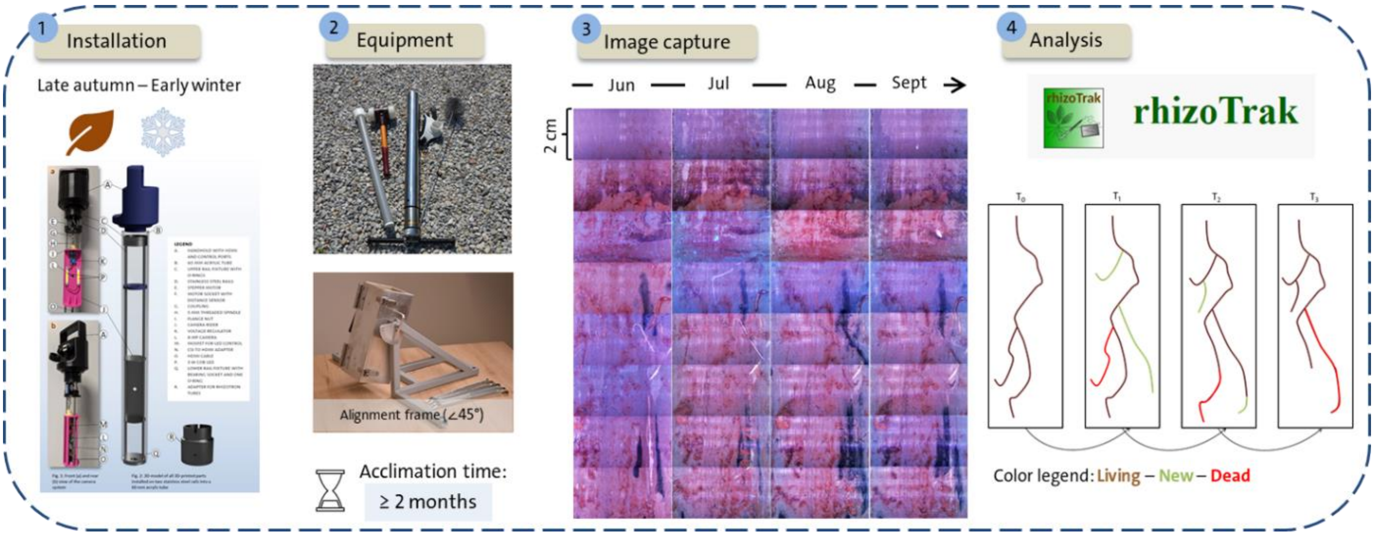


Figure BA.2. Theoretical workflow for installation and application of minirhizotron system for repeated *in situ* measurements.

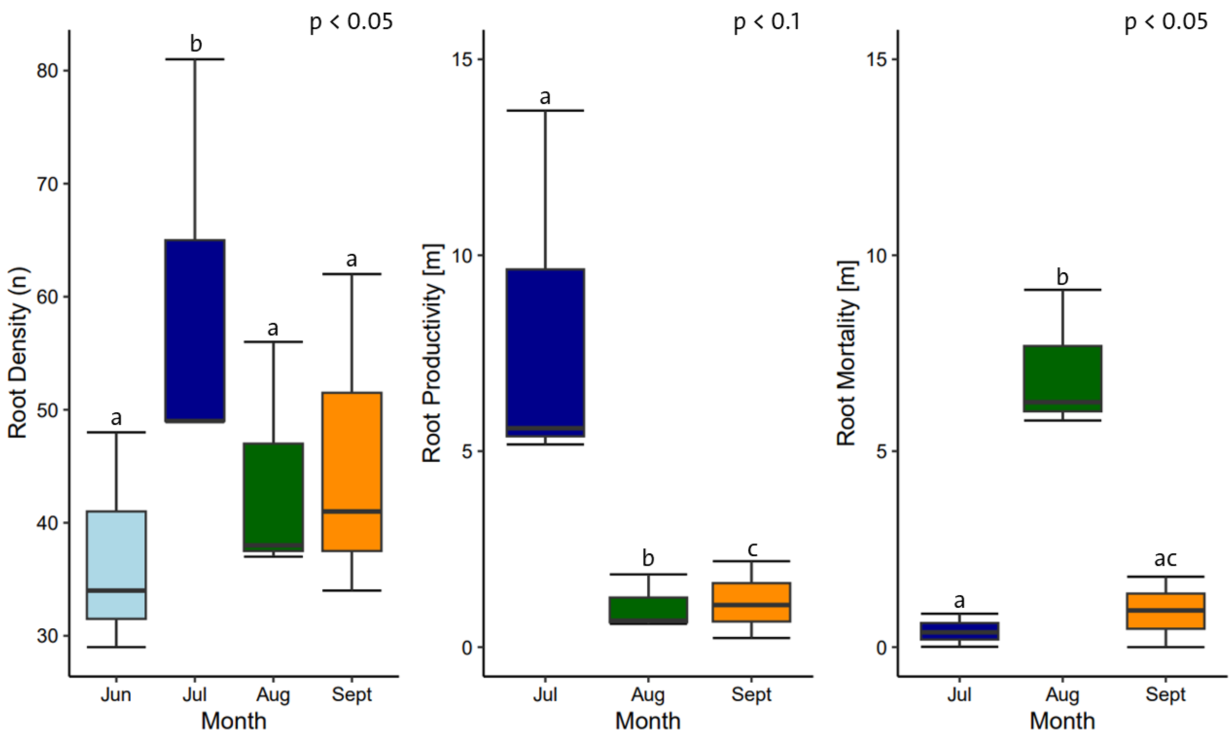


Figure BA.3. Box plots (n=3) for root density, root productivity and root mortality observed in minirhizotron tubes at 0-10 cm depth. Root productivity and root mortality could not be calculated for the first sampling month (June). Significance levels are indicated as above ($p > 0.05$ or > 0.1) based on one-way repeated measures ANOVA and Tukey's Significant Difference test. Root lengths were measured in pixels using *rhizoTrak* and converted to meters (m).

3 Contrasting gas exchange traits in aquatic and terrestrial life forms of *Littorella uniflora* (L.) Asch.

In preparation

Monica Wilson, Alina Frei, Nikola Lenzewski, Peter Mueller, Kai Jensen, Robert Johannes Meier, Ketil Koop-Jakobsen

Abstract

Amphibious plants display a large degree of phenotypic plasticity which enables them to swiftly adapt to both aquatic and terrestrial conditions via modifications of their morphology, physiology or metabolism. The range of trait variation within a single species makes it possible to evaluate plant performance under various environmental scenarios. This study compared functional root traits related to sediment gas exchange (CO_2 uptake and O_2 release) in two life forms of *Littorella uniflora*. The aim was to determine whether these functional root traits were maintained between life forms under fluctuating water level. Our findings show an insensitive response of aquatic *Littorella* plants to water level fluctuation and light-dependent response of terrestrial *Littorella* to maintain oxic rhizosphere conditions. CO_2 concentrations were lowest in aquatic *Littorella* rhizospheres (i.e. higher CO_2 uptake) and showed no significant response to light, while consistently higher CO_2 concentrations in terrestrial *Littorella* rhizospheres and accumulation under submerged conditions indicated limited CO_2 uptake. Similarly, aquatic *Littorella* rhizospheres showed the highest O_2 concentrations and remained oxic in the dark, while oxic rhizospheres in terrestrial *Littorella* were only maintained in the light phase under submergence or consistently after emergence. These findings indicate decreased efficiency of gas exchange between the sediment and rhizosphere of terrestrial *Littorella* upon reintroduced to aquatic conditions. In an oligotrophic environment, these observed limitations in the terrestrial form might be considered maladaptive. Further investigation is therefore required to determine the longevity of these responses, and the physiological trait or trait combinations give rise to them. Under future scenarios of extended dry periods or severe water level fluctuations, the implications of this study could be significant for occurrence of these isoetids and their role in maintaining the oligotrophic status of lakes.

Introduction

Oligotrophic lakes are important ecosystems in the global carbon budget due to their role as carbon sources (Duarte & Prairie, 2005; Morin et al., 2018; Wen et al., 2024), in contrast to many coastal wetlands that function as significant carbon sinks (Mcleod et al., 2011; Lu et al., 2017). Net carbon emissions are driven by higher contributions of respiration than photosynthetic assimilation, i.e. net heterotrophy. However, budget calculations for certain lake strata, e.g., the vegetated littoral zone of lakes, on their own can be autotrophic due to higher biomass and productivity (Andersson & Kumblad, 2006). Oligotrophic lakes are threatened by the spread of eutrophication, which results in nutrient-enrichment in the substrate and bulk water, increased turbidity, and increased light attenuation. For oligotrophic macrophytes, like isoetids, eutrophication can lead to long-term detrimental effects e.g. biomass reduction at high enrichment, uprooting and displacement by faster-growing macrophytes (Pedersen et al., 2006; Raun et al., 2010; Spierenburg et al., 2013). As isoetids are specifically adapted to nutrient-deficiency in these environments, their population sizes have seen significant decline or complete disappearance in formerly oligotrophic lakes (Pedersen et al., 2006; Ronowski et al., 2023). Furthermore, as ecosystem engineers, isoetids help to maintain the low nutrient status of their habitat through root-sediment interactions like P removal (Andersen & Olsen, 1994; Christensen & Andersen, 1996) and sediment oxygenation (Smolders et al., 2002).

Isoetids are a group of aquatic macrophytes distinguished by slow growth, an extensive root system, and small, stiff, evergreen leaves in a basal rosette form (den Hartog & Segal, 1964; Hutchinson, 1975; Raven et al., 1988). Altogether these traits help to reduce the energetic demand of its living tissues and conserve resources in infertile sediments, particularly deficient in nitrogen and phosphorous (Boston, 1986; Farmer & Spence, 1986; Murphy, 2002). They are generally distributed in soft-water (i.e. calcium-poor), infertile freshwater lakes in temperate and boreal areas of Northern and Southern Europe (Pedersen et al., 1995; Molina et al., 1999; Smolders et al., 2002).The characteristic fluctuating water levels along the margin of these lakes also means that the vegetation needs to be adapted to inconsistent CO_2 and light supply from overlying waters (Maberly & Madsen, 2002; Baastrup-Spohr et al., 2016). Isoetids are a paraphyletic group (Richardson et al., 1984; Boston, 1986) that is believed to have shared ancestry with terrestrial and emergent wetland species (Raven et al., 1988).

Isoetids predominantly uptake inorganic carbon from sediment porewater via their roots and transport it to their leaves for photosynthesis, which can account for 30-100% of carbon supply (Boston & Adams, 1987; Raven et al., 1988). No studies suggest that isoetids are capable of using bicarbonate as an inorganic carbon source (Maberly & Spence, 1983; Smolders et al., 2002), meaning CO_2 is the sole carbon species assimilated in photosynthesis. The diffusivity resistance of gases is 10^4 times greater in water (Armstrong et al., 1994), which deters accumulation of CO_2 in the overlying water around the leaves. In comparison, the sediment is a superior source of CO_2 with concentrations in the porewater nearly 100 times greater than in the water column (Wium-Andersek & Andersen, 1972). Examples of sediment-derived inorganic carbon uptake through the root system can be found in a number of submerged macrophytes (Winkel & Borum, 2009), and to a lesser extent in emergent aquatic macrophytes (and Stylites andicola, Keeley et al., 1984; e.g., Phragmites australis, Brix, 1990; Spartina alterniflora, Hwang & Morris, 1992). However, this mechanism is virtually absent in terrestrial plants (Graf & Aronoff, 1955; Stemmet et al., 1962). Although terrestrial plants are able to perform photosynthesis with their aerial tissues, increasing consideration has been given to flood-tolerance and aquatic photosynthesis in terrestrial species using comparisons from submerged and emergent macrophyte adaptations (Mommer & Visser, 2005; Pedersen et al., 2013; Guo et al., 2022).

Some isoetids, as e.g., *Littorella uniflora* (Boston & Adams, 1983, 1987) and species in the genus *Isoetes* (Keeley et al., 1981; Keeley, 1981, 1998), are additionally capable of aquatic Crassulacean Acid Metabolism (CAM) photosynthesis. Unlike traditional CAM photosynthesis for water conservation in terrestrial plants, aquatic CAM photosynthesis is an adaptation to daytime CO_2 limitations (Keeley, 1981; Aulio, 1985). During the daytime, photosynthetic organisms compete for CO_2 and may limit its availability. However, CAM photosynthesis allows plants to take advantage of elevated nighttime CO_2 concentrations from root and sediment respiration and perform dark CO_2 fixation. Even under dark conditions higher rates of fixation than respiration can therefore result in net CO_2 uptake (Richardson et al., 1984; Keeley, 1998). Furthermore aquatic CAM-plants can use their respired CO_2 as an additional source of CO_2 (Madsen, 1987; Keeley, 1998). CAM capabilities are confirmed by measured diurnal changes in malic acid content within the leaves (Boston & Adams, 1983; Surif & Raven, 1988). Because aquatic CAM photosynthesis temporally extends carbon capture, it is considered a carbon-concentrating mechanism (Maberly & Madsen, 2002).

Littorella uniflora (L.) Asch. (hereafter *Littorella*) typically grows in shallow areas of oligo- to mesotrophic lakes (Pietsch, 1995; Murphy, 2002; Holm, 2006; Kolář, 2014; Baastrup-Spohr et al., 2016). The relatively rapid growth rates of *Littorella* (Farmer & Spence, 1986; Boston & Adams, 1987) and ability to tolerate moderate (0.1-2.2. meq/L) levels of alkalinity (Vestergaard & Sand-Jensen, 2000) and soil organic enrichment (Pulido et al., 2011) support their wide distribution and competitive advantage over other isoetids, e.g. *Lobelia dortmanna* with a slower growth rate and higher sensitivity to enhanced sediment oxygen demand (Farmer & Spence, 1986; Møller & Sand-Jensen, 2012). *Littorella* is a phenotypically plastic, amphibious species that undergoes physiological and photosynthetic changes in response to environmental cues (e.g. changes in water level, CO_2 or light availability).

Under submerged conditions, *Littorella* develops thick, aquatic leaves with a thin epidermis and non-functional stomata. A well-developed lacunae system connects below- and aboveground biomass. The small stature of *Littorella* and the concentration of chloroplasts around the leaf lacunae, facilitates efficient gas transport across short diffusive distances of gases, toward or away from photosynthetic tissues along a concentration gradient (Smits et al., 1990). Belowground, the extensive root system features high root permeability, which is ideal for CO_2 uptake from the sediment and supply of photosynthetically produced O_2 to the respiring roots. Radial oxygen loss (ROL) is a well-recorded functional trait in aquatic plants including *Littorella* Sand-Jensen et al. (1982) in which the diffusion of O_2 into the surrounding sediment creates large oxic zones and raises redox potential in the rhizosphere (Wium-Andersen & Andersen, 1972; Holmer et al., 1998).

After prolonged aerial exposure, changes in leaf morphology enhance carbon capture from aerial tissues. *Littorella* develops thinner terrestrial leaves within the span of days to weeks (Robe & Griffiths, 1998) that have a smaller lacunae volume, increased density of (operational) stomata (Robe & Griffiths, 1998) and possibly thicker cuticle (Hostrup & Wiegleb, 1991). This rapid leaf development is accompanied by a switch from CAM to C_3 photosynthesis (Keeley, 1998; Robe & Griffiths, 2000) and results in a switch from solely vegetative reproduction (via stolons) to also include sexual reproduction via seeds (Arts & van der Heijden, 1990). In *Littorella*, it has been reported that the emergent form rapidly loses the ability to perform aquatic CAM photosynthesis within a few days under low humidity (Groenhof et al., 1988; Robe & Griffiths, 2000), yet it is still presumed to depend on sediment-derived CO_2 (Boston & Adams, 1987; Nielsen et al., 1991; Robe & Griffiths, 1998, 2000) without immediate differences in growth rate in the transition of the emergent form to aquatic conditions (Nielsen & Sand-Jensen, 1997).

For amphibious plants, retaining phenotypic plasticity can be an invaluable strategy for rapid acclimation to variable environmental conditions. Given these changes in leaf morphology and physiology in *Littorella* upon emersion, we aimed to understand their net effect on sediment characteristics. Because the dependence on sediment CO_2 is an adaptive response to carbon limitation, the co-occurrence of this trait with the carbon concentrating mechanism, CAM photosynthesis was especially considered (Madsen et al., 2002). Form this we assume that a lack of evidence supporting dark CO_2 uptake would indicate a shift from roots to shoots as the primary CO_2 source. Therefore, we evaluated the performance of aquatic and terrestrial *Littorella* with respect to the gas-related root traits, CO_2 uptake and O_2 release, under two immersion treatments (i.e. total submergence and emergence of aboveground biomass). Temporal dynamics in O_2 and CO_2 concentrations were in observed in the rhizosphere and bulk soil. Because of existing evidence for strong rhizosphere-mediated controls of sediment biogeochemistry, we expect a) concentrations of O_2 and CO_2 in the rhizosphere to be distinct from the bulk soil, b) higher concentrations of O_2 loss under light conditions, c) nocturnal reduction in rhizosphere CO_2 concentrations in aquatic *Littorella* and d) and insensitivity to immersion treatment in aquatic *Littorella*.

Material and methods

Plant and sediment material

Littorella plants and sediments were collected in 2016 from the mesotrophic lake Ihlsee near Bad Seeger in Schleswig-Holstein, Germany. Ihlsee has a surface area of 29 ha with a mean depth of 7.4 m and a maximum depth of up to 21.5 m. Its catchment area of 89 ha is dominated by forests to the west and south and by single, detached houses with private gardens to the north and east. This formerly oligotrophic lake has been colonized by typical oligotrophic isoetid species, such as *Lobelia dortmanna* L., *Isoetes lacutris*, *and Littorella* (Stuhr, 2006). Samples of *Littorella* were retrieved from drift deposits found at the eastern shoreline of the lake (53°57’30.16″N, 10°18’21.35″E) from 2016 to 2019. The plants were transported in plastic bags filled with lake water to the greenhouse (12:12 h, light:dark; approx. 200 µmol m^{-1} s^{-1}; 18°C) at the Institute of Plant Science and Microbiology, University of Hamburg. In the greenhouse, plants were either placed into an aquarium with native water and sediment from the Ihlsee (aquatic form) or transferred to plant pots containing a 50:50 mix of potting soil and sand under drained conditions with regular watering (terrestrial form). Sediment and lake water were routinely collected from the western shoreline of Ihlsee (53°57’39.27”N, 10°17’32.98”E) to replenish supplies throughout the course of measurements. The sediment of lake Ihlsee are predominantly sandy with <2% contributions of silty and clay, an overall low organic matter content (approx. 1%), and a pH of 6.3 (Lenzewski et al., 2018).

Experimental design

The study features a factorial design (Table and Figure 3.1) in which sediment gas exchange traits, root O_2-release and root CO_2-uptake, were investigated in aquatic and terrestrial *Littorella* under two immersion treatments (submergence and emergence of only aboveground biomass) with alternating light-dark cycles.

Table 3.1. Overview of factorial design to investigate root traits related to sediment gas exchange in aquatic and terrestrial *Littorella*. Root O_2 release and root CO_2 uptake was measured in the rhizospheres of each plant under submergence and subsequent emergence of aboveground biomass while the sediment remained waterlogged. Concentrations for each analyte were measured using four replicates of each life form (*n*=16).

		O_2-release	**CO_2-uptake**
		light/dark	light/dark
aquatic	submerged/ emerged	*n*=4	*n*=4
terrestrial	submerged/ emerged	*n*=4	*n*=4

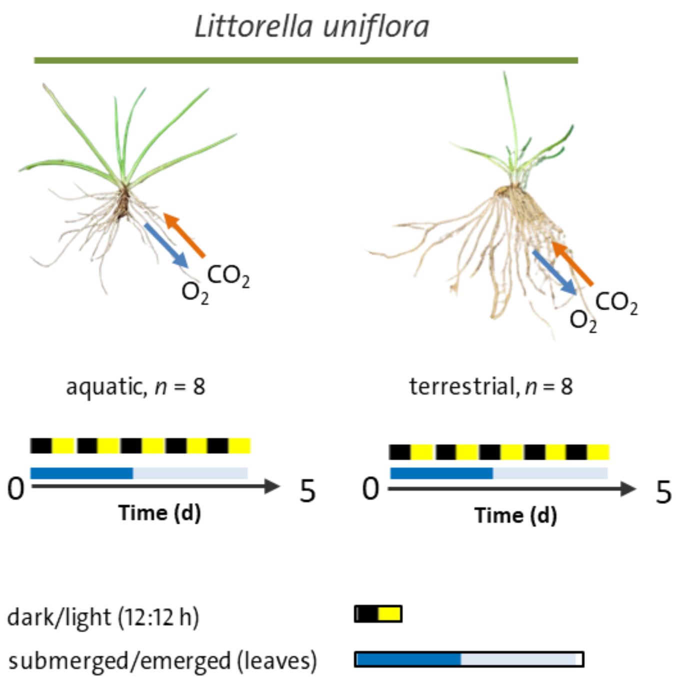


Figure 3.1. Visual overview of experimental design to investigate root traits related to gas transport in aquatic and terrestrial *Littorella*. Root O_2 release (blue arrow) and root CO_2 uptake (red arrow) was measured in the rhizospheres of each plant under submergence (solid blue line) and emergence of aboveground biomass (dashed blue line; leaf symbol) while the sediment remained waterlogged for a total time of 5 d. Concentrations for each analyte were measured using four replicates of each life form (*n*=16).

The experiment investigated O_2 and CO_2 dynamics in the rhizosphere of *Littorella uniflora* during five consecutive days with alternating light-dark cycles (12:12 h). The individual plant sample was fully submerged for the first 51 hours of CO_2 measurements and 55 hours of O_2 measurements. Hereafter, the water column was drained to the sediment surface to expose the aboveground biomass to atmospheric air while the sediment remained waterlogged for the remainder of the measurement.

Planar optode investigations

Planar optodes is a quantitative imaging technique that enables the visualization of biogeochemical processes in sediments or aqueous solution. Planar optode foils facilitate high resolution investigation of the spatial and temporal distribution of analytes such as O_2 and CO_2. When positioned at the root-sediment interface, i.e. the rhizosphere, these non-invasive observations can provide valuable information about chemical gradients around individual roots. Serial measurements further allow these dynamics analyte distributions to be studied under changing external factors.

O_2 and CO_2 dynamics were investigated in this study using the planar optode system VisiSens TD (PreSens Precision Sensing GmbH). A detailed description of the chemistry and imaging principles has been previously described by Tschiersch et al. (2011). Briefly, image analysis is based on dynamic quenching of a fluorophore immobilized in the optode foil following excitation. The fluorescent intensity is captured in regular intervals by a modified camera lens on a set timer. The VisiSens TD system combines the camera detector and internal excitation light source into one device. Measurements were conducted using O_2-sensitive optode foils (SF-RPSu4; 4x4 cm^2, 0-100% O_2 atm. sat., PreSens GmbH) and CO_2-sensitive foils (SF-CD1R, 4x4 cm^2, 1-25% O_2 atm. sat., PreSens GmbH). A custom timer ensured that a separate LED growth lamp (90W, 1000 µmol m^{-2} s^{-1} PAR; Roleadro) provided illumination during light periods with brief intermissions during imaging.

Images with a spatial resolution of 1292 x 964 pixels were captured every 30 minutes with the VisiSens TD system positioned approximately 20 cm from the rhizobox. Images were processed using the accompanying evaluation software VisiSens™ ScientifiCal (PreSens GmbH).

Planar optode foil calibration

Prior to optode investigations, optode foils were calibrated according to the manufacturer's recommendations. For the O_2-sensitive foils, a two-point calibration was performed using anoxic sediment for 0% and air-bubbled water for 100% at atmospheric saturation (atm. sat.). For the CO_2-sensitive foils, a four-point calibration was performed using mixtures of pure gases of CO_2 and N_2 (Air Products GmbH, Germany) in ascending order using the following concentrations: 0 vol. % (i.e. 100% N_2), 3 vol. %, 5 vol. % and 10 vol. % CO_2. Free CO_2 was measured as CO_2 partial pressure ($p$$CO_2$) at atmospheric pressure. All calibrations were performed in a similar manner as the investigations with *Littorella* samples (approx. 20°C) except that foils were not covered with water during calibration measurements. The raw values were evaluated using the ScientifiCal software for the respective analyte and based on the time point after which a saturation curve was reached (after approx. 10-20 minutes).

Preparation of Littorella *individuals for optode measurements*

Littorella individuals of similar size and leaf number (4-8 leaves) were selected for O_2 and CO_2 planar optode measurements. For O_2 measurements, the rhizobox design and preparation were as described by Lenzewski et al. (2018). For CO_2 measurements, a slightly larger rhizobox (2 x 25 x 25 cm^3) was constructed with a detachable front plate. This second design did not include a 2-mm neoprene round cord so that the rhizobox remained semi-permeable.

In both sets of measurements, the rhizoboxes were filled halfway with rewetted sediment from the sampling site. In a flat position, a small crevice was made in the center of the sediment and the *Littorella* sample was carefully inserted and fixed into the sediment. The roots were carefully arranged in a manner that the optode foil (O_2 or CO_2-sensitive) covered 2-5 roots as well as substantial area of the bulk sediment. Subsequently, the front plate was attached with water droplets between foil and plate to ensure an air-bubble-free water film was created. Thereafter, the closed rhizobox was lifted to an upright position and filled completely with lake water. For O_2 measurements, water was carefully added along the back plate of the rhizobox until complete submergence. In CO_2 measurements, the semi-permeable rhizobox was first transferred to an empty aquarium (20 x 30 x 20 cm^3) that was subsequently filled with water which slowly diffused into the rhizobox until complete submergence. In both instances, the porosity of the sandy substrate and slow addition of water allowed minimal sediment disturbance and prevention of air bubble entrapment along the optode foil.

Image and statistical analysis

One image of the entire optode foil (4 x 4 cm) was captured every 30 minutes for a total of 120 hours resulting in 240 images per measurement. Within each image, two regions of interest (ROIs) were selected around the roots and one representative area of the bulk soil (480 observations per time series). Images were visually inspected at the conclusion of each time series and the resulting dataset was filtered to exclude invalid images caused by technical interruptions, i.e. overexposed or unlit images. In total, 7261 observations (O_2: 3421, CO_2: 3840) were used for spatiotemporal analysis of O_2 and CO_2 in rhizosphere and bulk soil compartments. T-tests were used to determine statistical differences in gas concentrations between *Littorella* forms with respect to the immersion treatment and light availability as well as between samples of the same form. Additionally, t-tests were conducted to generally determine significant differences between bulk and rhizosphere soil compartments across all samples. All data was processed using R Studio version 4.2.1. (R Core Posit Team, 2024). Optode images for spatial analysis of O_2 and CO_2 concentrations around single roots were chosen from the end of the first and final light periods (i.e., 24 and 120 hours, respectively). Using the image manipulation program, GIMP (version 2.8.18 GIMP Development Team, 2019), geo-referencing tools were first used to generate merged images of the photographed roots with their respective optode images. Afterward root diameters were determined using a reference in the same image. Additional image analysis was performed for the final dark and light periods (i.e., 108 and 120 hours, respectively) and can be found in Supplementary Figures S3.1 and S3.2. For temporal analysis of O_2 and CO_2 over the five-day period, average concentrations of the entire ROI around the roots were compared to ROI mean concentrations from the respective bulk sediment in each sample.

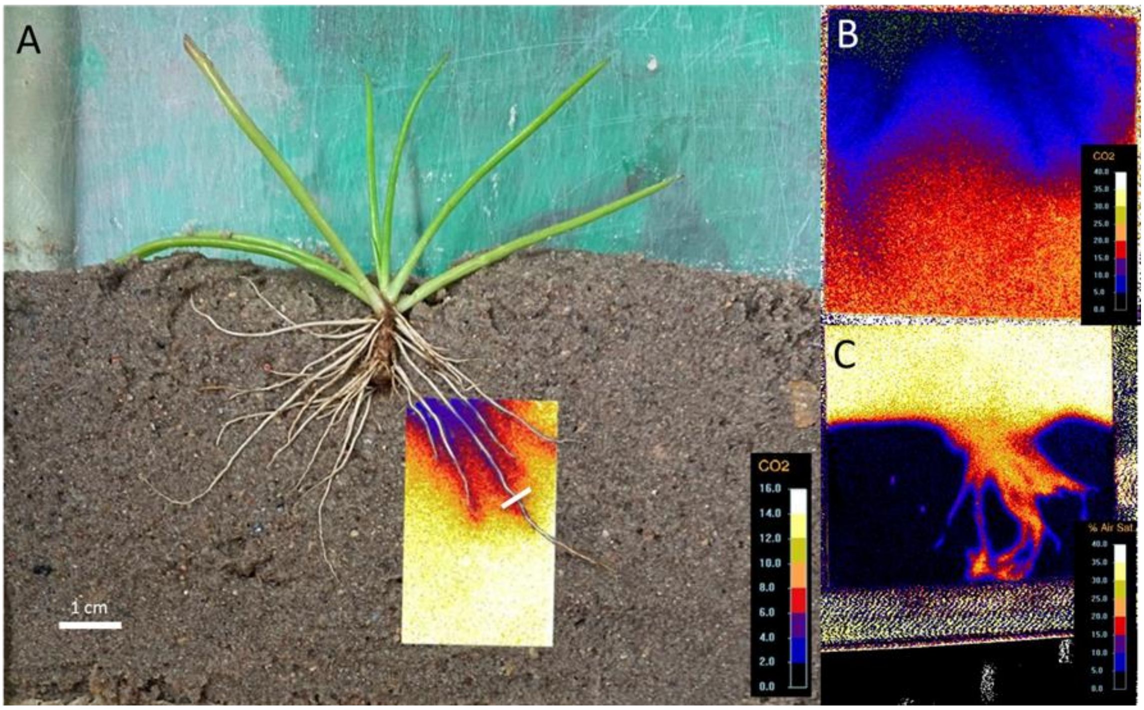


Figure 3.2. (A) Overlaid image of photographed plant sample with its corresponding optode image generated with georeferencing tools in GIMP (v.2.8.18). Example of (B) CO_2 concentrations (C) O_2 concentrations in rhizosphere and bulk sediment.

Results

Spatial O_2 and CO_2 distribution in the rhizosphere

The spatial distribution of %O_2 (Figure 3.3) and %CO_2 (Figure 3.4) in the rhizospheres of aquatic and terrestrial *Littorella* under submerged and emerged conditions were calculated for all samples (n=16). An exemplary optode image overlaid with the exact positions of the roots are provided in (Figure 3.2A). *Littorella* rhizospheres were visually distinct from the bulk sediment (Figure 3.2B-C). O_2 and CO_2 concentrations in the surrounding sediment were detected along a spatial gradient from the roots. Due to high variability in the CO_2 analyte, spatial analysis of rhizosphere radii was only conducted for the O_2 analyte (O_2-enriched zones; Table 3.2). The respective radii of O_2-enriched root areas (mm) are given in Table 3.2 as the mean values with standard deviations of the replicates (n=4) for each lifeform under emerged and submerged conditions. Rhizospheres of aquatic *Littorella* created O_2 -enriched zones that were larger and higher in concentration than rhizospheres of the terrestrial form. In response to immersion, O_2 rhizosphere concentrations in aquatic *Littorella* remained stable in both shape and magnitude, while these were lower for terrestrial *Littorella* under total submergence, and which recovered after emergence. A similar pattern could not be observed for CO_2 dynamics, which was inconclusive for aquatic and terrestrial forms.

Spatial O_2 distribution

Rhizosphere concentrations in aquatic *Littorella* did not respond to the immersion treatment (Table 3.2). During submergence the average radius of O_2-enriched soil around aquatic roots was 4.75 ± 1.18 mm with a maximum concentration of 51.80 ± 6.91%. After emergence O_2-release from aquatic roots did not significantly change in size (radius) or concentration (-1%). O_2-release from terrestrial roots showed a considerable response to the immersion treatment. During submergence, O_2-release from roots of the terrestrial life form were initially lower and narrower; only 1.375 ± 0.62 mm was O_2-enriched with a maximum concentration of 16.66 ± 8.04%. After emergence, roots of the terrestrial life form released 60% more oxygen (27.96 ± 7.29) over a larger distance (2.22 ± 1.55). The radii of the oxygen-enriched zones around the roots were highest for the aquatic life form, more than three times larger than that of the terrestrial plants.

Table 3.2. Summary of O_2-enriched zones in aquatic and terrestrial *Littorella* before (24h) and after (120h) emergence of aboveground biomass. The radii (mm), maximum and minimum concentrations in root-influenced sediment, i.e. the rhizosphere, is given as the mean value ± the standard deviation (*n*=4).

	aquatic			terrestrial		
	R (mm)	Max.	Min.	R (mm)	Max.	Min.
submerged	4.75 ± 1.18	51.80 ± 6.91	1.07 ± 0.85	1.375 ± 0.62	16.66 ± 8.04	0.29 ± 0.34
emerged	4.59 ± 1.19	50.18 ± 3.33	2.07 ± 2.41	2.22 ± 1.55	27.96 ± 7.29	0.46 ± 0.15

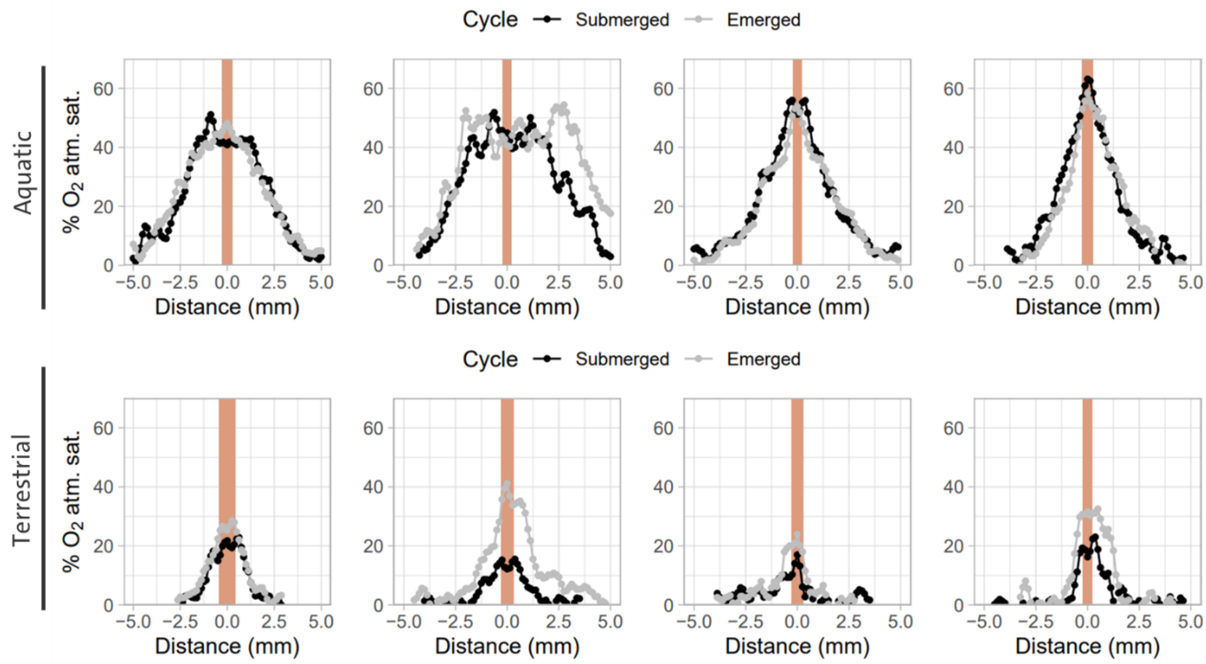


Figure 3.3. Spatial distribution of %O_2 (atm. sat.) around single roots from aquatic (*n*=4) and terrestrial (*n*=4) *Littorella* under submergence and after emergence (24 and 120 h, respectively). Brown bars represent the position and respective width of the root (centered at 0).

Spatial CO_2 distribution

With the exception of two replicates CO_2 concentrations around the roots of both the aquatic and terrestrial *Littorella* did not show a definitive pattern with respect to immersion. Generally, CO_2 concentrations were marginally lower in the immediate vicinity of the root, indicating net CO_2 removal relative to higher concentrations in the bulk sediment (Figure 3.4).

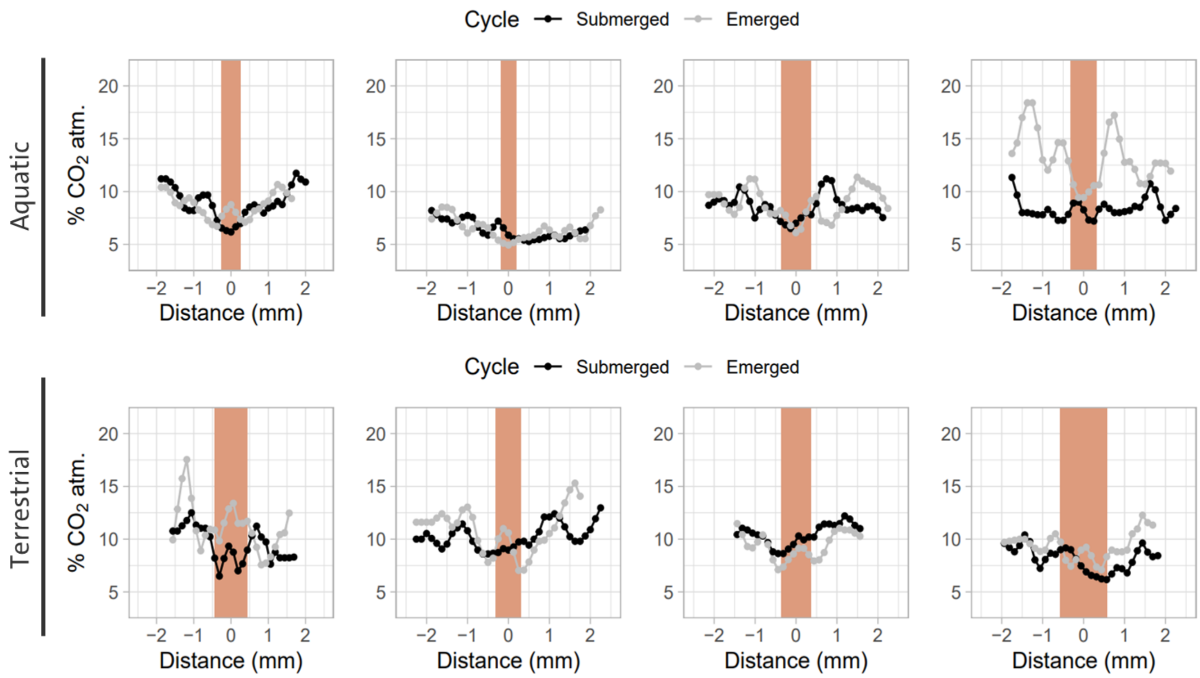


Figure 3.4. Spatial distribution of %pCO_2 (atm.) around single roots from aquatic (n=4) and terrestrial (n=4) *Littorella* under submergence and after emergence (24 and 120 h, respectively). Brown bars represent the position and respective width of the root (centered at 0).

Temporal O_2 and CO_2 dynamics in the rhizosphere

The temporal variation in %O_2 and %CO_2 in the rhizospheres of *Littorella* life forms under alternating light-dark cycles before and after leaf emergence are shown for all samples (n=16). Across all samples, there was a statistical difference (t-test) in CO_2 ($t=-67.65$, $p < 0.05$) and O_2 ($t=71.25$, $p < 0.05$) concentration between the rhizosphere and bulk soil compartments. These differences in O_2 or CO_2 between soil compartments remained significant with respect to life form, light availability and flooding treatments.

For both life forms, %O_2 in the rhizosphere significantly increased in the light and was consistently highest in aquatic *Littorella* rhizospheres. O_2 concentrations were significantly affected by immersion in the terrestrial life form (t=--27.22, $p < 0.05$) and to a lesser extent in the aquatic life form (t=--4.0496, $p < 0.05$). CO_2 concentrations in the rhizospheres of aquatic *Littorella* displayed a characteristic diel pattern and were distinct from bulk sediment concentrations. In contrast, rhizosphere CO_2 concentrations in terrestrial *Littorella* were similar to those from the bulk soil and no diel pattern could be observed here.

Temporal O_2 dynamics

Over consecutive (12 h)-dark and (12 h)-light periods, %O_2 in the rhizospheres was consistently higher in the light for both life forms. On average these values were 44.5 ± 7.77 % (dark) and 60.1 ± 10.4 % (light) in the aquatic life form (Figure 3.4A) and 13.3 ± 11.5 % (dark) and 27.6 ± 10.4 % (light) in the terrestrial life form (Figure 3.4B). At the onset of each dark period, O_2 concentrations in the rhizospheres of both life forms were rapidly depleted within the first hour and remained at equilibrium until the next light period. There was a statistical difference (*t*-test) between the aquatic and terrestrial rhizospheres with respect to their O_2 concentrations under submerged (t=-46.83, $p < 0.05$) and emerged conditions (t=-39.77, $p < 0.05$). Under submergence, the terrestrial life form showed O_2 concentrations approximately three times lower around their roots (10.27 ± 9.5%) than after emergence of the leaves (28.0 ± 9.7%). For the aquatic life form, these O_2 concentrations varied only slightly in response to emergence (Table 3.3.; 49.6 ± 14.1% vs. 52.9 ± 9.0%).

Table 3.3. Mean, median, minimum and maximum values of %O_2 (atm. sat.) measured in the rhizosphere and bulk soil for life forms under the immersion treatment.

Soil compartment	*Life form*	*Treatment*	*Mean*	*Median*	*Min*	*Max*	*SD*
Roots	Aquatic	Submerged	49.60	46.50	29.90	88.30	14.10
Roots	Aquatic	Emerged	52.90	51.80	34.70	80.80	9.00
Roots	Terrestrial	Submerged	10.30	5.14	0	32.0	9.51
Roots	Terrestrial	Emerged	28.10	27.90	3.81	55.90	9.68
Bulk	Aquatic	Submerged	0.34	0.12	0	1.54	0.43
Bulk	Aquatic	Emerged	0.63	0.52	0	4.20	0.69
Bulk	Terrestrial	Submerged	0.54	0.36	0	2.30	0.64
Bulk	Terrestrial	Emerged	0.90	0.80	0	3.00	0.83

Temporal CO_2 dynamics

Over consecutive (12 h)-dark and (12 h)-light periods, %CO_2 was slightly lower in the light for the aquatic life form (dark: 7.23 ± 1.53% vs. light: 7.04 ± 1.55 %) and lower in the dark for the terrestrial life form (dark: 11.6 ± 1.56 % vs. light: 12.2 ± 1.46 %). At the onset of each dark period, CO_2 concentrations in the rhizosphere of the aquatic life from showed the opposite pattern to O_2 and initially increased within the first hour, decreased to equilibrium, and increased again in the final 1-2 hours before onset of the next light period (Figure 3.5A). In the terrestrial life form, CO_2 concentrations varied significantly in response to immersion (t=-7.22, $p < 0.05$), were slightly lower under submergence (3.17 ± 3.09%) and stabilized upon emergence (4.38 ± 2.65%) (Figure 3.5B). No significant response to emergence could be detected in the aquatic life form (t=-1.8651, $p = 0.06248$). On average these values were 7.03 ± 1.34 under submergence and 7.21 ± 1.68 after emergence (Table 3.4).

Table 3.4. Mean, median, minimum and maximum values of %pCO_2 (atm.) measured in the rhizosphere and bulk soil for life forms under the immersion treatment.

Soil compartment	*Life form*	*Treatment*	*Mean*	*Median*	*Min*	*Max*	*SD*
Roots	Aquatic	Submerged	7.03	7.22	4.23	10.20	1.34
Roots	Aquatic	Emerged	7.21	7.60	3.87	10.50	1.68
Roots	Terrestrial	Submerged	11.50	11.80	6.72	14.20	1.70
Roots	Terrestrial	Emerged	12.20	12.10	9.72	15.40	1.34
Bulk	Aquatic	Submerged	17.40	18.50	8.95	23.10	4.71
Bulk	Aquatic	Emerged	18.70	17.40	12.00	23.50	3.63
Bulk	Terrestrial	Submerged	14.10	14.30	7.12	19.80	2.34
Bulk	Terrestrial	Emerged	17.00	16.00	13.30	23.80	3.09

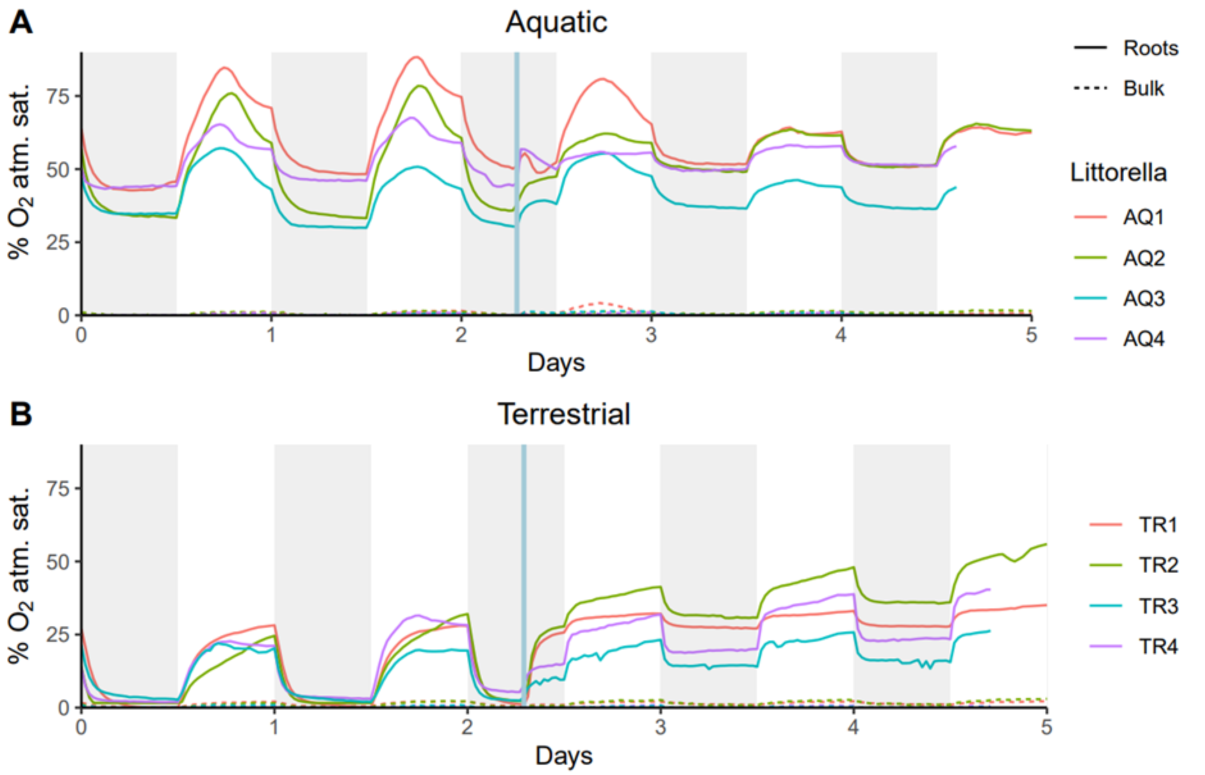


Figure 3.5. Temporal variation in %O_2 (atm. sat.) in aquatic (AQ, *n*=4) and terrestrial (TR, *n*=4) *Littorella* life forms before and after emergence of aerial leaves. Alternating gray and white boxes represent dark-light (12:12 h) cycles. The time point of leave emergence is indicated by a solid blue vertical line at 55 h. O_2 concentrations in the rhizosphere are depicted with solid lines; bulk soil in dotted lines.

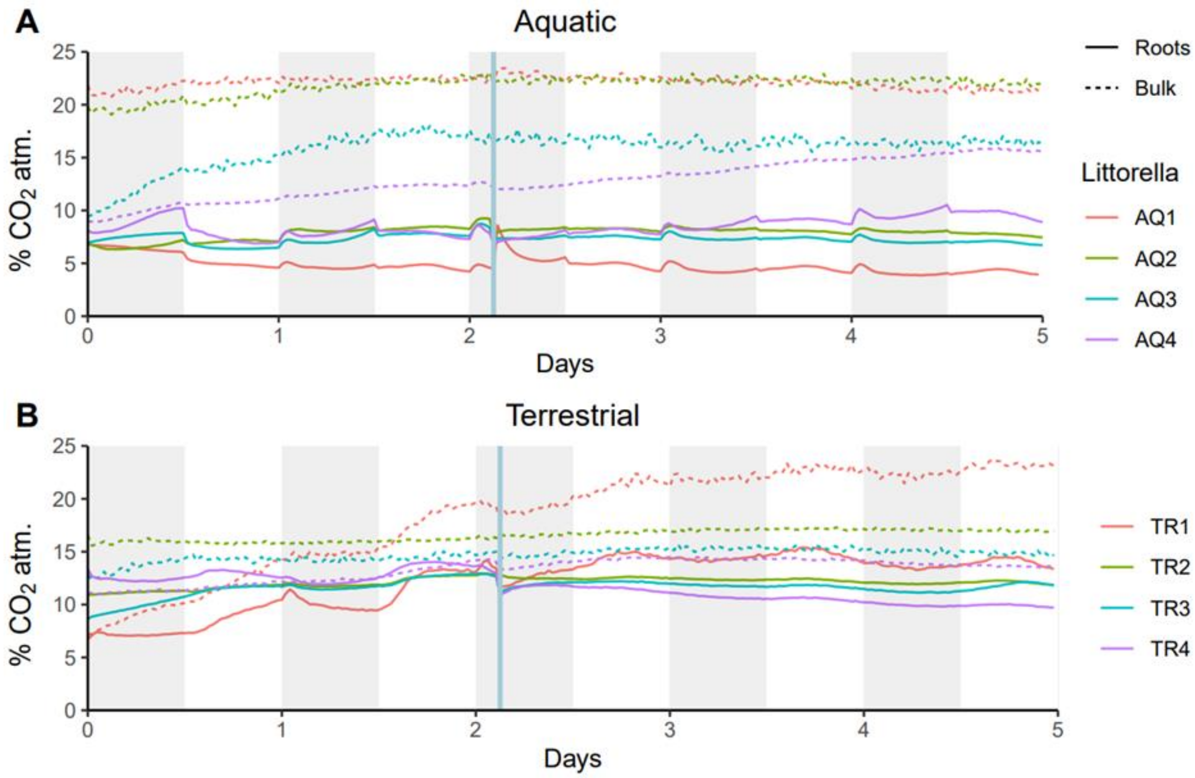


Figure 3.6. Temporal variation in %CO_2 (atm.) in aquatic (AQ, *n*=4) and terrestrial (TR, *n*=4) *Littorella* life forms before and after emergence of aerial leaves. Alternating gray and white boxes represent dark-light (12:12 h) cycles. The time point of leave emergence is indicated by a solid blue vertical line at 55 h. O_2 concentrations in the rhizosphere are depicted with solid lines; bulk soil in dotted lines.

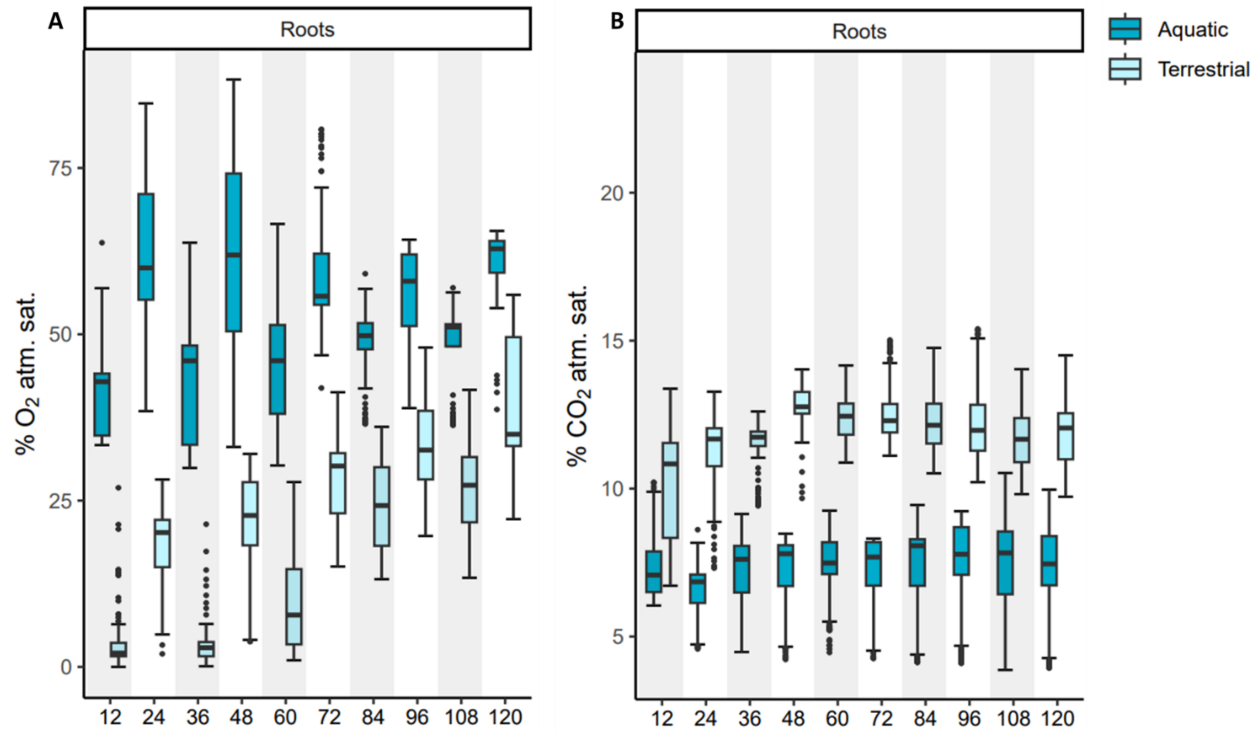


Figure 3.7. Temporal variation in %O_2 (atm. sat.) and %CO_2 (atm.) in aquatic (AQ, *n*=4) and terrestrial (TR, *n*=4) rhizospheres under submergence and after emergence of aboveground biomass. Alternating gray and white boxes represent dark-light (12:12 h) cycles.

Discussion

Our findings show an insensitive response of aquatic *Littorella* plants to water level fluctuation and light-dependent response of terrestrial *Littorella* to maintain oxic rhizosphere conditions. CO_2 concentrations were lowest in aquatic *Littorella* rhizospheres and light-independent while CO_2 concentrations were consistently higher in terrestrial *Littorella* rhizospheres and accumulated under submerged conditions. Similarly, aquatic *Littorella* rhizospheres showed the highest O_2 concentrations and remained oxic in the dark, while oxic rhizospheres in terrestrial *Littorella* were only present in the light under submergence and or after emergence of aboveground biomass. These findings suggest that the exchange of CO_2 and O_2 between the sediment and rhizosphere are somewhat limited in the terrestrial life form upon reintroduction to aquatic conditions. These results stand in contrast to previous findings from literature that state continued dependence on sediment-derived CO_2 in the emergent form. (Nielsen et al., 1991; Robe & Griffiths, 2000). Robe and Griffiths (2000) reported high leaf lacunae concentrations of CO_2 following emergence, likely as a result of enhanced CO_2 evolution under higher redox potentials in the aerated sediment. While diffusion into the still permeable root tissues along a concentration gradient is plausible, it does not reflect active uptake, as common for flood-adapted plants exhibiting a carbon concentrating mechanism (Madsen et al., 2002). Furthermore, a strong increase in CO_2 uptake as a result of higher bulk soil concentrations upon emergence was not observed in aquatic *Littorella* rhizospheres. CO_2 and O_2 measurements targeted fluxes to and from the rhizosphere by measuring directly at the roots. This method is particularly suited to isoetids who demonstrate gas exchange primarily in the rhizosphere or internal structures rather than in the sediment-water interface (Sand-Jensen et al., 1982).

Spatiotemporal O_2 Dynamics

O_2 rhizosphere concentrations in aquatic *Littorella* were consistently higher in this study and were maintained even during nighttime respiration of root and sediment. This efficient release of oxygen is likely helped by the outward diffusion of oxygen along the entire length of the root, rather than restriction to the apex (Smits et al., 1990). In terrestrial *Littorella*, oxidized rhizospheres were only detected during the light phase under submergence, indicating low sensitivity to submerged conditions. After removal of the diffusive barrier around the leaves following emergence, higher concentrations of oxygen release in terrestrial rhizospheres points to enhanced photosynthetic activity. The O_2 concentrations for the terrestrial form mirror the same diurnal pattern (low oxygen release in light and anoxia in dark) as *Lobelia dortmanna* (Møller & Sand-Jensen, 2012), whose high diffusive resistance across leaf surfaces prevented it from additional O_2 uptake from the water around the leaves therefore leading to rapid depletion of O_2 during nighttime respiration. Barriers to radial oxygen loss can reduce the capacity of the roots to oxidize reduced, phytotoxic substances such as iron plaque that further reinforce barriers to root permeability and increase stress. Temporary periods of anoxia have also been reported for the non-CAM isoetid, *Lobelia dortmanna* (Lenzewski et al., 2018). Efficient gas exchange at the root-sediment interface requires high root permeability (Smits et al., 1990), a well-developed internal lacunae system for efficient internal transport of gases to and from photosynthetic tissues, short diffusive distances, and barriers to outward diffusion to prevent leakage into the surrounding medium (Boston & Adams, 1987).

The width of the oxic zone created in the rhizospheres were slightly larger than those previously observed in literature . One explanation for the larger area in this study could be the use of planar optodes over Clark-type oxygen microelectrodes. The impermeable optode foil may exaggerate the spatial signal of the analyte by causing diffusive smearing (Jiménez et al., 2021). For aquatic roots, sufficient levels of oxygen were able to accumulate and be transported to the roots, establishing oxic zone that persisted in both the light and the dark (Christensen et al., 1994; Figure 3.4A). For terrestrial roots, much lower concentrations of oxygen release into the rhizosphere during illumination were not able to compensate for respiratory processes in the dark periods as these quickly became anoxic.

Temporal CO_2 Dynamics

Stable concentrations of CO_2 in the submerged, aquatic plants and initially higher CO_2 concentrations in the terrestrial form under submerged conditions echoes the trends in photosynthetic activity reported by Nielsen (1993). The morphology of the terrestrial life form likely makes it maladaptive to submerged photosynthesis and might require additional assimilation through the leaves to establish a CO_2 sink in at the root-sediment interface.

Aquatic *Littorella* did not demonstrate a significant change in rhizosphere concentrations of CO_2 or O_2 following air exposure of the leaves, suggesting that the relative contributions of gas exchange across leaf surfaces was negligible. Regarding CO_2 uptake this behavior demonstrates a consistent reliance on root-mediated assimilation from the comparatively richer substrate as was found in other studies (Søndergaard, 1979; Wetzel et al., 1985). However, a relatively thinner cuticle might have also enabled uptake from overlying water as well. Thinner cuticles in aquatic leaves has been observed in other plant species with varying flooding tolerances (Mommer et al., 2007) and (Robe & Griffiths, 2000) also suggested a change in permeability or cuticle thickness might have occurred in *Littorella* upon emergence, though this was not measured in our study.

Impact of light conditions

Variation amongst replicates within a life form, e.g. aquatic *Littorella* replicates, can be explained by age-related functional differences between single roots (Christensen et al., 1994). Because the light conditions were identical for all replicates, differences caused by variations in light intensity are not supported. During the first two hours of the dark period, CO_2 concentrations around aquatic roots increased before falling again (Figure 3.5A). This brief accumulation of CO_2 reflects a delayed shift in the activation of CAM metabolism, where respiratory processes (i.e. CO_2 generation) shortly dominate (Madsen, 1987; Pedersen et al., 2018). Still, the generally net CO_2 uptake in the dark from aquatic roots is congruent with their status as an aquatic CAM plant (Keeley, 1998). Although it was reported (Hostrup & Wiegleb, 1991) that submerged leaves of the aquatic *Littorella* die within 24 hours, this was not observed in our study. In the aquatic life form, CO_2 concentrations in the rhizosphere were lower in the dark than in the light, which is congruent with findings from Sand-Jensen and Søndergaard (1979), who also found that transport rates of CO_2 from the roots to the water in separated the leaf chamber were highest in the dark. As presumed in the literature and seen in this study, higher levels of oxygen release during illumination are likely stimulated by photosynthesis in which oxygen production likely outweighs respiratory demands of oxygen consumption. The lower rates of oxygen release during the dark periods compared to light periods are congruent with findings from other submerged macrophytes (Sand-Jensen et al., 1982).

Limitations of the study

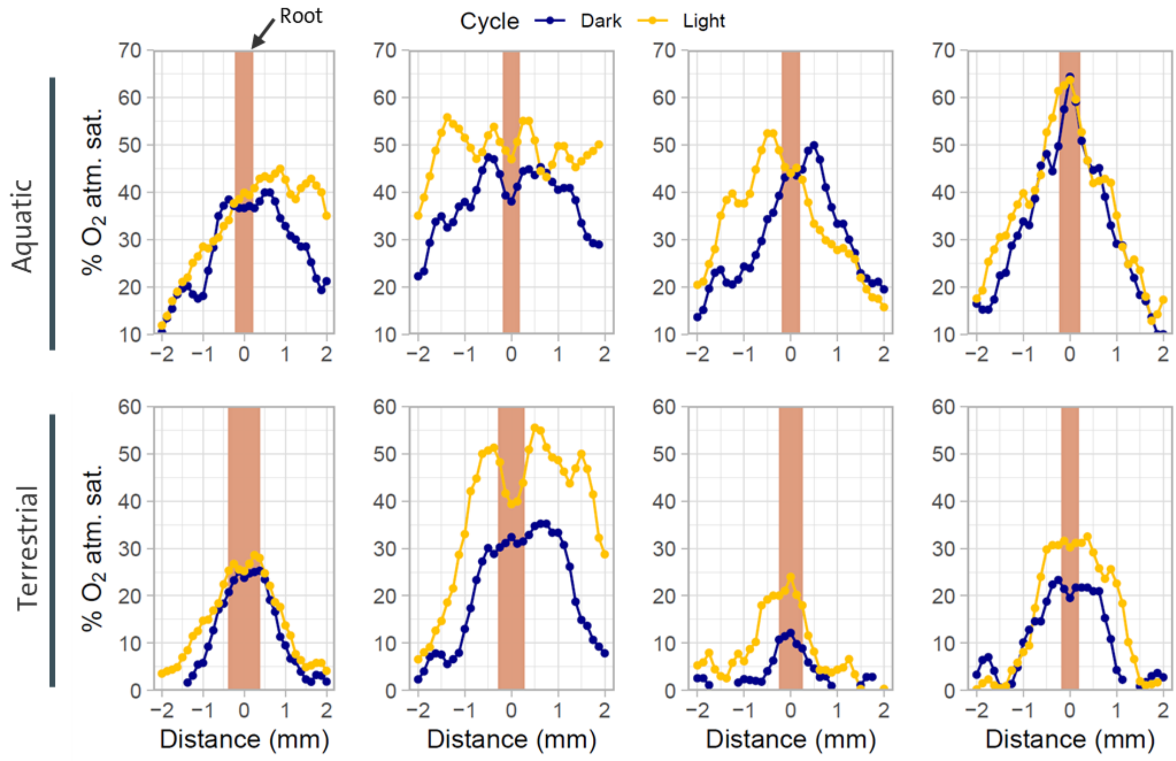
This study includes very few replicates (*n*=4) per analyte-life form pairing but is nevertheless comparable to other planar optode studies (e.g., Koop-Jakobsen et al., 2018; Lenzewski et al., 2018; Holz et al., 2020). More extensive replication and similarly longer observation windows could help to capture more variation associated with the examined root traits within each respective life form. Furthermore, this study could be augmented with additional measurements the DIC concentrations in the overlying water above the sediment. This could in part explain the large variation in bulk sediment CO_2 concentration between the life forms.

Conclusion

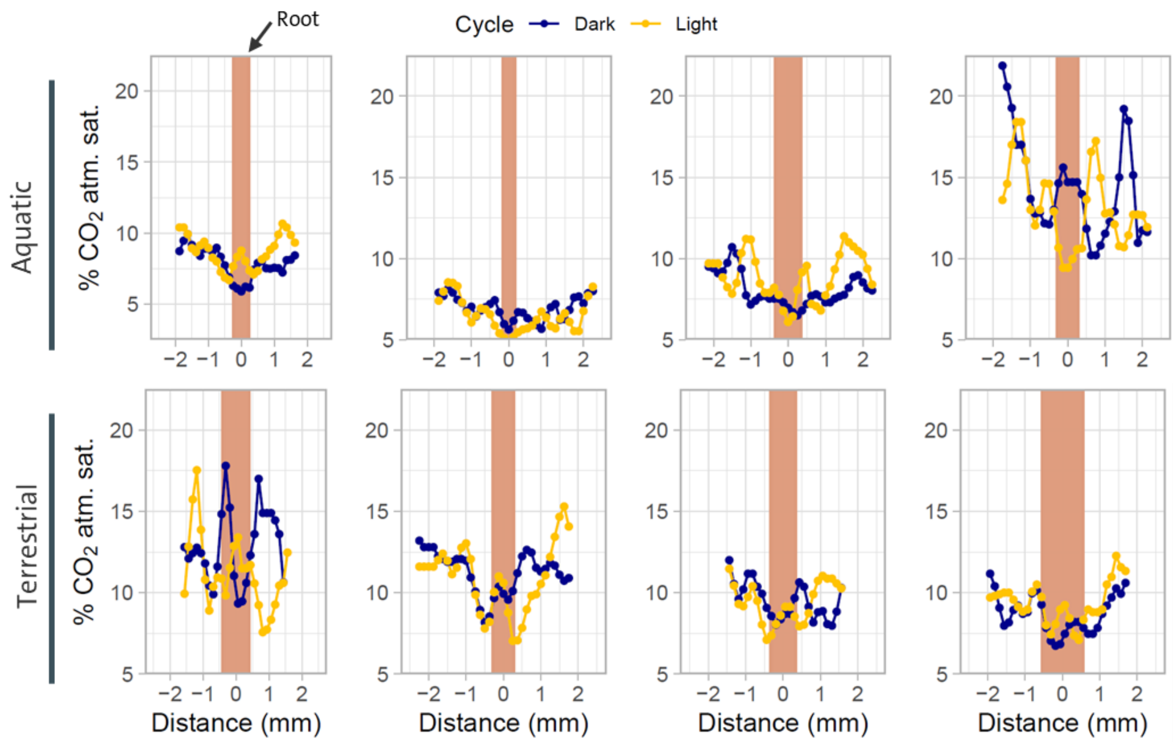
Although support for the reduction of bulk soil CO_2 concentrations could be demonstrated in all investigated plants of this study, CO_2 uptake as well as sediment oxygenation appeared most efficient in aquatic *Littorella*. The efficiency of the gas exchange with the sediment was heavily influenced by water level and light availability in the terrestrial life form. As sediment oxygenation and uptake of CO_2 are significant adaptive traits for oligotrophic macrophytes, these differences in phenotypic response to immersion may potentially have consequences for the overall fitness of the life forms.

The displacement of isoetid species in oligotrophic, softwater lakes is threatened by several processes including eutrophication and acidification. These processes are likely to result in the establishment of larger macrophytes, e.g. *Juncus bulbosus* (Smolders et al., 2002), phytoplankton (Sand-Jensen & Søndergaard, 1981) or epiphytic algae (Andersen et al., 2005) that further places small, slow-growing isoetids at a competitive disadvantage and reducing populations. The ecophysiology of isoetids like *Littorella uniflora*, however, make them an exemplary group to explore mechanisms controlling adaptive traits as well as the possible trade-offs associated with phenotypic plasticity in flood-adapted or even emergent wetland species.

Supplementary Figures



Supplementary Figure S3.1. Spatial distribution of $\%O_2$ (atm. sat.) in aquatic and terrestrial) *Littorella* life forms in the final dark (blue; 108 h) and light (yellow; 120 h) periods. Brown bars represent the position (centered at 0) and respective width of the selected roots.



Supplementary Figure S3.2. Spatial distribution of $\%CO_2$ (atm.) in aquatic and terrestrial *Littorella* life forms in the final dark (blue; 108 h) and light (yellow; 120 h) periods. Brown bars represent the position (centered at 0) and respective width of the selected roots.

Box B Patterns of radial oxygen loss in the rhizospheres of salt marsh plants

Planar optodes enable real-time, non-invasive observation of biogeochemical processes in the rhizosphere. Planar optodes were first introduced by Glud et al. (1996) for the study of oxygen distribution in benthic sediments but have since been advanced for use in controlled laboratory experiments targeting analytes such as pH (Larsen et al., 2011), O_2 (Askaer et al., 2010; Larsen et al., 2011; Lenzewski et al., 2018), CO_2 (Lenzewski et al., 2018; Holz et al., 2020). Inferring spatiotemporal O_2 dynamics from two-dimensional images replaces the need for conducting numerous one-dimensional point measurements with other electrodes. Measurements can be taken at a predetermined frequency over a time series provides information on the temporal variation of specific analyte concentration over the area of the foil or regions of interest within. Planar optodes work via dynamic quenching of fluorophore immobilized within the foil (Tschiersch et al., 2011). Upon excitation, the presence of e.g. oxygen diminishes or quenches the intensity of the fluorescent signal emitted by fluorophore which is simultaneously recorded with a modified camera system.

Methodological approach

Commercially available O_2-sensitive planar optode foils (4x4 cm^2, 0-100%; PreSens Precision Sensing GmbH) with a spatial resolution of up to 25 µm were used to quantify O_2 in the rhizosphere of salt marsh plants under waterlogged conditions. Images were taken with the VisiSens TD system every six minutes for a total of 24 hours. Calibration and software evaluation were conducted as described in Lenzewski et al. (2018) for temporal O_2 analysis. Plant samples were collected from the salt marsh in Kaiser-Wilhelm-Koog and cultivated for five months in the greenhouse (Universität Hamburg) prior to optode measurements. Plants were implanted into self-made rhizoboxes (2 x 25 x 25 cm) and inundated twice a week with freshwater to maintain flood-adapted characteristics. To investigate differences in radial oxygen loss based on substrate type, measurements were conducted on marsh soil (pioneer zone, Kaiser-Wilhelm-Koog), a mixed marsh-sand sediment (1:2), or sand.

Figure BB.1. Depiction of plant samples directly after implantation into rhizoboxes. Plants were incubated in tidal tanks for four months (Jan-Apr) that flooded twice a week. Rhizoboxes were angled and covered with aluminum foil to discourage light exposure of the roots (not depicted). Five biological replicates per species were cultivated (*n*=30)

For optode investigations, plant samples were removed from their rhizoboxes (depicted in Figure BB.1), rinsed and carefully re-arranged on freshly prepared substrate inside custom rhizoboxes on a flat horizontal surface. This was to avoid complications due to excessive growth and ensure that representative roots could be properly targeted for observation. Plant samples with low belowground or aboveground biomass, deformed or otherwise unsuitable roots were discarded (Figure BB.2).

Figure BB.2. Example of overgrown root system of *Aster tripolium* in rhizobox (A) and stunted *Spartina anglica* root system (B).

The optode foil was carefully attached in such a manner that air bubbles and sediment particles on the foil were painstakingly avoided. After placement of the optode foil on the targeted root(s), an additional larger transparent foil was placed on top to further discourage movement of substrate particles or water droplets onto the fluorescent optode foil. Following application of the optode foil, the entire closed rhizobox tightly secured, lifted to an upright position and transferred to an incubated basin for up to 24 hours prior to the start of the measurement (Figure BB.3). This incubation step helped to reduce the initial signal of background noise from sediment or temperature flucatuations.

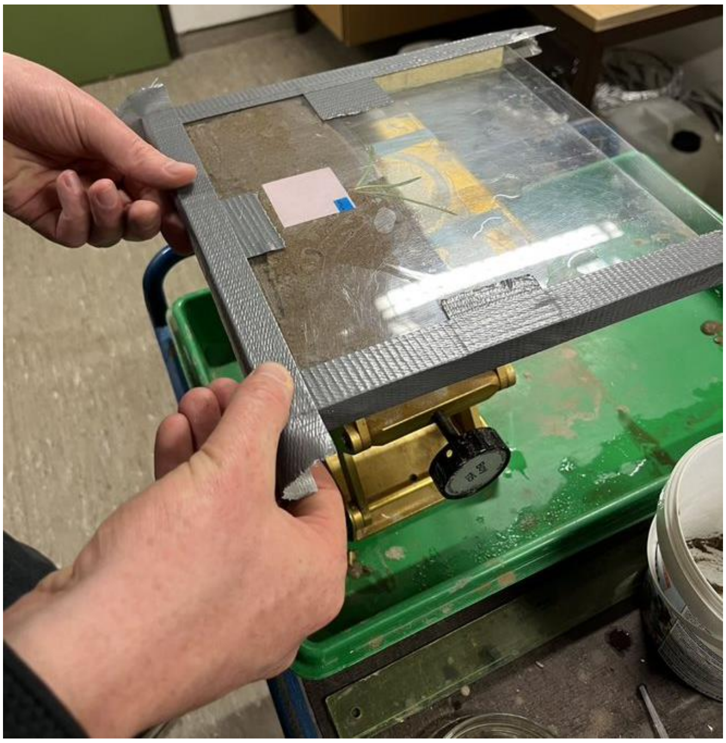


Figure BB.3. Depiction of sample preparation of plant material in rhizoboxes with optode foil and protective transparent foil. Before and after images were taken for each measurement to determine the position of the roots and possible movement over the 24-hour measurement.

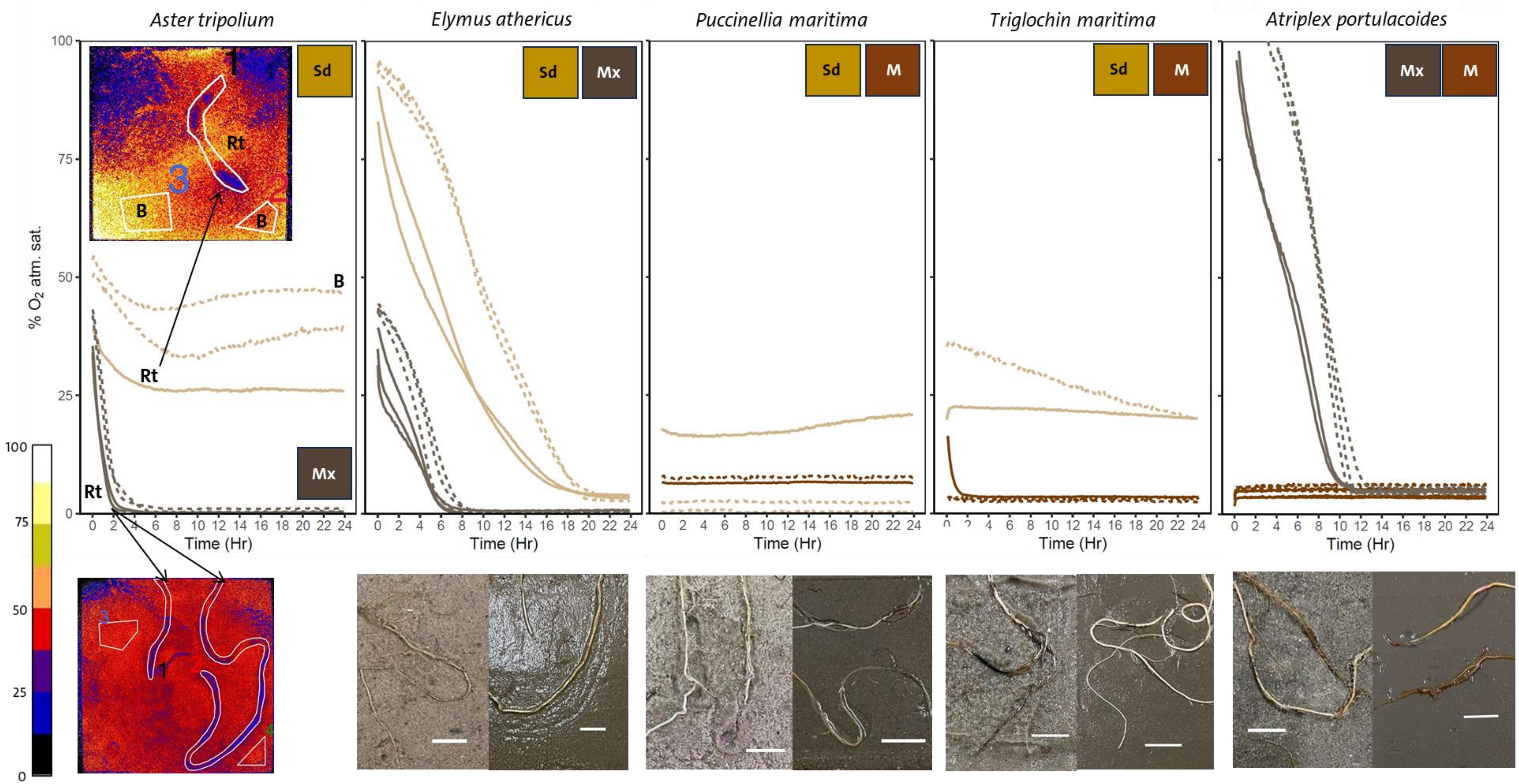


Figure BB.4. Temporal variation of sediment O_2 of rhizoboxes implanted with *Aster tripolium*, *Elymus athericus*, *Puccinellia maritima*, *Triglochin maritima*, *Atriplex portulacoides*. Various substrates were used to compare the ROL profiles within species (Sd = sand, Mx = mixed sediment, M = marsh sediment). For each planar optode observation, soil regions around single or multiple roots (Rt) and bulk sediment (B) were observed over 24 hours. A single exemplar of *Spartina anglica* is featured in Fig. 2.4a

4 Transcriptomic response of wetland microbes to root influence

Adapted from published article in *iScience*
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Abstract
Wetlands are crucial for carbon and nutrient cycling. The pivotal role of plant-microbe interactions in driving wetland biogeochemistry is widely acknowledged, prompting research into their molecular mechanisms for a deeper understanding of wetland biogeochemical processes. We analyzed soil microbes' transcriptomic responses to root exudates in coastal wetland soils using $^{13}CO_2$ pulse-labeling. Metatranscriptomics revealed 388 upregulated and 11 downregulated genes in response to root exudates. The Wood-Ljungdahl pathway and dissimilatory sulfate reduction/oxidation were the most active microbial pathways independent of root influence, whereas pathways with the strongest upregulation in response to roots were related to infection, stress response, and motility. We revealed shifts within the active community towards higher relative abundances of Betaproteobacteria, Campylobacterota, Kritimatiellota, Lentisphaerota, and Verrucomicrobiota in response to exudates. Overall, this study improves our mechanistic understanding of wetland plant-soil microbe interactions by revealing the phylogenetic and transcriptional response of soil microorganisms to root influence and exudate input.

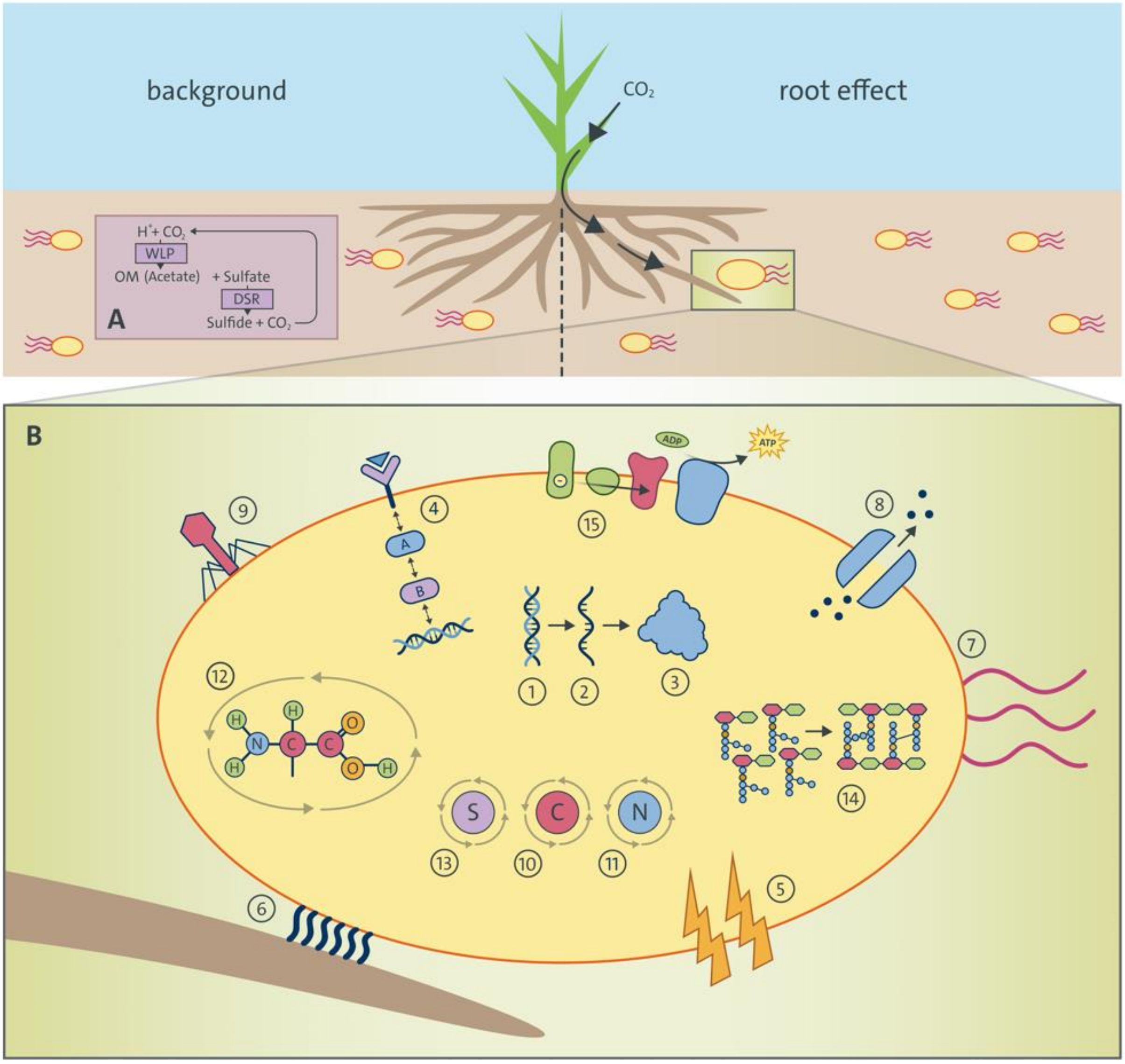


Figure 4.1. Differential gene expression in soil microbes under plant influence vs. independent conditions. Conceptual representation of upregulated microbial processes in response to plant influence (B) and independent of plant influence (A). Yellow objects represent the soil microbes. Independent of plant influence, key genes of the Wood-Ljungdahl pathway and dissimilatory sulfate reduction exhibit the highest transcript abundances (A). Frame B shows a zoom into one of the microbes influenced by exudate input. In response to exudate input, 338 genes show significant upregulation (adjusted $p < 0.05$) and can be categorized into the following metabolic pathways. 1) Transcription, 2) Translation, 3) Proteostasis, 4) Signal transduction and exchange, 5) Stress response, 6) Adhesion, 7) Motility, 8) Transport, 9) Infection, 10) Carbon metabolism, 11) Nitrogen metabolism, 12) Amino acid metabolism, 13) Sulfur metabolism, 14) Cell wall synthesis and 15) Electron transport. Image by courtesy of UHH/Alpen.

Introduction

The rhizosphere is defined as the volume of soil influenced by roots (Hiltner, 1904). Roots influence the physical and chemical properties of the soil matrix, the availability of soil resources (e.g., water and dissolved nutrients), and the activity of soil biota within the rhizosphere. The extent of root influence is a heterogeneous continuum that is characterized by strong biological, physical, and chemical gradients (Koop-Jakobsen et al., 2018; Koop-Jakobsen et al., 2021). These gradients are produced by a complex array of factors, including biota-mediated processes such as rhizodeposition, root and microbial respiration, plant nutrient uptake, microbial signaling and microbial degradation (Kuzyakov & Razavi, 2019; Ren et al., 2022). Competition over limited soil resources prompt intense interactions between plant roots and soil biota, rendering the rhizosphere a hotspot for biological activity and elemental exchange (Neumann & Römheld, 2012; Kuzyakov & Blagodatskaya, 2015).

Rhizodeposition is a key process shaping plant-soil microbe interactions in the rhizosphere (Dijkstra et al., 2021). While rhizodeposits encompass all root-derived carbon that enters the soil matrix regardless of origin and release mechanism (Oburger & Jones, 2018), organic inputs from intact, living roots, i.e. root exudates (Neumann, 2007) are particularly strong drivers of the microbial community (Nguyen, 2009; el Zahar Haichar et al., 2014; Eisenhauer et al., 2017). Root exudates include low-molecular-weight compounds (e.g., sugars, amino acids, B-vitamins and organic acids; Canarini et al., 2019) and secondary metabolites (e.g., phenolics, flavonoids and terpenoids; Gargallo-Garriga et al., 2018) as well as high-molecular-weight compounds (e.g. polymer-rich mucilage and proteins). Long-term tracer studies using isotopically labeled CO_2, e.g. continuous labeling, have been conducted to quantify net rhizodeposition (Kuzyakov & Domanski, 2000; Jones et al., 2004; Pausch & Kuzyakov, 2018). However, pulse labeling, applied over short periods, is best suited to trace the fraction of new assimilates (Studer et al., 2014; Oburger & Jones, 2018), dominated by low-molecular-weight compounds via root exudation into the rhizosphere (Meharg, 1994). Moreover, young roots have shown to be preferentially enriched in pulse labeling experiments (Stevenel et al., 2019).

There are a number of favorable and adverse effects of root exudates on soil microbial communities (Bais et al., 2006). These biotic interactions are enabled through complex chemical signaling both to and from the roots (Hirsch et al., 2003; Rasmann & Turlings, 2016; Wang et al., 2021). Root exudates provide carbon- and energy-rich metabolic substrates for microorganisms in an otherwise carbon-limited environment. The input of carbon and increased soil respiration can shift the microbial community composition towards rapidly growing bacteria, *r*-strategists (Sun et al., 2021). Numerous studies have demonstrated that the plant-controlled recruitment of rhizosphere microorganisms such plant growth-promoting bacteria (Samaras et al., 2022) and symbiotic mycorrhizal fungi (Jones et al., 2004) is regulated by root exudates (Herz et al., 2018; Schmid et al., 2019). Additionally, the exudation of secondary metabolites, antimicrobial compounds or chemo-attractants can be seen as a plant-defense mechanism to suppress pathogenic microbial groups (Bais et al., 2004; Bezerra et al., 2021). Finally, plant-derived B-vitamins and other metabolites can affect the growth of the soil microbes (Rovira & Harris, 1961; Streit et al., 1996).

The majority of rhizosphere studies on plant-soil microbe interactions has been conducted in upland terrestrial soil systems, with a particular focus on crops in agricultural soils. Studies on the rhizospheres of legumes (Phillips & Streit, 1996; Hartmann et al., 2008), wheat (Ji et al., 2023), barley (Kuzyakov & Domanski, 2000; Timmusk et al., 2011) and maize (Rudolph-Mohr et al., 2017; Rüger et al., 2021) have revealed many aspects of rhizosphere biology, such as the quantitative estimation of rhizodeposition (Kuzyakov & Schneckenberger, 2004; Qiao et al., 2017; Pausch & Kuzyakov, 2018), microbial substrate utilization (Oburger & Jones, 2009), nutrient competition between plants and soil biota (Dijkstra et al., 2013), and the regulation of rhizosphere processes under elevated temperature (Zhang et al., 2020). Yet, while there is a growing body of research refining our understanding of plant-soil microbe interactions in upland terrestrial soils, there remains a significant gap in our knowledge regarding the transferability of these findings to plant-soil microbe interactions in rhizospheres characterized by contrasting abiotic and biotic environmental conditions, such as those found in wetlands and marine sediments (Ren et al., 2022; Rolando et al., 2022). (Meta-)transcriptomic approaches are increasingly being used to elucidate key genes expressed in wetland plant-microbe interactions (Su et al., 2019; Cai et al., 2022; Rolando et al., 2023), however transcriptomic analysis of prokaryotes in wetland rhizospheres remains a definite challenge (Carvalhais et al., 2013) with markedly few studies available (e.g., Lu & Conrad, 2005; Rolando et al., 2023).

Frequent flooding and waterlogging in wetland soils dramatically reduces the availability of oxygen, thereby limiting terminal electron acceptors and leading to significantly lower rates of major microbial metabolic pathways as well as a shift towards anaerobic metabolism compared to well-aerated upland soils (Megonigal et al., 2004). The impact of oxygen limitation on metabolic diversity has been predominantly studied in hydric soils as anaerobic sites in upland systems are mainly restricted to the interior of aggregates (Keiluweit et al., 2017). However, in wetlands, microbial communities are majorly constrained by both carbon substrates (i.e., electron donors) and the availability of oxygen or alternative terminal electron acceptors (Sutton-Grier et al., 2011). Opposing gradients in wetland rhizospheres, however, may potentially result in fundamentally different mechanisms of plant-microbe interactions and magnitudes of root exudate effects. The low redox potential of wetland soils and their implications on soil metabolic diversity may also become more relevant for upland systems as wetland root traits become an increasingly sought-after solution to flooding tolerance in crop plants (Yamauchi et al., 2013).

The present study aims to improve our mechanistic understanding of wetland plant-soil microbe interactions by studying the phylogenetic and transcriptional response of soil microorganisms to root influence and exudate input. Using $^{13}CO_2$ pulse labeling and carbon tracing, we analyzed the microbial response to root influence in the anoxic rhizosphere of the common coastal wetland grass *Spartina anglica*. In particular, we aimed to determine the extent of root exudation on microbial gene expression, identify which metabolic pathways are affected, understand which microbial taxonomic groups are primarily involved, and compare these responses to the bulk soil microbial community. With this we aim to deepen our understanding of wetland ecosystem dynamics.

Material and Methods

To evaluate the influence of root activity and, particularly, exudate input on soil microbial gene expression, we conducted a $^{13}CO_2$ -pulse labeling experiment and traced the flux of recently formed photo-assimilates into the rhizosphere. We used the ^{13}C signal of soil samples as a proxy for the presence of root exudates (Figure S4.2) and distinguished soil and rhizosphere micro-environments that received root exudates (n = 3) from those that did not (n = 3) using isotope-ratio mass spectrometry. We conducted metatranscriptome sequencing of the soil microbial

RNA (of bacteria and archaea) and employed both functional annotation and a differential expression analysis for N = 6 metatranscriptomes.

Plant cultivation

Spartina anglica (also known as *Sporobolus anglicus*) is a common salt marsh plant along the European Atlantic coast (Granse et al., 2022). It is the direct allopolyploid descendent of the hybrid *Spartina x townsendii*, which formed in Southern England by hybridization between native *S. maritima* and the introduced *S. alterniflora* (originating from North America) at the end of the 19th century. All individuals of *Spartina anglica* and soil were sampled in June of 2021 from the pioneer zone of a Wadden Sea salt marsh situated at the Hamburger Hallig (54°36'06.2"N, 103 8°49'00.1"E) and cultivated thereafter in a greenhouse on a mixture of native pioneer zone soil, sand and fertilizer until the start of experimentation at the Institute of Plant Science and Microbiology (Universität Hamburg, Hamburg, Germany). Plants were given several weeks to adapt to greenhouse conditions upon sampling from the field. Two weeks prior to pulse labeling, three individuals of similar size and vitality (new shoot growth was observed) were rinsed and transferred into non-transparent plant pots without fertilizer and filled only with freshly homogenized and sieved native soil (pioneer zone; Hamburger Hallig). All pots, including unplanted control pots, were kept under waterlogged conditions prior to and during pulse labeling.

$^{13}CO_2$ pulse labeling

We used a plexiglass cylinder (thickness: 3 mm, diameter: 200/194 mm, height: 45 cm) with a removable lid to construct a labeling chamber. The chamber was equipped with a fan for air mixing and a sensor measuring atmospheric CO_2, temperature, and humidity (K33 LP T/RH Sensor; Senseair). The sensor was connected to an Arduino computer outside the chamber, which recorded the data on a SD card. Only the aboveground plant biomass was placed inside the chamber for $^{13}CO_2$ labeling and was sealed around the shoot base using clamps and plastic film (Figure S4.1). $^{13}CO_2$ was produced inside the chamber by adding 8 mL of 10% HCl to 0.1 g $NaH^{13}CO_3$ (Sigma-Aldrich, St. Louis, USA). We used a 300-W LED light (Roleadro) as a light source for plant photosynthesis (Figure S4.1). Plants were labeled daily over eight consecutive days. Label duration was 2-3 h and dependent on the time it took for the plants to draw chamber CO_2 concentrations (>1500 ppm) well below atmospheric concentrations (250-350 ppm).

Soil sample collection and quantification of ^{13}C enrichment analysis

After eight days of daily pulse-labeling, intact soil sods (2 liter) extracted from three planted pots and one unplanted control pot were vertically separated into two halves exposing the soils and rhizospheres for sampling. 10 soil subsamples (approximately 1.5 cm^3) were collected from each soil sod at random positions along a transect from the bulk soil toward the root mass using a spatula (N = 40). Subsequently, all 40 soil samples were frozen in 2-ml cryotubes using liquid nitrogen and stored at -80°C until further processing.

Soil samples were dried at 60°C to constant weight and ground using a ball mill (Retsch, Haan, Germany). Carbonates were removed through direct acidification of the soil samples using 10% HCl. Sample were weighed into tin capsules and analyzed for their isotopic ^{13}C signatures using an element analyzer (EURO-EA 3000, Euro Vector, Pavia, Italy) coupled to an isotope-ratio mass spectrometer (Nu Horizon, Nu Instruments, Wrexham, United Kingdom). Among these 40 samples, bulk and rhizosphere soil samples were not pre-determined by their proximity to the root but were operationally defined by their ^{13}C signature (Figure S4.2). Soil samples with isotopically enriched ^{13}C signatures were defined as impacted by root exudates, i.e. rhizosphere soil, whereas soil samples that showed no indication of ^{13}C enrichment in relation to control soils were defined as not affected by exudates, i.e. bulk soil. All soil samples were ranked by their ^{13}C enrichment in relation to control soils, and samples with the greatest ^{13}C enrichment (n = 3) were compared to samples that showed no ^{13}C divergence from the control (n = 3).

RNA and DNA extraction from soil
2 g of soil were used for RNA extraction using the RNeasy PowerSoil Kit (Qiagen, Venlo, Netherlands) following the manufacturer's protocol. RNA concentrations were quantified using the Qubit 2.0 Fluorometer and the RNA High Sensitivity Assay Kit (RNA HS, Thermo Fisher, Berlin, Germany). To efficiently assign the RNA sequences to the respective genes, we also sequenced the DNA of two soil samples (one from the rhizosphere and one from the unplanted soil). DNA was extracted from soil samples, and frozen at -80°C until analysis. 0.5 g of soil were used for DNA extraction using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. Subsequently, the isolated DNA was analyzed at a wavelength of 280 nm using a Nanodrop spectrophotometer (NanoDrop 2000, Thermo Scientific, Waltham, USA).

Metatranscriptome and metagenome analysis
First, the two metagenomes were combined and co-assembled. We used the ATLAS pipeline v2.12.0 (Kieser et al., 2020) that includes an extended workflow for quality control, contig assembly, gene prediction and functional annotation, binning of contigs into MAGs and taxonomic annotation of the MAGs. Among the included tools are several gold-standard tools, e.g. metaSPAdes v3.15.3 for read assembly (Nurk et al., 2017), eggNOG mapper v2.1 and eggNOG database v5.0 for functional gene annotation (Cantalapiedra et al., 2021), maxBin2 v2.2, metabat2 v2.15 and DAS_Tool v1.1.4 for binning and refinement of MAGs (Wu et al., 2016; Sieber et al., 2018; Kang et al., 2019), checkM v1.1.10 for MAG quality assesment (Parks et al., 2015) and GTDBtk tool v2.1.1 with GTDB database release207 for taxonomic annotation of the MAGs (Chaumeil et al., 2020). Default parameters were used, except for RAM (up to 1.5 TB) and CPU/threads (up to 80 threads). The co-assembled metagenome had a total of 38 million reads assembled into 87362 contigs with an N50 value of 14,475 (Table S4.1). 13 MAGs with contamination <10% and with completeness between 70 and 99.8% were extracted. Second, the metagenome data for individual samples were quality controlled using the ATLAS pipeline and the high-quality reads were mapped to the MAGs from the co-assembly using minimap2 v2.14 (Li, 2021) to assess the abundance of the MAGs within the single samples (Table S4.2). Third, metatranscriptomes were quality controlled using the ATLAS pipeline and high-quality reads were mapped to the genes detected on the assembled contigs from the metagenome co-assembly using minimap2 v2.14 (Li, 2021). Differential genes expression analysis was performed using R 4.0 and DESeq2 package v1.34 (Love et al., 2014). Transcript counts were normalized using the RPM (read per million mapped reads) normalization method to correct for different

sequencing depth between the sample. One sample (S3_10_low) has low total reads (Table S4.2), but this should be accounted for by DESeq normalization and reflected by a poorer p-value.

Future studies could increase sequencing depth to 40 million reads for better data accuracy. To reduce the large proportion of eukaryotic reads, removing polyA-tailed RNA during library preparation may enrich prokaryotic RNA.

Quantification and statistical analysis

All transcriptomes were filtered for only prokaryotic reads, to exclude reads that were assigned to the plant. We did this by employing the ATLAS pipeline, which includes the metaSPAdes tool optimized specifically for prokaryotic reads filtering. Despite the more complex gene structures of eukaryotes, such as exons and introns, making gene prediction more challenging, parts of eukaryotic genes could still be predicted and annotated by eggNOG. Subsequently, in the second step, we removed all gene queries annotated to eukaryota. This constituted 0.38% of all gene queries with at least one transcript assigned. Further, 3.77% of all gene queries could not be taxonomically assigned (Figure S4.3). Since these genes also could not be functionally assigned, they were excluded from the key gene analysis (Figure 4.3) and the differential expression analyses where we investigated the impact of root exudates on soil microbes (Figure 4.5). With this we ensured to only analyze the response of bacteria and archaea to root influence. The selection of genes for the key gene analysis (Figure 4.3) was modified after Yang et al. (2021), to cover the carbon, nitrogen and sulfur cycling of anoxic ecosystems, and Grueterich et al. (2024), to additionally cover key genes of dark CO_2 fixation. The key gene analysis is based on all transcripts independent of differential expression. The given transcript abundances depict the average of six metatranscriptomes.

For identification of differentially expressed genes, we applied an adjusted p-value < 0.05 and a $\log_2$ fold change > 2, < -2. To investigate which metabolic pathways are upregulated and which are downregulated in response to plant exudate influence, pathway annotation was performed on genes with a significant change in gene expression ($p < 0.05$) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and complemented by in depth literature search in order to cover most of the genes, that could not be assigned through the KEGG database. The distribution of all differentially expressed genes assigned to a given pathway subcategory was visualized in a box and whisker plot in the style of Tukey. All data points that extend beyond 1.5 * IQR of the upper hinge (upper whisker) or below 1.5 * IQR of the lower hinge (lower whisker) are defined as outliers.

Data availability

Raw reads of the metagenomic and metatranscriptomic analysis were deposited at the European nucleotide archive ENA under the project accession number PRJEB73855 (Biosamples: SAMEA115420425 - SAMEA115420432). The gene catalog is provided as supplementary table 2.

Results

Microbial gene expression

$^{13}CO_2$ pulse labeling and carbon tracing were employed to study the microbial response to root exudate input in the anoxic rhizosphere of the common coastal wetland grass *Spartina anglica*. A total of six metatranscriptomes of the root and rhizosphere microbiota (Tables S4.2, S4.3) were studied. For each sample, three independent biological replicates were analyzed. For all six metatranscriptomes an average of 16 million reads was obtained. In total, 85,886 genes showed a minimum of 1 transcript.

After setting the p-value and $\log_2$ fold change significance thresholds (see methods section *Metagenome and metatranscriptome analysis*), we observed that 388 of the resulting differentially expressed genes were significantly (adjusted $p<0.05$) upregulated and 11 were significantly (adjusted $p<0.05$) downregulated (Figure 4.2A) in response to root exudate influence. A principal component analyses (PCA) using normalized counts showed a clear distinction between the three replicates with high root exudate influence and the three replicates with no root exudate influence. The first principal component (PC1) explained 63% of the variance between the two depicted groups (Figure 4.2B).

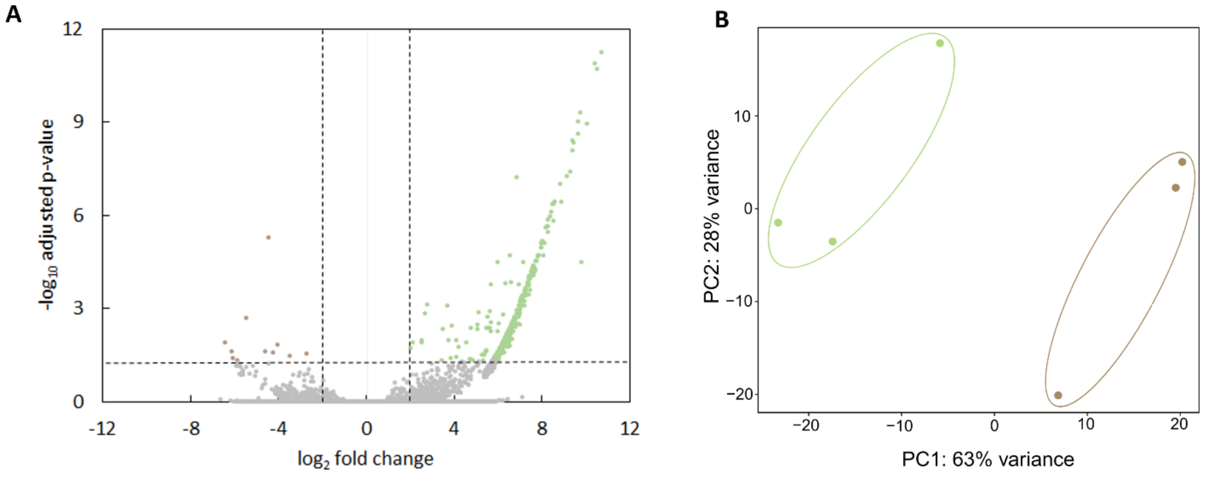


Figure 4.2. Volcano plot and PCA analysis of gene expression in response to root exudate influence in soil microbes. Volcano plot depicts all 85,886 genes with a minimum of 1 transcript assigned. An adjusted p-value < 0.05 and a $\log_2$ fold change > 2, < -2 were used as cutoff (dashed lines). Colored dots indicate genes with significant upregulation (green) or downregulation (brown) in response to root exudate influence. Grey dots indicate genes without significant differential expression (**A**). PCA using normalized counts shows a separation between the three replicates with high root exudate influence (green) and no root exudate influence (brown) (**B**).

Gene expression of the background microbial community

We conducted a key gene analysis on microbial processes independent of root exudate influence to establish the background environmental context of the bulk soil microbial community without root influence. This analysis examined the transcript abundances of indicator genes involved in the carbon, nitrogen, and sulfur cycles (Figure 4.3A) (Yang et al., 2021; modified after Grueterich et al., 2024)

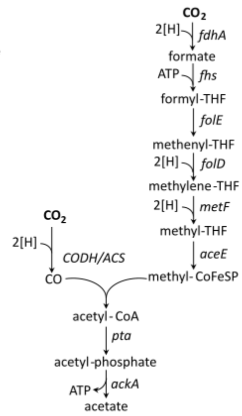
Key genes of the Wood-Ljungdahl pathway (WLP) (*CODH/ACS*) and dissimilatory sulfate reduction/oxidation (DSR) (*sat*, *aprA*, *aprB*, *dsrB*) exhibited the highest transcript numbers across all investigated pathways independently of differential expression with transcript abundances of 4093 (*aprA*), 967 (*dsrA*), 1235 (*dsrB*) and 1170 (*CODH/ACS*) (FIGURE 3A). We dissected both WLP and sulfur metabolism into their constituent genes and examined their respective transcript abundances (Figure 4.3B-C). We observed that within the pathways some genes are higher transcribed than others. For the WLP the last two genes (*pta, ackA*), that are responsible for the conversion of acetyl-CoA to acetate, showed nearly no transcripts. Regarding sulfur metabolism, only the full gene repertoire for DSR showed notable transcript numbers.

A

	genes	average transcript abundance
Denitrification	*nosZ*	9
	norB	3
	nirk	0
	nirS	4
	napA	22
	narG	6
Dissimilatory nitrate reduction	*nirB*	8
	nrfA	10
Methanogenesis	*mcrA*	0
Methylotrophy	*mdo*	0
Nitrification	*amoA*	0
	hao	0
Nitrogen fixation	*nifD*	1
	nifH	3
	nifk	2
Sulfur metabolism	*aprA*	4093
	dsrA	967
	dsrB	1235
Thiosulfate oxidation	*soxA*	4
	soxB	57
	soxY	34
Wood-Ljungdahl pathway	*CODH/ACS*	1170
rTCA cycle	*aclAB*	0
RuBisCO	*cbbL*	62
	rbcS	5
DC/4-HB cycle	*abfD*	0
3-HP bicycle	*accD*	22
	PCCB	5
3-HP/4-HB cycle	*K15019*	0
Fructose degradation	*xylA*	26
Glycolysis	*pfkA*	58
	pyk	105
Pentose phosphate pathway	*gnd*	33

B

Wood-Ljungdahl pathway	genes	average transcript abundance
formate dehydrogenase	*fdhA*	223
formyl-THF synthetase	*fhs*	3063
formyl-THF cyclohydrolase	*folE*	45
methylene-THF dehydrogenase	*folD*	263
methylene-THF reductase	*metF*	144
methyltransferase	*aceE*	141
CO dehydrogenase/acetyl-CoA synthase	*CODH/ACS*	1170
phosphotransacetylase	*pta*	38
acetate kinase	*ackA*	19



C

Sulfur metabolism		
Assimilatory sulfate reduction	*sat*	812
	cysNC	59
	cysH	7
	cysIJ	11
Dissimilatory sulfate reduction/oxidation	*sat*	812
	aprA	4093
	aprB	1066
	dsrA	967
	dsrB	1235
SOX system	*soxA*	4
	soxB	57
	soxC	5
	soxD	1
	soxX	17
	soxY	34
	soxZ	3

Figure 4.3. Average transcript abundance of key genes in carbon, nitrogen, and sulfur cycles. Average transcript abundance based on n=6 metatranscriptomes of selected key genes of the carbon, nitrogen, and sulfur cycles (**A**), entirety of genes involved in the WLP (**B**) and entirety of genes involved in sulfur metabolism (**C**). See also Table S4.4.

To obtain further insight into which microbial groups drove sulfate metabolism and WLP, we performed phylogenetic analysis. The top three microbial groups behind sulfate metabolism were Thermodesulfobacteriota with 34.7%, Gammaproteobacteria with 24% and

Deltaproteobacteria with 10.7%. The main microbial groups involved in the WLP were Thermodesulfobacteriota with 66.7% and Deltaproteobacteria with 25%. Community composition for the sulfate metabolism based on all 75 gene entries assigned to sulfur metabolism key genes. Community composition for the WLP based on all 12 gene entries assigned to the WLP key genes.

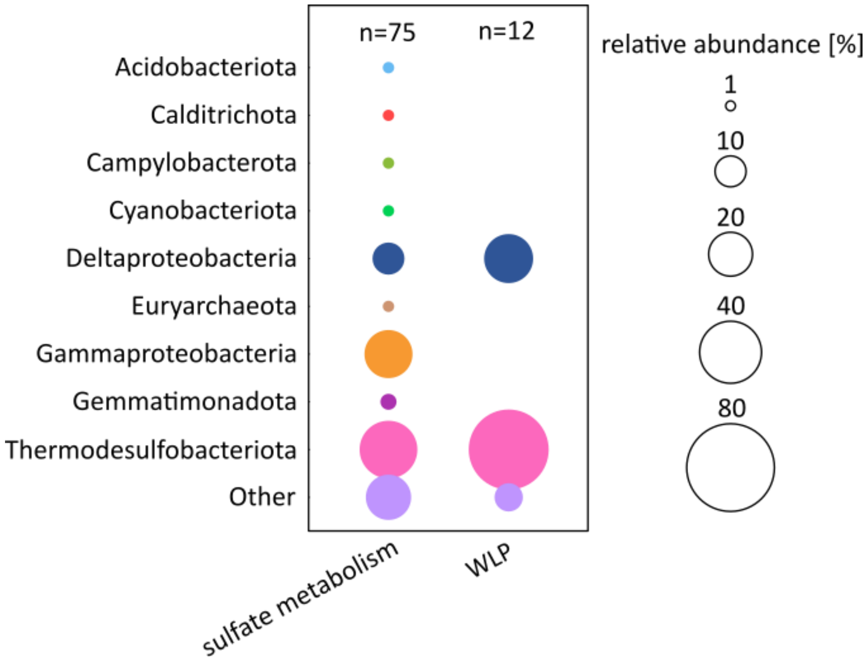


Figure 4.4. Microbial composition of sulfur metabolism and Wood-Ljungdahl pathway assigned transcripts. Microbial community composition based on transcripts of key genes assigned to sulfur metabolism (first bar; n=75) and based on transcripts of key genes assigned to the WLP (second bar; n=12).

Transcriptomic changes in response to root exudates

For 338 differentially expressed genes ($p < 0.05$), pathway annotation was possible. Notably, 327 of these genes were upregulated and only 11 were downregulated. These genes were sorted for functional categories into four pathway supercategories and 15 subcategories (listed in Table 4.1). ‘Infection’, ‘Stress response’ and ‘Motility’ showed with 7.3, 7.0 and 6.8 the highest $\log_2$-fold change median values indicating the strongest effect of root exudate influence on these pathway categories (Figure 4.5). Within the 'Stress response' and 'Transport' categories, genes associated with oxidative stress and iron transport showed strong upregulation. Rubrerythrin ($\log_2$-fold change = 8.54) and thioredoxin ($\log_2$-fold change = 6.63) were linked to oxidative stress, while the FecR protein ($\log_2$-fold change = 6.24) and TonB-dependent receptor ($\log_2$-fold change = 5.84) were linked to iron transport (Table S4.2, Queries: Gene184520, Gene169819, Gene12770, Gene015030).

Table 4.1. Functional categories for KEGG pathway enrichment analysis of significantly upregulated genes. List of functional categories for KEGG pathway enrichment analysis of 338 annotated gene queries with a significant change in expression (adjusted $p < 0.05$). Median $\log_2$-fold change values are shown.

Pathway supercategory	Pathway subcategory	Log_2-fold change	Queries (n)
Gene Expression and regulation	Transcription	6.4	15
	Translation	6.1	51
	Proteostasis	6.3	24
Cellular Processes in response to environmental stimuli	Signal transduction and exchange	6.0	7
	Stress response	7.0	11
	Adhesion	6.5	16
	Motility	6.8	31
	Transport	6.2	37
	Infection	7.3	10
Carbon, nitrogen and sulfur metabolism	Carbon metabolism	6.3	53
	Nitrogen metabolism	6.1	15
	Amino acid metabolism	6.1	25
	Sulfur metabolism	6.7	7
Cellular structure and energy supply	Cell wall synthesis	5.9	5
	Electron transport	6.5	31

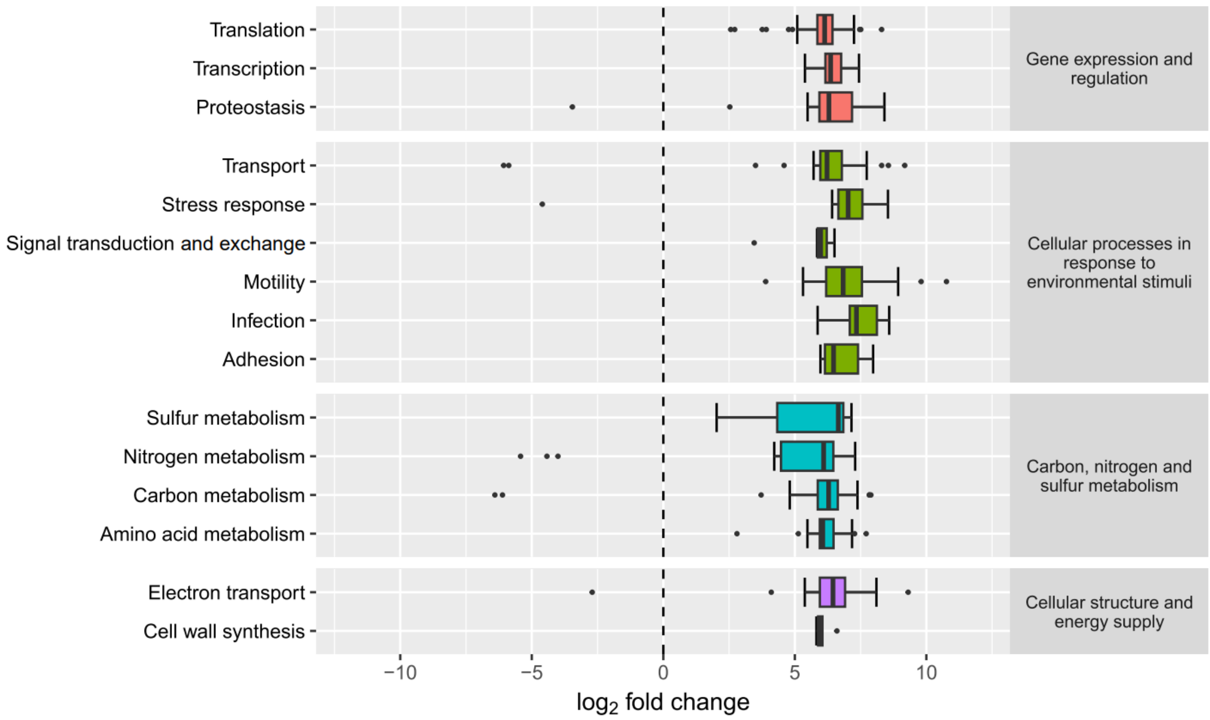


Figure 4.5. Boxplot of KEGG pathway enrichment analysis for significantly regulated genes under high root exudate influence. Boxplot of KEGG pathway enrichment analysis for 338 annotated gene queries with a significant change in expression (adjusted $p < 0.05$) grouped by functional categories (Table 4.1). Positive $\log_2$ fold changes reflect upregulation and negative $\log_2$-fold changes reflect downregulation of genes due to high root exudate influence. Outliers are displayed as individual points.

Table 4.2. Functional categories for KEGG pathway enrichment analysis of significantly downregulated genes. List of functional categories for KEGG pathway enrichment analysis of 338 annotated gene queries with a significant negative change in expression, i.e. downregulation (adjusted $p < 0.05$). Median $\log_2$-fold change values are shown. The downregulated genes shown in this table correspond to 11 gene queries.

Down regulated genes (Description)	Subcategory	$\log_2$-fold change
Glutamate dehydrogenase	Nitrogen metabolism	- 5.4
Glutamate dehydrogenase (*gdhA*)	Nitrogen metabolism	- 4.4
Glutamate dehydrogenase (*gluD*)	Nitrogen metabolism	- 4.0
Phycobilisome protein (*apcA*)	Carbon metabolism	- 6.4
Protein involved in exopolysaccharide biosynthesis	Carbon metabolism	- 6.1
PFAM ABC transporter	Transport	- 6.1
Predicted permease	Transport	- 5.9
COG1842 Phage shock protein A (IM30) (*pspA*)	Stress response	- 4.6
Peptidase S1C (*degP-2*)	Proteostasis	- 3.4
NADH ubiquinone oxidoreductase (*nuoF2*)	Electron transport	- 2.7
NA	NA	- 4.2

Microbial community shifts

A phylogenetic analysis was conducted to assess the shifts in the community caused by the presence and absence of root exudates, respectively. Initially, we assigned phylogenetic classifications to all transcripts present in the soil. The most abundant microbial groups, namely Gammaproteobacteria and Thermodesulfobacteriota collectively accounted for 51.4% of all transcripts.

In relation to the 388 transcripts that exhibited significant upregulation due to the influence of root exudates ($p < 0.05$), notable changes were observed in the proportions of certain taxa. Specifically, the proportion of Betaproteobacteria increased from 1% to 15.3%, Campylobacterota increased from 0.6% to 1.8%, Kritimatiellota from 0.8% to 13%, Lentisphaerota from 0.3% to 6.5%, and Verrucomicrobiota from 0.4% to 3.4%. 65.9% of all transcripts were assigned to Betaproteobacteria, Gammaproteobacteria and Kritimatiellota (Figure 4.6). Of the upregulated genes assigned to Betaproteobacteria, the majority were categorized into the pathway groups of translation, motility and transport. For Kritimatiellota these were carbon metabolism, translation and transport. For Lentisphaerota the upregulated pathway groups were carbon metabolism and translation and for Verrucomicrobiota they are motility and transport.

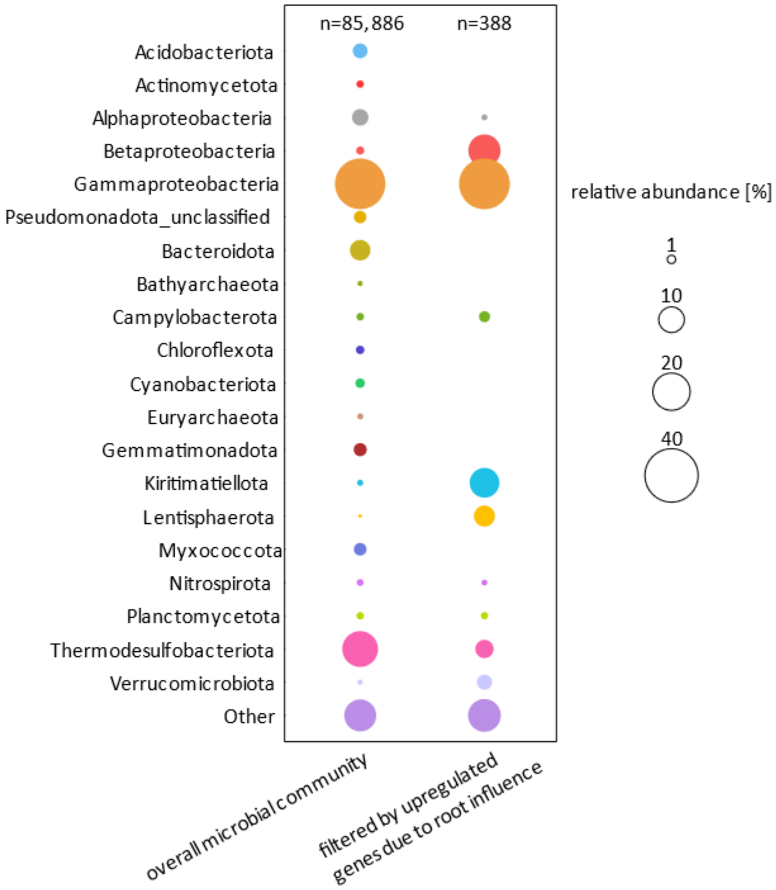


Figure 4.6. Microbial composition shift of root-influenced upregulated genes. Microbial community composition based on all transcribed genes (first column; n=91.563) and based on transcribed genes upregulated due to root influence (second column; n= 388; p adj. < 0.05).

Discussion

This study offers insight into the wetland microbial response to root exudation via metatranscriptomic analysis. We identified both upregulated and downregulated microbial metabolic pathways in response to exudate input (Figure 4.1, Tables 4.1, 4.2), shedding light on the complex plant-microbe interplay in wetland soils and assigned the microbial taxonomic groups which reacted to root exudates and compared them to those groups driving bulk soil microbial processes (Figures 4.4, 4.6). Notably, the upregulated fraction of genes was around 30-fold higher than the downregulated fraction.

Metabolic background of soil microbes independent of differential expression

We acquired metatranscriptomic data on microbial processes independent of root exudate influence (FIGURE 1A) to establish the background environmental context of the bulk soil microbial community subjected to root influence (FIGURES 1A and 1B).

The WLP and DSR were the primary metabolic pathways in the plant-independent soil microbial community of the bulk soil (Figure 4.3). It has been demonstrated in upland beech and pine forest soils that the WLP was one of the least predominant autotrophic pathways predicted (Akinyede et al., 2022). Further, in (semi-)arid soils it has been shown that the WLP had the lowest relative abundance of key genes compared to the other CO_2 fixing pathways (Yang et al., 2024). This study demonstrates that anaerobic wetland soils, in contrast, create conditions that favor the WLP. The prominence of WLP and DSR transcripts indicate the active utilization of these pathways under anoxic conditions, with a preference for low ATP consumption (Momper et al., 2017; Geng et al., 2022) While the dominance of sulfate reduction as an anaerobic microbial pathway in marine and coastal ecosystems is long established (Capone & Kiene, 1988; Lin et al., 2018; Jørgensen et al., 2019), recent metatranscriptomic work from our group and others also demonstrate a high prevalence of dark CO_2 fixation via WLP in the anoxic soils of these ecosystems (Yang et al., 2021; Grueterich et al., 2024). WLP and DSR are interconnected or mutually beneficial pathways, because the end product of the WLP, acetate, can flow into the DSR as carbon and energy source (Capone & Kiene, 1988). Conversely, the DSR end-product, CO_2, can serve as carbon source in the WLP (Figure 4.1A). It is therefore possible that a single bacterial species or organism is making use of both processes (Dörries et al., 2016). Indeed, our data show that Thermodesulfobacteriota and Deltaproteobacteria are associated with transcripts related to both DSR and WLP (Figure 4.4) and reveal several species transcribing for both WLP and DSR key genes at high frequencies.

The high transcription of *fhs* and *CODH/ACS* indicates high abundances of acetogenic bacteria that typically utilize the acetyl-CoA pathway to produce acetate as an end-product (Ragsdale & Pierce, 2008). However, low transcriptional levels of genes responsible for converting acetyl-CoA to acetate (*pta* and *ackA*, Figure 4.3B) may indicate anabolic incorporation of acetyl-CoA as the primary metabolic process, possibly reflecting a strategy to conserve energy. Acetyl-CoA is essential in various metabolic pathways, including CO_2 fixation pathways (Bährle et al., 2023), potentially making it more energy-efficient to channel it directly into other metabolic processes rather than converting it to acetate.

Anoxic, sulfate-rich wetland soils promote Deltaproteobacteria, Gammaproteobacteria and Thermodesulfobacteriota to be the drivers behind the main active microbial pathways, namely DRS and WLP (Figure 4.4). Gammaproteobacteria are known to be abundant in anoxic environments (Crump et al., 2007; Li et al., 2018) and increase towards marine conditions (Tebbe et al., 2022). The abundance of methanogenic microbial groups and transcripts encoding for methanogenesis was negligible in our study (Figure 4.3A). This high prevalence and activity of sulfate-reducing bacteria in comparison to methanogens is often explained by the higher free energy yield of sulfate reduction in relation to methanogenesis (Megonigal et al., 2004), which allows sulfate reducers to outcompete methanogens for energy or carbon substrates, like H_2 and acetate in sulfate-rich environments typically observed in coastal and marine ecosystems (Lovley et al., 1982; Oremland & Polcin, 1982; Poffenbarger, 2010).

Root activity-driven change in expression of soil microbial genes

The three replicates with root exudate influence and the three replicates without root exudate influence show a clear distinction in the expression of soil microbial genes (Figure 4.2B). The majority of differentially expressed genes is upregulated in response to root exudate influence (Figure 4.2A). This observation implies a strong stimulatory effect of the various plant exudates on the microorganisms and their metabolism and can be attributed to few pathway categories (Figure 4.5, Table 4.1). The metabolic categories with the strongest microbial response to root exudates were assigned to infection (log_2-fold change = 7.34), stress response (log_2-fold change = 7.03) and motility (log_2-fold change = 6.84) (Figure 4.5, Table 4.1). Notably, infection related genes also include those involved in establishing plant-beneficial symbiotic interactions. Thereby, microorganisms (pathogens and non-pathogens) are able to sense the root presence using root exudates and prepare to infect the plant (Büttner, 2016; Kloock et al., 2020).

Regarding stress response, genes can be part of defense mechanisms that bacteria activate to protect themselves from toxic environmental influences. This could, for example, be a response to the release of secondary plant compounds, oxygen, or reactive oxygen species upon pathogen recognition or response to abiotic stress (Chiang & Schellhorn, 2012; Huang et al., 2019). Concerning motility, bacteria direct their movement along chemical gradients in their environment and thus locate optimal conditions for growth and survival (Tan et al., 2013). Bacterial motility is also crucial for surface colonization and attachment to the root. The occurrence of transcripts relating to the cellular response to environmental stimuli, e.g. motility, aligns with finding from Wu et al. (Wu et al., 2023) , who also found motility, and additionally, polymer utilization to be enriched in the rhizosphere of maize, although, in contrast to our transcriptomic approach, the findings are based on metagenomics. Here, we demonstrated that also wetland plants influence the mobility of rhizosphere microbial communities.

Amino acid biosynthesis comes at a high cost for microorganisms and the corresponding biosynthesis pathways are tightly regulated. By utilizing amino acids via root exudates, soil microbes can save energy by abstaining from producing them internally. 3 of the 11 genes that were downregulated under the influence of plant exudates were assigned to the glutamate dehydrogenase (Table 4.2). The glutamate dehydrogenase catalyzes the conversion of glutamate to α-ketoglutarate and ammonium. This reaction is important for further downstream synthesis of other amino acids. In the presence of high levels of exudate-derived compounds, microbes may favor the use of these compounds over endogenous glutamate metabolism. By downregulating glutamate dehydrogenase, they can reduce competition for substrates and channel available resources toward other metabolic pathways that benefit from the exudates (Verhagen et al., 1995).

The metabolic categories translation, transport, and carbon metabolism have the highest query numbers (Figure 4.5, Table 4.1). Upregulation of translation genes typically indicate increased protein synthesis or enhanced efficiency in the translation process, in response to environmental changes (Tollerson & Ibba, 2020). Upregulated carbon metabolism genes indicate increased expression of proteins involved in utilizing carbon compounds, particularly in soils influenced by root exudates. Transport genes are upregulated to facilitate the movement of compounds between plant and microbial cells or the transport of enzymes for molecule degradation outside of the cell. Further, anaerobic wetland microbiota are known to rely on alternative terminal electron acceptors (Sutton-Grier et al., 2011). Impulses of oxygen via radial oxygen loss from roots into reduced soils can provide terminal electron acceptors for the oxidation of reduced Fe(II) (Megonigal et al., 2004). Additionally, it has been demonstrated by Yang et al. (2014), that wetland plants tend to have high Fe concentrations on root surfaces and in their rhizosphere. Our data suggest that, for example, Fe(III) deposition resulting from the oxidation of Fe(II) at the oxic-anoxic interface can be used as terminal electron acceptor by Fe-reducers, iron could be made available through plant exudates, as we observed a strong upregulation of genes directly related to iron transport in samples associated with high exudate influence (Table S4.2, Queries: Gene184520, Gene169819, Gene12770, Gene015030).

Higher relative abundance of transcripts assigned to certain microbial groups in response to root exudates compared to the abundance of these groups in the bulk soil reflect more favorable conditions for these taxa in the short-term and potentially increased competitive advantage in the long-term. The microbial community that reacts to root exudates by upregulation of genes differs from the community based on all transcripts (Figure 4.6). Specifically, the relative abundance of Betaproteobacteria, Campylobacterota, Kiritimatiellota, Lentisphaerota and Verrucomicrobiota increased based on assignment to upregulated genes due to root exudate influence (Figure 4.6). These results echo a similar metagenomic and metatranscriptomic rhizosphere study investigating rhizosphere effects of *Avena fatua* in a Bearwallow–Hellman loam soil (Nuccio et al., 2020), in which Betaproteobacteria and Verrucomicrobiota also significantly increased in the rhizosphere relative to the bulk soil based on 16S cDNA.

Method considerations and limitations

Our experimental approach aimed to capture microbial responses to exudates beyond their role as a carbon source, thus differing from RNA-SIP (RNA-Stable Isotope Probing) approaches, which rely on the uptake and metabolic utilization of exudates. Microbes can respond to exudates by different mechanisms without necessarily metabolizing them. For example, microbes may sense exudates and invest in flagella to move closer to or away from them. Also, exudates can act as repellents, toxins or inhibitors and influence microbial activity without directly serving as a carbon source. A drawback of our approach is, however, that it cannot clearly distinguish the effects of organic exudate release from those of other root processes, such as proton and oxygen release, as it uses a ^{13}C label to specifically track the exudate input to the soil. It is important to recognize that none of the currently available approaches alone are sufficient to fully capture the microbial response to exudates while excluding the influence of other root-related factors. To address this limitation, future research investigating microbial transcriptomic responses to root influence should assess options for combined approaches integrating multiple methods.

This study is further limited by its small sample size ($n = 3$ for each of ‘background’ and ‘root affected’ samples) and its focus on a single, albeit dominant and globally distributed species of coastal wetlands (Borges et al., 2021). These constraints may limit the generalizability of our findings. Therefore, future research should investigate larger sample sizes and wider geographic coverage to confirm these results at a larger scale.

Acknowledgements

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Supplementary Tables and Figures

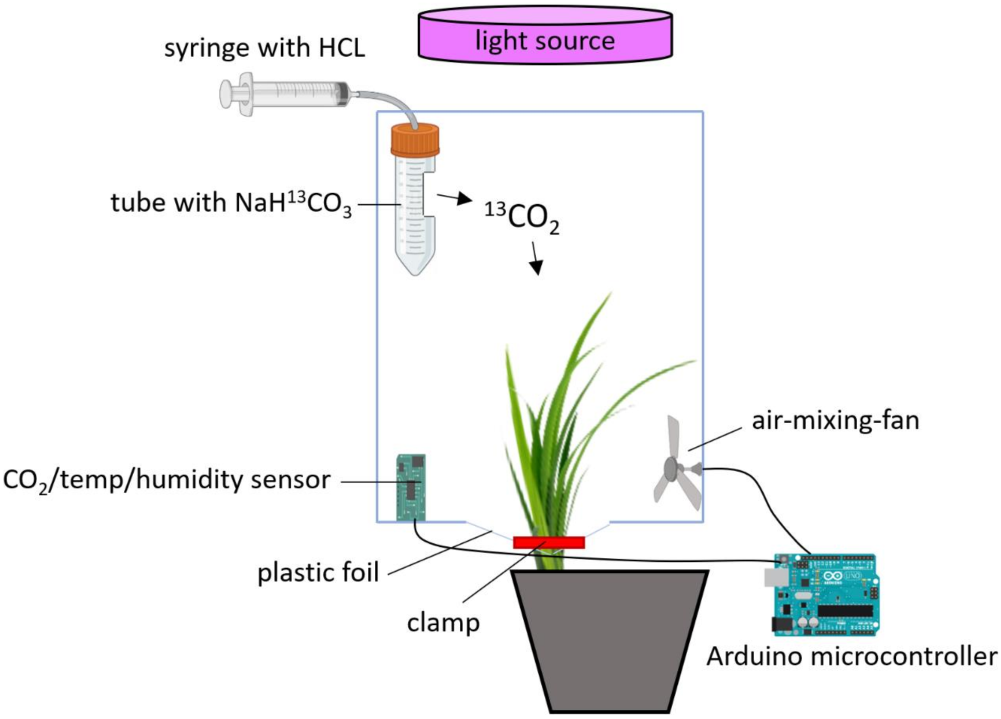

Figure S4.1. Schematic of $^{13}CO_2$ labeling chamber. Aboveground biomass is illuminated with an LED light source and exposed to $^{13}CO_2$ circulated in the transparent PVC chamber. Direct contact of labeled CO_2 with the soil was prevented by sealing the aboveground biomass at the shoot base using plastic film and clamps. A tight seal was accomplished and damage to shoots minimized by placing foam pads between clamp and shoots.

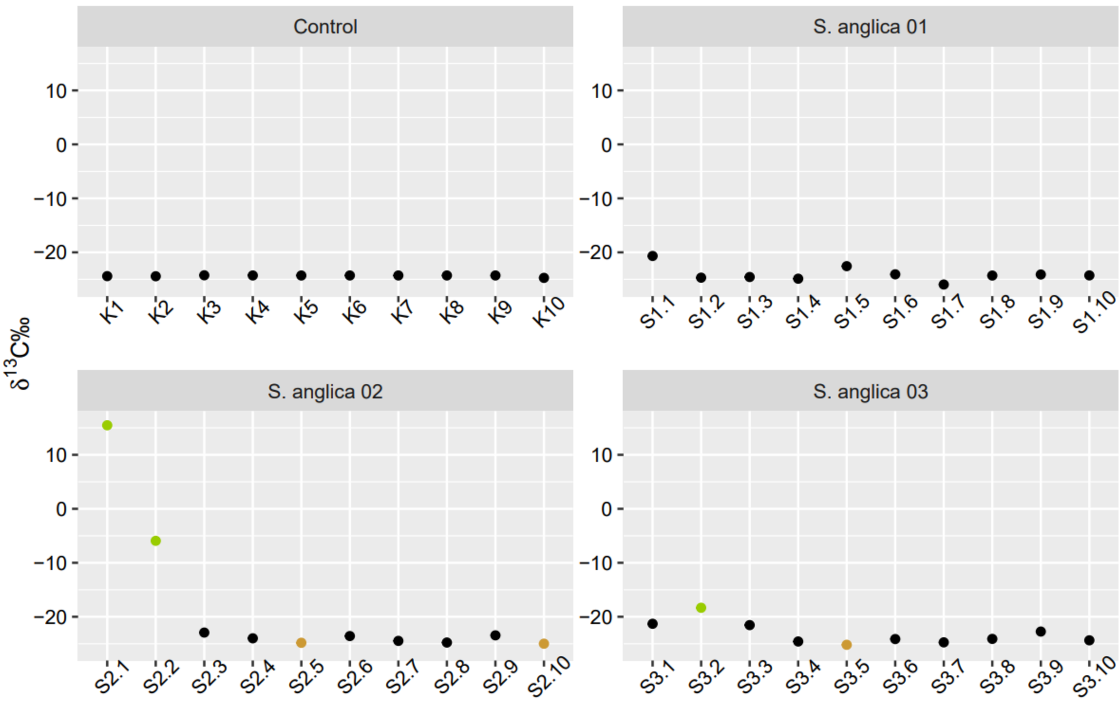

Figure S4.2. Scatterplot of delta ^{13}C signal in 40 soil samples after pulse-labelling (related to STAR methods). Colored are the samples used for metatranscriptomic sequencing. Soil samples with high ^{13}C signatures (i.e. rhizosphere soil) are in green; samples with low ^{13}C signatures (i.e. bulk soil) in brown.

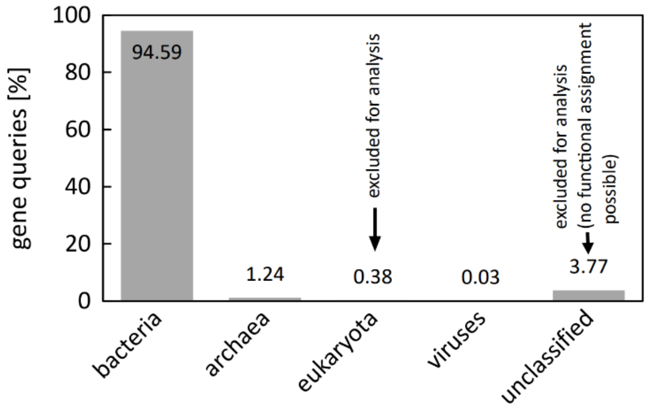

Figure S4.3. **Taxonomic distribution of gene queries.** Percentage of gene queries assigned to bacteria, archaea, eukaryota, viruses and genes that could not taxonomically be assigned (unclassified). Arrows indicate groups that were excluded for analysis.

Table S4.1. Bioinformatic output summary of the metagenome (metaG) that consists of a co-assembly of the metagenome 'NoRoot' and 'Root' (related to STAR methods).

	NoRoot	Root	co-assembled metaG
Total_Reads	74,148,310	80,603,444	37,965,400
Mapped reads	19,202,660	20,994,728	
Mapped [%]	25.90	26.05	
Num_of_scaffolds			80,894
Num_of_contigs			87,362
Contig [bp]			237,020,000
Gap [%]			0.145
N50			14,475
L50			3,372
N90			64,632
L90			1,217
N_Predicted_Genes			269,428
MAGs (compl. 70-99.8%)			13

Table S4.2. Bioinformatic output of metatranscriptomic reads (metaT) mapped to coassembled contigs (related to STAR methods).

	S2_1_high metaT	S2_2_high metaT	S3_2_high metaT	S2_7_low metaT	S3_6_low metaT	S3_10_low metaT
Total_Reads	11,926,662	20,677,854	13,468,226	16,843,094	27,279,520	5,420,742
mapped_Reads	828,063	1,421,828	1,031,738	1,046,490	1,721,926	387,670
Mapped [%]	6.94	6.88	7.66	6.21	6.31	7.15

Table S4.3. Bioinformatic output of mapped metatranscriptomic reads to the SILVA database (related to STAR methods).

	S2_1_high metaT	S2_2_high metaT	S3_2_high metaT	S2_7_low metaT	S3_6_low metaT	S3_10_low metaT
Total_Reads	59,183,872	92,628,710	66,058,376	71,013,190	106,000,000	20,247,024
mapped_Reads	33,114,620	50,674,333	38,172,098	33,699,450	49,910,414	9,508,265
Mapped [%]	55.95	54.71	57.79	47.46	47.03	46.96

5 Synthesis

Wetlands are important ecosystems for the global carbon cycle largely for their immense carbon sequestration capacity due to high net primary productivity and slow processes of microbially mediated OM decomposition. Plant-mediated effects on long-term carbon storage in wetland soils have been widely recognized, yet little is known about how these effects correlate to functional root traits. The aim of this thesis was to investigate rhizosphere processes in wetlands under the central hypothesis that plant-mediated effects on soil biogeochemistry (i.e. carbon cycling) can be explained by root trait variability. Due to the importance of soil redox potential for microbial mediated OM decomposition and therefore carbon fluxes in wetland soils, emphasis was given to root traits directly involved in redox process and plant-microbe interactions, i.e. the supply of oxygen (terminal electron acceptors), and organic carbon (i.e. electron donors) to the sediment.

Using a trait-based approach, physiological traits in the rhizosphere were examined to understand the relevance of functional root traits for whole-plant and ecosystem functioning. Specifically, a combined mesocosm-field study was conducted to quantify plant effects on soil redox potential along a flooding gradient (Chapter 2), variation in root traits responsible for root-sediment gas exchange was evaluated in two distinct life forms of an amphibious plant (Chapter 3) and root effects on transcriptional and compositional changes in the prokaryotic soil community (Chapter 4) were studied. The synthesis is divided into three sections beginning with a summary of the main insights gained from each of the previous chapters (*Key Findings*), followed by a discussion of how these findings relate to our broader understanding of root-mediated processes in wetland systems (*General Discussion*), and ending with further considerations for future research (*Perspectives and Outlook*).

Key Findings

Soil redox potential is controlled by plastic root responses to soil oxygen demand (Chapter 2)

Microbially-mediated oxidation of OM is impeded in wetland soils by thermodynamic constraints in the absence of oxygen. While it is known that wetland plants can modify oxidation-reduction states by providing terminal electron acceptors (i.e. oxygen) and electron donors (i.e. carbon-rich exudates, above- and belowground biomass) to the soil, roots of wetland plants are primarily believed to be soil oxidizers (Schreiner & Reed, 1909; Armstrong, 1967). Using a combined approach, soil reduction intensity values were collected from vegetated and non-vegetated plots of a field experiment and mesocosm study using the IRIS method. Additional plant traits (above-and belowground biomass) were measured to further relate ecological drivers to the observed plant effect on soil redox potential. In general, net plant reduction increased with increasing flooding frequency, though reduction intensity differed between species. The observed net plant effects on soil redox in the mesocosm study demonstrated both net reduction and net oxidation related to unvegetated soil. Vegetated plots in the field experiment consistently reduced the soil relative to control plots and plant soil reduction correlated positively with belowground biomass. A further investigation into radial oxygen loss from individual plant roots of two species revealed root trait plasticity in *Spartina anglica* that mirrored redox trends in the mesocosm and field experiment.

These findings indicate that i) net plant effects on soil redox potential are largely determined by existing redox gradients in the background soil to which plant roots exhibit a plastic response, ii) interspecific differences in plant soil reduction can help explain trends on the community-level from the *in situ* field experiment, and iii) plant effects on soil redox are likely linked to morphological (root biomass) and physiological (radial oxygen loss) root traits. These findings demonstrate the ability of plants to respond variably to soil oxygen demand at the community level and contribute as both net reducers and oxidizers of the system. The positive correlation of belowground biomass and soil reduction may help to identify other concomitant traits important for soil redox potential.

Belowground fine-root demography may be driven by seasonally induced changes in resource allocation (Box A)

The accessibility of belowground biomass for sequential measurements of root dynamics (i.e. production, mortality, turnover) is fraught with challenges such as sampling-related disturbance or lack of demographic root information using traditional methods like sequential coring or in-growth. In a pilot study, we addressed these challenges with the use of minirhizotrons, field-applicable transparent instruments, for repeated measurements of in situ root dynamics. The high turnover of fine roots makes them a potentially significant belowground carbon pool that fuels heterotrophic respiration and ultimately SOM formation. In the featured pilot study, minirhizotrons installed in the pioneer zone of a salt marsh (Kaiser-Wilhelm-Koog) were used to quantify root dynamics in the topsoil during the growing season.

The results indicate inverse relationships between productivity and mortality that may correlate with timely resource (re-)allocation into aboveground biomass. As similar studies in wetland soils are scarce, the parameters of this study can be expanded in the future to include additional elevation zones and salinity regimes of estuarine tidal marshes to better characterize the impact of abiotic factors on these trends.

Phenotypic variation in an amphibious plant affects sediment gas exchange efficiency in the rhizosphere (Chapter 3)

Amphibious plants display phenotypic plasticity in several plant traits such as leaf morphology, physiology or photosynthetic metabolism which enables them to swiftly adapt to aquatic and terrestrial conditions. Studying intraspecific trait variation enables one to relate phenotypic differences between life forms with physiological traits and make inferences about observed differences in function. This study compared physiological root traits of CO_2 uptake and O_2 release in distinct life forms (aquatic and terrestrial) of the amphibious isoetid plant *Littorella uniflora*. The aim was to determine the degree to which these traits were maintained between aquatic and terrestrial life forms under fluctuating water level and alternating light conditions. The findings revealed that aquatic *Littorella* plants did not respond to water level fluctuation and terrestrial *Littorella* demonstrated a light-dependent ability to maintain oxic rhizosphere conditions. CO_2 concentrations were lowest in aquatic *Littorella* rhizospheres and light-independent while CO_2 concentrations were consistently higher in terrestrial *Littorella* rhizospheres and accumulated under submerged conditions. Similarly, aquatic *Littorella* rhizospheres showed the highest O_2 concentrations and remained oxic in the dark, while rhizospheres in terrestrial *Littorella* went anoxic in the dark under submergence.

These findings indicate the root uptake of CO_2 from the sediment and root release of O_2 into the rhizosphere are somewhat limited in the terrestrial life form upon reintroduction to aquatic conditions. Under submerged conditions, impairment of efficient CO_2 uptake may restrict photosynthesis, while inadequate translocation of oxygen to roots may inhibit respiratory processes. Sediment oxygenation is further considered a key mechanism by which isoetids enhance soil CO_2 evolution and maintain oligotrophy within their environment. Further investigations are therefore required to determine the longevity of these responses and the physiological trait or trait combinations that give rise to them. Under future climatic scenarios of extended dry periods or severe water level fluctuations, the implications of this study could be significant for occurrence of isoetids and their role in maintaining oligotrophic lakes.

Species identity and substrate properties drive oxygen release patterns in the rhizosphere (Box B)

Wetland plant roots are subjected to a number of challenges in the soil under waterlogged conditions – as e.g., anoxia, accumulation of soil reductants and phytotoxins, and the precipitation of iron plaques on root surfaces. Wetland plants differ in their strategies to cope with these stressors such as the extent of aerenchyma development, diffusive barrier formation to ROL or mechanisms of internal gas transport. As these strategies all influence ROL, species-related variation in these factors may be detectable in ROL profiles. Additionally, substrate properties are also known to influence ROL patterns, though the relative importance of abiotic versus biotic factors are unclear. In a tidal tank experiment, six saltmarsh plant species: *Elymus athericus, Spartina anglica, Halimione (Atriplex) portulacoides, Aster tripolium, Puccinellia maritima,* and *Triglochin maritima* were cultivated in rhizoboxes and inundated bi-weekly with freshwater. Viable samples were later selected for ROL measurements using planar optode foils on various soil substrates (marsh, mixed, sand). Patterns of ROL varied among species and between substrates. ROL was generally higher on sandy substrates and often undetectable on more reducing soils (marsh, mixed) sediment. Iron deposition on the surface of or immediately surrounding roots was confirmed in this study and considered as further visual diagnostic tool for root oxidization (Armstrong, 1967). Despite technical challenges in the methodology, planar optodes are still a promising tool for high-resolution data on meaningful gas fluxes in the rhizosphere. As sediment oxygenation is seen as an adaptive strategy for wetland plants, examining physiological tolerance of changing substrate properties may help predict species-level responses and identify common strategies between plant functional types.

Roots reduce relative abundances of prokaryotic groups and stimulate microbial recruitment to the rhizosphere (Chapter 4)

Many studies have reported on transcriptional responses of root-associated microbial communities in aerated or agricultural soils, yet investigation in hydric soils is incredibly underrepresented (Bodelier & Dedysh, 2013). In this study, we combined stable isotope tracing with meta-transcriptomic sequencing to analyze root effects on prokaryotic gene expression and community composition. ^{13}C labeling was performed using a labeling chamber with a removable lid. The aboveground biomass of potted plants was inserted into the labeling chamber and sealed at the shoot base to prevent untargeted labeling of the soil. $^{13}CO_2$ was produced from the reaction of 10% HCl and $NaH^{13}CO3$ bicarbonate inside the chamber. A fan ensured circulation and CO_2 sensors monitored the evolution of CO_2. Pulse labeling of the aboveground biomass was limited to 2-3 hours at a time. Rhizosphere soil samples were operationally distinguished from bulk soil samples based on the strength of the ^{13}C isotopic signature following ^{13}C enrichment analysis. Enriched samples (relative to control samples) were regarded as being influenced by recent photosynthates from the plant (i.e. rhizosphere) and non-enriched samples (relative to control samples) were considered as bulk soil. Key gene analysis of bulk and rhizosphere soil showed a prevalence of two main metabolic pathways, Wood-Ljungdahl (WLP) and dissimilatory sulfate reduction-oxidation. In rhizosphere soil, microbial responses relating to chemotaxis and defense (motility, stress response and infection) as well as protein-synthesis and carbon metabolism were enhanced. Further shifts in the prokaryotic community composition could also be found in rhizosphere samples, though root influence rather acted on species evenness than on species richness.

These findings demonstrate the prevalence of energy-conserving metabolic pathways, indicating an energy-limited environment in both the bulk and rhizosphere soil. Nevertheless, root influence (i.e. root exudation) stimulated the movement of bacteria and archaea towards the rhizosphere for growth (protein synthesis) leading to increased relative abundances of certain prokaryotic groups which may have resulted in an enhanced competitive advantage.

General Discussion

Biogeochemical cycling of carbon in wetlands relies on the balance between OM input and OM output via degradation. Wetland plants play a key role in both sides of the equation through carbon sequestration and deposition into the soil (e.g. as litter, belowground biomass) and through indirect stimulation of soil microbial communities via oxygen release and root exudation. Variation in plant traits related to productivity, nutrient acquisition, and their capacity to exchange solutes with the soil are important regulators of these processes by controlling the degree of interaction between plants and their environment. A trait-based ecological approach attempts to link traits (e.g. morphological, physiological) to function across ecological scales (Shipley et al., 2016). Because variation in plant functional traits underlie plant responses, they also inform their adaptation potential across environmental gradients (Ackerly et al., 2000; Visakorpi et al., 2023). Trait variability – whether it occurs between species (interspecific variation), between genotypes of the same species (intraspecific variation) or in the differential expression of a single genotype (phenotypic variation) – becomes paramount in dynamic wetland ecosystems because high trait variability may facilitate the continuation of ecosystem functioning under changing environmental conditions, i.e. ecosystem resilience (Gladstone-Gallagher et al., 2019). Under strong selective pressures high root trait variability may give rise to adaptations on the individual level or become fixed at the community level (Sultan, 2000). However, one caveat is that plasticity as a root trait may itself be under selective pressure (Dawson et al., 2024) and is therefore not always necessarily advantageous (Grenier et al., 2016). Information on belowground traits and their changes along environmental gradients is scarce (Laliberté, 2017) with even fewer examples tailored to wetland systems (Moor et al., 2017; Pan et al., 2020).

Topic 1: Does root trait plasticity (to soil properties) facilitate adaptation to hydrological change?

ROL and barrier formation to oxygen leakage have been shown to be a plastic functional trait in some wetland plants (Colmer, 2003; Lemoine et al., 2012). As water poses a strong diffusive barrier to gas permeability, plants must develop strategies to fulfill their metabolic demands for oxygen and inorganic carbon in respiration and photosynthesis, respectively. However, trait expression may in some cases depend on substrate characteristics (grain size, porosity, organic content, soil redox potential) that modify the observed plant response.

Measured soil reduction in the field experiment located at the Wadden Sea showed a different pattern for vegetated plots compared to the mesocosm, highlighting the importance of physical soil properties on the observed plant effect. Compared to the substrate used in the mesocosm study (Figure 2.3b), the soils of the Wadden Sea were relatively well-aerated (Figure 2.5b) and were previously reported to have higher redox potentials (Mueller et al., 2020). In the field, plants consistently reduced the soil, whereas the mesocosm study demonstrated a bidirectional effect of vegetation on soil redox potentials. This observation exemplifies the ability of wetland plants to both oxidize and reduce the soil by responding to existing environmental redox conditions. In the mesocosm, where vegetated plots generally reduced the soil, a switch to net oxidation by plant roots was observed when redox levels in the unvegetated low marsh treatment was significantly reduced. This occurrence of net soil oxidation could reflect a potential induction (or enhancement) of ROL trigged by a particular redox threshold. This was also mirrored in the planar optode study with *Spartina anglica*, whose roots did not switch from net reduction to net oxidation until background O_2 concentrations in the bulk soil were already quite low (between approximately 2.5-8.5% O_2 atm. sat.; Figure 2.4a).

Plant-mediated sediment oxygenation is the result of two synergistic mechanisms – root porosity and diffusive barrier formation. Both aerenchyma formation and barrier formation (suberization, lignification) can be constitutively expressed or inducible (Colmer, 2002; Manzur et al., 2014). In *Spartina anglica*, aerenchyma are constitutively expressed and insensitive to further enhancement under flooded conditions (Maricle & Lee, 2002), while *Atriplex portulacoides* lacks aerenchyma (Rupprecht et al., 2015). While enhanced root porosity is unlikely to explain the switch in *Spartina anglica* roots from net reducing to net oxidizing (relative to bulk soil) in the planar optode study, these changes could be explained by inducible (plastic) ROL barrier formation. Investing in an inducible barrier to ROL with moderate strength, might be a preferable, 'cost-effective' strategy for *Spartina anglica* to cope with fluctuating water levels. Induction of barrier formation against ROL may carry investment costs due to lignin or suberin deposition that reduce root permeability (Manzur et al., 2014). However, an inducible barrier formation of moderate strength, i.e. ROL is not entirely inhibited, may reduce these costs. Under aerobic conditions, the suppression of barrier formation might enable unrestricted access for water and nutrients diffusion across the root surface, while moderate barrier formation under reducing conditions may protect the roots from phytotoxins while still ensuring an oxic niche at the tips for root respiration (Visser et al., 2000). Targeted oxygen release at the root tip, ensure efficient longitudinal transport to the root apex for growth by restricting unnecessary loss in the basal regions (Pedersen et al., 2021). In contrast, *Atriplex portulacoides* showed rapid oxygen depletion to anoxic levels and potentially endangered root vitality. As soil reduction correlates positively with increasing flooding frequency, root trait plasticity in oxygen leakage might be more advantageous for plants in low elevational zones of tidal marshes or where marsh boundaries are particularly vulnerable to increased flooding, subsidence or displacement.

In other plants, barrier formation to ROL might be maladaptive. As phenotypic plasticity is a source of intraspecific variation, investigating root traits in plant species known for their phenotypic plasticity can be useful in making inferences how these traits relate to an organism's ability to maintain their function (Sultan, 2000). In the investigation of sediment gas exchange traits in two life forms of *Littorella uniflora*, the terrestrial form demonstrated reduced efficiency of both CO_2 uptake and O_2 release into the sediment compared to the aquatic life form. It is assumed that phenotypic plasticity in *Littorella uniflora* is part of an adaptive strategy to keep them poised for acclimation to aquatic and terrestrial conditions. Previous literature proposes plasticity in leaf traits (e.g. heterophylly) that can be trigged by environmental cues help to prevent leaf desiccation and enable atmospheric uptake of CO_2, while the physiological root trait of sediment CO_2 uptake is conserved (e.g., Nielsen et al., 1991). The observations from the present study suggest reduced efficiency of this adaptation strategy in the terrestrial life form resulting from a loss of this conserved root trait. One hypothesis for the observed trait loss could be a reduction of root porosity under terrestrial conditions that becomes constitutively expressed after extended exposure of the plant to aerial conditions. Inducible changes in root porosity in the terrestrial form (i.e. retention of root trait plasticity) would enable it to more easily re-adapt to submerged conditions. Further research is needed to decipher the exact mechanisms behind this observed trait loss and their reversibility under longer observation.

Topic 2: Can a trait-based approach aid our understanding of plant responses in wetlands?

Mesocosms and controlled laboratory experiments provide the opportunity to investigate plant traits at the individual or species-level to gain a mechanistic understanding of how plant traits function and relate to *in situ* observations. By using soil redox indicators for single-species plots in the mesocosm study, it was possible to calculate net plant effects across all planting treatments and species-specific effects on soil redox conditions. The flooding treatment had a differential effect on distinct species which was shown in the variation of reduction intensity (Figure 2.3c). This is best exemplified by non-significant, but marked high soil reduction of *Atriplex portulacoides* compared to unplanted soil. In addition to relatively high belowground productivity (McMahon et al., 2023), plant litter of *Atriplex portulacoides* has also reportedly fast litter decomposition rates (Buth & De Wolf, 1985; Simões et al., 2011; Carrasco-Barea et al., 2022) which has been attributed to leaf litter quality (Simões et al., 2011; Carrasco-Barea et al., 2022). Microbial substrate use efficiency is directly linked to leaf litter quality as for example fewer extracellular enzymes are required by to depolymerize recalcitrant matter before assimilation (Cotrufo et al., 2013). Therefore, it is plausible that aboveground tissue quality may have contributed to higher soil reduction for this species, despite insignificant correlations in the quantity of dry-weight biomass. Comparatively high soil reduction for *A. portulacoides* at the individual level correlated with the soil redox pattern observed at the community level; the high relative soil reduction in the low marsh is dominated by the occurrence of *Atriplex portulacoides* and possibly reflects the disproportionate influence of this species-specific plant trait, i.e., soil reduction from *Atriplex portulacoides*. Interspecific variability was further highlighted in the corresponding planar optode investigation of ROL from single plant roots of *Spartina anglica* and *Atriplex portulacoides*. The plastic behavior of ROL in *Spartina anglica* roots relative to oxygen demand mirrored the plastic response in the mesocosm- and field experiments, thereby potentially identifying one of the mechanisms behind the net plant effects in the mesocosm study. Conducting investigations on various ecological levels therefore allowed us gain insight into interspecific root trait variability and its potential influence at the community-level. These findings are supported by other studies employing a trait-based approach that found interspecific differences in fine-root traits (decomposability) (Roumet et al., 2016) that correlated to patterns on the community-level (Prieto et al., 2015). From this I conclude that a trait-based approach is both applicable and informative in wetland soil systems and can benefit from the inclusion of additional functional traits to draw similarities between plant adaptation strategies to dynamic hydrology.

Topic 3: Exploring common ground – Expanding the root economic space

Plant-mediated soil reduction was correlated positively to belowground biomass, while aboveground biomass did not have a significant effect (Figure 2.6b). This underscores the importance of both physiological and morphological traits in elucidating the mechanisms behind plant responses to soil chemical gradients. Enhanced biomass resulting from new root growth (as opposed to root elongation) might increase the number of root tips capable of exudation. While observations from subtropical forests (Sun et al., 2021) give support for the positive correlation between root exudation and indicators of root metabolic activity (high root respiration and high root N content), a negative correlation was found between exudation and root density. Although this does not discredit the possibility of increased exudate-mediated soil respiration via enhanced belowground productivity for this study, enhanced soil reduction with increasing belowground biomass can also be explained by increased amount of substrate amount for degradation (i.e. turnover) or increased interaction with root endophytes such as mycorrhizal fungi that can also mineralize OM and reduce the soil environment. Both positive and negative correlations are essential to the development of new theories explaining trade-offs between traits – trait syndromes (Mommer & Weemstra, 2012; Dallstream et al., 2023) and connect economic spectra (spaces) between aboveground and belowground organs (Ye et al., 2024).

The root microbiome, the collective term all root-associated organisms, has increasingly become recognized as an integral component of the plant system, or holobiont (Vandenkoornhuyse et al., 2015; Carvalhais et al., 2017; Sapp et al., 2018). In this view, associated microorganisms of the rhizosphere can be considered as an extension of the root system and indicators of plant health and functioning. Trait-based approaches have already begun to use these biological traits (i.e. colonization of the rhizosphere by other organisms) as functional traits that can also be used to describe root trait syndromes concerning resource acquisition or stress tolerance in the root economic space. For example, mycelial networks developed by mycorrhizal fungi have a direct influence on nutrient acquisition for the plant and colonization of roots has been shown to increase under nutrient-deficient conditions. Therefore, the intensity of these biotic couplings can be used to make inferences of belowground nutrient pools and, when combined with other functional traits, reveal common strategies and trade-offs of functionally similar plants. This concept is demonstrated in the root economic space as a collaboration gradient (Bergmann et al., 2020; Figure 5.1), where colonization by mycorrhizal symbionts for nutrient acquisition ("outsourcing") is more likely in thicker roots with a larger root diameter, than in long roots with high specific root length for independent soil exploration ("do it yourself"). Combined with the conservation gradient, four quadrants of acquisition-growth strategies are presented: (1) "do-it-yourself-slow" (2) "outsourcing-slow" (3) "do-it-yourself-fast" and (4) "outsourcing-fast". While a proof of concept has been shown for grassland and forest species using trait data from the GRooT database (Rutten & Allan, 2023), the analysis did not cover physiological traits or those root traits in wetland species.

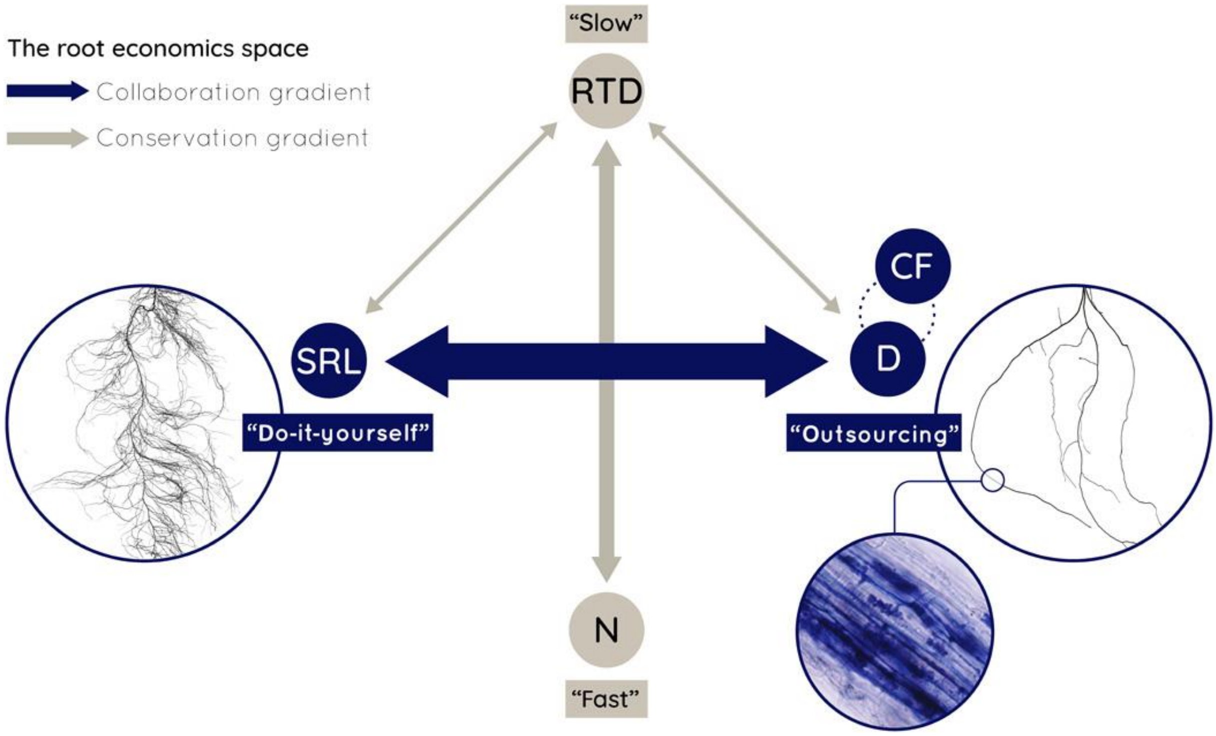

Figure 5.1. Visual conceptualization of collaboration gradient imposed on the conservation gradient for certain functional root traits as depicted by Bergmann et al. (2020). This conceptualization of the root economic space (RES) reflects a multidimensional framework for predicting the outcome of root trait interactions. This current view of the RES presents four quadrants of acquisition-growth strategies, (1) 'do-it-yourself-slow' (2) 'outsourcing-slow' (3) 'do-it-yourself-fast' and (4) 'outsourcing-fast'.

The prokaryotic fraction of the root microbiome may also hold similar potential to be integrating into the RES with regards to their functional diversity and tight association with the plant host (Bais et al., 2006; Ling et al., 2022). Using similar microbiology techniques as presented in Chapter 4 (isotopic pulse labeling and phylogenetic analysis), Hori et al. (2007) demonstrated a significant shift in the relative abundances of certain prokaryotic (bacterial and archaeal) classes (*Deltaproteobacteria*, *Acidobacteria*, *Planctomycetes*) that assimilated acetate from rice-influenced soil. Authors concluded that certain genera, known for iron reduction, were dominant in the oxic niches of rice paddy soil created by rhizosphere gradients of sediment oxygenation. Similarly, in the presented study on prokaryotic transcriptional activity in the rhizosphere, evidence of iron transport (S4.2) also support the possible activity of iron-reducers from high-exudate samples in the rhizosphere. In this way, microorganisms in the rhizosphere can act as bioindicators of soil quality (Singh et al., 2004; Singh & Sharma, 2020)

Recruitment of rhizosphere-associated microbes is commonly considered to be mediated by root exudates (Badri & Vivanco, 2009; Vives-Peris et al., 2020), which serve as chemoattractant and labile substrates for symbiotic and pathogenic microbes. This was validated by the transcriptomic analysis of prokaryotes in rhizosphere samples of *Spartina anglica* with elevated transcriptional activity of genes related to chemotaxis/motility as well as infection, the latter being applicable to beneficial and pathogenic prokaryotes (Figure 4.5). Although exudates are associated with the preference of fast-growing microorganisms (Sun et al., 2021), it has also been shown that microbial community structure changes be along gradients of environmental stress such as salt (Pan et al., 2022) or quality of root exudates (quality) (Dhungana et al., 2023) or other forms of rhizodeposition (Dennis et al., 2010). In salt marsh soils, key gene analysis revealed that metabolic activity was dominated by two energy-conserving pathways, WLP and DSR (Figure 4.3) that likely worked synergistically in a positive feedback loop. The necessity for energy-conservation might indicate heightened competition between roots and soil microorganisms for nutrients in the rhizosphere (Dijkstra et al., 2013), though further research is needed here to ascertain nutrient budgets and OM decomposition rates in the rhizosphere.

Future Research – Root-mediated processes of SOM formation

While the scope of this thesis addresses root-mediated mechanisms of carbon input and controls of microbially-mediated carbon decomposition, the mechanisms and root traits relevant for the formation and stability of stored carbon is another aspect that remains largely unexplored (Iversen et al., 2017), especially in wetlands (Mirabito & Chambers, 2023). Stabilization of organic carbon takes the form of SOM and the formation or destabilization of SOM determines the turnover rate of long-term carbon pools in wetlands. SOM primarily takes the form of mineral-associated organic matter (MAOM) in estuarine systems (Neiske et al., 2024 unpublished; Wu et al., 2024) and may be enhanced by rhizodeposition from roots. Specifically, low molecular weight compounds (i.e. root exudates) act as a source of easily degradable carbon for microbial decomposers that can increase soil respiration or enhance microbial biomass that ultimately forms tight associations with mineral surfaces resulting in an increase in MAOM (Sokol et al., 2019; Villarino et al., 2021; Chari & Taylor, 2022). Although some work has been done to uncover the chemical differences behind exuded compounds, most studies focus on sugars or organic acids, commonly believed to be the most abundant type of soluble exudates (Chari & Taylor, 2022). Because these labile exudates can also stimulate SOM decomposition through enhanced microbial respiration, the net effect of root exudates and other rhizodeposition regarding SOM formation is unclear (Dijkstra et al., 2021). The stimulation or depression of SOM decomposition by roots is referred to as the rhizosphere priming effect (RPE) and results in enhanced nutrient acquisition for the plant as well, either through reduction in competition for nutrients with soil microorganisms or increased nutrient availability (Carvalhais et al., 2011). Therefore, it would greatly benefit our conceptualization of the root economic spectrum to include physiological root traits (i.e. root exudation) and relate these to root nutrient content (e.g. root N or P). While great technological advancements have been made in rhizosphere research for the visualization and quantification of chemical gradients (Oburger & Schmidt, 2016; Neori & Agami, 2017; Wang et al., 2023), the proper isolation and characterization of root exudation is still extremely challenging (Oburger & Jones, 2018). New platforms offer hope for in-depth metabolomic studies under different environmental scenarios (Dietz et al., 2020; Steinauer et al., 2023). Rhizodeposition, including root exudates, comprises various compounds that vary in release mechanism, size, electrical charge, molecular weight, soil mobility, states of matter (Dennis et al., 2010; Vives-Peris et al., 2020). These compounds influence SOM or aggregate formation or destabilization through altered physicochemical soil properties such as water potential (mucilage) (Kroener et al., 2014; Sher et al., 2020). The nature of rhizodeposits (quantitative and qualitative characteristics) has been shown to be driven by genotypic diversity (Semchenko et al., 2021) and shape the microbial community (Paterson et al., 2007; Chapter 4). Still, much is left to be uncovered about their function in non-grassland systems (Henneron et al., 2020;

Semchenko et al., 2021), presenting an interesting research gap for wetland research that spans several disciplines including plant ecology, soil science, and microbiology.

6 References

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Author Contributions

This doctoral thesis consists of 5 chapters, including a general introduction (**Chapter 1**), three manuscripts (**Chapters 2**, **3** and **4**), and a synthesis (**Chapter 5**). The following text provides clarification regarding my personal contribution to the respective manuscripts featured in this dissertation. First authorship is denoted with an asterisk (*).

Chapter 2 J. Mittmann-Götsch, P. Mueller, and K. Jensen designed the field experiment. J. Mittmann-Götsch and M. Wilson set up the tidal tank mesocosm. M. Wilson conducted the planar optode study. J. Mittmann-Götsch conducted the IRIS study in both field and mesocosm, analyzed the data and wrote the manuscript together with P. Mueller, M. Wilson and K. Jensen.

Chapter 3 K. Koop-Jakobsen designed the experiment. K. Koop-Jakobsen conducted oxygen optode measurements and M. Wilson* and A. Frei conducted CO_2 optode measurements. R. Meier developed software applications for image analysis. M. Wilson analyzed the data and wrote the manuscript with contributions from K. Koop-Jakobsen, N. Lenzewski, P. Mueller and K. Jensen.

Chapter 4 P. Mueller and L. Grüterich designed the experiment. M. Wilson* and L. Grueterich* conducted the study, analyzed the data, and wrote the initial manuscript as equal contributors. M. Wilson and L. Grüterich finalized the manuscript with contributions from P. Mueller, K. Jensen, and W. Streit. P. Mueller, K. Jensen and W. Streit were responsible for funding acquisition and supervision.

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